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Utilization of Wood Biomass for Organic Soil Based on the Soil Fertility Index (SOFIX)

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Abstract

Possibility of wood biomass for preparing organic soil was examined to construct reproducible and stable organic standard soil. Seven organic soils were constructed from base soils and additive materials based on the recommended values of the soil fertility index (SOFIX) (total carbon ≥ 25,000 mg/kg, total nitrogen ≥ 1500 mg/kg, total phosphorus ≥ 1100, and total potassium of 2500 to 10,000 mg/kg). Base soils were prepared from two types of wood biomass (big- and small-sized wood chips) at 50%, 60%, and 70% (v/v) and other organic materials such as peat moss, black soil, and mountain soil. Additive materials (soybean meal, oil cake, cow manure, and bone meal) were amended into all organic soils at the same amount. Incubation experiment showed that bacterial biomass in all organic soil was greater than 6×10^8 cells/g-soil after addition of 30% of water content for 1 week. In addition, polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) analysis resulted in a stable bacterial diversity of the organic soil prepared from the small size wood chip at 70%. Chemical properties of all organic soils were within the recommended values of SOFIX. The plant cultivation experiment showed that fresh *Brassica rapa* var. *peruviridis* weights in the organic soils with 50%, 60%, and 70% of small-sized wood chip were 5%, 16%, and 27% higher than that of the chemical fertilizer-amended soil. The organic soil with 70% of small wood chip was the best in the seven organic soils in this study.

Keywords

Wood Biomass, Organic Soil, Soil Fertility Index (SOFIX)

1. Introduction

Over the last century, agrochemicals such as chemical fertilizers and pesticides

have been developed to enhance agricultural activities [1]. Crop and vegetable yields have been substantially enhanced but the chemicals have also increased the risk to human health and the environment. Chemical fertilizers and pesticides have the potential to cause a considerable environmental hazard, including the reduction in the numbers and activities of soil microorganisms [2] [3] [4] [5].

To protect soil microorganisms from the harmful effects of agrochemicals, it is necessary to either minimize the use of agrochemicals or increase the abundance and activities of soil microorganisms to accelerate the biodegradation process [6] [7]. Soil microorganisms represent one of the most important indicators for stable organic agriculture. Microorganisms play important roles in the decomposition of organic materials and the cycling of carbon, nitrogen, phosphorus, and several other nutrients in the soil [8] [9] [10] [11] [12].

Analysis of soils and organic materials can be used to determine the status of available nutrients. The soil fertility index (SOFIX) was developed considering the importance of physical, chemical, and biological soil characteristics [13]. More than 6000 agricultural soil samples (upland, paddy, and orchard fields) have been analyzed by the SOFIX. The suitable soil conditions for organic agriculture based on the SOFIX database are total carbon (TC) ≥ 25,000 mg/kg, total nitrogen (TN) ≥ 1500 mg/kg, total phosphorus (TP) ≥ 1100, total potassium (TK) 2500 to 10,000 mg/kg, and bacterial biomass ≥ 6×10^8 cells/g-soil.

Reproducible and stable organic soils with abundant microbial number and diversity are especially difficult to create, while it is willing to use for agriculture and scientific fields, because wood biomass is abundant not only in Japan but also over the world. Therefore, its utilization as soil amendment should be concerned. The previous experiments showed that wood chip from cedar leads to the increase of bacterial biomass [14]. This study aimed to construct a reproducible and stable organic soil based on the SOFIX database through testing a range of base soils and additive materials. This paper describes the process of control of the base soil and additive materials, the plant growth, and the bacterial analysis of the organic standard soil.

2. Materials and Methods

2.1. The Study Area

This study was carried out from November 2017 to September 2018 in the Faculty of Life Sciences, Ritsumeikan University, Kusatsu city, Shiga prefecture, Japan (34˚58'58.0"N 135˚57'49.2"E).

2.2. Materials

Black soil (Kanuma Kosan, Tochigi, Japan), vermiculite (Kanuma Kosan), peat moss (Kanuma Kosan), mountain soil (Toyo company, Aichi, Japan), wood chip 1 (particle size 1 cm; DaikenKogyo company, Osaka, Japan), and wood chip 2 (particle size 0.5 cm; DaikenKogyo company, Osaka, Japan) were used for the

base soil. Cow manure (Taniguchi Bokujo company, Shiga, Japan), horse manure from a horse ranch (Shiga, Japan), chicken manure from a chicken farm (Shiga, Japan), oil cake (JoY Agris company, Tokyo, Japan), soybean meal (Tamagoya company, Ibaraki, Japan), and bone meal (Tachikawa Heiwa Noen company, Tochigi, Japan) were used as the additive materials. The base soils and additive materials were air dried for 1 week, and then sieved through a 2-mm sieve. The chemical soil (Hanachanbaiyodo company, Nagoya, Japan), which is amended with chemical fertilizer, was considered as a control treatment.

