



Effects of Humic Acid Organic Fertilizer on Soil Environment in Black Soil for Paddy Field Under Water Saving Irrigation

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 13-05-2022

Revised: 14-06-2022

Accepted: 15-07-2022

Key Words:

Paddy field

Black soil

Humic acid

Microorganisms

Enzyme activities

Soil fertility

ABSTRACT

In the past decades, the application of organ fertilizer in agricultural soils has attracted wide attention. However, few studies have carefully explored the effects of humic acid organic fertilizer on soil microbial colonies, soil enzyme activities, and soil fertility. To provide a better growing environment for crops, we explore the best regulation mode of humic acid organic fertilizer in the farmland in the Songnen Plain Heilongjiang province. Through field experiment, we selected paddy as the test objective and applied humic acid organic fertilizer. Under the condition of water-saving irrigation, five fertilization levels were set up, which were NPK (local nitrogen level, 110 kg.hm⁻² pure nitrogen), NPKH1 (450 kg.hm⁻² humic acid organic fertilizer + 77 kg.hm⁻² nitrogen), NPKH2 (750 kg.hm⁻² humic acid organic fertilizer + 55 kg.hm⁻² nitrogen), NPKH3 (1050 kg.hm⁻² humic acid organic fertilizer + 33 kg.hm⁻² nitrogen) and PKH (1500 kg.hm⁻² humic acid organic fertilizer). The effects of different humic acid organic fertilizers on soil microbial colonies, soil enzyme activities, and soil fertility were discussed. The results showed that humic acid organic fertilizer could effectively change the structure of soil microbial colonies, soil enzyme activities, and soil fertility. Compared with NPK treatment, the bacteria, fungi, and actinomycete, urease, and catalase in PKH, NPKH3, NPKH2, and NPKH1 treatments increased, and significantly different under 0-10 cm layer conditions ($P < 0.05$). With the increase of humic acid organic fertilizer application, soil organic matter and soil fertility from superior to inferior was PKH>NPKH3>NPKH2>NPKH1>NPK. Therefore, the application of humic acid organic fertilizer was an effective measure to improve soil fertility and increase the amount of soil colony structure and enzyme activities.

INTRODUCTION

Heilongjiang, as an important province of output in China, plays the important role of “ballast stone” in ensuring national food security for the implementation of the strategy of grain storage on the land, and grain storage in technology. The protection of black soil for improving the grain production capacity, which is of great significance to further consolidate and enhance the advantages of agricultural green development and promote the construction of a strong agricultural province (Liang 2021). As an extremely precious agricultural resource, in the “fourteenth five-year plan” and the long-term goal of 2035 in China, the protection of black soil has been put forward, taking the effective protection of black soil as an important strategic task to promote the agricultural modernization in Heilongjiang province, China (Zhang 2021). In recent years, the application amount of organic fertilizer is insufficient, while the application amount of nitrogen fertilizer has increased greatly in Heilongjiang province (Chen et al. 2019). Therefore, it is necessary to reduce the input of nitrogen in farmland, scientifically reduce the application amount of chemical fertilizer, and reasonably

apply organic fertilizer, which is of great significance for improving soil fertility, the quality of agricultural products, and reducing agricultural non-point source pollution (Du et al. 2020).

In the process of agricultural planting, for the pursuit of high yield of crops, bringing the environmental problems of farmland ecological pollution because of increasing the amount of nitrogen fertilizer, therefore it is an inevitable demand for application of organic fertilizer in the sustainable development of agriculture in the future, also an important embodiment of agricultural green development, to adjust and solve the contradiction between the fertilizer of crop demand and soil fertilizer supply, and realize the balanced supply of nutrients, we can achieve the goal of reducing fertilizer and improving efficiency under the meeting the needs of crop growth conditions, and achieve the mutually beneficial goal of high crop yield and environmentally sustainable development. In the decades, due to a large amount of chemical fertilizer application, the fertilizer supply capacity and production capacity of black soil decreased significantly, so organic fertilizer instead of chemical fertilizer nitrogen is

