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Influence of Sewage Sludge Biochar on the Microbial Environment, Chinese Cabbage Growth, and Heavy Metals Availability of Soil

Guangwei Yu, Shengyu Xie, Jianli Ma, Xiaofu Shang, Yin Wang, Cheng Yu, Futian You, Xiaoda Tang, Héctor U. Levatti, Lanjia Pan, Jie Li and Chunxing Li

Abstract

The effects of sewage sludge biochar (SSB) on the microbial environment, Chinese cabbage yield, and heavy metals (HMs) availability of soil were comprehensively investigated in this study. Results showed that the concentrations of the dehydrogenase (DHA) and urease in the soil added with 10% SSB were 3.60 and 1.67 times as high as that of the control soil, respectively, after planting; the concentrations of the bacteria, fungi, ammonia-oxidizing archaea (AOA), and ammonia-oxidizing bacteria (AOB) in the soil added with 10% SSB after planting reached 2.84, 2.62, 1.76, and 2.23 times, respectively, compared with those of the control group; the weights of the aboveground and underground parts of Chinese cabbage were 5.82 and 8.67 times as high as those of the control group, respectively. Moreover, the addition of SSB enhanced the immobilization of Cr, Ni, and Cd. All in all, SSB can improve the microbial environment of soil and inhibit the availability of HMs, which is very important for their utilization in barren soil.

Keywords: sewage sludge biochar, soil, Chinese cabbage, microbial environment, heavy metals

1. Introduction

Because of rapid economic development, more than 30 million tons of wet sewage sludge (SS) are produced in China every year [1]. SS contains lots of organic pollutants, microorganisms, eggs of parasitic organisms, and heavy metals (HMs), which makes it an obvious threat to ecological environment [2]. Conventional disposal technologies such as landfill, incineration, and agricultural application encounter many environmental problems; so, they cannot be widely used [3]. Especially, the direct application of SS in agricultural production is strictly banned due to the problem of pathogens and contaminants [4].

The pyrolysis of SS is a technology in which SS is heated under zero or low-oxygen condition to produce sewage sludge biochar (SSB) and pyrolysis oil and gas. After conversion into SSB, all the pathogens and organic pollutants in SS are eliminated and the volume of SS is significantly reduced [5]. Also, the oil and gas produced by pyrolysis can save the input of external energy as supplemental fuel [6]. Apart from the applications mentioned above, SSB has numerous special advantages in improving soil quality and crop growth. First of all, biochar possesses a porous structure that can influence the soil's structure, porosity, particle size distribution, and density, which contributes to increasing the soil water-holding capacity and microbial activity [7]. Furthermore, biochar is alkaline and can improve the pH of soil [8]. Finally, biochar is rich in plenty of nutrients such as nitrogen, phosphorus, potassium, etc., exhibiting a positive effect on plant growth [9]. Song et al. [10] studied the influence of pyrolysis temperature and proportion of SSB on garlic yield and HMs accumulation and found that the SSB produced at 450°C and its addition at 25% could improve the yield of garlic well and inhibit HMs accumulation in garlic. Khan et al. [4] investigated the effects of SSB on rice yield, HMs bioaccumulation, and greenhouse gas emission and found that SSB amendments increased the pH, total nitrogen, organic carbon, and available nutrients of soil and crop yield, and decreased HMs bioavailability and N₂O emission. In addition, there are a large amount of studies on the influence of SSB on plant growth and HMs migration that have proved the positive effects of biochar addition [11–13].

Based on the pilot-scale plant on pyrolysis of SS with capacity 30 t/d in Xiamen, and our previous studies, it was found that the HMs in SS were converted into a more stable state after hydrothermal pretreatment combined with pyrolysis and the obtained SSB could be used to prepare ceramsite [14–16]. However, the study of the influence of SSB from the pyrolysis of hydrothermally treated SS on the microbial environment of soil during planting is still indispensable. On the one hand, the soil microorganisms are involved in many biochemical processes, including the degradation and conversion of organic matter, the mineralization and immobilization of nutrients, and the formation and stabilization of soil aggregates [17]. On the other hand, the soil microorganisms are also a repository of soil nutrients and an important nutrition source for plant growth [18]. In this study, we chose the common and easy-to-grow Chinese cabbage as the planting crop to investigate the influence of SSB from the pyrolysis of hydrothermally treated SS on the physical and chemical properties and microbial environment of soil before and after planting. Furthermore, the growth status of Chinese cabbage and HMs availability were also studied.

2. Materials and methods

2.1 Materials

The used soil was collected from a farmland near an abandoned mine in Longyan, Fujian Province, China. The soil was sieved and homogenized after collection. SS was obtained from a wastewater treatment plant in Xiamen, China. Then, the SS was disposed via hydrothermal treatment at 160°C for 1 hour, and followed by filtration and pyrolysis by a rotary furnace at 500°C for 3 hours to obtain SSB in the pilot-scale plant in Xiamen, Fujian Province [19]. The high-quality and early raping NO.5 seed of Chinese cabbage was chosen as the testing plant.

2.2 Chinese cabbage pot experiment

The Chinese cabbage pot experiment was carried out in a greenhouse located in Xiamen, Fujian province, China (24.36 N–118.3 E) and the height and diameter of

the polyethylene pot were 15 and 20 cm, respectively. To investigate the influence of SSB on the properties of soil, Chinese cabbage growth, and HMs availability, SSB was added with an SSB-to-soil mass ratio of 1:9 (10% SSB) in pot and the pure soil served as a control group. The total weight of soil or treated soil in each pot was 5.0 kg. Every pot experiment was assessed by four replicates. After seeding, each pot was treated with watering regularly and thinned out to ensure that only one Chinese cabbage grows. When the pot experiment finished (about 55 days), the soil and Chinese cabbage were collected to conduct relative tests, respectively.