2.3. Analytical Methods

Total carbon (TC) was analyzed using a total organic carbon analyzer (SSM-5000A, Shimadzu, Kyoto, Japan). Total nitrogen (TN), total phosphorus (TP), and total potassium (TK) contents were analyzed by extracting soil samples using the Kjeldahl digestion method followed by analysis using the indophenol blue method, molybdenum blue method, and atomic absorption spectrophotometry, respectively [15] [16]. The total bacterial biomass of the soil was analyzed by quantifying the environmental DNA (eDNA) extracted by the slow-stirring method [17]. Nitrogen (N) circulation and phosphorus (P) circulation activities were examined according to our previous studies [13] [18]. The maximum water holding capacity (WHC) and bulk density were measured by the standard methods [19]. Soil pH (1:2.5 soil-to-water suspension, w/v) was analyzed using a pH meter (LAQUA. F-71, Horiba, Kyoto, Japan).

2.4. Preparation and Analysis of the Soil

The base soils and additive materials were dried at 37˚C for 1 week, and then these materials were sieved through a 2-mm sieve. Seven organic soils were prepared by blending the base soils and additive materials. To activate the microbial activities, 200 g of each organic soil was preincubated in a 400 ml pot and maintained 30% of water content for 1 week. The soil sample of each organic soil treatment was subsequently collected for SOFIX analysis [13]. The bacterial biomass was measured on days 0, 3, 5, and 7, while the other parameters were measured on days 0 and 7. The bacterial diversity of the different lots of ideal standard organic soil and the different lots of chemical soils was analyzed on day 0 with polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) analysis. The organic soils were incubated in the plant factory with 12 h of light: 12 h of dark at 23˚C throughout the experimental period.

2.5. Plant Cultivation

Seven organic soils and the chemical soil (control) were used for plant cultivation experiment. A 2 L soil sample was put into a Wagner pot (1/5000a, Fujimoto Kagaku Kogyo company, Tokyo, Japan), and then preincubated at 30% of water content. *Brassica rapa* var. *peruviridis* (Komatsuna) seeds were sown in a nursery tray for 1 week, and four seedlings were then transplanted to each Wagner

pot. After 4 weeks of cultivation, *B. rapa* of each treatment were harvested and measured fresh weight, shoot length, root length, chlorophyll content, and the number of leaves. The parameters of *B. rapa* growth were determined using one-way analysis of variance (ANOVA).The leaf chlorophyll was analyzed by a chlorophyll meter (SPAD-502, Minolta, Tokyo, Japan) and described by SPAD reading values. The experiments were conducted in the plant factory (12 h of light and 12 h of dark; 23˚C).

2.6. PCR-DGGE Analysis

A best organic standard soil (among seven organic soils) and the chemical soil (base soils + chemical fertilizer) were used for PCR-DGGE analysis. The 16S rRNA bacterial gene was amplified using primers DGGE-F (5'-CGCCC GCCGC GCCCC GCGCC CGTCC CGCCG CCCCC GCCCG CCTAC GGGAG GCAGC AG-3') and DGGE-R (5'-CCGTC AATTC CTTTG AGTTT-3') [20]. The amplification reaction was carried out in a 50 µL PCR mixture containing 0.01 ng/µL of DNA template, 1.5 U rTaq DNA polymerase, 5.0 µL of 10× buffer, 5.0 µl of 2 mM dNTPs, 3.0 µl of $MgCl_2$, and 2.0 µL of 10 mmol/L of each primer. DNA polymerase, dNTPs, and PCR buffer were purchased from TOYOBO (Osaka, Japan), and all primers were synthesized by Sigma-Aldrich (Tokyo, Japan). The thermal PCR profile was as follows: initial denaturation at 95˚C for 1 min, followed by 35 cycles of denaturation at 95˚C for 1 min, primer annealing at 55˚C for 30 s, and extension at 72˚C for 1 min and then a final extension at 72˚C for 5 min. Finally, the amplified 16S rRNA bacterial genes were used for denaturing gradient gel electrophoresis (DGGE) analysis.

