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RESEARCH ARTICLE

Using manure for improving nitrogen fertilization and maize yield

Xucun Jia[1,2], Qinglong Yang[1], Shu-Ting Dong[1], Ji-Wang Zhang[1], Bin Zhao[1] and Peng Liu[1,*]

[1]College of Agronomy, Shandong Agricultural University/State Key Laboratory of Crop Biology, Tai'an, Shandong 271018, China and [2]College of Agronomy, Henan Agricultural University, Zhengzhou, Henan 450046, China
*Corresponding author. Email: liup@sdau.edu.cn or liupengsdau@126.com

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Abstract

Manure and chemical fertilizers have different effects on soil properties, the nitrogen cycle, and crop yield. This study aimed to investigate the effects of different fertilizer applications under the same N input on soil physicochemical properties and soil bacterial communities and to explain the contributions of soil properties to grain yield. Manure substitution of chemical fertilizer was conducted in leaching monitoring systems. The study began in 2009 and sampling was carried out in 2014 and 2016. Three fertilizer treatments with the same total N, P, and K application rates and one control treatment were designed as follows: (1) CK, without nitrogen fertilizer; (2) 100%U, whole nitrogen coming from urea; (3) 100%M, whole nitrogen coming from composted cattle manure; and (4) 50%U + 50%M, half nitrogen from composted cattle manure and half nitrogen from urea. Soil organic carbon (SOC) content was positively correlated with total N (TN), NO_3^-–N, and NH_4^+–N contents, the mean weight diameter of soil aggregates, and the Shannon diversity index of bacteria, whereas SOC content was not significantly correlated with grain yield. NO_3^-–N content was positively correlated with grain yield. Substituting half the amount of chemical fertilizer with manure as a nitrogen source improved soil stability, increased bacterial diversity, and enhanced nitrogen supply, while reducing nitrogen loss from ammonia volatilization and nitrogen leaching. Substituting half the amount of chemical fertilizer with manure as a nitrogen source was a more sustainable way to increase grain yield through a sustainable nitrate supply and to reduce N loss.

Keywords: Manure substitution; Soil organic carbon; Soil aggregate stability; Soil bacterial community; Nitrogen flow

Introduction

The expanding global population in combination with limited arable land has required yield increases per hectare of farmland, and this has come about through intensive chemical fertilization (Tilman *et al.*, 2011). However, the long-term excessive use of chemical fertilizer has resulted in nitrogen loss through leaching and ammonia volatilization (Jia *et al.*, 2014). Nitrogen leaching from field crops can pollute groundwater, which further threatens the safety of drinking water (Ju *et al.*, 2006). It has been widely reported that manure application may decrease nitrogen leaching compared with solo chemical fertilizer application (Fan *et al.*, 2017; Zhou *et al.*, 2014). However, repeated manure applications can increase nitrogen leaching, which may be due to the increased nitrogen mineralization of organic nitrogen (Shepherd and Newell-Price, 2013).

High-yielding crops are heavily dependent on soil nitrogen supplies, especially from synthetic fertilizers. Indeed, levels of nitrogen in the soil due to anthropogenic inputs are now far greater than those obtained from natural sources (~180 Tg N $year^{-1}$ versus 63 Tg N $year^{-1}$) (Fowler *et al.*, 2013). While the chemical fertilization of crops has led to low SOC and nitrogen levels compared to organic fertilizer input (Dong *et al.*, 2012), the levels of plant-available nitrogen from organic

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fertilizers are also inadequate, resulting from a slow rate of mineralization and leading to lower crop yields in fields treated with organic versus chemical fertilizers (Blatt *et al.*, 1991). The use of organic fertilizers only reaches 82% of the yield of chemical fertilizers, which varies heavily across crop species (Thorup–Kristensen *et al.*, 2012).

Given these shortcomings of chemical and organic fertilizers, recent studies have focused on the combined use of organic and inorganic fertilizers (Chen *et al.*, 2016; Li *et al.*, 2017). This combined treatment can improve the physicochemical properties of soil by increasing organic matter, moisture, and nitrogen content levels (Guo *et al.*, 2016), thereby increasing yields and nitrogen use efficiency (Zhou *et al.*, 2016a). According to Brar *et al.* (2013), both chemical and organic fertilizers have positive effects on SOC content in the subsurface layers of soil (15–60 cm). Organic amendment increases the pool of SOC, thus improving soil quality, and has better environmental outcomes than the application of chemical fertilizer alone (Guo *et al.*, 2016; Moharana *et al.*, 2012). However, most studies focus on the additional use of organic fertilizers without considering N input from organic fertilizers.

Increasing the SOC content also improves the physical properties of soil such as bulk density and the formation of soil aggregates, and this can strongly influence soil fertility, plant growth, and soil quality (Erktan *et al.*, 2016; Gentile *et al.*, 2013). Organic fertilization increases the formation of stable macroaggregates and of microaggregates within macroaggregates after decomposition (Golchin *et al.*, 1994; Volikov *et al.*, 2016). The contents of different sizes of aggregates are to some extent influenced by changes in SOC content (Abiven *et al.*, 2009). Meanwhile, other studies have indicated that chemical fertilization has no negative effects on soil physical properties such as the mean weight diameter (MWD) of aggregates (Xin *et al.*, 2016).

