Effects of fertilization methods in wheat on gases emission and environmental benefit in Mollisols of Northeast China

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Abstract

Aims Many previous studies have focused on crop yields, but there is a knowledge gap regarding the environmental benefits of phased fertilization and organic substitution. To study the effects of phased fertilization and organic substitution on soil gas emissions and spring wheat yields, a field experiment was conducted using Mollisols.

Methods Five treatments were set up: conventional nitrogen application (CF); no nitrogen application (N0); one-time (N1), two-stage (N2) and three-stage (N3) application of chemical nitrogen based on 25% of chemical nitrogen being replaced by organic fertilizer. The changes in NH_3 volatilization, N_2O and CO_2 emissions, soil-related carbon and nitrogen invertase activities and soil carbon and nitrogen contents under different treatments were analyzed and compared.

Result The results showed that N1 had the best emission reduction and yield increase effects. Compared with CF, N1 reduced NH_3 volatilization, N_2O and CO_2 emissions accumulation by 28.94%, 10.29% and 17.21%, respectively, and increased spring wheat yield by 21.33%. Compared with N2 and N3, N1 could better reduce soil urease, nitrate reductase, catalase and β -glucosidase activities, reduce the conversion rate of urea and organic carbon, increase the content of $\text{NH}_4^+\text{-N}$ in soil, and reduce the NH_3 volatilization rate and N_2O and CO_2 emissions rates.

Conclusions Comprehensive analysis showed that the N1 treatment had the best effect on reducing the soil gas emission rate, emission reduction rate and environmental cost as well as on increasing yield.

Introduction

Wheat is one of the top three crops worldwide, and nitrogen is one of the most restrictive nutrients for wheat production. Increasing the nitrogen application rate within a certain range can improve wheat yield (Herrera et al., 2016; Wen et al., 2019). Although high nitrogen fertilizer inputs can ensure crop yield, the nitrogen use efficiency in China is only approximately 30% (Lasisi et al., 2021). Due to the excessive application of chemical fertilizers, the traditional fertilization mode in agricultural production has increased NH_3 volatilization and emissions of greenhouse gases such as N_2O and CO_2 and reduced nitrogen use efficiency (Huang et al., 2016; Wang et al., 2014). Studies have found that nitrogen loss from NH_3 volatilization accounts for approximately 15% of the amount of fertilizer used, while this proportion is only 7% in developed countries but 18% in China, far higher than the world average (Rakshit et al., 2015). Moreover, the application of chemical fertilizers generally increases N_2O and CO_2 emissions (Rosa et al., 2018; Zamanian et al., 2018). Overall, direct and indirect N_2O emissions from agricultural soils account for more than 50% of global anthropogenic N_2O emissions (Lan et al., 2020). In addition, N_2O can be indirectly generated by NH_3 through atmospheric transport and deposition processes (Hayes et al., 2019; Min et al., 2021). N_2O is an important regulated greenhouse gas, second only to CO_2 and CH_4 in the atmosphere, and its potential to increase global temperature is very high. The 100-year warming potential of N_2O was nearly 300 times higher than that of CO_2 and 10 times higher than that of CH_4 (Lan et al., 2020). As one of the important branches of soil CO_2 emissions from terrestrial ecosystems (Bradford et al., 2016), soil CO_2 emissions from farmland ecosystems have a huge impact on global carbon emissions (Liu et al., 2018). Therefore, to achieve low-emission agricultural production, it is necessary to further study the effects of proper farming methods on gaseous nitrogen (NH_3 and N_2O) and gaseous carbon (CO_2) emissions from farmland soil.

Replacement of chemical fertilizer with organic fertilizer and phased fertilization by stages are important measures to reduce the amounts of chemical fertilizers applied. Replacing chemical fertilizer with organic fertilizer can effectively avoid soil acidification and improve soil nutrient contents and soil enzyme activities (Okami et al., 2016). Fertilization by stages can significantly improve the fertilizer utilization rate and increase crop yield (Zhou et al., 2017). The combined application of organic and inorganic fertilizers can not only increase wheat yield but also improve soil physical and chemical properties and significantly enhance nitrogen fertilizer use efficiency (Xia et al., 2017; Zhao et al., 2016). Replacing chemical fertilizers with organic fertilizers is an important method to reduce NH_3 volatilization (Ti et al., 2019). Studies have shown that in winter wheat and summer maize planting, appropriate replacement of chemical with organic fertilizer can significantly reduce NH_3 volatilization and N_2O emissions but increase CO_2 emissions (Wang et al., 2018; Zhou et al., 2011). Organic fertilizer can improve soil microbial content and soil enzyme activity. Soil enzyme activity is significantly correlated with carbon and nitrogen transformation. Urease and nitrate

reductase are important invertases of soil nitrogen, and β -glucosidase is an important invertase of the soil carbon cycle (Sinsabaugh et al., 2012). Studies have shown that compared with cellulase activity, an increase in ligninase activity can better catalyze the degradation of chemical refractory carbon in soil (Chen et al., 2020).

