

# Effects of background fertilization followed by co-application of two kinds of bacteria on soil nutrient content and rice yield in Northeast China

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**Abstract:** With the improvement of living standards, people pay more and more attention to the quality and safety of rice. Microbial agents are favored by the public because they can activate the nutrient supply in the soil, and reduce the residue and application amount of chemical fertilizers and pesticides. Based on the conventional fertilization in the field, *Bacillus mucilaginosus* and *Aspergillus niger* were applied, *Bacillus mucilaginosus* was inoculated at four levels in the paddy soil in the cold region of Heilongjiang Province of China. The effects of different proportions of *Bacillus mucilaginosus* and *Aspergillus niger* on the number of soil microorganisms, enzyme activity, microbial biomass, soil biochemical intensity, soil nutrient content, plant nutrient content and yield were studied, and the effects on the plant nutrient content of rice and the nutrient dynamics were discussed. The results showed that a 2.62%-21.20% higher yield of rice obtained from co-application treatments compared with that of the control-blank treatment. Furthermore, the highest yield obtained ( $10736 \pm 65 \text{ kg/hm}^2$ ) suggested that the optimized values for the two bacteria applied were  $120 \times 10^{11} \text{ CFU/hm}^2$  for *Bacillus mucilaginosus* and  $15 \times 10^{11} \text{ CFU/hm}^2$  for *Aspergillus niger*. *Bacillus mucilaginosus* can decompose minerals in soil, dissolve potassium and silicon, decompose apatite and release phosphorus into soil. *Aspergillus niger* can transform the phosphate which cannot be absorbed by plants into soluble phosphate which can be directly absorbed by plants by producing non-volatile acids. In particular, *Bacillus mucilaginosus* and *Aspergillus niger* have synergistic effect, and their combined application effect is greater than that of two bacteria alone. Co-application promoted the release of soil soluble silicon, and then increased the silicon content of plants. At the same time, soil microorganism, microbial biomass, enzyme activity and biochemical activity all increased significantly. This study provides an effective way to reduce the amount of chemical fertilizer applied in rice production in cold regions of China.

**Keywords:** microbial inoculants, microbial fertilizer, rice, co-application, *Bacillus mucilaginosus*, *Aspergillus niger*, nutrients

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## 1 Introduction

During the “Twelfth Five-Year Plan” period, Chinese agricultural sector clearly defined the basic goal of “One Regulatory, Two Reduction and Three Basic”, and actively built a supporting green output increase model around the target agricultural sectors at all levels<sup>[1-4]</sup>. Among them, the goal of reducing chemical fertilizer application is to achieve zero growth of chemical fertilizer application by 2020, improve the nutrient utilization rate of food production, soil fertility and comprehensive

benefits, and achieve green and sustainable food production development of reducing weight and increasing efficiency in food production, increasing fertility and “storing grain on the ground”. More than half of the world’s population is dependent on rice (*Oryza sativa* L.), which is a major global crop<sup>[5]</sup>. The total rice-cropped area in the world and global rice production are 163.25 million  $\text{hm}^2$  and 740.96 million t, respectively<sup>[6]</sup>. The yield of rice is significantly dependent on silicon (Si) in the soil. The growth and development of rice can be promoted by the absorption of Si; however, because of its existing form, most of the Si in soil cannot be directly absorbed and utilized. Furthermore, the amount of available Si in soil decreases during long-term planting due to continuous consumption by rice plants. Although conventional chemical fertilizers are mainly used in rice production in northeast China, unfortunately, most of them do not contain Si. Apart from Si, phosphorus (P) is also an essential element for plant and animal life. The utilization of P is heavily relied on in agriculture, but current practices are inefficient. Approximately 80%-90% of added P fertilizers become unavailable to plants in the first year due to run-off or adsorption by soil minerals<sup>[7-11]</sup>. Furthermore, the excessive use of chemical fertilizers contributes toward soil, air and water pollution<sup>[12]</sup>. Therefore, the gradual replacement of chemical fertilizers with biofertilizers and beneficial microorganisms is vital.

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Microbial fertilizer refers to a specific type of product containing live microorganisms, which can be used in agricultural production to obtain specific fertilizer effects<sup>[13]</sup>. In this effect, live microorganisms play a key role<sup>[14-16]</sup>. In 1885, Berthelot's basin experiments showed that microorganisms could convert nitrogen from the air into soil nitrogen that could be absorbed by plants through nitrogen fixation<sup>[17]</sup>. In 1895, the German scientist Noble developed the world's earliest microbial fertilizer "Nitragin" rhizobium inoculum patent product<sup>[18]</sup>, and since then, the microbial fertilizer has gradually come into people's attention. In the 1930s, the research on microbial fertilizer in China was just beginning. Zhang<sup>[17]</sup> took the lead in researching the inoculation technology of soybean rhizobia, and isolated 130 nitrogen-fixation bacteria strains from soil samples in northeast China<sup>[19]</sup>, which increased the average yield of soybean by more than 10%<sup>[20]</sup>. In the 1960s, "5406" antibiotic fertilizer and nitrogen-fixing blue-green algal fertilizer made from actinomycetes were popularized and applied in China<sup>[21]</sup>. In the 1990s, Liu et al.<sup>[22]</sup> developed a biological potassium fertilizer with *Bacillus circulans* as an effective strain<sup>[23]</sup>. The microbial fertilizer that appeared during this period is characterized by a single vegetative bacterium, and the content and effectiveness of major nutrient elements in the soil are mainly improved by utilizing microbial characteristics<sup>[24]</sup>.