In addition to improving SOC content, organic fertilizers improve other chemical properties of soil, such as nitrate and ammonia content, compared to chemical fertilizers (Li *et al.*, 2017). Soil treated with chemical fertilizers alone shows increased NO_3^-–N levels and higher NO_3^-–N leaching rates than soil fertilized with manure during the maize growing season (Jia *et al.*, 2014). Manure application decreases nitrogen loss through ammonia volatilization by 66.6% due to reduced soil NH_4^+–N concentrations relative to the application of chemical fertilizers (Guo *et al.*, 2016). In addition, manure application presents good residual effects such as higher available N and P (Cai *et al.*, 2018). Therefore, it is of interest to explore the N flow of available N under the same N input of different fertilizers.

Shifts in carbon and nitrogen availability affect microbial community composition (Zhao *et al.*, 2019) and the use of organic fertilizers in addition to chemical fertilizers increases the abundances of *Bacillus*, *Trichoderma*, and *Pseudomonas*, mainly due to increased SOC content (Li *et al.*, 2017). The microbial community is able to adapt to long-term organic fertilization (Tian *et al.*, 2017). The application of organic fertilizers benefits some microbes, increasing the abundance of ammonia-oxidizing bacteria (Fan *et al.*, 2011) and denitrifier communities (Chen *et al.*, 2012). Bacteria also increase soil aggregate formation through the production of exudates (Degens, 1997) and increase nutrient bioavailability via nitrogen mobilization (Rashid *et al.*, 2016).

While the effects of chemical and organic fertilization on SOC and nitrogen have been widely studied, most studies have focused on effects of the additional application of organic fertilizers along with chemical fertilizers on soil and plants. However, how do SOC and nitrogen affect grain yield under the same N inputs? In the current study, we compared the effects of three different nitrogen sources (urea, cattle manure, and half manure and half urea) on soil with the same input of total N, P, and K in a 7-year fertilization experiment using a previously described lysimeter setup (Gu *et al.*, 2014). Our objective was to determine the N flow, soil physicochemical properties, and bacterial communities under the same N inputs. We hypothesized that substituting half the amount of urea with manure would increase grain yields while maintaining a favorable physical and chemical soil environment.

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Materials and Methods

Study site, experimental design, and management

The experiments were conducted at the Maize Technological Innovation Center (MTIC) at the Northern China Plain (NCP), located at Shandong Agriculture University (36°09'N, 117°09'E, 128 m above sea level) in Tai'an.

Field studies were conducted in lysimeter facilities of the NCP MTIC. The research facility includes 12 lysimeters (2.5 m long × 2.5 m wide × 2 m deep) filled with soil as described previously (Jia *et al.*, 2014). The soil was classified as silt loam soil. From 2006 to 2009, no fertilizer was applied to the soil, and high levels of irrigation were applied with a rotation of wheat and maize planting to achieve uniform conditions of low soil fertility.

The study began in 2009. Initial soil status was: TN, 0.8 g kg^{-1}; NH_4^+–N, 3.4 mg kg^{-1}; NO_3^-–N, 14.2 mg kg^{-1}; organic matter, 10.8%; bulk density, 1.1 g cm^{-3}; pH, 7.3. Fresh manure (M; composted cow dung) and urea (U) were used as N fertilizers. Fertilizer treatments were applied using the same nutrient inputs (200 kg N ha^{-1}, 245.7 kg P_2O_5 ha^{-1}, and 202.5 kg K_2O ha^{-1}) for maize. Three fertilizer treatments were applied, including 100% urea (100%U), 100% manure (100%M), half manure and half urea (50%U + 50%M). The control soil (CK) did not receive any N input. The experiment was performed with a randomized block design with three replications.

Before sowing, the manure was analyzed to determine the concentrations of total N, P_2O_5, and K_2O (as shown in Supplementary Material Table S1 available online at https://doi.org/10.1017/S0014479720000393). These values were used to calculate and balance the amounts of phosphate and potassium to use in 100%M and 50%U + 50%M treatments. Based on the content of N in manure, we applied manure with the same amount of N, 200 kg N ha^{-1}. Then, we added additional P and K fertilizer (the same type with 100% urea) to ensure equal inputs of P and K (245.7 kg P_2O_5 ha^{-1} and 202.5 kg K_2O ha^{-1}). Manure was mixed with the top (0–10 cm) layer of soil with one application as the basal fertilizer, whereas for the mixed treatment 50% of the urea was applied at sowing, while the remaining 50% was applied at the 12-leaf stage. Calcium superphosphate and potassium fertilization were performed via surface application and manually mixed into the top (0–10 cm) layer of soil before crop planting.

Soil sampling

From 2009 to 2013, different fertilizer treatment as described above was applied to the soil with a rotation of wheat and maize. At the beginning and end of each growing season in 2014 and 2016, soil samples with three replications per treatment were randomly collected from each plot, to a depth of 0–180 cm, and divided into 20 cm increments. Each sample of 20 cm increments was divided into two portions. The first portion was air-dried, ground, passed through a 2 mm sieve, and used to analyze total nitrogen (TN) content, soil organic carbon (SOC) content, and soil pH in 0–20 cm soil layers. The first portion was stored in −20 °C and used to analyze NO_3^-–N and NH_4^+–N contents in 0–180 cm soil layers. Undisturbed soil was sampled using a steel 100 cm^3 volume cylinder (5 cm in diameter and 5.1 cm in height) for bulk density.

