Journal of Soils and Sediments (2020) 20:1253–1263
https://doi.org/10.1007/s11368-019-02524-y

SOILS, SEC 1 • SOIL ORGANIC MATTER DYNAMICS AND NUTRIENT CYCLING • RESEARCH ARTICLE

Effect of carbon rate and type amended with ammonium or nitrate on nitrous oxide emissions in a strong ammonia oxidation soil

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Received: 30 June 2019 / Accepted: 11 November 2019 / Published online: 7 December 2019

Abstract

Purpose The work aimed to (1) better understand how C rate and type affecting N_2O emissions when combined application with different N forms in a strong ammonia oxidation soil, (2) further explore the tradeoff and mechanism of C availability to N transformations, and (3) provide evidence for making targeted N_2O mitigation measures in these kinds of agricultural soils.

Materials and methods The soil was collected from a typical farmer's field in Quzhou, Hebei province, China, classified as calcareous Cambisols (FAO classification system) and characterized with high pH (7.72), low organic C (9.1 g kg^{-1}), and a strong ammonia oxidation potential. Two microcosm incubations were conducted in laboratory under 20 °C and 70% WFPS (water-filled pore space), and the soils were amended with different glucose rates (0, 0.5, 1.0, 2.0, 4.0 mg C kg^{-1}) in Exp I, and with different C sources (glucose, pectin, starch, cellulose, lignin, straw) in Exp II, meanwhile with NH_4^+ or NO_3^--based fertilizer addition. 480 g (equivalent to oven-dried weight) of pre-incubated soil was placed in a glass jar (1165 mL volume) and adjusted to a field bulk density of 1.34 g cm^{-3}. All the glass jars were placed randomly and incubated for 15 days under aerobic condition.

Results and discussion Alone application of NH_4^+ released more N_2O (peak at 16.27 μg N kg^{-1}) than alone NO_3^- application. However, combined application of glucose with NO_3^- could emit more N_2O than when combined with NH_4^+, and the highest N_2O peak value was found in 0.5 g $kg^{-1}$of glucose. When pectin is combined with NH_4^+, resulted in higher accumulated N_2O emissions than other C sources, while applying straw and cellulose had negligible effects on N_2O emissions but enhanced CO_2 emissions under the incubation conditions. Glucose and pectin were identified as two labile C sources, significantly enhanced N_2O emissions. As expected, C addition could stimulate mineral N immobilization.

Conclusions Combined application of C and N enhanced N_2O and CO_2 emissions albeit to different extents. A higher accumulated N_2O emission was found when C/N ratio was lower (12.5). The stimulating effect of glucose and pectin as labile C sources with N addition on N_2O production should be considered, especially in soils with high pH, low organic C, and a strong ammonia oxidation potential. Therefore, it may have the potential to mitigate N_2O emissions from the field when avoiding applying both N and C especially rich in glucose and pectin together.

Keywords Ammonium · Carbon rate and type · C/N ratio · Nitrate · Nitrous oxide emission

1 Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas (GHG), significantly contributing to a global warming, with about 300-fold higher global warming potential than carbon dioxide (CO_2) (IPCC 2007), and involved in the destruction of the protective ozone layer (Ravishankara et al. 2009). Nearly 65% of the total anthropogenic N_2O was emitted from agricultural section globally (IPCC 2007), and in China, up to 75% of N_2O was emitted from this section (UNEP 2013). Agricultural soils are the largest source of N_2O production

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Responsible editor: Zucong Cai

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11368-019-02524-y) contains supplementary material, which is available to authorized users.

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due to the large use of synthetic nitrogen (N) fertilizers and manure (IPCC 2013). Several biological processes involved in the soil N cycling contribute to N_2O emissions, including autotrophic/heterotrophic nitrification, heterotrophic denitrification, nitrifier denitrification, and dissimilatory nitrate reduction to ammonium (DNRA) and anammox, and nitrification and denitrification are considered as the major N_2O sources in soils (Butterbach-Bahi et al. Butterbach-Bahl et al. 2013). Nitrification is the main process of N_2O production under aerobic, high ammonia, low nitrate concentration conditions, while heterotrophic denitrification-related N_2O mainly occurs under oxygen-limited and high nitrate conditions (Wrage et al. 2001, 2005; Kool et al. 2010; Baggs 2011; Butterbach-Bahl et al. 2013; Pilegaard 2013; Zhu et al. 2013). Heterotrophic nitrification might also play an important role in pasture soils, and readily degradable carbon (C) addition could stimulate heterotrophic nitrification and thereby promote N_2O emissions (Lan et al. 2019).

The global consumption of fertilizer N was projected to increase at a rate of 1.4% annually from 2014 to 2018, because of meeting the growing population demand for the greater food production (FAO 2015). The extensive use of fertilizer N would greatly contribute to the apparent elevation in atmospheric N_2O concentrations (Shcherbak et al. 2014). An emerging body of research shows that N fertilizer type is the key affecting factor on N_2O emissions under certain O_2 and moisture condition. Greater N_2O emissions were reported from nitrate (NO_3^-)-based fertilizers than ammonium (NH_4^+)-based fertilizers with the high moisture and C soils (Dobbie and Smith 2003; Smith et al. 2012). However, the summary of available measurements from fertilized fields showed that (NH_4^+)-based fertilizers had greater N_2O emissions than that of (NO_3^-)-based fertilizers (Bouwman et al. 2002; Li et al. 2013). It is still not clear of the effects of N forms on N_2O emissions in the soils characterized with high pH, low organic C, and a strong ammonia oxidation potential, especially simultaneously combined with different C components.

Previous studies have shown that the application of readily degradable C, such as glucose, could inhibit nitrification-related N_2O by reducing NH_4^+ availability via immobilization (Freppaz et al. 2007; Ma et al. 2016), which also depended on the availability and application rate of external C input (Granli and Bøckman 1994). But N_2O production from the soil could also be stimulated by the application of readily degradable C possibly due to increasing denitrification (Wrage et al. 2001). Carbon could provide not only energy for soil microbe but also electron acceptor for denitrification (Yokoyama et al. 1992; Rivett et al. 2008; Zhu et al. 2015). The contrasting effects of readily degradable C on nitrification and denitrification might lead to discrepant results on the effect of C application on N_2O emissions from the soil. The effects of different C components (with different degradable extents) on N_2O emission and N transformation under aerobic condition, especially simultaneously combined applying with (NH_4^+) or (NO_3^-)-based fertilizer, are incompletely understood, and future research is urgently needed. To date, studies that combined examine the effect of C and N forms on N_2O emissions are also scarce. How the interaction of C availability and N forms affect N_2O emissions is still unclear.

