Nature Environment and Pollution Technology
An International Quarterly Scientific Journal
p-ISSN: 0972-6268 (Print copies up to 2016)
e-ISSN: 2395-3454
Vol. 19 | No. 4 | pp.1443-1452 | 2020
Original Research Paper
https://doi.org/10.46488/NEPT.2020.v19i04.010
Open Access Journal

Effects of Different Tillage Measures on Soil Microbes and Enzymatic Activity

Binglin Huang, Mengxue Wang†, Xinjun Jin, Yuxian Zhang and Guohua Hu
Agronomy College of Heilongjiang Bayi Agricultural University, Daqing, 163319, China
†Corresponding author: Mengxue Wang; wangmengxue1978@yeah.net

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

Received: 15-12-2019
Revised: 28-12-2019
Accepted: 01-03-2020

Key Words:
Tillage
Inter-tillage
Soil microorganisms
Soil enzymes

ABSTRACT

To reveal the effects of different tillage measures on soil microbes, enzyme activities and nutrients, eight different treatments were combined with different tillage methods to study the effects of different tillage measures on soil microbes, enzyme activities and nutrients. The results showed that in the flowering stage, the number of bacteria in the soil was higher than that in PT1 (ploughing + early ridging), RT1 (rotary tillage + early ridge) decreased by 22.6%. PT2 (ploughing + early subsoiling) was more than RT2 (rotary tillage + early subsoiling), PT3 (ploughing + conventional subsoiling) than RT3 (rotary tillage + conventional subsoiling), PCK (rotary tillage + conventional ridging) than RCK (rotary tillage + conventional ridging) increased by 13%, 22.9%, and 3.5%, respectively. The bacteria, fungi and actinomycetes in the whole rotary tillage treatment (rotary tillage) were higher than the tillage treatment (ploughing). Except for the invertase, the urease, phosphatase and catalase activities of the ploughing treatment were higher than those of the rotary tillage. When entering the pod-forming period, the number of soil bacteria, fungi and actinomycetes in rotary tillage were higher than tillage, the soil sucrase activity in rotary tillage was higher than that in ploughing. RT1 was significantly higher than PT1 and PT2 by 11.1% and 11.7%, but the soil urease, phosphatase and catalase activities were higher in ploughing than rotary tillage. The available nitrogen and the available potassium were opposite. At the stage of tillage, the number of bacteria, fungi, actinomycetes, urease activities and sucrase activities in the rotary tillage were higher than ploughing, while the activities of phosphatase and catalase were higher under ploughing. In the mature stage, the soil urease and catalase activities were higher in the tillage treatment, while the phosphatase and sucrase activities were opposite. On the whole, advance inter-tillage improved the soil environment to some extent on both tillage measures, especially T2 (subsoiling).

INTRODUCTION

Soil microorganisms is the generic term for all tiny organisms in the soil that are invisible or unclear to the naked eye, including bacteria, actinomycetes, fungi, etc. (Zhang et al. 2009). Soil microorganisms is the main active ingredient of the soil, gets involved with a variety of metabolic activities (Ryan & Adley 2010), promotes the cycle and transformation of soil organic matters and nutrients, and involves themselves in biochemical processes such as humus formation, so it plays a vital role in the crop productivity and the stabilization of soil ecosystem (Lucas et al. 2015). Produced by the interaction of microorganisms and crop roots, the soil enzyme is also one of the most important active ingredients of soil (Zhao et al. 2015). Getting involved in all biochemical processes of soil, the soil enzyme's activity level reflects the relative intensity of biochemical processes in soil. It can quickly respond to the effects that the short-term tillage measures have on soil quality and reflect the soil quality changes (Sui et al. 2016, Chen et al. 2014). The soil tillage method directly or indirectly affects the physical and chemical properties of the soil, mainly affecting the soil nutrient content and its effectiveness (Wang et al. 2015). The content level of soil nutrients reflects the soil health status and affects the circulation of energy and material in the whole ecosystem, guiding the decomposition and transformation of organic carbon (Yu et al. 2017, Chen et al. 2011), and the soil nutrient contents will directly affect the normal growth and development of crops (Zhang & Wu 2018). Tillage measures are the main factors affecting the soil environment. Soil microorganisms and enzymatic activities are particularly sensitive to their responses. At the same time, microorganisms and various soil enzymes play an important mediation role in the transformation of soil nutrients. Appropriate tillage measures can enrich the diversity of microbial community structures and improve the activities of various enzymes in the soil, thus favourable for the normal transformation and accumulation of soil nutrients, which in turn helps protect the soil environment and increase the crop yield.