Northeast China is an important grain production area. As farmers seemingly neglected the ecological environment and paid more attention to yield and efficiency, they blindly pursued yields in agricultural production and overinvested in chemical fertilizers such as urea, which caused environmental pollution. In this study, spring wheat fields were taken as the study object, and chemical nitrogen fertilizer was applied in stages while replacing part of the chemical fertilizer with organic fertilizer in accordance with the conventional tillage mode and fertilizer dosage in agricultural production in Heilongjiang Province. The objectives of this study were to (i) evaluate different fertilization modes optimize a fertilization mode for agricultural production in northeast China that can reduce environmental pollution, and (ii) explore the effects of phased chemical fertilizer application on soil NH_3 volatilization, N_2O and CO_2 emissions and soil enzyme activities when replacing 25% chemical nitrogen fertilizer with organic fertilizer.

Materials And Methods

Experimental site and soil description

The field experiments were carried out at the Northeast Agricultural University Experiment Station, Heilongjiang Province, P. R. China (45°773' N, 126°938' E). The site belongs to a typical temperate continental monsoon climate and the average annual temperature is 3.7°C. The annual precipitation is 500 mm. The soil is a typical black soil. The surface soil properties (0-20 cm) before the beginning of the experiment were as follows: pH 6.87, total N 1.7 g kg⁻¹, soil organic matter 25.3 g kg⁻¹, ammonium nitrogen (NH_4^+ -N) 5.55 mg kg⁻¹, nitrate nitrogen (NO_3^- -N) 5.85 mg kg⁻¹, alkali-hydrolyzable nitrogen 149.25 mg kg⁻¹, Olsen-P 53.01 mg kg⁻¹, and available potassium 198.81 mg kg⁻¹.

Experimental design and treatments

The experiment was divided into five treatments: (1) CF: conventional fertilization; (2) N0: no nitrogen fertilizer, while the amount of phosphorus and potassium was the same as in the CF treatment; (3) N1: 25% of chemical nitrogen fertilizer was replaced by organic fertilizer, while the amount of phosphorus and potassium was the same as in the CF treatment and all fertilizer was applied before sowing; (4) N2: 25% of chemical nitrogen fertilizer was replaced by organic fertilizer, while the amount of phosphorus and potassium was the same as that in the CF treatment, with 2/3 of chemical nitrogen fertilizer applied before sowing and 1/3 at the jointing stage; (5) N3: 25% of chemical nitrogen fertilizer was replaced by organic fertilizer, while the amount of phosphorus and potassium was the same as in the CF treatment, 1/3 of chemical nitrogen fertilizer was applied before sowing, and at the jointing and booting stages. Each treatment was repeated three times. The nutrient contents of the conventional fertilizer treatment were 75 kg N ha⁻¹, 75 kg P₂O₅ ha⁻¹ and 37.5 kg K₂O ha⁻¹, and the total application rates of P₂O₅ and K₂O in each fertilizer treatment were consistent with those in the conventional fertilizer treatment.

The spring wheat variety Kechun-9 was selected in this experiment. The plot area was 2.4 m×8.0 m=19.2 m². The seeds were sown on April 9, 2020, with chemical and organic fertilizer applied as basal fertilizers. The depth of seeding and fertilizer application was 5 cm, and the wheat density was 6.5 million hm⁻² plants. Chemical fertilizer consisted of urea (N, 46.0%), calcium superphosphate (P₂O₅, 46%) and potassium chloride (K₂O, 60%), and a commercial organic fertilizer was used (N-P₂O₅-K₂O, 2.33-1.8-2.35; organic matter, 46.1%). The fertilizer dosage for the spring wheat growing season is shown in Table 1.