DGGE was performed using the D Code System (BioRad Laboratories Inc., California, USA). A total of 20 µl of PCR product was loaded into 8% (w/v) poly acrylamide gel with a denaturant gradient of 27.5% - 67.5%. The gel was then run in 1 × Tris-acetate EDTA buffer at a constant voltage of 70 V at 60˚C for 15 h. The gel was stained using ethidium bromide for 30 min, then rinsed with distilled water. Cluster analysis of the DGGE band pattern was subsequently conducted using the FPQuest Bioinformatics Software (BioRad Laboratories Inc., California, USA).

3. Results

3.1. Selection of the Base Soils and Additive Materials

Base soils and additive materials were selected to construct suitable chemical, physical, and biological characteristics in the organic standard soil. The properties of candidates for the base soil (mountain soil, black soil, peat moss, vermiculite, and wood chips) were measured (**Table 1** and **Table 2**). The TC contents of peat moss and wood chips were higher than those of the other candidate base soils, while the TN and the TP contents of all candidates were low. The maximum WHC of black soil, vermiculite, and wood chips were relatively high but the bulk density of vermiculite, peat moss, and wood chips were low. The components

Table 1. The chemical properties of the base soils and additive materials.

Material		TC (mg/kg)	TN (mg/kg)	TP (mg/kg)	TK (mg/kg)	C/N ratio	C/P ratio
Base soil	Black soil	69,500	1770	2070	4000	39	34
	Mountain soil	300	90	410	8000	3	1
	Vermiculite	400	180	300	33,000	2	1
	Peat moss	412,200	2070	310	1300	199	1330
	Wood chip 1	445,100	700	270	2500	636	1649
	Wood chip 2	356,000	470	270	2600	757	1319
Additive material	Oil cake	416,900	51,200	18,200	14,000	8	23
	Soybean meal	405,900	66,800	7,350	24,200	7	55
	Bone meal	211,400	40,600	75,880	3600	5	3
	Chicken manure	194,000	34,600	17,500	24,400	6	11
	Horse manure	113,600	4729	3350	4330	24	34
	Cow manure	330,000	21,000	10,000	26,000	16	33

Table 2. The bacterial biomass and physical properties of the base soils and additive materials.

Material		Bacterial biomass ($\times 10^8$ cells/g-soil)	Water holding capacity (ml/kg)	Bulk density (g/cm^3)	Water content (%)
Base soil	Black soil	N.D.	980	0.84	1.2
	Mountain soil	N.D.	550	1.39	29.6
	Vermiculite	N.D.	3000	0.22	0.2
	Peat moss	N.D.	300	0.14	3.6
	Wood chip 1	2.7	1150	0.15	12.3
	Wood chip 2	8.8	1120	0.10	9.2
Additive material	Oil cake	N.D.	-	-	-
	Soybean meal	N.D.	-	-	-
	Bone meal	N.D.	-	-	-
	Chicken manure	7.8	-	-	-
	Horse manure	71.0	-	-	-
	Cow manure	132.4	-	-	-

N.D. = not detected.

difference sizes of wood chips (wood chips 1 and wood chips 2) were almost the same but the bacterial biomass of a wood chip 2 was higher than that of a wood chip 1.

The total nitrogen contents of oil cake, soybean meal, bone meal, chicken manure, and cow manure were above 20,000 mg/kg. The TP contents of oil cake, bone meal, chicken manure, and cow manure were high. The bacterial biomass of all manures was above 6×10^8 cells/g. Among the three types of manure, cow

manure was selected because of a well-balanced nutrient content and high bacterial biomass.

3.2. Construction and Characterization of the Organic Standard Soils

The candidates of a standard soil based on SOFIX recommended values (Table 3) were prepared to construct a stable and reproducible organic standard soil. Seven candidates of the organic standard soil were prepared using the base soils and additive materials at different ratios (Table 4 and Table 5). Cow manure, oil cake, soybean meal, and bone meal were added in base soil at 5%, 0.25%, 0.25%, and 0.05% w/w, respectively.

Chemical and physical properties of the seven prepared organic standard soils are shown in Table 6. The TC, TN, TP, and TK contents, and the C/N and C/P ratios of the seven candidate standard soils were 24,000 - 34,740 mg/kg, 1580 - 1840 mg/kg, 1040 - 1160 mg/kg, and 6450 - 9660 mg/kg, and 14 - 20 and 22 - 31, respectively. The bulk density and the WHC of the seven organic standard soils were above 0.5 g/cm^3 and 1200 ml/kg, respectively. The chemical and physical properties of the seven organic soils were around SOFIX recommended values. Among seven organic soils, T7 was showed the lowest bulk density but the highest WHC.