2.3 Analysis methods

The pH was measured according to the agricultural trade standard of China (NY/T 1377-2007) and the solution was analyzed with a UB-7 pH meter (Ultra Basic, US). Electrical conductivity (EC) was measured according to the national environmental protection standard of China (HJ 802-2016) and the solution was analyzed with a Cond 3110 conductometer (Teltracon 325, Germany). Surface area was calculated by the Brunauer-Emmett-Teller (BET) method after testing using nitrogen adsorption/desorption isotherms with an apparatus (TriStar II 3020 V1.01, USA). Elemental analysis was conducted by an elemental analyzer (Vario MAX, Germany). The concentrations of nutrient elements were analyzed by digestion in an acid mixture [15] and the solution was determined by ICP-OES (Optima 7000DV, USA). The concentrations of available HMs in the sample were measured by the DTPA extraction method [20] and the solution was determined by ICP-MS (Agilent 7500cx, USA). The surface functional group of SSB was analyzed by FTIR spectrometry (iS10, Thermo, USA) and the morphology of SSB was analyzed by scanning electron microscopy (SEM, S-4800, Hitachi, Japan).

The dehydrogenase (DHA) activity in soil was measured by the triphenyltetrazolium chloride (TTC) spectrophotometric method [21]. The urease activity was measured by Nesslerization [22]. The molecular target genes of bacteria, fungi, ammonia-oxidizing archaea (AOA), and ammonia-oxidizing bacteria (AOB) were measured by quantitative real-time polymerase chain reaction (RT-PCR) analysis [23] and the information of primers is shown in **Table 1**. A standard curve was obtained by tenfold dilution of recombinant plasmid acquired in each molecular target gene of the above microorganisms and each sample was repeated three times. The SYBR® Premix Ex Taq™ kit from Bao Biological Engineering (Dalian, China) Co. Ltd. was used for analysis at Roche Lightcycler® 480 PCR. The quantitative PCR reaction system was 20 μL, including 1 μL of tenfold diluted DNA template, 10 μL of SYBR® Premix Ex Taq™, 0.2 μL (20 μM) of forward and reverse primers

Target gene	Primer name	Primer sequence (5'-3')
Bacteria 16S rRNA	58F	CCTACGGGAGGCAGCAG
	517R	ATTCCGCGGCTGCTGGCA
Fungi 18S iRNA	ITS3	GCATCGATGAAGAACGCAGC
	ITS4	TCCTCCGCTTATTGATATGC
AOA amoA	Arch-amoAF	STAATGGTCTGGCTTAGACG
	Arch-amoAR	GCGGCCATCCATCTGTATGT
AOB amoA	amoA-1F	GGGGTTTCTACTGGTGGT
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC

Table 1.
RT-PCR amplification primers.

respectively, and 8.6 μL of sterilized distilled water. The procedure of PCR consisted of denaturation at 95°C for 5 min, denaturation at 94°C for 30 s, annealing at 55°C for 45 min, and extension at 72°C for 1 min, followed by 40 cycles of denaturation, annealing, and extension at 72°C for 10 min.

3. Results and discussions

3.1 Basic properties of the original soil and SSB

The physical and chemical properties of the original soil and SSB are listed in **Table 2**. SSB has higher pH, EC, and BET surface area compared with the soil, which shows that the addition of SSB can improve the physicochemical properties of soil, such as pH, salinity content, water retention, the adsorption of nutrient, and microbial population [24]. In particular, the change of pH in soil indicates the occurrence of some chemical and biological reactions. The contents of C, H, N, and S in biochar depend on the feedstock and pyrolysis condition. The H/C and C/N ratios represent the aromaticity of biochar and the capacity for organics to release inorganic N [10, 25]. In this study, the H/C ratio of SSB is lower (<0.1) than that of the soil, which suggests that SSB has higher aromaticity and can exist in the soil for many years [25]. However, the higher C/N ratio of SSB inhibits the release of inorganic N compared with the original soil. In addition, SSB contains higher concentrations of K, Na, P, and Ca compared with the soil, which indicates that the addition of SSB can increase the fertility of soil.

The FTIR spectra of SSB is shown in **Figure 1a**. The identified bands are assigned to the stretching vibrations of hydroxyl functionalities (3446 cm^{-1}), amide bond stretching (1637 cm^{-1}), bending vibration of methyl group (1385 cm^{-1}), carbon-oxygen single bond in phenol (1186 cm^{-1}), and carbon-oxygen double bond (1050 cm^{-1}) [10, 25, 26]. In addition, the stretching vibrations between 600 and 800 cm^{-1} can be related to the aromatic and heteroaromatic compounds, and the bands below 600 cm^{-1} can be attributed to the organic and inorganic halogen compounds [25]. The SEM micrograph of SSB is shown in **Figure 1b**. There are lots of lumps and holes

Parameters	Soil	SSB
pH	5.32 ± 0.03	10.00 ± 0.04
EC ($\mu\text{S}/\text{cm}$)	203.67 ± 2.22	871.33 ± 3.78
Moisture (%)	0.26 ± 0.00	ND ^a
BET surface area (m^2/g)	0.51	13.05
Carbon (%)	3.08 ± 0.02	7.84 ± 0.02
Hydrogen (%)	1.04 ± 0.03	0.63 ± 0.03
Nitrogen (%)	0.26 ± 0.00	0.34 ± 0.00
Sulfur (%)	3.96 ± 0.04	3.82 ± 0.08
K (mg/g)	8.37 ± 0.05	20.33 ± 0.06
Na (mg/g)	0.86 ± 0.01	10.57 ± 0.05
P (mg/g)	1.51 ± 0.02	7.28 ± 0.05
Ca (mg/g)	0.03 ± 0.00	39.66 ± 0.11

^aND, not detected.

Table 2.
Physical and chemical properties of soil and SSB.