In the present study, different forms and doses of C and N were applied to a strong ammonia oxidation soil in order to investigate the interaction of C availability and N forms affecting N_2O emissions and N transformation. We hypothesized that labile C with N addition would enhance N_2O emissions, and higher C/N ratio could decrease N_2O emissions due to enhancing N immobilization. The findings from this study would help us to develop better C and N managements for mitigating N_2O emissions in field conditions.

2 Materials and methods

2.1 Site and properties of soil samples

Soil samples were taken from a typical farmer's field where carried out winter wheat-summer maize double cropping system in the last many years at Quzhou research station (36.87°N, 115.02°E), Hebei province, China. The site is under warm temperate and semi-arid monsoon climatic conditions. The long-term (from 1980 to 2010) annual average temperature was 13 °C, and the annual average precipitation was 494 mm. Most rainfall occurred during summer (July to September) accounting for 60% of the total rainfall in a year. The soil is a typical calcareous fluvo-aquic soil belonging to calcareous Cambisols (FAO classification system), which has low NH_4^+ and dissolved C but with high NO_3^- and pH and is characterized by a high nitrification potential (Wan et al. 2009; Ju et al. 2011; Ju and Zhang 2017). Ten soil cores (0–20 cm) were randomly taken from the field (about half hectare) after summer maize harvest in 2011 to make a composited sample; the sample was stored in an ice box before returning to the laboratory within half a day. The sampled soil was homogenized, sieved (2 mm), air-dried, and stored at room temperature (20 °C) before the incubation commencement.

The soil was light loam containing 39.34% sand, 58.25% silt, and 2.41% clay, with a filed bulk density 1.34 g cm^{-3}, a pH of 7.72 (1:2.5, soil/water), a soil organic carbon concentration of 9.5 g kg^{-1}, a dissolved organic C of 45.0 mg kg^{-1}, a soil total N concentration of 0.7 g kg^{-1}, and NH_4^+ and NO_3^- concentrations of 0.38 and 31.82 mg kg^{-1}, respectively. The potential nitrification rate was 204.5 mg NO_3^--N kg^{-1} day^{-1} and measured by the method of Hart et al. (1994).

2.2 Experimental design

Two laboratory incubation experiments were conducted. Experiment I aimed to test the effects of different C application rates with N forms on N_2O emissions and N transformation. It was conducted with two N forms (ammonium sulfate, $(NH_4)_2SO_4$ and calcium nitrate, $Ca(NO_3)_2$) and four levels of C (glucose) addition. Apart from CK treatment, 40 mg N kg^{-1} as NH_4^+ or NO_3^-, was applied with or without glucose for each treatment. The four glucose rates were 0.5, 1.0, 2.0, and 4.0 g C kg^{-1}, respectively, and the corresponding C/N ratios of the applied C and N were 12.5, 25, 50, and 100, respectively. Each treatment had three replicates. Therefore, eight treatments plus three controls (no N or C addition and N addition only) were carried out in the Experiment I as follows:

1. CK (no N and C addition, CK)
2. $(NH_4)_2SO_4$ (NH_4^+)
3. $(NH_4)_2SO_4$ + 0.5 g C kg^{-1} glucose(NH_4^+ + Glu.0.5)
4. $(NH_4)_2SO_4$ + 1.0 g C kg^{-1} glucose(NH_4^+ + Glu.1.0)
5. $(NH_4)_2SO_4$ + 2.0 g C kg^{-1} glucose (NH_4^+ + Glu.2.0)
6. $(NH_4)_2SO_4$ + 4.0 g C kg^{-1} glucose (NH_4^+ + Glu.4.0)
7. $Ca(NO_3)_2$ (NO_3^-)
8. $Ca(NO_3)_2$ + 0.5 g C kg^{-1} glucose (NO_3^-+ Glu.0.5)
9. $Ca(NO_3)_2$ + 1.0 g C kg^{-1} glucose (NO_3^-+ Glu.1.0)
10. $Ca(NO_3)_2$ + 2.0 g C kg^{-1} glucose (NO_3^- + Glu.2.0)
11. $Ca(NO_3)_2$ + 4.0 g C kg^{-1} glucose (NO_3^-+ Glu.4.0)

Experiment II aimed to test the effects of different C types with N addition on N_2O emissions and N transformation. Six C sources (glucose, pectin, starch, cellulose, lignin and wheat straw) were used with C rate of 0.5 g C kg^{-1}. N rate was the same as experiment I as 40 mg N kg^{-1} of NH_4^+ or NO_3^-, respectively. Each treatment had three replicates. Therefore, 12 treatments plus three controls (no N or C addition and N addition only) were carried out in experiment II as follows:

1. CK (no N and C addition, CK)
2. $(NH_4)_2SO_4$ (NH_4^+)
3. $(NH_4)_2SO_4$ + 0.5 g C kg^{-1} glucose (NH_4^+ + glue)
4. $(NH_4)_2SO_4$ + 0.5 g C kg^{-1} pectin (NH_4^+ + pectin)
5. $(NH_4)_2SO_4$ + 0.5 g C kg^{-1} starch (NH_4^+ + starch)
6. $(NH_4)_2SO_4$ + 0.5 g C kg^{-1} cellulose (NH_4^+ + cellulose)
7. $(NH_4)_2SO_4$ + 0.5 g C kg^{-1} lignin (NH_4^+ + lignin)
8. $(NH_4)_2SO_4$ + 0.5 g C kg^{-1} straw (NH_4^+ + straw)
9. $Ca(NO_3)_2$ (NO_3^-)
10. $Ca(NO_3)_2$ + 0.5 g C kg^{-1} glucose (NO_3^- + glu)
11. $Ca(NO_3)_2$ + 0.5 g C kg^{-1} pectin (NO_3^- + pectin)
12. $Ca(NO_3)_2$ + 0.5 g C kg^{-1} starch (NO_3^- + starch)
13. $Ca(NO_3)_2$ + 0.5 g C kg^{-1} cellulose (NO_3^- + cellulose)
14. $Ca(NO_3)_2$ + 0.5 g C kg^{-1} lignin (NO_3^- + lignin)
15. $Ca(NO_3)_2$ + 0.5 g C kg^{-1} straw (NO_3^- + straw)

