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Pesticide Pollution Affects the Functional Diversity of Soil Microbial Community

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Abstract: With the development of China's economy and the acceleration of urbanization, the agricultural production structure has also been adjusted, and the protected cultivation agriculture has developed rapidly, in which the area of protected vegetable cultivation accounts for a large proportion. In the process of protected vegetable cultivation, due to the extensive use of agricultural film, pesticides, plastic greenhouses, as well as the return of urban sludge to the field and sewage irrigation, the soil organic pollution in China's protected vegetable cultivation bases has become increasingly serious, especially the ionic liquid pollution contained in pesticides is more common. This paper takes ionic liquids as the research object, carries out microbial experimental cultivation tests on them, and explores the impact of pesticide pollution(PP) on the functional diversity of soil microbial communities. It is concluded that the addition of ionic liquids to soil will have an impact on the soil ecosystem. The order of toxicity of the three ionic liquids to soil is [c10mim]br>[c6mim]br>[c4mim]br. These results can provide some theoretical and experimental basis for the screening of pesticide degrading strains, soil remediation and practical field application research in the future.

1. Introduction

The application of pesticides has become a routine in modern agricultural production, which can not only prevent diseases, but also promote the yield and income of food crops. For China with a large population, pesticides are undoubtedly a good way to increase grain production. However, on the one hand, the application of pesticides promotes the efficiency of agricultural production development, and on the other hand, with the increase of pesticide use, the degree of soil pollution is also more serious. Generally speaking, the effect of low-dose pesticides on non target soil microorganisms is not obvious, but if pesticides are applied continuously for many years, the microbial groups sensitive to such pesticides in the soil will be killed, while the tolerant micro

pesticide biological groups multiply in large numbers, and the physical and chemical properties of soil, the activity of soil enzymes and the diversity of soil microbial communities will change, thus affecting the soil ecosystem, This has affected the adjustment of agricultural planting structure.

Many scholars at home and abroad have studied and analyzed the impact of PP on the functional diversity of soil microbial communities. Andres pesticide P et al. Tested the medium-term effects of corn biochar on soil microorganisms and micro arthropods in Mediterranean vineyards. We applied biochar to three field plots with neutral sandy loam at a dose of 5 pesticides mg pesticide ha (-1). The abundance of soil micro arthropod functional groups was monitored, and the biomass of soil microbiota was estimated. During the monitoring period, biochar-c contributed little to the diet of most microbiota. However, two Gram-negative bacteria groups increased their biochar derived carbon absorption under extreme soil drought conditions, suggesting that biochar-c may help soil microorganisms overcome food shortages caused by drought [1]. Zhang et al. Studied the effect of salt gradient on soil microorganisms, nutrient availability and their relationship. The results showed that salt increased soil pH and exchangeable sodium percentage, but decreased soil organic matter, soil exchangeable potassium and soil microbial biomass. The abundance and community composition of soil bacteria and fungi are significantly different between non saline soil and saline soil. Soil salinity changes the abundance of soil bacteria, fungi and arbuscular mycorrhizal communities [2].

Due to the massive application of pesticides, the structure of soil microbial community(MC) will change accordingly, and the utilization ability of microorganisms to carbon sources will change. Therefore, the community structure determines the function of soil microorganisms. The effects of different concentrations of pesticides on soil microbial functions are different. Generally, any concentration of pesticides will stimulate the diversity of soil microbial functions. The effect of low concentration is short-term, and high concentration is long-term effective. Biolog method has been widely used to evaluate the change trend of soil microbial functional diversity under different treatments. Through this method, the metabolic characteristics of microbial communities in contaminated soil are measured to the greatest extent by using the differences in the utilization of 31 different carbon sources by microorganisms, so as to provide early warning for monitoring the changes of soil quality, and the impact of PP on the functional diversity of soil microbial communities is analyzed [3-4].