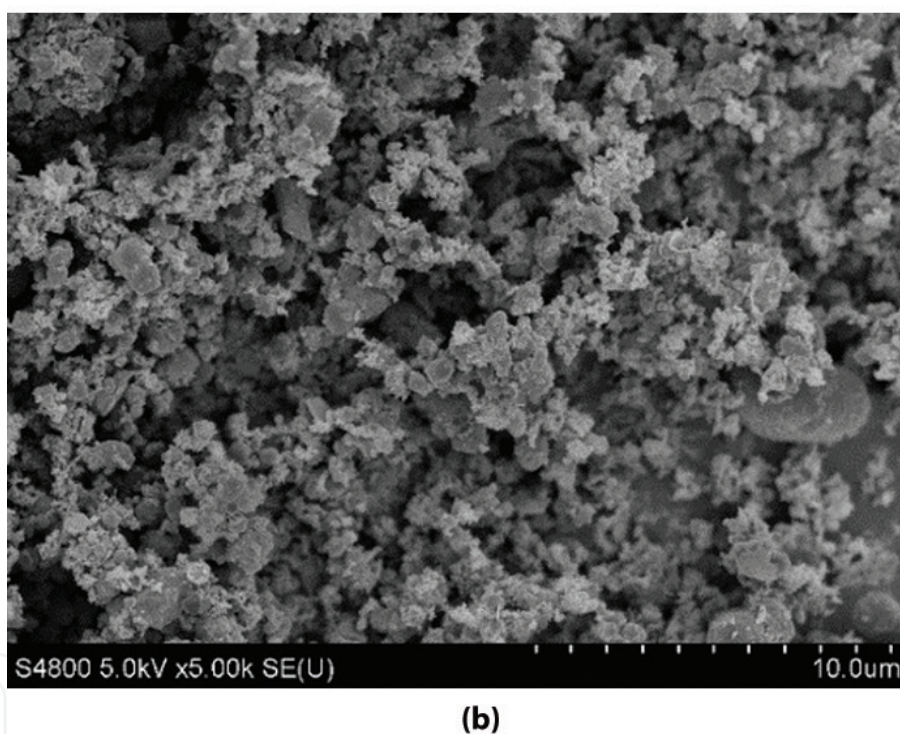
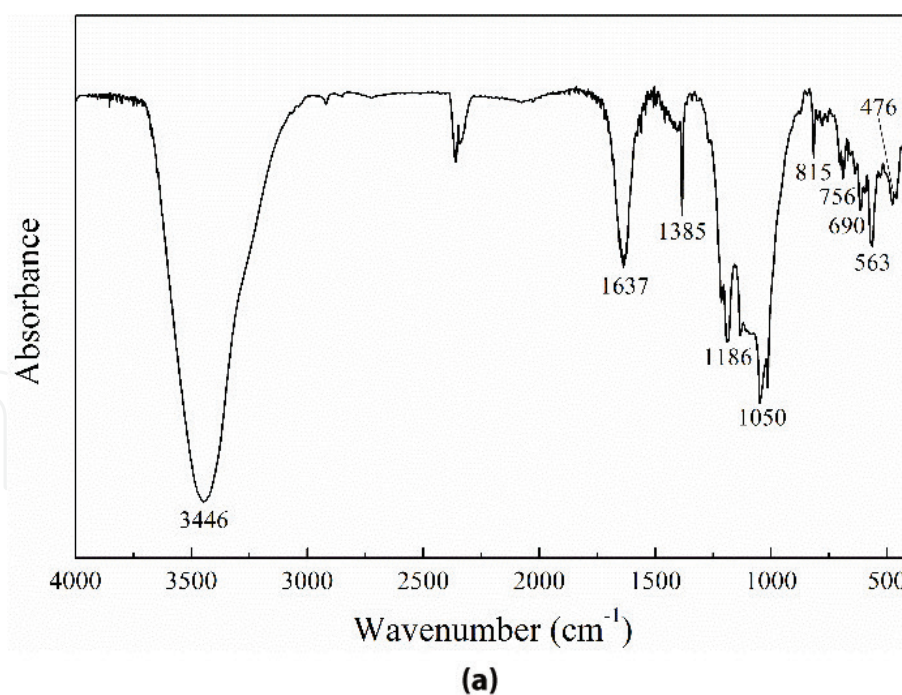


Figure 1.
 (a) FTIR spectra and (b) SEM micrograph of SSB.

in the SSB, and the size of holes is very large. These results indicate that the SSB with abundant functional groups and pore structure can also change the physical and chemical properties of soil and provide a survival shelter for microorganism [27].

3.2 Effects of SSB addition on the physicochemical property of soil

The effects of SSB addition on the pH and EC of soil are shown in **Figure 2**. The pH of the control soil increased remarkably after planting, which indicated that the acid organic matter in soil was decomposed during Chinese cabbage planting [28]. Also, the addition of SSB adjusted the pH of soil from acidic to neutral and the pH increased from 7.12 to 7.49 after planting. **Figure 2b** shows that the EC of the control soil increased slightly after cabbage planting, but it is just 382 μS/cm and close to the

