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Soil nitrogen sequestration in a long-term fertilizer experiment in central China

Xuemei Han (Han, XM)^{1,3}, Cheng Hu (Hu, C)², Yunfeng Chen (Chen, YF)², Yan Qiao (Qiao, Y)², Donghai Liu (Liu, DH)², Jun Fan (Fan, J)², Shuanglai Li (Li, SL)², Zhi Zhang (Zhang, Z)²

¹Nanyang Normal University, School of Life Science and Technology, Nanyang 473061, P. R. China. ²Hubei Academy of Agricultural Sciences, Institute of Plant Protection and Soil Fertilizer, Key Laboratory of Fertilization from Agricultural Wastes, Ministry of Agriculture and Rural Affairs, Wuhan 430064, P. R. China. ³Collaborative Innovation Center of Water Security for Water Source Region of Mid-line of South-to-North Diversion Project, Henan Province, Nanyang 473061, P. R. China.

Abstract

Aim of study: To evaluate the effects of a long-term manuring and fertilization experiment on the soil total N concentration and its storage and sequestration rates in the rice-wheat cropping system.

Area of study: A rice-wheat rotation area in central China.

Material and methods: A 35-yr long-term fertilizer experiment was conducted with 9 treatments: unfertilized (Control), N, P, and K fertilizers, manure (M) and M combined with N, P, and K fertilizers treatments. Soil total N input amount, total N concentration, total N storage amount and N sequestration rate in soil were calculated.

Main results: The soil total N input amount, N concentration, N storage amount and N sequestration rate were significantly influenced by M and chemical fertilizers. In total, 0.017-0.021 g N/kg soil accumulated in the organic M plots, whereas only 0.005-0.007 g in chemical fertilizer alone plots. The highest soil total N storage amount was 6.09 t/ha in the M alone plot, and the lowest value was 4.46 tN/ha in the N fertilizer alone plot. The highest N sequestration rate in soil was 0.061 t N/ha/yr in the high amount M plus NPK fertilizers plot, and the lowest value was 0.002 tN/ha/yr in the N fertilizer alone plot. A significant nonlinear regression relationship existed between the total N sequestration rate in soil and annual total N input amount. Moreover, the average soil total N concentration was significantly positively correlated with the average grain yield of crop and soil organic C concentration. The soil total N sequestration rate in M alone or M combined with inorganic fertilizer treatments were increased compared with inorganic fertilizer alone treatments.

Research highlights: Considering crop yields and total N sequestration rate in soil, the use of manure combined with inorganic fertilizer should be recommended in the rice-wheat cropping system.

Additional key words: soil total N concentration; N storage; N sequestration rate; long-term fertilizer experiment; rice-wheat cropping system.

Abbreviations used: hMNPK (1.67 times manure plus inorganic N, P and K fertilizers treatment); M (manure alone treatment); MN (manure plus inorganic N fertilizer treatment); MNP (manure plus inorganic N and P fertilizers treatment); MNPK (manure plus inorganic N, P and K fertilizers treatment); N (inorganic N fertilizer alone treatment); NP (inorganic N plus P fertilizer treatment); NPK (inorganic N, P plus K fertilizer treatment).

Authors' contributions: CH coordinated the research project, supervised the work and drafted the manuscript. XMH was responsible for writing and critical revision of the manuscript for important intellectual content. YFC and ZZ analyzed the data and performed statistical analysis. YQ and DHL were involved in lab analysis. JF and SLL were responsible for field experiment management. All authors read and approved the final revision of the manuscript.

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Correspondence should be addressed to Cheng Hu: huchenghxz@163.com

Introduction

Nitrogen is one of the most important plant-available nutrients and is the most vital crop yield-limiting factor in agricultural systems (Pandey *et al.*, 2017). Changes in soil total N concentration are largely responsible for variations in soil physical, chemical and biological properties and strongly influence crop productivity and environmental quality (Mazzoncini *et al.*, 2011). Increasing soil N immobilization could reduce the amount of N fertilizer use, N leaching, and N₂O emission, mitigating greenhouse gases causing global warming (Sainju *et al.*, 2008; Qiu *et al.*, 2016). However, when the N fertilizer application is neither completely assimilated by plants nor sequestered as soil organic N, it will result in N losses and related environmental problems, such as greenhouse gases, groundwater contamination, atmosphere pollution, water eutrophication, and reduced biodiversity (Lin *et al.*, 2016). Sainju *et al.* (2008) reported that N sequestration was influenced by long-term tillage, cropping systems, and N fertilizer sources in the cotton fields in the southeastern USA. Zhou *et al.* (2013) found that manure alone or manure combined with chemical fertilizer could sequester N in soil, whereas chemical fertilizer use alone decreased the soil N concentration. Similarly, Qiu *et al.* (2016) reported that only organic manure combined with chemical fertilizer could increase the soil total N concentration in monoculture maize fields. However, the result in the paddy-upland cropping system is similar to upland soil or not, as there is little study.

The rice (*Oryza sativa* L.) - wheat (*Triticum aestivum* L.) cropping system, which covers an area ranging from 9.5 to 13.5 million hectares, is a long-established food production system in China. It is mainly distributed in the south of the country, especially in the provinces of Jiangsu, Zhejiang, Hubei, Guizhou, Yunnan, Sichuan and Anhui (Han *et al.*, 2020). To ensure food security in China and people's livelihood, maintaining sustainable crop yields and ideal soil environments are crucial in the rice-wheat rotation regions (Hu *et al.*, 2015).

