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Quantifying soil N pools and N_2O emissions after application of chemical fertilizer and straw to a typical chernozem soil

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Abstract

An incubation experiment with equivalent N rates was conducted for 56 days using a typical black soil amended with chemical fertilizer with or without straw amendment using a ^{15}N cross-labeling technique. Compared with the chemical fertilizer treatment (^{15}NCF), chemical fertilizer combined with straw treatment (CF + S) showed a significantly higher ($P < 0.05$) contribution from applied N to microbial biomass N (BN) in the first 14 days and to particulate organic N (PON) and mineral-associated total N (MON) throughout the incubation. Straw application in the CF + S treatment significantly ($P < 0.05$) decreased the recovery of chemical fertilizer N as soil inorganic N except at day 3 but increased the recovery of chemical fertilizer N as BN before day 14 and as PON and MON from day 14 to the end of the incubation period. At the end of the incubation period, the total N_2O-N emissions in the CF + S treatment increased significantly ($P < 0.05$) compared with the CF treatment, and the increase in N_2O-N emissions was 73% from chemical fertilizer and 27% from straw N individually. Our results suggest that the combined application of chemical fertilizer and straw increased soil fertility together with an increase in N_2O emissions in the typical black soil and the N_2O emissions from straw cannot be ignored.

Keywords ^{15}N labeling · Inorganic N · Microbial biomass N · Dissolved organic N · Particulate organic N · Mineral-associated total N · Nitrous oxide

Introduction

The combined application of chemical fertilizer and straw is generally accepted as a promising practice to increase soil fertility (Chivenge et al. 2011; Liang et al. 2012; Pan et al. 2017). This practice can promote the immobilization of chemical fertilizer N in different soil N pools (Luxhøi et al. 2007; Qiu et al. 2012) and may also lead to an increase in greenhouse gas emissions (e.g., N_2O) (Li et al. 2013; Zhou et al. 2017) and this offsets the advantage of increased soil fertility to some extent. Understanding N immobilization in soil N pools and N_2O emissions is therefore necessary to evaluate and implement the practice of chemical fertilizer application combined with straw.

Soil N can be differentiated into labile and passive pools according to their turnover rates. The labile N pools respond sensitively to fertilization practices, and the passive N pools can reflect N retention capacity (Qiu et al. 2016). In agricultural ecosystems soil inorganic N (N_{inorg}), largely exchangeable NH_4^+-N and NO_3^--N is derived mainly from exogenous chemical fertilizers and can be immobilized into and released from soil organic N pools (Luce et al. 2014; Qiu et al. 2012). The labile organic N pools include soil microbial biomass N (BN), dissolved organic N (DON), and particulate organic N (PON) (Qiu et al. 2016). BN is both a source and a sink of soil N and the immobilization and mineralization of N by microorganisms controls the soil N supply (Brookes 2001; Sugihara et al. 2010). DON is generated from microbial decomposition and is utilized for microbial growth. Moreover, it can be

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mineralized to inorganic N and immobilized into passive N pools (Kalbitz et al. 2000; Marschner and Kalbitz 2003). PON represents partly decomposed plant residues and is an N source for soil microorganisms (Gregorich et al. 2006; Wander 2004). After PON removal using wet sieving method (Bronson et al. 2004), N in the residual soil particle is defined mineral-associated total N (MTN) (He et al. 2015). MTN holds the greater proportion of soil N than PON (Wander 2004) and retains the N substrates by mineral surface sorption or organic-mineral association formation (Lützow et al. 2006; Sokol et al. 2018). Consequently, the investigation of different soil N pools provides key information on soil N transformations and retention.

Chemical fertilizers and straw exert inconsistent effects on soil N pools. Chemical fertilizer application increases soil inorganic N (Ju et al. 2009), and the return of crop straw to the soil promotes the immobilization of inorganic N (Luxhøi et al. 2007). Several studies have documented an increase in the size of BN after chemical fertilizer and straw application (Blagodatskaya and Kuzyakov 2008; Said-Pullicino et al. 2014). Liang et al. (2011) report that DON increased when straw was applied alone or combined with chemical fertilizer but not when chemical fertilizer was applied alone. PON increased more under long-term applications of straw and chemical fertilizer than under chemical fertilizer applications (Qiu et al. 2016). He et al. (2015) investigated three Chinese agricultural soils after long-term fertilization and found that chemical fertilizer did not affect MTN and the effect of straw application on MTN was site-dependent. Overall, these studies have focused mainly on the changes in absolute contents of different soil N pools as affected by different long-term fertilization practices (He et al. 2015; Liang et al. 2011; Qiu et al. 2016). However, the contribution of chemical fertilizer N and straw N to different soil N pools is poorly known when the combination of chemical fertilizer and straw are compared.

Agricultural soils are important sources of N_2O, a key greenhouse gas that is greatly affected by agricultural management practices such as application of chemical fertilizer N or organic N (Smith et al. 2008; Snyder et al. 2009). Application of chemical fertilizer N generally increases N_2O emissions (Bouwman 1998; Shcherbak et al. 2014), but the effects of combinations of chemical fertilizer and straw on N_2O emissions remain elusive (Abalos et al. 2013; Frimpong and Baggs 2010; Li et al. 2013; Shan and Yan 2013; Wang et al. 2019). Thus, accurately distinguishing the N_2O emissions derived from different N sources (e.g., chemical fertilizer, straw, and soil) and directly quantifying the N_2O generated from straw N would make a valuable contribution to our understanding of the interactive effects between chemical fertilizer N and straw N after their combined application to soils.