one of the important measures to reduce fertilizer application, which not only improve soil fertility but also promote the growth of crops (Xu et al. 2018). Previous studies had shown that crop quality could be improved (Lu et al. 2015, Zhang et al. 2004), the crop photosynthesis and water and fertilizer utilization efficiency were increased (Huang et al. 2021, Zhang et al. 2019) by the application of organic fertilizer reasonably. It was also beneficial to the increase of plant height and stem diameter (He et al. 2019), reducing nitrogen leaching (Xie et al. 2021), and affecting the microbial community and enzyme activities in the root zone of crops (Liang et al. 2021), reducing greenhouse gas emissions from farmland effectively (Shu et al. 2021, Zhang et al. 2021), in the meantime, applying organic fertilizer in the soil would help to improve soil quality and maintain crop production. In addition, small-molecule organic matter in organic fertilizer had a positive effect on soil nutrient cycling and crop growth and development (Ma et al. 2021). Therefore, exploiting the environment-friendly fertilizer and reasonable fertilization measures is an important way to ensure crop growth, improve soil environmental effects and maintain sustainable agricultural development (Xu et al. 2020, Yu et al. 2020).

However, due to the influences of climate conditions, planting structure, and environmental effects of soil and water resources in Heilongjiang province, China, there are many uncertainties in soil environmental effects and organic fertilizer application in black soil areas. Therefore, how to further improve the quality of black soil and maintain crop productivity by increasing the application of organic fertilizer, is the main challenge of agriculture in Heilongjiang province. In our experiment, we have taken the paddy soil as an example, by the field experiment, under the water-saving irrigation condition, to study the impacts of humic acid organic fertilizer on the environmental effects of black soil, which is of great significance to seeking the technical mode of coordinated development of food production and agricultural sustainable utilization.

MATERIALS AND METHODS

Experimental Site

The experiment was performed at the National Key Irrigation Experimental Station located in Heping Town, Qing' an County, Suihua, Heilongjiang, China. The experimental site is located at 45°63' N and 125°44' E at an elevation of 450 m above sea level. This region consists of plain topography and has a semi-arid cold temperate continental monsoon climate, i.e., a typical cold region with a black glebe distribution area. The average annual temperature is 2.5°C, the average annual precipitation is 550 mm, and the average annual surface evaporation is 750 mm. The growth period of crops is 156-171d,

and there is a frost-free period of approximately 128 days. yr⁻¹. The soil at the study site is albic paddy soil, with a mean bulk density of 1.01 g.cm⁻³ and a porosity of 61.8 %. The basic physicochemical properties of the soil are as follows: the mass ratio of organic matter is 41.8 g.kg⁻¹, pH value is 6.45, the total nitrogen mass ratio is 15.06 g.kg⁻¹, the total phosphorus mass ratio is 15.23 g.kg⁻¹, total potassium mass ratio is 20.11 g.kg⁻¹, the mass ratio of alkaline hydrolysis nitrogen is 198.29 mg.kg⁻¹, the available phosphorus mass ratio is 36.22 mg.kg⁻¹ and the exchangeable potassium mass ratio is 112.06 mg.kg⁻¹ (Zheng et al. 2018).

Experimental Design

The plants were maintained under water-saving conditions, that was, at the re-greening stage, a water layer (0~30 mm) was maintained, but the soil was allowed to dry during the yellow ripeness stage; the water layer was not applied after the irrigation period. The upper limit of irrigation was taken as the saturated water content. In the early and middle tillering stages, jointing stage, heading stage, and milk-ripe stage, the lower limit of irrigation was 85% of the saturated water content. The soil moisture content was measured using a moisture content analyzer (TPIME-PICO64/32) every day (once at 07:00 and 18:00). When the soil moisture content was lower than or close to the lower limit of irrigation, it was necessary to irrigate to the upper limit.

In this experiment, Five fertilization treatments were applied: 100% urea which was 110 kg·ha⁻¹ (pure nitrogen) (NPK), 30% humic acid and 70% urea (NPKH1), 50% humic acid, and 50% urea (NPKH2), 70% humic acid and 30% urea (NPKH3), 100% humic acid which was 1500 kg·ha⁻¹ (PKH). Urea and humic acid organic fertilizer were applied according to the proportion of base fertilizer: tillering fertilizer: and heading fertilizer (5:3:2). The amount of phosphorus and potassium fertilizers was the same for all treatments, P₂O₅ (45 kg·ha⁻¹) and K₂O (80 kg·ha⁻¹) were used. Phosphorus was applied once as a basal application. Potassium fertilizer was applied twice: once as basal fertilizer and at the 8.5 leaf age (panicle primordium differentiation stage), at a 1:1 ratio (Zheng et al 2018).