Long-term agricultural field experiments provide valuable information regarding the effects of nutrient inputs on crop productivity and soil environment (Schlegel & Havlin, 2017; Hu *et al.*, 2018b, 2019). The problems of fertilization can be chronically, systematically, historically, and positionally investigated, providing scientific evaluation by means of long-term field experiments. Long-term experiments have incomparable advantages compared with routine trials and are important basic studies of agricultural sciences and could gain some information about the sustainable

agriculture and could assess human's influence on the soil fertility, landscape and agricultural environment (Cook & Trlica, 2016; Hu *et al.*, 2018a).

To date, few investigations have studied the effects of long-term manuring and fertilization on soil N inputs and total N sequestration in the rice-wheat cropping system of China. The aims of this study were to (1) evaluate the impacts of the long-term manuring and fertilization on the soil total N concentration and its storage and sequestration rates in the yellow-brown soil and (2) estimate the relationship between crop yields and soil N sequestration rates in the rice-wheat cropping system.

Material and methods

Experiment site and design

A 35-year long-term fertilizer experiment in a rice-wheat rotation was initiated for rice cultivation since 1981. The experimental site belongs to the National Fertilizer Experiment Monitoring Network at Nanhu Experimental Station, Hubei Academy of Agricultural Sciences in the Wuchang district of China, which is located at latitude 30°28'N, longitude 114°25'E and altitude 20 m in Central China. The experimental site lies in a subtropical monsoon zone, which is characterized by hot summers, severe winters, and occasional snowfall during the winter. The mean annual temperature is 13 °C ranging from a minimum of 3.7 °C in January to a maximum of 28.8 °C in July. The mean annual precipitation is 1300 mm, and the annual non-frost period is 240 days.

The soil at the experimental site is a yellow-brown soil, belonging to Albic Luvisol according to FAO classification. Soil properties at the beginning of the experiment in the topsoil were as follows: soil organic C 15.91 g/kg, total N 1.80 g/kg, total P 1.00 g/kg, total K 30.22 g/kg, alkaline hydrolysable N 150.70 mg/kg, available P 5.00 mg/kg, available K 98.50 mg/kg, pH 6.30, and bulk density 1.29 g/cm³.

The field experiment was conducted with nine treatments (in a randomized complete block design with three replicates), and each plot was 40 m² (5 m width × 8 m length). The nine treatments were as follows: unfertilized treatment (Control); nitrogen (N), phosphorus (P), and potassium (K) fertilizers; manure (M); manure combined with inorganic N, P, and K fertilizers; and 1.67 times manure plus inorganic N, P and K fertilizers (hMNPk). The unfertilized treatments that served as a control have not received any fertilizer and only crop stubble and roots were inputted. The N, P, and K fertilizers were ap-

plied with 150 kg N/ha, 75 kg P₂O₅/ha, and 150 kg K₂O/ha every year. The N, P, and K fertilizers were applied with urea, ammonium phosphate, and potassium chloride, respectively. In addition, 22,500 kg/ha of organic manure from pig dung compost was applied every year for M, MN, MNP and MNPK treatments and was equivalent to 105.18 kg N/ha, 145.36 kg P₂O₅/ha, 94.86 kg K₂O/ha, while 37,500 kg/ha was applied every year for the hMNPK treatment and was equivalent to 175.30 kg N/ha, 242.27 kg P₂O₅/ha, 158.10 kg K₂O/ha. The pig dung compost contained, on average, 282.05 g/kg organic C, 15.08 g/kg total N, 20.84 g/kg P₂O₅, 13.60 g/kg K₂O, and 69.00% water. Sixty percent of chemical fertilizers were applied during the rice growth season, and the remaining 40% was applied during the wheat growth season. Manure was applied equally (1:1) to the two crops. Forty percent of the N fertilizer was applied as a basal fertilizer, 40% was applied during the tillering stage and 20% was applied during the booting stage in the rice growth season. However, 50% of N fertilizer was applied as a basal fertilizer, 25% was applied during the wheat seedling stage, and 25% was applied during the jointing stage in the wheat growth season. The P and K fertilizer and manure were applied as basal fertilizers prior to plowing every year. All basal fertilizers and manure were evenly sprinkled on the soil surface by hand and were incorporated into the plow layer by tillage as soon as possible. Tillage was performed to 20 cm depth by plow followed by harrow. The fertilized and unfertilized plots were similarly tilled.

All plots were transplanted with rice seedlings in summer and were sown with wheat seeds in winter annually since 1981. The rice was transplanted in June and harvested in September, and then the wheat was directly sowed in November and harvested in May of the next year. The aboveground crops were harvested with a sickle and removed; thus, no straw returned to the soil in all plots. Nevertheless, rice or wheat stubbles and roots were incorporated into the soil with a plow before the subsequent rice and wheat cultivation. In addition to the fertilizer practice, all other agronomic managements were identical in fertilized and unfertilized plots. Rice and wheat grains were separated from straws using a plot thresher. Grains were weighed after sun-drying and recorded from the whole plot (40 m²).

