

RESEARCH ARTICLE

Adding NBPT to urea increases N use efficiency of maize and decreases the abundance of N-cycling soil microbes under reduced fertilizer-N rate on the North China Plain

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OPEN ACCESS

Citation: Liu G, Yang Z, Du J, He A, Yang H, Xue G, et al. (2020) Adding NBPT to urea increases N use efficiency of maize and decreases the abundance of N-cycling soil microbes under reduced fertilizer-N rate on the North China Plain. PLoS ONE 15(10): e0240925. <https://doi.org/10.1371/journal.pone.0240925>

Editor: Tim Hülsen, University of Queensland, AUSTRALIA

Received: May 12, 2020

Accepted: October 5, 2020

Published: October 28, 2020

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This study was supported by the National Key Research and Development Program of China (2018YFD0200605) and Henan Postdoctoral Science Foundation.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Urease inhibitor (UI) and nitrification inhibitor (NI) can reduce N losses from agricultural soils but effects of inhibitors on N cycle are unclear. A field experiment was conducted with maize to test effects of UI (N-(n-Butyl) thiophosphoric, NBPT) and NI (3,4-dimethylpyrazolephosphate, DMPP) on N uptake and N-cycling soil microbes. Five treatments were imposed: no N fertilizer input (CK), conventional fertilization (CF) and 80% of urea input with NBPT (80%U+UI), with DMPP (80%U+NI) and with half NBPT and half DMPP (80%U+1/2(UI+NI)). There were no significant differences in biomass between 80%U+UI, 80%U+NI and CF but harvest index was increased under 80%U+UI and 80%U+NI. Compared to CF, N use efficiency of grain under 80%U+UI was increased by 7.1%, whereas grain yield and N uptake under 80%U+1/2(UI+NI) were decreased by 8.2% and 9.4%, respectively. The peak soil NO_3^- -N content was at about 15 days after fertilization (DAF) under CF but 30 DAF under the inhibitor treatments. In soils of 80%U+UI, the activities of urease and nitrate reductase were decreased between 15–45 DAF and between 5–30 DAF. The abundance of N-cycling soil microbes was affected: 80%U+UI and 80%U+NI reduced the copies of the *amoA* AOA and *nirK* genes at about 15 days and reduced the copies of the *amoA* AOB gene at about 30 days. Correlation analysis indicated that there were significant positive relationships between *amoA* AOB gene and NH_4^+ -N, as well as between *nirK* gene and NO_3^- -N. Overall, urea applied with NBPT has greater potential for improving maize N use efficiency and inhibiting nitrification under reduced fertilizer-N applications.

Introduction

Worldwide demand for N fertilizer is predicted to increase at a rate of approximately 1.6 Tg yr^{-1} . About half of the increase is expected to be in China (18%), India (17%) and Latin

America (18%) [1]. Urea ($\text{CO}[\text{NH}_2]_2$) is the dominant form of N fertilizer used in Chinese agricultural soil as well as the world, because of its relative ease of transport and application [2, 3]. With surface applications of urea, N gaseous losses account for more than 40%-50% of the N applied under some environmental and edaphic conditions [4]. The processes of nitrification and denitrification are considered as the two major microbial NH_4^+ -N transformation pathways and also the main pathway of N loss from soil [5].

Nitrification inhibitor (NI) retards nitrification by deactivating ammonia oxidation [6–8]. Ammonia oxidation, a rate-limiting step in nitrification, catalyzed by the *amoA* gene encoding ammonia monooxygenase is carried out by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), which play important role in nitrification-derived N_2O [9]. NI can decrease the abundance of *amoA* genes [8], N_2O emissions and NH_3 volatilization [10] in soils. Urease inhibitor (UI) delays urea hydrolysis and consequently affects the processes of nitrification and denitrification [11]. In denitrification, nitrite reductase is encoded by the *nirK* and *nirS* genes and has been used as a molecular marker for denitrifying bacteria [12]. UI or/and NI are added to urea, which can improve crop N use efficiency (NUE) and N uptake. When UI combines with urea, its solubility and diffusivity values are similar to those of urea. This facilitates its inhibitory effects on urea hydrolysis by urease [13, 14]. For NI, it slows the first step of NH_3 oxidation and reduces NH_3 conversion to NO_3^- -N and thus the extent of soil leaching [15]. It is well known that plant N uptake generally depends on the soil NO_3^- -N, because NH_4^+ -N is rapidly converted to NO_3^- -N through nitrification followed by denitrification. However, there are few reports that focus on the effects of inhibitors on the dynamic changes in soil NO_3^- -N, not on the nitrifiers/denitrifiers. Additionally, there is also debate regarding the economic benefits of inhibitors because some studies in which yield increases are not always observed, despite the additional cost [16–18].

Maize (*Zea mays* L.) is an important oil and cash crop and China contributes nearly 25% of global production [19]. Meanwhile, around 30% of Chinese maize production is on the North China Plain (NCP) [20]. Simultaneous fertilization and sowing are key agricultural practice in maize planting on the NCP, as later topdressing is very difficult, especially after tasseling (VT stage). Overfertilization with N at sowing increases N loss and so leads to N deficiency after tasseling, such as has been found in some studies where the maize N recovery rate was less than 40% [21, 22]. Meanwhile, overuse N fertilizer application increases the risk of nitrate accumulation and leaching of soil, which leads to groundwater contamination. A survey of nitrate concentration of 1,139 groundwater samples on the NCP, Zhao et al. [23] reported that the average nitrate value was 11.9 mg L^{-1} , with 34.1% of samples exceeding the WHO standard due to overuse N fertilizer. Undoubtedly, this worrying result is associated with the overuse N fertilizer in the sampled area.

The objectives of this study were (i) to examine the effects of UI or/and NI on maize yield, N uptake and NUE under reduced fertilizer-N applications, (ii) to compare the dynamic changes in NO_3^- -N contents, enzyme activities and N-cycling genes in soils. It is hoped this study will provide optimal fertilization regime for increasing maize yield and improving soil environment under reduced fertilizer-N applications.

Materials and methods

Site description

Our experiment site was located in Gucheng town ($34^\circ 12' \text{ N}$, $113^\circ 33' \text{ E}$) of Yuzhou city, Henan province on the NCP. The field study was carried out on private land with the permission of the land owner and did not involve endangered or protected species. This region has a temperate, semi-humid monsoon climate, where the highest temperature and most of rainfall

simultaneously occur in summer. Annual precipitation is 673 mm, of which 70% occurs in June-August and annual evaporation is 946 mm. Mean annual temperature is 14.4°C and the frost-free period is 214 days. The soil taxonomy of the experiment site is an Aridic Haplustepts, which belongs to Inceptisols in USDA classification system. Before this study, the soil properties in the 0–20 cm depth band were: organic C 8.6 g kg⁻¹, total N 0.6 g kg⁻¹, available P 5.24 mg kg⁻¹, available K 121.4 mg kg⁻¹ and pH 7.94.