Microbial fertilizer also activates the soil nutrient supply, improve soil fertility, crop yield and quality, reduce the residues and dosages of chemical fertilizers, and protect the soil environment<sup>[25]</sup>. Insoluble Si and other elements in the soil can be transformed by *Bacillus mucilaginosus* (*B. mucilaginosus*) into available forms for plants to absorb and utilize. *B. mucilaginosus* could also produce hormones that promote plant growth and could also improve the P and potassium (K) nutritional status of the soil, which have beneficial effects on the growth of plants. Several soil fungi, particularly those belonging to *Aspergillus niger* (*A. niger*), possess the ability to bring insoluble P into soluble forms by secreting low molecular weight organic acids, leading to improvements in plant absorption and utilization of P<sup>[26-28]</sup>. The currently available research on biofertilizers has mainly focused on the application of single bacteria. Few studies have discussed the effect of co-applications of *B. mucilaginosus* and *A. niger* on the nutrient availability and utilization rates of paddy soil ecosystems, e.g., the changes in nutrient content and availability after interactions between *B. mucilaginosus* and *A. niger* take place.

In this study, Chinese paddy soil in the cold region of Heilongjiang Province, China, was investigated. Heilongjiang Province is the primary area of rice production in China and shoulders the heavy responsibility of 1/3 of China's total rice production<sup>[29]</sup>. *B. mucilaginosus* (silicate bacteria) and *A. niger* (phosphate-solubilizing bacteria) were studied based on fertilization conventions, and their effects on rice nutrient contents and soil nutrient dynamics were investigated. The objectives of this study were to identify the optimal proportions of microbial inoculants suitable for local use, to provide an effective way to reduce the amount of chemical fertilizers applied in rice production and to provide guidance for the research of Si and P availability in the rice-soil ecosystem.

## 2 Materials and methods

### 2.1 Plant material

The rice genotype used in the experiment was Daohuaxiang II. The growing period and accumulated temperature for Daohuaxiang

II were 147 d and 2850 °C, respectively. Daohuaxiang II is a late-maturing rice variety, which was planted on April 15 and transplanted on May 16.

The microbial inoculants used were *B. mucilaginosus* ( $-2 \times 10^8$  CFU/g soil) and *A. niger* ( $-1 \times 10^8$  CFU/g soil), which were selected from the Soil Environment Research Institute of Northeast Agricultural University. *B. mucilaginosus* exhibited properties of K-dissolution, Si-dissolution, P-solubilization and N-fixation, while *A. niger* had the ability of P-solubilization.

Traditional fertilizer was applied as background fertilization, which included di-ammonium phosphate (N 18%, P<sub>2</sub>O<sub>5</sub> 46%), urea (N 46%), calcium superphosphate (P<sub>2</sub>O<sub>5</sub> 13.5%), and potassium chloride (K<sub>2</sub>O 60%).

### 2.2 Experimental design

*B. mucilaginosus* and *A. niger* were applied in this experiment, with traditional fertilizer serve as control. After transplanting, before turning green tiller, the microorganisms were applied in solid granule media to 0-5 cm soil according to different testing proportions. *B. mucilaginosus* was inoculated at four levels 0 CFU/hm<sup>2</sup> (B0),  $60 \times 10^{11}$  CFU/hm<sup>2</sup> (B1),  $120 \times 10^{11}$  CFU/hm<sup>2</sup> (B2) and  $240 \times 10^{11}$  CFU/hm<sup>2</sup> (B3). *A. niger* was set up at three levels: 0 CFU/hm<sup>2</sup> (A0),  $7.5 \times 10^{11}$  CFU hm<sup>-2</sup> (A1) and  $15 \times 10^{11}$  CFU/hm<sup>2</sup> (A2). In general, 12 treatments (B0A0, B0A1, B0A2, B1A0, B1A1, B1A2, B2A0, B2A1, B2A2, B3A0, B2A1 and B3A2) with three replicates, i.e., a total of 36 test plots, were included in the experiment, as shown in Table 1. The area for each plot was 66.7 m<sup>2</sup>, and each plot was surrounded by a 45-cm-high plastic sheet with 10 cm buried in soil. The plots also had a single irrigation and single drainage system. The field management practices used in the experiment followed the local field management practices of the area.

**Table 1 Evaluation plan of experimental treatments in field**

Treatment	<i>B. mucilaginosus</i> /10 <sup>11</sup> CFU hm <sup>-2</sup>	<i>A. niger</i> /10 <sup>11</sup> CFU hm <sup>-2</sup>
B0A0 (control-blank)	0	0
B0A1	60	0
B0A2	120	0
B0A3	240	0
B1A0	0	7.5
B1A1	60	7.5
B1A2	120	7.5
B1A3	240	7.5
B2A0	0	15
B2A1	60	15
B2A2	120	15
B2A3	240	15

### 2.3 Field experiment

The experiment was carried out between 2014 and 2015 in Auspicious agricultural planting cooperative, Yagou town of Acheng district, Harbin city, which is located in southern Heilongjiang Province (45°31'N, 126°25'E). The location has a continental monsoon climate in the cold temperate zone. The annual mean temperature, sunshine, accumulated active temperature, average precipitation and frost-free days are 3.4 °C, 2442 h, 2946 °C, 569.1 mm and 162 d, respectively. The soil was a white pulp-type paddy soil on which rice had been grown for over 20 years. The soil fertility was medium, and the soil quality was uniform. The basic fertility of the tested soil is listed in Table 2.

**Table 2 Basic fertility properties of the tested soil**

Organic matter /g kg <sup>-1</sup>	Total N /g kg <sup>-1</sup>	Total P /g kg <sup>-1</sup>	Alkali-hydrolyzable N /mg kg <sup>-1</sup>	Available P /mg kg <sup>-1</sup>	Available K /mg kg <sup>-1</sup>	Available Si /mg kg <sup>-1</sup>	PH
21.65	1.52	0.9	125.1	24.2	139.0	242.4	6.1

## 2.4 Sample analyses

The quantity of soil microorganisms was determined by the dilution plate counting method<sup>[30]</sup>. The soil microbial biomass carbon and nitrogen were determined by the chloroform fumigation and extraction method<sup>[31]</sup>. The activities of urease, invertase and acid phosphatase in the soil were determined to be similar to that reported by Guan et al.<sup>[32]</sup>. The biochemical activity intensity was measured according to methods reported in the current literature<sup>[30]</sup>.