Soil solution was collected 72 h after irrigation under 30 kPa vacuum from the 30 cm soil layer when maize plants were at the 6-leaf (V6), 12-leaf (V12), tasseling (VT), and milking (R3) growth stages. Total leachate was collected from the 200 cm soil layer throughout the growth stage. Soil solutions were stored at –20 °C for nitrate determination.

After harvesting the maize in 2016, soil samples were randomly collected from each plot with 3 shovelfuls per plot from the 0–5, 5–10, 10–15, and 15–20 cm soil layers and carefully transported to the laboratory for aggregate analysis. In 2016, when the plants were at the flowering stage, five soil cores per plot were collected from the top (0–20 cm) soil layer, mixed, passed through a 2-mm sieve, and stored at –80 °C for DNA extraction and PCR amplification.

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Ammonia Volatilization

Ammonia volatilization was measured immediately after the application of base fertilizers and top dressing according to Wang *et al.* (2004). We collected the glycerin phosphate sponge into a 1 L cup every 2 days in the first week and every 3 days thereafter for 30 days. Samples were extracted by 1 mol L^{-1} KCl and then stored at −20 °C for analysis. Ammonia volatilization was measured with a Kjeldahl Analyzer (Kjeltec^{TM} 8200, Hillerød, Denmark).

Plant sampling and harvesting

Three maize plants per plot were sampled at the maturity stage, dried in an oven at 80 °C, and weighed. The dried tissue was then ground and passed through a 0.85- mm sieve for the TN content analysis. All plants in each plot were harvested at the maturity stage for yield measurements.

Chemical and physical analysis of soil

Soil pH in a 1:5 (w/v) soil/water suspension was measured using a digital pH meter. To measure TN concentration, the soil was digested by adding 10 mL H_2SO_4–H_2O_2 and TN content was determined with a continuous-flowing analyzer (Bran + Luebbe, Hamburg, Germany). NO_3^-–N and NH_4^+–N were extracted from the soil with 1 mol L^{-1} KCl and measured with a continuous-flow analyzer (Bran + Luebbe). Total soil inorganic N content of NO_3^-–N and NH_4^+–N at the beginning and end of each growing season was used to denote the initial N and residual N, respectively. SOC content was determined using the dichromate oxidation procedure (Yeomans and Bremner, 1988).

Soil aggregate analysis

Undisturbed soil samples were air-dried at room temperature to roughly achieve the plastic limit and separated into aggregates by hand along natural planes of weakness. The soil was cleared of visible plant residues and stones. Aggregates were separated by dry and wet sieving as described by Kemper and Rosenau (1986) and divided into six fractions: >5, 5–2, 2–1, 1–0.5, 0.5–0.25, and <0.25 mm. Dry-sieved aggregates ($R_{>0.25\,mm}$, dry-sieving), water-stable aggregates ($R_{>0.25}$ mm, wet-sieving), the MWD, and the geometric mean diameter (GMD) were analyzed as described by Pinheiro *et al.* (2004) and Regelink *et al.* (2015).

DNA extraction and PCR amplification

DNA was extracted from 250 mg soil samples using a QIAamp® DNA Stool Mini Kit (Qiagen, Valencia CA, USA). MiSeq technology was used to measure 16S rRNA transcript levels. PCR amplification from the v4–v5 region of the 16S rRNA gene from genomic DNA was performed using primers containing protruding end junctions designed based on the surrounding conserved region (515Fin: 5′–TTCCCTACACGACGCTCTTCCGAT CT–GTGCCAGCMGCCGCGGTAA–3′; 926Rin: 5′–GAGTTCCTTGGCACCCGAGA ATTCCACCGTCAATTCMTTTGAGTTT–3′). Phusion High-Fidelity PCR Master Mix with HF buffer (New England Biolabs, Hitchin, UK) was used to amplify each sample. Illumina sequencing junction box dual index bar codes were added to each amplicon using 5 μL of the subsequent finite cycle PCR of each PCR product (515Fout: 5′–AATGATACGGCGACCACCGGATCTACAC–NNNNNNNN–TCTTTCCCTAC AC GACGCTC–3′; 926Rot: 5′–CAAGCAGAAGACGGCATACGAGAT–NNNNNNNN–GT GACTGGAGTTCCTTGGCACCCGAGA–3′). After purification using an Axygen AxyPrep DNA Gel Extraction kit (Axygen Scientific, Union City, CA, USA), the expression data for each library were standardized using a real-time PCR system (FTC3000; Funglyn Biotech, Canada). Products from different samples were mixed at the same molar ratio prior to sequencing on the

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MiSeq platform using a 2 × 300 cycle V3 kit according to the standard Illumina sequencing protocol. Sequence analysis was carried out using the software QIIME (Quantitative Insights into Microbial Ecology) as described by Fadrosh *et al.* (2014). Sequence reads were extracted, primer sequences were removed, and the sequence reads without an average quality of 20 over a 30 bp were truncated. The sequence reads less than 75% of their original length and its paired read were trimmed. The trimmed and unique sequences were used to define the number of operational taxonomic units (OTUs) at the 97% similarity level.