2.3 Procedure of the incubation experiments

Fresh soil samples were pre-incubated under 50% WFPS (water-filled pore space) at 20 °C for a week in the dark to stabilize the microbial community and avoid fluctuations of soil respiration in the dry-wet process. A total of 480 g (equivalent to oven-dried weight) of pre-incubated soil was placed in a glass jar (1165 mL volume with 16.3 heights and 9.5 inner diameters) and adjusted to a field bulk density of 1.34 g cm^{-3}. The C sources like pectin, starch, cellulose, lignin, and straw were mixed directly with the soils, and the straw was dried and ground (passed through 1 mm mesh) before addition. The solutions of NH_4^+ or $NO_3^-$for each treatment were applied evenly to the soil in the glass jar using a syringe to achieve the target soil moisture of 70% WFPS, which was the moisture level as maximum N_2O emission from the previous studies (Huang et al. 2014). Each glass jar was covered with parafilm, which allowed air exchange but prevented water loss during the incubation. Soil moisture was replenished every 4 days. All the glass jars were placed randomly and incubated for 15 days at 20 °C in the dark. There were 33 glass jars (11 treatments *3 replicates) for experiment I and 45 jars (15 treatments * 3 replicates) for experiment II for gas samplings and the last destructive soil sampling, respectively. Totally, 132 glass jars (11 treatments * 3 replicates * 4 sampling times) for experiment I, and 135 glass jars for experiment II (15 treatments * 3 replicates * 3 sampling times) were incubated for destructive soil samplings and analysis.

2.4 Gas sampling and analysis

Gas samples (20 ml) were collected on days 1, 2, 3, 5, 8, 11, and 15 for N_2O and CO_2 concentrations analysis. Before gas sampling, parafilm was removed, and the air was allowed to exchange with the ambient air for 5 min. Subsequently, the jars were sealed with rubber seals equipped with a three-way valve, and this time point was defined as the zero point. Gas samples were collected at 0, 10, and 20 min with syringes. Samples were analyzed by a modified gas chromatography (Agilent 6820, Santa Clara, USA) with an ECD detector for N_2O and TCD detector for CO_2 concentration (Zheng et al. 2008) respectively. The ECD detector was fitted with a ^{63}Ni and operated at 350 °C. High-purity dinitrogen (99.999%) was used as the carrier gas for N_2O analysis. Ten percent of CO_2 in pure N_2 was used as a make-up gas for ECD. The GC was calibrated using known concentrations of mixed gas (0.354 ppm N_2O in pure N_2). Air conditioning was used in the laboratory during measurements to minimize the tendency of the ECD to drift with changing temperature.

2.5 Soil sampling and analysis

Destructive soil sampling was carried out for soil mineral N analysis on days 0, 3, 5, and 15 for experiment I, and on days 0, 3, and 15 for experiment II. Each soil sample was extracted with 1 M KCl (soil: 1 M KCl = 1:5) to determine the concentrations of NH_4^+ and NO_3^- using a continuous flow analyzer (AA3, Seal Analytical, Norderstedt, Germany).

2.6 Calculations and statistical analysis

N_2O and CO_2 fluxes were calculated according to the following equation:

$$F = \frac{273}{273+T} \times M \times 10^{-3} \times 60 \times 24 \times \frac{V}{22.4} \times \frac{dc}{dt} / 0.48$$

where F is the N_2O (μg N_2O-N kg^{-1} day^{-1}) or CO_2 (mg CO_2-C kg^{-1} day^{-1}) emission rate, T (°C) is the air temperature in the incubation glass jar, M is the molecular weight of N in molecular N_2O (28 g mol^{-1}) or molecular weight of C in molecular (CO_2 g mol^{-1}), 60 (min) is the number of minutes in an hour, 24 is the number of hours in a day, V (L) is the volume of the headspace, c is the concentration of N_2O (nL L^{-1}) or CO_2 (μL L^{-1}), t (min) is the time from jar sealing, dc/dt is the change in N_2O (nL L^{-1} min^{-1}) or CO_2 (μL L^{-1} min^{-1}) concentration per unit of time, 22.4 (L mol^{-1}) is the molecular volume at 101 kPa and 273 K, and 0.48 (kg) is the weight of oven-dried soil in the jar.

Accumulated N_2O and CO_2 emissions during the whole incubation were derived from the sums of measurement and no-measurement days estimated by linear interpolation (Mosier et al. 2006).

Differences in accumulated N_2O and CO_2 emissions were analyzed by one-way analysis of variance (ANOVA) and compared by Duncan's multiple range test at the 5% level using IBM SPSS Statistics 20; all the figures were made by Sigmaplot 12.5.

3 Results

3.1 Effects of C rates amended with ammonium or nitrate on N_2O and CO_2 emissions (experiment I)

3.1.1 N_2O and CO_2 emissions

Glucose addition significantly stimulated N_2O emissions regardless of applied N forms (Fig. 1). More N_2O produced after glucose addition combined with NO_3^- than with NH_4^+. N_2O emissions when glucose with NH_4^+ form reached the peak immediately in the first incubation day but followed by a repaid decline as the incubation proceeded (Fig. 1a). The highest peak value was found in the low rate of glucose treatment (0.5 g C kg^{-1}), while the lowest peak value was under the highest glucose rate (4 g C kg^{-1}). N_2O peak value from NH_4^+ + Glu.0.5 treatment was about 150 times higher than that from only NH_4^+-N addition treatment. However, a different trend was observed when glucose addition with NO_3^- form (Fig. 1b). Addition of high rate of glucose (4 g C kg^{-1}) significantly stimulated N_2O emissions on the first day and decreased afterward, but such response was not observed in lower rates (0.5, 1, and 2 g kg^{-1}) treatments. N_2O fluxes peaked on the second day of the incubation in the NO_3^- + Glu.0.5 and NO_3^- + Glu.1.0 treatments, and kept consistent during the first 2 days in NO_3^- + Glu.2.0 treatment. The N_2O peak values followed the order NO_3^- + Glu.0.5 > NO_3^- + Glu.1.0 > NO_3^- + Glu.4.0 > NO_3^- + Glu.2.0. N_2O peak from NO_3^- + Glu.0.5 treatment was about 235 times higher than that from only NO_3^--N addition treatment. N_2O flux from NO_3^- treatment was similar to CK treatment (from 0 to 6.7 μg N kg^{-1} day^{-1}) during the incubation, suggesting there were little N_2O emissions despite the presence of NO_3^- in the soil.