As one of the three major black soil belts in the world, with a total area of 7×104 km^2 (Yu et al. 2017), northeast China's soil environment has been seriously damaged due to the unduly pursuit of high crop yields, and improper tillage measures and field management methods, and the organic matters and total nitrogen content in the black soil has been decreasing year after year (Wu et al. 2017). Therefore, to look for a suitable tillage measure is particularly important for keeping the ecological environment of soil. According to the research report, no tillage + straw mulching can increase the organic matters on the soil surface, improve the composition and diversity of soil microbial community, thus optimizing the growing environment of crop roots (Wang et al. 2018). Other researches show that no tillage + straw mulching improves the enzymatic activity of the surface layer in a better way than traditional tillage (Peng et al. 2018). But the long-term no-tillage will increase the soil hardness, reduce the total porosity of the soil, and affect the growth of aerobic microorganisms and roots. The studies where the protective tillage, combined with other technical measures influence the ecological environment of soil and crop yields are frequently reported, but there are fewer reports on traditional farming combined with other technical measures.

In this study, a test was conducted in Heshan Farm Science Park, Heihe City, Heilongjiang Province to learn the effects of different tillage methods combined with different inter-tillage methods on soil microorganisms and enzymatic activities. The problems to be solved in this study are: (1) the effects of different tillage methods on soil microorganisms and enzymatic activities, (2) the effects of the same tillage methods and different inter-tillage methods on soil microorganisms and enzymatic activities, (3) according to the effects of different tillage measures on soil microorganisms and enzymes, the tillage and inter-tillage measures that are appropriate for the area can be determined.

MATERIALS AND METHODS

Overview of the Experimental Site

The experiment was carried out in Heshan Farm Science Park, Heihe City, Heilongjiang Province (48°43'N-49°03'N, 124°56'E-126°21'E), where the annual average temperature is ≥10°C, the annual effective accumulative temperature is from 2000 to 2300°C, and the frost-free period is 115-120 days, belonging to the continental climate in the cold temperate zone, where the rainy season is mostly concentrated in summer, with an annual rainfall of 500-600mm. The local soil type is dominated by the black soil, the cultivated land is faintly acidic, with the fertile soil and the balanced soil fertility. The 0-20cm basic physical and chemical properties in the experimental site are as given in Table 1.

Experiment Design

The experiment was carried out from October 2017 to October 2018, adopting the split-plot experiment design (Table 2). Among them, the main factors were ploughing and rotary tillage and the different inter-tillage methods were the second factors. That was, two different tillage methods were designed in the early period (the crop straws were returned to the field): Ploughing (P) and rotary tillage (R). After sowing, each tillage method was equipped with 4 different inter-tillage measures. As a whole, the comprehensive setting was conducted from different depths of deep scarification, different deep scarification and earthing up time, different deep scarification and earthing up number, a total of 8 treatments were designed, as given in Table 2. Deep scarification refers to a technique in which the deep scarification machines

Table 1: Basic physical and chemical properties of the soil of the test site.

Bulk weight ($g.cm^{-3}$)	Available nitrogen ($mg.kg^{-1}$)	Available phosphorus ($mg.kg^{-1}$)	Available potassium ($mg.kg^{-1}$)	Organic matter ($g.kg^{-1}$)	pH
1.19	138.9	20.79	179.35	14.3	6.25

Table 2: Different tillage methods.

Tillage measures	Treatments	Stage			
		4-5 days after the broadcast	V2-V3 stage	V4-V5 stage	V6-V7 stage
	PT1	Soil dressing		Middle ridging	Large banking up
Ploughing	PT2	25-30 cm subsoiling		30-35 cm subsoiling + Mid-banking up	Large banking up
	PT3		25-30cm Subsoiling		Large banking up
	PCK		Soil dressing	Mid-banking up	Large banking up
	RT1	Soil dressing			Large banking up
Rotary tillage	RT2	25-30 cm Subsoiling		30-35 cm Subsoiling + Mid-banking up	Large banking up
	RT3		25-30 cm Subsoiling		Large banking up
	RCK		Soil dressing	Mid-banking up	Large banking up

and tools are drawn by a cultivator to loosen the soil at different depths, improve the structure of the plough layer, and enhance the capacity of soil moisture conservation and drought resistance and drainage. Soil dressing: The width of the cultivator earthing knife is 20-30°. Mid-banking up: The width of the cultivator earthing knife is 80-90°, and the large banking up: The width of the cultivator earthing knife is 110~120°. Three times of earthing inter-tillage (common mode in rural areas) are set as contrast (CK).

The soybean was sowed with fertilizer on May 4, 2018. The local main variety Heihe 43 provided by the local seed company was selected. Each row area was 45 m, the row width was 62.5 cm, 8-row areas, repeated twice, and the harvest occurred on September 28, 2018. The preceding crop was corn, and the fertilization amount was consistent with the local fertilization level. There was 54 $kg \cdot hm^{-2}$ of pure nitrogen fertilizer, pure P_2O_5 67.5 $kg \cdot hm^{-2}$ and pure K_2O 30 $kg \cdot hm^{-2}$, and the other field managements were consistent with the local production.