Soil and plant sampling

Composite soil samples from all experimental plots were collected from the plow layer soil. Soil

augers (a 5-cm diameter and 0- to 20-cm depth) were used in each plot in October every year after rice was harvested and before soil was plowed. Each composite soil sample consists of ten mixed cores. Each soil sample was stored in a sealed plastic bag, transferred to the lab as soon as possible, air-dried for 14 days at room temperature and then ground. Subsamples were sieved through a 1-mm screen, mixed, and analyzed for the concentrations of alkaline-hydrolysable N, available P and available K and soil pH. Other subsamples were ground through a 0.25-mm sieve to determine soil organic C and total N concentrations.

After crop grains matured, 1 m² rice (September every year) or wheat (May every year) plants not from the boundary were manually harvested by sickle, removed from the fields, and then sun-dried. The grains were separated from straws by a plot thresher. Both of straws and grains were oven-dried at 65 °C for 72 h and then ground through a 0.5-mm sieve in order to analyze total N concentrations.

Laboratory analysis

The potassium dichromate external heating method determined soil organic C concentration (Page *et al.*, 1988). The micro-Kjeldahl method and the alkaline-hydrolysable diffusion method measured total N and alkaline-hydrolysable N concentrations, respectively (Bremner, 1996). Soil available P was extracted with 0.5 mol/L NaHCO₃ (soil: solution=1:20) and measured with the Olsen method (Olsen *et al.*, 1954). Soil available K was extracted with 1 mol/L NH₄Ac (soil: solution=1:10) and measured with the flame photometry method. Soil pH was measured with a 0.01 mol/L CaCl₂ slurry (soil: solution=1:2.5) with a glass electrode. Samples from plant tissues and manures were analyzed for total N using the micro-Kjeldahl method (Bremner, 1996). The P concentration of plant tissues and manures was determined by the ammonium molybdate method (Olsen & Sommers, 1982), and K concentration was determined by flame photometry (Helmke & Sparks, 1996). All the data were expressed on the basis of dry mass.

Calculation of nitrogen input amount

The straw biomass and grain yields of each crop were recorded from whole plots every year. The crop biomass N was calculated according to aboveground crop biomass and its N concentrations of harvestable

crop grains or straws. Namely, the rice biomass N was equal to the rice straw yield multiplied by its N concentration plus the rice grain yield multiplied by its N concentration. The wheat biomass N was calculated in the same way.

$$N_{\text{rice-biomass}} = \text{Yield}_{\text{rice-straw}} \times N_{\text{rice-straw}} + \text{Yield}_{\text{rice-grain}} \times N_{\text{rice-grain}} \quad (1)$$

$$N_{\text{wheat-biomass}} = \text{Yield}_{\text{wheat-straw}} \times N_{\text{wheat-straw}} + \text{Yield}_{\text{wheat-grain}} \times N_{\text{wheat-grain}} \quad (2)$$

where $N_{\text{rice-biomass}}$ and $N_{\text{wheat-biomass}}$ represent the biomass N of rice and wheat, respectively. $\text{Yield}_{\text{rice-straw}}$, $\text{Yield}_{\text{rice-grain}}$, $\text{Yield}_{\text{wheat-straw}}$ and $\text{Yield}_{\text{wheat-grain}}$ represent the straw and grain yields of rice and wheat, respectively. $N_{\text{rice-straw}}$, $N_{\text{rice-grain}}$, $N_{\text{wheat-straw}}$ and $N_{\text{wheat-grain}}$ represent the N concentration of straw and grain of rice and wheat, respectively.

The annual total N input amount was measured based on belowground roots and stubbles incorporated into the topsoil and applied N fertilizer and organic manure as follows:

$$N_{\text{input}} = N_{\text{fertilizer}} + N_{\text{manure}} + N_{\text{belowground}} + N_{\text{stubbles}} \quad (3)$$

$$N_{\text{belowground}} = R_{\text{rice-belowground}} \times N_{\text{rice-biomass}} + R_{\text{wheat-belowground}} \times N_{\text{wheat-biomass}} \quad (4)$$

$$N_{\text{stubbles}} = R_{\text{rice-stubbles}} \times \text{Yield}_{\text{rice-straw}} \times N_{\text{rice-straw}} + R_{\text{wheat-stubbles}} \times \text{Yield}_{\text{wheat-straw}} \times N_{\text{wheat-straw}} \quad (5)$$

where $N_{\text{fertilizer}}$ is the N from N fertilizer, and N_{manure} is the N from organic manure. $N_{\text{belowground}}$ is the underground biomass N mainly from rice or wheat roots. Both $R_{\text{rice-belowground}}$ and $R_{\text{wheat-belowground}}$ have a fixed value of 30%, which is the ratio of annual underground biomass N of each crop to aboveground biomass N (Kundu *et al.*, 2007). Approximately 75.3% of wheat roots and 100% of rice roots are distributed in 0-20 cm soil layers (Zhang *et al.*, 2012). $R_{\text{rice-stubbles}}$ account for 5.6%, and $R_{\text{wheat-stubbles}}$ account for 15% and both of these values are the ratios of each crop stubble biomass incorporated into soil to aboveground biomass (Zhang *et al.*, 2012).

