Doctoral Dissertation

Study on soil fertility and construction of new organic soil

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Graduate School of Life Sciences
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（土壌肥沃度および新規有機土壌構築に関する研究）

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Contents

General introduction

Soil is important as a reservoir of nutrients for plants [1]. In conventional agriculture mainly synthetic fertilizers, pesticides, and agrochemicals are used to have increased crop production [2][3][4]. However, application of synthetic fertilizers and agrochemicals have negative effects on soil fertility e.g. reduced abundance and activities of soil microorganisms and soil organic matter (SOM) content [5][6][7]. Considering the negative impact of conventional agricultural practice on environment, organic agricultural is being increased day by day as an alternative agriculture practice to keep environment healthy. Organic agriculture refers to a farming system that enhance soil fertility without the use of chemical fertilizers, pesticides, and agrochemicals [4][8]. However, crop yields under organic farming are lower than that under conventional farming [3][9]. Therefore, a new agricultural system based on biomass resources and biodiversity that provides high quality of soil has been proposed.

The soil fertility index (SOFIX) was developed considering the importance of physical, chemical, and biological soil characteristics [10]. More than 8,000 agricultural soil samples (upland, paddy, and orchard fields) have been analyzed by the SOFIX. SOFIX is the first system in the world to visually indicate soil health as a number through diagnosing and analyzing the 3 indicators e.g. microbial numbers, nitrogen activity, and phosphorous activity in the soil environment. Bacterial biomass, N circulation activity, and P circulation activity are closely related to total carbon (TC), total nitrogen (TN), and total phosphorus (TP). Improvement of TC, TN, TP, and other parameters will be effective for organic agricultural system. Recommended values (TC, TN, TP, etc.) in the upland field for enhance bacterial biomass and nutrient circulation activities levels are determined as TC $\geq$ 25,000 mg/kg, TN $\geq$ 1,500 mg/kg, TP $\geq$ 1,100 mg/kg, and TK 2,500 to 10,000 mg/kg.

Each agricultural field including orchard field, upland field and paddy field, represents different anthropogenic management practices and environmental conditions (e.g. aerobic and anaerobic) [4][11][12]. Soil management practices according to the cultivated crop types [13]. Orchard crops are cultivated as a monoculture growing for many years. The successive crops of the agricultural rotation are not typically carried out in orchard field [14]. Plowing is an agricultural practice done several times per year after crop rotation in the upland and paddy fields [15][16]. Plowing in the orchard field is practiced before permanent planting to avoid damage to root systems [17]. Based on soil fertility, orchard soil database was constructed to determine the features and suitable orchard soil conditions and to compare soil features with upland and paddy fields.

Upland soil at different soil types was examined. Chemical and biological properties of soil are influenced by management practices such as chemical or organic treatments [18][19][20][21][22]. Different long-term agricultural management practices have influenced soil properties. The effects of soil types and agricultural management practices on soil fertility, especially microbial biomass have been unknown. There has been a little information on biological properties of upland soils at different soil types in Japan. This study aims to investigate the tendency of soil fertility and effect of soil types on the soil fertility of upland field in Japan based on SOFIX analysis.

From the study on soil fertility of agricultural fields, new organic soil was constructed. Reproducible and stable organic soils with abundant microbial diversity and suitable nutrient values are especially difficult to create. There is a lack of understanding of decision-making processes underlying the use of processed and unprocessed organic fertilizers by farmers [23]. Soil organic amendments or fertilizers are mainly made from crop residues such as plant litter, wood chip, rice bran, and animal byproducts (bone meal and cow manure). These materials contain specifically high levels of nutrients such as high carbon contents in plant litter and wood chip, phosphorus contents in rice bran and bone meal, that is why they are commonly used as organic fertilizers [24][25][26]. However, incorrect application of organic materials effect to biodiversity and soil quality [27][28][29][30]. Over-application of nutrients in organic materials and organic fertilizers lead to nutrient excess require in soil [29][30]. The deficiency in the requirement of nutrients from organic fertilizer results in low crop yields than that of treated with chemical fertilizer [31]. Therefore, organic soil with abundant microbial diversity and suitable nutrient values based on the SOFIX database, a range of base soils and additive materials concentrations was constructed through experimental analysis.

Based on SOFIX, the standard organic soil using wood biomass database was constructed as incorrect application of organic materials can reduce biodiversity and affect soil quality. Reproducible and stable organic soils with abundant microbial numbers and diversity are especially difficult to create. The database of orchard field was constructed to know the features of orchard soils. An investigation on soil fertility of upland soil at different soil types was carried out to know about the tendency of soil fertility (bacterial biomass, total carbon (TC), and total nitrogen (TN)) and effect of soil types on the soil fertility in upland field in Japan. This study is divided into 3 chapters.

In chapter 1, the database of orchard field based on the SOFIX was constructed and compared with soil properties of orchard field with upland and paddy fields. Soil samples from 442 agricultural fields (139 orchard fields, 190 upland fields, and 113 paddy fields) in Japan

from 2014 to 2019 were collected and analyzed. To investigate orchard soil features and compared with upland and paddy soils. Moreover, the relationships of bacterial biomass, TC, TN, and TP were investigated.

In chapter 2, upland soils at different soil types were investigated and analyzed by SOFIX. Soil type is a vital determinant of soil fertility because of its biological, chemical, and physical properties. However, the soil fertility of upland soil is probably changed by different management practices regardless of soil type. This study was conducted to investigate the tendency of soil fertility (bacterial biomass, TC, and TN) and effect of soil types on the soil fertility in upland field in Japan. Soil samples from 1,000 upland soils at different soil types were collected and analyzed.

In chapter 3, the standard organic soil based on the SOFIX database was constructed by investigating base soils and additive materials and blending ratio. Seven organic soils were constructed from base soils (wood chips, peat moss, black soil, and mountain soil) and additive materials (soybean meal, oil cake, cow manure, and bone meal) based on the recommended values of the soil fertility index (SOFIX).

Chapter 1
Analysis of orchard soil for understanding suitable soil conditions

1.1 Introduction

Orchard crop cultivation is carried out under either conventional or organic agriculture systems. The development of conventional agriculture system using chemical fertilizers and pesticides has improved crop production and agricultural activities [2][3][32]. Conventional agricultural system has a higher yield of agricultural products than organic agriculture system, and more than 98.5 % of all crops are cultivated conventionally [33]. Long-term use of chemical fertilizers and pesticides has led to environmental impacts such as lower soil fertility, reduced biodiversity, and increased greenhouse gas emissions [32][34][35][36]. There are growing concerns about the negative impacts generated by conventional agricultural system [4][8]. As a response to these concerns, organic farming system that aims to reduce harm to the environment have been developed [37]. However, a significant obstacle in organic agricultural system is that the agricultural product yield is lower than those from conventional agricultural system [3][9][38][39][40].

