



Long-term appropriate N management can continuously enhance gross N mineralization rates and crop yields in a maize-wheat rotation system

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Abstract

A ¹⁵N tracing study was conducted with soils from a long-term nitrogen (N) fertilization experiment to quantify the concurrent gross N transformation rates in soil and the underlying mechanisms for crop yield and N₂O emission variability. The treatments were chemical fertilization (NPK, NP, PK, NK), organic fertilization (OF), half chemical/organic fertilization (HOF), and no fertilization (control, CK). The results showed that 30 years of repeated organic or chemical N applications significantly stimulated gross rates of N mineralization and autotrophic nitrification compared with CK. In addition, gross rates of N mineralization (except NP) and autotrophic nitrification (except CK, PK) in all treatments were significantly higher in 2019 (30 years) than in 2007 (18 years). However, the immobilization of mineral N decreased significantly (near to zero) after 30 years of repeated N applications compared to CK or the results of 18 years. The highest maize yields were observed in NPK, but they were not significantly different from HOF yields. The OM yields significantly increased with the duration of the experiment and reached values comparable to NPK yields after more than 12 years of repeated N applications. There was a logarithmic positive relationship between maize yields and gross N mineralization rates ($p < 0.01$), indicating that it was the key factor explaining the variations in yields. Long-term organic or chemical N applications significantly stimulated N₂O emission rates, in line with the stimulation of gross autotrophic nitrification rates. Highest N₂O emissions were associated with the organic fertilization treatments. Considering the most suitable climate-smart agricultural practice, taking into account yield, N₂O emissions, and carbon sequestration, the combination of organic and chemical N fertilizers can be recommended for the region where the study was carried out.

Keywords ¹⁵N tracing study · Crop yield · Gross N transformations · Long-term N management · N₂O emission

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Introduction

Long-term inappropriate use and management of nitrogen (N) have negative impacts on soil and the environment, such as declining soil fertility, soil acidification, soil structure degradation, greenhouse gas emissions, and eutrophication (Pernes-Debuyser and Tessier 2004; Long and Sun 2012). Thus, appropriate N management is required to maintain and improve soil fertility and crop production while minimizing N loss and N₂O emissions. Management practices, such as the balanced application of chemical fertilizers of N, P, and K, or mixed application of organic and inorganic fertilizers have been recommended in agroecosystem (Dong et al. 2014). Previous results have shown that appropriate N managements, as outlined above, can maintain soil productivity and increase soil organic carbon (SOC) and total N (TN) content, and crop yields (Cai and Qin 2006; Wang et al. 2015). However, crop yields are often significantly lower in long-term treatments using only organic fertilizers compared to a balanced application of chemical and organic fertilizers, although higher levels of SOC and TN are often observed in organic treatments. One of the possible reasons is the slow release of nutrients from organic matter, which affects crop growth at low temperatures (Cai and Qin 2006). Although this has been observed, the underlying mechanisms of long-term N management that regulate crop yield variability are still not well understood.

Soil N transformations, e.g., N mineralization-immobilization turnover (MIT), regulate the availability, conservation, and loss of N in soil, which are crucial for crop yields (Zhang et al. 2016). Soil substrates, e.g., SOC and TN, and microorganisms are the key factors regulating soil N transformations (Zhang et al. 2018). Many studies have shown that long-term N managements can significantly alter SOC and TN content, C/N ratio, pH, and microbial abundance and diversity (Zhong et al. 2015; Li et al. 2020; Liu et al. 2020; Wei et al. 2020), which in turn affects soil N transformations those are closely linked to crop yield variability and N₂O emissions (Wang et al. 2015, 2017; Dai et al. 2017). However, the opposite has also been reported that the addition of labile organic matter had no effect on gross soil N transformation rates whether under short-term (29 d) or long-term fertilization (Gibbs and Barraclough 1998; Zhang et al. 2012b). So far, it is not known whether long-term N managements can continuously influence gross soil N transformation rates.

Based on the previous studies outlined above, we hypothesized that (1) long-term N management can continuously enhance gross soil N transformations rates, especially N mineralization, nitrification, and immobilization, as previous investigations have shown that long-term N

management generally increases SOC and TN content as well as microbial abundance and diversity (Li et al. 2020; Liu et al. 2020; Wei et al. 2020); (2) the rate of N release from soil organic matter, i.e., gross N mineralization rate, is the key factor explaining the variation in crop yields, as the total N application rate is generally the same for all treatments in the long-term N management experiment; and (3) the stimulation of gross autotrophic nitrification rate is responsible for the variation in N₂O emissions. A ¹⁵N tracing technique (*Ntrace*) was used to quantify the gross soil N transformations rates in two sets of long-term fertilization soil samples that were collected in 2007 (18 years) and 2019 (30 years) of the same experiment, to identify continuous effects of long-term N managements on soil N transformations and the underlying mechanisms for the variations in crop yields and N₂O emissions in a maize-wheat rotation system.

Materials and method

Description of long-term N fertilization experiment

The experiment was initiated in 1990 in Fengqiu (35° 04' N, 113° 10' E), Henan Province, which is located in Huang-Huai-Hai Plain of China. The 30-year mean annual temperature is 13.9 °C, and the minimum and maximum monthly mean temperature is –1.0 °C in January and 27.3 °C in July, respectively. The mean annual precipitation (30-year) is 615 mm, most of which falls in summer (Ding et al. 2010). The soil with a sandy loam texture (approximately 9% clay, 21.8% silt) is derived from alluvial sediments of the Yellow River and classified as aquic inceptisol. The studied soil is P deficient. This area is a typical Chinese wheat–maize rotation area (i.e., winter wheat (*Triticum aestivum* L.) followed by summer maize (*Zea mays* L.)). Before treatments were established in 1990, the field had been cropped without fertilizer application from 1987 to 1989. The long-term experiment includes seven fertilizer application treatments: no fertilization (CK); chemical N, P, and K (NPK); chemical N and P without K (NP); chemical N and K without P (NK); chemical P and K without N (PK); organic fertilization without chemical fertilizers (OF); and half N from chemical fertilizer and another half from organic fertilization (HOF). N, P (as superphosphate), and K (as K₂SO₄) were applied at 150 kg N ha⁻¹, 32.7 kg P ha⁻¹, and 124 kg K ha⁻¹ for wheat and 150 kg N ha⁻¹, 26.2 kg P ha⁻¹, and 124 kg K ha⁻¹ for maize. In the OF treatment, organic fertilization was applied to provide the same amount of N as in NPK. The organic fertilization was made of wheat straw mixed with soybean cake and cotton seed cake. Chemical fertilizers were used as a basal fertilizer to provide the shortages of P and K in organic fertilizer treatments. In the treatments without