Soil Sampling and Analysis

Soil sampling: The soil sampling was conducted 4 times during the flowering period, pod-setting period, seed filling period, and mature period of soybean. 0-20 cm soil was sampled between the rows with a soil auger with a diameter of 5 cm. Six points were sampled randomly in the shape of "S" in each plot, with crop residues and stones removed, finally they were mixed evenly and put into the sealed pockets and immediately brought to the laboratory for the treatment. Some soil samples were stored under 4°C for the measurement and analysis of soil microbial quantity, others were air-dried, ground and sieved out from the soil by a 2-mm soil screen (organic matters sieved by a 0.25 mm soil sieve) to remove straw residues and plant roots, and finally, they were used to measure the soil enzymatic activity and soil nutrients.

Analysis of soil enzymatic activity: The determination of soil enzymatic activity is based on the method of Guan Songyin (Bao 1999). The activity of invertase was determined by 3,5-dinitro salicylic acid, expressed as the quantity of mg of glucose in 1 g of soil after 24 hours. The activity of urease was determined by sodium phenol colourimetry, expressed as the quantity of mg of ammonia nitrogen released from 1 g of soil after 24 hours. The phosphatase activity was determined by the disodium phenyl phosphate colourimetric method, expressed as the quantity of mg of phenol released in 1 g of soil after 24 h. The soil catalase activity was determined by the permanganimetric method, and all the treatments were repeated thrice.

Analysis of soil microbial quantity: The soil microorganisms were inoculated by spread-plate method (Pan 2015). The fungus was cultivated in Martin medium and its quantity was determined. The composition of the medium was 10 g of glucose, 5 g of peptone, 1 g of KH_2PO_4. 0.5 g of $MgSO_4.7H_20$, 1 L of distilled water, 18 g of agar, 3.3 mL of 10 g/L Bengal red solution. 0.3 mL of 10 g/L streptomycin solution was added into per 100 mL culture medium, at the using time after sterilization. The actinomycetes were cultivated in the modified Gao's No.1 culture medium and its quantity was determined. The composition of the medium was starch 20 g, KNO_3 1 g, K_2HPO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, NaCl 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, distilled water 1 L, agar 18 g, pH 7.2-7.4 at the using time after sterilization. 0.3 mL of 30 g/L $K_2Cr_2O_7$ solution was added per 100 mL of medium to inhibit the growth of fungi and bacteria (7.2-7.4). The bacteria were cultivated in the beef extract-peptone medium and then their quantity was determined (Medium composition: beef extract 3 g, protein 5 g, distilled water 1 L, agar 18 g, pH 7.0-7.2). All the treatments were repeated thrice. The fungus was cultivated under 28°C for 3 to 4 days, the bacteria were cultivated at 28°C for 2 to 3 days, and the actinomycetes were cultivated under 37°C for 6 to 8 days. After the completion of the cultivation, the number of bacterial colonies was determined by the plate count. Computational formulas are as follows:

CFU (forming unit of bacterial colonies/mL) = 3 repeated average quantity of bacterial colonies × dilution ratio.

Data Analysis

Data processing was conducted by Excel 2010, and the effects that each treatment has on soil microbial quantity and enzymatic activity are compared using one-way variance (ANOVA). All statistical analyses are completed by SPSS 17.0 and the plotting adopts Origin 2018.

RESULTS AND ANALYSIS

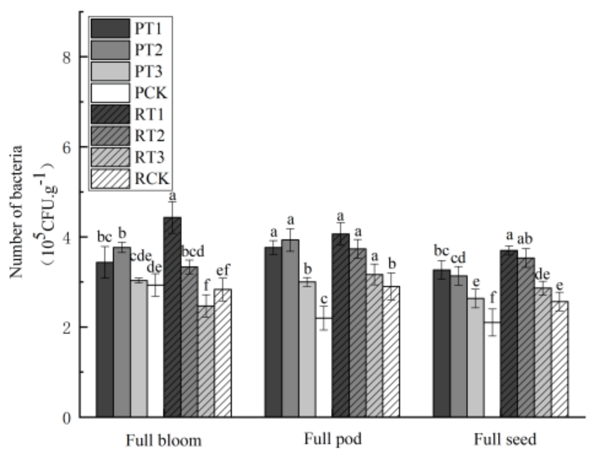
Effects of Different Tillage Measures on Soil Microbial Quantity