In a previous study, a soil fertility index (SOFIX) was developed to evaluate soil fertility and the efficiency of organic agricultural system [10]. SOFIX has been constructed considering the importance of biological, chemical, and physical soil characteristics. Following the concept of SOFIX, bacterial biomass and its activities (nitrogen and phosphorus circulation activities) are the main factors that determine soil fertility.

More than 8,000 agricultural soil samples were analyzed, and the SOFIX database was constructed from these samples. The suitable soil conditions for the upland fields based on the SOFIX database are total carbon (TC) ≥ 25,000 mg/kg, total nitrogen (TN) ≥ 1,500 mg/kg, total phosphorus (TP) ≥ 1,100 mg/kg, and total potassium (TK) 2,500 to 10,000 mg/kg to maintain bacterial biomass ≥ 6.0×10^8 cells/g-soil and their activities [10]. The suitable soil conditions for the paddy fields based on the SOFIX database are TC ≥ 20,000 mg/kg, TN ≥ 800 mg/kg, TP ≥ 650 mg/kg, and TK 2,500 to 10,000 mg/kg [41]. The environmental conditions between the upland and the paddy fields analyzed by the database were different because of differences in their respective soil environments [13].

In this chapter, the orchard soils were analyzed by SOFIX for the construction of the orchard database. The features and the suitable conditions of the orchard field were determined by comparing the databases of upland field and the paddy field.

1.2 Materials and methods

1.2.1 Soil collection

Soil samples from 442 agricultural fields were collected in Japan from 2014 to 2019. The soil samples included 190 upland fields, 113 paddy fields, and 139 orchard fields (Table 1). Soil samples from 5 random points were collected in each field and sieved them through a 2-mm sieve. We analyzed all soil samples within 2 weeks of sampling, and the samples were never dried.

Table 1. Analysis of orchard fields for construction of the database.

Field type	No. of sample
Apple	22
Grape	22
Tea	84
Others	11
Total	139

1.2.2 Analysis of soil biological properties

1.2.2.1 Analysis of bacterial biomass

The bacterial biomass in the soil samples was measured by quantifying the environmental DNA (eDNA) using the slow-stirring method [42]. To extract the eDNA from the soil, a 1.0 g soil sample was mixed with 8.0 mL of DNA extraction buffer (100 mM tris (hydroxymethyl) aminomethane (Table 2), 100 mM sodium EDTA, 100 mM sodium dihydrogen orthophosphate, 1.5 M sodium chloride, 1% (w/v) hexadecyltrimethylammonium bromide), and 1.0 mL of 20% (w/v) sodium dodecyl sulfate solution. The suspension was agitated with a propeller for 20 min. The suspension was centrifuged at 5,000 × *g* for 10 min, and then transferred about 700 μL of supernatant into a 1.5 mL microtube and 700 μL of chloroform-isoamyl alcohol was slowly added. The mixture was centrifuged at 18,000 × *g* for 10 min and then added 300 μL of isopropanol and separated the precipitate by centrifugation at 18,000 × *g* for 20 min. The pellet of crude nucleic acid was dissolved in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) after drying. The extracted eDNA was quantified based on the intensity of the eDNA bands after electrophoresis on an agarose gel using Kodak 1D 3.6 Image Analysis Software (Kodak, CT, USA). The bacterial biomass in the soil was estimated by using the equation ($Y = 1.70 \times 10^8 X$; $r^2 = 0.96$), where Y and X are the bacterial biomass g^{-1} soil and the amount of eDNA, respectively.

Table 2. Composition of DNA extraction buffer (pH 8.0).

Reagent	Concentration (g/L)
Ethylenediaminetetraacetic acid disodium salt dihydrate	37.22
Hexadecyltrimethylammonium bromide	10.00
Sodium chloride	87.66
Sodium dihydrogen phosphate	12.00
Tris(hydroxymethyl)aminomethane	12.11

1.2.2.2 Analysis of nitrogen (N) circulation activity

To estimate of nitrogen (N) circulation activity was analyzed based on the bacterial biomass, ammonium oxidation rate and nitrite oxidation rate in the soil [10]. Soil sample was analyzed by adjusted of water holding capacity to 60%. Then, ammonium sulfate or sodium nitrite was added to the soil (60 μg N g^{-1} dry soil) sample. Soil sample without the addition of ammonium sulfate or sodium nitrite was used as a control experiment and incubated at 25°C. After 3 days incubation, the percentage of reduction in the added N was defined as ammonium or nitrite oxidation rates. The bacterial biomass of 6.0 $\times 10^8$ cells/g-soil and 2.0 $\times 10^8$ cells/g-soil are defined as 100 points and 0 point, respectively. Using the scores of bacterial biomasses, ammonium oxidation rate and nitrite oxidation rate, a radar chart was constructed and relative area of the inner triangle is expressed as the N circulation activity (Figure 1).

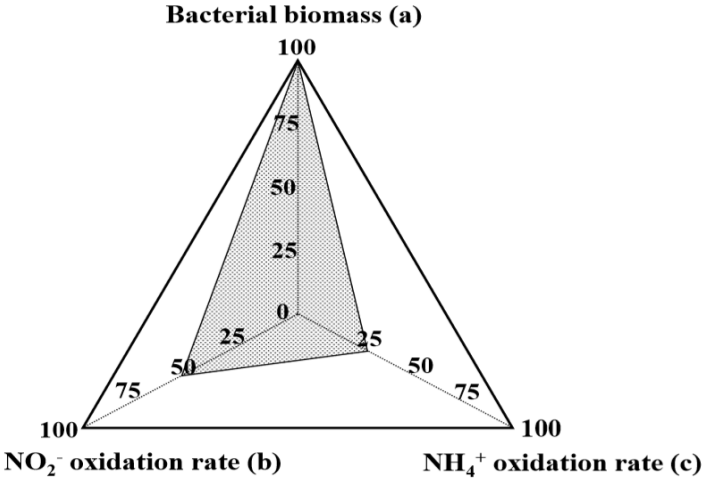

Figure 1. Radar chart used to calculate of N circulation activity [10].

The area of the inner triangle in the radar chart is calculated as follows:

$$\text{Area} = \frac{(a \times b) + (b \times c) + (c \times a)}{4} \times \frac{\sqrt{3}}{100}$$

Where a, b, and c denote scores of bacterial numbers, ammonium oxidation rate and nitrite oxidation rate, respectively. Nitrogen circulation activity was analyzed by calculating the relative area of inner triangle as follows:

$$\text{N } circulation\ activity\ (point) = \frac{Area\ of\ the\ inner\ triangle}{Area\ of\ the\ outer\ triangle} \times 100$$

1.2.2.3 Analysis of phosphorus (P) circulation activity

In this method, phytate (the most dominant form of soil organic P) is used as a substrate [43]. Firstly, phytate solution (pH 7.0) containing 3.9 mg of P was added to 1.0 g of soil sample and incubated for 3 days at room temperature at 6% water holding capacity. Control experiment (without phytate) was carried out simultaneously. Soluble phosphorous (SP) was extracted from the incubated 1.0 g soil sample with 20 mL distilled water and analyzed by the molybdenum blue method. The increment in soluble phosphorous in phytate added soil after 3 days was defined as the P circulation activity in the soil. The circulation activity was expressed in points assigning 0 point for no circulation activity and 100 points for full circulation activity of the added phytate P.