Northeast China is known nationally as the “corn belt” and is responsible for 35% of total Chinese maize production (China Agriculture Yearbook 2014). However, the indigenous highly fertile black soils (Udic Mollisols) typical of this region are currently subject to substantial soil degradation by long-term irrational intensive fertilization practices (Xie et al. 2007). It has recently been recognized that the management practice of applying chemical fertilizer combined with straw is effective in maintaining or increasing soil fertility and soil N storage in these soils (Qiu et al. 2016; Wang et al. 2013; Wei et al. 2016).

However, it remains unclear how the chemical fertilizer- or straw-N is distributed among different soil N pools and what is the contribution to N_2O emissions in black soils of Northeast China. By using ^{15}N cross-labeled tracer techniques, we can understand quantitatively how straw application affects the N transformations and N contribution to different soil N pools and to N_2O emissions from different fertilizers. Here, we therefore aimed to (1) quantify the distribution and recovery of chemical fertilizer N and straw N in the soil N pools, such as N_{inorg}, BN, DON, PON, and MTN and in N_2O, and (2) elucidate the dynamics of the different soil N pools under chemical fertilizer alone or under the combined application of chemical fertilizer and straw. Overall, we hypothesized that chemical fertilizer combined with straw leads to different distribution patterns of soil N pools and produces greater N_2O emissions than application of chemical fertilizer alone and that straw is an important contributor to N_2O emissions.

Materials and methods

Soil sampling

The soil studied was collected after the maize harvest from the top 20 cm of the profile in a field located at the Modern Agricultural Science and Technology Demonstration Park of Heilongjiang Academy of Agricultural Sciences in Harbin city, Heilongjiang province, Northeast China (45°50’ N, 126°51′ E; elev. 130 m). The local climate is cold temperate continental monsoon with a mean annual temperature of 3.6 °C and mean annual precipitation of 486 mm. The soil type is Mollisol (Typic Hapludoll, Soil Survey Staff 2003) developed from Quaternary loess-like parent materials. A total of 20 soil cores from the field were collected using a 10-cm-diameter soil auger and a serpentine sampling pattern. The cores were thoroughly mixed to give one composite sample which was divided into two subsamples, one was stored at 4 °C for the incubation experiment, and the other was air-dried and ground for determining soil physicochemical properties. Selected soil physicochemical properties are shown in Table 1.

Table 1 Selected physicochemical properties of the soil

Soil property	Value
pH	6.9
Organic C (g kg^{-1})	19.4
Total N (g kg^{-1})	1.8
Available P (mg kg^{-1})	9.6
Available K (mg kg^{-1})	155.9
Exchangeable NH_4^+-N (mg kg^{-1})	1.0
NO_3^--N (mg kg^{-1})	21.8
Soil texture	
Sand (%)	27.4
Silt (%)	48
Clay (%)	24.6

^{15}N labeling of straw

^{15}N-labeled straw was prepared in a maize (*Zea mays* L. cv. ZD958) pot experiment with a low fertility soil in a greenhouse. As reported by Qiu et al. (2012), the maize was planted and fertilized with 30.16 atom% $(^{15}NH_4)_2SO_4$ at a rate of 150 mg N kg^{-1} to obtain maize straw with a relatively high ^{15}N abundance. In addition, NaH_2PO_4 at 65 mg P kg^{-1} and KCl at 125 mg K kg^{-1} were applied on 29 April 2012. Additional ^{15}N labeled chemical fertilizer (1 g $plant^{-1}$) was applied on 26 May and (2 g $plant^{-1}$) on 3 July as topdressing fertilizer to prevent maize N deficiency. At the maize tasseling stage, the ear was covered with a paper bag to prevent pollination. After the maize harvest on 20 August, the ^{15}N labeled straw was dried at 60 °C and ground. The labeled maize straw had an organic C content of 434.8 g kg^{-1}, a total N content of 9.6 g kg^{-1}, and a ^{15}N abundance of 15.24%. The corresponding unlabeled maize straw was planted in the field near the greenhouse with the same soil type and maize cultivar. The maize sowing and harvest dates of the unplanted maize were the same as those of the labeled plants. The total organic carbon, total N, and ^{15}N abundance in the unlabeled plants were 448.6 g kg^{-1}, 11.3 g kg^{-1}, and 0.37%, respectively.

Experimental design

An equivalent N amendment incubation experiment with a randomized complete design was carried out in the dark in an incubator at 20 °C for 56 days. The treatments were (1) control (CK); (2) ^{15}N labeled chemical fertilizer (^{15}NCF); (3) chemical fertilizer combined with straw (CF + S). The CF + S treatment had two sub-treatments: (i) ^{15}N labeled chemical fertilizer combined with straw (^{15}NCF + S) and (ii) ^{15}N labeled straw combined with chemical fertilizer (^{15}NS + CF). The N rate was 70 mg kg^{-1} in treatments with chemical fertilizer N amendment, and the ratio of chemical fertilizer N to straw N was 6:4 in the CF + S treatment. The chemical fertilizer used was $(NH_4)_2SO_4$, and the ^{15}N isotope abundance of the $(^{15}NH_4)_2SO_4$ was 20.2%. Stones, straw, and roots were removed from the freshly collected soil, and the soil was sieved (< 2 mm) before incubation. The sieved soil was pre-incubated for 1 week under controlled conditions (in the dark, at 20 °C, and at 40% water-filled pore space) to activate soil microbial activity. The pre-incubated soil was then divided into four parts to set up the four treatments. Chemical fertilizer N solution was sprayed onto the pre-incubated soil in the fertilizer N treatments, and the 0.25 mm sieved straw powder was uniformly mixed with the pre-incubated soil in the CF + S treatment. The moisture content of the pre-incubated soil in each treatment was adjusted to 45% of water-filled pore space with deionized water. Finally, a 220-g aliquot of soil of each treatment at 45% WFPS soil moisture was placed in a 1-L glass bottle. Three replicate bottles of each treatment were prepared for each sampling time (1, 3, 7, 14, 21, 28, 42, and 56 days of incubation), giving a total of 96 glass bottles for the four fertilizer treatments. All glass bottles were sealed with stoppers and transferred to an incubator. Aerobic conditions were maintained by opening the glass bottles for up to 15 min every day.