organic fertilizer application, two-thirds of N (as urea) was applied as basal fertilization and one-third as top-dressing for both crops.

The experiment has four replicates in a random block design plot (45.5 m²) that was isolated by cement banks (60-cm deep and 10 cm above the soil surface). Winter wheat was sown in October and harvested in early June of the next year. Maize was sown in June and harvested in October. Bulk soil (10 cores each 5 cm in diameter) from the plough layer (0–20 cm) were collected in October 2007 (Zhang et al. 2012b) and 2019 from each plot, respectively. Samples from the same treatment at the same time were pooled together into a composite sample and sieved immediately (2 mm). After that, each sample was divided into three sub-samples. The majority was stored at 4 °C for < 1 month before the start of the incubation experiment. About 50 g of fresh soil was stored at – 80 °C for DNA extraction and 100 g soil sample was air-dried for determination of soil chemical properties (e.g., pH, SOC, TN, C:N ratio).

¹⁵N tracing experiment

A ¹⁵N tracing experiment was carried out as described by Zhang et al. (2012b), except the ¹⁵N enrichment was adjusted from 20 ¹⁵N atom% excess to about 10 ¹⁵N atom% excess. Two ¹⁵N treatments were applied (each in triplicate): an ammonium label (¹⁵NH₄NO₃, 9.75 ¹⁵N atom% excess) and a nitrate label (NH₄¹⁵NO₃, 9.81 ¹⁵N atom% excess). For each soil, a series of 250-mL Erlenmeyer flasks was prepared with 20 g moist soil (oven-dry basis). After soil pre-cultivation for 1 day, a volume of 2 mL of ¹⁵NH₄NO₃ or NH₄¹⁵NO₃ solution was added to each of the flasks to provide 50 mg NH₄⁺-N kg⁻¹ soil or 50 mg NO₃⁻-N kg⁻¹ soil. The soil was adjusted to 60% water holding capacity and incubated for 72 h at 25 °C. The flasks were sealed with parafilm, which was pierced to prevent excessive evaporation of water but allow gas exchange. The soils were extracted with 2 M KCl at 0.5, 24, 48, and 72 h after NH₄NO₃ application to determine the concentration and isotopic composition of the NH₄⁺-N and NO₃⁻-N.

Three flasks of each treatment were used to measure N₂O emission rates at 24, 48, and 72 h after adding NH₄NO₃ solution. Then, the flasks were sealed with silicone rubber stoppers at 18, 42, and 66 h. Before each measurement, bottles were ventilated and then sealed for 6 h. The headspace gas was sampled using a syringe. N₂O concentrations were determined on a gas chromatograph (Agilent 7890, Wilmington, USA).

¹⁵N tracing model

The ¹⁵N tracing model (*Ntrace*) used in this study was an updated version based on Müller et al. (2007) (Fig. S1). It

was used to re-analyze the 2007 data (Zhang et al. 2012b) and the new data from 2019. The model considered 10 simultaneously occurring gross N transformations: M_{Nrec} , mineralization of recalcitrant organic-N to NH₄⁺; M_{Nlab} , mineralization of labile organic-N to NH₄⁺; I_{NH4_Nlab} , immobilization of NH₄⁺ to labile organic-N; I_{NH4_Nrec} , immobilization of NH₄⁺ to recalcitrant organic-N; R_{NH4ads} , release of adsorbed NH₄⁺; A_{NH4} , adsorption of NH₄⁺ on cation exchange sites; O_{NH4} , oxidation of NH₄⁺ to NO₃⁻; O_{Nrec} , oxidation of recalcitrant organic-N to NO₃⁻ (heterotrophic nitrification); I_{NO3} , immobilization of NO₃⁻ to recalcitrant organic-N; and D_{NO3} , dissimilatory NO₃⁻ reduction to NH₄⁺. The concentrations and ¹⁵N enrichment of NH₄⁺ and NO₃⁻ (average ± standard deviations) for each treatment were supplied to the model to calculate soil gross N transformation rates (μg N g⁻¹ soil day⁻¹).

The gross N transformation rates were calculated by simultaneously optimizing the kinetic parameters (setting as zero-order, first-order, or Michaelis–Menten kinetics) to minimize the degree of misfit between the modeled and observed values. The Markov Chain Monte Carlo (MCMC) algorithm is the key numerical technique of the model, which determines the probability density function (PDF) for each parameter. Thus, the MCMC method provides an average ± standard deviation for each gross N transformation rate (Müller et al. 2007).

Soil property analyses

Soil pH was measured using a DMP-2 mV/pH detector (soil:water, 1:2.5) (Quark Ltd, Nanjing, China). SOC was analyzed by wet-digestion with H₂SO₄-K₂Cr₂O₇, and TN was determined by semi-micro Kjeldahl digestion using Se, CuSO₄, and K₂SO₄ as catalysts (Weaver et al. 1994). Alkali-hydrolyzable N (AHN) was determined by alkaline diffusion method (Weaver et al. 1994). Olson P was determined by NaHCO₃ method and available K content was determined by CH₃COONH₄ extraction method (Chang and Jackson 1957). The soils were extracted with 2 M KCl at a soil:solution ratio of 1:5 on a mechanical shaker for 60 min at 300 rpm and 25 °C. NH₄⁺-N and NO₃⁻-N concentrations of soil extracts were measured by a continuous-flow analyzer (Skalar, Breda, the Netherlands). The ammonia-oxidizing bacteria (AOB) abundance was determined using real-time PCR (Zhang et al. 2020). Since ammonia-oxidizing archaea (AOA) were considered to be the main driver of nitrification in acidic soils (Burton et al. 2007), we focused only on AOB in this study.