1.2.3 Analysis of soil chemical properties

1.2.3.1 Analysis of total carbon

Total carbon (TC) was analyzed using a total organic carbon analyzer (SSM-5000A, Shimadzu, Kyoto, Japan).

1.2.3.2 Kjeldahl digestion method

Total nitrogen (TN), total phosphorus (TP), and total potassium (TK) contents were analyzed by extracting soil samples using the Kjeldahl digestion method (Gerhardt, Königswinter, Germany). A 0.5 g of soil sample was mixed with 0.5 g of $CuSO_4$ in a Kjeldahl tube. Subsequently, 5 mL of H_2SO_4 and 5 mL of H_2O_2 were added to the mixture in a fume hood. The mixture was digested at 420°C for 1.5 h. After that, allow cooling for 30 min and filtered the extract using ADVANTEC filter paper no. 6 (Toyo Roshi Kaisha, Ltd., Tokyo, Japan).

1.2.3.3 Analysis of total nitrogen

The TN of soil samples was measured by using the indophenol blue method [44]. Indophenol blue solution (Table 3) and sodium hypochlorite solution (Table 4) were prepared to analyze of TN. Subsequently, 1 mL of Kjeldahl extract was mixed with 400 µL of Indophenol blue solution and 600 µL of sodium hypochlorite solution. The mixture was incubated at room temperature for 45 minutes for the color development. The absorbance of TN was observed using UV visible spectrophotometer (U-1900 spectrophotometer; Hitachi, Tokyo, Japan) at 635 nm. Ammonium sulfate was used for preparing the standard solution [$(NH_4)_2SO_4$, (0.56 g/50 mL)] (Table 5). The standard curve for TN analysis is shown in Figure 2.

Table 3. Composition of indophenol blue solution.

Reagent	Quantity (g/L)
Trisodium phosphate ($Na_3PO_4 \cdot 12H_2O$)	30.0
Trisodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)	30.0
EDTA	3.0
Sodium pentacyano-nitrosulferrate(III) dihydrate	0.2
Phenol granules	60.0

Table 4. Composition of sodium hypochlorite solution.

Reagent	Quantity (mL)
Sodium hypochlorite	20.0
1 M NaOH solution	400.0

Table 5. Absorbance of standard ammonium solutions.

Concentration (mg–NH_4^+–N/L)	Absorbance (at 635 nm)
0.0	0.043
0.1	0.200
0.2	0.313
0.4	0.499
0.8	0.894
1.6	1.641

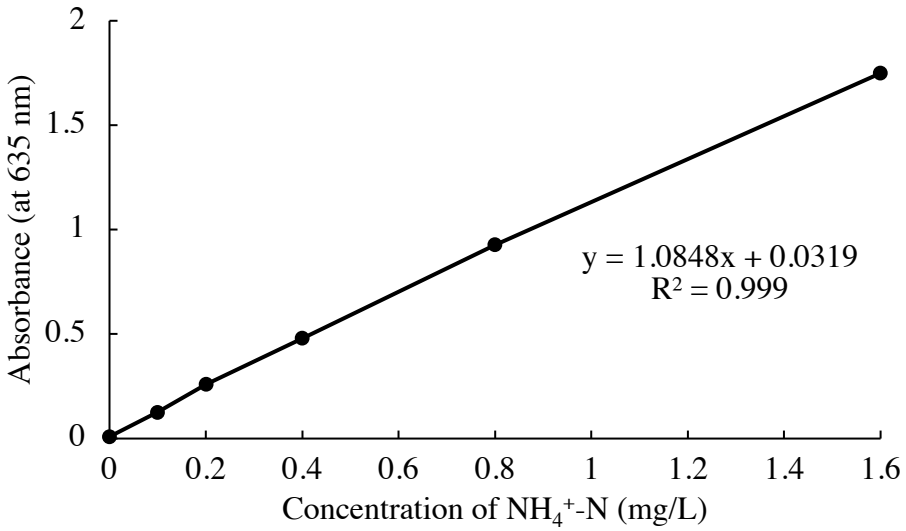

Figure 2. Standard curve of total nitrogen (TN).

1.2.3.4 Analysis of total phosphorus

The TP of soil samples was measured by using the molybdenum blue method [45]. Ammonium molybdate solution (Table 6) and ascorbic acid solution (Table 7) were prepared

for the TP analysis. A 1 mL of Kjeldahl extract was mixed with 100 µL of 5:1 ammonium molybdate and ascorbic acid solution. The mixture was incubated at 30°C for 30 min to obtain color development. The absorbance was observed using UV visible spectrophotometer at 710 nm. Standard curve was obtained from serial dilution of 1000 mg/L (Table 8). The standard curve for TP analysis is shown in Figure 3.

Table 6. Composition of ammonium molybdate solution.

Reagent	Quantity
Ammonium molybdate tetrahydrate	6.00 g
Antimony potassium tartrate	0.24 g
1:2 H_2O:H_2SO_4	120 mL
Distilled water	up to 500 ml

Table 7. Composition of ascorbic acid solution.

Reagent	Quantity
L ascorbic acid	7.2 g
Distilled water	up to 100 mL

Table 8. Absorbance of standard P solutions.

Concentration (mg–P/L)	Absorbance (at 710 nm)
0.0	0.005
0.1	0.055
0.2	0.246
0.5	0.491
1.0	0.765
1.5	0.958

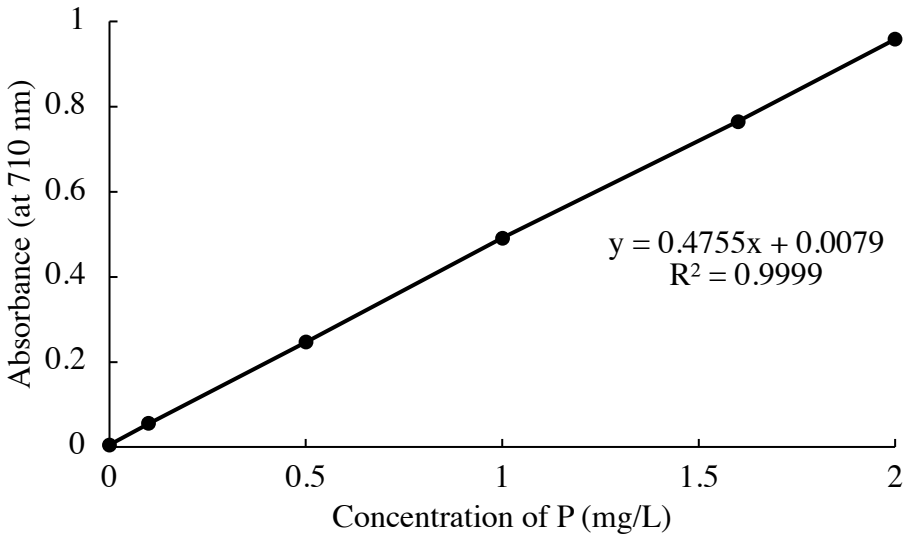

Figure 3. Standard curve of total phosphorous (TP).