2. Experimental Test on the Effect of PP on the Functional Diversity of Soil MC

2.1. Soil Microorganisms

Soil is known as the "black box" of nature. It is the living place of producers and decomposers. It controls the semi initiative of nature and maintains the whole biogeochemical cycle. Soil microorganism is the general name of all micro organisms in soil. Soil microorganisms have high species richness, large number, wide distribution and various ecological functions. Different types of soil contain different types and numbers of microorganisms, and the distribution range of microorganisms in the same type of soil is different. The closer to the surface of the soil, the richer the microbial species are. In addition, the number of soil microorganisms is related to vegetation types and soil quality [5-6].

Soil microorganism is the bridge connecting the above ground part and the underground part; On the one hand, it helps plants absorb and transport mineral elements, improves plant growth and increases crop yield; On the other hand, soil microorganisms can degrade environmental pollutants that are not conducive to vegetation growth and affect soil quality. In addition, soil microorganisms

can also reflect the quality of soil. The functional diversity and genetic diversity of soil microorganisms can earlier reflect the change process of soil quality and evaluate the safety of ecological environment. Soil microbial ecology is an important part of soil ecosystem, so the study of soil microbial functional diversity is a key link to evaluate the environmental safety of transgenic crops [7-8].

2.2. Experimental Test of PP on Soil Microorganisms

2.2.1. Materials and Methods

Adjust the soil moisture content to close to 60% of the maximum field water capacity, and pre incubate it in a constant temperature incubator at 25 ± 1 ℃ for one week. According to the concentration determined in the preliminary experiment, [c4mim]br, [c6mim]br set three ionic liquid treatment concentrations of 1.0mg/kg, 10.0mg/kg and 100.0mg/kg and a deionized water control, [c10mim]br set three ionic liquid treatment concentrations of 1.0mg/kg, 10.0mg/kg and 50.0mg/kg and a deionized water control. During poisoning, three repetitions are set for each concentration. Each repeated treatment is divided into four brown vials (corresponding to four sampling cycles), and each vial is poisoned separately [9].

After poisoning and bottling, weigh each brown bottle, label and record the quality, and then put all soil samples into a constant temperature biochemical incubator at 25 ±1 ℃ for cultivation in the dark, take them out and weigh them every two days, and supplement sterile deionized water according to the difference method to maintain the constant water holding capacity of the soil. Take out three vials for each concentration on the 10th, 20th, 30th and 40th days of culture, and measure each index [10].

2.2.2. Microbial Colony Count Experiment

Effect of ionic liquid on the number of bacteria: see Table 1 for the change of the number of culturable bacteria in the soil after the addition of ionic liquid (different letters indicate the significant differences between the treatment groups with different concentrations at the same exposure time).

Table 1. Changes in the number of culturable bacteria

	10	20	30	40
a	0.75	0.76	0.85	0.64
b	0.66	0.78	0.62	0.53
c	0.60	0.54	0.71	0.63
d	0.68	0.51	0.57	0.67

The [c6mim] br high concentration treatment group (100mg/kg) showed significant differences on the 10th day of the experiment, and a short recovery on the 20th day, and then gradually showed differences with the progress of the experiment. The number of bacteria in the medium concentration treatment group (1.0mg/kg and 10mg/kg) changed slowly. Compared with the control group, there was no difference in the early stage of the experiment. On the 40th day, there was a significant difference in the 1.0mg/kg treatment group. Compared with the control group, the number of bacteria in each concentration of [c10mim] br treatment group was significantly inhibited, and this inhibition showed a gradually obvious trend in the first three cycles of the

experiment. On the 40th day of the experiment, the inhibition trend of each treatment group began to gradually reduce, and there was no significant difference between the 50mg/kg treatment group and the 10mg /kg treatment group. Compared with the three ionic liquids, [c10mim]br has the greatest impact on the number of bacteria in the soil. On the 40th day of the experiment, the number of bacteria in each ionic liquid treatment group did not return to the control level [11-12].