Statistical analyses and calculations

Total gross N mineralization rate (M) = $M_{Nlab} + M_{Nrec}$

Total gross N immobilization rate = $I_{NH4} + I_{NO3}$

where M_{Nlab} is mineralization of labile organic N to NH_4^+ rate, M_{Nrec} is mineralization of recalcitrant organic N to NH_4^+ rate, I_{NH_4} is NH_4^+ immobilization rate, and I_{NO_3} is NO_3^- immobilization rate.

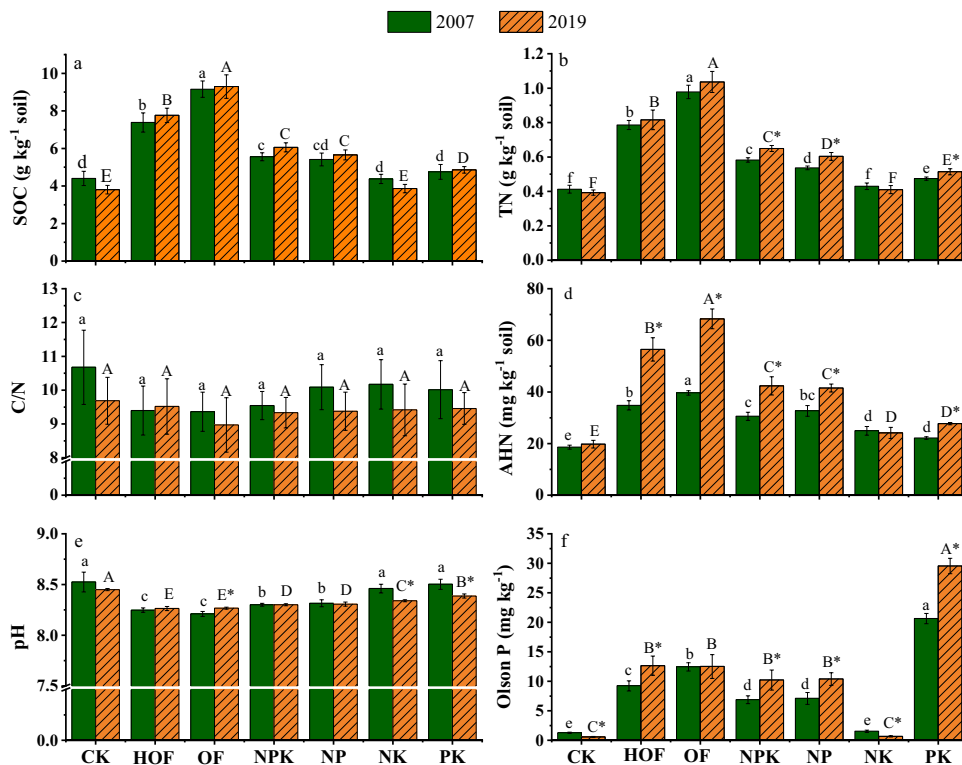
In this study, significant differences between treatments were estimated using one-way analysis of variance (ANOVA), based on the averages and standard deviations of the individual N transformation rates. Statistical calculations were performed using SigmaStat 4.0 (Systat Software Inc., San Jose, CA, USA). Based on the actual experimental repetitions (three repetitions), the least significant differences at the 5% significance level ($\text{LSD}_{0.05}$) were calculated for each N transformation rate, according to treatment (Müller et al. 2011). This presents the most conservative way to calculate LSDs. The observed error in the observations was linked to the number of actual repetitions and was reflected in the probability density function (PDF) of each parameter (Müller et al. 2007). Correlation and multiple stepwise regression analyses were carried out to test measured soil properties which were significantly related to soil N transformation rates, maize yields, and N_2O emission rates (SPSS 23.0, Inc., USA).

Results

Soil properties

Long-term repeated chemical or organic N application resulted in a significant change in SOC, TN, C/N, AHN, soil pH, and Olson P (Fig. 1). In both years 2007 or 2019, the highest SOC was observed in OF, i.e., 9.16 g kg^{-1} and 9.30 g kg^{-1} , respectively, and significantly higher than those in the other treatments. Compared with 2007, SOC was higher, but not significantly, in all treatment of 2019 except NK and CK. The dynamics of TN followed that of SOC with a decreasing trend of C/N in 2019 compared with 2007, except for HOF (Fig. 1c). The lowest AHN content was observed in CK with average of 18.6 and 19.8 mg kg^{-1} in 2007 and, respectively. The highest AHN was in OF with an average of 39.6 and 68.3 mg kg^{-1} in 2007 and 2019, respectively (Fig. 1d). AHN content was significantly higher in 2019 than in 2007, except CK and NK. Soil pH values ranged from 8.2 to 8.5 and from 8.3 to 8.4 in 2007 and 2019, respectively (Fig. 1e). The highest pH was observed in CK. The pH values decreased from 8.5 to 8.2 after long-term N application, especially in OF and HOF. The Olson P content in CK and NK was very low ($< 1.5 \text{ mg kg}^{-1}$), and was even lower in 2019 than in 2007 (Fig. 1f). The Olson P content was significantly higher in the chemical P fertilizer treatments than in CK and NK and increased with the duration

Fig. 1 Soil organic C (SOC) (a), total N (TN) (b), the ratio of soil organic C and total N (C/N) (c), alkali-hydrolyzable nitrogen (AHN) (d), soil pH (e), and Olson P (f) under different fertilization treatments in 2007 and 2019. CK, no fertilization; NPK, chemical N, P, and K; NP, chemical N and P without K; NK, chemical N and K without P; PK, chemical P and K without N; OF, organic fertilization without chemical fertilizers; HOF, half N from chemical fertilizer and another half from organic fertilization. Error bars refer to standard deviations. Different lowercase and capital letters above bars indicate significant differences among the treatments of 2007, and 2019, respectively ($p < 0.05$). * above bars of 2019 indicates significant differences between 2007 and 2019 in the same treatment ($p < 0.05$)



of the experiment. The highest Olson P was observed in PK with 20.6 and 29.5 mg kg⁻¹ in 2007 and 2019, respectively.

