Analysis of Physicochemical and Biological Characters by Applying Mushroom Substrate into Soil in Cold Region

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Keywords: Mushroom Substrate, Soil Physicochemical.

Abstract: After soil reclamation in cold regions, the basic fertility, tillage, and biological activity of creatures all decrease. As a waste of edible fungus medium, the mushroom substrate contains quite a lot of nutrients that are beneficial to the growth of crops, which are capable of soil improvement and cultivation and make a promotion to yields. Therefore, it is of great significance to research the application of mushroom substrate to improve the physicochemical and biological characteristics of soil in a cold region. The experiment was implemented in 2016 as a randomized block design with micro plots. The soil hardness of 0 - 20 cm horizon, the ratio of three phases, field capacity, soil nutrients content, microbe amounts, soil enzymatic activity, and the influence of yield are discussed. In these tests, the writer added black mushroom substrate into plowing horizon soil at different rates: 0 kg m^{-2} (CK), 2.5 kg m^{-2} (T1), 5 kg m^{-2} (T2), 7.5 kg m^{-2} (T3), and 10 kg m^{-2} (T4). The results show that the addition of mushroom substrate can decrease the penetration resistance of soil, improve soil three-phase, increase soil fertility and activate the activity of soil microorganisms, earthworms, and soil enzymes, and thus increase crop yield. Compared with CK, the potato production correspondingly increased by 12.82% (T1), 27.90% (T2), 50.74% (T3), and 63.41% (T4). The treatments with the mushroom substrate at 7.5 kg m^{-2}, and 10 kg m^{-2} stand out in improving soil physicochemical properties and activating the activity of soil microorganisms.

1 INTRODUCTION

Logs were traditionally used as raw material to cultivate edible fungus. At present, logs are replaced by straw, rice husk, sawdust, and other raw materials, which will be edible fungus hypha residues and compounds of crude fibers decomposed by edible fungi and qualitatively changed after the harvest of edible fungus. These compounds are known as edible fungus cultivation waste, residue, or oddment, which is referred toas the mushroom substrate in this article. China ranks first in total production and export of edible fungus in the world, and the annual production of edible fungi accounts for more than 75% of the world's total output (Zhang et al., 2016; Deng 2016), which results in an enormous amount of fungus waste. It is calculated that about 5kg of the mushroom substrate can be produced by each 1kg of edible fungus (Nakatsuka et al., 2016; Lau et al., 2003). After grain, vegetable, fruit tree, and oil production, the edible fungus is the fifth largest industry in China's agricultural industry. Traditional ways of dealing with edible fungus are wasting or burning them, which not only waste agricultural organic resources but also seriously pollute the surrounding environment (Lou et al., 2016; Zhang et al., 2017). The pressure on the economy and environment caused by the mushroom substrate in agricultural and forestry areas has become an urgent problem.

The utilization ratio of the nutrients in the medium is about 70%, so it remains a large number of edible mycelia in the mushroom substrate after the

Meng, Q., Zhu, B., Zhang, C., Wang, N., Feng, H., Yang, X. and Li, C.
Analysis of Physicochemical and Biological Characters by Applying Mushroom Substrate into Soil in Cold Region.
DOI: 10.5220/0011596500003430
In *Proceedings of the 8th International Conference on Agricultural and Biological Sciences (ABS 2022)*, pages 83-91
ISBN: 978-989-758-607-1; ISSN: 2795-5893

harvest of edible fungus, including organic matters, nitrogen, phosphorus, potassium, and other nutrients needed by crops (such as protein, crude fiber, fat, amino acid, many vitamins, microelements, and special enzymes) (Jordan et al., 2008; Medina et al., 2012; Roy et al., 2015). Apart from the existing beneficial nutrients, the mushroom substrate is loose, permeable, and can preserve water and fertilizer. Due to the special physicochemical properties of mushroom substrate, the main reusing ways are making it into medium (Liu et al., 2016; Picornell et al., 2016; Li et al., 2015; Mao et al., 2015), organic fertilizer (Paredes et al., 2016; Hidayat 2017; Cao et al., 2017; Zeng et al., 2015), feed (Rangubhet et al., 2017; Li et al., 2016; Hassan et al., 2014; Seok et al., 2016), soil improver or restorer (Shi et al., 2014; Wang et al., 2017).