Experiment design

A field experiment had five treatments: (i) no N fertilizer input (CK), (ii) conventional fertilization (CF) and (iii) 80% of urea input with NBPT (80%U+UI), (iv) with DMPP (80%U+NI) and (v) with half NBPT and half DMPP (80%U+1/2(UI+NI)). The area of each plot was about 45 m² (5.6 m × 8.0 m), and plots were completely randomized with respect to treatment and each treatment was repeated three times. The plot distribution, buffer area and guard rows are illustrated in [S1 Fig](#). The application levels of fertilizers and inhibitors are listed in [Table 1](#). Fertilizer application and sowing were carried out simultaneously using an integrated machine. No topdressing fertilizers were applied in any treatments. The maize cultivar was Zhengdan 1002, and it was sown in Jun 2019 at a density of 67500 plants hm⁻² and a row spacing of 60 cm. All rows in each plot were harvested in late September 2019. Field managements were consistent with local practice for the control of pests, diseases and weeds, and for irrigation.

Sampling

At 5, 15, 30, 45, 60 and 90 days after fertilization (DAF), three soil samples were taken with an auger from each plot, respectively. Each sample was split into three subsamples from soil depths of 0–20, 21–40 and 41–60 cm. Samples were combined between replicate treatments to give a bulk sample at the same depth band and immediately stored in a cooler at 4°C to determine NO₃⁻-N content. In laboratory, subsamples (50 g) from the 0–20 cm depth band were separated and stored at 4°C for analysis of enzyme activity. Meanwhile, some of subsamples at 15, 30 and 45 DAF from the 0–20 cm depth band were stored at -20°C used for later analysis of microbial abundance and the rest were air-dried for analysis of physiochemical property. The grain and straw from each plot were collected separately by hand to determine above-ground biomass. At harvest, 500 g samples of air-dried grain and subsamples (10 plants) of air-dried straw were taken from each plot and carried to the laboratory to determine N uptake and NUE.

Sample analysis

The mass yields of grain and straw were determined at the moisture levels of 12% and 11%, respectively. Harvest index was obtained by the ratio of grain yield to biomass. The N contents of grain and straw were measured by the method of concentrated H₂SO₄ digestion. The NUE

Table 1. Application levels of fertilizers and inhibitors.

Treatment	Fertilizer (kg·km ⁻²)			Inhibitor
	N	P ₂ O ₅	K ₂ O	
CK	0	60	60	/
CF	225	60	60	/
80%U+UI	180	60	60	1.5% of urea dosage
80%U+NI	180	60	60	1.5% of urea dosage
80%U+1/2(UI+NI)	180	60	60	0.75% of urea dosage

<https://doi.org/10.1371/journal.pone.0240925.t001>

was calculated as:

$$NUE (\%) = \frac{N_1 - N_2}{N_i} \times 100\%$$

where N_1 and N_2 are the N uptake under N and no-N fertilizer treatment (kg hm^{-2}), respectively, and N_i is net N input from fertilizer (kg hm^{-2}).

The analyses of organic C, total N, NH_4^+ -N, available P, available K and moisture in soils were according to the methods described by Lu [24]. The NO_3^- -N content was determined using a continuous flow analyzer (CleverChem 200, DeChem-Tech, GmbH Corporation, Germany) after being extracted with 2M KCl [25]. Enzyme activities were determined by the methods described by Hu et al. [26]. For urease, the samples were incubated with 1 mL methylbenzene, 10 mL of 10% urea solution and 20 mL of citrate buffer at 30°C for 24 h, NH_4^+ -N concentration in the mixture was determined using an UV-visible spectrophotometer (UV-2600, SHIMADZU, Tokyo). For nitrate reductase, the samples were incubated with 1 mL of 0.8 mM 2,4-dinitrophenol solution, 1 mL of 0.05% KNO_3 solution, 1 mL of 1% glucose solution and 7 mL of anaerobic deionized water at 30°C for 24 h, NO_2^- -N concentration in the mixture was determined using the UV-visible spectrophotometer.

Total DNA was extracted using an E. Z. N. A. Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. Extracted DNA were stored at -20°C until used in downstream analyses. The quantity and quality of the extracted DNA were determined using a Nanodrop Spectrophotometer (Nanodrop 2000, Thermo Fisher Scientific, USA). The primers of the Arch-amoA:Arch-amoA (amoA AOA), amoA-1F:amoA-2R (amoA AOB), nirScd3A:nirSR3cd and nirKF1aCu:nirKR3Cu were used to amplify the amoA AOA, amoA AOB, nirS and nirK gene fragments, respectively [27–29]. All gene quantitative PCR (qPCR) amplifications were performed with a Rotor-Gene[®] Q (CFX96Touch, BIORAD, USA) in 20 μL of the reaction mixture containing 10 μL of 2 \times SYBR Green Master Mix (BioTeke, Beijing), 0.4 μM of forward and reverse primer, 1 μL of template DNA and 8.2 mL of sterile distilled water. Target genes were quantified using the primers and qPCR programs [30].

Statistical analysis

The differences in biomass between treatments were tested with a one-way ANOVA in SPSS v. 25.0, as well as N uptake, NUE, harvest index and gene copies. An LSD analysis was conducted to compare the difference in NO_3^- -N content and in enzyme activity in SAS v. 9.4. The Spearman's coefficient and *p*-value were performed to quantify correlations between enzyme activities, N-cycling genes and physiochemical parameters using functions in psych package of R v. 3.6.0, and the corrplot package in R was used to display the results.

Results

Maize N uptake, N use efficiency and harvest index

Maize N uptake, NUE and harvest index were analyzed under different fertilization treatments (Table 2). Results showed that the differences in biomass between 80%U+UI, 80%U+NI and CF were not significant but that under 80%U+1/2(UI+NI) was significantly lower than under CF (grain 8.2% and straw 17.0%). Meanwhile, grain N uptake under 80%U+1/2(UI+NI) was significantly decreased by 9.4% than that under CF. Compared to CF, straw N uptake under the inhibitor treatments was decreased by between 18.9% and 31.3%. The grain NUE under 80%U+UI was significantly increased by 7.1% than that under CF, whereas the differences were not significant between 80%U+NI, 80%U+1/2(UI+NI) and CF. For harvest index, it was

Table 2. Maize N uptake, NUE and harvest index under different fertilization treatments.