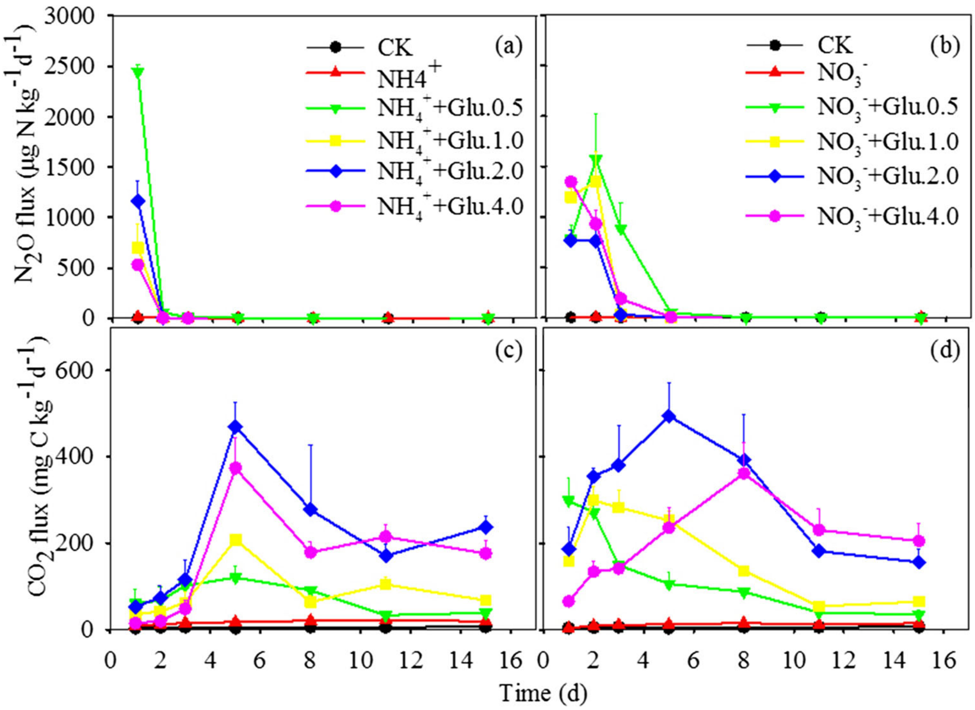
CO_2 fluxes increased with the addition of glucose and NH_4^+ (Fig. 1c) or NO_3^--forms (Fig. 1d). CO_2 fluxes of NH_4^+ + glucose treatments showed an uptrend in the first 5 days of the incubation, then decreasing gradually afterward. CO_2 flux was the highest in 2.0 g C $kg^{-1}$of glucose rate and increased by about 20-fold compared with only NH_4^+ addition. As for CO_2 fluxes in treatments with glucose plus NO_3^--based fertilizer addition (Fig. 1d), glucose addition enhanced CO_2 fluxes to different degrees, and the order of the CO_2 peak value was NO_3^- + Glu.2.0 > NO_3^- + Glu.4.0 > NO_3^- + Glu.0.5 > NO_3^- + Glu.1.0.

Accumulated N_2O and CO_2 emissions were regulated by the ratios of external C and N inputs (Fig. 2, Table A1—Electronic Supplementary Material-ESM). The accumulated N_2O emission was the highest when C/N ratio was 12.5 (Fig. 2a) and decreased along with increasing C/N ratio under both N forms. The N_2O emissions were significantly higher ($P < 0.05$) when the N source was NO_3^- than NH_4^+ with equal glucose addition in most treatments except for Glu.2.0 treatment (Table A1 – ESM). The trends of accumulated CO_2 emissions (Fig. 2b) were different from N_2O emissions, and it increased along with increasing C/N ratio and reached the peak when C/N was 50, and then decreased when C/N ratio was 100 in both N forms. The accumulated CO_2 emissions were higher but not significantly ($P > 0.05$) when N form was NO_3^- than NH_4^+ with equal glucose addition in most treatments except for Glu.2.0 treatment ($P < 0.05$) (Table A1—ESM).

3.1.2 Dynamics of NH_4^+ and NO_3^- concentration and N balance

The concentrations of NH_4^+ decreased after NH_4^+ and glucose addition in the first 5 days of incubation, indicating high

Fig. 1 N_2O and CO_2 fluxes from different treatments during the incubation in experiment I. Nitrogen source for **a**, **c** was $(NH_4)_2SO_4$, for **b**, **d** was $Ca(NO_3)_2$, and the carbon source was glucose. Error bars are the standard error of three replicates



nitrification or immobilization occurred in the soil (Fig. 3a). The concentrations of NO_3^- generally increased under NH_4^+ treatment without glucose addition (Fig. 3c), but it decreased quickly to zero on the third day of the incubation in all the addition of glucose treatments regardless of how much C added. Few NH_4^+ was detected, and it remained consistently low (below 1 mg N kg^{-1}) during the whole incubation in all NO_3^- treatments (Fig. 3b). The NO_3^- concentrations were relatively stable during the whole incubation in CK and NO_3^- treatments, indicating low processes of N transformations in the soil. Whereas with the application of glucose (Fig. 3d), the NO_3^- concentrations decreased sharply to zero in the first 3 days of incubation in all glucose treatments, indicating high denitrification or immobilization occurred in the soil. The increased trend of NO_3^- concentrations in NO_3^- + Glu.0.5 treatment after 3 days showed mineralization again with low dose of glucose addition.