The humic acid organic fertilizer was produced by Yunnan Kunming Grey Environmental Protection Engineering Co., Ltd, China. The organic matter≥61.4%, the total nutrients (nitrogen, phosphorus, and potassium) ≥18.23%, of which N≥3.63%, P₂O₅≥2.03%, K₂O≥12.57%. The moisture was ≤2.51%, the pH value was 5.7, the worm egg mortality rate≥95%, and the amount of fecal colibacillosis≤3%. The fertilizer contained numerous elements necessary for plants. The contents of harmful elements including arsenic, mercury, plumbum, cadmium, and chromium were≤2.8%, 0.01%,

7.6%, 0.1%, and 4.7%, respectively; these were much lower than the test standard.

Plant Management

This study was performed with a randomized complete block design with three replications. The length and width of each plot were 10.0 m and 10.0 m, respectively (area = 100 m²). The rice was also planted around the cell as a protection row. A 40 cm deep plastic plate was embedded between the plots to prevent underground water-fertilizer exchange in each plot. The plant protection and pesticide application measures and field management conditions in each plot were consistent.

Sampling and Measurements

The determination of soil enzymatic activities was based on the method of Huang et al. (2021). The activity of urease was determined by sodium phenol colorimetry, expressed as the quantity of mg of ammonia nitrogen released from 1 g of soil after 24 hours. The acid phosphatase activity was determined by the disodium phenyl phosphate colorimetric method, expressed as the quantity of mg of phenol released in 1 µg of soil after 24 hours. The soil catalase activity was determined by the permanganometric method, expressed as the quantity of mg of phenol released in 1 g of soil after 1 hour, all the treatments were repeated thrice. The soil microorganism was inoculated by the spread-plate method of Huang et al. (2021), The fungus was cultivated in Martin medium and its quantity was determined, the actinomycetes were cultivated in the modified Gao's No. 1 culture medium and its quantity was determined, the bacteria were cultivated in the beef extract-peptone medium and then their quantity was determined, the soil fertility was based on the method of Song (2019).

Statistical Analysis

For statistical analysis, data processing was completed using Microsoft Excel 2010 followed by analysis with SPSS 19 software. The statistical results are reported as the mean value and were confirmed with an LSD (least significant difference) test.

RESULTS

Effects on Soil Microorganisms

The humic acid organic fertilizer had a significant impact on soil microorganisms (Fig. 1). Under the different soil layers, the bacteria, fungi, and actinomycetes from superior to inferior were 0-10 cm > 10-20 cm > 20-30 cm. However, the microorganism of each treatment was not significant under the 10-20 cm and 20-30 cm layers, but in the 0-10 cm soil layer, it was significant at a 5% probability level. In the 0-10 cm soil layer, the microorganism in NPKH4 and PKH treatments was higher than in NPK, NPKH1, and NPKH2 treatments ($P < 0.05$), and the number of bacteria, fungi, and actinomycetes in NPKH4 and PKH treatments increased by 13.68%, 17.68%, 7.34% on average compared with NPK, NPKH1 and NPKH2 treatments ($P < 0.05$), the increase of fungi was more than bacteria and actinomycetes.

Effects on Soil Enzyme Activities

The humic acid organic fertilizer could affect the soil enzyme activities (Fig. 2), the urease and catalase activities in the 0-10 cm soil layer were significant at a 5% probability level, while acid phosphatase activity was not significant. In the 0-10 cm soil layer, the urease activity in NPK treatment decreased by 29.91% ($P < 0.05$), 27.05% ($P < 0.05$), 18.91% ($P < 0.05$) and 18.84% ($P < 0.05$) as compared with PKH,

