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Microorganism Quantity and Enzyme Activities in Wheat Field Subjected to Different Nitrogen Fertilizer Rate

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Abstract: Field experiments are described involving nitrogen fertilizer in the wheat field to analyze its effect on microbial quantity and enzyme activity. The results showed that 250 kg N hm^{-2} applied in the wheat field (1) can increase bacteria, fungi and the quantity of actinomycetes by 39.9%, 56.7% and 70.5% compared to 0 kg N hm^{-2}. (2) Similarly, the activities of soil urease, catalase and FDA exhibited 60.8%, 18.3% and 49.1% improved variation compared to 0 kg N hm^{-2}, respectively. (3) Moreover, Shannon-Wiener diversity index (*H*) and Evenness index (*E*) reached the peak (*H*=0.37, *E*=0.33). Correlation analysis of microbial quantity with enzyme activity indicated that they were related to each other. These findings suggested that soil microbial quantity and enzyme activity were significantly influenced by nitrogen fertilizer and the application of 250 kg N hm^{-2} in the wheat field was observed to be the best.

Keyword: Enzyme activity, microorganism quantità, nitrogen fertilizer, wheat field.

1. INTRODUCTION

Nitrogen (N) fertilizer applied to agricultural soil influences the crop and soil system, which has made the current nitrogen fertilizer management practices lead to negative environmental effects [1]. The North China Plain as the major wheat production area in China plays an important role in securing national food security [2]. It is well known that in the last three decades, in this region, farmers increased the use of nitrogen fertilizer in the soil which caused an abundance of microorganisms [3] (bacterial, fungal and actinomycetes) and enzyme activities (urease, phosphatase, catalase, dehydrogenase activities) [4] which impaired the nutrient (especially nitrogen, carton and phosphorus) cycles [5, 6] of soil.

Microorganisms perform a critical role in nutrient transformation, cycling, and in many soil biochemical processes. Microbial quantity, biomass and enzyme activity express the functional relationship among microbial compositions [7] when different nitrogen fertilizer rates are applied to the soil [8]. The quantity of microorganisms, especially of bacteria is directly and indirectly related to the wheat field in the soil organic matter decomposition [9] and soil respiration [10], which significantly influence the absorption of nutrients of wheat. The difference in the population of soil fungal was due to nitrogen fertilizer [11]. A number of bacteria, fungi and actinomycetes are associated with the amount of nitrogen fertilizer, and the optimum nitrogen fertilizer in the soil results in larger populations. In addition, their diversity is an important soil microbial parameter. Shannon-Wiener diversity index is sensitive to changes in the microbial quantity, the more the number of species, the higher is the value of microbial diversity [12]. Soil enzyme activities can be associated with soil properties, active cells and climate which are used as the indicators of soil fertility [13, 14]. Urease plays an important role in nitrogen cycling, which catalyzes urea to carbon dioxide and ammonia [15]. Catalase is used to catalyze hydrogen peroxide (H_2O_2) to water (H_2O) and molecular oxygen (O_2) in the soil, and prevent the toxicity of H_2O_2 in the biological body [16]. Fluorescein diacetate (FDA) activity was observed to be directly related to microbial activity. Previous studies suggested that nitrogen addition increases enzyme activities [17, 18], but other studies have shown that excessive nitrogen fertilizer reduces the enzyme activity [19] and microbial diversity [20]. As a consequence, the quantity of microorganisms and enzyme activity in the soil need to be better understood.

We selected a wheat field in the North China Plain to study the relationships between microbial quantity and enzyme activity. Our aims were to analyze the changes on the soil under different nitrogen fertilizers from biological perspective and investigate the microbial diversity of the soil to determine its correlation with the nitrogen fertilizer.

2. MATERIALS AND METHODS

2.1. Experiment Sites

The experiment site is located in Qingyuan county, Hebei province, China (38°5'N, 115°30'E). The area is a temperate monsoon climatic region, with annual average temperature of 12°C and annual precipitation of 550mm. The experiment field was established with a wheat-maize rotation. Before the experiment, the physicochemical properties of initial soil

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samples were collected from the surface layer (0-20cm) in May 2010, while the content of soil organic matter, total N, Olson-P and Olson-K was 16.8g kg^{-1}, 0.9g kg^{-1}, 16.6 mg kg^{-1} and 99.3 mg kg^{-1}, respectively.

2.2. Experiment Design and Soil Sampling

A randomized block design was used with three replicates of each treatment, with the area of each plot being 40 m^2 (8m ! 5m). The experiment comprised of five treatments: 0 (N0), 100 (N100), 180 (N180), 250 (N250), and 300 (N300) kg N hm^{-2}. Fertilizers used were urea (46%), phosphorus pentoxide (12%) and potassium sulfate (60%). Urea was applied three times during the whole stage; 40% of urea was applied as the basal fertilizer, 40% of urea was applied at the reviving stage and20% was applied at the blossoming stage. Total P and K fertilizers were applied as basal fertilizers, in the quantity of 120 kg P_2O_5 hm^{-2} and 120 kg K_2O hm^{-2}.