Effects on the bacterial population: As shown in Fig. 1, the soil bacterial population reaches 10^5 orders of magnitude. Different tillage measures have significant effects on bacteria. In the flowering period, T1 and T2 are significantly higher than CK with the same tillage method, and T3 is less significantly different from CK. Among them, PT1 and PT2 are significantly higher than PCK by 17.05% and 28.41%. RT1 and RT2 were significantly higher than RCK by 56.48% and 17.65%. PT2 is higher than PT3 by 24.47% and RT2 is higher than RT3 by 35.16%, indicating that the deep scarification or earthing up in advance contributes to the increase of the soil bacterial population. In the pod-setting period, except for

PCK and RT1, the bacterial population treated respectively increased in some degree, and in the same tillage method, T1, T2 and T3 were significantly higher than CK, which was consistent with that in the flowering period. In the seed filling period, the bacterial population treated respectively tends to decrease to some degree, compared with the previous period. And with the same tillage method, the bacterial population is consistent with the previous trend, i.e., T1 and T2 are significantly higher than T3 and CK treatment. In the same inter-tillage method, the bacterial population treated by the rotary tillage is higher than that treated by the ploughing. Among them, RT1 is increased by 13.29% compared with PT1, and RT2 is increased by 12.78% compared with PT2, both of which reach a significant level, indicating that the rotary tillage combined with other tillage measures is more conducive to bacterial survival, and RT1 has been kept at a higher level. This may be caused by the earthing up ahead of time in the RT1 treatment, increasing the soil temperature during the flowering period, and creating a more suitable soil environment for the bacteria.

Effect on the number of fungi: As shown in Fig. 2, the soil fungi number reaches 10^4 orders of magnitude. In the flowering period, the fungi number in the rotary tillage was higher than that in the ploughing on the whole. By the same tillage, T1, T2 and T3 all show a decreasing trend. In the ploughing, PT1, PT2 and PT3 are decreased by 12.71%, 21.82% and 14.48%, compared with PCK. RT1, RT2, RT3 are significantly lower than RCK by 20.91%, 47.76% and 28.35% respectively. The fungi number by the earthing-up treatment with the two ploughing methods (T1, CK) is higher than that of deep scarification (T2, T3), indicating that the deep scarification is more conducive to reducing the fungi number in the soil and creating a better soil environment for the soil than earthing up, in the pod-setting period and seed filling period, the two have the same variation tendency. In the same cultivating conditions, the fungi number produced by ploughing is lower than that produced by the rotary tillage, and both tillage methods show CK>T1>T3>T2. In the seed filling period, PT1 is decreased by 12.67% compared with PCK, while RT1 is decreased by 4.69%, compared with RCK. Compared with PT3, PT2 is decreased by 17.91%. Compared with RT3, RT2 is decreased by 27.63%, which is similar to the result in the flowering period, showing that deep scarification can reduce the soil fungi number and make the soil microbial community structure more reasonable.

Effect on the actinomycete number: As shown in Fig. 3, the soil actinomycete number reaches 10^5 orders of magnitude. In the flowering period, the soil actinomycete number treated by the tillage is higher than that treated by the rotary tillage. In the tillage, PT1, PT2 and PT3 are significantly increased by 32.5%, 37.68% and 27.3% compared with PCK. In rotary tillage, RT1 and RT2 are less different from RCK, RT2 is significantly higher than RCK by 50%. In the seed filling period, the two tillage methods have the same trend, i.e., T2>T1>T3>CK. In ploughing, PT1, PT2 and PT3 are significantly higher than PCK by 28.4%, 38.62% and 11.36%. Similarly, in rotary tillage, RT2 is significantly increased by 17.61% compared with RCK, while RT1 and PT3 are less significantly different from RCK, but the actinomycete number treated by the rotary tillage is higher than that treated by the ploughing, which may be because the rotary tillage has less soil disturbance than ploughing. In the



Note: Different letters indicate significant difference among treatments (P < 0.05).

Fig. 1: Effects of different tillage measures on soil microbial population.

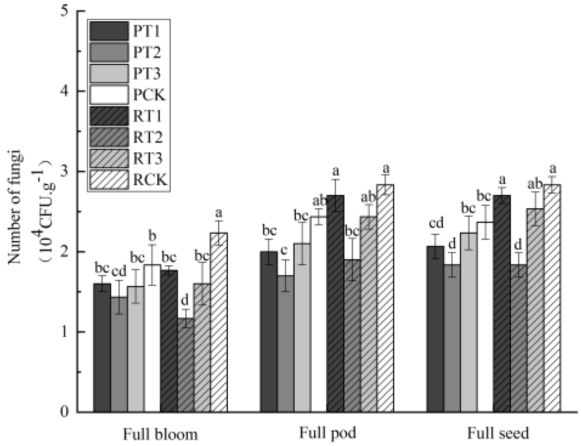


Fig. 2: Effects of different tillage measures on soil fungal population.