Long-term N application increased AOB abundance compared with CK and PK ($p < 0.01$) (Fig. 2) with a large increase over the 12 years since 2007 ($p < 0.01$). Chemical N fertilizer had a greater impact on AOB abundance than organic fertilizer.

Gross N transformation rates

Long-term application of organic or chemical N enhanced gross N mineralization rates (M), compared with CK and PK (Fig. 3a). The highest M was observed in NPK averaging 3.76 and 6.57 $\mu\text{g N g}^{-1} \text{d}^{-1}$ in 2007 and 2019, respectively. The M in all treatments in 2019 was significantly higher than those in 2007, except NP. A particularly high stimulation of M was observed in OF (+91%), while it only increased 50% and 75% in HOF and NPK in 2019 compared to 2007. There was no significant difference between OF and HOF in 2019. Dissimilatory NO_3^- reduction to NH_4^+ (DNRA) rate, another NH_4^+ supplying process, was negligible (data not shown). There was a positive relationship between M and TN ($p < 0.05$) (Fig. 4a) and a negative correlation between M and C/N ($p < 0.01$) (Fig. 4b). Moreover, M increased with increasing AHN ($p < 0.01$) (Fig. 4c). The results of the stepwise regression analysis, including TN, C/N, AHN, Olson P,

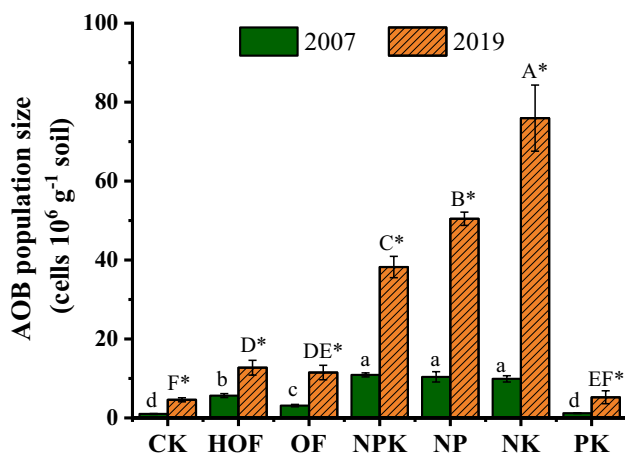


Fig. 2 Soil AOB abundance under long-term fertilizer management in 2007 and 2019. Error bar is standard deviation. CK, no fertilization; NPK, chemical N, P, and K; NP, chemical N and P without K; NK, chemical N and K without P; PK, chemical P and K without N; OF, organic fertilization without chemical fertilizers; HOF, half N from chemical fertilizer and another half from organic fertilization. Different lowercase letters above bars indicate significant differences between different treatments of 2007 ($p < 0.01$). Different lowercase and capital letters above bars indicate significant differences among the treatments of 2007, and 2019, respectively ($p < 0.01$). * above bars of 2019 indicates significant differences between 2007 and 2019 in the same treatment ($p < 0.01$). Data of AOB abundance in 2007 are from Chu et al. (2008)

and pH, showed that AHN alone could explain 49% of the variation in M ($p < 0.01$).

Similarly, long-term application of organic or chemical N also significantly stimulated gross autotrophic nitrification rate (O_{NH_4}) compared with CK and PK (Fig. 3b). The highest O_{NH_4} was observed in NPK and HOF. The O_{NH_4} in OF was significantly lower than in NPK and HOF. O_{NH_4} in HOF, OF, NPK, NP, and NK were 1.2, 1.5, 1.3, 1.3, 1.1, 1.3, and 1.5 times higher in 2019 than in 2007, respectively. O_{NH_4} was negatively related to pH ($p < 0.01$) (Fig. 4d) and positively correlated with M ($p < 0.01$) and AOB abundance ($p < 0.01$) (Fig. 4e, f). The results of the stepwise regression analysis, which included M , pH, TN, C/N, Olson P, and AOB abundance, showed that M and pH could explain 86% of the variations in O_{NH_4} ($p < 0.01$). Judging by the standardized coefficients, M was a more important variable (0.607) in explaining the variations in O_{NH_4} than pH (-0.403). The oxidation of recalcitrant organic N to NO_3^- (heterotrophic nitrification) was negligible in all treatments of both years (data not shown).

Interestingly, the NH_4^+ immobilization rate (I_{NH_4}) was significantly lower for all N fertilization treatments in 2019 than in 2007 (Fig. 3c). In 2007, the highest I_{NH_4} was observed in HOF, OF, and NK with an average of 6.73, 6.05, and 5.75 $\mu\text{g N g}^{-1} \text{d}^{-1}$, respectively, which was significantly higher compared to CK, NPK, and PK. The lowest I_{NH_4} was in PK and NPK with an average of 1.14, and 2.26 $\mu\text{g N g}^{-1} \text{d}^{-1}$. In 2019, the I_{NH_4} was near zero in HOF, OF, and NPK, and less than 0.3 $\mu\text{g N g}^{-1} \text{d}^{-1}$ in NP and NK. The highest I_{NH_4} (4.22 $\mu\text{g N g}^{-1} \text{d}^{-1}$) in 2019 occurred in CK. Similarly, NO_3^- immobilization rates were also significantly lower in 2019 compared with 2007 (Fig. 3d). Total gross immobilization rates ($I_{\text{NH}_4} + I_{\text{NO}_3}$) were significantly lower in organic or chemical N fertilizer treatments in 2019 than those in 2007. No significant relationships were found between immobilization rates, measured soil properties, and gross N transformation rates.

