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Effects of Long-Term Fertilization on Different Nitrogen Forms in Paddy along Soil Depth Gradient

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Abstract

The combined application of organic fertilizer and chemical fertilizer is an effective measure to increase nutrient content of soil plough layer, which must have a profound impact on the deep soil nutrients, especially the contents of nitrogen forms. The purpose of this study was to explore the characteristics of soil nitrogen forms in plough layer and along depth gradient in different fertilization treatments, so as to evaluate the soil quality in spatial dimension, further providing a theoretical basis for scientific fertilization and improvement of paddy soil fertility. Here, a 34-year field experiment was conducted with three treatments: without any fertilizer (CK), pure chemical fertilizer (NPK) and chemical fertilizer combined with organic fertilizer (NPKM). We analyzed the content of nitrogen forms in 0 - 100 cm soil depth and their ratios to total nitrogen (TN), and discussed the correlation between nitrogen forms contents and pH, CEC. Results showed that, compared with CK, both NPK and NPKM significantly increased the contents of nitrogen forms in topsoil (soil layer of 0 - 20 cm), especially nitrate nitrogen (NO_3^--N) content increased by 70% (NPK) and 111% (NPKM), respectively. Although the contents of different nitrogen forms decreased gradually along soil depth gradient, NPKS slowed down the decline rate of TN and alkali-hydrolysable nitrogen (AN) in 0 - 60 cm soil layer, compared to CK. Compared to NPK, NPKM significantly increased the NO_3^--N/TN ratio in 0 - 20 cm soil layer, but also decreased the content of NO_3^--N in 20 - 40 cm, which was beneficial to reduce the risk of nitrogen leaching caused by nitrate leaching into deep layer. The increase of soil pH in NPKM treatment obviously alleviated the problem of soil acidification caused by long-term application of chemical fertilizer. Correlation analysis showed that there was a significant positive correlation between soil nitrogen forms and cation exchange capacity (CEC), but no significant correlation with soil pH. In conclusion, NPKM ensured the

nutrients of soil plough layer (0 - 20 cm), also reduced the risk of nitrogen infiltration and nitrogen loss, thus ensuring the fertility of soil profile.

Keywords

Long-Term Fertilization, Soil Depth Gradient, Total Nitrogen, Nitrogen Form

1. Introduction

Rice is the main food crop in China, and its planting area has increased from 25.4 million·hm^2 to 31.4 million·hm^2 with per unit yield that has increased from 1.9 t to 6.3 t in the past 50 years [1]. Rice belongs to ammonium-loving plants. When nitrogen supply is sufficient, the roots and tillers of rice will increase rapidly, so nitrogen plays a key role in the yield and quality of rice [2]. Therefore, fertilization is one of the most important agronomic measures to increase grain production, resulting in an increase in the amount of chemical fertilizer year by year. However, excessive chemical fertilizer may cause nutrient loss and reduce fertilizer use efficiency, which is not conducive to higher crop yield and soil fertility [3]. Rational application of nitrogen fertilizer has been a key measure to obtain higher target yield in crop production in the world, and it is of great significance to alleviate the problems of soil quality degradation, rice yield and quality decline, environmental pollution and so on [4].