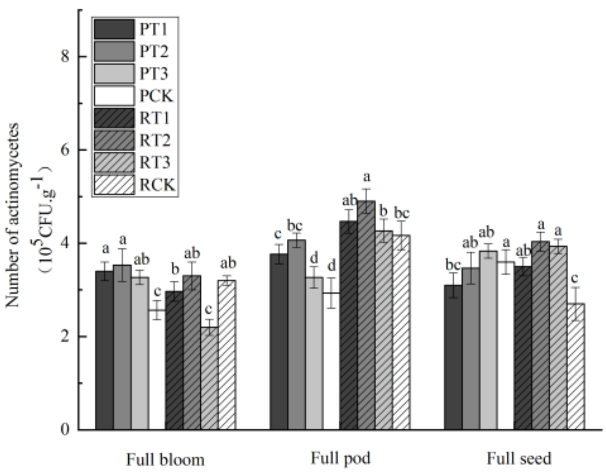


Fig. 3: Effects of different tillage measures on soil actinomycetes population.

seed filling period, the actinomycete number with the two tillage methods is decreased compared with the pod-setting period, especially PT1 and PT2 are decreased significantly compared with those in the previous period, both of which are lower than PCK. In rotary tillage, RT1, RT2 and RT3 are significantly higher than RCK by 29.63%, 49.37% and 45.66%. In general, the actinomycete number treated by the rotary tillage is higher than that treated by the ploughing in the later period, which is consistent with bacteria but opposite to the fungus, indicating that the rotary tillage may be more conducive to creating a more reasonable soil microbial community structure.

Effects of Different Tillage Measures on Soil Enzymes

Effect on soil urease: Soil urease is often an important indicator used to characterize the soil nitrogen supply. As shown in Fig. 4, different tillage measures have a significant impact on it. During the flowering period, both tillage measures show a consistent variation trend (T2>T3>T1>CK). In the same tillage, PT2 is increased by 15.91%, 3.99% and 14.14% compared to PT1, PT3 and PCK. Compared with RT1, RT3 and RCK, RT2 is increased by 6.81%, 5.64% and 8.22%. Generally, the urease activity by the tillage is higher than that by the rotary tillage, which may be because the ploughing improves the soil's respiratory ability, accelerates the metabolism between rhizosphere and microorganisms, and increases the rate of secretory urease. During the pod-setting period, the basic trends of the two tillage measures are the same, T1 and T2 are higher than CK, and the urease activity of each treatment is generally improved. In the seed filling period, the urease activity of each treatment in rotary tillage has no significant difference. But the urease activity treated

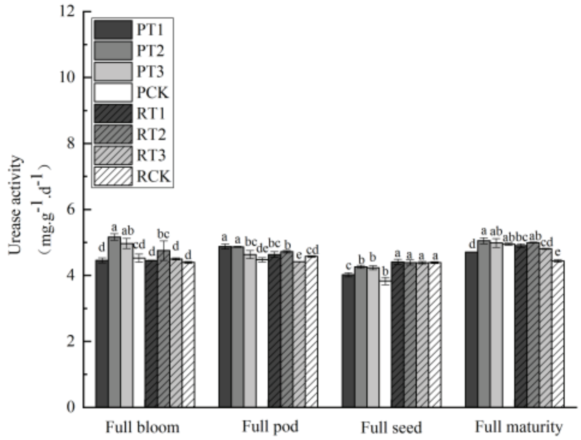


Fig. 4: Effects of different tillage measures on soil urease activity.

by rotary tillage is significantly higher than that treated by the ploughing; the urease activity in the mature period of soybean is increased compared with that in the seed filling period. That may be because the soybean roots break away from the putrefactive root nodules, and then release the rhizobia, which increases the activity of soil urease. Moreover, the urease activity treated by PT2 and RT2 is high all the time, indicating that the deep scarification is positively significant in improving and keeping the soil urease activity.

Effects on soil phosphatase: Soil phosphatase has a positive effect on the increase of the availability of soil phosphorus. As shown in Fig. 5, soil phosphatase activity gradually increases with the progress of soybean growth period. In the flowering period, the soil phosphatase activity treated by ploughing is higher than that treated by the rotary tillage but the differences are not significant. Similarly, whether treated by the ploughing or the rotary tillage, the treatment difference with the same tillage measures is not significant. In the soybean pod-setting period, except for RT1 and RT2, the phosphatase activity treated by each method is improved significantly. In the seed filling period, the soil phosphatase activity increases greatly, and the treatment difference is significant. Among them, the soil phosphatase activity treated by the tillage is higher than that treated by the rotary tillage, but the changing trend treated by each method is similar. That is, T2>T3>T1>CK. In the tillage, PT1, PT2 and PT3 increase by 11.26%, 53.62% and 26.38%, reaching a significant level compared with PCK. In rotary tillage, RT1, RT2 and RT3 increase by 5.96%, 25.5% and 12.21% compared with PCK. In the mature period, the variation trend in this period is consistent with that in the seeding filling period, and the phosphatase activity reaches the highest value. What differs from the seeding filling period is that the phosphatase activity treated by the deep scarification is lower than that treated by the earthing up, probably it is because the deep scarification improves the total porosity of the soil, the