The total concentrations of Si, N, P and K in the rice plants were measured after the samples were crushed<sup>[33]</sup>. The concentrations of Si in the rice plants were determined by silicon molybdenum blue colorimetry. The total N in the rice plant was determined by the Nesslerization method. The total P in the rice plant was determined by the *vanadium* molybdate *yellow colorimetric method*.

The theoretical rice yield was calculated as follows: 5 points along the diagonal line of each plot were selected. For each point, 21 lines were subsequently applied, and the space between the lines was measured. The distance between each rice plant was further calculated, and the number of hills per square meter was calculated. Then, 20 hills were selected to calculate the number of effective leaves per hm<sup>2</sup>. Finally, the number of grains per leaf and the ripening rate in 2-3 hills were investigated. The detailed calculation is shown below.

Theoretical yield (kg) = effective spikes (spike) per hm<sup>2</sup> × number of grains per spike (grain) × ripening rate (%) × thousand-grain weight (g) × 10<sup>-6</sup> × 85%.

## 2.5 Data analyses

The experimental data were statistically analyzed by SPSS 19.0 software and Excel 2007. The differences between treatments were analyzed by Duncan at a 0.05 confidence level.

## 3 Results and discussion

### 3.1 Effects of *B. mucilaginosus* and *A. niger* on soil microorganisms.

Bacteria, fungi and actinomycetes are the main groups of soil microorganisms that play an important role in soil energy conversion, nutrient cycling and humus formation<sup>[34]</sup>. In general, the amount of soil microorganisms showed significant changes with the applications of *B. mucilaginosus* and *A. niger*, as shown in Table 3. The number of bacteria in the soil increased with the application of both *B. mucilaginosus* and *Aspergillus niger*. For the treatments with single applications of *B. mucilaginosus*, the number of soil bacteria increased steadily with increasing amounts of *B. mucilaginosus*, whose maximum was 14.0±2.0% (10<sup>7</sup> CFU/g) for B3A0. This trend is similar to that reported in the literature<sup>[35]</sup>. For the treatments with a single application of *A. niger*, the maximum was 14.6±1.0% (10<sup>7</sup> CFU/g) for B0A2. This is attributed to the fact that *A. niger*, being a phosphate-solubilizing bacterium, might activate the phosphorus in the soil, resulting in increased organic phosphorus concentrations and consequently increased fungal concentration of the soil<sup>[36]</sup>. However, for the treatments with co-applications of *B. mucilaginosus* and *A. niger*, the amount of soil bacteria was significantly higher than that for treatments with single applications by 6.67%-82.25%. Note that this figure was also 167.4% higher than that of the control-blank treatment

(B0A0). The maximum number of bacteria was 19.2±1.0% (10<sup>7</sup> CFU/g) for B2A2.

**Table 3 Effects of *B. mucilaginosus* and *A. niger* on soil microorganisms**

Treatment	Bacteria (10 <sup>7</sup> CFU g <sup>-1</sup> )	Fungi (10 <sup>2</sup> CFU g <sup>-1</sup> )	Actinomycetes (10 <sup>5</sup> CFU g <sup>-1</sup> )
B0A0	7.18±0.62e	49.3±1.1ef	34.9±1.1e
B1A0	10.5±0.4c	50.0±1.0ef	39.4±0.8c
B2A0	13.0±0.2b	47.0±0.8f	44.7±0.3b
B3A0	14.0±2.0b	48.8±1.5f	48.5±0.9a
B0A1	10.6±0.7c	57.7±0.8cd	35.1±1.3e
B1A1	10.7±0.8c	55.3±1.0d	39.1±0.8c
B2A1	14.7±0.8b	56.7±0.5cd	44.5±0.8b
B3A1	18.0±0.7a	62.7±1.8c	48.6±0.7a
B0A2	14.6±1.0b	73.3±1.6a	36.8±1.3de
B1A2	12.9±1.1b	59.3±0.8c	39.8±0.7c
B2A2	19.2±1.0a	67.7±0.6b	49.5±2.1a
B3A2	14.6±1.1b	51.3±0.6e	45.3±0.9b

The treatments with single application of *A. niger* and coapplications of *B. mucilaginosus* and *A. niger* all showed a significant increase in the number of fungi, which reached a maximum of 73.3±1.6% (10<sup>2</sup> CFU/g) for B0A2, followed by 67.7±0.6% (10<sup>2</sup> CFU/g) for B2A2. In contrast, treatments with single applications of *B. mucilaginosus* were associated with a slight decrease in the number of fungi.

For actinomycetes, in general, there were no significant influences on their numbers by single applications of *A. niger*. Both coapplications of *B. mucilaginosus* and *A. niger* and single applications of *B. mucilaginosus* increased the number of actinomycetes, and the maximum appeared in B2A2 (49.5±2.1% 10<sup>5</sup> CFU/g).

Our work also suggested the presence of a synergistic effect for the treatments with co-applications of *B. mucilaginosus* and *A. niger*<sup>[37]</sup>. This is reflected by an initial increase in the amount of soil microorganisms with increasing co-application concentrations and a decrease after the concentrations reached optimization.

### 3.2 Effects of *B. mucilaginosus* and *A. niger* on soil microbial biomass

The soil microbial biomass has extremely important functions in soil ecosystems, such as converting organic matter into nutrients that could be absorbed and utilized by plants, promoting organic carbon metabolism, and transferring nutrients in plants<sup>[38-40]</sup>. The soil microbial biomass carbon (MBC) and nitrogen (MBN) could be increased to various degrees by applications of *B. mucilaginosus* and *A. niger*, as shown in Table 4. High MBC and MBN were generally found in treatments with coapplications of *B. mucilaginosus* and *A. niger*, with maximum values of (928.2±28.3%) mg/kg and (105.2±2.4%) mg/kg, respectively, for B2A2. Under single applications of *B. mucilaginosus*, the highest MBC and MBN were (848.1±36.2%) mg/kg and (78.0±0.4%) mg/kg, respectively, for B3A0. For single applications of *A. niger*, the highest MBC and MBN were (529.5±8.4%) mg/kg and (75.5±3.5%) mg/kg, respectively, for B0A2. The results suggested that the high MBC and MBN obtained from the

treatments with co-applications were likely attributed to interactions between *B. mucilaginosus* and *A. niger*<sup>[41]</sup>. However, an optimized amount existed for the co-applications, and when this value was surpassed, poisoning and inhibition of MBC and MBN occurred, e.g., the amounts of MBC and MBN were higher in B2A2 than in B3A2.