Table 1

The schedule of fertilization during wheat growing season (kg ha⁻¹)

Treatment	Before sowing				Jointing stage	Booting stage
	Organic Fertilizer	Urea	Triple superphosphate	Potassium chloride	Urea	Urea
CF	—	163	163	63	—	—
N0	—	—	163	63	—	—
N1	805	123	131	31	—	—
N2	805	82	131	31	41	—
N3	805	41	131	31	41	41

Calculation of cumulative soil NH₃, N₂O and CO₂ emissions

The NH₃ volatilization flux was measured by the boric acid absorption-continuous airflow sealing method, N₂O and CO₂ were collected by the static incubator method, and the concentrations of N₂O and CO₂ were analyzed by gas chromatography (Shang et al., 2014). During the test, an airtight chamber made of polyvinyl chloride (PVC, diameter 16 cm, height 16 cm, 5 cm into the ground, with an air extraction device attached to the top of the chamber) was inserted into the test area to measure NH₃ volatilization and N₂O and CO₂ emissions. The temperature and air temperature in the closed chamber were measured with a thermometer at the same time the gas samples were collected: three days after seeding and fertilization (April 11, SD-1), 8 days after seeding (April 16, SD-2), at the wheat seedling stage (May 1, SS), before the jointing stage fertilization (June 2, JS-1), after the jointing stage fertilization (June 7, JS-2), before the booting stage fertilization (July 3, BS-1) and after the booting stage fertilization (on July 9, BS-2).

Calculation formula of NH₃ volatilization rate:

$$v = M \times A^{-1} \times D^{-1} \times 10^{-2} \quad (1)$$

v is the volatilization rate of NH₃-N (kg ha⁻¹ d⁻¹); M is the ammonia quantity (NH₃-N, mg) measured each time by a single device of the closed method; A is the cross-sectional area (m²) of the capturing device; D is the time of each successive capture (d).

Calculation formula of N₂O and CO₂ emission fluxes:

$$F = M \times \rho \times (K + T)^{-1} \times H \times R^{-1} \times (dc/dt) \quad (2)$$

F is the emission flux (μg m⁻² h⁻¹), M is the molar mass of carbon (12 g mol⁻¹), ρ is the density of N₂O (g L⁻¹), H is the height of the collection box (cm), R is the universal gas constant (8.314 J mol⁻¹ K⁻¹), K is the standard temperature (K), T is the average temperature in the chamber at the time of sampling (°C), and dc/dt is the N₂O emission rate (μL L⁻¹ min⁻¹).

Calculating formula of NH₃, N₂O and CO₂ emission accumulation:

$$Ma = 0.5 \times (Fi + Fi+1) \times n \quad (3)$$

The cumulative emission of soil NH₃, N₂O and CO₂ (t ha⁻¹) were denoted by M_{ai} , where the emission of soil NH₃, N₂O and CO₂ is presented by Fi at initial time; and the emission of soil NH₃, N₂O and CO₂ is presented by $Fi+1$ after the following time i ; n is the number of interval days for soil NH₃, N₂O and CO₂ emission.

Gas emission coefficient calculation formula:

$$\rho = (M_N - M_{N0}) / 163 \times 100\% \quad (4)$$

ρ is the gas emission coefficient; M_N is the cumulative amount of gas volatilization under different treatments; M_{N0} is the volatile accumulation of blank treatment gas.

Calculation formula of gas emission reduction ratio:

$$R = (M_{CF} - M_N) / (M_{CF} - M_{N0}) \times 100\% \quad (5)$$

R is the proportion of gas emission reduction; M_{CF} is the cumulative emission of gas volatilization from conventional treatment. M_N is the cumulative emissions of gas volatilization from other fertilization treatments. M_{N0} is the cumulative emission of volatilization of blank treatment gas.

The calculation formula of environmental loss benefit caused by gas volatilization:

$$EC = M \times DC \quad (6)$$

EC is environmental loss, Yuan ha^{-1} ; M is the accumulation of soil gas volatilization, kg ha^{-1} ; DC is the environmental loss of gas volatilization, NH_3 is 37.5 Yuan kg^{-1} , N_2O is 310 $\times 0.4$ Yuan kg^{-1} , CO_2 is 0.4 Yuan kg^{-1} (Gu et al., 2012; Li et al., 2021).

Soil Sampling and Analysis

Soil samples were collected at the seedling stage (May 1, SS), jointing stage (June 7, JS), booting stage (July 9, BS) and maturity stage (July 28, MP). Soil samples from the 0-10 cm layer were collected with a soil drill, and 5 points were taken and evenly mixed to form a composite soil sample. Some of the fresh samples were stored, and the contents of NH_4^+ and NO_3^- in the soil were determined by 0.01 mol L^{-1} CaCl_2 and flow analysis. Some samples were dried, ground and screened, and pH, organic matter and total nitrogen were determined according to conventional soil agrochemical laboratory methods. Urease was determined by the phenol-sodium hypochlorite colorimetric method, nitrate reductase was determined by the phenol-disulfonic acid colorimetric method, β -glucosidase was determined by the p-nitrophenol colorimetric method, and catalase was determined by the potassium permanganate titration method (Mei et al., 2020; Saiya et al., 2002).