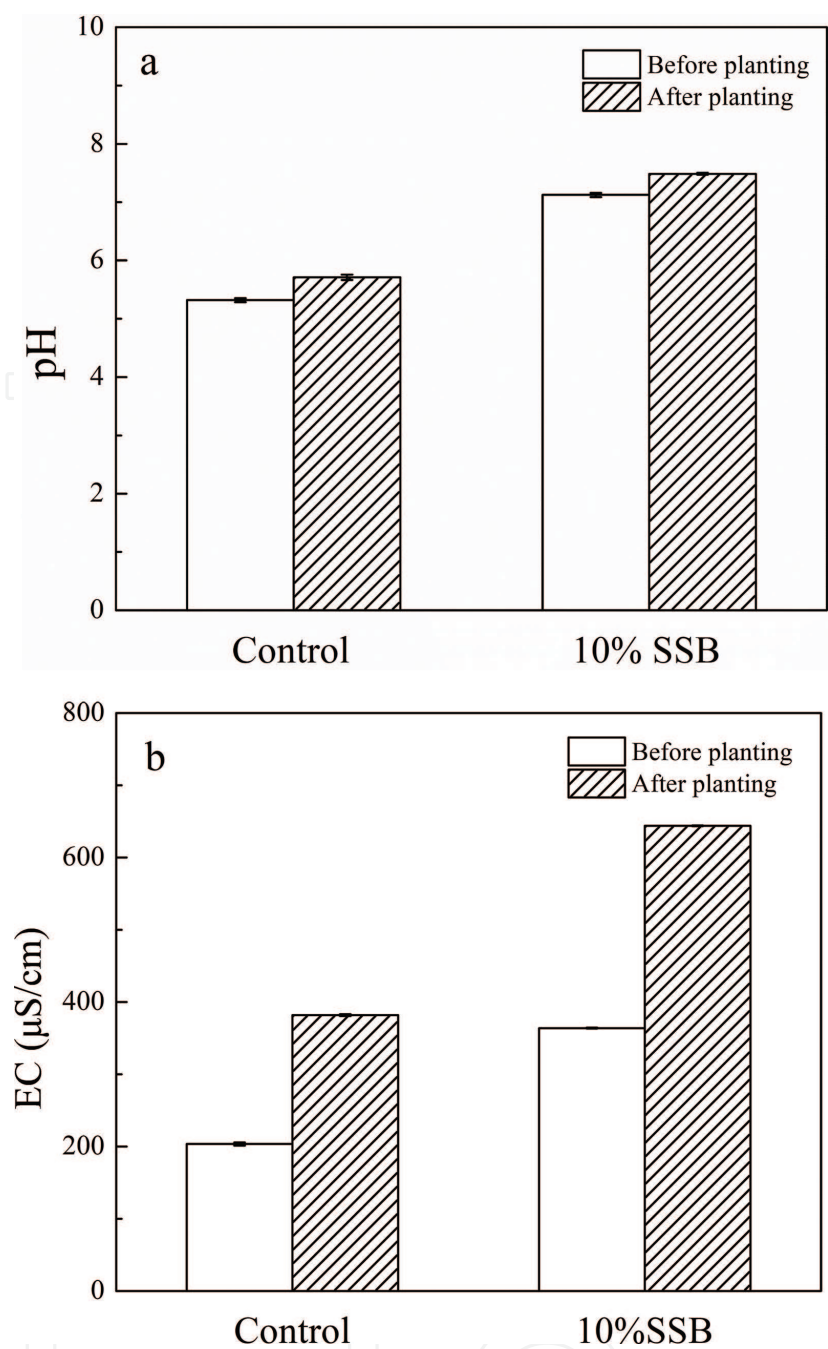


Figure 2.
Effects of SSB addition on the pH (a) and EC (b) of soil.

EC of the soil added with 10% SSB before planting. The EC of the soil with 10% SSB addition increased from 364 to 644 $\mu\text{S}/\text{cm}$ after planting and the increase rate was 76.92%. When EC is lower than 500 $\mu\text{S}/\text{cm}$ or higher than 2000 $\mu\text{S}/\text{cm}$, the phenomenon of lacking nutrient or seedling burning will occur during planting [29]. Therefore, adding SSB in soil could adjust the EC to a suitable range (500–2000 $\mu\text{S}/\text{cm}$) for plant growth. The above results are because a number of alkaline ions such as hydrocarbon anion, bicarbonate, carbonate, and phosphate in SSB were released during planting and increased the pH and EC of soil effectively [30, 31].

3.3 Effects of adding SSB on the microbiological property of soil

3.3.1 Effects of adding SSB on the DHA and urease in soil

DHA plays a key role in the decomposition process of organic matter and can be used as an indicator for the evaluation of total cell oxidation activity [32]. Therefore,

DHA activity is used to characterize the intensity of microbial activity. Urease can convert urea into ammonia and carbon dioxide or ammonium carbonate, and it reflects the intensity of nitrogen relevant reactions in the soil system [33]. The effects of SSB addition on the concentrations of DHA and urease in soil are shown in **Figure 3**. The addition of SSB increased the concentrations of DHA and urease in soil before planting, which rose from 3.83 $\mu\text{g IPTF}/(\text{g h})$ and 16.53 $\mu\text{g NH}_3\text{-N}/(\text{g h})$ to 14.33 $\mu\text{g IPTF}/(\text{g h})$ and 32.00 $\mu\text{g NH}_3\text{-N}/(\text{g h})$, respectively. Whether SSB is added or not, the concentrations of DHA and urease in soil increased after planting, and the concentrations of the DHA and urease in the soil added with 10% SSB reached 3.60 and 1.67 times as high as those of the control soil. These results implied that adding SSB could improve the activities of DHA and urease in soil, promote anaerobic microbial growth and synthesis of enzymes, and enhance microbial activity. This is because SSB influenced enzyme activity with the changes of physiochemical properties