Calculation of soil total nitrogen storage amount and sequestration rate

The total N storage amount of the soil is the soil total N concentration multiplied by the bulk density and the depth. The sequestered soil N amount of each treatment in the 0-20 cm soil depth is the difference between the soil present N storage amount (2015) and

the initial N storage amount (1981). The soil N sequestration rate is equal to the sequestered amount of soil total N in each treatment divided by fertilization time (35 years).

Statistical analysis

One-way variance analysis (one-way ANOVA) was used to detect differences between treatments. Pearson linear correlation (two tailed) was used to evaluate the relationships between soil total N concentration and fertilization years. Nonlinear regression was used to estimate the relationships between N sequestration rate and annual N input as well as between crop yields and soil total N concentration. All statistical and regression analysis were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Differences at the $p < 0.05$ level were considered statistically significant according to the least significant difference (LSD) test.

Results

Soil total nitrogen input

The total N input amount of soil was significantly ($p < 0.001$) affected by fertilization during the study period. The dynamics of the annual total N input amount are depicted in Fig. 1. The highest average total N input amount was 0.389 t N/ha/yr in the hMNPk plot, and the lowest value was 0.027 t N/ha/yr in the control plot. The average annual N input amount in the manure plots (MN, MNP, MNPK, hMNPk) was 61.07-101.14% higher than that in the NPK plot.

Total N concentration dynamics

Statistical analysis indicated that the soil total N concentration was significantly ($p < 0.001$) influenced by fertilization during the study period. The changing tendency of soil total N concentration under different fertilization treatments is shown in Fig. 2. For the duration of experiments, the soil total N concentration significantly ($p < 0.05$) increased in all the fertilization plots. Nevertheless, the increasing rates were diverse in the different fertilization plots. The slopes of linear regression equations in soil total N concentration indicated that 0.017-0.021 g N/kg soil accumulated in the appended organic manure plots every year, whereas only 0.005-0.007 g N/kg soil accumu-

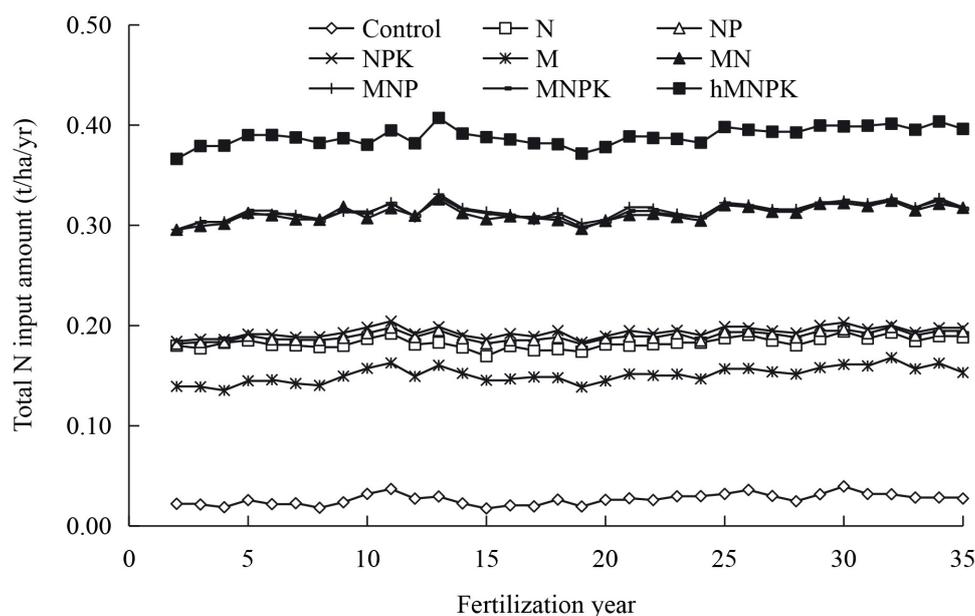


Figure 1. Annual total N input amount in different fertilization treatments. Control: unfertilized treatment; N: inorganic N fertilizer treatment; NP: inorganic N plus P fertilizer treatment; NPK: inorganic N, P plus K fertilizer treatment; M: manure treatment; MN: manure plus inorganic N fertilizer treatment; MNP: manure plus inorganic N and P fertilizers treatment; MNPK: manure plus inorganic N, P and K fertilizers treatment; hMNPK: 1.67 times manure plus inorganic N, P and K fertilizers treatment.

lated in chemical fertilizer alone plots. On average, the soil total N concentrations in the manure combined with inorganic fertilizer (MN, MNP, MNPK) plots were 27.07%, 21.53%, and 15.19% higher compared with the corresponding application of chemical fertilizer alone (N, NP, NPK) plots, respectively. The soil total N concentration in the NPK fertilizer plot was 10.55% and 7.01% higher than that in the N and NP fertilizer alone plots, respectively.