In 2007, the adsorption of NH_4^+ on cation exchange sites rate (A_{NH_4}) varied from 0.55 to 2.67 $\mu\text{g N g}^{-1} \text{d}^{-1}$ with no significant differences in all treatments. In 2019, A_{NH_4} significantly reduced to less than 0.2 $\mu\text{g N g}^{-1} \text{d}^{-1}$ in all fertilizer treatments (Fig. 2e). The highest release of absorbed NH_4^+ rates (R_{NH_4}) were found in NP (5.89 $\mu\text{g N g}^{-1} \text{d}^{-1}$) and HOF (3.99 $\mu\text{g N g}^{-1} \text{d}^{-1}$) in 2019. The R_{NH_4} in CK, HOF, OF, NPK, NP, NK and PK treatments were 6.5, 1.5, 13.2, 9.2, 143.5, 13.3 and 23.2 times higher, respectively, than those in 2007. No significant relationship between A_{NH_4} or R_{NH_4} and measured soil properties were found.

Crop yields

In general, maize yields increased with significant fluctuation in HOF, OF, NPK, and NP in the duration of the

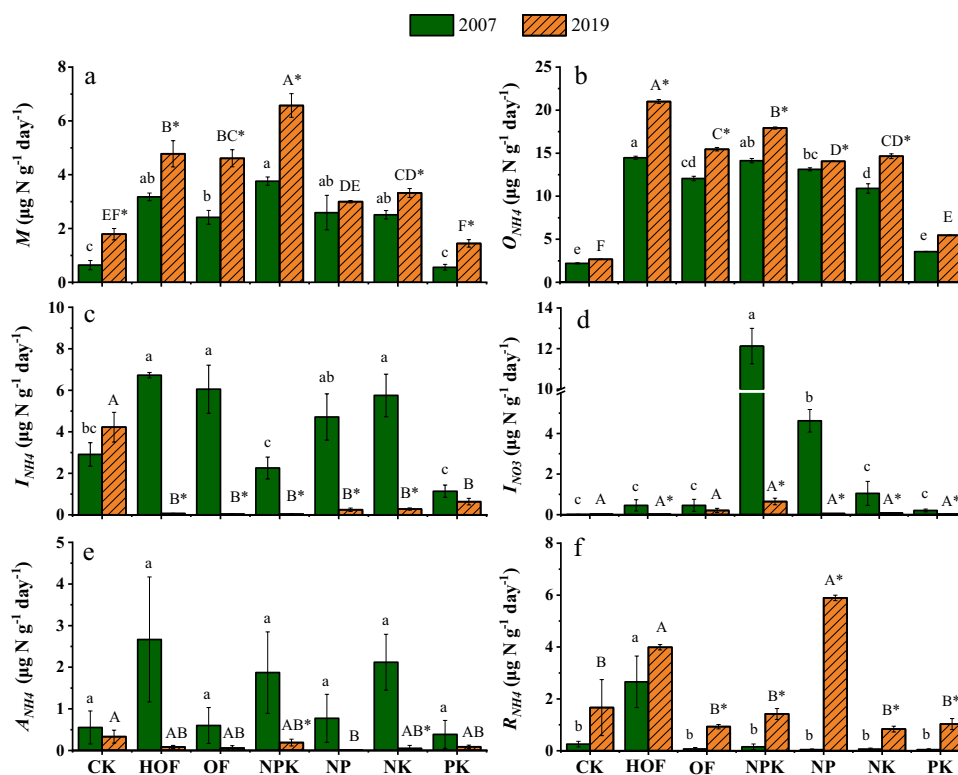


Fig. 3 Gross N mineralization rate (M) (a), gross autotrophic nitrification rate (O_{NH_4}) (b), immobilization rate of NH_4^+ (I_{NH_4}) (c), immobilization rate of NO_3^- (I_{NO_3}) (d), adsorption of NH_4^+ on cation exchange rates (A_{NH_4}) (e), and release of NH_4^+ on cation exchange rates (R_{NH_4}) (f) under different fertilization treatments of 2007 and 2019. CK, no fertilization; NPK, chemical N, P, and K; NP, chemical N and P without K; NK, chemical N and K without P; PK, chemi-

cal P and K without N; OF, organic fertilization without chemical fertilizers; HOF, half N from chemical fertilizer and another half from organic fertilization. Error bars refer to standard deviations. Different lowercase and capital letters above bars indicate significant differences among the treatments of 2007, and 2019, respectively ($p < 0.05$). * above bars of 2019 indicates significant differences between 2007 and 2019 in the same treatment ($p < 0.05$)

experiment and were significantly higher than in PK, NK, and CK (Fig. 5a). As the soils in this studied region were extremely P deficient, the lowest maize yields were observed in NK and CK with no significant difference between them. Maize yields in PK were significantly higher than those in NK and CK. Generally, maize yields were significantly higher in NPK than those in other treatments, especially prior to 2002. Only the yields in NP in 1992, HOF in 1993 and 1994, and OF in 1998 were significantly higher than in NPK. Maize yields in HOF were generally lower or not significantly different from NPK except in 1993 and 1994. Maize yields in OF increased significantly with increasing experimental duration and, with the exception of 1998, were significantly lower than in HOF, NPK, and NP until 2002. After that, maize yields in OF were higher or not significantly different from NPK. In NP until 2001, maize yields were similar to NPK; thereafter, the yields were significantly lower than in NPK. Available K content in NP was only 51 mg kg^{-1} , which was even lower than in CK (65 mg kg^{-1}) in 2019, indicating that soil became gradually K deficient.

