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 wheat field to research its effect on microbial quantity and enzyme activity. The results showed that 250 kg N hm$^{-2}$ applied in wheat field (1) can increase bacteria, fungi, actinomycetes quantity 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 expressed 60.8%, 18.3% and 49.1% of the improvement variation compared to 0 kg N hm$^{-2}$, respectively. (3) Shannon-Wiener diversity index ($H$) and Evenness index ($E$) reached the peak ($H=0.37$, $E=0.33$). Correlation analysis microbial quantity with enzyme activity indicated they were related to each other. These findings suggested that soil microbial quantity and enzyme activity were significantly influenced by nitrogen fertilizer and application of 250 kg N hm$^{-2}$ in wheat field was the best.

Keyword: Microorganism quantity, enzyme activity, nitrogen fertilizer, wheat field.

1. INTRODUCTION

Nitrogen (N) fertilizer applied to agricultural soil would be influenced crop and soil system, which current nitrogen fertilizer management practices had negative environment effects [1]. The North China Plain as the major wheat production area in China plays an important role on securing national food security [2]. It is well known, in the last three decades, in this region farmers increased nitrogen fertilizer rate in soil caused abundance of microorganism quantity [3] (bacterial, fungal and actinomycetes) and enzyme activities (urease, phosphatase, catalase, dehydrogenase activities) [4] reduction then impaired nutrient (especially nitrogen, carbon and phosphorus) cycles [5, 6] of soil.

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

We selected a wheat field in North China Plain to research the relationships between microbial quantity and enzyme activity. Our aims were to analyze the changes of soil under different nitrogen fertilizer from soil biological perspective and investigate the soil microbial diversity whether be correlated to 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 climate, with annual average temperature 12°C and...
annual precipitation 550mm. The experiments field was established with a wheat-maize rotation. Before experiment the physicochemical properties of initial soil samples were collected from the surface layer (0-20cm) in May, 2010, the content of soil organic matter, total N, Olson-P and Olson-K were 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 treatments, the area of each plot was 40 m² (8m × 5m). The experiment comprised five treatments: 0 (N0), 100 (N100), 180 (N180), 250 (N250), 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 basal fertilizer, 40% of urea was applied at reviving stage, 20% of urea was applied at blossoming stage. Total P and K fertilizer was applied both as basal fertilizer, which comprised 120 kg P₂O₅ hm⁻² and 120 kg K₂O hm⁻².

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

2.3. Microbial Population

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

2.4. Enzyme Activity

Soil urease activity was based on the colorimetric determination [22], 2.5g soil with 0.5 mL toluene 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 constant temperature incubator (38°C) for 24h, then the soil sample with 38°C distilled water dilution to 25 mL (toluene should float in the scale above) mixing, 1ml filtrate in 50mL volumetric flask with 10 mL distilled water, then added 3 sodium phenate and 3 mL sodium hypochlorite, constant volume, 20 min later, determination at 578nm, and expressed as mg NH₃-N g⁻¹ 24h⁻¹.

Soil catalase activity was based on permanganate titration method [23], 2 g of soil with 40 mL of distilled water and 5 mL of 0.3% H₂O₂ were added into the 100 mL triangular flask vibrated for 20 min, then 5 mL of 3 mol L⁻¹ H₂SO₄ terminated the reaction, 25 mL of filtrate with 0.1 mol L⁻¹ potassium permanganate titration. The catalase was expressed as mL 0.02 mol L⁻¹ KMnO₄ g⁻¹ 20min⁻¹.

Soil fluorescein diacetate (FDA) hydrolysis activity was based on colorimetric determination [24], 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, then 15 mL of chloroform/methanol solution terminated the reaction, and measuring 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 is the proportion of each taxon in the total quantity. Evenness index (E), \( E = -H/I \) lnS, 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. Soil bacteria quantity increased by 20.4%, 31.95, 39.9% and 35.6% compare to N0, respectively (Fig. 1A). Application of N fertilizer brought significantly increase to soil fertility, as well as cause changes in microbial quantity. Soil fungi quantity increased by 22.0%, 53.3%, 56.7% and 49.4% compare to N0, respectively (Fig. 1B). Soil actinomycetes quantity increased by 12.9%, 54.25, 70.5% and 59.1% compare to N0, respectively (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 compare to N 250 (Fig. 1D). N0 had the lowest quantity in all treatments, it can be attributed to less N nutrition that there was not enough demands 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 protected the diversity of microorganisms was to sustain the soil development in agroecological system.