More than 90% of the nitrogen in the soil exists in organic form, and only 10% of the inorganic nitrogen can be directly absorbed and utilized by plants. Inorganic nitrogen is mainly mineral nitrogen like nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N), which is not only easy to leach and volatilize from soil, but also can be fixed by soil clay minerals and organic matter. So the nitrogen in soil that can be directly absorbed and utilized by plants is often insufficient [5]. Field management measures such as tillage and fertilization significantly changed the distribution of soil inorganic nitrogen [6], and then affected the overall soil quality along the soil depth gradient. Li *et al.* [7] pointed out that the content of total nitrogen (TN) in red soil decreased gradually with soil depth and tended to be stable after 60 cm. There was a positive correlation between nitrogen application rate and soil alkali-hydrolysable nitrogen (AN), but long-term application had no significant effect on soil TN [8]. In addition, long-term nitrogen fertilizer accumulated NO_3^--N rather than NH_4^+-N content in 100 - 180 cm soil layer of dryland [9] [10]. Scientific fertilization can provide effective nitrogen source in time, ensure higher yield of rice and improve soil fertility. Organic fertilizer can increase the AN content of brown soil and avoid a large amount of nitrogen loss due to the rapid increase of soil nitrogen [11]. The input of organic fertilizer also greatly reduced the NH_4^+-N and NO_3^--N in the runoff of the tea garden, which effectively improved the fertilizer use efficiency and reduced the environmental pollution [12]. The change of nitrogen in the surface layer of soil will inevitably have a profound impact on the nitrogen forms in the deep layer. However, due to the lack of attention to the distribution of nitrogen in soil profile, the change of soil quality cannot be better evaluated.

The long-term positioning experiment adopts the special research method of both "long-term" and "positioning", and cultivates the farmland ecosystem with remarkable advantages such as long-term time, climate repeatability, and geographical location and so on. It is a key platform for carrying out scientific research and discovering major scientific problems [13] [14] [15]. Field management measures such as fertilization and tillage have a great impact on the soil properties. Positioning experiment with long-term, stable and systematic can observe these significant changes and reduce individual errors [16] [17]. At present, many scholars have done more research in dryland soil and purple soil of long-term fertilization [18] [19], but there are few reports on the distribution of nitrogen forms along the depth gradient of paddy, especially combined with long-term positioning experiment. Therefore, this experiment mainly studied the effect of 34-year positioning fertilization on nitrogen forms in paddy soil profile, and explored the influence factors and vertical change law of soil nitrogen content in fertilization treatments. This result is expected to systematically understand the changes of paddy fertility, further providing scientific basis for scientific fertilization and improvement of soil quality.

2. Materials and Methods

2.1. Site Description

The experiment was established in 1984 at the "National cultivated Land quality Monitoring Point" in Deyang City, Sichuan Province, Southwest China (30˚58'19"N, 104˚44'23"E). The experimental site, which is located in the hilly area of the Sichuan Basin, belongs to the subtropical humid monsoon climate zone, at an altitude of 332 meters, with a mean annual temperature of 16.7˚C, mean annual precipitation of 900 - 950 mm and annual average sunshine hours of 1000 - 1300 hours. The parent material of soil formation belongs to the alluvial alluvium weathered by thin sand and thick shale of Lower Cretaceous, and the soil type is paddy soil.

2.2. Experimental Design

In the field experiment with three plots: without any fertilizer (CK), pure chemical fertilizer (NPK) and chemical fertilizer combined with organic fertilizer (NPKM). There is no repetition, with the area of 866 m^2 (**Figure 1**). The planting system is wheat-rice rotation. Wheat is sown in October and harvested in May; rice is planted in May and harvested in September every year. In 2017-2018, in NPK plot, the fertilizer application was N 132.23 $kg\cdot hm^{-2}$, P_2O_5 34.54 $kg\cdot hm^{-2}$ and K_2O 48.88 $kg\cdot hm^{-2}$ in wheat season, and N 183.51 $kg\cdot hm^{-2}$, P_2O_5 34.54 $kg\cdot hm^{-2}$ and K_2O 48.88 $kg\cdot hm^{-2}$ in rice season. In NPKM plot, on the basis of the same fertilizer as NPK, the application of organic fertilizer was about 5000 $kg\cdot hm^{-2}$ (**Table 1**).