2.2.3. Activity Determination

Weigh 1.00G dry soil weight of fresh soil sample passing 1mm sieve into a 50ml triangular flask, add 0.2ml toluene, 4mlmub buffer solution (pH6.5) and 1ml 0.05mol/l disodium p-nitrophenyl phosphate solution, shake gently, mix well, plug the bottle, and incubate at 37 ℃ for 1H. After the culture, add 1ml of 0.5mol/l CaCl2 and 4ml of 0.5mol/l NaOH, shake gently for a few seconds and then filter. Dilute the filtrate 10 times with pH6.5 mub buffer solution, and measure the absorbance value of the diluent at 400nm with a spectrophotometer. PNP (p-Nitrobenzene) is used as enzyme activity unit μ G/ (GH) indicates. Control experiment: add 0.2ml toluene and 4ml buffer solution to 1.00G soil sample, and put them into the incubator together with the samples of the treatment group for culture. After taking them out, add 1ml of 0.5mol/l CaCl2 solution and 4ml of 0.5mol/l NaOH solution to terminate the enzyme reaction, and then add 1ml of 0.05mol/l disodium p-nitrophenyl phosphate solution. After shaking, filter immediately, and use a spectrophotometer to compare colors at 400nm [13-14].

2.2.4. Result Calculation

The mass of phenol released from 1g soil after 1h(μ g) Indicates soil acid phosphatase activity.

$$pho = \frac{m1}{Hm2} \tag{1}$$

$$H = \begin{cases} k_{ij} + \sum_{a_j}^{a_i} m_{b_{ij}}^{m_1} \\ \sum_{k_{m\times ab}} m_1 + m_2 \end{cases} \tag{2}$$

Where: pho is the yield of p-nitrophenol per unit time: μ g.g-1.h-1; M1 is the mass of p-nitrophenol in the test solution, μ g; M2 is the sample mass, G; K is the moisture coefficient.

3. Analysis of Experimental Test Results

The effect of the addition of three ionic liquids on the number of culturable fungi in soil is shown in Figure 1.

It can be seen from Figure 1 that due to the addition of [c4mim]br, the number of fungi in each treatment group showed an overall trend of inhibition, and with the increase of concentration, the inhibition trend was obvious; The low concentration treatment group had no significant difference in the whole experimental cycle. On the 40th day of the experiment, the 10mg/kg treatment group

had the most obvious inhibition trend, but there was no significant difference compared with the control group.

At the beginning of the experiment, compared with the control group, the number of fungi in the [c6mim] br high concentration treatment group (10mg/kg and 100mg/kg) was significantly inhibited. This inhibition trend gradually weakened with the experiment, and gradually returned to the control water in the later stage of the experiment; The low concentration treatment group showed no obvious inhibition trend throughout the experimental cycle [15].