Heilongjiang province is the third largest edible fungus production base in China after Henan and Shandong provinces. There is a growing threat caused by enormous fungi to the economy, especially the ecological environment in agricultural and forestry areas. As an agricultural area in a cold region, parts of cultivated land of the Sanjiang plain in the eastern part of this province saw a reduction in tillage land, and soil fertility declined, which were caused by many improper methods of tillage, such as continuous cropping, single management, and extensive farming. Water and soil erosion, soil acidification, soil pollution, and other problems stand out (Zhou 2015; Zhang et al., 2014; Yang et al., 2017). To solve the soil problems, improve soil quality and restore soil productivity in this area, the researches on soil improvement have received wide attention.

Many reports on the use of mushroom substrate mainly focus on soil physicochemical properties. By adding different proportions of the black mushroom substrate to the soil to improve soil, the writer aimed at studying soil physicochemical properties, as well as soil microorganisms, animals, and enzymes, which provide a comprehensive reference basis for comprehensively improving soil, increasing soil fertility, and improving the economic efficiency of crops.

2 MATERIALS AND METHODS

2.1 Experimental Site

Located in Jiamusi branch of Heilongjiang academy of agricultural sciences (130° 24' 29.5056" E, 46° 47' 29.9328"N) in Sanjiang plain, China, the test site is subjected to the temperate zone continental monsoon climate, with rain and hot over the same period, and the annual average temperature is 3 ℃. Here, winter is long and summer is short, and the annual average precipitation is 527 mm.

2.2 Experimental Design

The test began in 2019. The tested soil was meadow soil. The basic physicochemical properties of soil were the organic matter of 31.41 g kg^{-1}, total nitrogen of 1.10 g kg^{-1}, total phosphorus of 1.68 g kg^{-1}, total potassium of 26.26 g kg^{-1}, and the pH was 6.17.

The component of the mushroom substrate used for test was black mushroom substrate. Its main components were: wood chips 53%, wheat bran 15%, corncob 30%, gypsum 1%, and lime 1%. The basic physicochemical properties of the mushroom substrate are shown in table 1.

Table 1: Basic physicochemical properties of the mushroom substrate.

Total nitrogen (g kg^{-1})	Total phosphorus (g kg^{-1})	Total potassium (g kg^{-1})	Organic matter (g kg^{-1})	pH
9.47	2.09	1.89	216.5	6.2

The mushroom substrate was added in five different treatments, and the amounts (dry weight) were 0 kg m^{-2} (CK), 2.5 kg m^{-2} (T1), 5 kg m^{-2} (T2), 7.5 kg m^{-2} (T3), and 10 kg m^{-2} (T4). Randomly distributed, they were tested in small areas and each is 6 m^{-2}, and repeated 3 times. A 50 cm isolation belt was set between two areas. The mushroom substrate was fully mixed with soil (0 - 20 cm), which was shown in Figure 1.

Notes: 1-a The mushroom substrate; 1-b The mushroom substrate covered soil; 1-c The mushroom substrate and the plowing horizon soil were fully mixed.

Figure 1: The mushroom substrate was added to the soil.

Growing crops: potato (Netherlands 14). The row spacing was 80cm and the plant distance was 18cm. Fertilizer: base fertilizer and potato special fertilizer (N: P: K=15: 12: 13) 800 kg hm^{-2}. The mixed fertilizer was well dug in the space between every two lines, supplied with natural precipitation, and the test areas were managed like normal fields. The potato special fertilizer was used at 800 kg hm^{-2} when the crops were in the growing period.