Total N storage and sequestration in soil

The soil total N storage amounts (0-20 cm) were significantly ($p < 0.05$) influenced by long-term manure and chemical fertilizer use. The soil N storage amount was increased by 3.24-40.97% in all treatments compared with the initial N storage amount (4.32 t N/ha) (Fig. 3a). The highest soil N storage amount was 6.09 t N/ha in the M plot, and the lowest value was 4.46 t N/ha in the N plot. Soil N storage amounts in the manure plots were significantly ($p < 0.05$) higher than those in the chemical fertilizer alone and control plots; moreover, the soil N storage amount in the NPK fertilizer plot was significantly ($p < 0.05$) higher than those in the N and NP fertilizer alone and control plots. However, there was no significant difference between N and NP fertilizer alone and control plots (Fig. 3a).

The total N sequestration rate in soil (0-20 cm) was also significantly ($p < 0.05$) influenced by long-term manure and chemical fertilizer use. The highest N sequestration rate in soil was 0.061 t N/ha/yr in the hMNPK plot, and the lowest rate was 0.002 t N/ha/yr in the N alone plot (Fig. 3b). Soil N sequestration rates in the manure plots were significantly ($p < 0.05$) higher than those in the inorganic fertilizer alone and control plots. Furthermore, the soil N sequestration rate in the NPK fertilizer plot was significantly ($p < 0.05$) higher than those in the N and NP fertilizer alone and control plots. However, there was no significant difference among the N, NP and control plots (Fig. 3b).

Regression analysis

A significant ($p < 0.05$) nonlinear regression was noted between the soil N sequestration rate and the annual total N input amount (Fig. 4a). A significant ($p < 0.001$) nonlinear regression was noted between the average crop yields and average total N concentration of the soil (Fig. 4b). The average grain yields of wheat and rice were significantly ($p < 0.001$) nonlinearly correlated with the average total N concentration of the soil (Fig. 4c and Fig. 4d). Similarly, a significant ($p < 0.001$) linear regression was noted between the average soil total N concentration and average organic C concentration (Fig. 5).

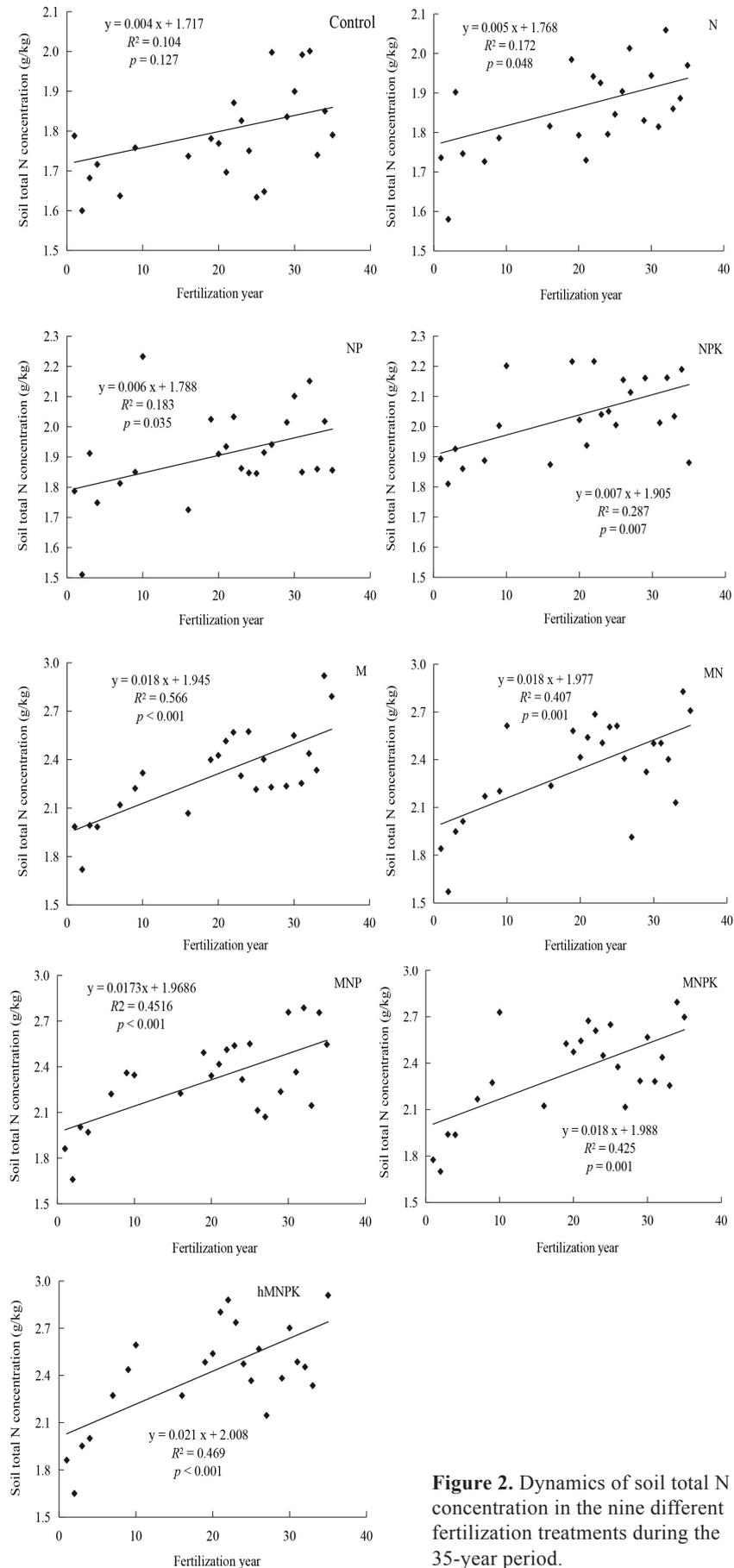


Figure 2. Dynamics of soil total N concentration in the nine different fertilization treatments during the 35-year period.