Dates used in this study were collected from the surface layer (0-30cm) in May, 2013. The fresh soil samples were sieved 2mm and preserved for further experiment and analysis.

2.3. Microbial Population

The microbial populations were determined by the dilution plate method [21]; bacteria were determined using beef extract peptone medium; fungi, using Martin medium, and actinomycetes using Gause's I medium.

2.4. Enzyme Activity

Soil urease activity was based on the colorimetric determination [22], where 2.5g soil with 0.5 mL toluene was added in 50mL volumetric flask for 15 min; then 2.5 mL of 10%urea and 5mL of citrate buffer (pH 6.7) were added in the constant temperature incubator (38°C) for 24h. Following this, the soil sample with 38°C distilled water was diluted to 25 ml (toluene should float in the scale above) mixture, containing 1ml filtrate in 50ml volumetric flask with 10 ml distilled water. In this mixture, 4 ml sodium phenate and 3ml sodium hypochlorite were added in constant volume, and determined at 578nm 20 min later and expressed as mg NH3-N g^{-1} $24h^{-1}$.

Soil catalase activity was based on permanganate titration method [23]. In this method, 2 g of soil with 40 mL of distilled water and 5 mL of 0.3% H_2O_2 were added into 100 mL triangular flask vibrated for 20 min. Following this, 5 mL of 3 mol L^{-1} H_2SO_4 terminated the reaction, leaving 25 mL of filtrate with 0.1 mol L^{-1} potassium permanganate titration. The catalase was expressed as mL 0.02 mol L^{-1} $KMnO_4$ g^{-1} $20min^{-1}$.

Soil fluorescein diacetate (FDA) hydrolase activity was based on colorimetric determination [24], where 5 g of fresh soil with 15 mL of 60 mmol L-1 phosphate buffer (pH 7.6) and 0.2 mL of 1000μg mL^{-1} fluorescent diacetate (FDA) reserves were added into the 50 mL triangular flask vibrated for 20 min at 30°C. Following this, 15 mL of chloroform/methanol solution terminated the reaction, with the absorbance of the released fluorescein at 490 nm.

2.5. Data Analysis

Shannon-Wiener index (*H*), $H = -\sum P_i ! [\ln P_i]$, where P_i is the proportion of each taxon in the total quantity. Evenness index (*E*), $E = -H/\ln S$, where *S* is the total number of species [25]. Correlation analysis was analyzed with SPSS 18.0 and other data analysis was performed with origin 9.0.

3. RESULTS AND DISCUSSION

3.1. Microbial Population

Soil microbial quantity varied greatly under the different N fertilizer treatments used in this study. The quantity of soil bacteria was increased by 20.4%, 31.95, 39.9% and 35.6% compared to N0 (Fig. **1A**). Application of N fertilizer brought significant increase in the soil fertility, as well as caused changes in the microbial quantity. The quantity of soil fungi was increased by 22.0%, 53.3%, 56.7% and 49.4% compared to N0, (Fig. **1B**). Similarly, the quantity of soil actinomycetes was increased by 12.9%, 54.25, 70.5% and 59.1% compared to N0, (Fig. **1C**). The increase in the total microbial quantity (bacteria, fungi and actinomycetes) was greater in N250. However, the microbial quantity in N300 showed a slight decrease compared to N 250 (Fig. **1D**). N0 had the lowest quantity in all treatments, which can be attributed to less N nutrition suggesting that there was not enough demand for soil microorganism [26]. Some researchers have already reported that microbial quantity had no significant change at low fertilizer application in the black soil [27]. In addition, the response of fungi number to N fertilizer was highly variable [28]. After 13 years' fertilizer experiment [29], the bacteria, actinomycetes and fungi in the red soil changed greatly and indicated that protecting the diversity of microorganisms sustained the soil development in the agroecological system.

3.2. Enzyme Activity

The enzyme activity was significantly influenced by N fertilizer [30], as the activity of urease increased with increase in the N fertilizer rate and N300 had the highest urease activity in five treatments; being significantly higher than other treatments (Table **1**). This result was similar to a maize-wheat experiments in India [31]. Compared to the N0, N100, N180, N250 and N300 increased the catalase activity by 15.7%, 18.3%, 16.5% and 11.3%, respectively. But there was no significant effect on catalase activity, which was different from other studies [32]. FDA activity increased by 10.9%, 45.5%, 49.1% and 40.0% in N100, N180, N250 and N300 compared to N0, respectively, and closely related to bacteria, fungi and actinomycetes quantity (Table **3**). An equation from Nayak indicated that FDA hydrolysis activity was one of the important factors in the soil biochemical possesses [30].

Higher enzyme activities in N250 probably resulted from optimum N fertilizer rate, which increased the soil fertility. On the other hand, crops growing better not only directly benefited the proper fertilizer [33], but alsocreated more root exudation (carbohydrate, amino acids and organic acids) [34] and the turnover indirectly stimulated the soil microbiological metabolism [35].