1.2.3.5 Analysis of total potassium

The TK of soil samples was performed using Z-2300 atomic absorption spectrophotometer (Hitachi High-technologies Corporation, Tokyo, Japan). Standard solution was obtained from Potassium standard solution 1000 mg/L (Table 9). The standard curve for TK analysis is shown in Figure 4.

Table 9. Absorbance of standard K solutions.

Concentration (mg-K/L)	Absorbance (at 248.3 nm)
0.00	0.0006
1.00	0.0444
2.00	0.1054
5.00	0.3073
10.00	0.6523

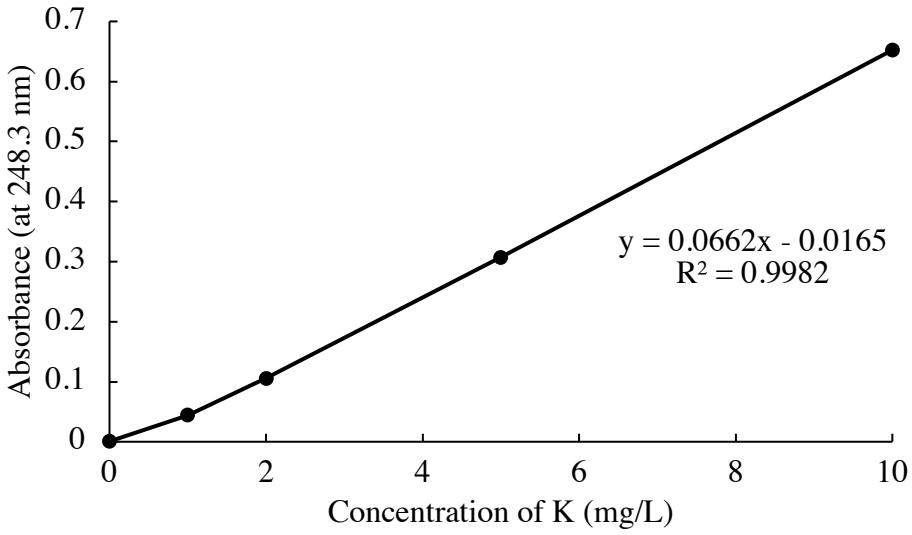

Figure 4. Standard curve of total potassium (TK).

1.2.3.6 Analysis of water soluble phosphorus (SP) and potassium (SK)

To analyze SP and SK, the soil-water suspension (1:20, w/v) was shaken reciprocally at 100 rpm for 1 h and the extracts were analyzed by the molybdenum blue method and the atomic absorption spectrophotometry, respectively [45].

1.2.3.7 Analysis of ammonium-nitrogen

Ammonium-nitrogen (NH_4^+-N) was analyzed by a 4.0 g of soil sample was extracted with 1M KCl solution. Then, suspension was shaken reciprocally at 100 rpm for 1 h and the extracts were analyzed by the indophenol blue method [44].

1.2.3.8 Analysis of nitrate-nitrogen

Nitrate-nitrogen (NO_3-N) was analyzed by soil was extracted with 1M KCl solution followed by brucine method [44]. A 200 µL of soil extract was mixed with A 100 µL of brucine aminobenzene sulfonic acid solution. Then, a 1.0 mL of H_2SO_4: H_2O solution was added and mixed. Sample was kept in a dark place with cold temperature for 10 min. Sample was mixed with a 1 mL of deionized water and in a dark place with cold temperature for 30 min. The absorbance was observed using UV visible spectrophotometer at 410 nm.

1.2.3.9 Analysis of pH and EC

Soil pH and EC were determined in 1:2.5 soil-water mixture (w/v). Soil pH was measured by a pH meter (LAQUA. F-71, Horiba, Kyoto, Japan). Soil EC was measured by EC meter (5LE1-408, Kenis, Hyogo, Japan).

1.2.4 Analysis of soil physical properties

1.2.4.1 Analysis of water holding capacity of soil

Water holding capacity of soil was measured using a volumetric method [46]. A known amount of air-dried soil was put in a funnel connected with a tube, while distilled water was added to completely submerge the soil and left for 30 min to saturate the soil. The water was drained from funnel for 30 min that was collected. Adding water and draining water were weighed to get the weight of the water held in soil for soil water holding capacity calculation.

1.2.4.2 Analysis of water content in soil

Water content in soil is measured as the difference between the moist soil and the soil dried. A moist soil sample was weighed and then oven dried at 105°C for 48 hours and then re-weighed. Water content (%) = [mass of moist soil (g) − mass of oven-dried soil (g)/mass of oven-dried soil (g)] × 100 [47][48].

1.2.5 Statistical analysis

The parameters of soil properties were determined using a one-way analysis of variance (ANOVA). The differences were evaluated at the 5% significance level when significance was observed at the $P < 0.05$ level.

1.3 Results

1.3.1 Analysis of orchard soils from apple, grape, tea, and other fields

Soil samples from the orchard fields were analyzed with SOFIX. The values of biological, chemical, and physical parameters of apple, grape, tea, and other orchard fields are shown in Table 10. The averages of the bacterial biomass of apple, grape, tea, and other field types were 1.7×10^9, 7.0×10^8, 4.9×10^8, and 7.9×10^8 cells/g-soil, respectively. Bacterial biomass in apple fields was the highest, while that in the tea fields was the lowest. The higher bacterial biomass

Table 10. Average values of biological, chemical, and physical parameters of the orchard field.