The apparent soil nitrification rate (SNR) was calculated according to the following equation:

$$\text{SNR} = A / (A+B) \times 100\%$$

where A indicates the concentrations of NO_3^- in the surface soil after different fertilizer applications, and B indicates the concentrations of NH_4^+ in the surface soil after different fertilizer applications.

Crop yield

At the mature stage, a 0.9 m^2 quadrat (3 rows and 2 meters) was randomly selected from each plot to calculate the planting density. The aboveground plant parts were collected and put into nylon net bags, dried naturally and threshed for yield measurement.

Data analysis

Excel 2010 software was used for data calculation. SPSS 20.0 software was used for single factor analysis of variance (LSD) among all processes at the 0.05 probability level. The figures were plotted by software Origin 2019b.

Results

Soil NH_3 volatilization

As shown in Fig. 1, in general, the NH_3 volatilization rate decreased significantly from fertilization at the seeding stage to the jointing stage, and the NH_3 volatilization rate of the N1 treatment was significantly lower than that of the CF treatment during the wheat growth stage ($P < 0.05$). Staging fertilizer applications reduced the NH_3 volatilization rate before the jointing stage but significantly increased the NH_3 volatilization rate under the N2 treatment after the jointing stage and under the N3 treatment at the jointing stage. The volatilization accumulation of NH_3 in the CF treatment was significantly higher than that in the organic replacement treatments, and NH_3 volatilization from the fertilization to seedling stages accounted for the largest proportion of the total accumulation. Compared with the CF treatment, the N1, N2 and N3 treatments reduced NH_3 emission accumulation by 28.94%, 19.63% and 19.70%, respectively. Compared with the N1, N2 and N3 treatments, fertilization at different stages reduced

NH₃ emission accumulation at the seedling stage, but fertilization at different stages significantly increased NH₃ emission accumulation at the jointing stage and booting stage, so the N1 treatment had the lowest NH₃ emission accumulation.

Soil N₂O emissions

As shown in Fig. 2, the N₂O emission rate was the highest after fertilization at the seeding stage and decreased significantly from the fertilization to the jointing stage. In general, the N₂O emission rate of the CF and N1 treatments decreased gradually during the growth stages of wheat. The N₂O emission rate before the jointing stage was decreased by applying chemical fertilizer in stages, but the N₂O emission rate at the jointing stage and under the N3 treatment after fertilization at the jointing and booting stages was significantly increased. The N₂O emission accumulation under the CF treatment was significantly higher than that under the other treatments, and the N₂O emissions from the fertilization to seeding stages accounted for the largest proportion of the total accumulated. Compared with that under CF, the accumulation of N₂O emissions under N1, N2 and N3 decreased by 10.29%, 11.52% and 16.87%, respectively. Compared with the N1 treatment with one-time fertilization, the N2 and N3 treatments with phased fertilization significantly reduced N₂O emissions at the seeding stage, but they did not significantly increase N₂O emissions at the jointing and booting stages, so the accumulation of N₂O emissions under the N1 treatment was higher than that under the N2 and N3 treatments.

Soil CO₂ emissions

As shown in Fig. 3, during the growth stage, the CO₂ emission rate under the N1 treatment was significantly lower than that under the CF treatment. The staged application of chemical fertilizer reduced the CO₂ emission rate before the jointing stage but significantly increased the CO₂ emission rate under the N2 treatment after the jointing stage and under the N3 treatment at the jointing stage. The CO₂ emission accumulation of the CF treatment was significantly higher than that of the other treatments, and the CO₂ emission under the N1 treatment was lower than that under the CF treatment in all stages. Compared with CF, the CO₂ emission accumulation under the N1, N2 and N3 treatments decreased by 17.21%, 7.27% and 11.74%, respectively. Compared with N1, N2 and N3, which employed phased fertilization could reduce CO₂ emission accumulation from the seedling stage to the jointing stage, but phased fertilization could significantly increase CO₂ emission accumulation at the jointing stage and booting stage, so the N1 treatment resulted in the lowest CO₂ emission accumulation.