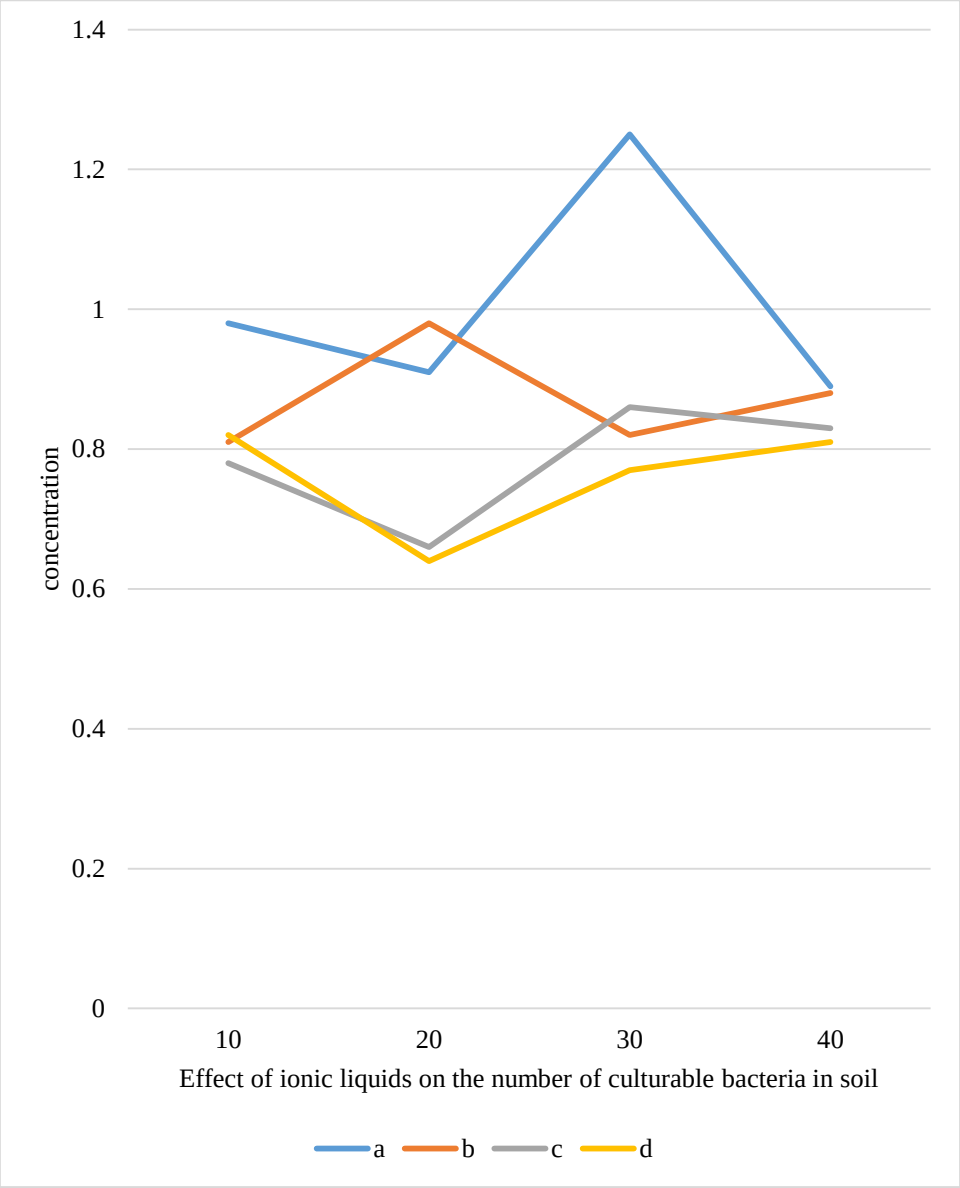

Figure 1. Effect of ionic liquids on the number of culturable bacteria in soil

3.1. Effect of Ionic Liquids on Soil MC Diversity

The structural diversity of soil microorganisms refers to the diversity of microbial communities in the soil in terms of cell structural components, which is the direct reason for the diversification of

microbial physiological functions and metabolic modes. Next, the impact of ionic liquids on the diversity of soil microbial communities was tested, and the test results are shown in table 2 and Figure 2.

Table 2. Changes in the relative abundance of microbial communities in soil

	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4	D1	D2	D3	D4
92	0.18	0.17	0.21	0.23	0.17	0.12	0.21	0.23	0.19	0.21	0.20	0.22	0.15	0.21	0.18	0.18
138	0.36	0.31	0.42	0.43	0.38	0.29	0.42	0.43	0.31	0.32	0.31	0.30	0.31	0.32	0.34	0.39
148	0.73	0.68	0.74	0.75	0.68	0.58	0.72	0.72	0.63	0.65	0.63	0.76	0.65	0.64	0.62	0.61
292	0.89	0.90	0.93	0.96	0.85	0.85	0.89	0.89	0.83	0.82	0.80	0.82	0.83	0.79	0.78	0.79
490	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