Data analysis

The experimental data were statistically analyzed by ANOVA with SPSS Statistics (SPSS 20.0, IBM Corp., Armonk, NY, USA). One-way ANOVA was used for comparisons, and Tukey's honest significant difference method ($\alpha = 0.05$) was used for multiple comparisons. Linear regression analysis and Pearson correlation coefficients were calculated with Sigmaplot (Sigmaplot 12.5, Systat Software Inc., San Jose, CA, USA). The diversity analysis of the microbial community for each treatment was conducted using the Chao and Shannon indices based on the Illumina MiSeq sequencing data.

Results

Soil physical properties

The proportion of aggregate fractions, MWD, and GMD was affected after 11 years of various fertilization treatments (Figure 1; Table 1). At 0–5 cm soil depth, aggregate fractions of >5 and 5–2 mm in dry sieving were major proportion, which showed a trend of 100%M > 50% M + 50%U > 100%U (Figure 1a). These differences in aggregate size distribution were reflected in MWD and GMD. Treatment with 100%M and 50%M + 50%U significantly increased MWD and GMD, whereas treatment with 100%U led to the lowest MWD and GMD (Table 1).

At 5–10 cm soil depth, treatment with 100%M and 50%M + 50%U significantly increased $R_{>0.25}$ in soil subjected to both dry and wet sieving, while the lowest $R_{>0.25}$ values were detected in soil treated with 100%U (Table 1). These treatments resulted in the same trends for MWD and GMD, even though the values after 100%U treatment were significantly lower than those of CK, that is, 1.61 and 0.179 mm versus 2.07 and 0.253 mm, respectively (Table 1). At 10–15 and 15–20 cm, there was no significant difference in $R_{>0.25}$ among treatments, while the 100%U treatment caused a significantly lower MWD and GMD compared to the 100%M, 50%M + 50%U, and CK (Table 1).

In 2014 and 2016, treatment with 100%U significantly increased the bulk density in the 0–20 cm layer of soil, whereas treatments with 100%M and 50%M + 50%U had no significant effect on this parameter compared with CK (Table 2).

SOC and nitrogen content

In 2014 and 2016, treatment with 100%M and 50%M + 50%U significantly increased the content of SOC, TN, and NO_3^-–N in the 0–20 cm soil layer compared to 100%U and CK (Table 2). The NO_3^-–N content of 50%M + 50%U in soil solutions was high throughout the growth stage, while that of 100%U was lower than that of 100%M and 50%M + 50%U at R2 and R3 stages (Figure 2).

SOC and N content significantly differed among soil layers in 2016 (Table 1). At 0–5 cm soil depth, 100%U induced a very low SOC content of approximately 7.48 g kg^{-1} (Table 1). The 100% M and 50%M + 50%U caused a significantly higher SOC content, increasing SOC by 79.8 and 59.8% when compared to 100%U, respectively (Table 1). The TN content in the 0–5 cm soil layer followed a similar trend to that of SOC; treatment with 100%M and 50%M + 50%U led to a significantly higher TN of approximately 1.99 and 1.83 g kg^{-1}, increased by 75.2 and 61.0%

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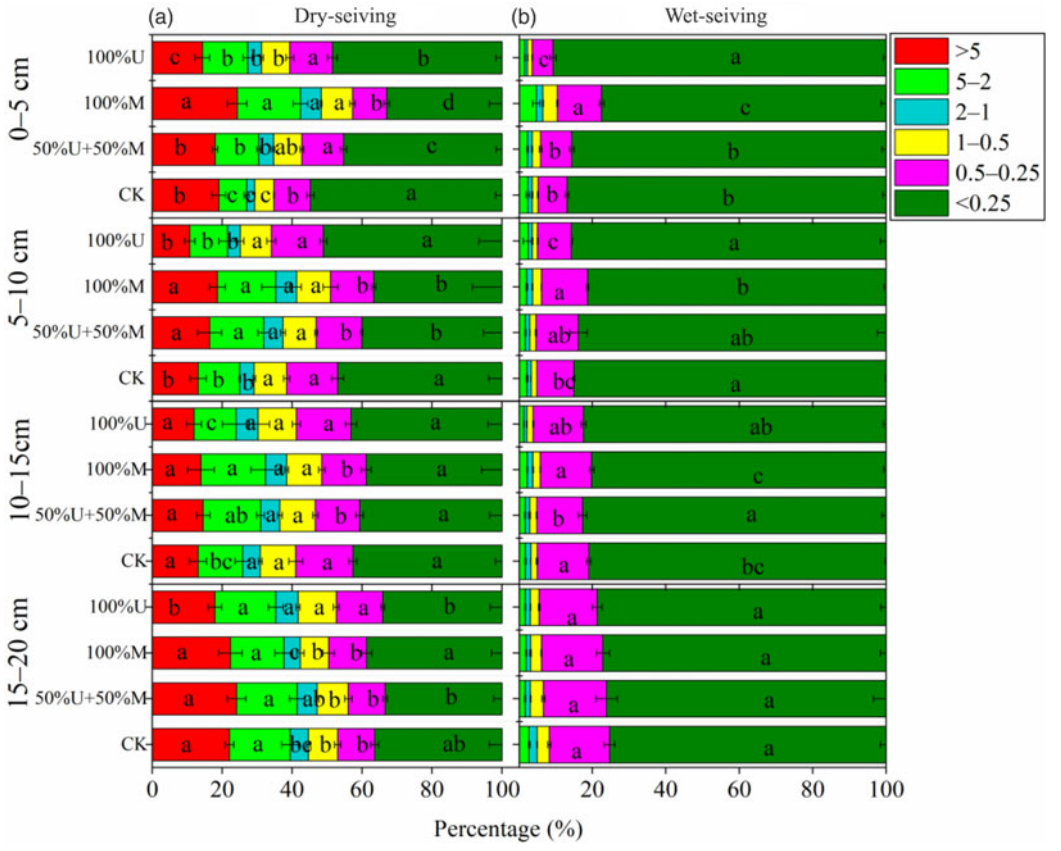