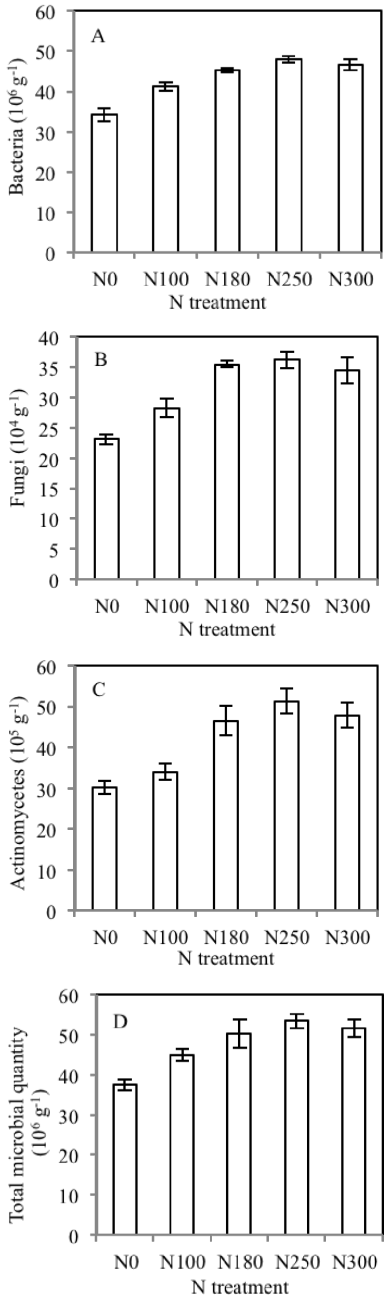

Fig. (1). Effects of different nitrogen fertilizer rate on soil microbial quantity (CFU/g soil).

3.3. Microbial Diversity Index

Fertilizer in the agricultural soil is a major impact factor that influences the diversity of microorganisms [36]. In the results of this study, *H* and *E* (Table **2**) did not show a trend of increase with increased N fertilizer rate. They both reached the peak (*H*=0.37, *E*=0.33) at N250, and decreased at N300, which indicated that applying low and high rate was not good for microbial diversity. Sarathchandra reported that N fertilizer affected soil microbial functional diversity and with increased N fertilizer, *H* was significantly reduced [8]. This trend also appeared in the enzyme activity. Because NH_4 and NO_3 as the nutrients of soil microorganism were obtained from N fertilizer, thus, different rate resulted in different microbial diversity [8]. Based on our study, the application of 250 kg N hm^{-2} can promote microbial diversity in the wheat field.

3.4. Relationships between Microbial Quantity and Enzyme Activity

According to the correlational analysis (Table **3**), microbial quantity was positively correlated with the soil enzyme activity. All microbial quantity was significantly correlated with FDA activity. In few of the analyses, the quantity of bacteria was significantly positively correlated with FDA ($r=0.982$, $P<0.05$), along with the quantity of fungi and actinomycetes, which was significantly positively related to FDA ($r=0.985$, $r=0.975$, $P<0.01$). These positive correlations were observed through biochemical processes [37]. In addition, this result showed that increasing the total quantity of microorganisms increased the FDA activity of the soil [38]. No significant relationship was found between microbial quantity, urease and CAT activity.

CONCLUSION

In our study, we tested differences in microorganism quantity, enzyme activities and soil microbial diversity. All these parameters were related to each other and more importantly, were directly influenced by the addition of nitrogen fertilizer. Applying 250 kg N hm^{-2} in this region not only improved soil enzyme activity but also the soil microbial quantity as well as the soil microbial diversity.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

Table 1. Effects of different nitrogen fertilizer rate on soil enzyme activity.

Treatment	Urease Activity mg/kg·24h	CAT Activity 0.002 mol/L $KMnO_4$ mL/g	FDA Activity μg/ g·20min
N0	7.39ab	1.15a	0.55a
N100	8.04ab	1.33a	0.61a
N180	7.63ab	1.34a	0.80a
N250	11.88a	1.36a	0.82a
N300	11.96a	1.28a	0.77a

Table 2. Effect of nitrogen fertilizer on Shannon-Wiener diversity and Evenness index.

Diversity Index	N0	N100	N180	N250	N300
H	0.32	0.31	0.34	0.37	0.35
E	0.29	0.28	0.31	0.33	0.32

H:Shannon-Wiener diversity index, *E*: Evenness index.

Table 3. Correlation analysis microbial quantity and enzyme activity.

	Bacteria	Fungi	Actinomycetes	Urease	CAT	FDA
Bacteria	1					
Fungi	0.960**	1				
Actinomycetes	0.884*	0.974**	1			
Urease	0.500	0.642	0.751	1		
CAT	0.846	0.794	0.654	0.256	1	
FDA	0.928*	0.985**	0.975**	0.589	0.727	1

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

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