Sample analysis

Soil inorganic N (N_{inorg}, exchangeable NH_4^+-N, and NO_3^--N) was extracted with 1-M KCl solution (1:5, *w*/*v*) on a reciprocal shaker for 1 h and then determined with a continuous flow analyzer (FIAstar5000, Foss, Hillerød, Denmark). The ^{15}N abundance of exchangeable NH_4^+-N and NO_3^--N was determined using an isotope ratio mass spectrometer (Finnigan MAT251, Thermo Fisher, Waltham, MA) after oxidization of exchangeable NH_4^+ to N_2 with sodium bromate (NaBrO) (Laughlin et al. 1997) and reduction of NO_3^- to N_2O with Cd-Cu (Stevens and Laughlin 1994).

Soil microbial biomass N was determined by the chloroform fumigation-K_2SO_4 extraction method (Brookes et al. 1985). Briefly, the incubated soil was subjected to $CHCl_3$ fumigation for 24 h and then extracted with 0.5 M K_2SO_4 solution at a soil:water ratio of 1:4 (*w*/*v*) for 0.5 h. The extracted solutions from fumigated and unfumigated soils were treated by the alkaline persulfate oxidation method as described by Cabrea and Beare (1993), and the NO_3^--N concentrations in the fumigated or unfumigated solutions were determined using a continuous flow analyzer (FIAstar5000, FOSS, Hillerød, Denmark). Soil microbial biomass N was calculated using the formula $(N_f\text{-}N_{uf})/K_{EN}$, where N_f and N_{uf} are the N concentrations in soil extracts using 0.5 M K_2SO_4 from fumigated and unfumigated soils, respectively, and K_{EN} is the conversion factor of 0.45 (Jenkinson 1988). Soil dissolved organic N (DON) was calculated by subtracting N_{inorg} from the N concentration in the unfumigated solution extracted with 0.5 M K_2SO_4 (Cookson et al. 2007; Qiu et al. 2016). In accordance with Hauck et al. (1996), the fumigated and unfumigated solutions were acidified with 0.5 M H_2SO_4 and then oven-dried at 60 °C for the determination of total ^{15}N abundance by isotope ratio mass spectrometry (Finnigan MAT 251, Thermo Fisher, Waltham, MA).

Particulate organic matter (POM) and mineral-associated matter were fractionated as described by Bronson et al. (2004). Briefly, 25 g of air-dried soil (< 2 mm) were dispersed in 5% sodium hexametaphosphate solution at a soil:solution ratio of 1:4 (w/v) on a reciprocal shaker for 1 h. The suspension was sieved using a 53-μm mesh until the deionized water became clear, and both < 53- and > 53-μm soil fractions were transferred to beakers separately for drying at 60 °C. Nitrogen in both isolated soil fractions is termed particulate organic N (PON, > 53 μm) and mineral-associated total N (MTN, < 53 μm), and their N concentrations and ^{15}N abundances were determined using an elemental analyzer (Macrocube, Elementar, Hanau, Germany) and isotope ratio mass spectrometry (Delta Plus XP, Thermo Finnigan, Waltham, MA), respectively.

Gas samples were collected on days 1, 3, 7, 14, 21, 28, 42, and 56 of the incubation period. At each sampling time, 20- and 40-ml samples were taken from the headspace of the incubated glass bottles using a gas-tight syringe connected to a two-way valve. The 20-ml gas samples were used for analysis of N_2O concentration using gas chromatography (6890 N, Agilent Technologies, Santa Clara, CA). The 40-ml gas samples were used to determine the ^{15}N abundances of N_2O using a gas chromatograph linked to an isotope ratio mass spectrometer system (Finnigan MAT 251).

Calculations

N_2O-N emission flux was calculated according to the following equation:

$$E = (A - B)/1000000 \times V \times P/(R \times T) \times 28 \times 1000 \times 1000/m \tag{1}$$

where E is the N_2O emission flux (μg N kg^{-1} d^{-1}), A is the N_2O concentration in the natural air (ppmv), B is the N_2O concentration in the sample air (ppmv), V is the headspace volume (0.81 L), P is standard atmospheric pressure (101.3 Pa), R is the universal gas constant (8.314), T is the absolute temperature (291 K), 28 is the mass number of N per mol N_2O molecule, and m is the weight of dry soil used in the incubation (0.182 kg).

Cumulative N_2O-N emission was calculated according to the following equation:

$$C_{t'} = C_t + (E_t + E_{t'})/2 \times (t' - t) \tag{2}$$

where t and t′ are the two neighboring sampling days, respectively (d), C_t and $C_{t'}$ are cumulative N_2O-N emissions at t and t′ (μg N kg^{-1}), and E_t and $E_{t'}$ are N_2O-N emission fluxes at t and t′ (μg N kg^{-1} d^{-1}).

The content of N derived from chemical fertilizer or straw in a specific soil N pool or N_2O emission was calculated as follows:

$$N_{dff} = CON_p \times APE_p \times 100/APE_a \tag{3}$$

where CON_p is the content of a specific soil N pool (mg N kg^{-1}) or N_2O-N emission (μg N kg^{-1}), APE_p is the ^{15}N atom percent excess of a specific soil N pool or N_2O-N emission flux (%), and APE_a is the ^{15}N atom percent excess of the applied chemical fertilizer or straw N (%).