Parameter	Average value			
	Apple	Grape	Tea	Others
Bacterial biomass (× 10^8 cells/g-soil)	17.0 ^{a} (± 8.7)	7.0 ^{b} (± 15.5)	4.9 ^{b} (± 7.4)	7.9 ^{b} (± 5.3)
NH_4^+ oxidation rate (point)	52.6 ^{b} (± 26.2)	49.9 ^{b} (± 32.3)	76.4 ^{a} (± 38.1)	41.0 ^{b} (± 29.5)
NO_2^- oxidation rate (point)	42.3 ^{abc} (± 25.8)	35.4 ^{bc} (± 26.9)	41.0 ^{ab} (± 44.6)	56.9 ^{a} (± 23.9)
N circulation activity (point)	38.4 ^{a} (± 20.4)	20.3 ^{b} (± 12.5)	19.4 ^{b} (± 19.6)	33.8 ^{a} (± 21.4)
P circulation activity (point)	1.0 ^{c} (± 1.0)	3.2 ^{b} (± 3.0)	24.7 ^{a} (± 32.9)	15.9 ^{a} (± 12.6)
TC (mg/kg)	40,900 ^{a} (± 12,930)	19,600 ^{b} (± 14,070)	21,470 ^{b} (± 18,900)	18,330 ^{b} (± 9,110)
TN (mg/kg)	1,900 ^{a} (± 670)	1,710 ^{ab} (± 1,570)	1,340 ^{b} (± 1,210)	1,080 ^{b} (± 260)
TP (mg/kg)	1,190 ^{a} (± 600)	820 ^{b} (± 580)	970 ^{ab} (± 960)	1,550 ^{a} (± 580)
TK (mg/kg)	4,350 ^{b} (± 1,710)	2,990 ^{c} (± 710)	6,240 ^{a} (± 4,170)	5,540 ^{ab} (± 3,730)
C/N ratio	22 ^{a} (± 4)	14 ^{b} (± 7)	19 ^{ab} (± 12)	18 ^{ab} (± 8)
C/P ratio	39 ^{a} (± 12)	26 ^{b} (±8)	26 ^{b} (± 16)	14 ^{c} (± 8)
NO_3^--N (mg/kg)	6 ^{b} (± 7.2)	0 ^{c} (± 0.6)	18 ^{a} (± 10.9)	11 ^{b} (±7.7)
NH_4^+-N(mg/kg)	7 ^{c} (± 21.3)	108 ^{a} (± 95.3)	71 ^{b} (± 59.5)	2 ^{c} (± 1.8)
Soluble P_2O_5 (mg/kg)	332 ^{a} (± 376)	372 ^{a} (± 633)	123 ^{b} (± 249)	434 ^{a} (± 521)
Soluble K_2O (mg/kg)	923 ^{a} (± 412)	278 ^{b} (± 317)	110 ^{c} (± 109)	244 ^{b} (± 246)
pH	6.5 ^{a} (± 0.5)	6.3 ^{a} (± 0.5)	4.0 ^{b} (± 0.8)	6.5 ^{a} (± 0.9)
EC (ds/m)	0.3 ^{a} (± 0.2)	0.3 ^{a} (± 0.3)	0.2 ^{a} (± 0.1)	0.2 ^{a} (± 0.1)
Water content (%)	27 ^{a} (± 11)	29 ^{a} (± 19)	24 ^{a} (± 8)	19 ^{a} (± 6)
Water-holding capacity (ml/kg)	1,130 ^{a} (± 329)	1,316 ^{a} (± 736)	759 ^{b} (± 325)	577 ^{b} (± 260)

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

enhances nitrogen circulation. The results indicate that nitrogen circulation activity and bacterial biomass were related to each other, while phosphorus circulation activity and bacterial biomass were not.

The average values of TC in the apple, grape, tea, and other fields were 40,900 mg/kg, 19,600 mg/kg, 21,470 mg/kg, and 18,330 mg/kg, respectively. The average values of TN in the apple, grape, tea, and other fields were 1,900 mg/kg, 1,710 mg/kg, 1,340 mg/kg, and 1,080 mg/kg, respectively. The apple fields had the highest TC and TN values. The water-holding capacity of the apple fields (1,130 ml/kg) was also relatively high. The soil pH in the tea fields was acidic (pH 4.0) lower than those in the other fields was less so. No significant differences for EC were detected within the apple, grape, tea, and other fields. These results indicate that TC, TN, and water-holding capacity in the soil are related to each other.

1.3.2 Analysis and comparison of TC and bacterial biomass in the orchard, upland, and paddy fields

The relationship between the bacterial biomass and TC in the orchard, upland, and paddy fields were investigated. The average values of biological, chemical, and physical parameters of the 3 field types are shown in Table 11. The average bacterial biomass in the orchard fields (7.4×10^8 cells/g-soil) was almost the same in the upland fields (8.0×10^8 cells/g-soil), but the value was lower than that in the paddy fields (12.9×10^8 cells/g-soil). The bacterial biomass of 90 orchard soil samples (64.7%) was lower than 6.0×10^8 cells/g-soil (Figure 5a). The bacterial biomass of many tea soil samples was not detected ($< 6.6 \times 10^6$ cells/g-soil), indicating that agrochemicals use in the tea fields seem to be relatively high.

The average values of TC in the orchard, upland, and paddy fields were 24,000 mg/kg, 33,120 mg/kg, and 15,420 mg/kg, respectively. The TC value of the orchard fields was between those of the upland and paddy fields. The TC value of 50 orchard soil samples (35.9%) was higher than 24,000 mg/kg (Figure 5a), and about 50% of the upland soil samples exhibited high TC (above 25,000 mg/kg) (Figure 5b). The range of TC values in the paddy fields was narrow (8,000 to 25,000 mg/kg) (Figure 5c). Among the SOFIX parameters, bacterial biomass and TC are two of the most important factors that determine soil fertility. The relationships between bacterial biomass and TC in the orchard (R^2= 0.34), upland (R^2= 0.09), and paddy fields (R^2= 0.01) (Figure 5a, 5b, and 5c) were observed. The accumulation level of carbon in the orchard fields was similar to that in the upland fields, indicating that an agricultural system using biomass for organic fertilizer is reasonable. In addition, a relatively aerobic condition in both soil environments creates similar microbial diversity.

Table 11. Average values of biological, chemical, and physical parameters of the orchard, upland, and paddy fields.