Nitrogen balance, as seen in Table A2 (ESM), reflects soil N transformations and their relationship with N_2O emissions. The positive N balance could be explained by immobilization of mineral N (NH_4^++NO_3^-) or other gaseous loss such as NH_3 or N_2, while the negative N balance could be explained by mineralization of organic N in the soil. Different C/N ratios due to different amount of glucose addition affected soil N mineralization and mobilization in various ways. In control (CK) and NO_3^- treatments, low negative N balance was observed while a low positive N balance was observed in NH_4^+ treatment. However, all treatments with glucose addition had relatively large positive values of N balance, demonstrating high mineral N immobilization or other gaseous losses. With the glucose addition, the N

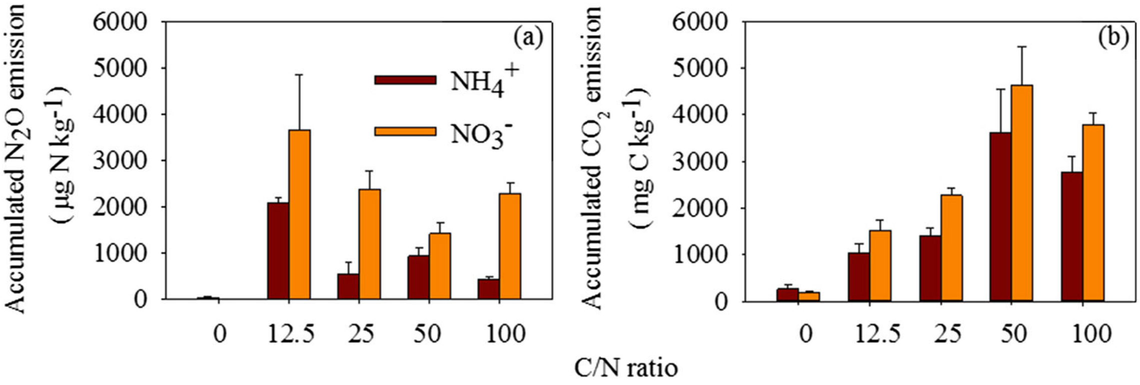


Fig. 2 Effects of different C/N ratios on accumulated N_2O (**a**) and CO_2 (**b**) emissions. C/N ratio was calculated by rates of external C and N addition. Error bars are the standard error of three replicates

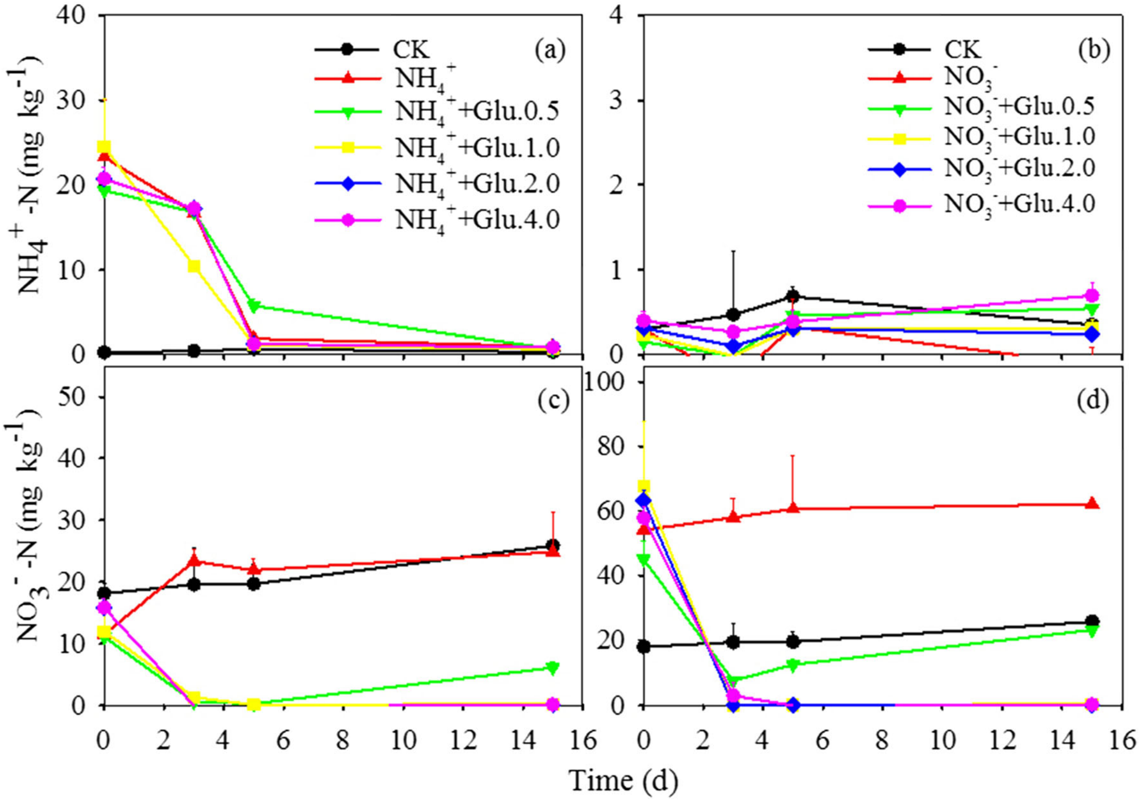


Fig. 3 Ammonium and nitrate concentrations of different treatments on sampling days in experiment I. Nitrogen source for **a**, **c** was $(NH_4)_2SO_4$, for **b**, **d** was $Ca(NO_3)_2$, and carbon source was glucose. Error bars are the standard error of three replicates

balance values of NO_3^- form were higher than that of NH_4^+form except NO_3^- + Glu.0.5 treatment .

3.2 Effects of C types amended with ammonium or nitrate on N_2O and CO_2 emissions (experiment II)

3.2.1 N_2O and CO_2 emissions

The highest N_2O flux was found when pectin is applied with NH_4^+ (Fig. 4a, b); however, when N form changed to NO_3^-, the highest N_2O was emitted from glucose treatment (Fig. 4c, d). N_2O fluxes from all N treatments reached the peak on the first day of the incubation, and followed by a quick decline afterward. The accumulated N_2O emissions are shown in Fig. 5a and Table A3 (ESM). During the whole incubation, total N_2O emissions of pectin and glucose treatments were higher than other C treatments combined with either NH_4^+ or NO_3^-. The accumulated N_2O emissions of other four C treatments followed the order NH_4^+ + lignin > NH_4^+ + starch > NH_4^+ + straw > NH_4^+ + cellulose, and NO_3^- + starch > NO_3^- + straw > NO_3^- + lignin > NO_3^- + cellulose, but the differences were not significant ($P > 0.05$). Accumulated N_2O emissions of NO_3^- + cellulose were the lowest among the different C treatments combined with either NH_4^+ or NO_3^-.