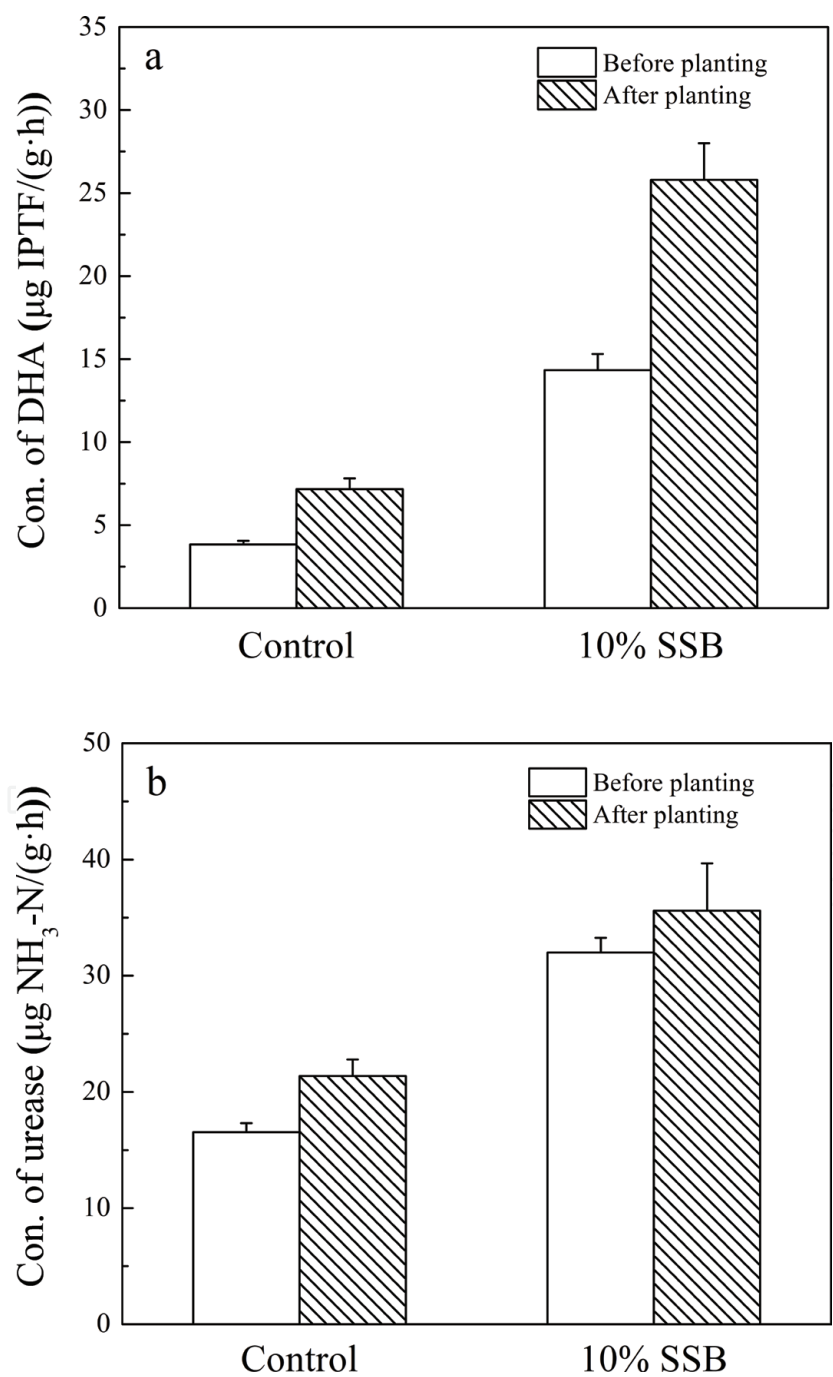


Figure 3.
Effects of SSB addition on the concentrations of DHA (a) and urease (b) in soil.

(especially pH) in soil, and the adsorption of enzymes and soil organic matter on SSB also changed the kinetic properties of enzyme activity [17].

3.3.2 Effects of SSB addition on bacteria and fungi in soil

In the planting process, bacteria play an important role in the transformation of organic and inorganic matter in soil, while fungi have significant effects on the carbon and energy cycle in soil [18]. The bacteria and fungi counts are important indicators of microbial activity intensity, and effectively reflect whether the environment of soil is suitable for crop growth or not. The effects of SSB addition on the concentrations of bacteria and fungi in soil are shown in **Figure 4**. The addition of SSB increased the concentrations of bacteria and fungi in soil before planting, which rose from 2.43×10^6 and 0.77×10^6 CFU/g to 20.60×10^6 and 3.67×10^6 CFU/g, respectively. Whether SSB is added or not, the concentrations of both bacteria and fungi in soil increased after planting, and the bacteria and fungi

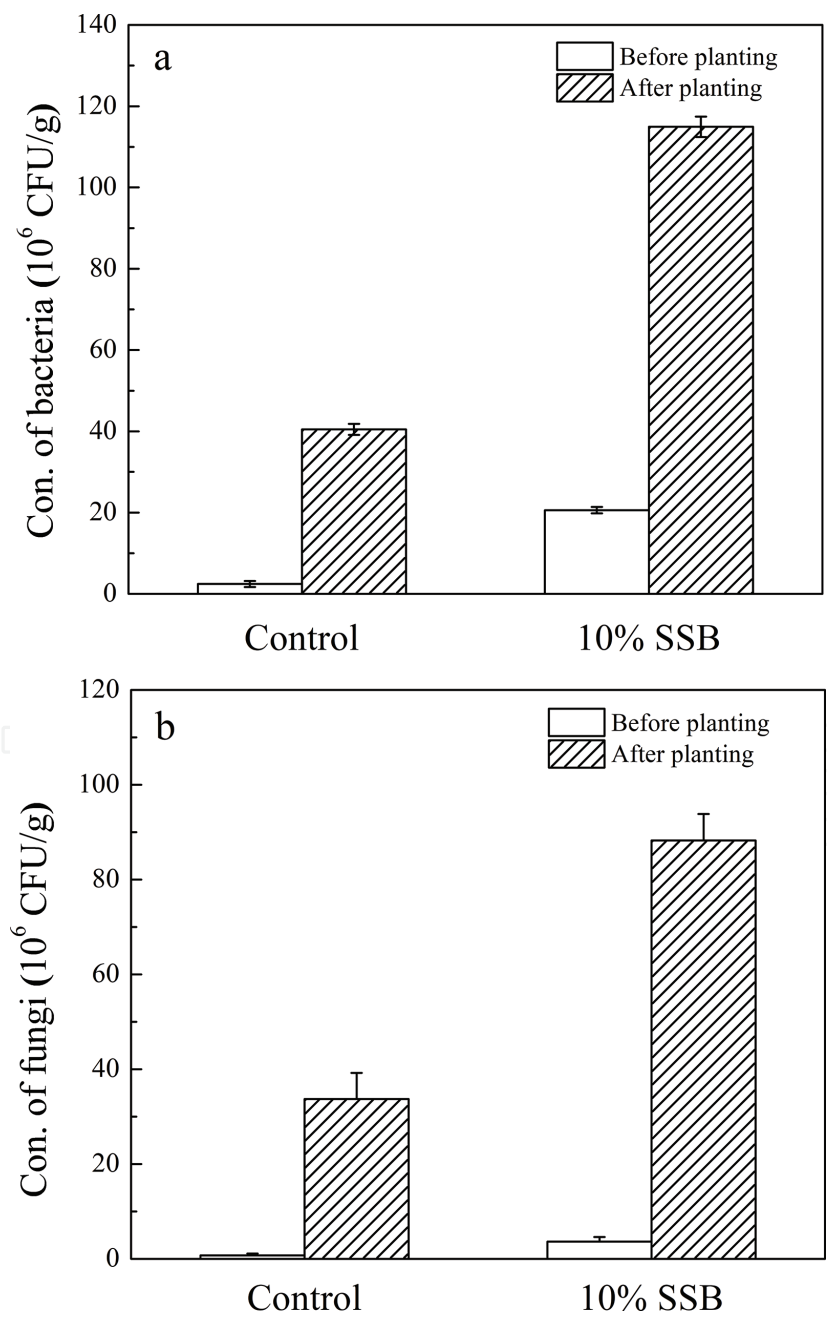


Figure 4.
Effects of SSB addition on the concentrations of bacteria (a) and fungi (b) in soil.

concentrations in soil added with 10% SSB reached 2.84 and 2.62 times as high as those of the control soil, respectively. These results showed that the addition of SSB had beneficial modulation effects on the concentrations of bacteria and fungi during planting, and it could effectively enhance the microbial property of soil.