Although the highest maize yield was measured in NPK (9903 kg ha^{-1}), it was not significantly different from OF and HOF in 2019. Maize yields in 2019 were generally higher than 2007 except PK, NK, and CK. There was a logarithmic, positive relationship between maize yields and M ($p < 0.01$) (Fig. 5b) except NK. No significant relationships were found between maize yields and the other soil properties measured. The results of the stepwise regression analysis, including M , I_{NH_4} , O_{NH_4} , A_{NH_4} , R_{NH_4} , TN, C/N, AHN, and Olson P, showed that M and TN could explain 77% of the variations in maize yields ($p < 0.01$). Judging by the standardized coefficients, M was a more important variable (0.527) in explaining the variation in maize yields than pH (0.471). Wheat yields showed a similar trend to maize in all treatments (Fig. S2), but the relationship between wheat yields and M was weaker ($R^2 = 0.46$) than for maize ($R^2 = 0.69$), mainly due to water availability (drought often occurs during the wheat growth season in the studied region), which was another important factor affecting wheat growth.

Fig. 4 Relationships between gross N mineralization rate with total nitrogen (TN) (a), C/N ratio (b), and alkali-hydrolyzable nitrogen (AHN) (c) and relationships between gross autotrophic nitrification rate with pH (d), gross N mineralization rate (e), and population of ammonia-oxidizing bacteria (f). Error bars refer to standard deviations

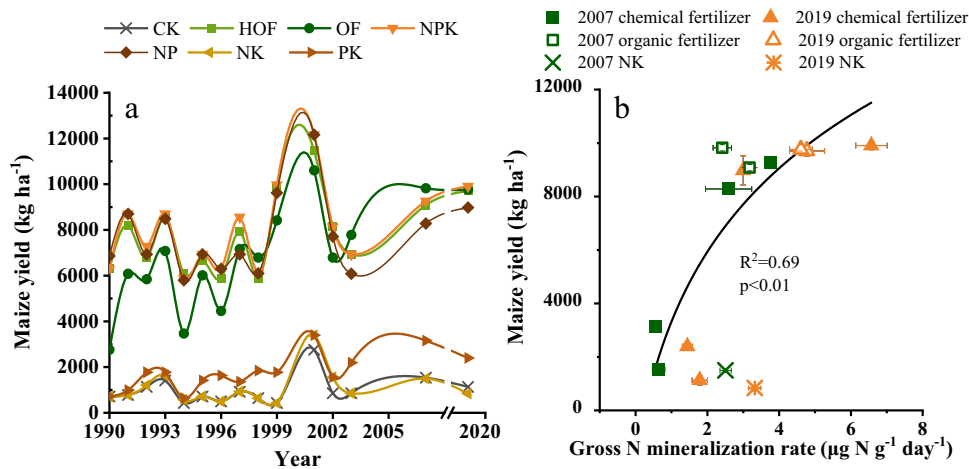
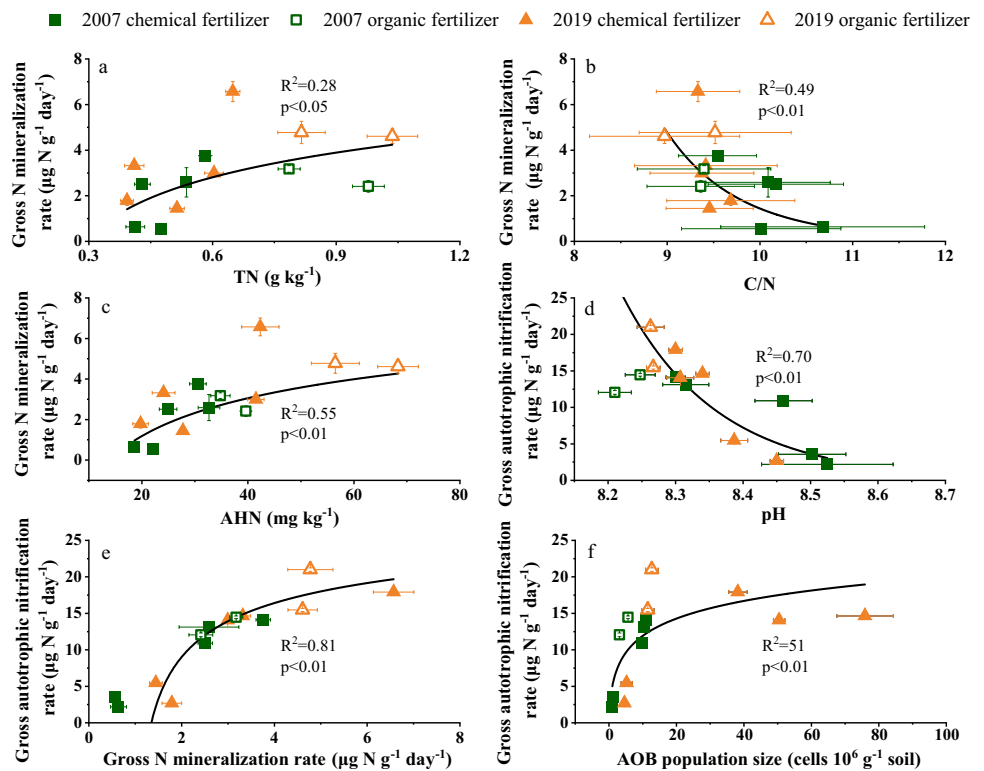


Fig. 5 Dynamic of maize yield in different fertilization treatment in Fengqiu, Henan Province of China from 1990 to 2019 (a) and relationship between maize yield and gross N mineralization for all treatment of 2007 and 2019 (b). CK, no fertilization; NPK, chemical N, P, and K; NP, chemical N and P without K; NK, chemical N and K

without P; PK, chemical P and K without N; OF, organic fertilization without chemical fertilizers; HOF, half N from chemical fertilizer and another half from organic fertilization. Error bars refer to standard deviations. Data of maize yield from 1990 to 2003 are from Cai and Qin (2006)