The CO_2 fluxes reached the peak at different incubation times after different C addition with NH_4^+ form (Fig. 4e, f). With the glucose addition, CO_2 fluxes peaked on the first incubation day up to 175.8 mg C kg^{-1} day^{-1}, and followed by a decrease trend. Whereas for other C treatments, the CO_2 emissions peak occurred after 2 days and even after 11 days for the straw treatment, indicating that different C types stimulated the microbial respiration to different degrees. Cellulose and straw addition significantly ($P < 0.05$) increased accumulated CO_2 emissions during the whole incubation (Fig. 5b; Table A3), and more CO_2 produced when N form was NO_3^- compared to NH_4^+.

When applied N as NO_3^- form (Fig. 4g, h), the dynamic of CO_2 fluxes were similar to that of NH_4^+ treatments. With the addition of glucose and pectin, higher CO_2 fluxes were observed than other treatments especially for the first 8 days and peaked on the first day of the incubation. The CO_2 emissions reached the peak in the rest of the C treatments lagged behind the treatments of NO_3^- + Glu and NO_3^- + pectin and lasted longer during the incubation. NO_3^- + Glu and NO_3^- + pectin treatments produced the highest accumulated CO_2 during the incubation (Table A3). The lowest CO_2 fluxes were observed in lignin treatment combined with either NH_4^+ or NO_3^-.

3.2.2 Dynamics of NH_4^+ and NO_3^- concentration and N balance

When the N forms as NH_4^+, the soil NH_4^+ concentrations decreased (Fig. 6a), and while the NO_3^- concentrations

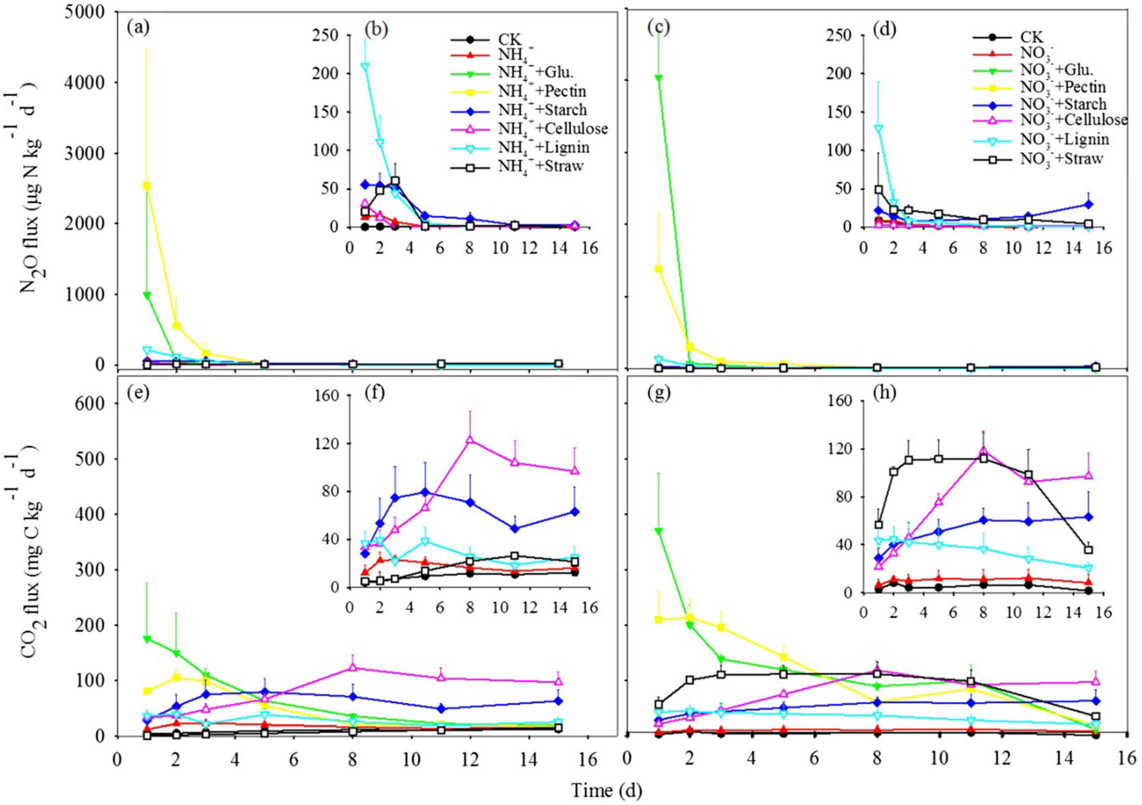


Fig. 4 N_2O and CO_2 fluxes of different treatments during the incubation in experiment II. Nitrogen source for (**a**, **b**, **e**, **f**) was $(NH_4)_2SO_4$, for (**c**, **d**, **g**, **h**) was $Ca(NO_3)_2$. The insert panel (**b**, **d**, **f**, **h**) are shown without NH_4^++Glu. and NH_4^++Pectin to make the figure more clear; Error bars are the standard error of three replicates

increased before the third day(Fig. 6c), and then slightly decreased till the end of the incubation. The concentrations of NH_4^+ in the NH_4^+ + Glu treatment was the lowest among C addition treatments as glucose might increase the immobilization of NH_4^+rapidly. At the end of the incubation, there were various amounts of NO_3^- detected in the treatments with the range of about 10 in NH_4^+ + Glu treatment to about 40 mg N kg^{-1} in NH_4^+ + starch treatment. When applied N as NO_3^- form, the NH_4^+ concentrations were low and had a slight uptrend at the end of the incubation probably due to mineralization (Fig. 6b). Apart from cellulose addition, NO_3^- concentrations decreased to different degrees among other C treatments (Fig. 6d).