Figure 1. Aggregate fractions of dry sieving (a) and wet sieving (b) in 0–20 cm soil depth as affected by fertilization: 100% urea (100%U), 100% manure (100%M), half manure and half urea (50%U + 50%M), no nitrogen fertilizer (CK). Aggregate fractions include those > 5, 5–2, 2–1, 1–0.5, 0.5–0.25and <0.25 mm.

compared to 100%U, respectively (Table 1). In the 5–10 cm soil layer, SOC and TN under 100%M treatment were 58.4 and 22.5% higher compared to 100%U, and those under 50%M + 50%U treatment were 36.8 and 36.4% higher compared to 100%U, respectively (Table 1). SOC and TN were also much higher in each aggregate fraction in 0–5 cm soil depth under 100%M and 50%M + 50%U treatment compared to 100%U (Supplementary Material Figures S1 and S2).

Microbial abundance and diversity

We analyzed the microbial abundance and diversity of soil samples collected in 2016. Significant differences in richness and diversity were observed in terms of OTUs and the ACE, Chao, Shannon, and Simpson indices (Supplementary Material Table S2). Compared with CK, treatment with 100%M and 50%M + 50%U significantly increased microbial diversity (higher Shannon and Chao indices and lower Simpson index), whereas 100%U had no significant effect on microbial diversity (Table S2). The dominant phyla across all treatments were Nitrospira, Bacteroidetes, Acidobacteria, Proteobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Firmicutes, Armatimonadetes, Latescibacteria, and Verrucomicrobia (>1%), accounting for more than 98% of bacterial sequences (Figure 3). The populations of Nitrospira, Proteobacteria, Gemmatimonadetes, Latescibacteria, and Chlorobi were significantly affected by fertilizer treatments (Figure 3).

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Table 1. Macroaggregate ($R_{>0.25}$, dry-sieving and wet-sieving), aggregate geometric diameter (GMD), aggregate weight diameter (MWD), soil organic carbon (SOC), and N contents as affected by fertilization: 100% urea (100%U), 100% manure (100%M), half manure and half urea (50%U + 50%M), no nitrogen fertilizer (CK)

Soil layer (cm)	Treatment	$R_{>0.25}$ dry-sieving (%)	$R_{>0.25}$ wet-sieving (%)	MWD (mm)	GWD (mm)	SOC content (g kg^{-1})	N content (g kg^{-1})
0–5	100%U	51.5c	9.2d	2.08c	0.223c	7.48c	1.13c
	100%M	67.0a	22.5a	3.22a	0.636a	13.46a	1.99a
	50%U + 50%M	54.7b	17.2b	2.66b	0.341b	11.96b	1.83b
	CK	45.2d	12.9c	2.30c	0.238c	7.66c	1.02d
5–10	100%U	48.9b	14.4c	1.61c	0.179d	7.80c	1.07c
	100%M	63.3a	18.6a	2.69a	0.661a	10.31a	1.31b
	50%U + 50%M	59.9a	17.0ab	2.42a	0.426b	8.91b	1.46a
	CK	52.1b	14.9bc	2.07b	0.253c	7.18 d	0.97c
10–15	100%U	56.8a	16.3b	1.71b	0.261d	6.51b	0.94b
	100%M	61.2a	19.8a	2.28a	0.516a	7.47a	1.05a
	50%U + 50%M	59.3a	18.1ab	2.41a	0.411b	7.58a	1.14a
	CK	58.2a	18.6a	2.14a	0.361c	6.66b	0.78c
15–20	100%U	65.9a	21.3a	2.42c	0.351c	6.42ab	0.87b
	100%M	61.3b	22.8a	3.43b	0.449b	7.00a	0.98a
	50%U + 50%M	66.6a	23.8a	3.81ab	0.573a	6.25b	0.79b
	CK	63.6ab	24.9a	3.82a	0.525a	6.48ab	0.78b

Different letters represent significant differences ($p \leq 0.05$). Samples were taken in 2016.