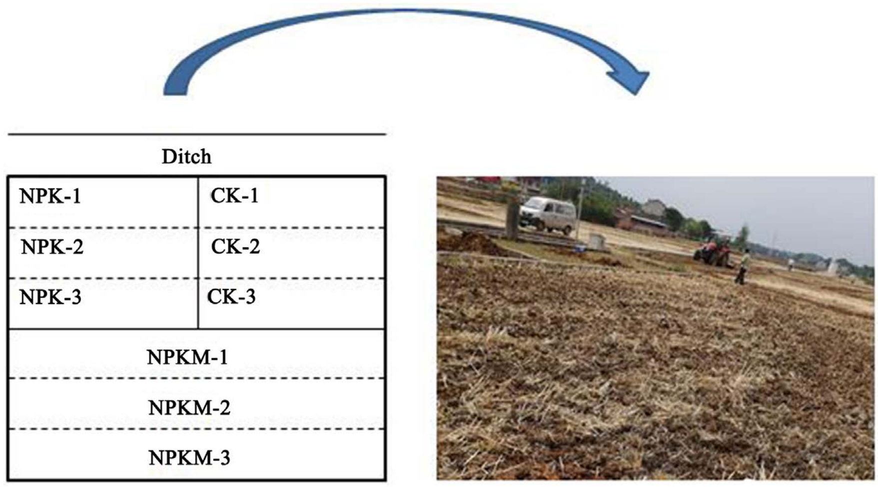

Figure 1. Field trial layout. Note: CK, without any fertilizer, NPK, pure chemical fertilizer, and NPKM, chemical fertilizer combined with organic fertilizer.

Table 1. The application of fertilizer dose ($kg \cdot hm^{2}$).

Crop season	Treatment	N	P_2O_5	K_2O	Organic fertilizer
Wheat	CK	0	0	0	0
	NPK	132.23	34.54	48.88	0
	NPKM	132.23	34.54	48.88	5000
Rice	CK	0	0	0	0
	NPK	183.51	34.54	48.88	0
	NPKM	183.51	34.54	48.88	5000

2.3. Sampling and Measurements

After the rice harvest in late May 2018, each plot was divided into three parts. Mixed soil samples in each part were gathered from five evenly distributed points, and a total of 45 mixed samples were collected from 0 to 100 cm (20 cm per layer, a total of 5 layers). The soil sample was separated into two parts after removing all visible animal and plant residues, stones and other debris, some of them were freshly stored at 4˚C for the determination of soil NH_4^+-N and NO_3^--N, and the rest were ground and screened (0.15, 2 mm) after air-drying in a cool and ventilated place.

Refer to "soil investigation Laboratory Analysis method" [20]: Soil TN was determined by Kjeldahl nitrogen meter, Soil AN was hydrolyzed by 1mol L^{-1} NaOH and absorbed by boric acid, NH_4^+-N and NO_3^--N were determined by indophenol blue colorimetry and dual-band colorimetry, soil pH was determined by pH meter, and cation exchange capacity (CEC) was determined by ammonium acetate method [21].

2.4. Statistical Analysis

All statistical analyses were performed using the SPSS19.0 software and plotted with Excel 2010. Two-way ANOVA was performed to test the effects of fertilization treatments and soil depth gradient on nitrogen forms, pH and CEC. Data were expressed as mean values ± S.E. (standard error). All the results were considered statistically significant at $P < 0.05$ unless otherwise stated. Multiple comparisons were performed by LSD method.

3. Results

3.1. Nitrogen Forms in Topsoil

It can be seen from Table 2 that long-term fertilizations significantly affected the content of nitrogen forms in topsoil (soil layer of 0 - 20 cm). Compared with CK, the soil TN content in NPK and NPKM treatments increased by 64% and 59%, soil AN increased by 65% and 61%, soil NO_3^--N content increased by 70% and 111%, and NH_4^+-N content increased by 45% and 42%, respectively. The contents of AN and NO_3^--N in soil treated with NPKM were significantly better than those of NPK.