When applied N form as NH_4^+, relatively larger values of N balance were observed with the addition of glucose, cellulose, and straw treatments (Table A4—ESM), indicating higher mineral N immobilization or other gaseous losses in these treatments. When applied N form as NO_3^-, the values of N balance showed positive in all treatments except the relatively high negative N balance only in NO_3^- + cellulose

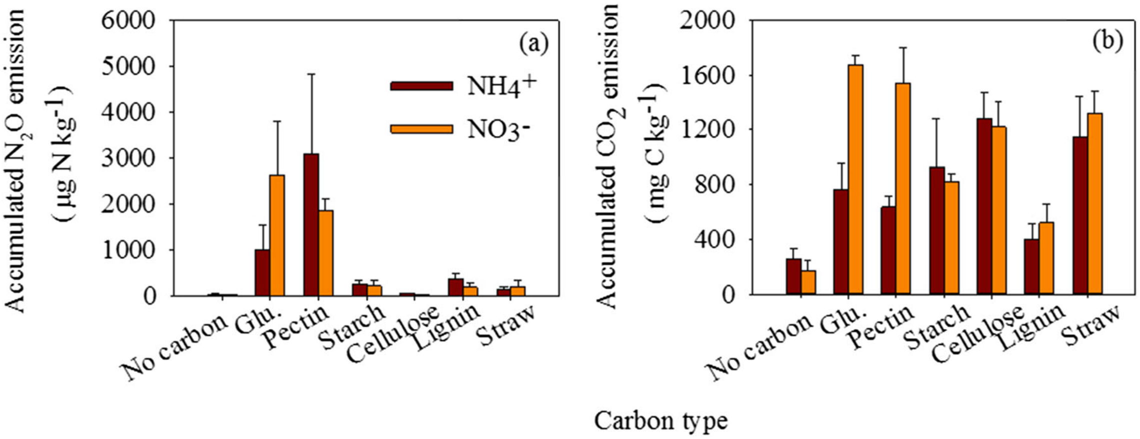


Fig. 5 Effects of different C types on accumulated N_2O (**a**) and CO_2 (**b**) emissions. Error bars are the standard error of three replicates

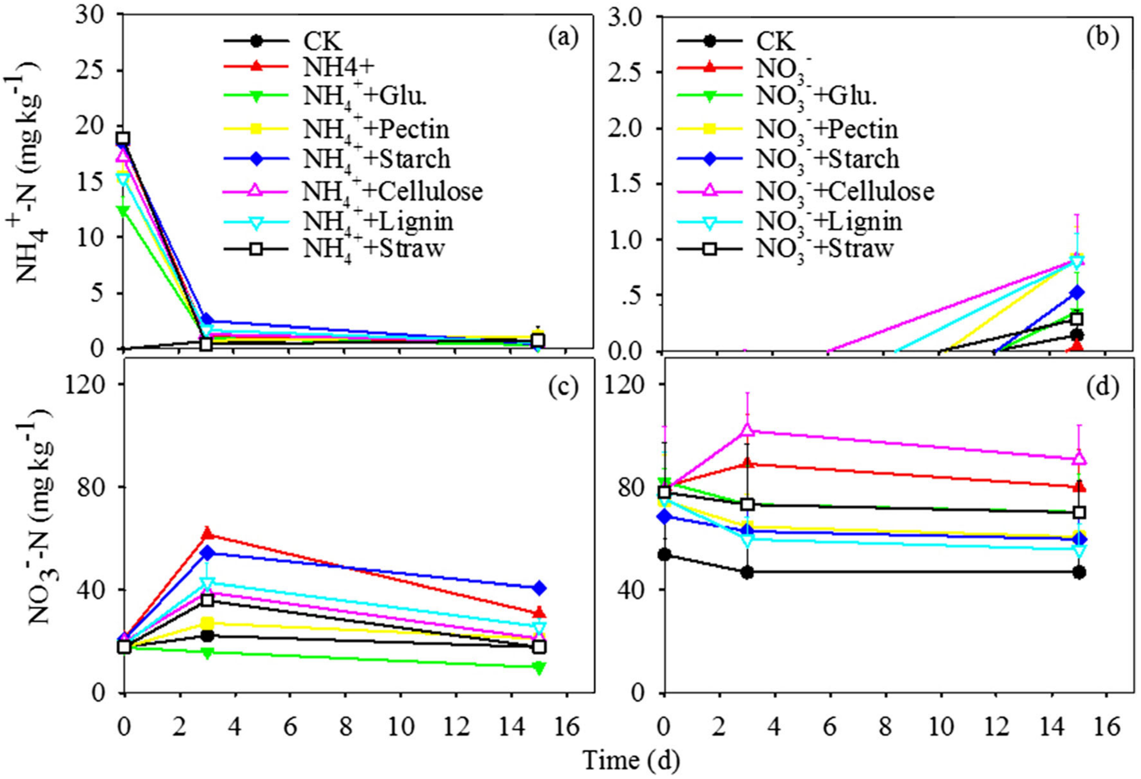


Fig. 6 Ammonium and nitrate concentrations of different treatments on sampling days in experiment II; Nitrogen source for (**a**) and (**c**) was $(NH_4)_2SO_4$, for (**b**) and (**d**) was $Ca(NO_3)_2$; Error bars are the standard error of three replicates

treatment, indicating enhanced mineralization with addition of cellulose.

4 Discussion

In the current incubation study, the combined application of C and N significantly enhanced N_2O and CO_2 emissions, albeit to varying degrees, which was consistent with other field results (Ju et al. 2011; Bao et al. 2012; Cui et al. 2012; Gao et al. 2014). Moreover, different types of C and N had different effects on increasing N_2O and CO_2 emissions. For example, when applied C source was pectin or glucose, more accumulated N_2O were emitted than other C sources regardless of applied N forms. Several previous studies revealed that soil N_2O production rate could be stimulated by adding readily degradable C, possibly due to increasing denitrification (Wang et al. 2005; Cheng et al. 2012; Ameloot et al. 2016; Mehnaz et al. 2018). In our study, NO_3^- concentrations sharply decreased after C and N addition indicating denitrification occurrence or immobilization. C addition could greatly enhance O_2 consumption and microorganism respiration in the soil, where generated anaerobic microsites which was favorable for denitrification (Huang et al. 2014; Song et al. 2019). We did not think that DNRA could occur because the NH_4^+ concentration did not increase at the end of the incubation in both experiments. However, the immobilization of NO_3^- is actually a possible process of NO_3^- decrease. A meta-analysis also showed that specific exogenous organic C input at a high rate (> 0.5 g C kg^{-1}) or with a high C/N ratio (> 18) could enhance microbial NO_3^- immobilization (Cheng et al. 2017).