**Table 4 Effect of *B. mucilaginosus* and *A. niger* on soil microbial biomass**

Treatment	Microbial biomass carbon /mg kg <sup>-1</sup>	Microbial biomass nitrogen /mg kg <sup>-1</sup>
B0A0	433.5±25.9e	54.1±1.5e
B1A0	556.5±35.4d	57.6±1.6d
B2A0	776.0±38.9bc	55.6±3.5de
B3A0	848.1±36.2b	58.7±2.0d
B0A1	480.6±13.5e	61.9±2.0d
B1A1	618.6±34.6cd	71.1±0.9c
B2A1	848.8±35.5b	78.0±0.4b
B3A1	919.2±21.1a	79.3±0.2b
B0A2	529.5±8.4de	75.5±3.5b
B1A2	691.0±19.4c	83.8±4.1b
B2A2	928.2±28.3a	105.2±2.4a
B3A2	837.9±32.5b	101.4±2.9a

**3.3 Effects of *B. mucilaginosus* and *A. niger* on soil enzyme activity**

Soil enzyme (urease, acid phosphatase and invertase) activities reflect the direction and intensity of soil biochemical processes and are important biological indicators of soil fertility and the quality of the ecological environment<sup>[29]</sup>. Urease, acid phosphatase and invertase activities were all increased by the co-applications of *B. mucilaginosus* and *A. niger*, as shown in Table 5. The treatment B3A0 showed the highest urease, acid phosphatase and invertase activities under single application of *B. mucilaginosus*, whose trend is in agreement with previous work<sup>[37]</sup>. The treatment B0A2 exhibited the highest urease, acid phosphatase and invertase activities under the condition of a single application of *A. niger*. This may be due to the secretions of rice plant roots and microorganisms, which are correlated with the transformation of soil P, since rice plants require large amounts of nutrients during maturity. When *A. niger*, a phosphate-solubilizing bacteria, was applied, a large amount of available P was transformed and could be absorbed and utilized by the rice plants. The soil enzyme activity was the strongest in the coapplications of *B. mucilaginosus* and *A. niger*, which increased by 11.24%-15.49% and 14.90%-26.52%, respectively, compared with that of single applications of *B. mucilaginosus* and *A. niger*, respectively. Note that the treatments with coapplications of *B. mucilaginosus* and *A. niger* were also 39.87% higher than the control-blank treatment (B0A0). In addition, the activity of the soil enzymes began to decrease slightly with an excessive amount of the co-applications, i.e., higher activity for B2A2 than B3A2, also indicating that a poisoning effect occurred<sup>[42]</sup>.

**3.4 Effects of *B. mucilaginosus* and *A. niger* on soil biochemical activity**

Soil biochemical activity refers to the ability of soil microbes and enzymes to decompose substrates or form products at a certain temperature per unit time. Therefore, the application of an appropriate number of microorganisms will have an impact on the life activities and strength of microorganisms in the soil and will also indirectly affect the intensity of various biochemical activities in soil<sup>[43]</sup>.

**Table 5 Effects of *B. mucilaginosus* and *A. niger* on soil enzyme activities**

Treatments	Urease /mg L <sup>-1</sup>	Acid phosphatase /mg L <sup>-1</sup>	Invertase /mg L <sup>-1</sup>
B0A0	4.74±0.14e	25.70±1.02d	7.1±0.99d
B1A0	7.81±0.3a	31.31±1.49c	10.57±0.78c
B2A0	7.21±0.22ab	32.66±1.93bc	11.28±1.27c
B3A0	7.47±0.3a	39.72±0.73a	11.57±1.15c
B0A1	5.24±0.35de	34.77±0.73bc	12.21±0.57bc
B1A1	4.81±0.53e	32.57±0.51bc	12.46±0.12bc
B2A1	5.25±0.58cde	33.25±2.04bc	12.16±0.45bc
B3A1	6.24±0.38a	34.36±0.80bc	11.83±1.29bc
B0A2	5.77±0.14cd	42.83±4.44a	13.65±0.37b
B1A2	4.88±0.22e	31.60±2.09c	12.29±1.34bc
B2A2	6.63±0.75b	36.09±3.58b	15.58±1.07a
B3A2	5.85±0.26c	34.11±1.97bc	11.21±1.02c

Applications of *B. mucilaginosus* and *A. niger* could affect the soil biochemical activity (organic P and inorganic P, K-releasing ability), as shown in Table 6. For single applications of *B. mucilaginosus*, the highest intensity of organic P appeared in B3A0 (17.53±0.91) mg/L. The increase is due to the P-releasing and K-releasing abilities of *B. mucilaginosus*, consistent with previous works<sup>[44,45]</sup>. For single applications of *A. niger*, the highest intensity of conversion of organic P appeared in B0A2 (19.11±1.01) mg/L, which is due to the organic and inorganic P in the soil being dissolved by *A. niger* and then absorbed by the rice plants. For coapplications of *B. mucilaginosus* and *A. niger*, the intensity of conversion of organic P increased by 24.30%-39.2.3% and 14.02%-26.46%, respectively, compared with that with single applications of *B. mucilaginosus* and *A. niger*. This figure was also increased by 37.04% compared with that of the control-blank treatment (B0A0). The results indicated a synergistic effect between *B. mucilaginosus* and *A. niger*, leading to increased soil organic P conversion intensities.