Treatment	Biomass (kg hm ⁻²)		N uptake (kg hm ⁻²)		Grain NUE (%)	Harvest index
	Grain	Straw	Grain	Straw		
CK	7639±204 c	6472±192 c	77.9±3.4 c	5.8±1.8 d	/	0.53±0.02 a
CF	10090±333 a	10856±478 a	124.4±7.1 a	40.5±4.4 a	18.2±1.9 b	0.48±0.02 b
80%U+UI	10394±598 a	10084±535 ab	123.5±5.9 a	32.9±2.8 b	25.3±2.6 a	0.51±0.01 a
80%U+NI	10265±224 a	9919±561 ab	119.3±8.2 ab	31.8±3.6 bc	23.0±2.8 ab	0.51±0.00 a
80%U+1/2(UI+NI)	9265±369 b	9008±348 b	112.8±4.0 b	27.8±1.9 c	19.4±1.4 b	0.50±0.01 ab

Values are means±SD (n = 3). Values in the same column followed by different lowercase letters are significantly different ($p < 0.05$). CK, no N fertilizer input; CF, conventional fertilization; 80%U+UI, 80%U+NI and 80%U+1/2(UI+NI) indicate 80% of urea input with NBPT, with DMPP and with half NBPT and half DMPP, respectively.

<https://doi.org/10.1371/journal.pone.0240925.t002>

significantly increased under 80%U+UI and 80%U+NI compared to CF, but not under 80%U+1/2(UI+NI).

Dynamic change of soil NO₃⁻–N content

In the 0–60 cm soil depth band, the mean NO₃⁻–N content for each treatment was calculated from 5 to 90 DAF (Fig 1). Under CF, the peak NO₃⁻–N content appeared at about 15 DAF, when it was significantly increased by 121.2%, 92.5% and 36.7% than under 80%U+UI, 80%U+NI and 80%U+1/2(NI+UI), respectively. With additions of the inhibitors, the peaks of NO₃⁻–N content appeared at about 30 DAF. Those peaks were obviously increased compared to CF, with a range of 10.4%–26.6%. At 45 DAF, the NO₃⁻–N content under 80%U+UI was still higher (30.9%, $p < 0.05$) than under CF. During the period 60 to 90 DAF, the differences in NO₃⁻–N contents between N fertilizer treatments were not significant.

Activities of urease and nitrate reductase

Compared to CF, the urease activity under 80%U+UI was significantly decreased by 17.3% between 15–45 DAF, with a range of 10.2%–27.9%. However, over the period 30–90 DAF, no significant differences were observed between 80%U+NI, 80%U+1/2(UI+NI) and CF (Fig 2A). The nitrate reductase activities under the inhibitor treatments were substantially decreased from 5 to 30 DAF compared to CF, and the reduction rate was between 18.6% and 27.1% (Fig 2B). During the period 45 to 90 DAF, the nitrate reductase activities under different treatments showed a downward trend but no significant differences were found between N fertilizer treatments (Fig 2B).

Abundance of N-cycling genes

The abundance of N-cycling genes was markedly affected by inhibitors, especially when UI or NI was used on their own (Fig 3). Compared to CF, the *amoA* AOA gene copies under 80%U+UI and 80%U+NI were significantly decreased by 22.3% and 40.1% at 15 DAF, respectively (Fig 3A). Meanwhile, the *amoA* AOB gene copies under 80%U+UI and 80%U+NI were significantly decreased by 44.5% and 65.6% at 15 DAF and by 36.8% and 29.3% at 30 DAF, respectively (Fig 3B). No significant differences in *amoA* genes were observed between 80%U+1/2(UI+NI) and CF from 30 to 45 DAF (Fig 3A and 3B).

Compared to CF, at 15 DAF, the copies of *nirS* and *nirK* genes were significantly decreased by 19.4% and 31.4% under 80%U+UI and by 28.7% and 49.2% under 80%U+NI, respectively (Fig 3C and 3D). However, no significant differences in *nir* genes were found between 80%U+1/2(UI+NI) and CF at 15 DAF. Additionally, the differences in *nir* genes between N fertilizer treatments were not significant at either 30 or 45 DAF (Fig 3C and 3D).