Table 2. Physical and chemical properties in 0–20 cm in 2014 and 2016 as affected by fertilization: 100% urea (100%U), 100% manure (100%M), half manure and half urea (50%U + 50%M), no nitrogen fertilizer (CK)

Year	Treatment	Bulk density (g cm^{-3})	pH	SOC (g kg^{-1})	Soil N (g kg^{-1})	NO_3^-–N (mg kg^{-1})	NH_4^+–N (mg kg^{-1})
2005	Initial	1.10	7.3	3.83	–	–	–
2014	100U%	1.35a	7.11b	7.25c	0.79c	9.30b	4.13ab
	100M%	1.20b	7.29a	9.47a	1.30a	15.27a	6.37a
	50%U + 50%M	1.24b	7.27a	8.79b	1.12b	13.63a	3.82b
	CK	1.38a	7.28a	7.13c	0.70c	4.63c	4.29ab
2016	100U%	1.34a	7.08c	7.47c	0.84c	11.55b	1.69a
	100M%	1.23b	7.31a	9.03a	1.56a	16.92a	1.43a
	50%U + 50%M	1.25b	7.2b	8.28b	1.35b	16.56a	1.87a
	CK	1.35a	7.28ab	7.37c	0.78c	5.18c	1.75a

SOC, the content of soil organic carbon; Soil N, total nitrogen content in soil in 0–20 cm; NO_3^-–N, nitrate content in soil; NH_4^+–N, ammonium content in soil; different letters represent significant differences ($p \leq 0.05$).

Nitrogen and grain yield

The application of fertilizer significantly increased the crop uptake (CU) of nitrogen compared to the CK, whereas treatment with 100%M led to significantly lower CU of nitrogen among the three fertilization treatments in 2014 and 2016 (Supplementary Material Table S3). Nitrogen leaching accounted for 2.0–26.9% of TN input and 100%U significantly increased nitrogen leaching, with levels 1027.6 and 477.8% higher than those of CK in 2014 and 2016, respectively. The treatments with 100%M and 50%M + 50%U did not significantly differ from those of CK for nitrogen leaching in 2014 (Table S3). Nitrogen loss from ammonia volatilization (AM) showed a trend of 100% U > 50%M + 50%U > 100%M > CK in 2014. In 2016, nitrogen leaching and AM showed the same trends as in 2014.

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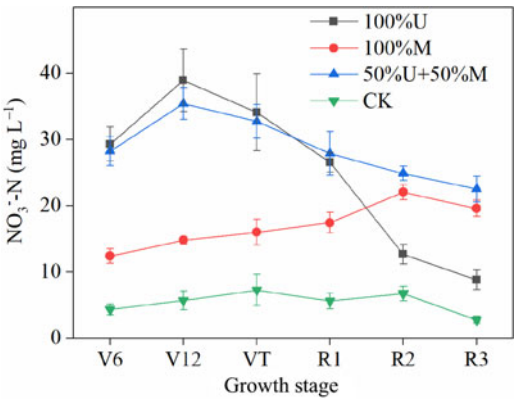


Figure 2. Nitrate content in soil solution extracted from 0 to 30 cm soil layer as affected by fertilization: 100% urea (100%U), 100% manure (100%M), half manure and half urea (50%U + 50%M), no nitrogen fertilizer (CK). V6, jointing stage; V12, 12th leaf stage; VT, tasseling stage; R1, silking stage; R2, blister stage; R3, milk stage.

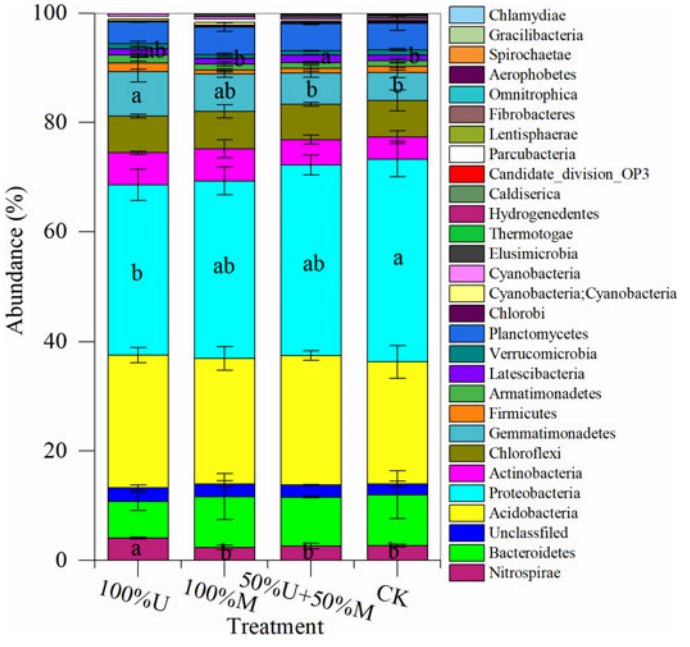


Figure 3. Phylum-level bacterial community structure as affected by fertilization: 100% urea (100%U), 100% manure (100% M), half manure and half urea (50%U + 50%M), no nitrogen fertilizer (CK) in 2016.

In 2014 and 2016, all three fertilization treatments significantly increased the grain yield of summer maize (Table 3). Treatment with 50%M + 50%U produced the highest grain yield, increasing by approximately 10 and 20% in 2014 and 3.5 and 5.2% in 2016 when compared to 100%U and 100%M, respectively (Table 3). Treatment with 100%U led to a significantly higher grain yield than 100%M in 2014, whereas the effects of 100%U and 100%M did not significantly differ in 2016 (Table 3). Grain yield was positively correlated with soil NO_3^-–N content in 2014 and 2016 (Table 4).