3.2. Effects of Long-Term Fertilization on Nitrogen Forms along Soil Depth Gradient

3.2.1. The Contents of TN, AN, NH_4^+-N and NO_3^--N

The distribution of nitrogen forms in paddy soil profile is shown in Figure 2. It is not difficult to find that fertilization increased the content of soil nitrogen forms. The contents of soil TN and AN decreased gradually with soil depth. While obvious faults appeared in the 40 cm soil layer, and the nitrogen compositions in the 0 - 40 cm soil layer were significantly higher than those below 40 cm soil layer. In 20 - 40 cm, the contents of TN and AN in NPK treatment were significantly higher than those in NPKM treatment, which increased by 62% and 20%, respectively. The content of NO_3^--N decreased gradually with soil depth, and the differences of below 60 cm soil layer were not significant. NH_4^+-N had an obvious downward migration tendency, and NPK and NPKM in 0 - 100 cm soil layer were significantly higher than CK, especially NPKM significantly increased the NH_4^+-N of 60 - 80 cm soil, compared with NPK.

3.2.2. Vertical Change of the Proportion of Nitrogen Forms

The ratios of all forms of nitrogen are shown in Figure 3. The ratios of AN/TN and NH_4^+-N/TN in soil profiles fluctuated in a certain range. The ratios of AN/TN in CK, NPK and NPKM treatments fluctuated in the range of 4.31% to

Table 2. The content of soil nitrogen forms in fertilization.

Treatments	TN ($g \cdot kg^{-1}$)	AN ($mg \cdot kg^{-1}$)	NO_3^--N ($mg \cdot kg^{-1}$)	NH_4^+-N ($mg \cdot kg^{-1}$)	AN/TN (%)	NO_3^--N/TN (%)	NH_4^+-N/TN (%)
CK	0.54 ± 0.01b	35.22 ± 2.46b	12.52 ± 0.23c	14.73 ± 0.46b	6.47 ± 0.47a	2.30 ± 0.05b	2.70 ± 0.11a
NPK	0.89 ± 0.04a	43.78 ± 2.18a	21.27 ± 2.32b	21.43 ± 1.73a	4.91 ± 0.17b	2.38 ± 0.95a	2.40 ± 0.19a
NPKM	0.87 ± 0.03a	47.39 ± 2.37a	26.46 ± 1.72a	20.91 ± 1.28a	5.46 ± 0.25b	3.07 ± 0.91a	1.72 ± 0.20b

Note: different letters in the same column indicate significant differences between different treatments ($P < 0.05$).

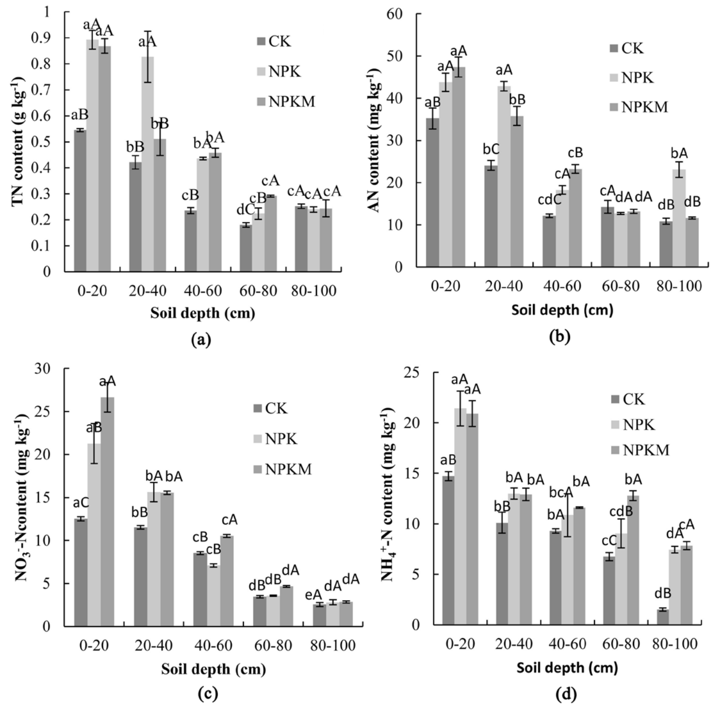

Figure 2. Distribution of nitrogen forms in 0 - 100 cm profiles of paddy. Note: Different lowercase letters indicate significant differences between soil layers; different uppercase letters indicate significant differences between treatments (P < 0.05).