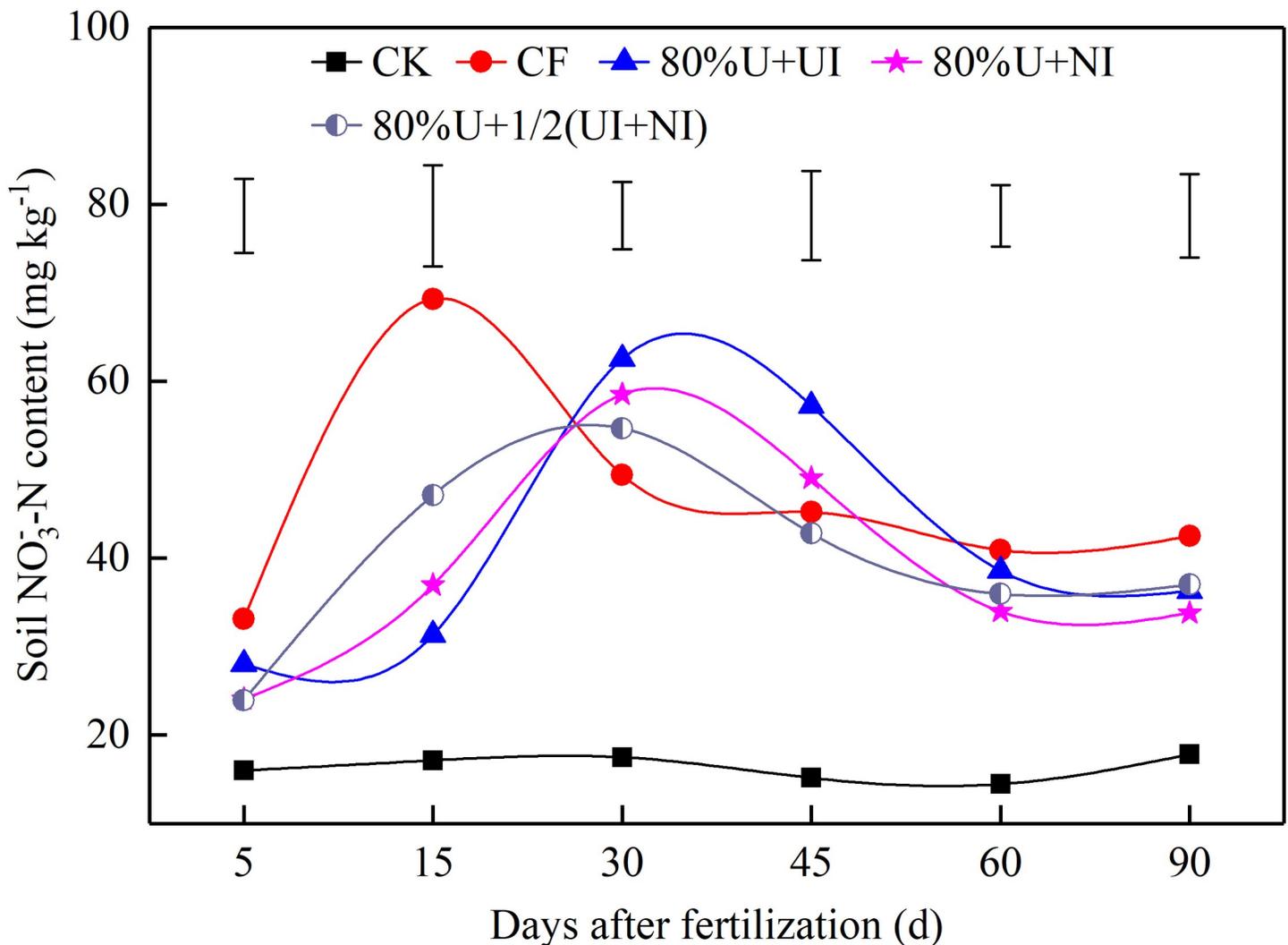


Fig 1. Mean NO₃⁻-N content in the 0–60 cm soil depth band from 5 to 90 days after fertilization. CK, no N fertilizer input; CF, conventional fertilization; 80%U+UI, 80%U+NI and 80%U+1/2(UI+NI) indicate 80% of urea input with NBPT, with DMPP and with half NBPT and half DMPP, respectively. Vertical bars represent LSD_{0.05} between treatments.

<https://doi.org/10.1371/journal.pone.0240925.g001>

Correlation analysis

The correlation analysis revealed significant relationships between enzyme activities, nitrifying/denitrifying genes and physiochemical parameters when different fertilization treatments were considered (Fig 4). There were significant positive relationships between *amoA* AOB gene and NH₄⁺-N, as well as *nirK* gene and NO₃⁻-N. In contrast, the abundance of *amoA* AOA and *nirS* genes was relatively little affected by physiochemical properties. Additionally, there was a significant positive relationship between urease activity and NO₃⁻-N.

Discussion

Using meta-analysis, Linquist et al. [31] have found that additions of the inhibitors led to an 8.0% increase in field crop N uptake. Similarly, our results indicate that under 80%U+UI and 80%U+NI, maize NUE was increased by 8.5% and 5.9%, respectively (Table 2). Previous

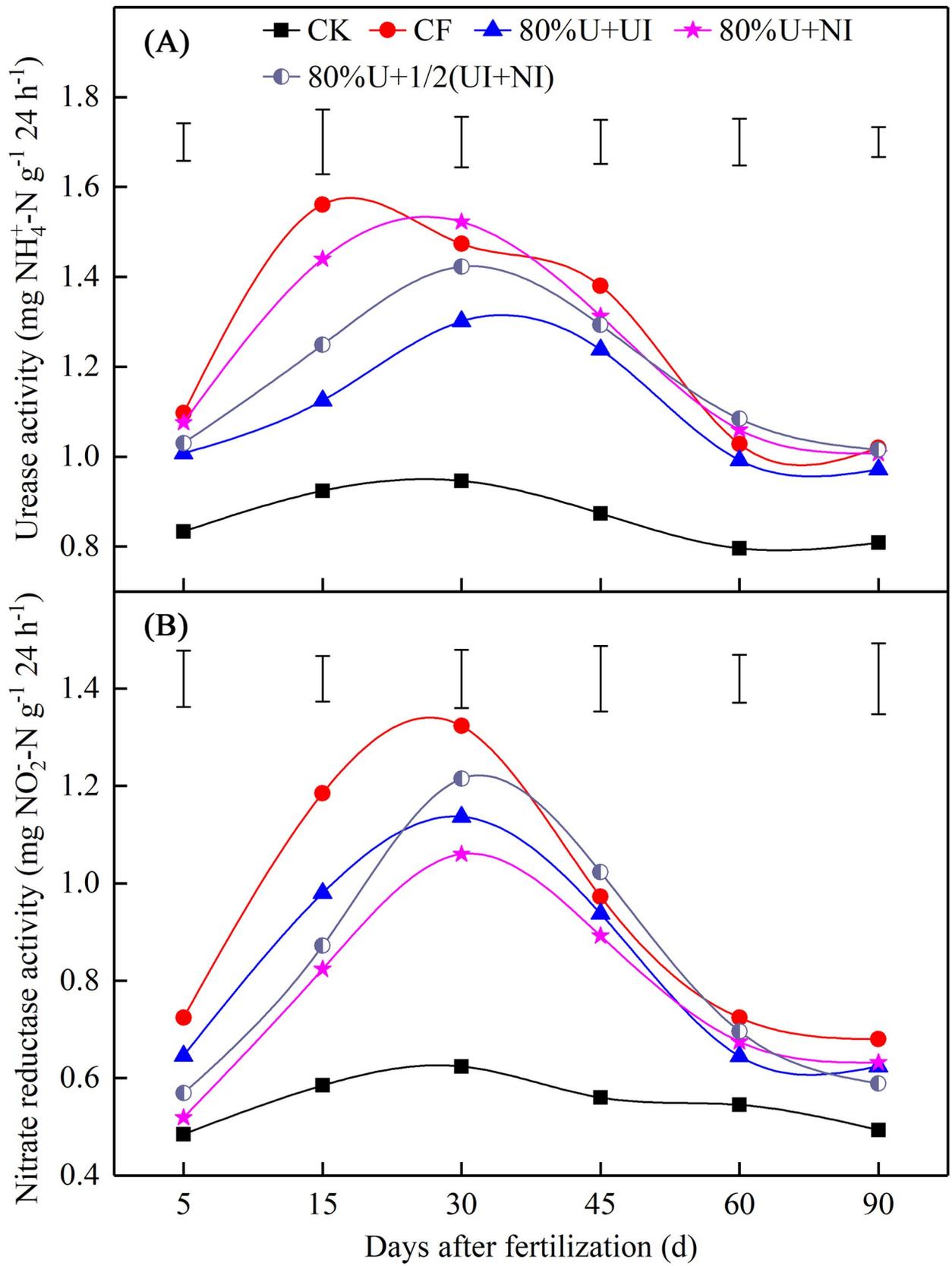


Fig 2. The activities of urease (A) and nitrate reductase (B) under different fertilization treatments. CK, no N fertilizer input; CF, conventional fertilization; 80%U+UI, 80%U+NI and 80%U+1/2(UI+NI) indicate 80% of urea input with NBPT, with DMPP and with half NBPT and half DMPP, respectively. Vertical bars represent $LSD_{0.05}$ between treatments.