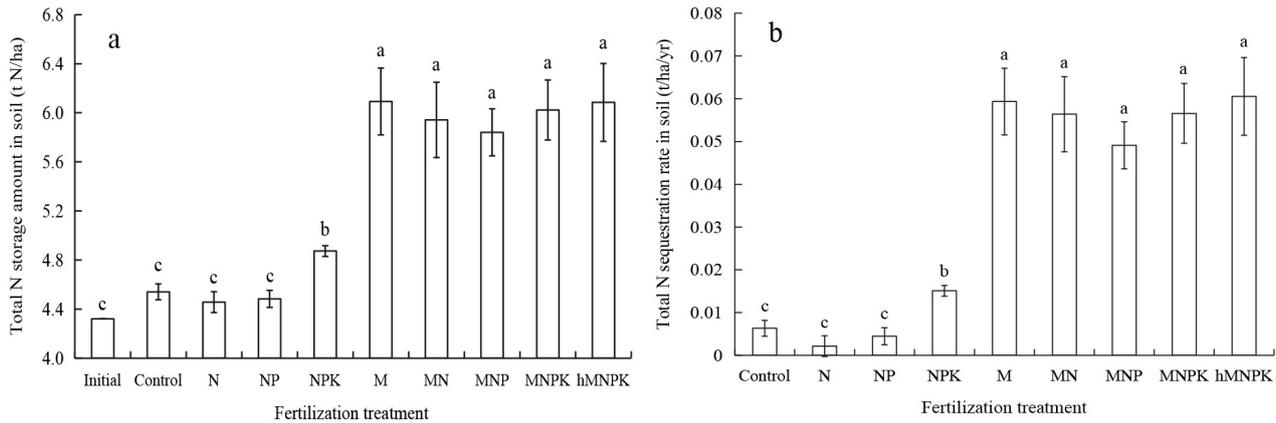


Figure 3. Total N storage amount (a) and total N sequestration rate (b) in soil (0-20 cm) in different fertilization treatments during the 35-year period. The values are reported as means \pm standard deviations. Different letters indicate significant differences ($p < 0.05$) among treatments according to LSD multiple comparison.