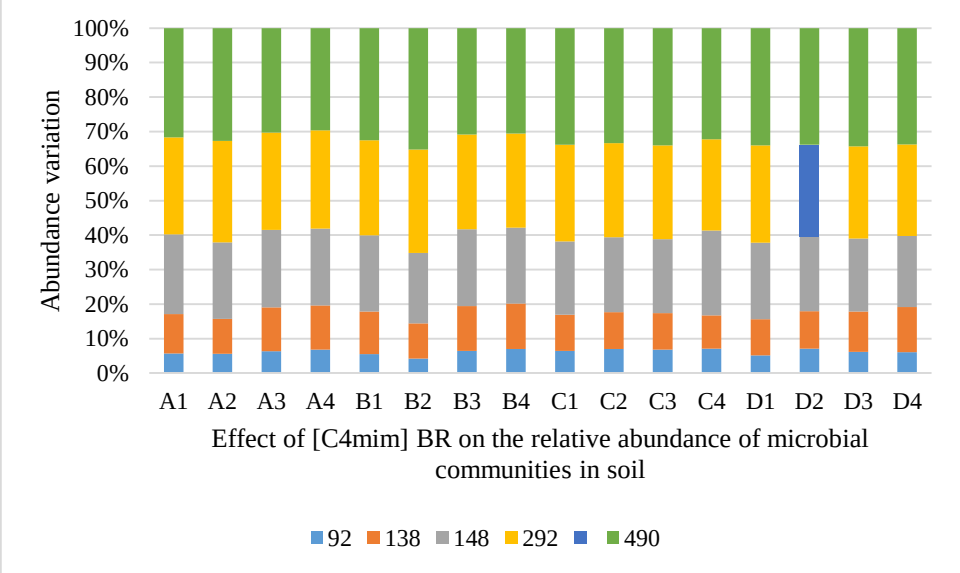

Figure 2. Effect of [C4mim] BR on the relative abundance of microbial communities in soil

It can be seen from the chart that on the 10th day after the addition of ionic liquid [c4mim]br, compared with the control group, the number of bacteria with fragment length of 148 in each concentration treatment group changed significantly, except for the number of bacteria with fragment length of 148. Among them, the relative proportion of bacteria with fragment length of 92, 138 and 400 in the 1mg/kg treatment group decreased, while the number of fragments of 292 increased significantly; The change trend of high concentration treatment group (10mg/kg, 100mg/kg) is similar, the relative proportion of flora with fragment length of 92, 138, 292 increases

significantly, and the number of flora with fragment length of 400 decreases significantly [16-17].

3.2. Effect of [c6mim]Br on Functional Diversity of Microbial Communities in Soil

According to T-RFLP data, the changes of Shannon index and Simpson index in soil exposed to ionic liquid [c4mim]br are analyzed. The results are shown in Table 3.

Table 3. Changes of Shannon index and Simpson index in soil exposed to ionic liquids

Concentration mg/kg	Shannon index				Simpson index			
	10d	20d	30d	40d	10d	20d	30d	40d
0	1.52±0.06a	1.57±0.04a	1.54±0.03a	1.52±0.05a	0.67±0.04a	0.63±0.03a	0.64±0.01a	0.60±0.01a
1	1.51±0.03a	1.55±0.02ab	1.54±0.05a	1.55±0.02ab	0.66±0.08a	0.62±0.02a	0.63±0.02a	0.60±0.2a
10	1.53±0.02a	1.52±0.03b	1.52±0.04a	1.55±0.04b	0.66±0.03a	0.60±0.04a	0.58±0.01b	0.60±0.03a
100	1.51±0.04a	1.55±0.02a	1.53±0.04a	1.56±0.02b	0.66±0.03a	0.61±0.05a	0.60±0.03ab	0.61±0.01a

It can be seen from table 3 that on the 40th day of the experiment, except for the 100mg/kg treatment group, it decreased significantly compared with the control, and did not return to the control level. On the 10th and 20th day of the experiment, the Simpson index in the soil of the ionic liquid treatment group decreased, and there was no significant change compared with the control. On the 30th day of the experiment, the Simpson index of the 1mg/kg treatment group was significantly different from the control; On the 40th day of the experiment, Simpson index of each concentration treatment group decreased, and showed a significant dose-effect relationship. Among them, Simpson index of 100mg/kg treatment group decreased significantly compared with the control group [18].

4. Effects of PP on the Functional Diversity of Soil Microbial Communities

4.1. Effect of Pesticides on Soil Enzyme Activity

When the soil is stressed, compared with the physical and chemical properties of the soil, the reaction of soil enzyme activity is more rapid. Because the soil enzyme activity is directly affected by soil microorganisms, it can more accurately characterize the fertility level of the soil and can be used as an index to predict the change of soil quality. At present, a large number of studies have found that there are significant differences in soil enzyme activity after pesticide application. Through the determination of soil urease and dehydrogenase activities after the application of three pesticides at different concentrations and different time periods, it was found that after the application of fomesafen, soil urease was first inhibited and then stimulated, and the dehydrogenase activity was positively correlated with the concentration of the application. The application of pesticides will significantly affect the activities of soil urease and dehydrogenase, which shows that soil urease and dehydrogenase can be used as indicators to monitor soil quality.