<https://doi.org/10.1371/journal.pone.0240925.g002>

studies also concluded that additions of the inhibitors improve maize yields in comparison to conventional urea alone [15, 32]. In present study, although the N fertilizer applications under 80%U+UI and 80%U+NI were reduced by 20%, their yields were not affected while harvest indexes were increased (Table 2). In other words, the addition of UI or NI increases maize yield and thus commercial return under reduced N fertilizer application. We also found that maize biomass and N uptake under 80%U+1/2(UI+NI) did not perform better than the addition of UI or NI alone. Also, these were obviously decreased compared to CF (Table 2). This finding has been described by Zaman et al. [11] and Frame [33] who attributed it to the addition of NI to urea with UI increasing NH_3 volatilization in the soil.

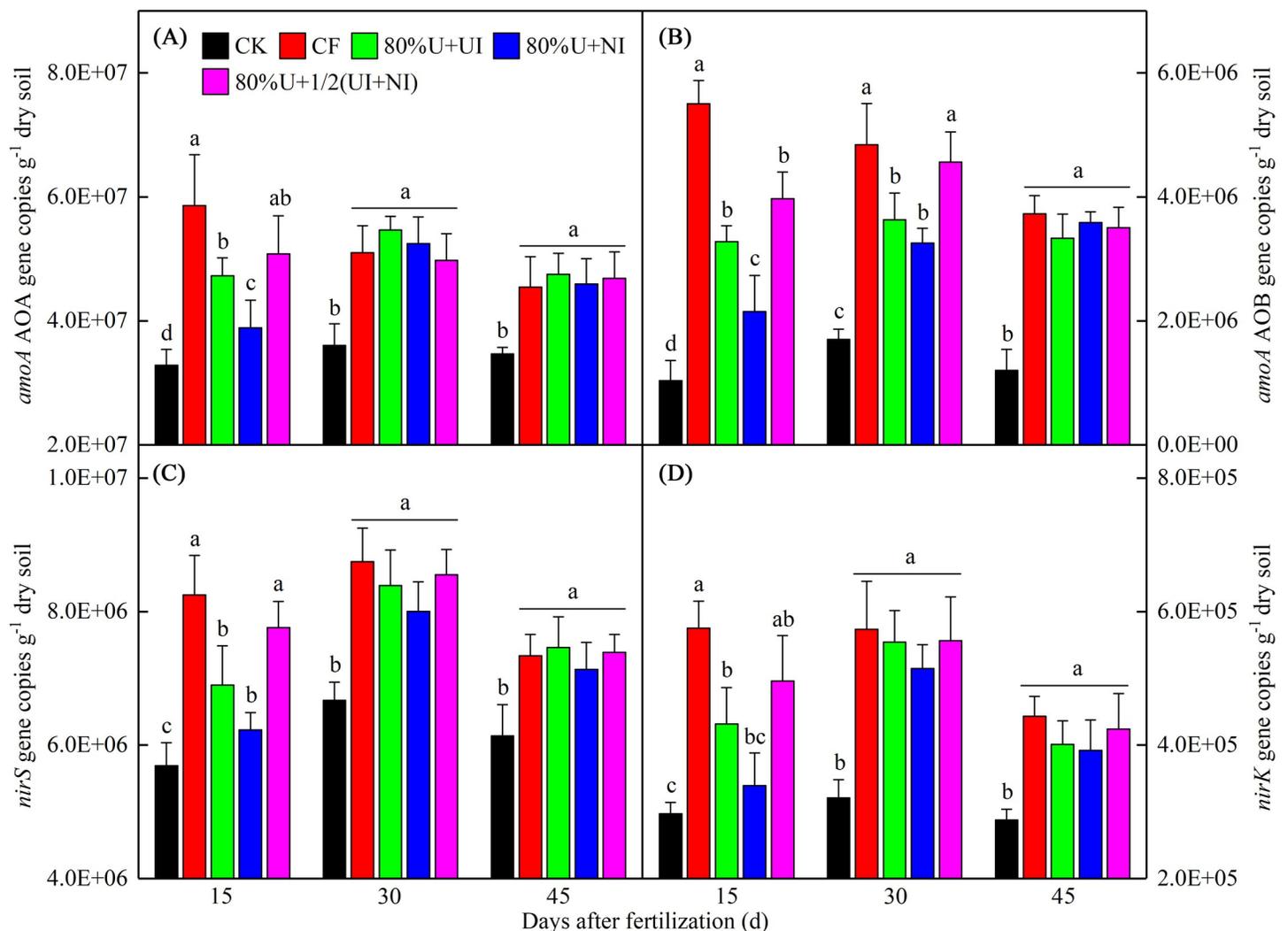


Fig 3. The N-cycling gene copies under different fertilization treatments. CK, no N fertilizer input; CF, conventional fertilization; 80%U+UI, 80%U+NI and 80%U+1/2 (UI+NI) indicate 80% of urea input with NBPT, with DMPP and with half NBPT and half DMPP, respectively. Different lowercase letters above the bars represent significant differences ($p < 0.05$) between treatments.