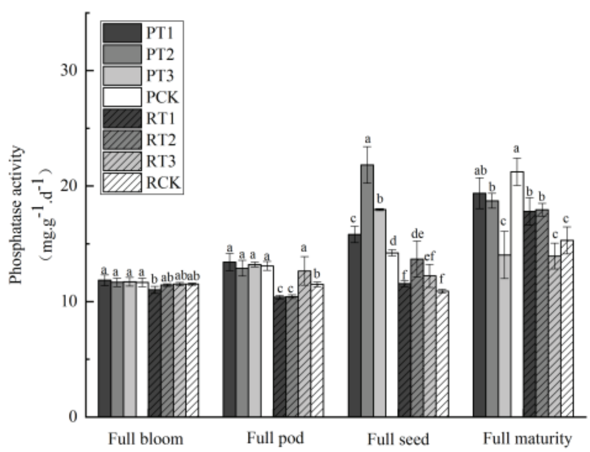


Fig. 5: Effects of different tillage measures on soil phosphatase activity.

thermal conductivity of soil decreases, causing the surface soil temperature to be closer to the surface temperature, thus affecting the soil phosphatase activity.

Effect on soil invertase: Widely distributed in soil, the soil invertase is often used to characterize the degree of soil maturity. It can catalyse and boost the sucrose in the soil to hydrolyse into glucose and fructose. As shown in Fig. 6, the soil invertase is significantly affected by the tillage methods. In the flowering period, the invertase activity gained by the ploughing is higher than that gained by the rotary tillage. The difference between two tillage methods may be caused because the crop stalks are returned to the field and the ploughing makes the soil respiration and the mineralization rate of organic matter stronger than those gained by the rotary tillage, allowing roots and microorganisms to secrete more active enzymes. By ploughing, PT1 and PT2 are significantly higher than PCK by 10.88% and 14.77%, respectively. PT1 and PT2 are both significantly higher than PT3, but the difference between PT1 and PT2 is not significant. By the rotary tillage, RT1 is significantly increased by 10.77 %, compared to RCK. RT2 is increased significantly by 19.92%, 27.92% and 31.19%, compared to RT1, RT3 and RCK, indicating that the advanced inter-tillage is beneficial to the increase of the activity of soil invertase, especially the effect through the advanced deep scarification is more significant. The variation tendencies in the pod-setting period, the seed filling period, and the mature period are basically similar. Both tillage measures show T2>T1>T3>CK. Under the same inter-tillage conditions, the invertase activity gained by the rotary tillage is higher than that gained by the ploughing, which may be related to a higher mineralization rate of organic matter in the flowering period. A large quantity of organic carbon is mineralized and decomposed, which causes the soil sucrose to be decreased relatively, thus the invertase activity is indirectly reduced. Especially in the mature period, the activity of invertase treated by the ploughing is lower than that treated by the rotary tillage. Among them, RT2 is increased by 27.56%, compared to PT2; RT1 is increased by 27.46%, compared to PT1, RT3 is increased by 29.47%, compared to PT3; and RCK is increased by 24.02%, compared to PCK, and all of them reach a significant level, indicating that the rotary tillage is more conducive to the improvement of invertase activity.

Effect on soil catalase: Catalase can transform hydrogen peroxide into H_2O and O_2 through the enzymatic reaction, thereby removing the harm of hydrogen peroxide to soil and soybean roots. Therefore, the activity level of catalase seriously affects the soil environment and the health of soybean roots. As shown in Fig. 7, two different tillage measures have a significant effect on catalase. Generally speaking, in the same inter-tillage treatment, the catalase activity treated by the ploughing is higher than that treated by the rotary tillage. In the flowering period of soybean, through the same tillage method, on the whole, each treatment is not significantly different from CK, and the soil catalase activity treated by each method is higher. It is probably because the soybean roots grow and develop more quickly in the previous and middle period of soybean growth. The root metabolism is relatively strong, causing an increase of secretions. The metabolic activities of microorganisms and enzymes are active during this period, which leads to an increase in soil catalase activity. In the seed filling period, through the same tillage treatment, T1 treatment is significantly higher than other treatments. Among them, PT1 is significantly higher than PCK by 25.7%, RT1 is significantly higher than RCK by 10.3%, and the difference through other treatments are