The contribution from applied N to a specific soil N pool and the recovery of applied N as a specific soil N pool were calculated using following equations:

$$P_{con} = APE_p \times 100/APE_a \tag{4}$$

$$P_{re} = CON_p \times APE_p \times 100/APE_a/CON_a \tag{5}$$

where P_{con} is the contribution from applied N to a specific soil N pool or N_2O (%), P_{re} is the recovery of applied N as a specific soil N pool or N_2O (%), CON_a is the N amount of applied chemical fertilizer or straw N (mg), and CON_p, APE_p, and APE_a are as described above.

Statistical analysis

The effects of different fertilizer amendment treatments on the contribution from applied N to the specific N pools (exchangeable NH_4^+, NO_3^-, N_{inorg}, BN, DON, PON, MTN, and N_2O), the recovery of applied N as the specific N pools, and the accumulated N_2O-N emissions were assessed using analysis of variance (ANOVA) with the SAS 8.01 statistical software package and the mean values were compared by least significant difference (LSD) at the 5% protection level.

Results

Soil exchangeable NH_4^+-N, NO_3^--N, and total inorganic N

The contribution from applied N to exchangeable NH_4^+-N in the CF + S treatment was significantly lower ($P < 0.05$, Fig. 1a) from days 1 to 3 than in the CF treatment and

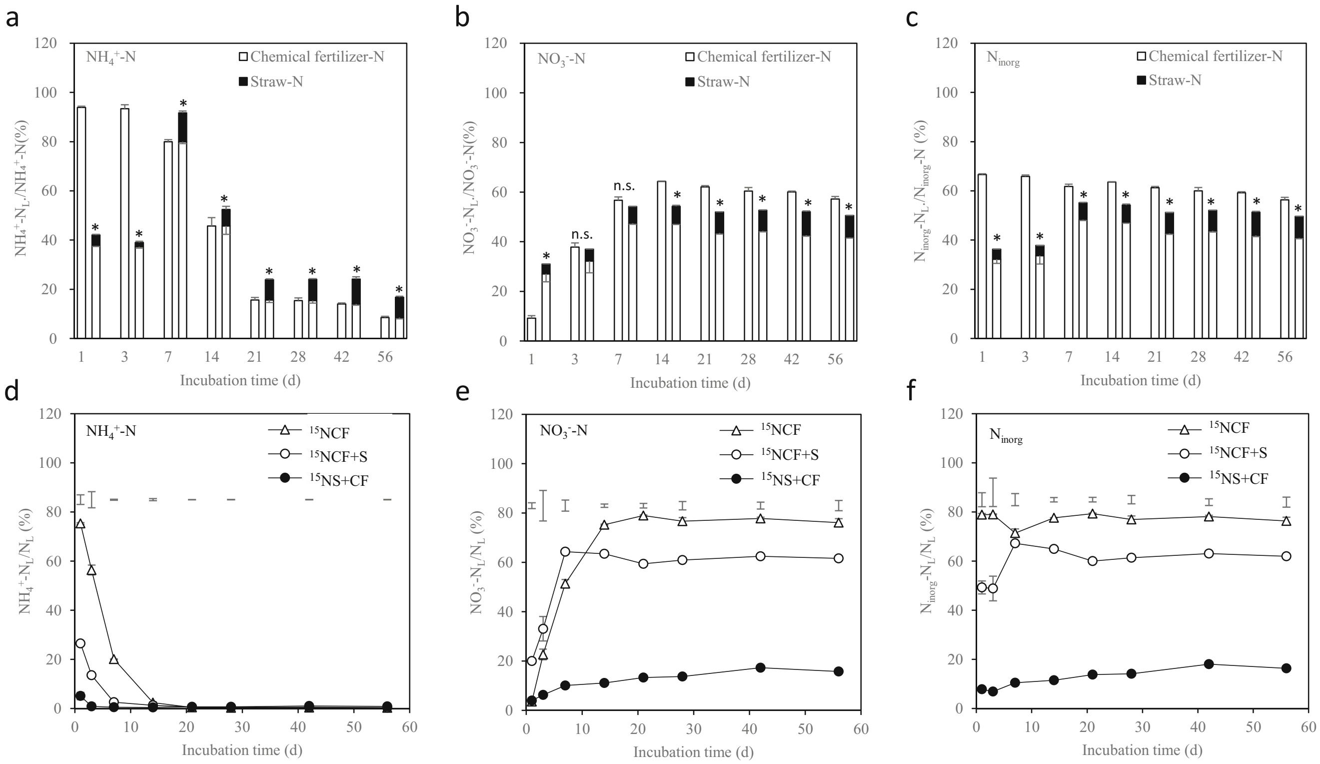

Fig. 1 The contribution from applied N (a, b, c) to exchangeable ammonium (NH_4^+-N), nitrate (NO_3^--N), and inorganic N (N_{inorg}) and the recovery of applied N (d, e, f) as exchangeable ammonium (NH_4^+-N), nitrate (NO_3^--N), and inorganic N (N_{inorg}) under different fertilization treatments during a 56-day incubation period. In the bar charts (a, b, c), white-only bars represent chemical fertilizer treatment (CF). White plus black bars represent chemical fertilizer combined with straw treatment (CF + S). (*) indicates significant difference ($P < 0.05$). In the line graphs (d, e, f), the symbols in the legend indicate the fertilizer treatments. The vertical lines represent $LSD_{0.05}$ values.
Error bars for all data represent one standard error of the mean ($n = 3$). Subscript L indicates labeled fertilizer.

significantly higher ($P < 0.05$, Fig. 1a) from days 7 to 56, and the contribution from applied N to NO_3^--N and N_{inorg} in the CF + S treatment was significantly lower ($P < 0.05$, Fig. 1b, c) except for NO_3^--N on day 3.