<https://doi.org/10.1371/journal.pone.0240925.g003>

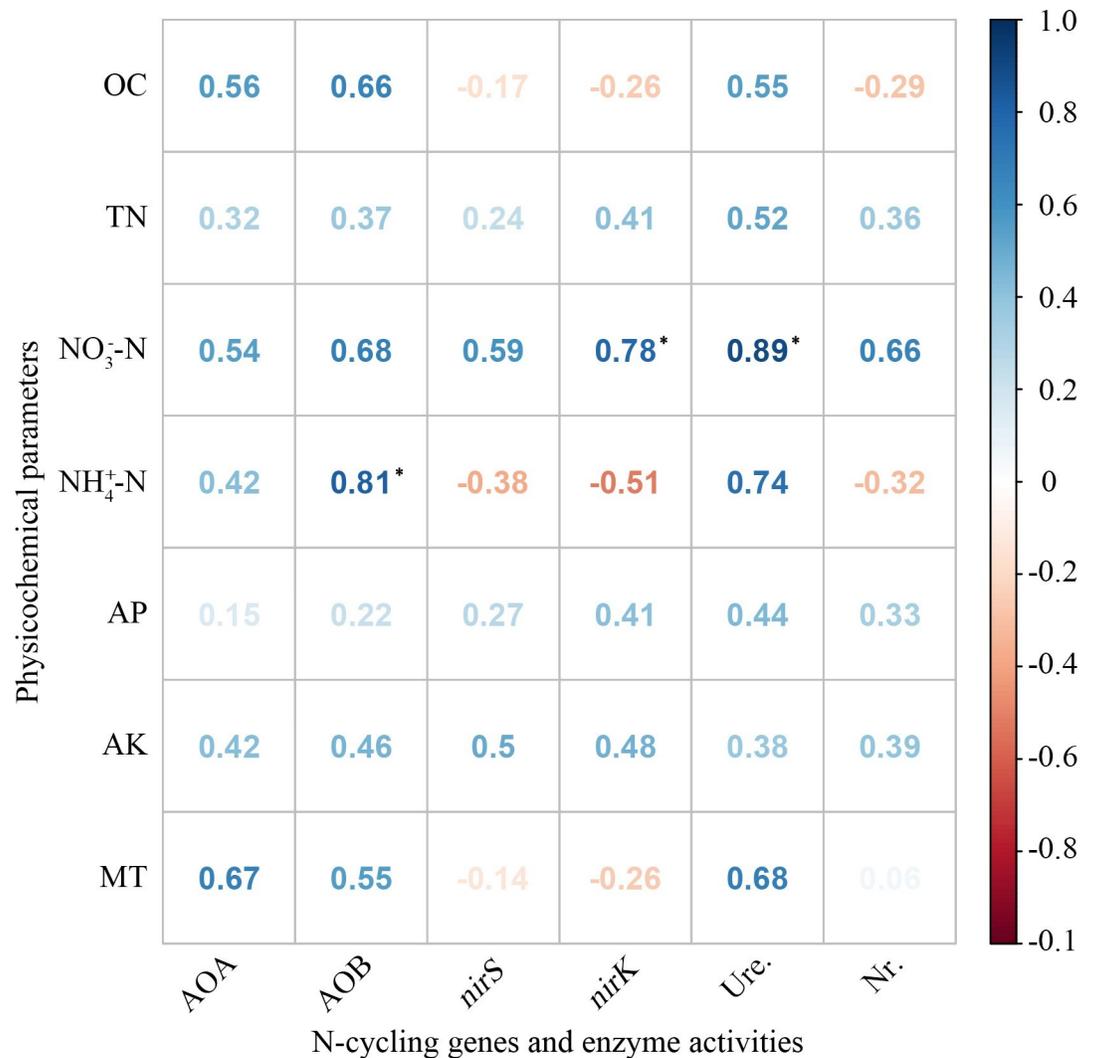


Fig 4. Correlation analysis between N-cycling genes, enzyme activities and physicochemical parameters in the soil. OC, organic C; TN, total N; AP and AK, available P and K; MT, moisture; AOA, *amoA* AOA; AOB, *amoA* AOB; Ure., urease activity; Nr., nitrate reductase activity. * represents significant correlation ($p < 0.05$).

<https://doi.org/10.1371/journal.pone.0240925.g004>

Urea stabilized by inhibitor retards the N transformation process and extends the period over which N is available to the crop in the soil [34–36]. In our study, the NO₃⁻-N contents under the inhibitor treatments were decreased at 15 DAF but then increased at 30 DAF (Fig 1). This result is consistent with that described by Azam et al. [5], who reported that when urea was applied with inhibitor, the enzyme activity and NH₃ volatilization were reduced but the NO₃⁻-N accumulation was increased. Moreover, our result showed that the NO₃⁻-N content at 45 DAF under 80%U+UI was higher than that under CF (Fig 1)—this says urea applied with NBPT has a longer N supply period. This may be related to the reduction of urease activity at about 45 DAF (Fig 2A).

To some extent, UI retarded the urea hydrolysis by decreasing urease activity and so slowed the conversion of urea-N to NH₄⁺-N, which led to a slow-down in the reduction of NO₃⁻-N to NO₂⁻-N [37, 38]. In our study, 80%U+UI decreased the urease activity between 15 and 45 DAF and decreased the nitrate reductase activity between 5 and 30 DAF (Fig 2). The inhibitory

effect of 80%U+NI and 80%U+1/2(UI+NI) on urease activity was relatively weak in comparison to 80%U+UI (Fig 2A). The N-conversion related enzyme is the primary factor affecting inhibitory effect under dryland conditions [32]. Therefore, we speculate that urea applied along with NBPT has greater potential as an inhibitor of urea hydrolysis.

Fu et al. [38] reported that there were no significant differences in *amoA* AOA genes between urea only and with additions of the inhibitors in an acid red soil. In our study, the *amoA* AOA genes under 80%U+UI and 80%U+NI were decreased at about 15 DAF (Fig 3A). This result can be explained by differences in soil pH, inhibitor dosage and sampling times between our study and theirs. Our result showed that the *amoA* AOB genes under 80%U+UI and 80%U+NI were decreased between 15 and 30 DAF (Fig 3B). This is due to additions of the inhibitors which inhibited the growth of AOB [6]. Additionally, the inhibitory effect of 80%U+1/2(UI+NI) on the *amoA* genes was relatively weak in comparison to UI or NI alone (Fig 3A and 3B). We speculate that this is related not only to inhibitor dosage, but also to the negative effect of NI addition to urea with UI [39]. The *nir* genes increase more with N fertilizer dosages in an alkaline soil [40]. Our result showed that the *nir* genes in the treatments with 80% fertilizer-N were obviously reduced at about 15 DAF, especially under 80%U+UI and 80%U+NI (Fig 3C and 3D). The results can be explained as that the increases in fertilizer-N provided sufficient substrate for denitrification but the additions of the inhibitor limited the conversion rate of $\text{NH}_4^+ - \text{N}$ to $\text{NO}_3^- - \text{N}$ [41, 42]. Additionally, we found that the inhibitor treatments did not affect the *nir* genes between 30 and 45 DAF (Fig 3C and 3D). This finding suggests that urea applied with NBPT and/or DMPP inhibits denitrification about 15 days, after that, the inhibitory effect has weakened, somewhat.

Nutrient availability, particularly of C and N, are the main factors affecting microbial abundance in the soil [43]. In our study, a significant positive relationship was observed between *amoA* AOB gene and $\text{NH}_4^+ - \text{N}$ (Fig 4). This result has previously been confirmed by Chen et al. [44], who attributed it to added $\text{NH}_4^+ - \text{N}$ to the soil which provided abundant N substrate and energy for the growth of AOB. Meanwhile, we found that there was a significant positive relationship between *nirK* gene and $\text{NO}_3^- - \text{N}$ (Fig 4). It can be explained as that $\text{NO}_3^- - \text{N}$ supplies a rich reaction substrate for the *nir* denitrifiers in the soil and so stimulates their reproduction, where the *nirK* denitrifiers are more sensitive to $\text{NO}_3^- - \text{N}$ than the *nirS* denitrifiers [45].