The biological properties of the seven candidate organic soils after controlling the water (30% of water content) for 1 week are shown in Figure 1 and Table 7. The bacterial biomass of all candidate organic soils exceeded 6×10^8 cells/g-soil on day 3, and the bacterial biomass of T2, T3, T4, T5, T6, and T7 was greater than 11×10^8 cells/g-soil on day 7. This result indicates that the wood chips increase the bacterial biomass. Among the seven organic soils, T7 showed the highest value of the bacterial biomass. The nitrogen and phosphorus circulation activities of the seven candidates of the organic soil were close to the SOFIX recommended values.

3.3. Plant Growth in the Organic Standard Soils

To compare the plant growth, *B. rapa* cultivation experiment was conducted (Table 8). The performance of *Brassica rapa* in the seven organic soils was similar

Table 3. The SOFIX recommended value.

Parameter	Recommended value
Total carbon (TC) (mg/kg)	≥25,000
Total nitrogen (TN) (mg/kg)	≥1500
Total phosphorus (TP) (mg/kg)	≥1100
Total potassium (TK) (mg/kg)	2500 - 10,000
C/N ratio	8 - 25
C/P ratio	23 - 46
N circulation activity (point)	≥38
P circulation activity (point)	30 - 70

Table 4. The blend of the organic soils.

Organic soil	Base soil (% v/v)					
	Mountain soil	Black soil	Vermiculite	Peat moss	Wood chip 1	Wood chip 2
T1	30	10	50	10	-	-
T2	30	10	-	10	50	-
T3	30	10	-	-	60	-
T4	20	10	-	-	70	-
T5	30	10	-	10	-	50
T6	30	10	-	-	-	60
T7	20	10	-	-	-	70

Table 5. The blend of the organic soils.

Organic soil	Additive material (% w/w)			
	Cow manure	Oil cake	Soybean meal	Bone meal
T1 - T7	5	0.25	0.25	0.05

Table 6. The chemical and physical properties of the organic soils (Unit: mg/kg air dried soil).

Organic soil	TC (mg/kg)*	TN (mg/kg)	TP (mg/kg)	TK (mg/kg)	C/N ratio	C/P ratio	Bulk density (g/cm^3)	Water holding capacity (ml/kg)
T1	31,550	1650	1040	9660	19	30	0.69	1328
T2	34,400	1740	1130	7510	19	31	0.59	1340
T3	24,000	1580	1090	7120	15	23	0.63	1297
T4	26,120	1840	1120	6460	14	24	0.51	1332
T5	34,740	1690	1130	7550	20	31	0.58	1362
T6	25,350	1650	1160	7920	15	22	0.55	1338
T7	26,350	1690	1120	6450	15	24	0.50	1407

*TC was determined without wood.

Table 7. N and P circulation activites of the organic soils.

Organic soil	N circulation (point)		P circulation (point)	
	Day		Day	
	0	7	0	7
T1	31	36	54	47
T2	22	32	57	31
T3	47	37	54	58
T4	34	42	54	49
T5	34	45	39	72
T6	35	54	63	37
T7	14	50	60	53

Table 8. Parameters of *Brassica rapa* growth in the organic soils and the chemical soil.