**Table 6 Effects of *B. mucilaginosus* and *A. niger* on soil biochemical activity intensity**

Treatment	Conversion of Organic P/mg L <sup>-1</sup>	Conversion of Inorganic P/mg L <sup>-1</sup>	K-releasing ability /mg L <sup>-1</sup>
B0A0	15.9±0.99d	99.5±6.89a	11.83±0.91e
B1A0	15.65±1.05d	100.5±4.8a	13.21±0.34d
B2A0	16.75±1.12cd	101.5±6.6a	15.01±0.9bc
B3A0	17.53±0.91cd	106.3±4.5a	17.00±1.5a
B0A1	17.23±1.09cd	100.9±3.8a	11.62±1.23e
B1A1	18.06±0.89c	99.9±7.6a	14.8±1.23bc
B2A1	19.13±1.06b	104.8±6.5a	15.81±0.91ab
B3A1	19.84±0.88ab	102.4±2.9a	16.581.24ab
B0A2	19.11±1.01b	103.0±4.6a	12.51±0.90de
B1A2	19.73±1.13ab	104.3±5.1a	13.79±1.19cd
B2A2	21.79±1.17a	104.5±6.9a	16.98±0.59a
B3A2	20.41±0.93a	101.9±3.8a	16.53±1.19ab

There were no significant effects on the intensity of conversion of soil inorganic P by applications of *B. mucilaginosus* and *A. niger*, indicating that they did not have the ability to convert or dissolve inorganic P.

Both *B. mucilaginosus* and *A. niger* showed some effects on the soil K-releasing ability to varying degrees. The highest K-releasing ability occurred in B3A0 (17.00±1.5), followed by B2A2 (16.98±0.59). This is because *B. mucilaginosus*, a

K-soluble functional bacterium, had the ability to decompose potassium aluminum silicate minerals in the soil<sup>[46,47]</sup>.

### 3.5 Effects of *B. mucilaginosa* and *A. niger* on soil nutrient contents

The soil nutrient contents (available Si, alkali-hydrolyzable N, available P and available K) could be affected by the applications of *B. mucilaginosa* and *A. niger* to various extents. As shown in Table 7, the contents of soil available Si were the highest for the treatments with coapplications of *B. mucilaginosa* and *A. niger*, followed by the treatments with single application of *B. mucilaginosa*. The highest content of soil available Si appeared in treatment B2A2 (344.6±2.5 mg/kg), which was mainly attributed to the effect of *B. mucilaginosa*, similar to the trend reported previously<sup>[48]</sup>. Many previous reports suggested that *B. mucilaginosa* can dissolve minerals and release Si from the crystal lattice of minerals in soil<sup>[49,50]</sup>. For the treatments with single application of *A. niger*, the soil available Si was relatively low. This is because the available Si content in the soil is dependent on pH. However, the pH range of *A. niger*, being a phosphate-solubilizing bacterium, is between 2 and 3. When *A. niger* is applied, the pH is reduced, resulting in the inhibition of the dissolution of available Si in the soil<sup>[51]</sup>. For coapplications of *B. mucilaginosa* and *A. niger*, the available Si content in the soil still increased, suggesting that the negative effects of the application of *A. niger* were relieved by coapplications. Note that the trend of soil available Si first increased and then decreased with the concentration of the coapplications, which is also due to the antagonistic effect induced when excessive amounts of *A. niger* were applied.

**Table 7 Effects of *B. mucilaginosa* and *A. niger* on soil nutrient contents**

Treatments	Available Si /mg kg <sup>-1</sup>	Alkali-hydrolyzable N /mg kg <sup>-1</sup>	Available P /mg kg <sup>-1</sup>	Available K /mg kg <sup>-1</sup>
B0A0	308.7±6.9cd	90.2±4.1d	6.8±1.2d	166.6±1.8bc
B1A0	312.4±8.3c	103.4±3.5cd	10.5±1.3cd	188.4±6.3b
B2A0	336.2±10.9a	107.3±6.2c	11.5±1.0c	217.6±11.8a
B3A0	315.9±11.0cd	100.6±6.1cd	9.0±0.6cd	216.6±13.7a
B0A1	301.0±7.8de	100.9±4.1cd	12.6±1.0bc	157.0±3.6c
B1A1	306.2±7.5de	111.3±6.0bc	11.9±1.3c	159.1±11.0c
B2A1	320.3±7.5bc	120.1±2.6b	12.8±1.0bc	179.0±1.8b
B3A1	340.2±8.4a	131.3±7.7ab	14.5±1.4ab	208.7±0.9a
B0A2	297.0±11.4de	107.6±4.0c	10.9±1.6c	143.9±2.5d
B1A2	335.1±4.7ab	113.2±0.5bc	14.6±0.8ab	169.5±7.9bc
B2A2	344.6±2.5a	133.8±5.8ab	15.9±1.3a	180.1±16.2b
B3A2	337.1±12.6a	145.0±14.5a	14.9±1.6ab	184.6±9.3b

The content of alkali-hydrolyzable N was increased significantly by applications of *B. mucilaginosa* and *A. niger*. In general, the contents for the treatments with coapplications of *B. mucilaginosa* and *A. niger* were higher than those for treatments with the single applications of *B. mucilaginosa* and *Aspergillus niger*. Treatment B2A3 had the highest alkali-hydrolyzable N (145.0±14.5 mg/kg), followed by treatment B2A2 (133.8±5.8 mg/kg).

The content of soil available P was improved to various degrees by the applications of *B. mucilaginosa* and *A. niger*. The effect is expressed as coapplications of *B. mucilaginosa* and *A. niger* > single applications of *A. niger* > single applications of *B. mucilaginosa*. The soil available P content was the highest for the B2A2 treatment (133.8±5.8 mg/kg).