4.2. Effect of Pesticide Application on Functional Diversity of Soil Microorganisms

The functional diversity of soil microorganisms can be fed back through the differences of microbial metabolic functions. The principle of microbial functional diversity is that different microbial communities use a single carbon source differently. Generally, the effect of low concentration pesticides on microbial metabolic activity is short-term and small, while the treatment of high concentration can significantly affect the long-term. This is consistent with the results of this study. The effects of fomesafen, enoxate and mixed pesticides on soil microbial metabolic activity are positively correlated with the application concentration.

The awcd of soil microorganisms after pesticide treatment is significantly different from that of the control group. The reason may be that the addition of pesticides changes soil organic matter and physical and chemical properties, resulting in changes in the living environment of soil microorganisms, which changes the structure of soil MC and affects the metabolic function. In the results of this study, the awcd value of f500 treated microplate showed an "inflection point" when cultured for 3D and X10 microplate for 4D, indicating that at this time, the overall utilization rate of soil microorganisms for different carbon sources in biologeco microplate suddenly decreased, and the analysis may be due to the deterioration of microbial growth at this time

The species richness and species dominance of soil treated with high concentration of fomesafen were higher than those of the control group, because the application of fomesafen increased the number of dominant bacteria in the soil; However, the diversity index of soil microorganisms was significantly reduced by the treatment of high concentration of enoxate and mixed pesticides, because the application of pesticides reduced the total number of soil microorganisms, and the number of microorganisms with strong metabolic activity and the ability to use specific carbon sources was small.

The application of pesticides will significantly affect the physical and chemical properties of soil. Among them, the soil pH of f500, X500 and FX500 in the high concentration treatment group is lower than that in the control group. The analysis may be that the application of pesticides makes the soil environment weakly acidic, which is conducive to the growth of some acidophiles, which is consistent with the results of high-throughput sequencing in this experiment, and this bacterium is an acidophilic bacterium that can both acidophilic and acidogenic, Therefore, the long-term excessive application of pesticides may increase the number of acidobacteria in the soil, gradually reduce the soil pH, cause soil acidification, cause soil hardening, destroy the physical structure of the soil, and cause the loss of soil nutrients. The content of soil organic carbon in the treatment group was significantly higher than that in the control group, while the pH value was lower than that in the control group. The content of soil organic carbon was inversely proportional to the soil pH value, which may be compensated by inorganic carbon in the process of pesticide application. The contents of soil total nitrogen and alkali hydrolyzable nitrogen are in direct proportion to the content of organic carbon. Therefore, when pesticides are applied, the contents of soil total nitrogen and alkali hydrolyzable nitrogen are higher than those of the control. The application of pesticides reduces the soil pH value, the soil nitrification rate and the alkali hydrolyzable nitrogen content, while the soil is weakly acidic and neutral after high concentration pesticide treatment, resulting in the highest availability of soil alkali hydrolyzable nitrogen. At present, the research focus of enoxate and mixed pesticides at home and abroad is still on the weeding effect. The results of this experimental study show that the treatment of enoxate and fomesafen ·enoxate 21% oil suspension will significantly affect the physical and chemical properties of soil, and then affect the microbial diversity of soil.

5. Conclusion

Soil is not only a complex ecosystem, but also a living space for microorganisms. In this paper, the effects of PP on the functional diversity of soil microbial communities were studied and analyzed through microbial culture experiments, and phased results were achieved. However, the soil studied in this experiment is only cultured indoors, and the soil itself is very complex. There are some deficiencies and limitations in the research, which need to be further improved. In the future, we can continue to explore the following aspects: monitoring the changes of soil microbial communities under field conditions to achieve an accurate understanding of soil health; Search for a large number of degrading strains in the dominant flora found by high-throughput sequencing, in order to achieve better degradation effect; Try to study the effect of pesticide application on the growth of subsequent plants in order to apply pesticides more reasonably and reduce pollution in the future.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

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