Treatment	Fresh weight (g/plant)	Shoot length (cm)	Root length (cm)	Chlorophyll (SPAD reading)	Number of leaves
T1	$3.4^{a} \pm 0.8$ (98%)	$19.0^{a} \pm 2.2$ (117%)	$11.5^{a} \pm 3.1$ (85%)	$25.3^{b} \pm 3.1$ (76%)	$6^{a} \pm 1.1$ (85%)
T2	$3.5^{a} \pm 1.1$ (96%)	$18.4^{a} \pm 1.9$ (113%)	$10.3^{a} \pm 2.1$ (76%)	$24.4^{b} \pm 3.2$ (73%)	$7^{a} \pm 1.0$ (100%)
T3	$3.4^{a} \pm 1.6$ (98%)	$17.1^{a} \pm 7.1$ (105%)	$11.0^{a} \pm 4.3$ (81%)	$23.8^{b} \pm 4.0$ (71%)	$6^{a} \pm 0.7$ (85%)
T4	$4.4^{a} \pm 1.0$ (118%)	$18.9^{a} \pm 4.1$ (116%)	$14.3^{a} \pm 5.4$ (105%)	$25.7^{b} \pm 4.1$ (77%)	$7^{a} \pm 1.4$ (100%)
T5	$3.9^{a} \pm 1.1$ (105%)	$18.7^{a} \pm 2.7$ (115%)	$13.4^{a} \pm 4.7$ (99%)	$23.8^{b} \pm 1.8$ (71%)	$6^{a} \pm 0.9$ (85%)
T6	$4.3^{a} \pm 1.0$ (116%)	$20.9^{a} \pm 2.2$ (129%)	$13.1^{a} \pm 2.3$ (97%)	$24.5^{b} \pm 3.9$ (73%)	$7^{a} \pm 0.9$ (100%)
T7	$4.7^{a} \pm 2.1$ (127%)	$19.7^{a} \pm 2.5$ (121%)	$13.3^{a} \pm 2.4$ (98%)	$27.0^{b} \pm 3.5$ (81%)	$6^{a} \pm 0.7$ (85%)
Chemical (control)	$3.7^{a} \pm 1.7$ (100%)	$16.2^{a} \pm 2.7$ (100%)	$13.5^{a} \pm 2.1$ (100%)	$33.2^{a} \pm 2.5$ (100%)	$7^{a} \pm 0.5$ (100%)

Means followed by the same letter do not significantly differ ($p < 0.05$). Value followed by ± is standard deviation.

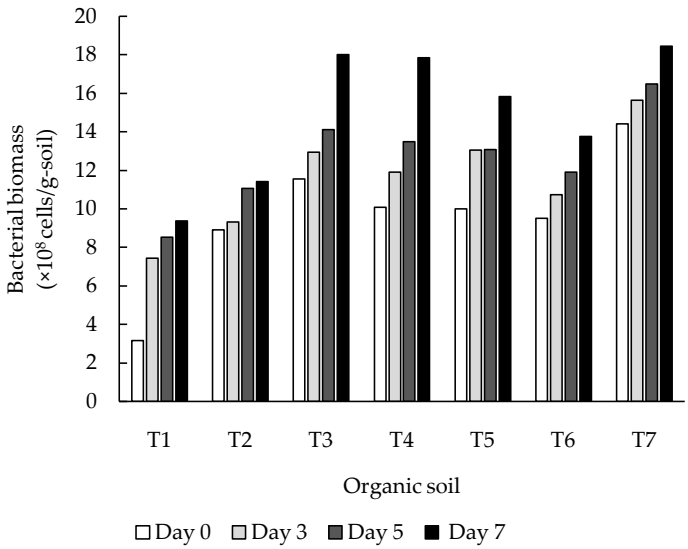

Figure 1. The bacterial biomass in the seven organic soils (T1-T7) during 7 days.

or better than that in the chemical soil. An increase of wood chip 2 led to a higher fresh weight and shoot length of *B. rapa* than that in the chemical soil and in the organic soils with wood chip 1. Especially, *B. rapa* growth in the organic soil T7 containing 70% (v/v) of wood chip 2 was the highest. These findings suggest that wood chip 2 is the most suitable for *B. rapa* cultivation. Chlorophyll of plants in the chemical soil used was 19% - 29% higher than those in the organic soils, suggesting that the inorganic nitrogen in the chemical soil was richer than that in the organic soil.

As a result, the organic soil T7 was identified as the best organic standard soil. In the next experiment, comparison of the bacterial diversity between the organic standard soil (T7) and the chemical soil was conducted.

3.4. Analysis of the Bacterial Diversity in the Organic Standard Soil

The comparison of the bacterial diversity between the organic standard soil (T7) and the chemical soil were conducted in this study. The bacterial diversities of different lots of the organic standard soil and different lots of the chemical soil were compared (Figure 2). The bacterial diversities of the organic standard soil and the chemical soil were different, even though the same base soil was used in the organic standard soil and the chemical soil. The bacterial diversities of the organic standard soil were similar, but those of different lots of the chemical soil were unstable. The number of bacterial species in the organic standard soil was higher than that in the chemical soil. The organic standard soil was controlled not only by the bacterial biomass but also by the bacterial diversity, suggesting that the bacteria biomass and bacterial diversity seem to be a positive relationship.