3.3.3 Effects of adding SSB on the AOA and AOB in soil

AOA and AOB associated with the nitrification of soil are called the nitrifying bacteria. The higher concentrations of AOA and AOB can improve the conversion of other forms of nitrogen into available nitrogen fertilizer so as to enhance the fertility of soil and promote plant growth [34]. The effects of SSB addition on the concentrations of the AOA and AOB in soil are displayed in **Figure 5**. The addition of SSB increased the concentrations of AOA and AOB in soil before planting, which rose from 4.83×10^6 and 2.47×10^6 amoA copies/g to 8.63×10^6 and 6.07×10^6 amoA copies/g, respectively. Whether SSB is added or not, the concentrations of both AOA and AOB in soil increased after planting, and the AOA and AOB

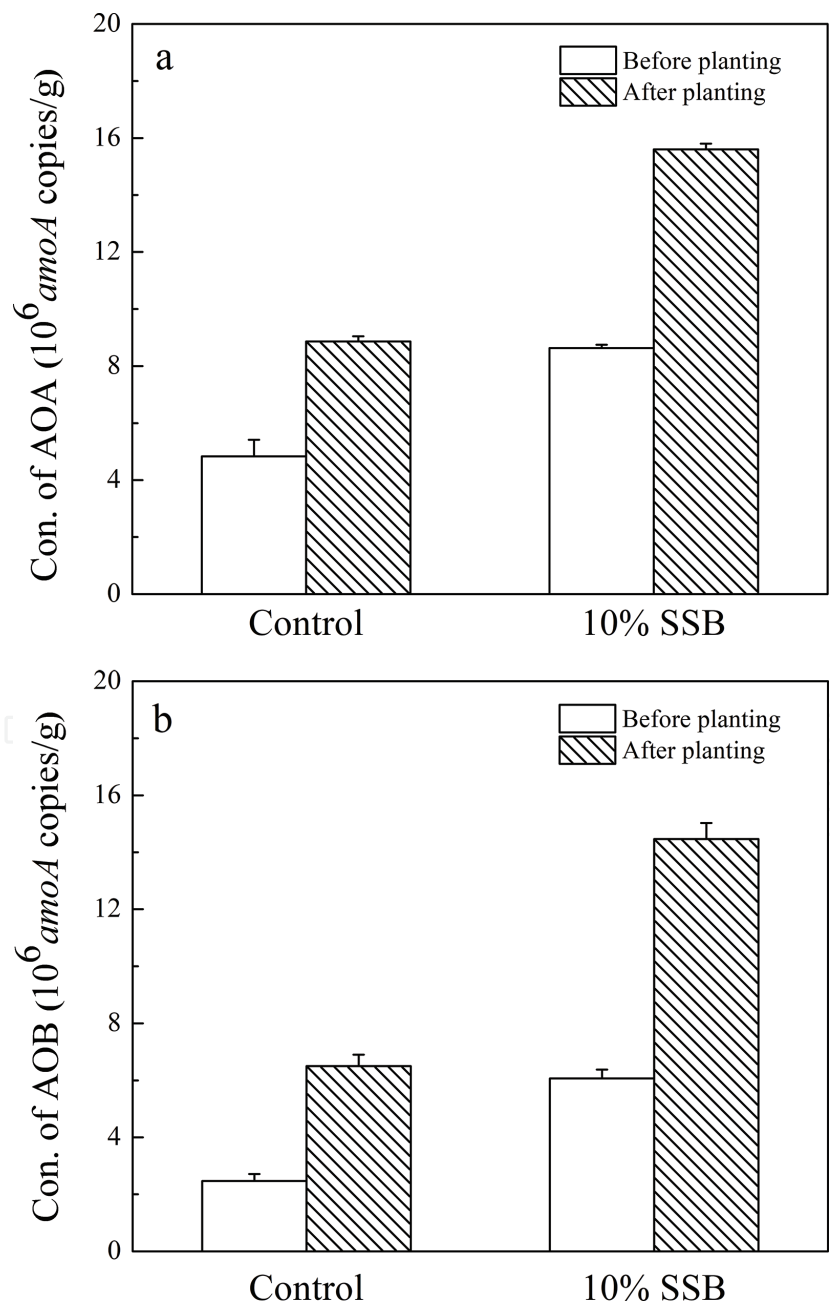


Figure 5.
Effects of SSB addition on the concentrations of AOA (a) and AOB (b) in soil.

concentrations in soil on adding 10% SSB reached 1.76 and 2.23 times as high as those of the control soil, respectively. These results show that SSB addition could effectively increase the concentrations of microorganisms associated with soil nitrification before and after planting.

To sum up, the influence of SSB on the microbiological property are as follows: on the one hand, SSB stored and supplied a large amount of nutrients by the bonding of nutrient cations and inorganic anions in soil with its surface functional groups; on the other hand, SSB changed the physiochemical property of soil and reduced the toxicity of contaminants to soil microorganisms [17].