The recovery of applied N as exchangeable NH_4^+-N showed a rapid decrease during the first 7 days in the fertilizer treatments (Fig. 1d), and the sequence of significance ($P < 0.05$) was ^{15}NCF > ^{15}NCF + S > ^{15}NS + CF. Correspondingly, the recovery of applied N as NO_3^--N showed the opposite trend (Fig. 1e). A significant sequence ($P < 0.05$, Fig. 1e, f) similar to exchangeable NH_4^+-N was also found for NO_3^--N after day 14 and for N_{inorg} throughout the incubation period with the exception of day 7.

Soil microbial N and dissolved organic N

The contribution from applied N to BN was significantly higher ($P < 0.05$, Fig. 2a) in the CF + S treatment than in the CF treatment except for days 21 to 42 during the incubation period. The recoveries of applied N as BN in ^{15}NCF + S and ^{15}NS + CF treatments were both significantly higher ($P < 0.05$, Fig. 2c) than in the ^{15}NCF treatment during the first 14 days of incubation. Compared with the ^{15}NS + CF treatment, ^{15}NCF + S treatment showed significantly greater ($P < 0.05$, Fig. 2c) recovery of applied N as BN in the first 3 days.

The contribution from applied N to DON in the CF + S treatment ranged from 0% to 16.38% (Fig. 2b). In most cases, no contribution from chemical fertilizer N to DON was detected (Fig. 2b, d).

Particulate organic N and soil mineral-associated total N

Compared with the CF treatment, the CF + S treatment increased the contribution from applied N to soil particulate organic N (PON) and soil mineral-associated total N (MTN) significantly ($P < 0.05$, Fig. 3a, b). There were significant differences ($P < 0.05$, Fig. 3c) among fertilizer treatments in the recovery of applied N as PON with a sequence of significance of ^{15}NS + CF > ^{15}NCF + S > ^{15}NCF and a similar sequence ($P < 0.05$, Fig. 3d) was also found for recovery of applied N as MON during the incubation period after day 1.

N_2O-N flux and cumulative N_2O-N emission

The CF + S treatment increased the contribution from applied N to N_2O-N significantly ($P < 0.05$, Fig. 4a) compared with

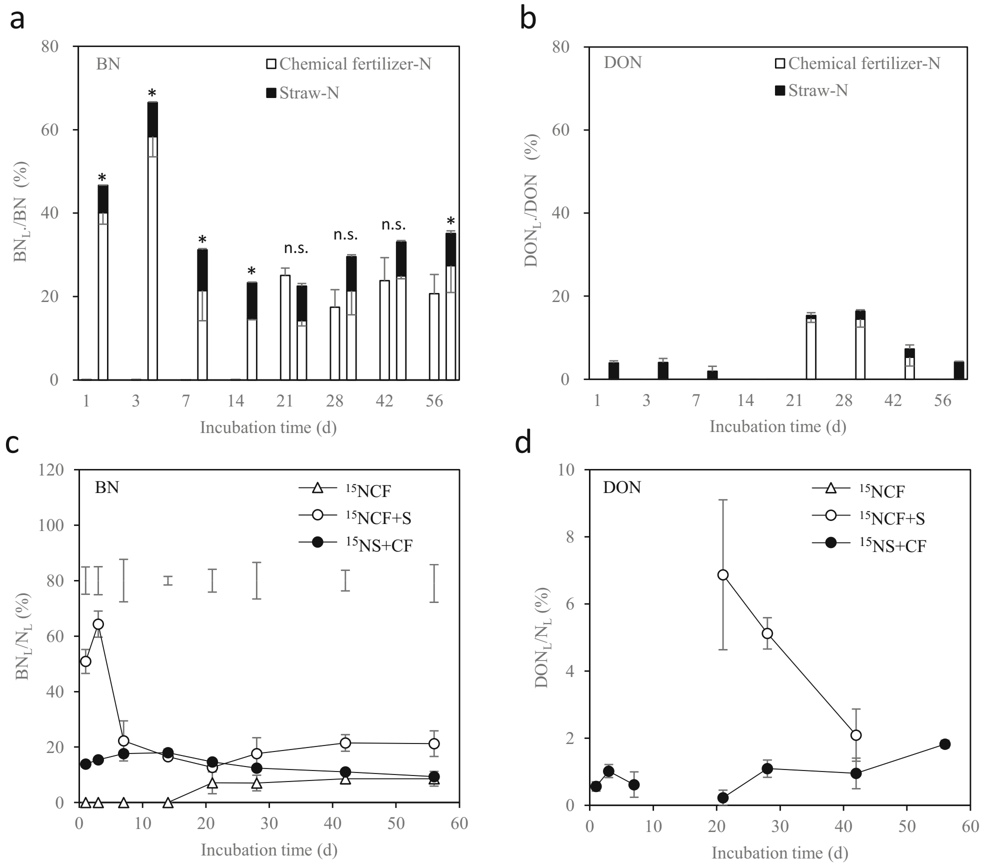

Fig. 2 The contribution from applied N (a, b) to microbial biomass N (BN) and dissolved organic N (DON) and the recovery of applied N (c, d) as microbial biomass N (BN) and dissolved organic N (DON) in different fertilization treatments during a 56-day incubation period In the bar charts (a, b), white-only bars represent chemical fertilizer treatment (CF). White plus black bars represent chemical fertilizer combined with straw treatment (CF + S). (*) indicates significant difference ($P < 0.05$). In the line graphs (c, d), the symbols in the legend indicate fertilizer treatments. The vertical lines represent $LSD_{0.05}$ values. Error bars for all data represent one standard error of the mean ($n = 3$). Subscript L indicates labeled fertilizer.

the CF treatment, except for days 28 and 56. The ^{15}NCF + S treatment showed a significantly greater ($P < 0.05$, Fig. 4b) recovery of applied N as N_2O-N than did the ^{15}NS + CF treatment or the ^{15}NCF treatment throughout the incubation period.