The content of the soil available K was significantly increased by single applications of *B. mucilaginosa* and coapplications of *B.*

*mucilaginosa* and *A. niger*. For the treatments with single applications of *B. mucilaginosa*, the content of soil available K increased steadily with increasing amounts of the bacteria, which is consistent with the positive correlation between soil K-releasing strength and the amount of *B. mucilaginosa* applied. In contrast, a single application of *A. niger* resulted in a decreased content of available K in the soil, suggesting that it had an antagonistic effect. However, for coapplications of *B. mucilaginosa* and *Aspergillus niger*, improvement of the available K content suggested that this antagonistic effect could be mitigated.

Many previous studies showed that in applying microbial inoculants, the contents of alkali hydrolyzable N, available P and available K in the soil could be increased<sup>[52,53]</sup>. This is because the microbial inoculants applied can increase the N in soil by N-fixation and the conversion rate and utilization rate of poorly soluble P, K and other nutrients in the soil through microbial activities<sup>[52,53]</sup>. Our results also showed that the applications of *B. mucilaginosa* and *A. niger* resulted in similar trends. Furthermore, among the three types of treatments, the content of soil alkali-hydrolyzable N and available P in the treatments with co-applications of *B. mucilaginosa* and *A. niger* showed the highest increase, indicating that co-applications played a mutually reinforcing role, consistent with a trend reported in previous work<sup>[54]</sup>.

### 3.6 Effects of *B. mucilaginosa* and *A. niger* on the nutrient content in the above-ground parts of the rice plant

The nutrient contents (Si, N, P and K) in the above-ground parts of the rice plant (stem, leaf and spike) could be affected by the application of *B. mucilaginosa* and *A. niger* to various extents, as shown in Figure 1. The highest nutrient contents generally appeared in the treatments with coapplications of *B. mucilaginosa* and *A. niger*.

Our study showed that applications of both *B. mucilaginosa* and *A. niger* could affect the content of available Si in various organs of the above-ground part of the rice plant (Figure 1). The absorption and accumulation of Si could be promoted, and the distribution of Si in the stems, leaves and spikes could be affected<sup>[55]</sup>. The content of available Si from high to low is ranked as: leaves, stems, spikes. This is because the deposition of Si in rice plants is a passive physical process that is affected by transpiration. When water is evaporated from the epidermis of the leaves, stems and other organs, the silicic acid reaches super saturation and spontaneously polymerizes into insoluble silica gel, resulting in immobilization and inability of redistribution<sup>[56]</sup>. Since the main organs for plant transpiration are the leaves, the Si content of the leaves is significantly higher than in other organs<sup>[57,58]</sup>. The content of available Si in the stems of rice plants was the highest for the B2A2 treatment (53.1±2.1 g/kg), followed by the B2A0 treatment (53.0±1.3 g/kg). The content of available Si in the leaves was the highest for the B2A2 treatment, followed by the B2A3 treatment. The content of the available Si in the spike of the rice plant was lower than that of the conventional fertilization (B0A0) treatment.

N, P and K are the three major nutrients of rice plants, which closely interact with each other. The application of one of the nutrients can promote the absorption and utilization of the other two nutrients by the soil. Applications of both *B. mucilaginosa* and *A. niger* resulted in an increase in the N content in the above-ground tissues of the rice plants by various degrees (Figure 2). The absorbed and accumulated N in rice plants further affected the distribution of N in the stems, leaves and spikes. In

general, the distribution of N was spikes > leaves > stems. This is because N content and nutrients in rice plants gradually transfer to the spikes as the growth period progresses, promoting grain formation<sup>[13,59,60]</sup>. For the stems of the rice plants, the highest content of N appeared in B2A2 (6.95±0.26 g/kg), followed by B1A2 (6.72±0.15 g/kg). For the leaves of the rice plants, the highest content of N appeared in B2A2 (13.85±0.28 g/kg), followed by B3A2 (13.85±0.28 g/kg). For the spikes of the rice plants, the highest content of N appeared in B2A0 (23.67±1.03), followed by B2A2 (23.42±0.32 g/kg).

The P content of the above-ground parts of the rice plants

could also be improved by applications of both *B. mucilaginosa* and *A. niger* (Figure 3). In the stems of the rice plants, the content of P was the highest for B2A2 (2.23±0.13 g/kg), which was 12.62% higher than that of the conventional treatment (B0A0). In the leaves of the rice plants, the content of P was the highest for B2A3 (1.56±0.09), followed by B2A2 (1.54±0.08 g/kg). In the spikes of the rice plants, the content of P was the highest for B2A2 (2.30±0.16 g/kg), followed by B3A1 (2.24±0.11 g/kg). The applications of *B. mucilaginosa* significantly increased the P content in the stems, leaves and spikes of rice plants observed here, similar to that reported previously<sup>[53,60,61]</sup>.

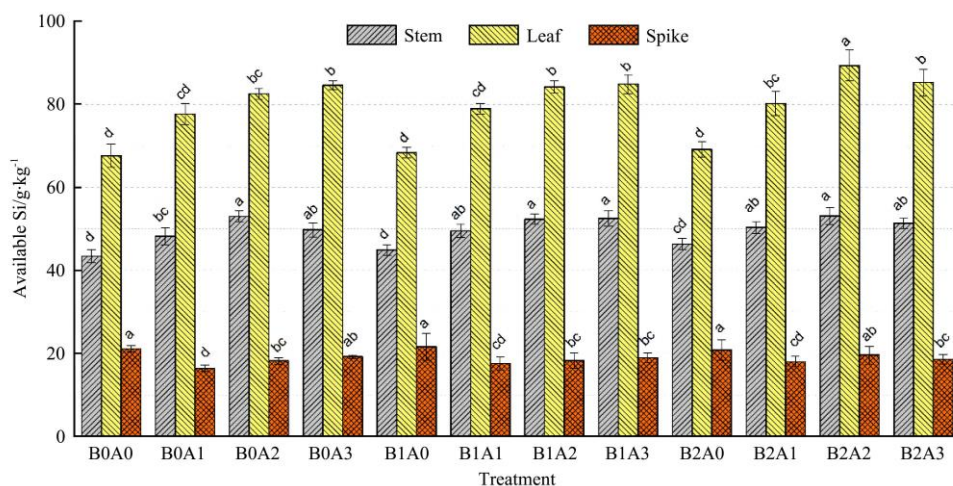


Figure 1 Effects of *B. mucilaginosa* and *A. niger* on the available Si of the above-ground (stems, leaves and spikes) parts of the rice plant