N₂O emissions potential

The results showed long-term application of organic or chemical N stimulated N₂O emission rates (Fig. 6a). N₂O emission rates of HOF, OF, NPK, NP, and NK were significantly higher than those of PK and CK in both 2007 and 2019, except for NK in 2019. The N₂O emissions of the N

fertilizer treatments in 2007 did not differ significantly. N₂O emission rates in CK, HOF, OF, NPK, NK, and PK in 2019 were 5.4, 2.7, 5.4, 1.5, 1.4, and 6.7 times higher than those in 2007, respectively. In 2019, the highest N₂O emission rate (71.3 µg kg⁻¹ d⁻¹) was observed in OF that was much higher than those in HOF (32.4 µg kg⁻¹ d⁻¹) and the chemical N treatments (9–19.6 µg kg⁻¹ d⁻¹). The N₂O emission rate in

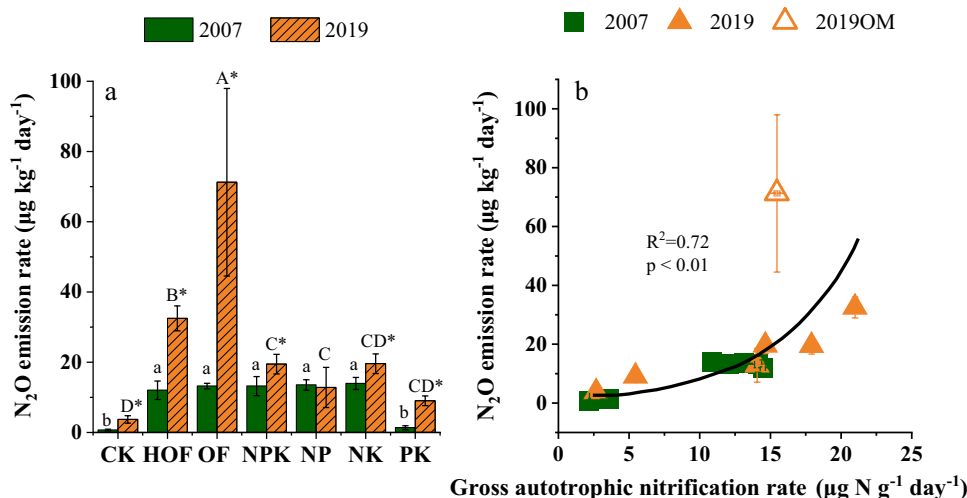


Fig. 6 N₂O emission rates in different fertilization treatments (a) and relationship between N₂O emission rate and gross autotrophic nitrification rate (b) in 2007 and 2019. CK, no fertilization; NPK, chemical N, P, and K; NP, chemical N and P without K; NK, chemical N and K without P; PK, chemical P and K without N; OF, organic fertilization without chemical fertilizers; HOF, half N from chemical fertilizer and

another half from organic fertilization. Error bars refer to standard deviations. Different lowercase, and capital letters above bars indicate significant differences among the treatments of 2007, and 2019, respectively ($p < 0.05$). * above bars of 2019 indicates significant differences between 2007 and 2019 in the same treatment ($p < 0.05$). Data of N₂O emission rate of 2007 are from Zhang et al. (2012b)

HOF was also significantly higher than the chemical N treatments in 2019. There was no significant difference among the chemical N fertilizer treatments. The N₂O emission rate increased with the increasing gross autotrophic nitrification rate ($p < 0.01$) (Fig. 6b).

Discussion

Effect of long-term N fertilization on gross N transformation rates

Our results showed that 30 years of repeated organic or chemical N applications significantly enhanced gross N mineralization rates compared to the control (no fertilizer application). In addition, gross N mineralization rates in all treatments (except NP) were significantly higher in 2019 than in 2007. These results indicated that long-term N managements could continuously increase gross N mineralization rates, which partially confirmed the first hypothesis of this study. The quantity and quality of substrates in soil were considered to be the key factors regulating N mineralization rate (Booth et al. 2005; Zhang et al. 2018). Our results showed that there was a positive relationship between gross N mineralization rate and TN content ($p < 0.05$), which was in line with functional relationships developed by others (Booth et al. 2005; Wang et al. 2015; Dai et al. 2017). A negative relationship between gross N mineralization rate and C/N was also observed ($p < 0.01$), indicating that the quality of substrates also affected N mineralization (Booth et al. 2005;

Cordovil et al. 2005). Furthermore, gross N transformation rate was closer correlated with the labile N pool concentration, e.g., AHN in this study ($p < 0.01$), than with TN ($p < 0.05$), further confirming that the quality of substrates played a more important role in regulating N mineralization rate than its quantity. In this study, AHN alone was able to explain 49% of the variations in *M*. Thus, the increasing AHN could explain well the higher gross N mineralization rate in 2019 compared to 2007. However, the gross N mineralization rate was still significantly higher in NPK than OF, although the AHN content was highest in OF. One of the possible reasons was that microbial activities also play an important role in controlling N mineralization. Previous investigations in the same long-term experiment have shown that enzyme activities, e.g., invertase, cellobiohydrolase, β -glucosidase, xylosidase, and polyphenol oxidase, in the bulk soil and in the different aggregate size fractions were all higher in NPK than in OF, while anaerobic bacterial biomass was significantly lower in NPK than in OF (Yu et al. 2012). These changes in microbial biomass and activities also could affect N mineralization.

