Difference of Soil Microbial Populations under Long-Term Different Fertilizer Application and Impact of Herbicide Acetochlor on Them

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Abstract

Soil microorganism plays an important role in quality and health of soil, long term different fertilizer application patterns would have differential effect on microorganism populations. The experimental results indicated that manure and chemical fertilizer + manure treatments could maintain higher fertility levels and microbial populations than chemical fertilizer and control treatments, increment of soil organic matter is benefit to growth of microorganisms. Application of herbicide acetochlor had no significant positive or negative effects on the populations of soil microorganisms and species, and also did not display inconsistent results among the soils with different fertilizer application patterns.

Keywords: Microbial population; Paddy soil; Long-term fertilizer application; Herbicide

Introduction

Fertilizer application is an essential measure to improve crop production in modern agriculture. However, different fertilization patterns have obvious effect on soil property and fertility, and optimum pattern can maintain a high soil fertility level and crop yield [1]. Soil microorganism is one of indicators of soil health and fertility, and long-term fertilizer application experiments indicated that different fertilizers lead to great impact on soil microbial population [2-5]. It is thought that organic fertilizer application has a promoting effect on increasing soil microorganism community.

In the meantime, currently herbicides are commonly applied to control weed in crop systems. Differential toxicity of herbicides in soil may cause changes in microbial community structure and function, and concomitantly influence soil health and ecosystem processes [6]. But, there is a serious lack of information that herbicide application whether has differential effects on microbial population of soils with long-time different fertilizer application treatments so far.

Materials and Methods

Experimental soil

The paddy soils sampled from a long-term fertilizer application experimental site of over 35 years at Qiyang red soil experimental station, Hunan province, China (111°52'32" E,26°45'12" N). The basic properties of the soil are shown in Table 1.

Experimental herbicide

The herbicide acetochlor is commonly applied in agriculture. The acetochlor used in this experiment is 50% active ingredient emulsifiable concentrate (EC), which was produced by Qingfeng Agro-chemical Co. Ltd. Hangzhou, China.

Experimental design

Our investigation on the influence of different fertilizer treatments and acetochlor application on soil microorganisms was conducted in the laboratory with a 14-day incubation period. Ten mg active ingredient (a.i) kg⁻¹ was applied to paddy soil with four replicates. The fresh soils sampled from the experimental site were placed into a 300mL wide-mouth bottle. Subsequently, soil samples were adjusted to 60% of water holding capacity with double deionized water in which acetochlor could be dissolved. Control soils were also prepared only with double deionized water. All soil samples were incubated at 25 °C, and 10g soil was sampled each time by destructive sampling from each bottle at 3, 7 and 14 days after incubation.

Methods for soil microbial populations

Microbial numbers were determined using conventional methods and calculated. Ten grams of each soil sample incubated
was suspended in 90mL sterile water and shaken in a shaker for 30min. Then, these soil suspensions were diluted in 10-fold series.

The Colony counting method

0.05mL soil suspensions of $10^{-1} - 10^{-7}$ dilutions were used to inoculate in PDA medium, gauzerime synthetic agar medium, beef extract peptone medium [7] and acetamide agar medium [8] respectively, which determined the numbers of fungi, actinomycete, aerobic bacteria and heterotrophic nitrifying bacteria. Each microorganism was cultured with four dilutions and every concentration had four parallel culture dishes. All the dishes were incubated in dark at 25 °C. Total number of microorganisms on the agar mediums just need to be counted.

The Most probable number (MPN) method

One mL soil suspensions of $10^{-3} - 10^{-7}$ dilutions were used to inoculate tubes containing selective liquid media. Ammonifier were cultured in peptone ammonification medium [9], nitrosomonas medium reported by Kihn et al. [10], autotrophic nitrifying bacteria medium described by Lin et al. [11], and denitrifying bacteria medium followed by Johns et al. [12]. Each microorganism was cultured with four dilutions and every concentration has four parallel tubes. All the tubes were incubated in dark at 25 °C. After incubation, the pattern of positive and negative tubes is noted, and a standardized MPN table was consulted to determine the most probable number of organisms (causing the positive results) per unit volume of the original sample.

Statistical analysis

All statistical analyses were performed using SPSS19.0 (SPSS for Windows, Version 13.0). The differences of microbial populations among treatments were analyzed using ANOVA, and the differences were considered significant at p<0.05.