The cumulative N_2O-N emission in the different fertilizer treatments followed the significance order CF + S > CF > CK throughout the incubation period ($P < 0.05$, Table 2). A significantly higher ($P < 0.05$) N_2O-N from soil sources was found in the CF and CF + S treatments compared with CK. The N_2O-N from chemical fertilizer in the CF + S treatment increased significantly ($P < 0.05$) compared with the CF treatment. The straw N in the CF + S treatment emitted 5.6% of total N_2O-N.

Discussion

Soil N pools

A rapid decrease in the contribution from applied N to exchangeable NH_4^+-N and a quick increase to NO_3^--N during the first 7 days (Fig. 1a, b) indicates that rapid nitrification of exchangeable NH_4^+-N occurred. Our soil moisture content of 45% WPFS also supports the soil nitrification according to Bouwman (1998). Straw amendment provides C sources for soil microorganism (Fontaine et al. 2011; He et al. 2011), further promoting the nitrification in the CF + S treatment compared with the CF treatment. For example, the nitrification rate in the CF + S treatment was 1.4 times that in the CF treatment in the first 7 days (Fig. 1e).

Soil inorganic N can be generated by mineralization and immobilization turnover (MIT), and C source amendment can accelerate this process (Chen et al. 2014a). Straw C stimulated chemical fertilizer N immobilization by microorganisms in the first 14 days (Gentile et al. 2009; Nicolardot et al. 2001) as shown by the significantly higher contribution from applied N to BN in the CF + S treatment than in the CF treatment (Fig. 2a). The transformation of microbially immobilized N to the other soil N pools (Quan et al. 2016) further resulted in a significantly lower recovery of labeled chemical fertilizer N as NO_3^--N (Fig. 1e). After incubation for 7 days, the microbial mineralization of immobilized fertilizer N stimulated by straw C resulted in a significantly higher contribution of fertilizer N to exchangeable NH_4^+-N in the CF + S treatment than in the CF treatment. From day 3 to day 7 in the CF + S treatment, as the easily decomposed C in the straw was consumed, microbiome died and the chemical N immobilized by microorganisms was re-mineralized (Chen et al. 2014a), as shown by the decrease in chemical fertilizer N in BN (Fig. 2a, c) and

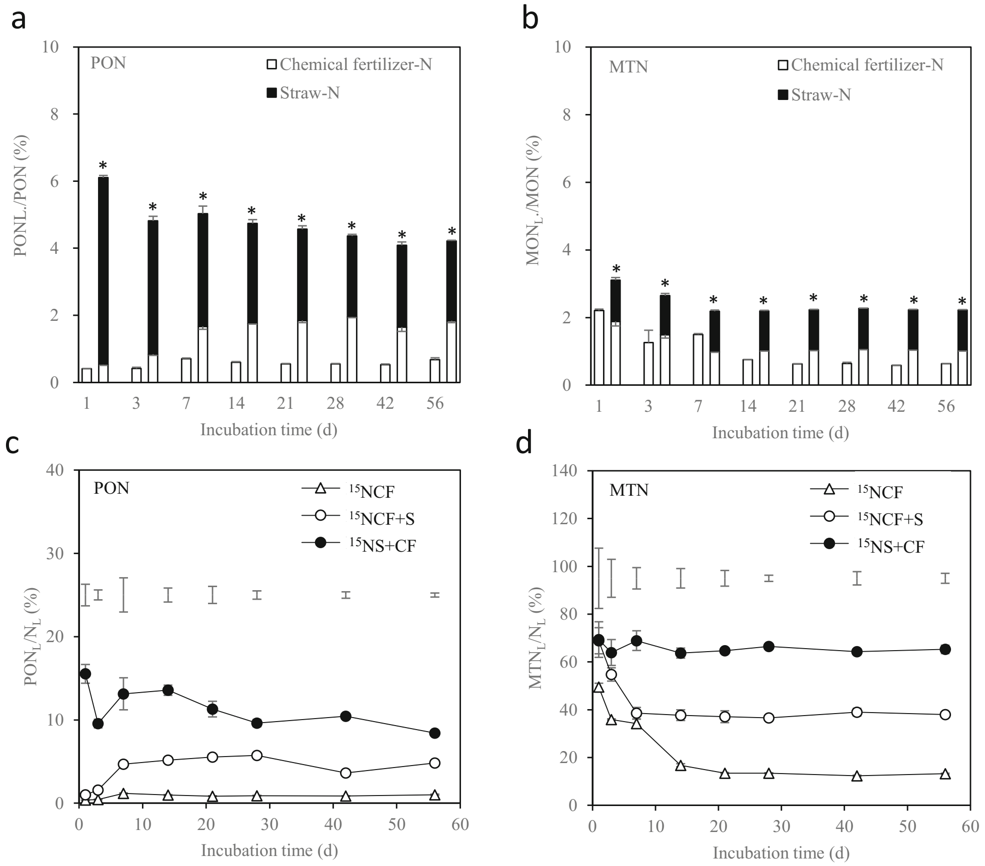

Fig. 3 The contribution from applied N (a, b) to particulate organic N (PON) and mineral-associated total N (MTN) and the recovery of applied N (c, d) as particulate organic N (PON) and mineral-associated total N (MTN) in different fertilization treatments during a 56-day incubation period In the bar charts (a, b), white-only bars represent chemical fertilizer treatment (CF). White plus black bars represent chemical fertilizer combined with straw treatment (CF + S). (*) indicates significant difference ($P < 0.05$). In the line graphs (c, d), the symbols in the legend indicate fertilizer treatments. The vertical lines represent $LSD_{0.05}$ values. Error bars for all data represent one standard error of the mean ($n = 3$). Subscript L indicates labeled fertilizer.

the increase in chemical fertilizer N in exchangeable NH_4^+-N (Fig. 1a). In addition, straw has a high C/N ratio, and a large amount of recalcitrant N (Luxhøi et al. 2007) generally resulted in significantly lower inorganic N in the CF + S treatment than in the CF treatment (Fig. 1 b, c, e, f) and in a significantly lower recovery of straw N than of chemical fertilizer N as inorganic N in the CF + S treatment (Fig. 1d–f).