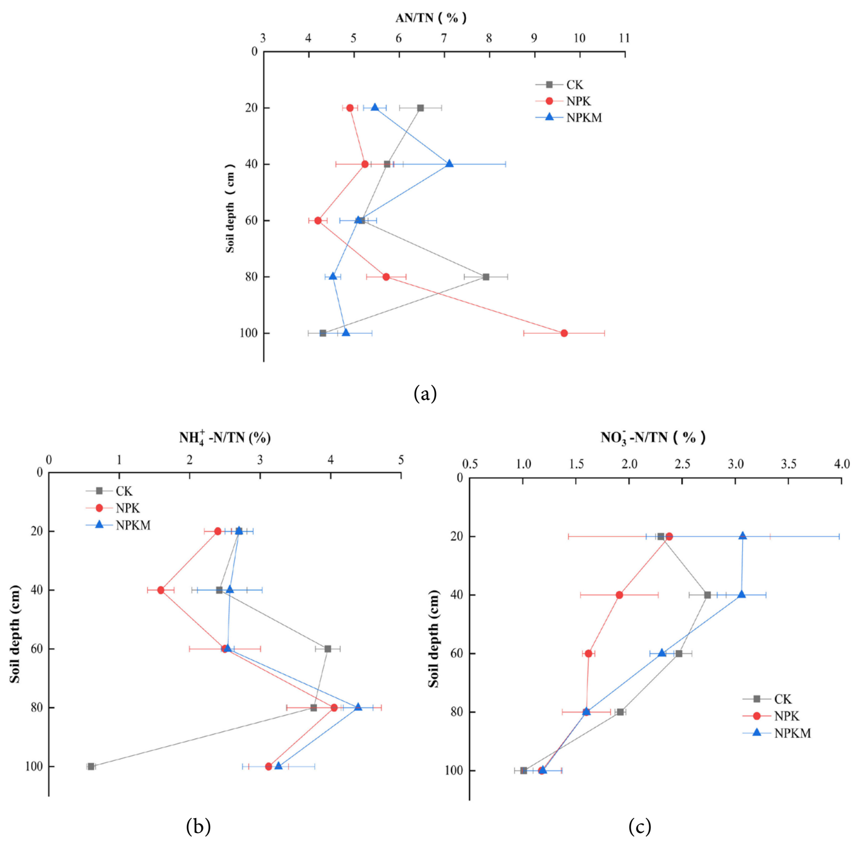

Figure 3. Vertical variations of the ratios of nitrogen in long-term fertilization.

7.92%, 4.2% to 9.65% and 4.53% to 7.11%, respectively. The AN/TN ratio in NPK and NPKM treatments increased at first and then decreased in 0 - 60 cm soil layer. The trend of NH_4^+-N/TN ratio was opposite to that of AN/TN, and it decreased at first and then increased in 0 - 80 cm soil layer. The NO_3^--N/TN ratio decreased gradually with the soil depth. It was worth mentioning that in 0 - 60 cm soil layer, the ratios of AN/TN, NH_4^+-N/TN and NO_3^--N/TN in NPKM were significantly higher, relative to NPK.

3.3. Effects of Long-Term Fertilization on the Contents of pH and CEC along Soil Depth Gradient

It can be seen from Table 3 that compared with NPK, the increase of soil pH in NPKM treatment significantly alleviated soil acidification caused by long-term chemical fertilizer application. The soil pH in NPK treatment increased with the soil depth, but there was no significant difference. Different from soil pH, soil CEC of the three fertilization treatments decreased gradually along soil depth gradient. Compared with CK treatment, NPKM significantly increased the content of CEC in 0 - 20 cm soil, while NPK treatment significantly decreased it.