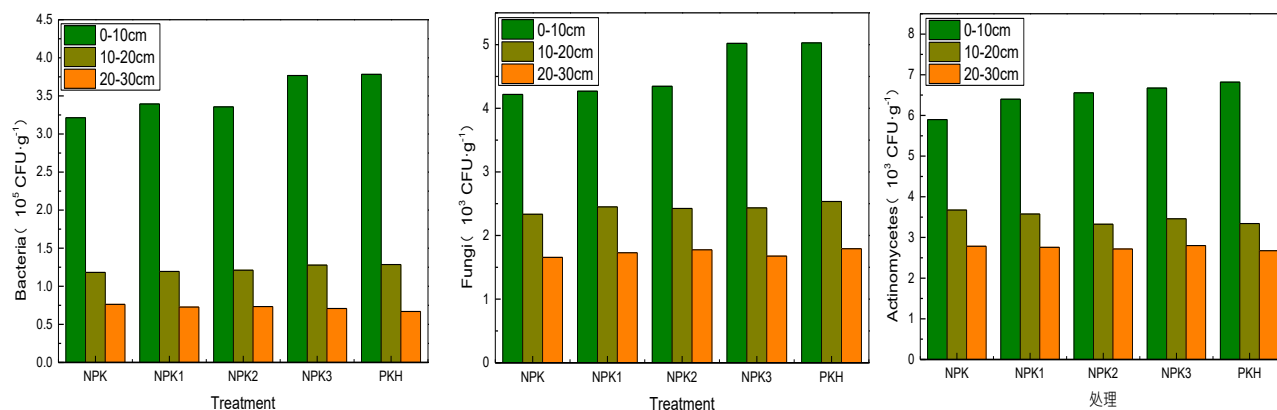


Fig.1: Change of soil microbial content in different soil layers under different fertilization treatments.

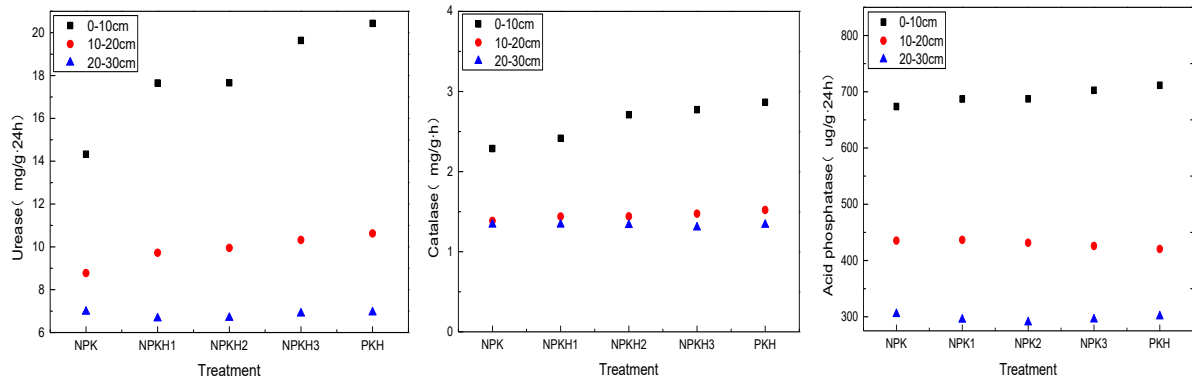


Fig. 2: Change of soil enzyme activities in different soil layers under different fertilization treatments.

NPKH3, NPKH2 and NPKH1; the catalase activity in NPK treatment decreased by 20.15% ($P < 0.05$), 17.49% ($P < 0.05$), 15.59% ($P < 0.05$) and 5.31% ($P > 0.05$) as compared with PKH, NPKH3, NPKH2 and NPKH1; the acid phosphatase activity in NPK treatment decreased by 5.31% ($P > 0.05$), 4.11% ($P > 0.05$), 1.98% ($P > 0.05$) and 1.94% ($P > 0.05$) as compared with PKH, NPKH3, NPKH2 and NPKH1. In the vertical direction of the soil, the soil enzyme activities decreased with the increase of soil depth, and there were no significant differences between the different treatments of 10-20 cm and 20-30 cm.

Effects on Soil Organic Matter

The soil organic matter increased with the increase of humic acid amount (Table 1), from superior to inferior was $PKH > NPKH3 > NPKH2 > NPKH1 > NPK$. In the vertical direction of the soil, the soil organic matter in 0-10 cm soil was higher than 10-20 cm and 20-30 cm soil layers. Compared with NPK treatment, in 0-10 cm soil layer, the soil organic matter in PKH, NPKH3, NPKH2, and NPKH1 increased by 22.07% ($P < 0.05$), 19.62% ($P < 0.05$), 12.02% ($P < 0.05$) and 6.42%. In the 10-20 cm soil layer, the soil organic matter in PKH and NPKH3 treatments was higher than in the others. However, in 20-30 cm soil layer, across all treatments, had no significant difference.