The contribution from chemical fertilizer N to BN (Fig. 2a) in the CF treatment was very low (< 1%) in the first 14 days and clearly increased from day 21, and there are two possible

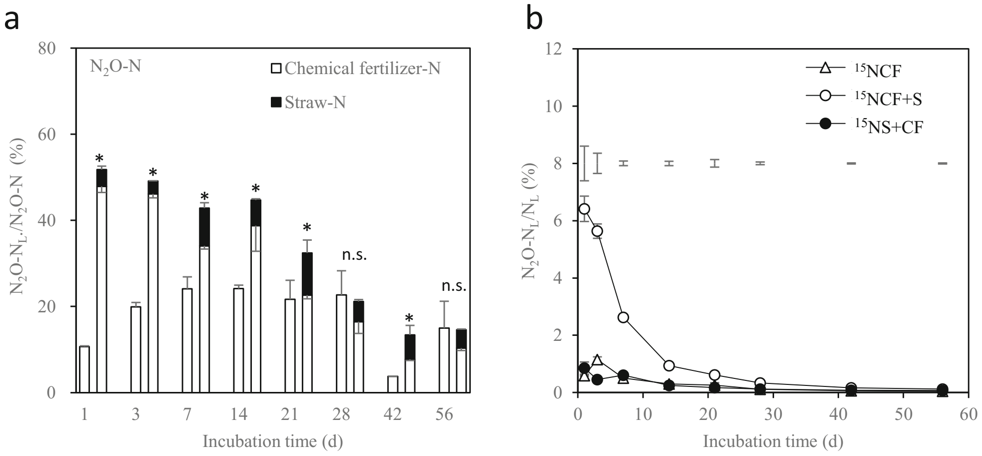

Fig. 4 The contribution from applied N (a) to nitrous oxide (N_2O-N) and the recovery of applied N (b) as nitrous oxide (N_2O-N) in different fertilization treatments during a 56-day incubation period. In the bar chart (a), white-only bars represent chemical fertilizer treatment (CF). White plus black bars represent chemical fertilizer combined with straw treatment (CF + S). (*) indicates significant difference ($P < 0.05$). In the line graph (b), the symbols in the legend indicate fertilizer treatments. The vertical lines represent $LSD_{0.05}$ values. Error bars for all data represent one standard error of the mean ($n = 3$). Subscript L indicates labeled fertilizer.

Table 2 The accumulated N_2O-N emissions from soil, chemical fertilizer, and straw sources in CK, CF, and CF + S treatments at the end of the incubation period

Treatment	Accumulated N_2O-N emission (μg kg^{-1})			
	Total	Soil-N	Chemical fertilizer-N	Straw N
Control	38.3c	38.3b	NA	NA
CF	57.4b	48.1a	9.3b	NA
CF + S	72.9a	46.4a	22.4a	4.1

NA no data; different letters within columns indicate significant differences at $P < 0.05$ between treatments

explanations for this. Firstly, the application of chemical fertilizer to soil can stimulate the mineralization of soil organic N (Kuzyakov et al. 2000; Liu et al. 2017), and the mineralized soil N can be preferentially assimilated by soil microorganisms (Burger and Jackson 2003; Nicolardot et al. 2001). This is supported by the undetected contribution from chemical fertilizer N to DON in the chemical fertilizer treatment (Fig. 2b) and < 50% contribution from applied N to BN in both treatments in most cases (Fig. 2a). Secondly, the lack of available C sources in the chemical fertilizer treatment may have limited exogenous N assimilation by microorganisms (Pan et al. 2017). With the soil native available N exhausted, the applied chemical fertilizer N would have been assimilated by microorganisms as shown by the contribution from chemical fertilizer to BN increasing in the CF treatment after day 14 (Fig. 2a). In addition, DON utilization by soil microorganisms can be enhanced by fertilizer application to agricultural soils (Ma et al. 2018). Theoretically, the assimilation of DON by microorganisms requires less energy than the assimilation of exchangeable NH_4^+-N (Daniel et al. 2010). This can be confirmed by the disappearance of the contribution from applied N to DON in the CF treatment throughout the incubation period and that from the CF + S treatment at day 14 (Fig. 2b), although a relatively higher contribution from applied N to exchangeable NH_4^+-N than to DON was found simultaneously (Fig. 1a). After straw amendment, the available C sources of straw rapidly increased microbial biomass (Fig. 2a, c) and promoted the assimilation of straw N into the microbial biomass (Luce et al. 2014). Straw N in dead microbial biomass was transferred to other soil N pools as a result of microbial turnover (Luce et al. 2014), such as exchangeable NH_4^+-N (Fig. 1a, c) and DON (Fig. 2b, d).

Fertilizer N appeared in the PON since the first day of incubation (Fig. 3a, c). Generally, POM is easily decomposed and is regarded as an unprotected soil fraction (Wander 2004). The mineralization of POM therefore readily occurs when chemical fertilizer is added to the soil (Yang et al. 2012). On the other hand, POM is regarded as a nucleus for aggregates (Marriott and Wander 2006), and it can be associated with mineral particles and small aggregates through microbial secretions (Chantigny et al. 1999). Moreover, chemical fertilizer can be adsorbed on the surfaces of mineral particles (Qiu et al. 2012). This was especially likely to have occurred in the recovery of chemical fertilizer N as PON in the CF + S treatment (Fig. 3c). In the CF + S treatment, the decrease in the contribution of straw N in PON and recovery of straw N as PON (Fig. 3a,c) were likely related to the decomposition of the part of the straw that is available to soil microorganisms (Luce et al. 2014).