3.4. Correlation between Nitrogen Forms and Soil Properties

The correlation between nitrogen forms (TN, AN, NO_3^--N, NH_4^+-N) and soil properties (pH and CEC) were analyzed, and the results were shown in Table 4 below. There was a strong correlation among different soil nitrogen forms ($P < 0.01$). There was no significant correlation between soil nitrogen forms and pH, but there was a significant positive correlation between soil nitrogen forms and CEC ($P < 0.05$).

4. Discussion

Nitrogen is one of the factors that determine the yield of crops and the status of soil fertility in farmland [22]. The availability of nitrogen in surface soil is a direct reflection of human activities like fertilization and tillage [23] [24]. Nitrate nitrogen is the most active nitrogen forms that can be directly absorbed and

Table 3. Effect of fertilization treatments on pH and the content of CEC.

Indicators	treatments	0 - 20 cm	20 - 40 cm	40 - 60 cm	60 - 80 cm	80 - 100 cm
pH	CK	7.59 ± 0.02aA	7.58 ± 0.04aA	7.39 ± 0.07bA	7.65 ± 0.01aA	7.49 ± 0.13abA
	NPK	6.45 ± 0.01bB	6.46 ± 0.01abB	6.49 ± 0.02abB	6.49 ± 0.06abB	6.56 ± 0.07aB
	NPKM	7.56 ± 0.02aA	7.63 ± 0.05aA	7.45 ± 0.03bA	7.45 ± 0.05bA	7.63 ± 0.01aA
CEC ($cmol \cdot kg^{-1}$)	CK	15.38 ± 0.12aB	13.53 ± 0.52bA	10.95 ± 0.43cB	11.07 ± 0.46cB	10.56 ± 0.47cAB
	NPK	14.05 ± 0.42aC	13.88 ± 0.16aA	12.75 ± 0.27bA	11.18 ± 0.53cB	11.04 ± 0.15cA
	NPKM	16.7 ± 50.29aA	14.2 ± 0.55bA	12.4 ± 0.27dA	13.26 ± 0.28cA	10.18 ± 0.11eB

Note: Different lowercase letters indicate significant differences between soil layers; different uppercase letters indicate significant differences between treatments ($P < 0.05$).

Table 4. Correlation between soil nitrogen forms and soil properties.

Indicators	TN	AN	NO_3^--N	NH_4^+-N	pH
AN	0.925**		.		
NO_3^--N	0.889**	0.814**			
NH_4^+-N	0.777**	0.754**	0.774**		
pH	−0.122	0.057	−0.108	-0.152	
CEC	0.803**	0.818**	0.825**	0.734**	0.061

Note: ** Significant correlation at 0.01 level (bilateral).

utilized by crops [25], and it is easy to leach into the ground with Rain Water. Therefore, high concentrations of NO_3^--N accumulated in the soil, which greatly increased the risk of nitrogen leaching [26]. This study showed that fertilization was closely related to the content of soil nitrogen forms in paddy (**Table 2**). Compared with non-fertilization plot, long-term fertilization significantly increased the content of active nitrogen forms in topsoil (soil layer of 0 - 20 cm), which was consistent with Hao *et al.* [27]. Zhu *et al.* [28] also showed that the topsoil NO_3^--N content in long-term chemical fertilizer combined with organic fertilizer plot was higher, compared to pure chemical fertilizer, indicating that combined application of organic-inorganic fertilizer promoted nitrogen mineralization and provided sufficient nitrogen for rice growth. Due to organic fertilizer increased the number of soil microorganisms, which was largely offset by the nitrogen residues fixed by microorganisms. Chemical fertilizer can stimulate soil nitrogen mineralization and increase nitrogen content [29]. Therefore, it was also observed that the TN content in 0 - 40 cm soil layer of pure chemical fertilizer plot was higher (**Figure 2(a)**).