2.3 Soil Sample Collection and Measurement Method

2.3.1 Soil Sample Collection

After removing plant residues and stones, plowing horizon soil (0 - 20 cm) samples were collected in the serpentine sampling method in the autumn of 2016 during the potato maturation period. Fully mixed and sifted with a sieve (size: 2 mm), the soil was put in the aseptic bags to be used for the measurement of soil nutrients, soil enzymes, and the number of soil microorganisms. The plowing horizon soil samples collected in the ring knife collection method were to be used for the measurement of soil three-phase. The soil samples collected from different depths (0 - 5 cm, 5 - 10 cm, 10 - 15 cm, and 15 - 20 cm) were to be used for the measurement of soil field capacity.

2.3.2 Soil Sample Measurement and Method

Soil physicochemical properties measurement and method: soil penetration resistance (DIK-5521 hardness tester, Japan, and the conical area was $2cm^2$); soil three-phase (DIK-1120 soil three-phase instrument, Japan); soil field capacity content (dry method, 105 °C, 24 h); soil nutrient measurement and organic matters (potassium dichromate-sulfuric acid external heating method); soil total nitrogen (Kjeldahl determination); soil full phosphorus (acid soluble molybdenum antimony colorimetry); soil full potassium (sodium hydroxide melt-flame photometer); soil alkaline solution nitrogen (alkali-diffusion method); effective phosphorus (sodium bicarbonate leaching-molybdenum anticolorimetry); rapidly available potassium (ammonium acetate extraction - flame photometric method) (Lu, 2000).

Measurement and methods of soil microorganisms and soil enzymes: soil bacterium (beef extract peptone medium), fungus (improved gauzeImedium), and the plate counting method were used for the number counting of actinomycetes (rose bengal medium); the number of earthworms in soil was measured by sample method (quadrat size: 30 cm × 30 cm × 20 cm). Soil enzymes measurement included soil catalase (0.1 N $KMnO_4$ titration), urease (indophenol blue colorimetry), and phosphatase (3, 5 dinitrosalicylic acid colorimetric method) (Guan, 1986).

2.3.3 Potato Yield

In each test area, whole potatoes were measured, and the single tuber of potato, significantly more than 200 g, was named big one, and 100 - 200 g medium potato, less than 100 g small potato. Potato starch content was measured by the method of specific gravity.

2.4 Statistical Analysis

The experimental data were calculated and statistically analyzed using SPSS 20.0, and LSD multiple comparison method was adopted. The significant level was 0.05.

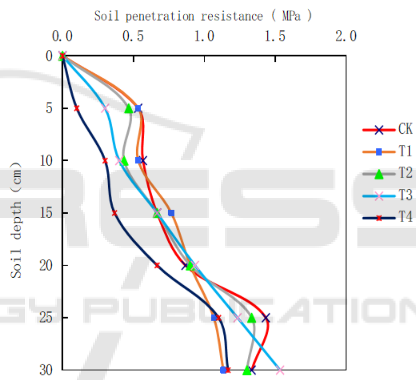

Figure 2: Effect of soil improved by the mushroom substrate on soil penetration resistance.

3 RESULTS AND ANALYSIS

3.1 Effects of Soil Improved by The Mushroom Substrate on Physicochemical Properties of Soil

3.1.1 Soil Penetration Resistance

Soil penetration resistance has a direct effect on plant growth and crop yield. With soil depth increased, the penetration resistance of processed soil increased (Figure 2); with the increase of adding the amount of mushroom substrate, the penetration resistance value significantly lowered at each measured point of soil. Decrease of soil penetration resistance value at each horizon in T4 treatment shows that the addition of mushroom substrate can reduce soil penetration resistance and loosen the soil. At depth of 10 cm, the values of soil penetration resistance were respectively T2 - 0.43 MPa, T3 - 0.40 MPa, T4 - 0.30 MPa, and all were lower than CK - 0.57 MPa.