Effects on N, P, K

The humic acid organic fertilizer increased soil total nitrogen, available phosphorus, and available potassium contents (Table 2). In 0-10 cm soil layer, the total nitrogen in PKH, NPKH3, NPKH2 and NPKH1 treatments increased by 30.10%, 25.10%, 23.43% and 9.47%, respectively than NPK; the available phosphorus increased by 38.30%, 36.49%, 29.67% and 8.54%, respectively; the available potassium increased by 20.32%, 19.30%, 12.59% and 6.17%, respectively. In 10-20 cm soil layer, total nitrogen in PKH, NPKH3, NPKH2 and NPKH1 treatments increased by 20.32%, 16.09%, 4.59% and 3.79%, respectively; the available phosphorus increased by 30.95%, 30.29%, 24.86, and 10.91%, respectively; the available potassium increased by 13.71%, 13.33%, 8.63% and 6.54%, respectively. In the 20-30 cm soil layer, the total nitrogen, available phosphorus, and available potassium increased by less than 10% as compared with the NPK treatment, and the increase was not significant.

Principal Component Analysis of Each Index

Two principal components were obtained with basic characteristic values greater than 0.5. The contribution rate of the first principal component (urease, bacteria, fungi, soil organic matter, total nitrogen, and available potassium) to

Table 1: Contents of soil organic matter under different fertilization treatments ($\text{g}\cdot\text{kg}^{-1}$).

| Treatment | 0-10cm | 10-20cm | 20-30cm |
|-----------|---------|---------|---------|
| NPK | 47.53c | 44.99b | 42.57a |
| NPK1 | 50.59bc | 45.55b | 42.61a |
| NPK2 | 53.25b | 45.32b | 42.74a |
| NPK3 | 56.86a | 47.79a | 42.78a |
| PKH | 58.02a | 48.54a | 43.21a |

Note: Different letters within columns have significant differences at $P < 0.05$ according to the LSD test.

Table 2: Content of soil fertility elements under different fertilization treatments.

| | Treatment | 0-10cm | 10-20cm | 20-30cm |
|--|-----------|---------|----------|---------|
| Total nitrogen (g.kg ⁻¹) | NPK | 2.40b | 2.17b | 1.91a |
| | NPK1 | 2.62b | 2.25b | 1.99a |
| | NPK2 | 2.96a | 2.27b | 1.96a |
| | NPK3 | 3.01a | 2.52a | 2.07a |
| | PKH | 3.12a | 2.65a | 2.10a |
| Available phosphorus (mg.kg ⁻¹) | NPK | 47.65b | 44.30c | 41.37a |
| | NPK1 | 51.72b | 49.14b | 43.18a |
| | NPK2 | 61.79a | 55.32ab | 44.51a |
| | NPK3 | 65.04a | 59.73a | 45.01a |
| Available potassium (mg.kg ⁻¹) | PKH | 65.90a | 61.96a | 45.49a |
| | NPK | 153.15d | 143.23c | 132.40a |
| | NPK1 | 162.61c | 152.60b | 132.94a |
| | NPK2 | 172.44b | 155.61ab | 132.39a |
| | NPK3 | 182.71a | 162.33a | 132.11a |
| PKH | 184.27a | 162.88a | 134.17a | |

Note: Different letters within columns have significant differences at $P < 0.05$ according to the LSD test.

the total variance was 90.08%, and the second principal component (catalase, acid phosphatase, and available phosphorus) was 6.47%. The cumulative contribution rate of the two principal components reached 96.55%, indicating that the two principal components could represent 96.55% of all soil environmental effects information. The order of the comprehensive evaluation scores of the two principal components, from superior to inferior was PKH>NPKH3>NPKH2>NPKH1>NPK (Fig.3), and the comprehensive score in PKH treatment was the highest.

DISCUSSION

Soil microorganism is an important component of soil (Schutter & Fuhrmann 2001). The results showed that the bacteria, fungi, and actinomycetes in the 0-10cm soil layer increased significantly with the increase of humic acid organic fertilizer. Maybe the humic acid organic fertilizer improved the physical and chemical properties of soil, leading to the permeability and water retention of soil was improved, promoting the growth of crops and the metabolism of plant roots, stimulating the beneficial microbial activity of the soil, and speeding up the reproduction speed of soil microorganisms. The response of different microorganisms to humic acid organic fertilizer was also different. The increase of fungi was greater than that of bacteria and actinomycetes, the results from the humic acid organic fertilizer were acidic, and the fungi were dominant in an acidic environment, at the same

time, the fungi were strictly aerobic, the humic acid organic fertilizer could change the permeability of the soil, improve the gas exchange between the soil and the outside, therefore it provided a good living environment for the fungi. Through the application of humic acid organic fertilizer, the microorganisms increased, indicating that the humic acid organic fertilizer could improve the soil's environmental effects.