Conclusion

Our results show that, compared to conventional fertilization, the application of NBPT increased maize grain NUE and harvest index when the urea rate was reduced by 20%, although biomass and N uptake were not affected. With additions of the inhibitors, the peak $\text{NO}_3^- - \text{N}$ contents in the 0–60 cm depth band were delayed by 15 days. The addition of NBPT to urea decreased the urease activity for about 45 days, which was longer than under the other treatments. The abundance of N-cycling soil microbes was affected by additions of NBPT or DMPP to the urea, decreasing the copies of the *amoA* AOA, *amoA* AOB and *nir* genes during the growth period. These results suggest that urea applied with NBPT has greater potential for improving maize NUE and inhibiting nitrification under reduced fertilizer-N applications on the NCP.

Supporting information

S1 Fig. The plot distribution, buffer area and guard row in this experiment.
(TIF)

Acknowledgments

We are grateful to Gaiping Zhang, Shujun Zhang, Yunzheng, Tong, Jingyang Xu and Zhiqian Li for sampling. We would like to thank Sandy Lang for language editing. We also thank editors and reviewers for their valuable comments.

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References

1. FAO. World Fertilizer Trends and Outlook to 2018. Rome: Food and Agricultural Organization of the United Nations; 2015.
2. Staley C, Breuillin-Sessoms F, Wang P, Kaiser T, Venterea RT, Sadowsky MJ. Urea admendment decreases microbial diversity and selects for specific nitrifying strains in eight contrasting agricultural soils. *Front Microbiol.* 2018; 9: 634. <https://doi.org/10.3389/fmicb.2018.00634> PMID: 29670600
3. Zhang Y, Luan S, Chen L, Shao M. Estimating the volatilization of ammonia from synthetic nitrogenous fertilizers used in China. *J Environ Manage.* 2011; 92: 480–493. <https://doi.org/10.1016/j.jenvman.2010.09.018> PMID: 20932634
4. Gioacchini P, Nistri A, Marzadori C, Giovannini C, Vittori Antisari L, Gessa C. Influence of urease and nitrification inhibitors on N losses from soils fertilized with urea. *Biol Fert Soils.* 2002; 36: 129–135.
5. Azam F, Müller C, Weiske A, Benckiser G, Ottow J. Nitrification and denitrification as sources of atmospheric nitrous oxide—role of oxidizable carbon and applied nitrogen. *Biol Fert Soils.* 2002; 35: 54–61.
6. Dong D, Kou Y, Yang W, Chen G, Xu H. Effects of urease and nitrification inhibitors on nitrous oxide emissions and nitrifying/denitrifying microbial communities in a rainfed maize soil: A 6-year field observation. *Soil Till Res.* 2018; 180: 82–90.
7. Ding W, Yu H, Cai Z. Impact of urease and nitrification inhibitors on nitrous oxide emissions from fluvo-aquic soil in the North China Plain. *Biol Fert Soils.* 2011; 47: 91–99.
8. Dai Y, Di H, Cameron KC, He J. Effects of nitrogen application rate and a nitrification inhibitor dicyandiamide on ammonia oxidizers and N₂O emissions in a grazed pasture soil. *Sci Total Environ.* 2013; 465: 125–135. <https://doi.org/10.1016/j.scitotenv.2012.08.091> PMID: 23021462
9. Liu H, Ding Y, Zhang Q, Liu X, Xu J, Li Y, et al. Heterotrophic nitrification and denitrification are the main sources of nitrous oxide in two paddy soils. *Plant Soil.* 2019; 445: 39–53.
10. Zaman M, Saggari S, Blennerhassett JD, Singh J. Effect of urease and nitrification inhibitors on N transformation, gaseous emissions of ammonia and nitrous oxide, pasture yield and N uptake in grazed pasture system. *Soil Biol Biochem.* 2009; 41: 1270–1280.
11. Zaman M, Nguyen M, Blennerhassett J, Quin B. Reducing NH₃, N₂O and NO₃—N losses from a pasture soil with urease or nitrification inhibitors and elemental S-amended nitrogenous fertilizers. *Biol Fert Soils.* 2008; 44: 693–705.
12. Chen Z, Luo X, Hu R, Wu M, Wu J, Wei W. Impact of long-term fertilization on the composition of denitrifier communities based on nitrite reductase analyses in a paddy soil. *Microb Ecol.* 2010; 60: 850–861. <https://doi.org/10.1007/s00248-010-9700-z> PMID: 20563573