Soil pH, SOC, total N, NH₄⁺-N and NO₃⁻-N contents

Soil fertility is one of the important factors affecting crop yield, and soil pH, organic matter, total nitrogen, ammonium nitrogen and nitrate nitrogen contents are important indexes to measure soil fertility. As shown in Fig. 4, the application of organic fertilizer increased the soil total nitrogen content but had no significant effect on the soil organic matter and pH. Compared with the CF treatment, the N1 treatment had the best effect on increasing soil nitrogen nutrients. Applying chemical nitrogen fertilizer by stages improved the soil nitrogen nutrient content at the later growth stage of wheat but decreased the soil nitrogen nutrient content at the seedling stage and jointing stage of wheat. The application of organic fertilizer can increase the content of available nutrients in soil, and the content of ammonium and nitrate nitrogen in soil may be significantly improved. Compared with the N2 and N3 treatments, the N1 treatment had a better effect on increasing the ammonium nitrogen and nitrate nitrogen contents.

The SNR value under the N1 treatment was the lowest at all stages and was significantly lower than that under the N0 and CF treatments (Table 2; $P < 0.05$). The N1, N2 and N3 treatments were superior to the N0 and CF treatments in inhibiting NH₄⁺ to NO₃⁻ transformation. The maximum value of NH₄⁺/NO₃⁻ appeared in the jointing stage under the N0 treatment and was significantly higher than that of the N0 and CF treatments (Table 2; $P < 0.05$). NH₄⁺/NO₃⁻ under the N3 treatment was significantly increased by 80.9% and 130.3% at the jointing and booting stages, respectively. In general, with the growth of wheat, the value of NH₄⁺/NO₃⁻ decreased under urea application alone. The NH₄⁺/NO₃⁻ value could be increased by applying organic fertilizer and by phased fertilization at different periods.

Table 2

The SNR and $\text{NH}_4^+/\text{NO}_3^-$ as affected by the application of Substitute organic fertilizer for chemical fertilizer and stage fertilization. Different lowercase letters in the same column indicate a significant difference between treatments at $P < 0.05$ level.

Treatment	SNR(%)				$\text{NH}_4^+/\text{NO}_3^-$			
	SS	JS	BS	MP	SS	JS	BS	MP
CF	71.7±2.9a	16.1±1.4b	22.4±5.1a	22.7±0.8b	0.40±0.06c	5.23±0.53ab	3.58±1.04a	3.40±0.16ab
N0	78.4±1.1a	12.3±2.6b	25.7±5.6a	34.5±6.6a	0.28±0.02c	7.34±1.76a	2.98±0.86a	1.96±0.56c
N1	36.2±2.5c	19.8±0.9b	25.9±4.6a	19.6±0.8b	1.77±0.19a	4.05±0.22bc	2.92±0.69a	4.12±0.20a
N2	53.1±0.5b	38.4±1.8a	32.8±2.5a	21.5±1.9b	0.89±0.02b	1.61±0.12d	2.05±0.23a	3.67±0.42ab
N3	73.4±5.5a	34.8±5.5a	32.2±6.6a	27.3±5.3ab	0.37±0.10c	1.91±0.46cd	2.21±0.75a	2.75±0.67bc
Note: SS, seeding stage; JS, jointing stage; BS, booting stage; MP, mature stage.								

Discussion

Soil NH_3 volatilization

In this study, compared with conventional fertilization, replacing 25% of chemical fertilizer with organic fertilizer can reduce the emission rates and amounts of NH_3 and N_2O , mainly because a large number of organic acids are generated during the decomposition of organic fertilizer. Organic acids can reduce soil pH and increase the soil retention of NH_4^+ , and the volatilization rate and volatilization accumulation of NH_3 are reduced (Liang et al., 2012; Pant et al., 2017). After fertilization at the sowing stage, the NH_3 volatilization rate in the treatments with staged fertilization was lower than that in the one-time fertilization treatment, but the volatilization peak appeared at the jointing and booting stages, and the emission peak showed a decreasing trend. This is because the input of exogenous nitrogen promoted the process of soil denitrification, and a large amount of NH_3 was generated in a short time (Vincent et al., 2017). Rational application of organic fertilizer significantly enhances soil carbon and nitrogen conversion capacity and enzyme activity (Liu et al., 2018; Okami et al., 2016), as shown in Fig. 5. This study found that replacement of 25% of chemical nitrogen with organic fertilizer treatments significantly increased the contents of ammonium nitrogen and nitrate nitrogen in soil because organic fertilizer increased the number and activity of soil microorganisms and soil enzymes, promoted nitrogen transformation, and was conducive to the transformation of soil nitrogen into forms that can be absorbed and utilized by plants. In this study, the content of ammonium nitrogen in soil under the N1, N2 and N3 treatments was generally higher than that of nitrate nitrogen at the harvest stage because the organic substances contained in the organic fertilizer could adsorb NH_4^+ in soil, thus slowing nitrification of NH_4^+ (Thomsen et al., 1998), reducing NH_3 volatilization and increasing the NH_4^+ content in soil. This is beneficial to the absorption and utilization of nitrogen in spring wheat and increases yield.