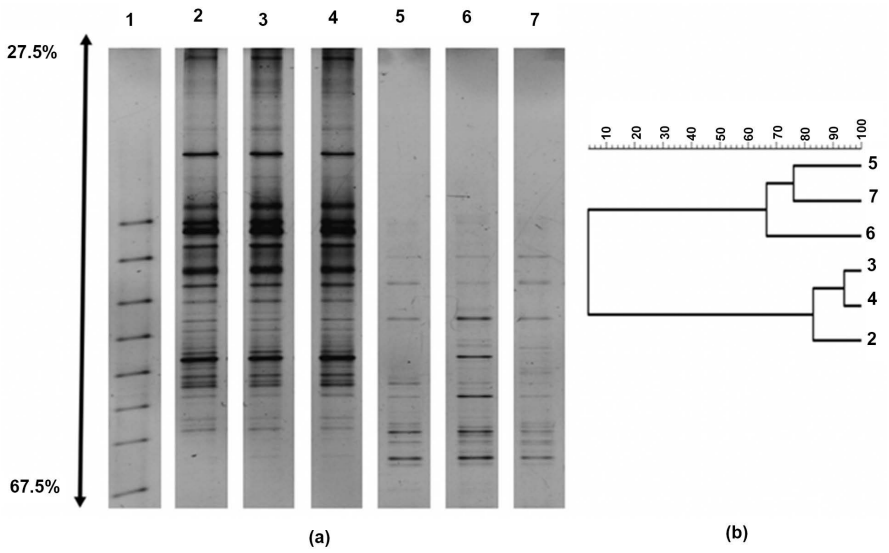

Figure 2. PCR DGGE analysis of 16S rRNA bacterial genes: image of electrophoresis (1: Marker, 2 - 4: Different lots of the organic standard soil, and 5 - 7: Different lots of the chemical soil) (a) and cluster analysis (b).

4. Discussion

Based on SOFIX database [13], the values of TC (≥25,000 mg/kg), TN (≥1500 mg/kg), TP (≥1100 mg/kg), TK (2500 to 10,000 mg/kg), and C/N ratio (8 to 25) were controlled by mountain soil, black soil, wood chips, peat moss, vermiculite, and additive materials. The bacterial biomass of the organic soils with wood chips was higher than 6×10^8 cells/g-soil after controlling the water content to 30%. Wood chips, especially the small particle size (wood chips 2), were found to

be most suitable for the bacteria growth and diversity. The surface area and pore size of wood chips may be suitable for soil microorganisms [21] [22]. In fact, the bacterial biomass in the organic soils with wood chips 2 were obviously higher ($\geq 14 \times 10^8$ cells/g) than that in vermiculite after 7 days. Moreover, the bacterial biomass in the organic soils was also higher than those in organic farming soils [23].

The growth of *B. rapa* in the organic standard soil was higher than that in the chemical soil. Soil microorganisms play an important role in soil nutrientcycling [24] [25]. The supply of nitrogen, phosphorus, potassium, and other minerals in organic materials for plants via the material circulations in soil seems to be as sufficient for growth of the plant as that of chemical fertilizers [23] [24] [25] [26]. The organic standard soil could be used in limited areas of agricultural fields such as greenhouse.

The bacterial biomass was low under the dry conditions in the organic standard soil. However, the bacterial biomass was drastically increased after controlling the water content in the short term [27] [28] [29]. Subsequently, nitrogen and phosphorus circulation activities based on the additive materials occurred after increasing the bacterial biomass. Our results indicate that the organic standard soil led to increased richness and diversity of soil microbes relative to the chemical soil. Many studies have confirmed that the soil microbes are often more diverse and abundant under organic than conventional systems [30] [31] [32] [33]. In addition, the bacterial diversities in the organic standard soil became almost the same within the PCR-DGGE experiment [34], indicating that the preparation of the organic standard soil was reproducible. The bacterial diversity was also controlled reproducibly by the addition of the water.

In this study, the main elements (nitrogen, phosphorus, and potassium) in the organic standard soil were successfully controlled by biomass resources based on the SOFIX database. Other factors, such as micronutrients, will be considered in the next stage of the organic soil construction, which is currently in progress.

5. Conclusion

The reproducible and stable organic standard soil was constructed in this study. All organic soils showed the suitable values of chemical and biological properties according to SOFIX recommended values. Out of those, T7 (with 70% of small-sized wood chip) had the highest bacterial biomass and stable bacterial diversity. In addition, T7 led to the increase of the fresh weight of *B. rapa*.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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