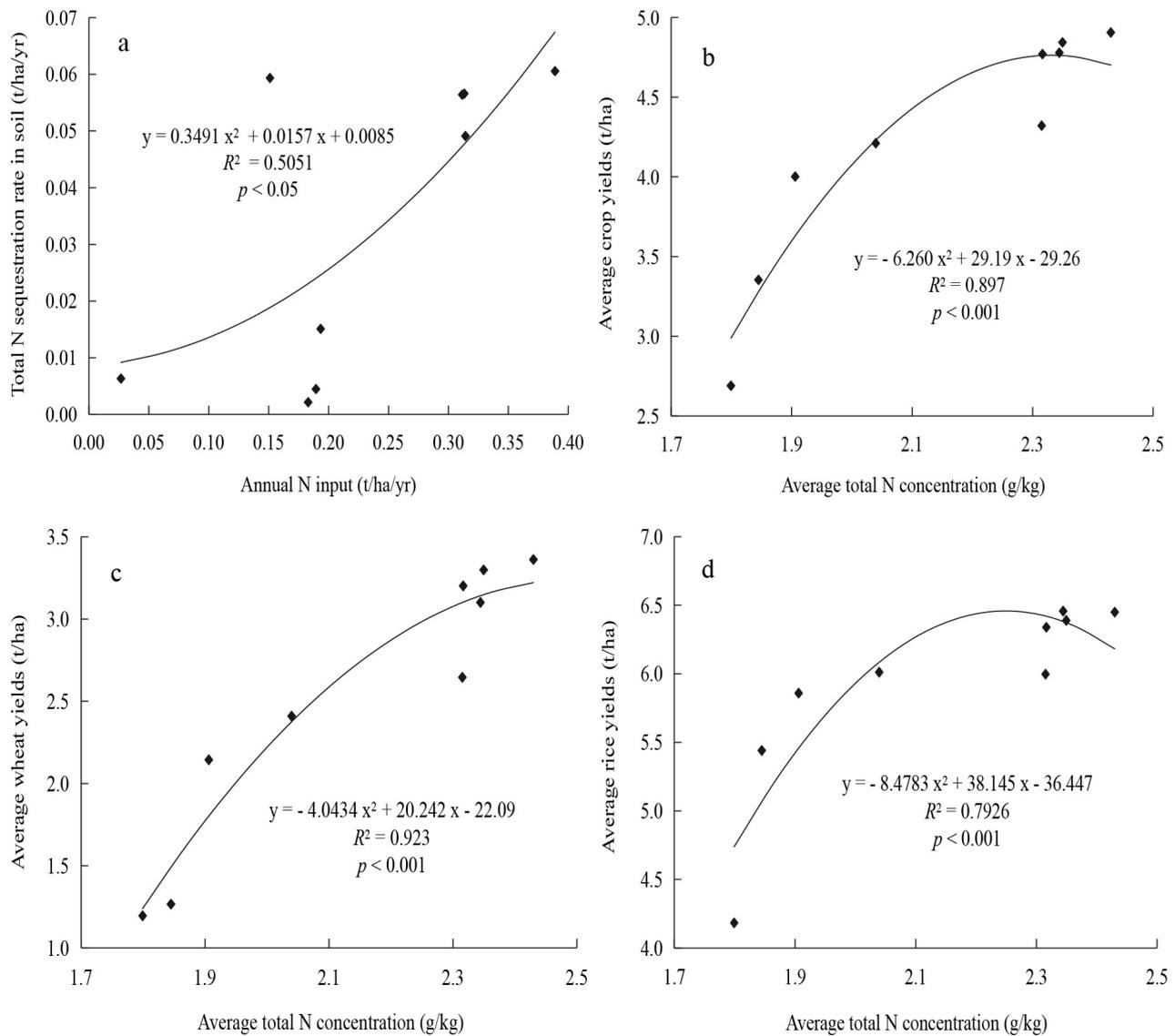


Figure 4. Nonlinear regression relationship, during the 35-year period, between: (a) total N sequestration rate in soil (0-20 cm) and annual N input; (b) average crop yields and total N concentration of soil (0-20 cm); (c) average wheat yields and total N concentration of soil (0-20 cm); and (d) average rice yields and total N concentration of soil (0-20 cm)

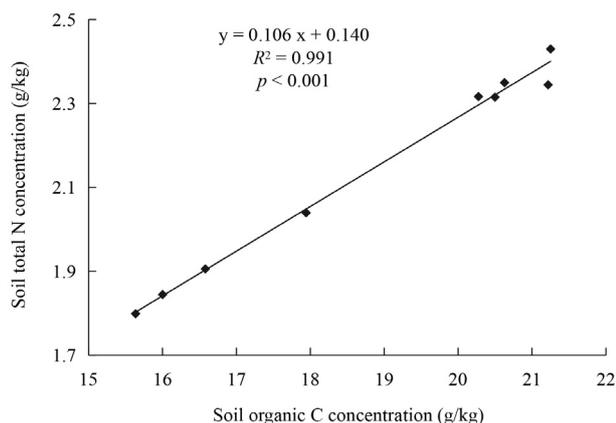


Figure 5. Linear regression relationship between average total N concentration of soil and soil organic C concentration (0-20 cm) during the 35-year period.

Discussion

Total N input and sequestration in soil

The aim of this study was to evaluate the influence of long-term organic manure and inorganic fertilizer use on soil total N input amount and N sequestration. On average, the annual total N input amounts of soil in the manure and chemical fertilizer plots were 6.86-14.58 times greater than that in the unfertilized plot. The total N input amounts in the NPK fertilizer without manure plot were considerably increased compared with the N and NP fertilizer without manure and control plots; however, inorganic N input amounts were the same. These findings show that fertilized NPK without manure treatment aided in sequestering more N in soil than soil fertilized with N or NP without manure treatments. A possible reason involves more organic N input in the NPK treatment due to considerably increased crop stubbles and roots biomass compared with the N and NP treatments. The observed soil total N concentration variations were correlated with N returned to the soil via crop residues, aboveground cover crop biomass and weeds (Mazzoncini *et al.*, 2011).

In general, the soil total N concentration in the manure-treated plots was significantly higher than that in the chemical fertilizer-treated plots; other reports supported these results (Gami *et al.*, 2001; Regmi *et al.*, 2002; Mandal *et al.*, 2007; Zhou *et al.*, 2013). Although all the fertilization treatments resulted in significant increases in the total N concentration compared with initial values at the beginning of the experiment, the elevated rates were considerably increased in the manure plots compared with the chemical fertilizer alone plots. As such, the soil total N concentration in all fertilization treatments showed an increasing tendency during the study period; furthermore, manure-treated

plots maintained higher levels of soil total N concentrations in contrast to the chemical fertilizer-treated plots. In contrast, other studies found that only the application of organic manure could increase the soil total N concentration; however, the application of chemical fertilizer alone reduced the soil total N concentration (Zhang *et al.*, 2009; Congreves *et al.*, 2017). N accumulation was higher when manure or straw mixed with NP fertilizers was applied, which is partially due to a slow release of N from organic materials that results in smaller losses of N (Hu *et al.*, 2015). The organic material treatments created higher amounts of rhizodeposition, which are residues of crop root and stubbles, and may have contributed to high N concentrations (Blair *et al.*, 2006).

Regression analysis showed that the slopes of linear regression equations were not significant in the unfertilized plot over time, suggesting that N established an equilibrium. These results are in accordance with other observations (Gami *et al.*, 2001; Fan *et al.*, 2005). Nevertheless, soil total N concentration was decreased in the unfertilized control compared with initial values at the beginning of experiment in the other sites (Bhandari *et al.*, 2002; Zhang *et al.*, 2009; Zhou *et al.*, 2013).