Urease is an important soil hydrolase that catalyzes the hydrolysis of urea and organic fertilizer to produce NH_3 , N_2O and CO_2 (Hagin et al., 1990). After hydrolysis, NH_4HCO_3 is rapidly transformed into $\text{NH}_4^+\text{-N}$ and HCO_3^- , part of which is absorbed by the soil as adsorbed NH_4^+ , and the other part enters the soil solution to rapidly improve the concentration of NH_4^+ and HCO_3^- in the soil, providing sufficient substrates for NH_3 volatilization and CO_2 emission. In this study, the soil urease activity was lower under organic than under conventional fertilization treatment, because reducing the urea content in soil reduces urea hydrolysis to produce nitrogen, and when the nitrogen needed for soil microbes is adequate, soil microbial activity changes, indirectly changing soil urease activity (Chen et al., 2017; Liu et al., 2017). As shown in the heat analysis in Fig. 5, N1 promoted urease activity more than N2 and N3, with higher urease activity at the booting stage than at the jointing stage; this is because the organic fertilizer promoted the stable and sustained release of nitrogen in the soil, providing an ample nitrogen source for microbial activity, and chemical fertilizers increased the nitrogen loss in stages, failing to provide nutrients in time.

Under the action of soil urease, urea is hydrolyzed into NH_4HCO_3 and then rapidly transformed into $\text{NH}_4^+\text{-N}$. Part of the urea is absorbed by the soil colloids as adsorbed NH_4^+ , and the other part enters the soil solution so that the concentration of NH_4^+ increases rapidly, providing sufficient substrate for ammonia volatilization. However, a large number of organic nitrogen components in organic fertilizer can participate in the process of NH_3 volatilization only after a long time of mineralization and decomposition (Shan et al., 2015). In addition, organic matter will release a large number of organic acids and form humus during the decomposition process, which inhibits the increase in soil pH during urea hydrolysis. Thus, NH_3 volatilization in soil was significantly inhibited.

Soil N_2O emissions

In this study, the treatments in which organic fertilizer replaced chemical fertilizer significantly reduced N_2O emissions because organic matter consumes oxygen in soil during microbial decomposition, inhibits nitrification, and thus reduces the release of N_2O in soil. In this study, the soil nitrate nitrogen content of the CF, N2 and N3 treatments was higher than that of ammonium nitrogen at the jointing stage, which might have been due to the transformation of NH_4^+ into NO_3^- under the action of nitrifying microorganisms, which would also lead to an increase in N_2O emissions. As shown in Fig. 6, the positive effects on nitrate reductase activity were $\text{N1} > \text{N2} > \text{CK} > \text{N3}$ in this study, which may have been due to the organic C/N value being lower, which is suitable for microbes, than those under the chemical fertilizer (Rashid et al., 2020). The organic fertilizer contained mushroom residue that can more quickly provide soil organic matter, increasing the available substrate for microbes in the soil, while nitrate reductase activity suppresses soil nitrification, and thus N_2O emissions are reduced. Therefore, soil nitrate reductase activity is significantly positively correlated with N_2O emissions (Chen et al., 2017; Liu et al., 2017).

In this study, N1 showed the highest nitrate reductase activity in soil, which may have been because the C/N value of organic fertilizer was lower than that of the single chemical fertilizer and was suitable for microbial decomposition (Rashid et al., 2020). Moreover, organic fertilizer can provide soil organic matter more quickly than fungal residue, and thus the available substrate of soil microbes increases, and the denitrifying enzyme activity, in particular, nitrate reductase activity, increases (Chen et al., 2014; Elzobair et al., 2016). Soil nitrate reductase decreased gradually with wheat growth, which may have been related to the choice of spring wheat as the experimental object. Spring sowing improves soil aeration, the nitrifying bacteria in the soil increased, and denitrification resulted in the highest nitrate reductase activity in May-June. As temperatures rose gradually, wheat soil water evaporation increased. The suitable soil hydrothermal environment formed in May and June was broken, the metabolic activities of denitrifying microorganisms decreased, and the activity of soil nitrate reductase decreased (Van et al., 2014).