3.1.2 Soil Penetration Resistance

The three-phase ratio of soil is the volume percent of soil solid phase, liquid phase, and gas phase. The solid phase mainly refers to various kinds of detritus of rocks, mineral particles, and residue of animals, plants, and microorganisms in the soil; the liquid phase mainly refers to soil solution or soil water; the gas phase refers to soil air or void that is not occupied by water. Different distribution and ratios of soil three phases influence soil aeration, permeability, water supply, water conservation, and other physical properties, as well as the soil pH, exchange number of cation ions, base saturate rate, and other chemical properties. So it is used as an important parameter to value the relationship between water, fertilizer, gas, and heat in the soil. An ideal soil three-phase ratio is: the solid phase is 50%, the liquid phase is 20% ~ 30%, and the gas phase is 20% ~ 30%.

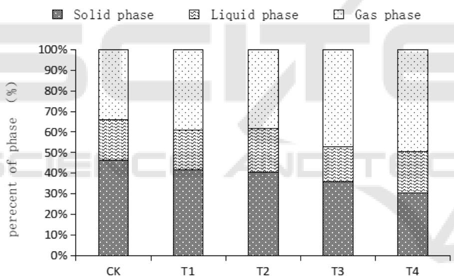

Figure 3: Effects of soil improved by mushroom substrate on the three-phases of soil.

The results of the three-phase of the soil adding ripe mushroom substrate (Figure 3) show that with the increase of the mushroom substrate, the solid phase value decreased, and the soil gas and liquid value increased. The comparison of solid phase value is: CK - 46.43% > T1 - 41.62% > T2 - 40.63% > T3 - 35.77% > T4 - 30.34%. Compared with CK, solid phase value decreased by 26.38% in T4 treatment. The results show that the addition of the mushroom substrate can change the soil three-phase, reduce the soil solid phase value effectively, and increase the gas and liquid phase value, which can effectively change the soil compaction, hardening, and other problems caused by the long-term tillage.

3.1.3 Soil Field Capacity

Soil field capacity is one of the most important components of soil. It plays an important role in the formation and development of soil and the migration of matter and energy in the soil. Soil water is the material basis for the survival and growth of plants, and is the main source of crop water (Sun et al., 2007).

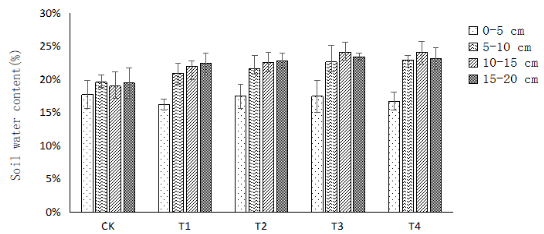

Figure 4: Effect of soil improved by mushroom substrate on soil field capacity.

The plowing horizon soil was sectionally collected every 5cm, and the soil field capacity was measured. As shown in Figure 4, the soil field capacity increased with the increase of the soil depth of plowing horizon soil. Except for the soil of 0-5cm, mushroom substrate and soil field capacity were almost the same. soil field capacity was increased in the soil horizons of 5 - 10 cm, 10 - 15 cm, and 15 - 20 cm. With the mushroom substrate increasing, soil field capacity also tended to increase. In the depth of 5 - 10 cm and compared with CK, soil field capacity in treatments respectively increased by 6.60%, 10.69%, 15.73%, 17.19%; in the depth of 10 - 15 cm and compared with CK, soil field capacity in test areas respectively increased by 15.46%, 19.00%, 26.77%, 17.19%; in the depth of 15 - 20 cm and compared with CK, soil field capacity content in treatments respectively increased by 14.98%, 17.36%, 20.31%, 18.90%. The results show that the addition of mushroom substrate can increase the soil field capacity of plowing horizon soil and then improve soil field capacity conservation and the efficiency of water utilization.

3.1.4 Soil Nutrients and pH

Soil fertility degradation mainly refers to the barren soil nutrient and the loss of soil's effective nutrient supply mechanism. Element content and effectiveness of soil nutrients are closely related to the composition and the content of the nutrient elements in the plants (Sun et al., 2007).

Table 2: Effect of the soil improved by the mushroom substrate on soil nutrients and pH.