Parameter	Average value		
	Orchard	Upland	Paddy
Bacterial biomass ($\times 10^8$ cells/g-soil)	7.4 ^{b} (± 10.1)	8.0 ^{b} (± 9.0)	12.9 ^{a} (± 13.4)
NH_4^+ oxidation rate (point)	65.6 ^{a} (± 37.4)	40.9 ^{b} (± 32.2)	15.5 ^{c} (± 15.5)
NO_2^- oxidation rate (point)	41.6 ^{b} (± 38.7)	63.0 ^{a} (± 34.4)	43.6 ^{b} (± 27.7)
N circulation activity (point)	23.7 ^{b} (± 20.4)	34.4 ^{a} (± 30.4)	21.8 ^{b} (± 14.9)
P circulation activity (point)	16.8 ^{b} (± 27.8)	40.7 ^{a} (± 37.7)	36.9 ^{a} (± 33.5)
TC (mg/kg)	24,000 ^{b} (± 18,300)	33,120 ^{a} (± 29,650)	15,420 ^{c} (± 4,910)
TN (mg/kg)	1,460 ^{b} (± 1,190)	2,010 ^{a} (± 2,580)	1,080 ^{c} (± 450)
TP (mg/kg)	1,030 ^{b} (± 860)	3,250 ^{a} (± 5,300)	880 ^{b} (± 430)
TK (mg/kg)	5,370 ^{b} (± 3,700)	8,600 ^{a} (± 8,340)	3,270 ^{c} (± 1,820)
C/N ratio	19 ^{a} (± 11)	20 ^{a} (± 16)	16 ^{a} (± 7)
C/P ratio	27 ^{a} (± 15)	31 ^{a} (± 78)	24 ^{a} (± 33)
NO_3^--N (mg/kg)	13 ^{b} (± 11.6)	44 ^{a} (± 123.3)	5 ^{c} (± 8.3)
NH_4^+-N(mg/kg)	61 ^{a} (± 69.3)	15 ^{b} (± 36.0)	9 ^{b} (± 34.7)
Soluble P_2O_5 (mg/kg)	220 ^{a} (± 400)	60 ^{b} (± 80)	18 ^{c} (± 21)
Soluble K_2O (mg/kg)	276 ^{a} (± 371)	273 ^{a} (± 393)	43 ^{b} (± 74)
pH	5.0 ^{b} (± 1.4)	6.4 ^{a} (± 1.0)	7.5 ^{a} (± 8.4)
EC (ds/m)	0.2 ^{b} (± 0.2)	0.9 ^{a} (± 1.8)	0.8 ^{a} (± 1.9)
Water content (%)	25 ^{c} (± 11)	42 ^{a} (± 36)	33 ^{b} (± 23)
Water-holding capacity (ml/kg)	891 ^{a} (± 477)	804 ^{a} (± 947)	609 ^{b} (± 400)

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

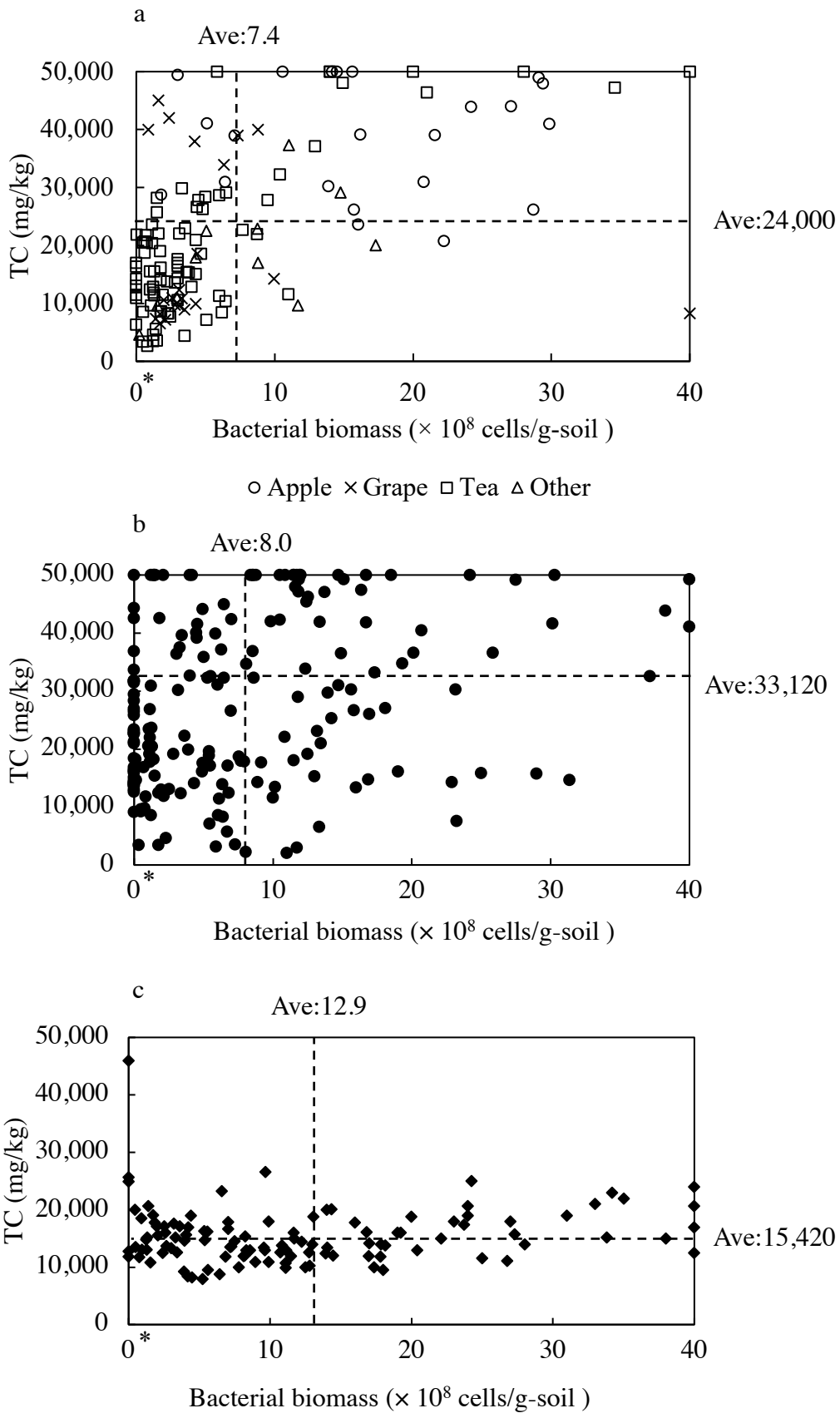

Figure 5. Relationship between TC and bacterial biomass of orchard field (a), upland field (b), and paddy field (c). Dashed lines indicate the average values of TC and bacterial biomass in each field. *Not detected bacterial biomass < 6.6 × 10^6 cells/g-soil.

1.3.3 Analysis and comparison of TC and TN in the orchard, upland, and paddy fields

Figure 6 shows the relationship between TC and TN. The average values of TN and the C/N ratio in the orchard fields were 1,460 mg/kg and 19, respectively (Table 11). The average TN value in the orchard fields was lower than that in the upland fields (2,010 mg/kg), but the value was higher than that in the paddy fields (1,080 mg/kg). A significant positive relationships between TC and TN in the orchard (R^2= 0.64), upland (R^2= 0.55), and paddy fields (R^2= 0.45) were observed. Organic materials such as manures and unfermented materials possess a similar ratio to TC and TN. This finding indicates that C/N ratios of the orchard fields, the upland fields, and the paddy fields were resembled.