The total N sequestration rate in soil (0-20 cm) was 0.002-0.015 t N/ha/yr under the inorganic fertilizer plots in the present study. The total N sequestration rate in soil (0-20 cm) was 0.015 t N/ha/yr due to NH_4NO_3 applications with the tilled systems (Sainju *et al.*, 2008). Notably, the total N sequestration rate in soil (0-20 cm) was 0.049-0.061 t N/ha/yr due to organic manure use in this study. Similarly, it was 0.066-0.103 t N/ha/yr (0-20 cm) in the NPK fertilizer combined with manure plots in maize fields (Qiu *et al.*, 2016) and 0.019-0.032 t N/ha/yr (0-20 cm) in the chemical fertilizer mixed with manure plots in wheat fields (Zhou *et al.*, 2013).

A significant nonlinear regression relationship between average crop yields and average soil total N concentration is observed, which is similar to other reports of a significantly positive correlation between crop grain yields and total N concentration of the soil (Zhou *et al.*, 2013). Numerous researchers have also reported that soil organic C and soil total N concentration are highly related and that sequestration of soil C also leads to sequestration of soil N (Sainju *et al.*, 2008; Triberti *et al.*, 2016; Gai *et al.*, 2018). Chen *et al.* (2010) reported that cyclic utilization of farmland organic materials increased soil organic C concentration and accordingly improved soil total N concentration, so that soil N was deposited. For example, regression analysis showed that the soil total N stock was significantly correlated with soil organic C stock in the manure combined with NPK fertilizers treatments, indicating greater se-

questration of N with increasing soil organic C (Gai *et al.*, 2018). The alteration of soil C/N ratio could reflect the N retention capacity by microbial communities (Mooshammer *et al.*, 2014). The low soil C/N ratio indicated inadequate C concentration in the farmland, which might limit N immobilization and lead to high N losses (Gundersen *et al.*, 1998). Therefore, it is vital to increase soil organic C concentration, accordingly improving the soil N immobilization potential through optimal manure application (Gai *et al.*, 2018). The manure use can affect soil N turnover by regulating soil organic C concentration and the increase of soil organic C concentration can help to enhance the N immobilization potential and decrease N losses (Ren *et al.*, 2014).

Affecting factors on total N sequestration in soil

Losses or sequestration of soil total N depend on the balance between N inputs (N fixation, and imports) and outputs (crop exports, erosion, leaching, run off and gaseous losses) (Gong *et al.*, 2011; Sainju *et al.*, 2016, 2018). Therefore, total N sequestration in soil was mainly influenced by fertilization, fertilizer types, climates, and soil types. In general, total N sequestration in soil was increased along with manure use (Regmi *et al.*, 2002); however, soil total N concentration often did not increase with chemical fertilizer use, depending on climates, soil types (Manna *et al.*, 2005; Bedada *et al.*, 2014), land uses, and tillages (Congreves *et al.*, 2017). With regard to fallow, contradictory results were noted. For instance, Qiu *et al.* (2016) reported that the N sequestration rate in soil (0-20 cm) was 23.0 kg N/ha/yr through 22 years of fallow; nevertheless, long-term fallow led to N concentrations decreasing in a semiarid cropland (Zhou *et al.*, 2013), which may be mainly attributed to climate differences. Long-term straw returning to soil could increase soil total N concentrations in paddy-upland cropping systems (Shen *et al.*, 2007), whereas it decreased soil N concentrations in the upland soil (Zhang *et al.*, 2009). Mazzoncini *et al.* (2011) observed that under the Mediterranean climate, it is easier to conserve or increase soil total N by adopting no-tillage, but the conventional tillage system requires higher N fertilization rates. The land use is an important factor that affects the soil N sequestration rate. For example, some researchers reported that soil total N could be sequestered due to fertilizer application in the paddy or paddy-upland cropping systems (Regmi *et al.*, 2002; Shen *et al.*, 2007). However, the soil total N concentration decreased due to long-term inorganic fertilizer application in the upland systems (Zhang *et al.*, 2009; Zhou *et al.*, 2013; Qiu *et al.*, 2016).

As conclusions, soil total N input amount and total N concentration were significantly influenced by long-term manure and chemical fertilizer use. The average annual N input amount in the manure (MN, MNP, MNPK, hMNPK) plots was 61.07-101.14% higher than that in the NPK alone plot. Similar to the soil total N input amount, the total N concentration of soil was significantly affected by fertilization during the study period. For the duration of experiments, the soil total N concentration exhibited a significantly ascending trend in the all fertilization plots. Soil total N concentration was significantly higher in the manure plots compared with the chemical fertilizer alone plots. The N sequestration rates in soil (0-20 cm) in the manure plots were significantly higher than that in the chemical fertilizer and control plots; furthermore, the soil N sequestration rate in the NPK fertilizers plot was significantly higher than those in the N, NP fertilizer and control plots. The average grain yields of crops were significantly positively correlated with the average total N concentration of soil. Moreover, the average total N concentration of soil was significantly positively correlated with the average soil organic C concentration. Long-term organic manure or chemical fertilizer application could sequester N in soil in the rice-wheat cropping system; however, sequestration efficiency in manure or manure combined with chemical fertilizer use is higher than that in the chemical fertilizer alone use. Considering crop yields and the total N sequestration rate in soil, manure mixed with chemical fertilizer use should be recommended.

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