Urease, catalase, and acid phosphatase are closely related to plant nutrition (Zhang 2019). The results showed that the activities of urease and catalase in the 0-10cm soil layer increased with the increase of humic acid organic fertilizer, and acid phosphatase increased not significantly. The reasons may be that the soil aggregate structure was improved be-

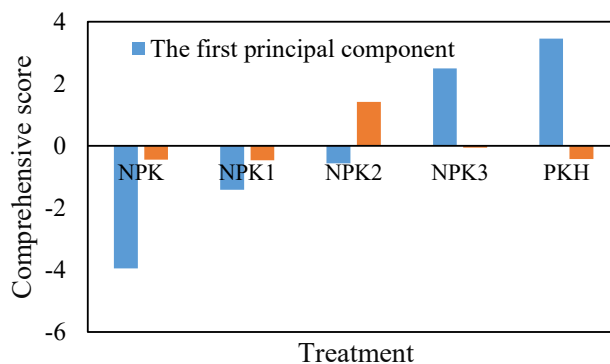


Fig.3: Score of principal components under different treatments.

cause of the humic acid organic fertilizer applied in the soil, which could combine well with the enzyme, therefore the enzyme activities were improved. Studies have shown that (Wang et al. 2012), the activities of soil protease and urease were positively correlated with soil aggregate structure, this also proved that the enzyme activities in urea treatment were lower than that in humic acid organic fertilizer treatments. In our experiment, the soil microorganisms increased significantly, it also could improve the enzyme activities. Fan and Hao (2019) showed that organic fertilizer had the greatest effects on urease, which was consistent with our conclusion. In our experiment, the activities of urease, catalase, and acid phosphatase in soil were measured. Combined with the previous conclusions, the results showed that the enzyme had a very sensitive response to soil fertilization, and the organic fertilizer could significantly improve the activities of soil enzymes. Therefore, in a sense, the application of organic fertilizer was a measure of adding enzyme.

As one of the components of soil, the soil organic matter plays an important role in the formation of soil, the protection of the soil environment, and the sustainable development of agriculture (Zhu 2012). In our experiment, we found, that the humic acid organic fertilizer could improve the soil organic matter, compared with the NPK treatment, the soil organic matter in the other treatments increased significantly, which was similar to the literature (Zeng et al. 2002, Wang et al. 2005). The Physical and chemical properties of soil could be improved because the soil organic matter increase, therefore the soil fertility also could be enhanced. while, the humic acid organic fertilizer also had significant effects on the total nitrogen, available phosphorus, and available potassium. The humic acid organic fertilizer contained a large number of water-stable groups with different particle sizes, which could reduce the leaching. And the humic acid organic fertilizer also contained a lot of available elements such as carbon, hydrogen, oxygen, nitrogen, phosphorus, and potassium, which was a good supplement to the soil elements. The application of organic fertilizer could improve soil organic matter and nutrient elements, the results of our study also confirmed the conclusion, that it was similar to the literature (Li et al. 2012, Ailinc et al. 2011, Gong & Lü 2014).

CONCLUSION

The application of humic acid organic fertilizer in black soil had a positive impact on improving soil environmental effects. The number of soil microorganisms, enzyme activities, soil organic matter, total nitrogen, available phosphorus, and available potassium were all increased. According to the analysis of different soil layers, humic acid organic fertilizer had affected 0-10cm soil. In 0-10 cm soil, the soil

microorganisms increased significantly in NPKH3 and PKH treatments, while the soil enzyme activities were similar but inconsistent, showing that the acid phosphatase was no significant difference across all treatments, while the urease and catalase were obvious increased with the increase of humic acid organic fertilizer application. The contents of organic matter, total nitrogen, available phosphorus, and available potassium increased with the increase of humic acid organic fertilizer application and reached the maximum value in PKH treatment. The principal component analysis showed that humic acid organic fertilizer application had positive impacts on the improvement of soil environmental effects, and the PKH treatment was the best.

ACKNOWLEDGEMENT

All authors thank the Basic Scientific Research Fund of Heilongjiang Provincial Universities: (2020-KYYWF-1042). We are grateful to the staff of the National Key Irrigation Experimental Station for their technical assistance.

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