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Table 3. Grain yield in 2014 and 2016 as affected by fertilization: 100% urea (100%U), 100% manure (100%M), half manure and half urea (50%U + 50%M), no nitrogen fertilizer (CK)

	Grain yield (kg ha^{-1})	
Treatment	2014	2016
100U%	11,080.27b	13,394.31ab
100M%	10,072.80c	13,169.34b
50%U + 50%M	12,187.92a	13,860.35a
CK	7119.41d	8633.09c

Different letters represent significant differences ($p \leq 0.05$).

Table 4. Pearson correlations of soil parameters with soil organic carbon (SOC) and grain yield

		SOC		Grain yield	
Year	Soil parameters	Correlation coefficient	*p* value	Correlation coefficient	*p* value
2014	SOC	–	–	0.456	0.136
	pH	0.520	0.083	−0.306	0.334
	TN	0.975	<0.001	0.492	0.104
	NO_3^-–N	0.874	<0.001	0.687	0.014
	NH_4^+–N	0.376	0.228	−0.109	0.736
	Bulk density	−0.934	<0.001	−0.521	0.082
2016	SOC	–	–	0.535	0.073
	pH	0.461	0.131	−0.385	0.217
	TN	0.881	<0.001	0.776	0.003
	NO_3^-–N	0.815	0.001	0.872	<0.001
	NH_4^+–N	−0.425	0.168	−0.057	0.861
	Bulk density	−0.893	<0.001	−0.538	0.071
	MWD	0.916	<0.001	0.298	0.346
	Shannon index	0.845	<0.001	0.293	0.355
	Simpson index	−0.892	<0.001	−0.265	0.404

SOC, the content of soil organic carbon; TN, total nitrogen content in soil; NO_3^-–N, nitrate content in soil; NH_4^+–N, ammonium content in soil; MWD, the mean weight diameter of soil aggregates.

Discussion

Strategies for achieving high crop yield will be essential in addressing food security challenges due to the increasing global population and limited farmland availability (Smith *et al.*, 2013). Herein, the use of 50%U + 50%M caused the highest grain yield (Table 3), which was positively correlated with crop N uptake (Table 4). This may be attributed to the residual effect of manure application (Shen *et al.*, 2007) and the rapid fluctuation in NO_3^-–N content of chemical fertilizer (Lee, 2010). Indeed, organic fertilizer was shown to maintain a stable NO_3^-–N content throughout the growing season, while chemical fertilizer application led to a rapid decrease in NO_3^-–N content after R1 stage (Figure 2). The content of soil NO_3^-–N was positively correlated with grain yield ($p < 0.01$), whereas SOC content was not, implying that an increased SOC indirectly increases grain yield by increasing soil nitrate supply throughout the growing season. The 100%M generated the highest initial N before sowing and residual N after harvest. Manure application alone barely achieved the same grain yield compared with solo chemical fertilizer application under the same N input (Chen *et al.*, 2018). Accordingly, the grain yield under 100%M was lower than that of 50% U + 50%M and 100%U (Table 3), whereas chemical fertilizer application alone after 25-year fertilization indirectly affected grain yield and yielded less than manure application alone under nitrogen input of 210 kg ha^{-1} (Cai *et al.*, 2018). This was due to the contrast effect that chemical fertilizer application decreased the soil pH from 5.70 to 4.35, while solo manure application increased the soil pH from 5.70 to 6.66 (Cai *et al.*, 2018). Although the soil pH showed no correlation with grain yield in the present study, chemical fertilization alone led to soil acidification (Table 2).

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These findings suggest that substituting half of the urea with manure may ensure a sustainable, good nitrate supply throughout the maize growing season, thereby increasing grain yield.

The physical and chemical status of the studied soil markedly changed after 7 years of fertilization. Treatment with manure led to a marked decrease in soil bulk density (Guo *et al.*, 2016). Guo *et al.* (2016) found soil bulk density to be significantly lower after treatment with 100% cattle manure compared to 50% cattle manure combined with 50% chemical fertilizer. Here, 100%M and 50%U + 50%M treatment significantly decreased the soil bulk density compared with CK and 100%U treatment (Table 2). In addition to reducing bulk density, organic amendment can also increase soil porosity (Bronick and Lal, 2005). In general, treatment with manure and the combined application of manure and chemical fertilizer increase the formation of macroaggregates (Xie *et al.*, 2015). We found the treatment with 100%M and 50%U + 50%M significantly increased macroaggregate formation compared to 100%U and CK in the 0–10 cm soil layer (Table 1). In addition, both MWD and GMD significantly increased in response to the application of 100%M and 50%U + 50%M in the 0–10 cm soil layer, reflecting soil aggregate stability (Table 1). The enhanced stabilization of soil aggregates observed might be due to the considerable adhesive properties supplied by SOC (Tisdall and Oades, 1982). Although Lu *et al.* (2018) have demonstrated that long-term chemical fertilization also increases the formation of soil macroaggregates, our treatment with 100U% reduced macroaggregate formation compared to CK (Table 1).