Treatment	Organic matter (g kg^{-1})	Total nitrogen (g kg^{-1})	Total phosphorus (g kg^{-1})	Total potassium (g kg^{-1})	Alkaline nitrogen (m g kg^{-1})	Alkaline phosphorus (mg kg^{-1})	Available potassium (mg kg^{-1})	pH
CK	36.15 c	1.63 b	1.65 b	22.57 a	98.46 b	111.33 c	388.06 c	6.23 a
T1	38.79 b	1.65 b	1.70 b	22.57 a	100.57 b	113.00 c	405.10 b	6.24 a
T2	39.15 b	1.68 b	1.85 b	23.41 a	106.90 b	126.67 b	383.24 c	6.29 a
T3	40.22 b	1.82 a	1.89 a	21.88 a	109.01 b	134.00 b	405.18 b	6.24 a
T4	42.42 a	1.85 a	1.89 a	23.20 a	127.29 a	146.00 a	427.50 a	6.27 a

Note: Different loweercase letters in the same column mean a significant difference bwtween treatments. (LSD, $P < 0.05$). The same below.

Table 3: Effects of the soil improved by the mushroom substrate on soil microorganisms, earthworms, and activity of soil enzymes.

Treatment	Bacteria (×10^4)	Fungi (×10^4)	Actinamyces (×10^4)	Earthworms (Each quadrat)	Catalase (0.1N $KMnO_4$ mL g^{-1})	Ourease (NH_3-N mg 100g^{-1})	Intervase (Glucose mg g^{-1})
CK	24.44 d	1.07 c	6.87 c	3.00 d	5.07 b	5.02 c	6.66 b
T1	34.00 cd	1.73 b	7.88 c	6.33 c	5.29 ab	5.52 c	6.95 b
T2	42.30 bc	1.30 c	8.16 c	8.67 bc	6.03 ab	10.05 b	7.72 a
T3	51.70 b	2.01 b	12.92 b	11.00 b	6.49 a	10.18 b	8.50 a
T4	79.64 a	3.98 a	23.89 a	17.33 a	6.56 a	14.13 a	8.90 a

After the harvest of the potatoes, the soil nutrients influence of the mushroom substrate addition is shown in table 2. Different mushroom substrate addition made no significant difference to soil total potassium and pH, while making a great difference to the soil organic matter, total nitrogen, total phosphorus, total potassium, alkaline nitrogen, alkaline phosphorus, and alkaline potassium. Compared to CK, with the increase of the amount of the mushroom substrate added in, soil organic matter, total nitrogen, total phosphorus, alkaline nitrogen, and alkaline phosphorus content tended to increase. The reasons for the phenomenon above are1) edible fungus cultivation material itself contains large amounts of organic matter; 2) the addition of the mushroom substrate improves the soil physical properties, which improves the soil aeration and the permeable performance, influences the activity of the soil microorganisms, and then activates the nutrient content in the soil.

Compared to CK, organic matter increased by 17.34%, total nitrogen by 13.50%, total phosphorus by 14.55%, total potassium by 2.79%, alkaline nitrogen by 29.28%, alkaline phosphorus by 31.14%, and alkaline potassium by 10.16% in treatment T4. After the growth of edible fungus mycelium, there are many residual nutrients, from which plants can absorb organic matter, carbon, and nitrogen nutrient for the growing need. Therefore, the edible mushroom substrate can supply good organic matter for the crops. Soil organic matter is an important source of a variety of nutrients in the soil, especially NPK. Because soil organic matters can absorb many positive ions, the soil can conserve the fertilizer well and has good buffering. The content of organic matter in the soil is an important index of the soil fertility. The mushroom substrate plays little role in the soil pH and there is no significant difference among the treatments.