Many studies have shown that long-term fertilization significantly affected the distribution of nitrogen forms in the vertical plane of soil [30] [31]. Our study also showed that the content of soil nitrogen forms decreased gradually with soil depth, and long-term fertilization slowed down the downward trend (**Figure 2**). This is because fertilization and tillage mainly act on the plough layer, the less the effect is, the nitrogen content in the soil profile showed the phenomenon of "surface aggregation" [32]. While nitrogen fertilizer increased nitrogen source and promoted nitrogen mineralization. The accumulation of a large amount of nitrogen in surface soil increased the infiltration of nutrients [33]. In the 20 - 40 cm soil layer, compared with pure chemical fertilizer plot, the application of organic-inorganic fertilizer significantly reduced the content of soil NO_3^--N and delayed its leaching into the deep soil, which indicated that the organic fertilizer had a better fertilizer conservation performance. This may be due to the fact that organic fertilizer could dissociate H^+ in the solution, so that the soil had a strong cation exchange capacity [34]. In addition, long-term application of organic-inorganic fertilizers may increase the biological residues of crops and promote the metabolism of microorganisms [35]. It was also found that relative to pure chemical fertilizer, organic fertilizer increased the content of NO_3^--N in 0 - 20 cm soil layer. However, in 20 - 40 cm soil layer, NPK fertilization increased the content of TN and AN. This showed that soil nitrogen forms showed different responses to different fertilization treatments. The ratios of soil active nitrogen to total nitrogen can better reflect the quality of soil. In this study, AN/TN, NH_4^+-N/TN and NO_3^--N/TN treated by organic-inorganic fertilizer were higher in 0 - 60 cm soil layer, compared with pure chemical fertilizer (**Figure 3**). Similarly, Li *et al.* [36] studied the double-cropping rice soil and found that inorganic combined with organic fertilizer increased the proportion of active nitrogen forms to TN.

Soil physical and chemical properties like CEC and pH play important roles in soil quality evaluation. Soil CEC can directly reflect soil nutrition supply, nutrition conservation and buffering capacity [37]. Soil CEC increased after application of organic fertilizer [38]. This study also showed that the organic-inorganic fertilizers significantly increased the soil CEC (**Table 3**). This is probably because the long-term application of organic fertilizer increased the content of organic matter, thus reducing the amount of variable negative charge. As most of the common chemical fertilizers are acidic, long-term application tends to reduce soil pH, resulting in soil consolidation, nutrient imbalance, fertilizer use efficiency and other problems. The combined application of organic fertilizer alleviated soil acidification, mainly because organic functional groups can absorb more H^+ and Al^{3+}, organic matter decarboxylation in the process of mineralization, and release basic substances such as calcium and magnesium, thus increasing soil pH value. In addition, the correlation analysis between soil nitrogen forms and pH, CEC showed that there was a significant positive correlation among different soil nitrogen forms (**Table 4**). This showed that AN may reflect the status of soil nitrogen nutrients to some extent. There was a significant positive correlation between soil CEC and nitrogen forms, which was related to the easy leaching of available nutrients such as nitrate nitrogen and ammonium nitrogen [39].

5. Conclusions

The main results were as follows:

1) Compared with the non-fertilization treatment, long-term fertilization treatment significantly increased the content of nitrogen forms in topsoil (soil layer of 0 - 20 cm). Compared with chemical fertilizer alone, organic-inorganic fertilizer significantly increased the content of NO_3^--N in topsoil, which was beneficial to promote the mineralization of nitrogen fertilizer.

2) The contents of nitrogen forms decreased gradually with soil depths. However, fertilization alleviated the decline to some extent, especially organic fertilizer effectively increased the proportion of NH_4^+-N/TN and NO_3^--N/TN in 0 - 60 cm soil layer. In addition, organic fertilizer increased soil pH and CEC, preventing soil acidification and improving soil fertilizer conservation.

3) There was a significant positive correlation among different soil nitrogen forms. Compared with other nitrogen forms, there was a very significant positive correlation between AN and CEC. The effect of long-term fertilization on soil fertilizer conservation could be observed through the change of AN.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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