1.3.4 Analysis and comparison of TC and TP in the orchard, upland, and paddy fields

Figure 7 shows the relationships between TC and TP in the orchard, upland, and paddy fields. The average value of TP and C/P ratio in the orchard fields were 1,030 mg/kg and 27, respectively. The average value of TP in the orchard fields was lower than that in the upland fields (3,250 mg/kg), but the value was higher than that in the paddy fields (880 mg/kg) (Table 11). The weak relationship between the C/P ratio in the orchard (R^2= 0.32) and the upland fields (R^2= 0.20) were observed (Figures 7a and 7b). However, no relationship with the C/P ratio in the paddy fields (R^2= 0.036) were observed (Figure 7c). The relationships between TC and TP and the 3 field types were variable. In addition, the relationship between the C/P ratio in the orchard and the upland fields was distributed over a wide range, while the range of the C/P ratio in the paddy fields was narrow. This finding suggests that TC was not related with TP compared with the relationship between TC and TN.

1.3.5 Suitable soil conditions of the orchard field

To determine the minimum and recommended values for the orchard fields, the TC, TN, TP, and TK values were compared with the upland and paddy fields (Figures 8, 9, 10, and 11). The TC, TN, and TK levels in the orchard fields were the same as those in the upland fields but different from those in the paddy fields, while the levels of TP in the orchard and upland fields are different. This finding suggests that the minimum and recommended values of TC, TN, and TK in the orchard and upland fields should be similar. In addition, the suitable values of bacterial biomass ($> 6.0 \times 10^8$ cells/g-soil), N circulation activity (≥ 20 points), and P circulation activity (20~80 points) were considered to decide the recommended values of orchard field (Tables 12, 13, and 14). The minimum required values in the orchard fields are TC: ≥ 12,000 mg/kg, TN: ≥ 1,000 mg/kg, and TK: ≥ 1,500 mg/kg.

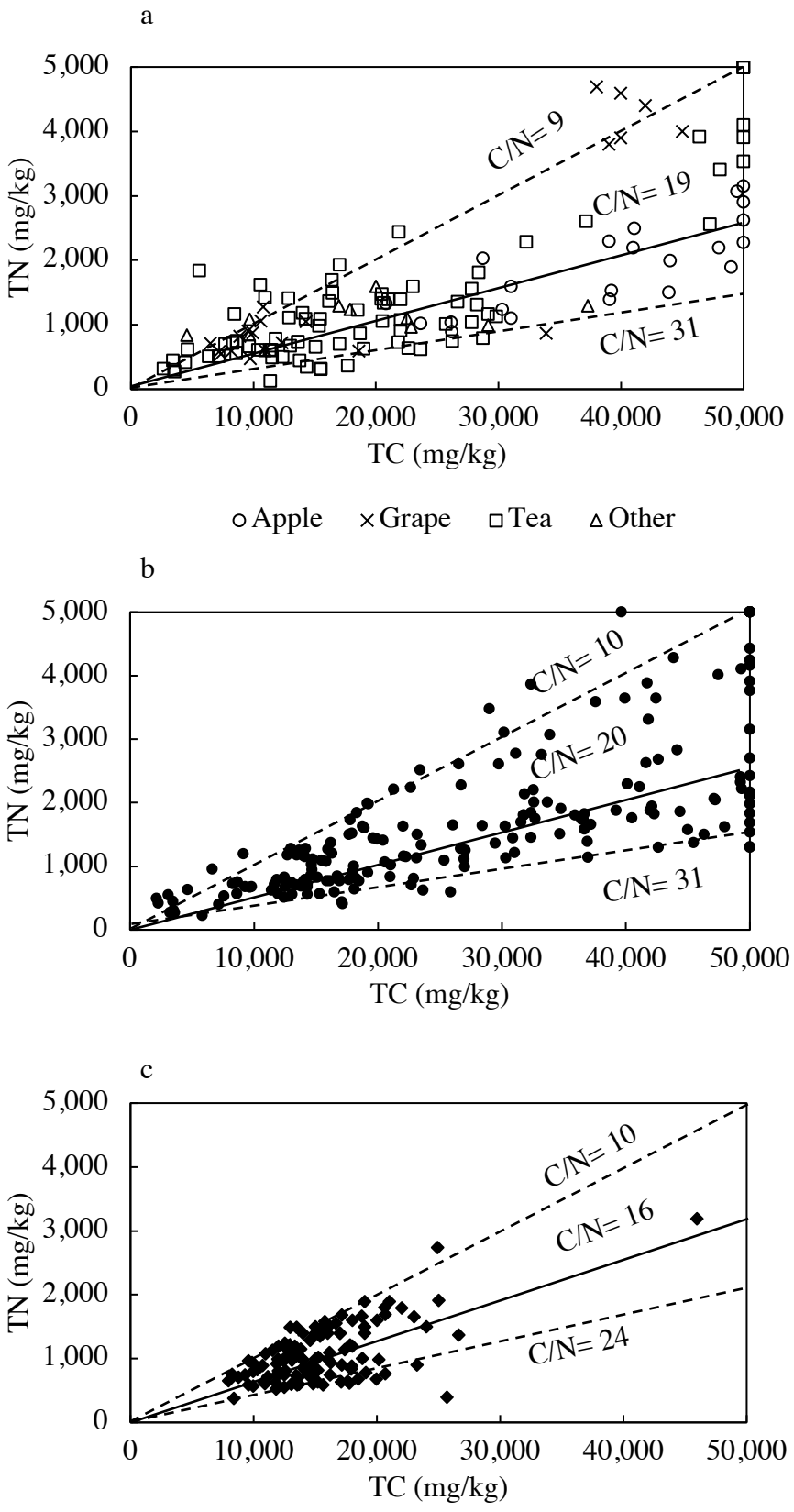

Figure 6. Relationship between TC and TN of orchard field (a), upland field (b), and paddy field (c). Solid line indicates the average values between TC and TN. Dashed lines indicate C/N ratio of 80% of samples.