Results and Discussion

Table 1: Physical and chemical properties of the soil under different fertilizer treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>OM (g/kg)</th>
<th>Available N (mg/kg)</th>
<th>Available P (mg/kg)</th>
<th>Available K (mg/kg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>34.2</td>
<td>140.8</td>
<td>12</td>
<td>129</td>
<td>6.3</td>
</tr>
<tr>
<td>NPK</td>
<td>26.2</td>
<td>117.9</td>
<td>34</td>
<td>102</td>
<td>6.1</td>
</tr>
<tr>
<td>NPKM</td>
<td>36.9</td>
<td>147</td>
<td>47</td>
<td>137</td>
<td>6.2</td>
</tr>
<tr>
<td>CK</td>
<td>20.7</td>
<td>99.9</td>
<td>11</td>
<td>94</td>
<td>6.4</td>
</tr>
</tbody>
</table>

The experimental data in Table 2 showed that fungi, bacteria and actinomyces populations in soils appeared great differences among fertilizer application treatments, their numbers were in order M>NPKM>NPK>CK. Soil organic matter (OM) is benefit to growth of microorganism, the higher soil OM are the higher the populations relatively are (Table 1 & 2). And, chemical fertilizer application also could increase the populations compared to control. The long term mineral fertilizer increases microbial biomass in cropping systems [4].

Table 2: The population of soil microorganisms under long term different fertilizer application.

<table>
<thead>
<tr>
<th></th>
<th>Fungi($\times 10^4$ cfu/g dry soil)</th>
<th>Bacteria($\times 10^7$ cfu/g dry soil)</th>
<th>Actinomycetes($\times 10^4$ cfu/g dry soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>4.23±0.51 d</td>
<td>1.36±0.04 c</td>
<td>7.25±0.95 d</td>
</tr>
<tr>
<td>NPK</td>
<td>5.28±0.43 c</td>
<td>1.42±0.03 c</td>
<td>8.95±0.61 c</td>
</tr>
<tr>
<td>M</td>
<td>9.23±0.68 a</td>
<td>1.65±0.06 a</td>
<td>13.85±0.71 a</td>
</tr>
<tr>
<td>NPKM</td>
<td>8.00±0.46 b</td>
<td>1.57±0.02 b</td>
<td>11.43±0.74 b</td>
</tr>
</tbody>
</table>

Table 3: Functional microbial populations under long term different fertilizer application.

<table>
<thead>
<tr>
<th></th>
<th>Ammonifying bacteria($\times 10^4$ cfu/g dry soil)</th>
<th>Nitrosomonas($\times 10^4$ cfu/g dry soil)</th>
<th>Autotrophic nitrifying bacteria($\times 10^4$ cfu/g dry soil)</th>
<th>Heterotrophic nitrifying bacteria ($\times 10^6$ cfu/g dry soil)</th>
<th>Denitrifying bacteria ($\times 10^4$ cfu/g dry soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>0.42±0.05c</td>
<td>0.17±0.05b</td>
<td>0.26±0.05c</td>
<td>0.26±0.05c</td>
<td>0.53±0.13b</td>
</tr>
<tr>
<td>NPK</td>
<td>0.45±0.00c</td>
<td>0.21±0.08b</td>
<td>0.36±0.00c</td>
<td>0.36±0.00c</td>
<td>0.68±0.19b</td>
</tr>
<tr>
<td>M</td>
<td>1.31±0.06a</td>
<td>0.39±0.05a</td>
<td>0.75±0.06a</td>
<td>0.75±0.06a</td>
<td>1.54±0.52a</td>
</tr>
<tr>
<td>NPKM</td>
<td>0.75±0.06b</td>
<td>0.27±0.08ab</td>
<td>0.53±0.13b</td>
<td>0.53±0.13b</td>
<td>1.09±0.26ab</td>
</tr>
</tbody>
</table>

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Similarly, the numbers of functional microbe (Table 3) included ammonifying bacteria, nitrosomonas, autotrophic nitrifying bacteria, heterotrophic nitrifying bacteria, denitrifying bacteria also showed M>NPKM>NPK>CK, and significantly greater in two manure application treatments (P<0.05), however, have no apparent differences between chemical fertilizer treatment and control.

Microorganisms. However, these researches had drawn different conclusions that the impacts depended on herbicide types [6,13], applied concentrations [14,15], soil types [16,17], and microbial species [18, 19]. The results in Figure 1 & 2 indicated that herbicide acetochlor application has no influence on microbial populations and species in four long term fertilization treatments under this experimental condition.

**Conclusion**

Soil microbial populations, include fungi, bacteria and actinomycetes, as well as ammonifying bacteria, nitrosomonas, autotrophic nitrifying bacteria, heterotrophic nitrifying bacteria and denitrifying bacteria, showed great variation among long term different fertilizer application treatments, the order of numbers displayed as fellows M>NPKM>NPK≥CK. Herbicide acetochlor did not significantly inhibit microbial populations, and also had no differences of responses to soils with long term different fertilizer application.

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