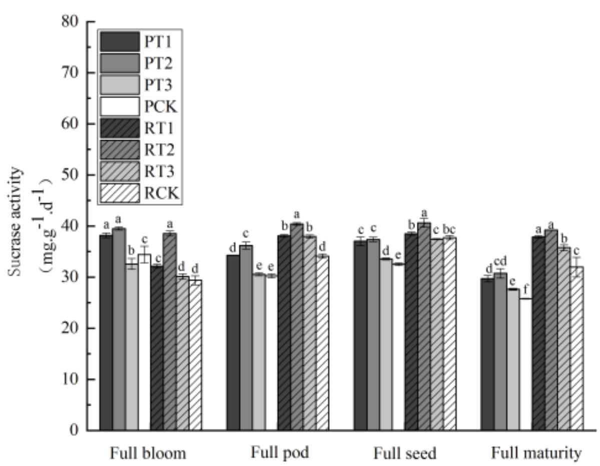


Fig. 6: Effects of different tillage measures on soil sucrose activity.

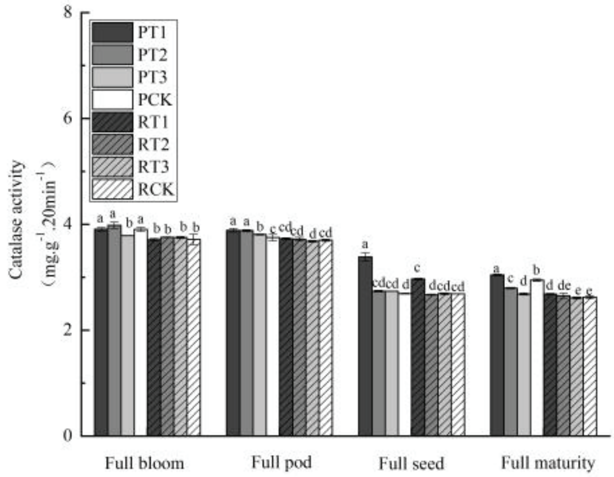


Fig. 7: Effects of different tillage measures on soil catalase activity.

not significant. In the mature period, the changing trend of soil catalase is the same as that in the seed filling period, and the soil catalase of PT1 and RT1 has a higher activity, which may be caused by the fact that, with the abundant rain this year, the soil treated by deep scarification (T2, T3) retains more water, which is not conducive to the metabolism of roots and soil microbes, resulting in the decrease of the activity. However, the earthing up treatment (T1, CK) just avoids this situation. The fact that PT2 and RT2 (addition of soil for V4 to V5) are higher than T3 can further indicate that the earthing up can increase the soil catalase activity.

DISCUSSION

The soil microbial quantity, soil enzyme activity and nutrient have a great impact on the growth and development of soybean (Wei et al. 2018, Gai et al. 2019), and different farming measures will all affect greatly the soil environment (Zhang et al. 2019). Soil microbes are the main decomposers of soil micro-environment, and play a positive role in the transformation of soil nutrients and increase of soil moisture retention (Wei et al. 2018, Gai et al. 2019, Zhang et al. 2019, Li et al. 2018). A study by Vida et al. (2016) shows that the rotary tillage is more conducive to the increase of soil microbial quantity than the ploughing, and the microbial community structure can be improved. The study of Zhang (2018) indicates that deep scarification helps to improve the living environment of soil microbes, thereby contributing to the increase of soil microbial quantity, and thus affecting the improvement of genetic and functional diversity of soil microbes. Similarly, this experiment shows that in the flowering period of soybean, the quantity of soil bacteria and actinomycetes by the ploughing is higher than that by the rotary tillage. But the fungi hold the opposite direction, this is because, in the early period of soybean growth, the ploughing can cause the soil gap big and the soil temperature and humidity to be increased. Therefore, the mineralization rate of soil organic carbon is increased, which is beneficial to the survival of microorganisms. After entering the pod-setting period and seed filling period of soybean, the quantity of soil bacteria, fungi and actinomycetes produced by the rotary tillage is higher than that of actinomycetes. It is because the rotary tillage has less soil disturbance than the ploughing, and the mineralization speed of organic carbon through the ploughing in the early period is too fast. The relatively loose soil layer increases the soil moisture and weakens the circulation of soil air. Therefore, the rotary tillage can provide a more stable habitat, which is inconsistent with the study of Jia et al. (2018), maybe which is caused by the differences in rainfall and other issues in different years. Similarly, through the same tillage method, the microorganisms treated by the advanced inter-tillage (T1, T2) are higher than CK, which is because the advanced deep scarification or earthing up can increase the soil temperature in the soybean seedlings period and achieve the purpose of soil moisture conservation. Therefore, it provides a suitable habitat for the soil microorganisms. On the other hand, the quantity of microorganisms treated by the deep scarification (T2, T3) is also higher than that treated by the earthing up (T1, CK). It is because the deep scarification can change the surface weeds into the soil layer, which not only improves the permeability of the soil but also provides more nutrients for the soil microorganisms, contributing to the increase of the number of soil microorganisms.