Fertilizer N also appeared in the MTN after the first day of incubation (Fig. 3b, d), because this < 53-μm soil fraction has high specific surface area which can adsorb more N, and the ammonium fixation can occur if ammonium-fixing clays are present in the soil (Nannipieri et al. 1999; Nieder et al. 2011). The labeled N recovery as MTN was ranked as ^{15}NS + CF > ^{15}NCF + S > ^{15}NCF (Fig. 3d) because straw contains recalcitrant N (Duong et al. 2009); the microbial biomass was higher in the CF + S treatment than in the CF treatment (Fig. 2a), and likely microbial immobilized N was present into the < 53 μm soil fraction (Lavallee et al. 2018). A clear increase in the contribution of applied N to exchangeable NH_4^+-N was found from day 3 to day 7 and to N_{inorg} after the 7th day in the CF + S treatment (Fig. 1a, c). The highest chemical fertilizer N recovery in MTN occurred on the first day in the ^{15}NCF + S treatment (Fig. 3d). This further indicates that the CF + S treatment was more conducive to soil N increase than the CF treatment.

The fate of fertilizer N in soil N pools was likely affected by the fate of straw C because straw used in our study had high C/N ratio, provided C sources for microorganisms, and further drived soil N transformation in soil different N pools. For example, the CF + S treatment showed straw N mineralization, chemical fertilizer N immobilization into PON, as well as straw N in PON during the incubation (Figs.1, 3). Besides, the CF + S treatment promoted the recovery of chemical fertilizer N in MTN (< 53 μm), as shown the significantly higher chemical fertilizer N recovery in MTN (< 53 μm) in the ^{15}NCF + S treatment than in the ^{15}NCF treatment (Fig. 3d). Ghafoor et al. (2017) found 87.6% of the total SOC stored in < 53 μm fraction with straw treatment with or without chemical fertilizer, and Yan et al. (2012) found the largest proportion C and N in the < 53 μm fraction after long-term fertilization experiment. However, Angers et al. (1997) reported that the largest straw C occurred in the soil 53–250 μm fraction, and the highest straw N was present in the < 53 μm fraction using $^{13}C^{15}N$ labeled straw.

N_2O emissions

Fertilizer N amendment stimulates N_2O emission (Shcherbak et al. 2014; Xia et al. 2018), and the N_2O emitted is derived from both fertilizer N and soil N (Di and Cameron 2008;

Garcia-Ruiz et al. 2012; Gentile et al. 2008). Li et al. (2013) reported that the contribution of soil N to N_2O is dominant when fertilizer N is applied to black soils in Northeast China. For example, we found that N_2O-N from soil N accounted for 64% in the CF treatment and 84% in the CF + S treatment (Table 2). Crop straw application may alleviate the limitation on soil N_2O emissions by providing C and N sources (Chen et al. 2014b; Frimpong and Baggs 2010; Huang et al. 2004). Correspondingly, the significant higher accumulated N_2O-N emission from chemical fertilizer in the CF + S treatment than in the CF treatment (Table 2) indicates that straw application in the CF + S treatment stimulated the contribution of the chemical fertilizer N to N_2O-N emissions because straw C provided energy for the growth of soil N_2O-producing microbes (e.g., nitrifiers) (Huang et al. 2017) and increased the nitrification rate of exchangeable NH_4^+-N (Fig.1). In addition, although straw N in the CF + S treatment contributed to only 5.6% of the total N_2O-N emissions (Table 2), the recovery of straw N as N_2O-N emissions in the CF + S treatment was comparable to that of chemical fertilizer N as N_2O-N emissions in the CF treatment (Fig. 4b). Combined application of chemical fertilizer and straw therefore led to more N_2O emissions from chemical fertilizer N, and the N_2O emissions from straw N cannot be ignored. In contrast with our results in the dryland soil, Wang et al. (2019) reported that N_2O emission decreased in a paddy soil when chemical fertilizer N was partly replaced with straw N. N_2O emission from the chemical fertilizer and straw treatment may depend on the specific field conditions.

It is well known that N_2O is an important byproduct of nitrification or denitrification (Bremner 1997; Ma et al. 2012). In our study, N_2O-N from chemical fertilizer N was mainly derived from the nitrification process because the decrease in the amount of newly formed NH_4^+-N was much lower than the increase amount of newly formed NO_3^--N in the CF + S treatments, and both amounts were close in the CF treatment from day 1 (Fig. 1 d, e). In parallel, N_2O-N emissions from straw N were mainly derived from the mineralization of PON during the incubation period (Fig. 3a). Previous studies report that PON was the major source of inorganic N in soil amended with straw N (Boone 1994; Luce et al. 2014; Whalen et al. 2000). The PON from straw N in the present study decreased by 7.1% from the start to the end of the incubation period (Fig. 3c), and changes in straw N in the other soil N pools were much smaller than in the PON.

Conclusions

Compared with chemical fertilizer alone, the combination of chemical fertilizer and straw significantly promoted the retention of applied N in soil N pools such as microbial biomass N, particulate organic N, and mineral-associated total N, which were related to the immobilization of chemical fertilizer N stimulated by straw and the accumulation of straw N in these N pools. However, chemical fertilizer application with straw led to significantly higher total N_2O emissions mainly from chemical fertilizer compared with chemical fertilizer application alone, and straw N was also a definite source of N_2O emissions which were produced mainly through the decomposition of straw-derived PON. Our study highlights the necessity of evaluating and managing the tradeoffs between soil N increase and N_2O emissions before the widespread application of chemical fertilizer with straw can be recommended to black soils in Northeast China, and straw is an important source of N_2O emissions.

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