3.4 Effects of adding SSB on Chinese cabbage growth

The weights of the aboveground and underground parts of Chinese cabbage are considered as important indicators that directly reflect the influence of the physical, chemical, and microbial properties of soil on plant growth. **Figure 6** shows the effects of adding SSB on the weight of Chinese cabbage. The weights of the aboveground and underground parts of Chinese cabbage increased with 10% SSB added to soil. The weight of edible aboveground part was 5.82 times and that of the underground part was 8.67 times as much as those from the control soil. These results can be explained by the fact that the addition of SSB brought the pH and EC of the original soil to suitable ranges for plant growth, and that the increases of the DHA activity, urease activity, bacteria concentration, and fungi concentration provided appropriate metabolic environment for soil microorganisms. This favorable metabolic environment further improved the microbial characteristics and forms a virtuous cycle [17]. In addition, SSB contains nutritive elements like K, P, and N at high concentrations, which increased the fertility of barren soil [9]. Therefore, the weights of Chinese cabbage increased significantly after SSB addition. This also showed that SSB had a positive effect on the growth of crop in barren soil.

3.5 Effects of SSB addition on HMs availability in Chinese cabbage and soil

Figure 7 shows the concentrations of HMs in the aboveground and underground parts of Chinese cabbage, respectively. For the aboveground part, the addition of SSB to soil significantly decreased the concentrations of Mn and Cd, and reduced the toxicity of Chinese cabbage in the edible part compared with the control group. For the underground part, the addition of SSB significantly decreased the

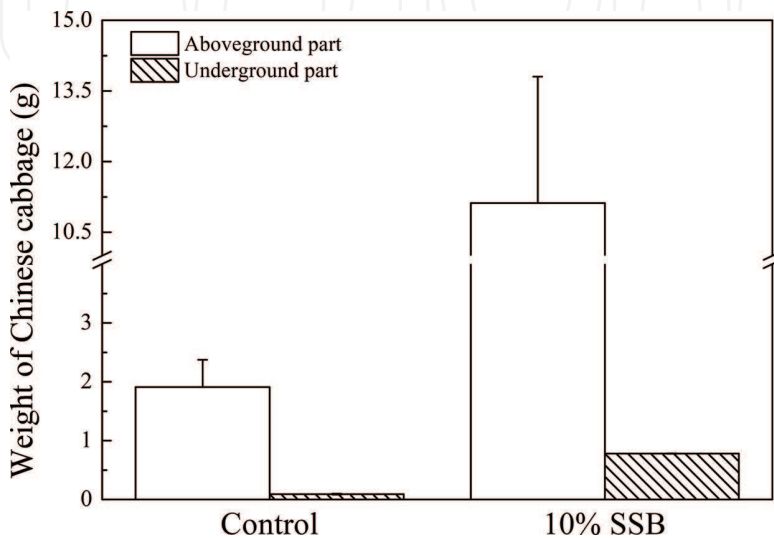


Figure 6.
Effects of adding SSB on the weight of Chinese cabbage planted.

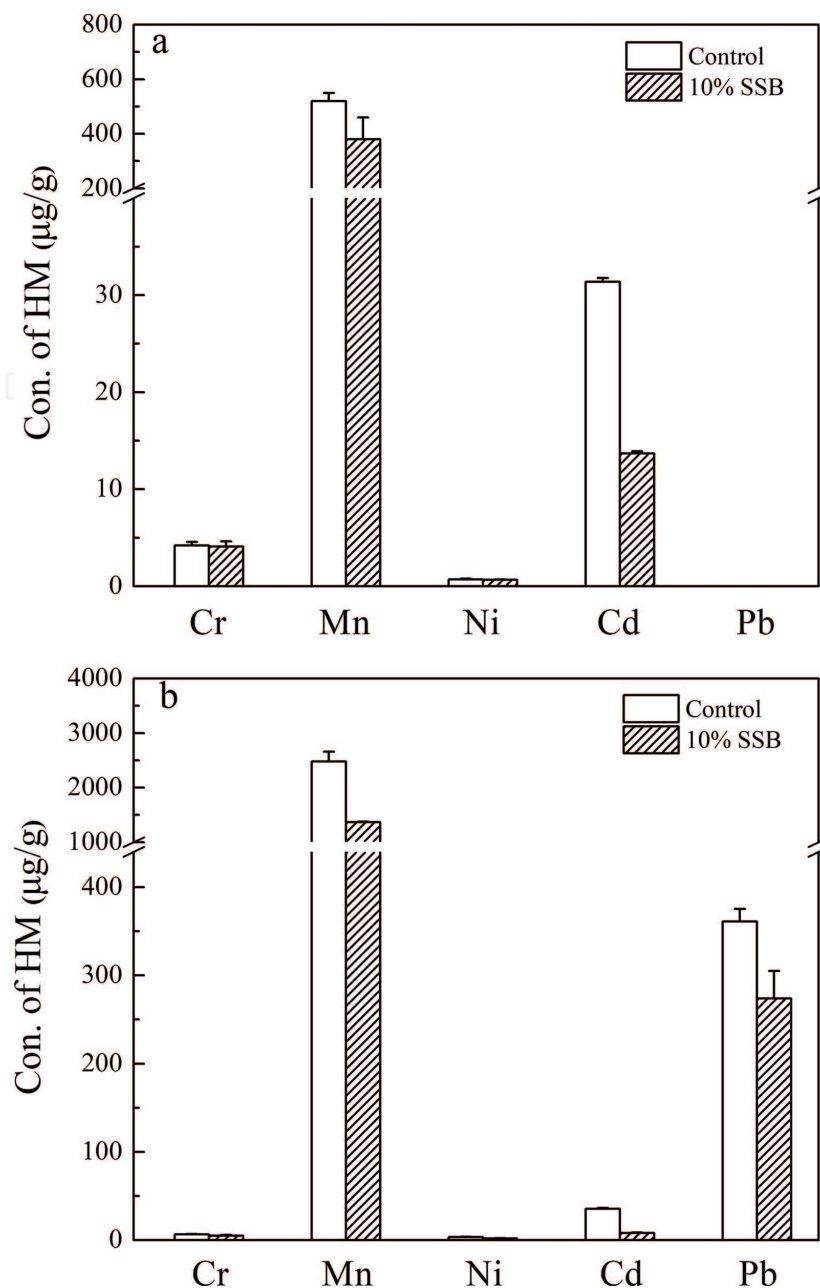


Figure 7. Effects of adding SSB on the concentrations of HMs in aboveground part (a) and underground part (b) of Chinese cabbage planted.

concentrations of Mn, Pb, and Cd compared with the control group, which implied that the addition of SSB in soil inhibited the migration of HMs from soil to the underground part of Chinese cabbage.