Previous studies have shown that both organic and chemical fertilization increased the SOC content (Brar et al., 2013; Xie *et al.*, 2015). Accordingly, treatments with 100%M and 50% U + 50%M significantly increased the SOC content compared with 100%U and CK in the top (0–10 cm) soil layer, and soil treated with 100%U had a significantly higher SOC content than CK soil in the 5–10 cm soil layer (Table 1). Manure treatment involves the direct input of organic material, whereas chemical fertilization involves the indirect input of plant biomass residues as an organic source (Tripathi *et al.*, 2014). While the same SOC contents were obtained in 100%U and CK treatment, 100%U significantly reduced MWD and GMD in the 10–20 cm soil layer compared to CK (Table 1), which was likely due to decreased soil pH induced by 100%U (Table 2). Treatment with chemical fertilizer alone significantly decreased soil pH compared with CK, while manure and manure plus urea had no significant effect on soil pH (Table 2). As a possible explanation, the buffering effects of the carboxyl and phenolic hydroxyl groups in manure balanced the acidity generated by urea (Garcia–Gil *et al.*, 2004). Soil N status also varied after 7 years of fertilization. Although the TN input was the same under all fertilizer treatments, 100%M and 50% U + 50%M caused significantly higher soil TN contents than the other treatments (Table 2). These results are consistent with the findings of a previous study, in which the manure input ratio of >50% caused higher TN content than chemical fertilization alone (Guo *et al.*, 2016), likely due to the lower release rate of nitrogen from manure (Liu *et al.*, 2013).

Soil microbial abundance and diversity are also important factors affecting soil quality (Murugan and Kumar, 2013). In recent years, many studies have focused on the effects of manure amendment on soil bacteria (Chen *et al.*, 2016; Mocali *et al.*, 2013). Manure amendment significantly increases the abundance of Gemmatimonadetes and decreases the abundance of Acidobacteria and Cyanobacteria (Chen *et al.*, 2016). Tu *et al.* (2018) demonstrated that organic manure increases the abundance of members of the bacterial phylum Firmicutes compared to inorganic fertilizer. Here, 100%U increased the abundance of Nitrospirae compared to 100%M and 50%U + 50%M treatment, leading to a higher leaching risk. Soil pH (Zhalnina *et al.*, 2015) and SOC content (Li *et al.*, 2018) are thought to be the main factors leading to different soil bacterial abundances in response to fertilizers. In fact, SOC content was positively correlated with the Shannon index (Table 4). Both inorganic and organic fertilizers have important effects on bacterial community structure and diversity (Geisseler and Scow, 2014). Inorganic N fertilizer was previously found to markedly reduce bacterial diversity compared to manure amendment (Chen *et al.*, 2016). Consistent with this finding, 100%M and 50%U + 50%M treatment significantly

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increased the alpha diversity of bacteria compared to CK and 100%U treatment based on the ACE, Chao, and Shannon indices (Table S2). Meanwhile, 100%U significantly reduced bacterial diversity compared to CK, as indicated by the Shannon index (Table S2). These results suggest that adding manure to the soil alters the composition of bacterial communities.

The loss of soil nitrogen is significantly affected by the type of fertilizer applied, even with the same level of nitrogen input. Globally, ammonia volatilization accounts for an average of 18% (up to 64%) of nitrogen loss after chemical fertilization (Pan *et al.*, 2016). We found ammonia volatilization to account for 6.8–20.0% of the loss of nitrogen input and the highest ammonia volatilization was found under 100%U (Table S3). Indeed, Xia *et al.* (2017) demonstrated that substituting manure for chemical fertilizer significantly decreased ammonia volatilization by 26.8%. The presence of highly buffered soils due to high concentrations of soil organic matter may partly explain the low rates of ammonia volatilization observed in soil treated with manure application alone (Jones *et al.*, 2013). By contrast, higher ammonia volatilization was detected in soil treated with manure plus chemical fertilizer compared to chemical fertilizer alone (Huang *et al.*, 2017; Zhou *et al.*, 2016b). This difference might be due to the different soil pH conditions and fertilizer application depths in soil utilized in these studies. In addition, N loss from ammonia volatilization could be higher than that measured in this experiment, as we only collected ammonia nitrogen for the first 30 days after fertilization.

Minimizing nitrogen leaching is also critical for reducing nitrogen pollution in agroecosystems. In the present study, soil treated with 100%U exhibited the highest rate of nitrogen leaching, while treatment with 100%M and 50%U + 50%M had no significant effect on nitrogen leaching compared to CK (Table S3). This may be partly due to the small mineral nitrogen pool and high moisture retention capacity of manure (Blanco–Canqui *et al.*, 2015; Iqbal et al., 2019; Wang *et al.*, 2015). Zhalnina *et al.* (2015) demonstrated that high relative abundance of Nitrospirae bacteria increases the leaching risk. Thus, significantly higher relative abundance of Nitrospirae after 100% U application might contribute to high rates of nitrogen leaching from soil (Figure 3). Additionally, MWD was negatively related to nitrogen leaching under all three fertilizer treatments ($R = -0.778$, $p = 0.014$). This finding is consistent with the notion that the enhanced stability of soil aggregates induces the immobilization of mineral nitrogen in soil, thus reducing nitrogen leaching (Cui *et al.*, 2014).

Conclusion

Manure application significantly increased the contents of SOC, TN content, NO_3^-–N, and, NH_4^+–N, increased the formation of macroaggregates and aggregate MWD, enhanced soil bacterial abundance and diversity, and decreased nitrogen loss through ammonia volatilization and nitrogen leaching. Treatment with half manure and half urea led to the highest grain yield in summer maize, due to the improved nitrogen supply throughout the plant growth stage.

Supplementary materials. For supplementary material for this article, please visit https://doi.org/10.1017/S0014479720000393

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