3.2 Effects of Soil Improved by the Mushroom Substrate on Soil Microorganisms and the Activity of Soil Enzymes

The quantity, distribution, and activity of microorganisms in the soil reflect soil fertility, so they are commonly used as biological indicators for the evaluation of soil quality, maintaining productivity, environment protection quality, and a healthy system maintenance (Zhou, 1987). The addition of the mushroom substrate significantly increased the number of bacterium, fungus, and actinomycetes in the soil. As shown in Table 3 comparing to CK, the total number of microorganisms respectively increased by 34.68% in T1, 59.83% in T2, 105.77% in T3, and 232.06% in T4.

The earthworms can improve the soil, promote the decomposition and the mineralization of organic matters, and the nutrients cycling, which help the potatoes grow. Furthermore, the earthworms can reflect the soil fertility status and soil productivity. That is to say, they are soil environmental indicators. Due to long-term tillage, there was no earthworm in the tested soil before treatment. The number of earthworms in the soil significantly increased with the increase of the amount of the mushroom substrate addition, as shown in Table 3. Soil enzymes are the core of the soil ecosystem (Huang and Xu, 2013), and it mainly comes from the secretion of soil microorganisms, the secretion of the plant root system, residues of plants, and the decomposition of soil fauna. The activity of soil enzymes are a potential indicator of soil biological activity and maintenance of soil fertility. Soil catalases come from fungus and bacterium, and it may come from the plant roots. Soil catalase can promote the decomposition of hydrogen peroxide in the soil (Sun et al., 2007), and is conducive to preventing hydrogen peroxide from hurting the plant roots in the soil (Xue et al., 2005). Urease is an obligate enzyme, and urea can only be hydrolyzed under the function of urease. Nitrogen, product of urease's enzymatic reaction, is one of the nitrogen sources the plants need. Its activity can reflect the amount of nitrogen in soil. Soil sucrase is widely found in the soil and it directly participates in the metabolism of soil organic matters (He et al., 2003). In general, intervase the higher the soil fertility is, the stronger the enzyme activity is. The activity of the intervase can not only reflect the soil biological activity, but also can be used as an indicator of soil maturation and the soil fertility. mushroom substrate added to the soil significantly strengthened the activity of soil catalase, urease and intervase. As shown in table 3, the mushroom substrate added to the soil strengthened the activity of soil microorganisms and changed the environment of soil microorganisms.

3.3 Effects of Soil Improved by the Yield Traits

Adding mushroom substrate to the soil can increase the yield of potatoes. As shown in table 4, with the increase of the adding amount of the mushroom substrate, the increasing rate of potato yield also increased. Compared to CK, yield in T1 increased by 12.82%, T2 by 27.90%, T3 by 50.74%, and T4 by 63.41%. The mushroom substrate added to the soil increased the big and medium size of the potato, reduced the small one, and increased the starch content of the potato.

Table 4: Effects of soil improved by mushroom substrate on potato yield and quality.

Treatment	Rate of big tuber (%)	Rate of medium tuber (%)	Rate of small tuber (%)	Starch content (%)	Yield (kg hm$^{2)}$	Increasing rate (%)
CK	23.27 d	45.38 a	31.35 a	12.59 c	25423.90 c	-
T1	33.02 c	36.58 b	30.40 ab	14.57 b	28684.17 bc	12.82
T2	35.89 bc	40.04 ab	24.08 abc	14.57 b	32517.67 b	27.90
T3	39.72 b	38.76 ab	21.52 bc	15.91 ab	38323.58 a	50.74
T4	47.41 a	32.80 b	19.80 c	16.59 a	41544.74 a	63.41