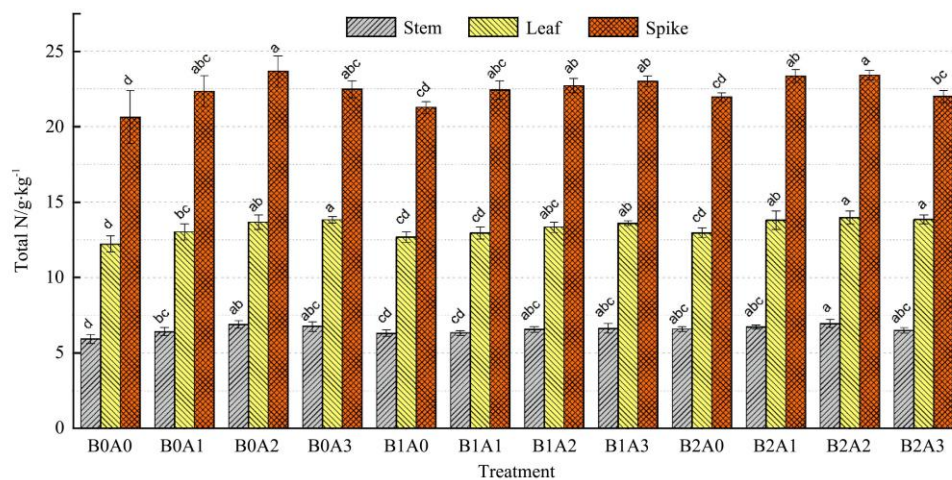


Figure 2 Effects of *B. mucilaginosa* and *A. niger* on the total N of the above-ground (stems, leaves and spikes) parts of the rice plant

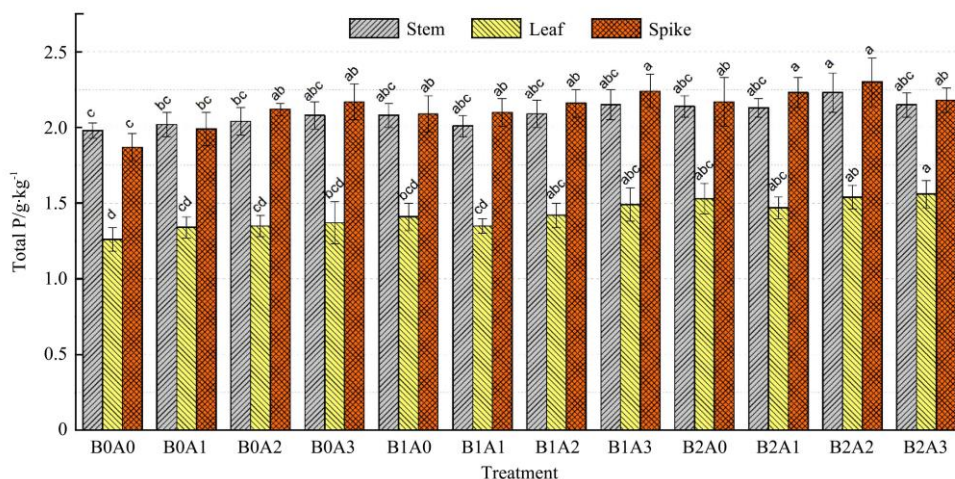


Figure 3 Effects of *B. mucilaginosa* and *A. niger* on the total P of the above-ground (stems, leaves and spikes) parts of the rice plant

The application of *B. mucilaginosus* significantly increased the K content in the stems, leaves and spikes of the rice plants (Figure 4). The application amount was positively correlated with the K content of various organs. The single applications (B1A0 and B2A0) of *A. niger* had no effect on the aboveground K content of

rice plants compared with that of the control-blank treatment (B0A0). For the treatments with co-applications of *B. mucilaginosus* and *A. niger*, the K content in the above-ground parts of the rice plants was increased, but the extent was slightly lower than that of the single applications of *B. mucilaginosus*.

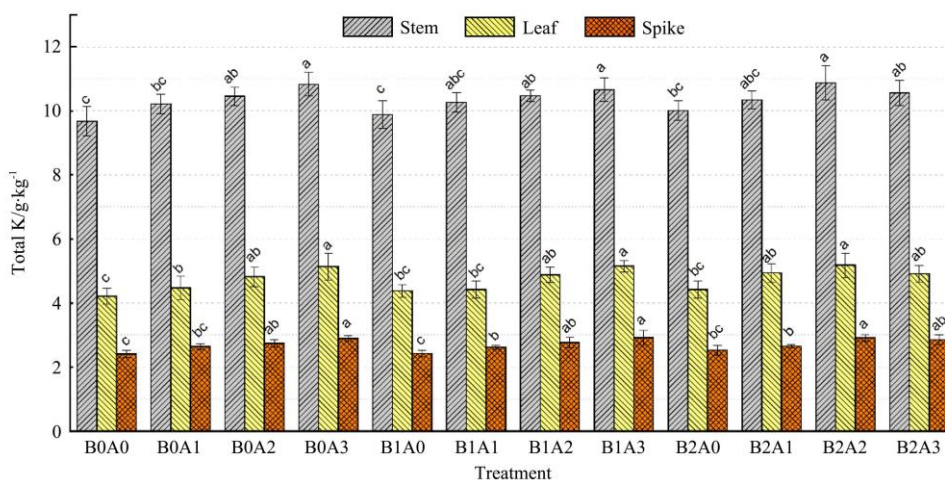


Figure 4 Effects of *B. mucilaginosus* and *A. niger* on the total K of the above-ground (stems, leaves and spikes) parts of the rice plant

### 3.7 Effects of *B. mucilaginosus* and *A. niger* on the yield of rice

Our results showed that applications of *B. mucilaginosus* and *A. niger* resulted in increased yields of the rice plants to various degrees (2.62%-21.20%) compared with that of the control-blank treatment (B0C0) (Figure 5). The highest increase was observed in the treatments with co-applications of *B. mucilaginosus* and *A. niger* (B2A2, 10736±65 kg/hm<sup>2</sup>), followed by B1A2 (10640±105 kg/hm<sup>2</sup>). In contrast, single applications of *A. niger* had almost no effect on the rice yield, indicating that the application of *B. mucilaginosus* was the main cause of the rice yield increases.

conditions. Meanwhile, the phytohormone produced by the metabolic activities of *B. mucilaginosus* promotes the growth of the roots of the rice plant, thereby increasing the absorption of soil elements and consequently increasing the yield.