Soil CO_2 emissions

Urease is an important soil hydrolase that catalyzes the hydrolysis of urea and organic fertilizer to produce CO_2 (Hagin et al., 1990). After hydrolysis, NH_4HCO_3 will rapidly transform into HCO_3^- and enter the soil solution, rapidly increasing the concentration of HCO_3^- in the soil and providing sufficient substrate for CO_2 emission. Urea hydrolysis is the main source of CO_2 emissions, while the replacement of chemical fertilizer by organic fertilizer reduces the urea content in soil, so the CO_2 emissions under the replacement treatments were significantly reduced. In this study, as shown in Fig. 7, the activities of β -glucosidase and catalase under the N1 treatment were higher than those under the other treatments, mainly because organic fertilizer improved soil fertility, and the increase in carbon, nitrogen and oxygen contents promoted the growth of soil microorganisms and the secretion of microbial enzymes and increased the activities of β -glucosidase and catalase. The application of chemical and organic fertilizers increased the content of organic matter, led to an increase in soil microbial species and number, and increased the activities of hydrolytic enzymes such as β -glucosidase and catalase, while phased fertilization failed to provide carbon, nitrogen and oxygen in time, so the enzyme activities decreased. Compared with β -glucoside, catalase was more conducive to the degradation and transformation of soil refractory carbon (Chen et al., 2020). As shown in Fig. 7, N2 and N3 increased soil catalase activity at the later stage of wheat growth, thus improving microbial carbon utilization and transformation and promoting CO_2 emissions.

Soil NH_3 , N_2O and CO_2 environmental benefits and wheat yields

As shown in Table 3, there were significant differences in NH₃, N₂O and CO₂ emission rates (ρ), emission reduction rates (R) and environmental costs (EC) among the different treatments. The emission rates and environmental costs of NH₃, N₂O and CO₂ under the CF treatment were the highest. Compared with those of the CF treatment, the NH₃ emission rates of N1, N2 and N3 decreased by 53.56%, 36.29% and 36.50%, respectively, and the N₂O emission rates decreased by 21.13%, 23.94% and 35.21%, respectively. The CO₂ emission rate decreased by 32.76%, 13.83% and 22.36%; the N1 treatment had the highest emission reduction rates for NH₃ and CO₂. Compared with N1, N2 and N3 reduced the emission reduction rates of NH₃ by 32.14% and 31.90% and those of CO₂ by 57.78% and 31.75%, respectively. N3 had the highest N₂O emission reduction rate. Compared with that of N3, the N₂O emission reduction rates of N1 and N2 were 38.30% and 30.45%, respectively. The environmental costs of NH₃ in the N1, N2 and N3 treatments decreased by 28.94%, 19.64% and 19.71%, the environmental costs of N₂O decreased by 10.32%, 11.64% and 16.73%, and the environmental costs of CO₂ decreased by 17.20%, 7.27% and 11.74%. Compared with N2 and N3, N1 had the lowest total environmental cost of gas emissions, with a 9.25% and 5.46% reduction in total environmental cost compared with N2 and N3.

Table 3

NH₃, N₂O and CO₂ average emission rates (ρ, %), emission reduction rate (R, %), environment cost (EC, Yuan ha⁻¹) under different treatments, total environment cost (Total EC, Yuan ha⁻¹) and wheat yield (kg ha⁻¹).

Treatment	NH ₃			N ₂ O			CO ₂			Total EC	Yield
	ρ	R	EC	ρ	R	EC	ρ	R	EC		
CF	4.63	—	523.63	0.71	—	301.40	515.88	—	640.40	1465.44	4159±166b
N0	—	—	240.58	—	—	157.37	—	—	304.05	702.00	2813±129c
N1	2.15	53.54	372.09	0.56	21.60	270.30	346.89	32.76	530.22	1172.60	5046±253a
N2	2.95	36.33	420.81	0.54	24.35	266.33	444.52	13.83	593.87	1281.02	4851±116a
N3	2.94	36.46	420.43	0.46	35.01	250.97	400.53	22.36	565.19	1236.59	3864±153b