The soil enzymes get involved in the circulation of materials and the flow of energy in the soil environment. The activity level of soil enzymes reflects the relative intensity of various biochemical processes in the soil (Martin et al. 2006). Its activity is one of the important indicators to comprehensively evaluate the change of soil quality and detect the activity and quantity of soil microorganisms (Zhou et al. 2018, Li et al. 2018). The studies of Li et al. (2018) indicate that, compared with the ploughing, the rotary tillage can increase the soil enzymes in the soil surface layer (0~20 cm), but for the soil enzymes in the 20~40 cm soil layer, the case is opposite. The studies of Ding et al. (2017) and others show that the rotary tillage combined with other technical measures increases the activity of soil enzymes, compared with ploughing. But the study of Zhang & Wu (2018) shows that the activities of soil urease, phosphatase, invertase and catalase treated by the ploughing are higher than those treated by the rotary tillage, which is the same as the results of this experiment. In this experiment, except for the invertase, the enzymatic activities of soil urease, phosphatase and catalase in each period all indicate that the ploughing > rotary tillage, which may be caused by the fact that the ploughing can turn the preceding crops and other plant residues into the deeper soil layer, which not only increases the permeability and fertility of the soil, but also breaks the plough sole to create greater habitat for soybean roots and microorganisms, thus contributing to the improvement of soil enzyme activity. The invertase shows an opposite rule because invertase is often positively related to the content of soil organic matter. The soil ploughing increases the mineralization rate of soil organic carbon, accelerates soil respiration, decreases the content of organic matter, which reduces the catalytic substrate of the invertase, resulting in a decrease in the activity. The study of Zhang (2017) shows that the soil deep scarification in different tillage measures increases the activity of soil enzyme, but the catalase activity decreases, which is consistent with the experimental result. Similarly, in the experiment, under the same farming conditions, except for catalase, the enzyme activity through the deep scarification treatment is also improved to some extent, compared to the earthing up treatment. Luo (2009) also has a similar report. It is probably because the deep scarification reduces the unit density and hardness of the soil, making the damp heat condition of soil more reasonable, and thus provides a better growing environment for soybean roots and soil microorganisms, which in turn makes the roots and the metabolic activities of microbes more active, and finally, the enhancement of organisms' secretion increases the enzyme activity. The experiment also shows that the enzyme activity gained by the advanced inter-tillage (T1, T2) is increased more greatly than that gained by the corresponding treatment (CK, T3), indicating that the advanced inter-tillage and the "release the coldness" and the soil moisture conservation in the early period are beneficial to the rooting of soybeans and also play a positive role in the improvement of the soil environment.

CONCLUSIONS

The tillage method affects the soil environment greatly, and then the growth and development of the soybean are affected, ultimately affecting the increase in yield. The frequent disturbance to the soil is not conducive to the growth of microorganisms, resulting in a decrease of the bacteria, fungoid and actinomycetes in the soil. Among them, the quantity of the bacteria, fungi and actinomycetes through the ploughing and treatment of PT1 in the maturity period reduces respectively by 11.7%, 23.4% and 11.4%, compared with the treatment of RT1 by the rotary tillage.

Compared with the microorganism, except for the invertase, the ploughing increases the activity of various enzymes in the soil than the rotary tillage, as well as the activities of the urease, phosphatase and catalase. PT2 treated by ploughing increases by 1.12%, 4.32%, 5.47% respectively than that treated by the rotary tillage in the mature period, but the invertase PT2 reduces by 21.6%, compared to RT2.

Besides, the advanced inter-tillage improves the soil environment significantly, the number of the soil microorganisms and the activity of enzymes are improved in different degrees, at the same time, deep scarification has a greater improvement than soil heaping, especially deep scarification reduces the number of the soil fungi and increases the number of bacteria, a more rational soil microbial community structure is created.

Therefore, no matter which tillage method is used, the properly advanced inter-tillage (especially deep scarification) after sowing can be conducted, which will have a positive effect and significant role in the improvement of the soil environment.

ACKNOWLEDGMENTS

This work was supported by the "National Key R＆D Projects" (2018YFD1000905), "Natural science foundation of Heilongjiang province"（C2017049), "Key Scientific Research Projects of Heilongjiang Farms and Land Reclamation administration (HKKY190206-1)" and "School start-up plan" (XBD-2017-03).

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