Our results showed that long-term supply of organic or chemical N also stimulated gross autotrophic nitrification rates, indicating that autotrophic nitrification was very sensitive to various N forms and the inherent network of soil N dynamics (Schimel and Bennett 2004; Dai et al. 2017). Nitrogen substrates and soil pH were generally considered the main factors controlling autotrophic nitrification rate. In the present study, we found a significant, positive relationship between gross autotrophic nitrification

rate and gross N mineralization rate ($p < 0.01$), indicating a stimulatory effect of long-term N supply from organic N sources. It is generally known that the rate of autotrophic nitrification rate is positively correlated with soil pH (Booth et al. 2005; Zhao et al. 2007; Zhang et al. 2013). However, a negative relationship was observed in this study ($p < 0.01$). The pH values of studied soil ranged from 8.2 to 8.5, which were above the optimum pH for autotrophic nitrification (6.6–8.0). With the long-term N fertilization, a net increase of protons reduced soil pH to the optimum near 8.0, which could explain the stimulation of gross nitrification rate with decreasing pH (Pernes-Debuyser and Tessier 2004; Enwall et al. 2005). Moreover, at pH above 8, the equilibrium of ammoniacal N is shifted towards ammonia (NH_3) with large volatilization competing with oxidation and a negative effect on specific growth rates of nitrifiers (Prosser 1990). Judging from the stepwise regression analysis, the gross N mineralization rate was a more important factor in explaining the variations in the gross autotrophic nitrification rate than pH in this study. Ammonia-oxidizing bacteria (AOB) were considered to be the main driver of autotrophic nitrification in the alkaline soils (Chu et al. 2007, 2008). The results of this study also found that long-term N application could significantly stimulate AOB abundance. The gross autotrophic nitrification rate was positively correlated with AOB abundance, which could partly explain the stimulation of gross autotrophic nitrification rate in long-term N fertilization. These results also partly confirmed the first hypothesis of this study.

Interestingly, both mineral N immobilization and NH_4^+ adsorption on cation exchange sites decreased significantly (near to zero) after 30 years of repeated organic or chemical N applications, compared to the control or the results of 2007 (after 18 years) when relatively high immobilization rates were observed, especially for organic fertilizer applications. These results indicated that long-term repeated organic or chemical N applications (e.g. 30 years in this study) decreased the mineral N retention capacity. It was generally considered that N immobilization was closely related with C/N ratio and it was comparatively high in soils with high C/N ratios (Mary et al. 1996; Li et al. 2021). In the present study, C/N ratios were lower in all treatments in 2019 than in 2007, which caused decreasing immobilization rates (Hartmann et al. 2014). The decreasing immobilization of mineral N rate could increase the potential N losses via N leaching and gaseous N emissions in the long-term N fertilization soils. This result was not in line with our first hypothesis. The repeated assessments of soil N transformations should be considered in long-term fertilization experiments, since the response of soil N transformation processes to fertilization are likely to change with experiment duration.

Effect of long-term N application on crop yields and N_2O emissions

Long-term repeated organic or chemical N applications increased crop yields compared with the control. Previous investigation have proved that soil organic N mineralization provided mineral N for crops, contributing to more than 50% of total N uptake by crops (Wang et al. 2015). As the total N application rate was the same in all N fertilizer treatments, the stimulation of N release from soil organic matter was the key factor explaining the variations in yields. The positive relationship between maize yields and gross N mineralization rates ($p < 0.01$) supported this conclusion, in line of the second hypothesis of this study. Higher crop yields were observed when all elements (NPK, HOF, OF) were supplied suggesting that only this practice was able to alleviate nutrient limitations for crop production in the studied region. This was also supported by the results that unbalanced chemical fertilization treatments (without K, or P or N) were not able to maintain the same productivity levels.

However, SOC content in the long-term balanced application of chemical fertilizers treatment (NPK) was significantly lower than those in the OF or HOF treatments. Furthermore, the yields in OF or HOF were generally not significantly different from NPK in the long-term repeated N fertilizer experiment. Thus, the treatments including organic fertilizer were the preferred practices, maintaining both food security and enhancing soil C sequestration in the studied region.

Many previous studies have shown that application of N fertilizer could significantly increase N_2O emissions from arable soils (Kaiser et al. 1998; Ding et al. 2007; Zhang et al. 2012a). The results of the present study also showed that long-term application of organic or chemical N could stimulate N_2O emissions, with a tight correlation to gross autotrophic nitrification rate, testing the third hypothesis of this study. The N_2O emission rate in organic fertilizer application treatments, especially in OF, was higher than from the chemical N treatments. Meng et al. (2005) working on the same experiments also reported that highest N_2O emissions were observed from OF treatments during the maize and wheat growth seasons of 2002–2003. Some other studies also report similar results that N_2O emissions from soils supplied with organic fertilizers were much higher than those from chemical N fertilizer (Stevens and Laughlin 2001; Khalil et al. 2002; Maljanen et al. 2003). These additional N_2O emissions could offset the enhanced C sequestration in those organic treatments. Therefore, mixed application of organic and chemical N fertilizers provided a suitable compromise to maintain high yields, and enhance soil C sequestration, while N_2O emissions only moderately increased. However, further studies would be helpful to evaluate the most suitable balance between the various fertilizer types for the most sustainable agricultural practice that overall

maintains the most suitable balance between food security, soil C sequestration, and N₂O emission.

Conclusion

The results of the present investigation clearly showed that 30-year long-term organic or chemical N managements stimulated soil gross N mineralization rates, mainly due to the increasing total N and alkali-hydrolyzable N contents. The soil N supply capacity via N mineralization was the key factor affecting the variation in crop yields. Long-term organic or chemical N applications could potentially stimulate N₂O emissions, especially when organic fertilizers were applied in the long-term. The stimulations of gross autotrophic nitrification rates by N application were the main factor controlling N₂O emissions. To identify the most suitable balance between food security, N₂O emission control, and soil C sequestration, a combination of organic and chemical N fertilizers appeared to be most suitable. The investigations combining ¹⁵N tracing approach with amplicon sequencing technique would be suitable to determine the microbial drivers of the studied N reactions and elucidate the underlying mechanisms.

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Declarations

Conflict of interest The authors declare no competing interests.

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