It is widely accepted that the HMs in plant are entirely from the migration of the available HMs in the mixed soil during planting [4, 35]. Therefore, the concentrations of available HMs in soil before and after planting were measured to investigate the influence of SSB addition on the transfer of HMs, as shown in **Table 3**. The change rate of available HM concentration in soil after planting compared with that before planting was defined as:

$$\alpha = \frac{c_{s2} - c_{s1}}{c_{s1}} \times 100 \tag{1}$$

where, α is the change rate of available HM concentration in soil after planting compared with that before planting, %; c_{s2} is the concentration of available HM in

HM	Condition	Before planting (μg/g)	After planting (μg/g)	α (%)
Cr	Control	8.24 ± 0.01	8.20 ± 0.38	−0.49
	10% SSB	6.80 ± 0.06	6.68 ± 0.29	−1.76
Mn	Control	0.35 ± 0.02	0.34 ± 0.02	−2.86
	10% SSB	0.34 ± 0.00	0.31 ± 0.01	−8.82
Ni	Control	7.59 ± 0.02	7.92 ± 0.03	+4.35
	10% SSB	7.10 ± 0.09	7.08 ± 0.68	−0.28
Cd	Control	4.88 ± 0.11	5.57 ± 0.22	+14.14
	10% SSB	4.62 ± 0.47	4.75 ± 0.15	+2.81
Pb	Control	0.36 ± 0.05	0.35 ± 0.06	−2.78
	10% SSB	0.27 ± 0.03	0.25 ± 0.02	−7.41

Table 3.
Concentrations and change rates of available HMs in soil before and after planting.

the soil after planting, μg/g; and c_{s1} is the concentration of available HM in the soil before planting, μg/g.

The addition of SSB decreased the concentrations of available Cr, Mn, Ni, Cd, and Pb in soil before planting, which is mostly because the fractions of HMs in SSB are more stable than those in soil. After planting, the concentrations of available Cr, Mn, and Pb in control soil decreased by 0.49, 2.86, and 2.78%, respectively, which indicated that these HMs were taken up by cabbages or migrated to more stable fractions during planting. Compared with the control soil, the addition of SSB reduced the transfer of the available HMs in soil during planting and the α value of Cr, Mn, Ni, Cd, and Pb decreased from −0.49, −2.86, 4.35, 14.14, and −2.78 to −1.76, −8.82, −0.28, 2.81, and −7.41%, respectively.

In order to investigate the effects of SSB addition on the migration of the available HMs in soil, the conversion rate of the content of available HM was defined as:

$$\eta = \frac{c_{s2} \cdot m_{s2} + c_{ca} \cdot m_{ca} + c_{cb} \cdot m_{cb} - c_{s1} \cdot m_{s1}}{c_{s1} \cdot m_{s1}} \times 100 \tag{2}$$

where η is the conversion rate of the content of available HM, %; c_{ca} and c_{cb} are the concentration of available HM in the aboveground and underground parts of Chinese cabbage after planting, respectively, μg/g; m_{s1} and m_{s2} are the mass of soil before and after planting, respectively, g; and m_{ca} and m_{cb} are the mass of the aboveground and underground parts of Chinese cabbage, respectively, g. When $\eta > 0$, the HM in soil transforms from the stable state to the available state after planting; and when $\eta < 0$, the HM in soil transforms from the available state to the stable state after planting. The conversion rates of the available HMs are shown in **Table 4**. The planting of Chinese cabbage in control soil promoted the immobilization of Cr and Pb and inhibited the immobilization of Mn, Ni, and Cd. Moreover, the addition of SSB increased the conversion rate of Mn compared with the control soil, which indicated that SSB addition could improve the migration of Mn to the available state. Mn plays an important role in the process of photosynthesis, respiration, protein synthesis, and hormone activation [36], which explains partly the effects of SSB addition on the weights of the aboveground and underground parts of Chinese cabbage. In addition, the conversion rates of Cr, Ni, and Cd after adding SSB decreased from −0.46, 4.35, and 14.40 to −1.62, −0.26, and 3.50% compared with the control soil, which indicated that the addition of SSB could promote the migration of Ni, Cd, and Cr from the available state to the stable state. In general, this

Condition	η (%)				
	Cr	Mn	Ni	Cd	Pb
Control	-0.46	66.65	4.35	14.40	-0.97
10% SSB	-1.62	302.60	-0.26	3.50	8.42

Table 4.
Conversion rates of the content of available HMs.

immobilization effect was closely related to the biochar properties and its effects on the microbial environment in soil. The surface of SSB has numerous rich alkaline groups such as alkyl negative ion, bicarbonate, carbonate, and phosphate [31], and its application in soil increased the pH, which led to the immobilization of the available HMs in soil [37, 38]. And, the interactions of SSB with the available HMs promoted the more stable transformation of HMs, and included the ion exchange between metal in soil and exchangeable metal in SSB, electrostatic attraction of anionic metal, electrostatic attraction of cation metal, and precipitation of metal [39]. Also, SSB has a good porous structure and can improve the microbial activity, which enhanced the transformation of microorganism on HMs [17]. Therefore, the addition of SSB could improve the immobilization of available HMs in soil.

4. Conclusions

SSB has better pH and EC, more developed pore structure, and higher concentrations of nutrient elements compared with the original soil. The addition of SSB could adjust the pH of mine soil from acidic to neutral and increase the EC of soil. Also, the addition of SSB increased the concentrations of enzyme and microorganisms. Therefore, the changes of the physiochemical property and microbial environment improved the growth of Chinese cabbage. The edible aboveground and the underground parts of cabbage in SSB-amended soil weighed 5.82 times and 8.67 times as much as those from the control group. Moreover, the addition of SSB promoted the migration of Cr, Ni, and Cd from the available state to the more stable state due to the special properties of SSB and changes of soil environment. To sum up, SSB has positive effects on the planting in barren soil.

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