4 DISCUSSION

The mushroom substrate is light in weight and small in density, which can improve the soil structure. For example, Shi et al. (2014) found that Hericium Erinaceus substrate could reduce the soil bulk density and increase the soil porosity as a saline-alkali soil modifier; Dai et al. (2014) improved saline-alkali soil with Agaricus Bisporus substrate and the results showed that the substrate could reduce soil bulk density. This study demonstrated that mushroom substrate addition reduced the soil hardness, in which the water capability increased and the soil pore was enlarged with a decrease in solid phase ratio. This is beneficial for the water intake and usage of the crops. General methods for soil fertility are organic fertilizer addition and straw returning in China (Zhang et al., 2019). The low-temperature condition in northern China, especially in Heilongjiang Province, reduces the decomposition rate of straw seriously, so the straw is an improper material for soil improvement. The mushroom substrate in which cellulose and lignin have been largely decomposed plays an active role in soil improvement and makes a positive significance for chemical fertilizer reduction and rapid soil fertilizing (Wang et al., 2017; Sun et al., 2007). The soil nutrient content increased after the mushroom substrate addition in this research. The possible reasons are as follows: 1) the mushroom substrate produces plenty of organic matters such as polysaccharides, monosaccharides, amino acids, and other nutrients with the mycelia growth; 2) biological enzymes and other secondary metabolites are secreted to the cultivation matrix. Zhang et al. (2019) believed that mushroom substrate could promote plant growth and root development, improve the root exudates, and then increase the content of soil organic carbon. A large number of studies showed that mushroom substrate was capable of increasing the content of soil organic matter and nutrients (Zeng et al., 2015; Sun et al., 2007). The soil organic matter fertilized the soil and affected the microbiological characteristics. The addition of mushroom substrate increased the number of soil microorganisms and soil enzyme activity during potato maturity. On the one hand, the mushroom substrate contained a large number of microorganisms, especially fungi with high-energy cellulose degradation, which was a good biological agent (Wang et al., 2007). On the other hand, it increased the water capacity and organic matter, which also provided raw materials and growth conditions for the growth of soil microorganisms. The nutrients in the mushroom substrate provided a carbon source and energy for microorganisms and enhance the activity and quantity of microorganisms (Feng et al., 2019). Cao et al. (2017) thought that the high microbial content improved and optimized the growth environment of plants effectively, reduced diseases and pests greatly, and formed a good growth mode. The results above generally showed the addition of mushroom substrate could effectively increase the yield of potatoes.

Adding Organic fertilizer, returning straw in soil and other methods are adopted in China to enrich the soil. While low temperature in the cold region in northern China seriously obstructs the decomposing of straw. Greatly degraded cellulose and lignin in the mushroom substrate are of positive significance for the rapid fertilization of the soil. The physical and chemical properties of different edible fungus wastes differ a lot. If we use the mushroom substrate as a material to improve the soil, we should do the research according to the physicochemical properties of the mushroom substrate and soil. The mushroom substrate itself contains a large number of microorganisms, and there was no sterilization in this research. We should analyze the change in the diversity of the soil microorganisms in the soil with a long-term addition of the mushroom substrate, as well as the comprehensive evaluation of the effect on the environment at the same time. There will be further research into the conversion process of organic carbon contained in the mushroom substrate in the future.

5 CONCLUSION

In this study, by adding different amounts of the mushroom substrate into the soil to improve soil, the conclusions are as follows:

1. The mushroom substrate added to the soil improves the soil physicochemical properties in the cold region. With the increase of the adding amount of the mushroom substrate, the penetration resistance in the plowing horizon soil is reduced, the soil gas and liquid phase are increased, soil field capacity in the plowing horizon is increased, the soil fertility is heightened, and organic matters, nitrogen, phosphorus, and potassium tend to rise generally.
2. The mushroom substrate added to the soil in the cold region activates the soil microorganisms, earthworm, and the activity of soil enzymes.
3. The mushroom substrate added to the soil increases the crop yield and related the yield traits. The yield of potato increased by 12.82%, 27.90%, 50.74%, and 63.41%. The rate of the big potato increased, as well as the content of starch.
4. The results show that when the mushroom substrate was added to the soil at 7.5 kg m^{-2}, 10 kg m^{-2}, soil physicochemical properties, the activity of soil microorganisms, and the crop yield were significantly increased.

ACKNOWLEDGEMENTS

This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDA28100202, XDA28010403). The Agricultural Science and Technology Innovation Leaping Project of Heilongjiang Academy of Agricultural Sciences (HNK2019CX1304, HNK2019CX14, HNK2019CX07-12).

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