Compared with CF, one-time fertilization and two-stage fertilization based on 25% of chemical fertilizer being replaced by organic fertilizer can significantly increase the yield of spring wheat by 21.33% and 16.64%, respectively. One-time fertilization has the best yield increase effect (Table 3) because spring wheat in Northeast China has a short growth period, and its demand for nitrogen is concentrated from the jointing stage to the booting stage (Ladha et al., 2016). The release of nutrients by the organic fertilizer was slow, and the yield of spring wheat in this experiment was N1>N2>N3, further indicating that phased fertilization caused a large amount of nitrogen fertilizer to remain unavailable for too long, resulting in insufficient nitrogen supply in the early spring wheat growth. Compared with that under one-time fertilization, nitrogen use efficiency was reduced, affecting the growth of spring wheat vegetative organs. In this study, replacement of 25% of chemical fertilizer with slow nitrogen-release organic fertilizer reduced early nitrogen loss, and since spring wheat shows an increased demand for nitrogen in the middle and late growth periods, proper fertilizer staging can more effectively meet the demands for nitrogen for spring wheat growth; thus, two applications significantly increased yields (Redding et al., 2016). However, the N3 treatment, which included substitution with organic fertilizer and application in three stages, decreased yield. This is because fertilizer can be absorbed and utilized by crops only after it is transformed and migrates into the soil. However, multiple spray or broadcast applications of nitrogen fertilizer in a short period of time easily causes fertilizer loss and reduces the fertilizer utilization rate. Therefore, the three-stage fertilization treatment could not supply nitrogen in a timely manner during the most critical stage for plant nutrient absorption (Li et al., 2018; Wang et al., 2018), resulting in a yield reduction in spring wheat.

Conclusions

Based on soil characteristics and climate conditions in the black soil region of Northeast China, compared with conventional application of chemical fertilizer and staging applications of chemical nitrogen fertilizer while substituting organic fertilizer for 25% of the chemical nitrogen fertilizer, the one-time fertilization before sowing with a substitution of 25% of chemical nitrogen with organic fertilizer could effectively improve soil carbon and nitrogen nutrient contents and spring wheat yield; moreover, under this treatment, the activities of urease, nitrate reductase, β-glucosidase and catalase in soil were decreased, the content of NH₄⁺-N in

soil was increased, the amount of NH_3 volatilization, N_2O and CO_2 emissions were significantly reduced, and the economic and environmental benefits were increased.

Declarations

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Competing Interests:

The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors have no relevant financial or non-financial interests to disclose.

Author Contributions:

All authors contributed to the study conception and design. Liu Chunzhu: Data curation, Writing-original draft, Visualization, Investigation. Zhu Yingxue: Conceptualization, Methodology, Writing-review & editing, Software. Wang Qi: Formal analysis. Xu Lianzhou: Conceptualization, Methodology. Ma Xianfa: Writing- review & editing, Funding acquisition. The first draft of the manuscript was written by Liu Chunzhu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability:

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1

Substitute organic fertilizer for chemical fertilizer and fertilization with stage application method affected (a) NH_3 volatilization rate and (b) NH_3 volatilization accumulation during wheat growth.

Note: growth stage; SD-1, the third day after fertilization; SD-2, the eighth day after fertilization; SS, seeding stage; JS-1, before jointing stage fertilization; JS-2, after jointing stage fertilization; BS-1, before booting stage fertilization; BS-2, after booting stage fertilization; The same below.

Figure 2

Substitute organic fertilizer for chemical fertilizer and fertilization with stage application method affected (a) N₂O emission rate and (b) N₂O emission accumulation during wheat growth.

Figure 3

Substitute organic fertilizer for chemical fertilizer and fertilization with stage application method affected (a) CO₂ emission rate and (b) CO₂ emission accumulation during wheat growth.

Figure 4

Changes of (a) soil pH, (b) soil organic matter, (c) soil total nitrogen, (d) soil $\text{NH}_4^+\text{-N}$ and (e) soil $\text{NO}_3^-\text{-N}$ in spring wheat fields under the different nitrogen fertilizer managements.

Figure 5

(a) Changes in soil urease activity in different growth stages of wheat, (b) Heat map analysis of soil urease activity in different treatments.

Figure 6

(a) Changes in soil nitrate reductase activity in different growth stages of wheat, (b) Heat map analysis of soil nitrate reductase activity in different treatments.

Figure 7

(a) Changes in soil β -glucosidase activity in different growth stages of wheat, (b) Heat map analysis of soil β -glucosidase activity in different treatments, (c) Changes in soil catalase activity in different growth stages of wheat and (d) Heat map analysis of soil catalase activity in different treatments.