13. Martins MR, Sant'Anna SAC, Zaman M, Santos RC, Monteiro RC, Alves BJR, et al. Strategies for the use of urease and nitrification inhibitors with urea: Impact on N₂O and NH₃ emissions, fertilizer-¹⁵N recovery and maize yield in a tropical soil. *Agr Ecosyst Environ*. 2017; 247: 54–62.
14. Trenkel ME. Slow-and controlled-release and stabilized Fertilizers: an option for enhancing nutrient use efficiency in agriculture. International Fertilizer Industry Association (IFA). Paris; 2010.
15. Qiao C, Liu L, Hu S, Compton JE, Greaver TL, Li Q. How inhibiting nitrification affects nitrogen cycle and reduces environmental impacts of anthropogenic nitrogen input. *Global Change Biol*. 2015; 21: 1249–1257. <https://doi.org/10.1111/gcb.12802> PMID: 25380547
16. Akiyama H, Yan X, Yagi K. Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N₂O and NO emissions from agricultural soils: meta-analysis. *Global Change Biol*. 2010; 16: 1837–1846.
17. Abalos D, Jeffery S, Sanz-Cobena A, Guardia G, Vallejo A. Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. *Agr Ecosyst Environ*. 2014; 189: 136–144.
18. Hu X, Su F, Ju X, Gao B, Oenema O, Christie P, et al. Greenhouse gas emissions from a wheat-maize double cropping system with different nitrogen fertilization regimes. *Environ Pollut*. 2013; 176: 198–207. <https://doi.org/10.1016/j.envpol.2013.01.040> PMID: 23434574
19. FAO. FAOSTAT; 2017. Available from: <https://www.fao.org>.
20. Zhao Y, Lobell DB. Assessing the heterogeneity and persistence of farmers' maize yield performance across the North China Plain. *Field Crop Res*. 2017; 205: 55–66.
21. Xu C, Huang S, Tian B, Ren J, Meng Q, Wang P. Manipulating planting density and nitrogen fertilizer application to improve yield and reduce environmental impact in Chinese maize production. *Front Plant Sci*. 2017; 8: 1234. <https://doi.org/10.3389/fpls.2017.01234> PMID: 28747925
22. Meng Q, Cui Z, Yang H, Zhang F, Chen X. Chapter Three—Establishing high-yielding maize system for sustainable intensification in China. In: Sparks DL, editor. *Advances in Agronomy*. Salt Lake City: American Academic Press; 2018. pp. 85–109.
23. Zhao T, Zhang C, Du L, Liu B, An Z. Investigation on nitrate concentration in groundwater in seven provinces(city) surrounding the Bo-Hai Sea. *Journal of Agro-Environment Science*. 2006; 26: 779–783.
24. Lu R. *Analytical methods for soil agrochemistry*. Beijing: Chinese Agricultural Science and Technology Publishing House; 2000.
25. Rock L, Ellert BH, Mayer B. Tracing sources of soil nitrate using the dual isotopic composition of nitrate in 2 M KCl-extracts. *Soil Biol Biochem*. 2011; 43: 2397–2405.
26. Hu B, Liang D, Liu J, Lei L, Yu D. Transformation of heavy metal fractions on soil urease and nitrate reductase activities in copper and selenium co-contaminated soil. *Ecotox Environ Safe*. 2014; 110: 41–48. <https://doi.org/10.1016/j.ecoenv.2014.08.007> PMID: 25193883
27. Hayden HL, Drake J, Imhof M, Oxley APA, Norng S, Mele PM. The abundance of nitrogen cycle genes *amoA* and *nifH* depends on land-uses and soil types in South-Eastern Australia. *Soil Biol Biochem*. 2010; 42: 1774–1783.
28. Henry S, Baudoin E, López-Gutiérrez JC, Martin-Laurent F, Brauman A, Philippot L. Quantification of denitrifying bacteria in soils by *nirK* gene targeted real-time PCR. *J Microbiol Meth*. 2004; 59: 327–335. <https://doi.org/10.1016/j.mimet.2004.07.002> PMID: 15488276
29. Kastl EM, Schloter-Hai B, Buegger F, Schloter M. Impact of fertilization on the abundance of nitrifiers and denitrifiers at the root-soil interface of plants with different uptake strategies for nitrogen. *Biol Fert Soils*. 2015; 51: 57–64.
30. Ligi T, Truu M, Truu J, Nõlvak H, Kaasik A, Mitsch WJ, et al. Effects of soil chemical characteristics and water regime on denitrification genes (*nirS*, *nirK*, and *nosZ*) abundances in a created riverine wetland complex. *Ecol Eng*. 2014; 72: 47–55.
31. Linquist BA, Liu L, van Kessel C, van Groenigen KJ. Enhanced efficiency nitrogen fertilizers for rice systems: Meta-analysis of yield and nitrogen uptake. *Field Crop Res*. 2013; 154: 246–254.
32. Feng J, Li F, Deng A, Feng X, Fang F, Zhang W. Integrated assessment of the impact of enhanced-efficiency nitrogen fertilizer on N₂O emission and crop yield. *Agr Ecosyst Environ*. 2016; 231: 218–228.
33. Frame W. Ammonia volatilization from urea treated with NBPT and two nitrification inhibitors. *Agron J*. 2017; 109: 378–387.
34. Ni K, Pacholski A, Kage H. Ammonia volatilization after application of urea to winter wheat over 3 years affected by novel urease and nitrification inhibitors. *Agr Ecosyst Environ*. 2014; 197: 184–194.
35. Zanin L, Tomasi N, Zamboni A, Varanini Z, Pinton R. The urease inhibitor NBPT negatively affects DUR3-mediated uptake and assimilation of urea in maize roots. *Front Plant Sci*. 2015; 6: 1007. <https://doi.org/10.3389/fpls.2015.01007> PMID: 26635834

36. Li Y, Chapman SJ, Nicol GW, Yao H. Nitrification and nitrifiers in acidic soils. *Soil Biol Biochem.* 2018; 116: 290–301.
37. Castellano-Hinojosa A, González-López J, Vallejo A, Bedmar EJ. Effect of urease and nitrification inhibitors on ammonia volatilization and abundance of N-cycling genes in an agricultural soil. *J Plant Nutr Soil Sc.* 2020; 183: 99–109.
38. Fu Q, Abadie M, Blaud A, Carswell A, Misselbrook TH, Clark IM, et al. Effects of urease and nitrification inhibitors on soil N, nitrifier abundance and activity in a sandy loam soil. *Biol Fert Soils.* 2020; 56: 185–194.
39. Liu Z, Gao J, Gao F, Dong S, Liu P, Zhao B, et al. Integrated agronomic practices management improve yield and nitrogen balance in double cropping of winter wheat-summer maize. *Field Crop Res.* 2018; 221: 196–206.
40. Yang Y, Zhao J, Jiang Y, Hu Y, Zhang M, Zeng Z. Response of bacteria harboring *nirS* and *nirK* genes to different N fertilization rates in an alkaline northern Chinese soil. *Eur J Soil Biol.* 2017; 82: 1–9.
41. Philippot L, Hallin S, Schloter M. Ecology of denitrifying prokaryotes in agricultural soil. In: Sparks DL, editor. *Advances in Agronomy.* Salt Lake City: American Academic Press; 2007. pp. 249–305.
42. Hallin S, Jones CM, Schloter M, Philippot L. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. *ISME J.* 2009; 3: 597–605. <https://doi.org/10.1038/ismej.2008.128> PMID: 19148144
43. Das S, Jeong ST, Das S, Kim PJ. Composted cattle manure increases microbial activity and soil fertility more than composted swine manure in a submerged rice paddy. *Front Microbiol.* 2017; 8: 1702. <https://doi.org/10.3389/fmicb.2017.01702> PMID: 28928727
44. Chen Y, Hu H, Han H, Yue D, Wan S, Xu Z, et al. Abundance and community structure of ammonia-oxidizing Archaea and Bacteria in response to fertilization and mowing in a temperate steppe in Inner Mongolia. *Fems Microbiol Ecol.* 2014; 89: 67–79. <https://doi.org/10.1111/1574-6941.12336> PMID: 24712910
45. Fiorentino N, Venterino V, Bertora C, Pepe O, Giancarlo M, Grignani C, et al. Changes in soil mineral N content and abundances of bacterial communities involved in N reactions under laboratory conditions as predictors of soil N availability to maize under field conditions. *Biol Fert Soils.* 2016; 52: 523–537.