Our results indicated that the soils treated with high concentrations (> 0.5 g C kg^{-1}) of glucose produced lower N_2O than NH_4^+ + Glu 0.5 during the incubation (Table A1—ESM, Fig. 1). It is possibly because glucose was easy to be used by soil microorganism, and high concentrations might stimulate the growth and activity of heterotrophic microorganisms (Schimel and Bennett 2004; Booth et al. 2005), thus assimilation of mineral N (Burger and Jackson 2003; Myrold and Posavatz 2007), which reduced the NH_4^+ substrate for nitrification and then reduced related N_2O production as well. And it was also reflected by higher CO_2 production in this treatment (Table A1). Another reason for lower amounts of N_2O emissions from high application rate of glucose (> 0.5 g kg^{-1}) than NH_4^+ + Glu 0.5 was that it might stimulate the anaerobic conditions which enhanced the activity of the enzyme responsible for reduction of N_2O to N_2 (Murray et al. 2004; Kool et al. 2010).

NH_4^+ and NO_3^-- N forms are two main N fertilizer types in crop production, which are also substrates for nitrification and denitrification respectively, and have various effects on N_2O emissions from different soil types. Bouwman et al. (2002) reported that more N_2O was emitted when applied NO_3^- than NH_4^+-based fertilizers from New Zealand and Germany grassland soil due to fine texture and high clay content of the soil. However, in the present study, we found opposite results that application of NH_4^+-based fertilizer alone produced more N_2O than NO_3^--based fertilizer due to high nitrification capacity of our studied soil (Ding et al. 2015; Ju and Zhang 2017); NH_4^+-based fertilizer could be immediately proceeded by nitrifiers and produce large amount of nitrification-related N_2O (Wan et al. 2009; Ju et al. 2011; Ju and Zhang 2017; Zhu et al. 2019a, b).

Different C types had various effects on N_2O and CO_2 emissions, indicating different interactions between C and N with respect to N_2O and CO_2 production. In our study, the application of glucose and pectin with NH_4^+ or NO_3^- resulted in the highest N_2O emissions (Table A3—ESM, Fig. 6). Probably because they were more readily degradable C to stimulate growth of soil microorganisms. In contrast, complex C compounds like straw had low ability of NO_3^- immobilization and produced lower N_2O and CO_2 than glucose and pectin addition, which was in line with previous studies (Miller et al. 2008; Huang et al. 2013; Qiu et al. 2013). The soluble C types like glucose and pectin were more efficiently used by soil microorganisms in a soil with pH 7.4 under anaerobic condition (de Catanzaro and Beauchamp 1985). Although our incubation condition was aerobic, the C addition might enhance O_2 consumption and respiration in soil matrix, which could create anaerobic condition (Song et al. 2019).

The CO_2 fluxes and its duration are mainly related with the structure and composition of various organic materials. In our study, the straw treatment combined with NH_4^+ or NO_3^- produced small amount of CO_2 in the first few days but showed sustained production over time as the straw contained much lower soil labile C and required a longer decomposition time. The limited available C and aerobic condition might inhibit denitrification, resulting in less N_2O emissions (Table A3—ESM) and higher final NO_3^- (Fig. 6) concentrations in NO_3^- + straw treatment. Only readily available C sources (e.g., glucose and pectin) together with either NH_4^+ or NO_3^- stimulated N_2O emissions. Therefore, from our current study under the aerobic conditions, the possible mitigation strategies of N_2O emission would be avoiding the combination addition of N fertilizers and easily accessible C.

Soil NH_4^+ and NO_3^- were involved in many N transformation pathways, including nitrification, denitrification, N immobilization, and mineralization. Nitrification and denitrification depended on NH_4^+ and NO_3^- concentrations and O_2 status, and soil N mineralization and immobilization were related to the C/N ratio of added substrate (Larsen and McCartney et al. 2000). Higher C/N ratio (27) led to a strong immobilization of N, while lower C/N ratio (15–17) led to mineralization (Bengtsson et al. 2003), which explained soil NH_4^+ kinetics in the present study (Fig. 2, Table A2—ESM). Moreover, C types could also affect N transformations, and the organic materials with higher C/N ratio (45.5) could have a slower decomposition rate than that with lower C/N ratio (18.8), which stimulated N immobilization (Trinsoutrot et al. 2000; Moritsuka et al. 2004). The immobilization was greater in soils incubated with glucose amendments than those incubated with more complex C compounds (Magill and Aber 2000). The returned C sources in the field are crop roots, straw, manure or compost, which are composed with different C components. As we did the model experiments in laboratory, we are interested in the effects of different C components on N transformations and N_2O emissions. The results would help us understand the interactions of C and N when applying the integrates of complex OM in situ.

Previous studies regarding application of C and N rates and types on N_2O emissions were almost all under strict anaerobic condition, but our study was aerobic which would be a better representation of real field conditions in upland soils (Ju and Zhang 2017). We still have no direct evidences to show nitrifier denitrification or denitrification in our current treatments (Yang et al. 2017), but measuring O_2 concentration in soil microsites might be helpful to further explain the mechanisms of N_2O production and differentiate N_2O processes in the soil.

5 Conclusions

We observed from the incubation study that the application of mixed C and N substrates enhanced N_2O and CO_2 emissions albeit to different extents. A higher accumulated N_2O emission was found when C/N ratio was lower (12.5). NO_3^- together with glucose treatments produced more N_2O than NH_4^+-treated soils. The stimulating effect of glucose and pectin as labile C sources with N addition on N_2O production should be considered, especially in soils with high pH, low organic C, and a strong ammonia oxidation potential. Under field conditions, therefore, avoiding applying both N and C especially rich in glucose and pectin together could be the suitable targeted strategy for mitigating N_2O emissions.

Funding information This work was supported by the National Natural Science Foundation of China (41830751, 41807036) and the National Key Research and Development Program of China (2017YFD0200105).

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