For the treatments with co-applications of *B. mucilaginosus* and *A. niger*, the yield of rice increased significantly, which was higher than in the treatments with single applications, indicating strong interactions between *B. mucilaginosus* and *A. niger*. However, when the amount of *B. mucilaginosus* was low in the coapplications (e.g., B1A1, B1A2), the yield only increased slightly. This is because rice plants have a certain competitive effect on the absorption of Si and P, i.e., excessive Si in the soil can inhibit the excessive absorption of P by rice plants, and deficient Si in soil can promote the absorption of P by rice plants<sup>[65,66]</sup>. When P is present in excessive concentrations in the soil, increasing Si can reduce the absorption of P by rice plants to avoid the excessive P affecting the normal maturity of the rice plants. Another factor contributing to the increase in crop yield is that by improving the nutrient environment of the crop, releasing hormones and plant stress resistance being enhanced, then plant diseases and pests can be inhibited to some extent, thereby reducing yield loss.

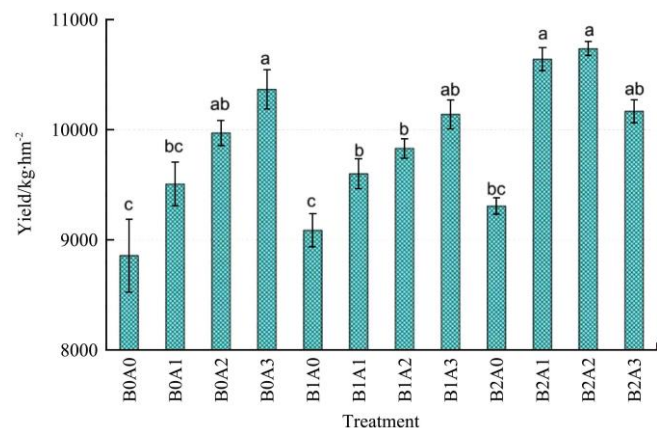


Figure 5 Effects of *B. mucilaginosus* and *A. niger* on the yield of rice

It has been suggested that applications of Si fertilizer on rice plants can significantly promote the development of rice roots, resulting in larger stems, improved disease resistance, enhanced lodging resistance, increased grain numbers per spike, increased 1000-grain weight and significantly increased yield<sup>[62-64]</sup>. Our work showed that applications of *B. mucilaginosus* could increase the Si content in rice plants, resulting in an effect similar to that of Si fertilizer. This is attributed to the fact that *B. mucilaginosus* can decompose K and P minerals such as phosphorus lime and feldspar in the soil into available K and P that can be absorbed and utilized by the rice plants, resulting in improved nutritional

To ensure the realization of the goal of “One Regulatory, Two Reduction and Three Basic” by 2020, effectively guarantee the food supply security in China, agricultural product quality and agricultural environment, especially the production environment, and achieve the goal of reducing weight and increasing efficiency, we have promoted our research results into parts of northeast China, currently, farmers have achieved good economic benefits. In 2017, Shangzhi city rice cooperative have applied the bacterial agent on 10 hm<sup>2</sup> of land, based on microbial agents for 6 Yuan/kg, when rice is 4 Yuan/kg. After testing, the yield of conventional fertilization was 8625 kg/hm<sup>2</sup>, and the yield of the plots with microbial inoculum was 9574 kg/hm<sup>2</sup>, the application of microbial agents increased yield by 11%, economic benefit increased by 3378 Yuan/hm<sup>2</sup>. In 2016, Qianpaoshou village of Shuangcheng city applied the bacterial agent on 3.3 hm<sup>2</sup> of land, the application of microbial agents increased yield by 12.5%, economic benefit increased by 5558 Yuan/hm<sup>2</sup>.

## 4 Conclusions

In this work, the results showed that co-applications of *B. mucilaginosus* and *A. niger* had significant effects on the improvement of soil microorganisms, microbial biomass, enzyme and biochemical activities and plant nutrient contents. Furthermore, the combination of *B. mucilaginosus* and *A. niger* had a synergistic effect, which resulted in better effect than single applications of *B. mucilaginosus* or single applications of *A. niger*. Considering the yields, plant nutrient contents, availability of Si in paddy fields and soil ecological effects, the optimized combination was  $120 \times 10^{11}$  CFU/hm<sup>2</sup> for *B. mucilaginosus* and  $15 \times 10^{11}$  CFU/hm<sup>2</sup> for *A. niger*.

The development of microbial fertilizers should focus on the following aspects: research on the molecular basis of microbial inoculants, screening and mutagenesis of excellent strains, improving the stability and competitiveness of bacterial species; clarifying the mechanism and ecological role of microbial inoculants; improving the product standard of microbial fertilizer, strengthen the quality supervision and management of microbial fertilizer; standardize the detection methods of microbial fertilizers and develop rapid detection techniques.

Through this study, it can be found that fertilizer and microbial agents can improve the utilization rate of nutrients in food production. In the future, the research will be focused on developing a one-off organic or inorganic compound fertilizer and bio-fertilizer, in order to provide a theoretical basis for the protection and utilization of soil in northeast China and developing more environmental efficient green bio-bacterial manure.

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