3.2. Enzyme Activity

The enzyme activity was significantly influenced by N fertilizer [30], the activity of urease increased with increasing of N fertilizer rate and N300 had the highest urease activity in five treatments, 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). A 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 can increase soil fertility. On the other hand, crops growing better not only were benefited to proper fertilizer directly [33], but also crop growing better can create more root exudation (carbohydrate,
Fig. (1). Effects of different nitrogen fertilizer rate on soil microbial quantity (CFU/g soil).
### Table 1. Effects of different nitrogen fertilizer rate on soil enzyme activity.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urease Activity (mg/kg·24h)</th>
<th>CAT Activity (0.002 mol/L KMnO₄ mL/g)</th>
<th>FDA Activity (µg/g·20min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N0</td>
<td>7.39ab</td>
<td>1.15a</td>
<td>0.55a</td>
</tr>
<tr>
<td>N100</td>
<td>8.04ab</td>
<td>1.33a</td>
<td>0.61a</td>
</tr>
<tr>
<td>N180</td>
<td>7.63ab</td>
<td>1.34a</td>
<td>0.80a</td>
</tr>
<tr>
<td>N250</td>
<td>11.88a</td>
<td>1.36a</td>
<td>0.82a</td>
</tr>
<tr>
<td>N300</td>
<td>11.96a</td>
<td>1.28a</td>
<td>0.77a</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Diversity Index</th>
<th>N0</th>
<th>N100</th>
<th>N180</th>
<th>N250</th>
<th>N300</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>0.32</td>
<td>0.31</td>
<td>0.34</td>
<td>0.37</td>
<td>0.35</td>
</tr>
<tr>
<td>E</td>
<td>0.29</td>
<td>0.28</td>
<td>0.31</td>
<td>0.33</td>
<td>0.32</td>
</tr>
</tbody>
</table>

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

### Table 3. Correlation analysis microbial quantity and enzyme activity.

<table>
<thead>
<tr>
<th></th>
<th>Bacteria</th>
<th>Fungi</th>
<th>Actinomycetes</th>
<th>Urease</th>
<th>CAT</th>
<th>FDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td>0.960**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actinomycetes</td>
<td>0.884*</td>
<td>0.974**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urease</td>
<td>0.500</td>
<td>0.642</td>
<td>0.751</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td>0.846</td>
<td>0.794</td>
<td>0.654</td>
<td>0.256</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>FDA</td>
<td>0.928*</td>
<td>0.985**</td>
<td>0.975**</td>
<td>0.589</td>
<td>0.727</td>
<td>1</td>
</tr>
</tbody>
</table>

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

3.3. Microbial Diversity Index

Fertilizer in agricultural soil as a major impact factor that influenced the diversity of microorganism [36]. In our results, $H$ and $E$ (Table 2) did not show a trend of increasing with increased N fertilizer rate. They both reached the peak ($H=0.37$, $E=0.33$) at N250, then reduced at N300, which indicated that applying low and high was not good for microbial diversity. Sarathchandra reported that N fertilizer affected soil microbial functional diversity and with increased N fertilizer $H$ significantly reduced [8]. This trend was also appeared in the enzyme activity. Because NH$_4$ and NO$_3$ as nutrient of soil microorganism came from N fertilizer, thus, different rate resulted in different microbial diversity [8]. Based on our study, application of 250 kg N hm$^{-2}$ can be promoted microbial diversity in wheat field.

3.4. Relationships between Microbial Quantity and Enzyme Activity

According to correlation analysis (Table 3) microbial quantity were positively correlated with soil enzyme activity. All microbial quantity was significantly correlated with FDA activity. Some of which bacteria quantity was significantly positively correlated with FDA ($r=0.982$, $P<0.05$), fungi and actinomycetes quantity high significantly positively related to FDA ($r=0.985$, $r=0.975$, $P<0.01$). These positive correlations would be occurred through biochemical processes [37]. In addition, this result showed that increased the total quantity of microorganism being increased soil FDA activity [38]. No significant relationship was found between microbial quantity and urease and CAT activity.
CONCLUSION

In our study, we tested differences in microorganism quantity, enzyme activities and soil microbial diversity, all these parameters was related to each other and more importantly, was directly influenced by the addition of nitrogen fertilizer. Applied 250 kg N ha⁻¹ in this region improved not only soil enzyme activity but also the soil microbial quantity as well as the soil microbial diversity.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

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