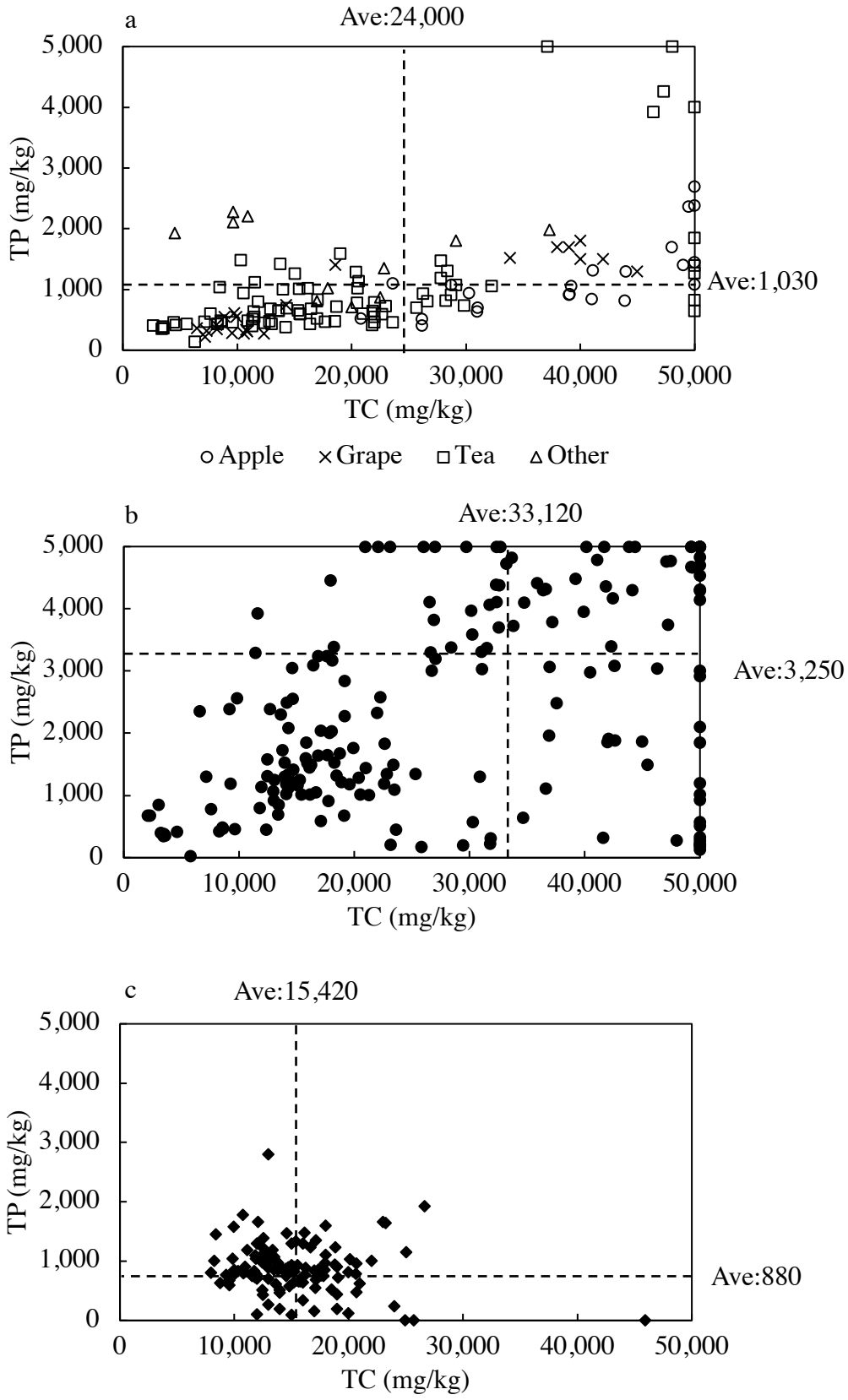

Figure 7. Relationship between TC and TP of orchard field (a), upland field (b), and paddy field (c). Dashed lines indicate the average values of TC and TP in each field.

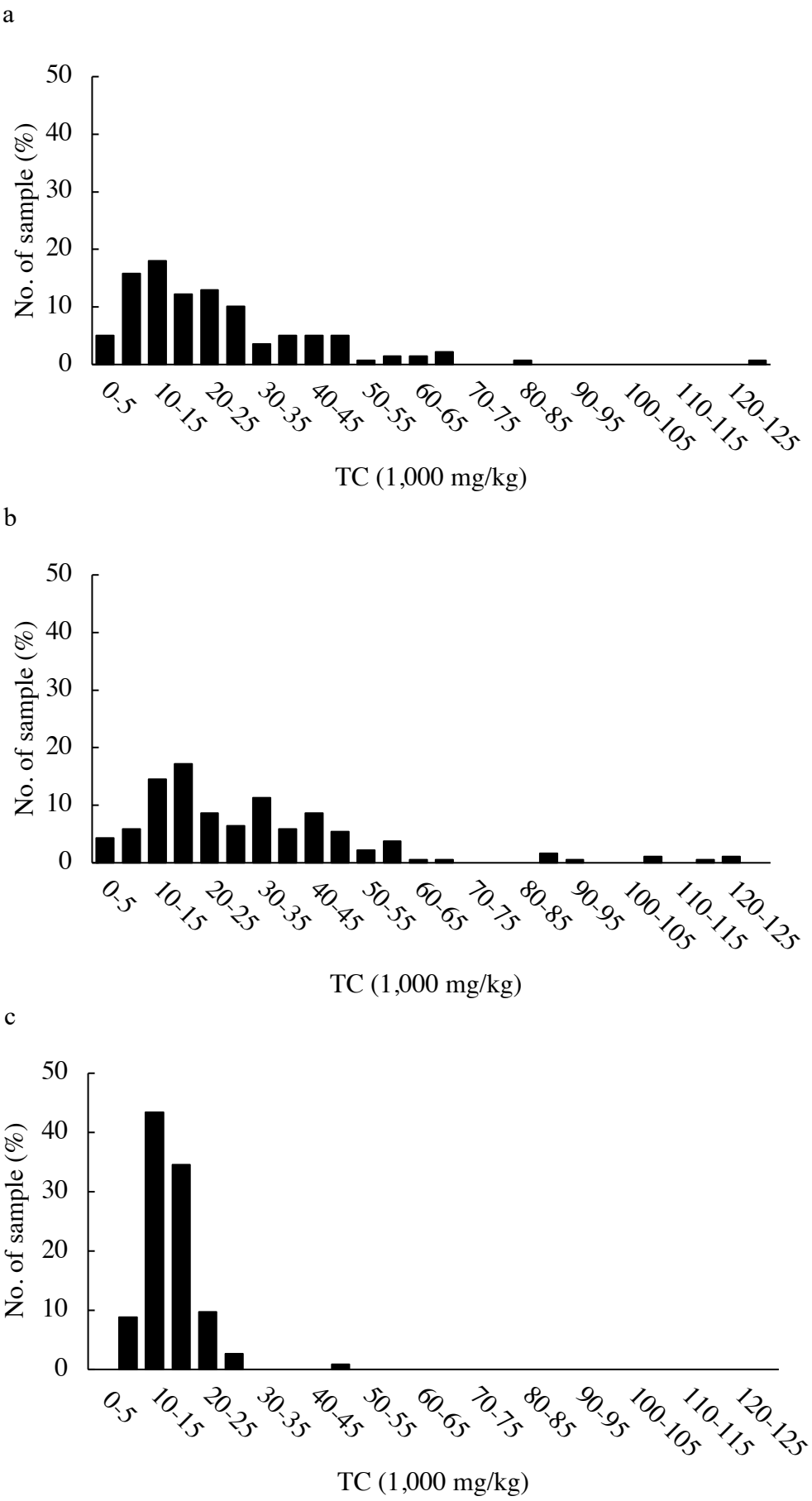

Figure 8. Frequency distribution of TC in the orchard (a), upland (b), and paddy (c) fields.

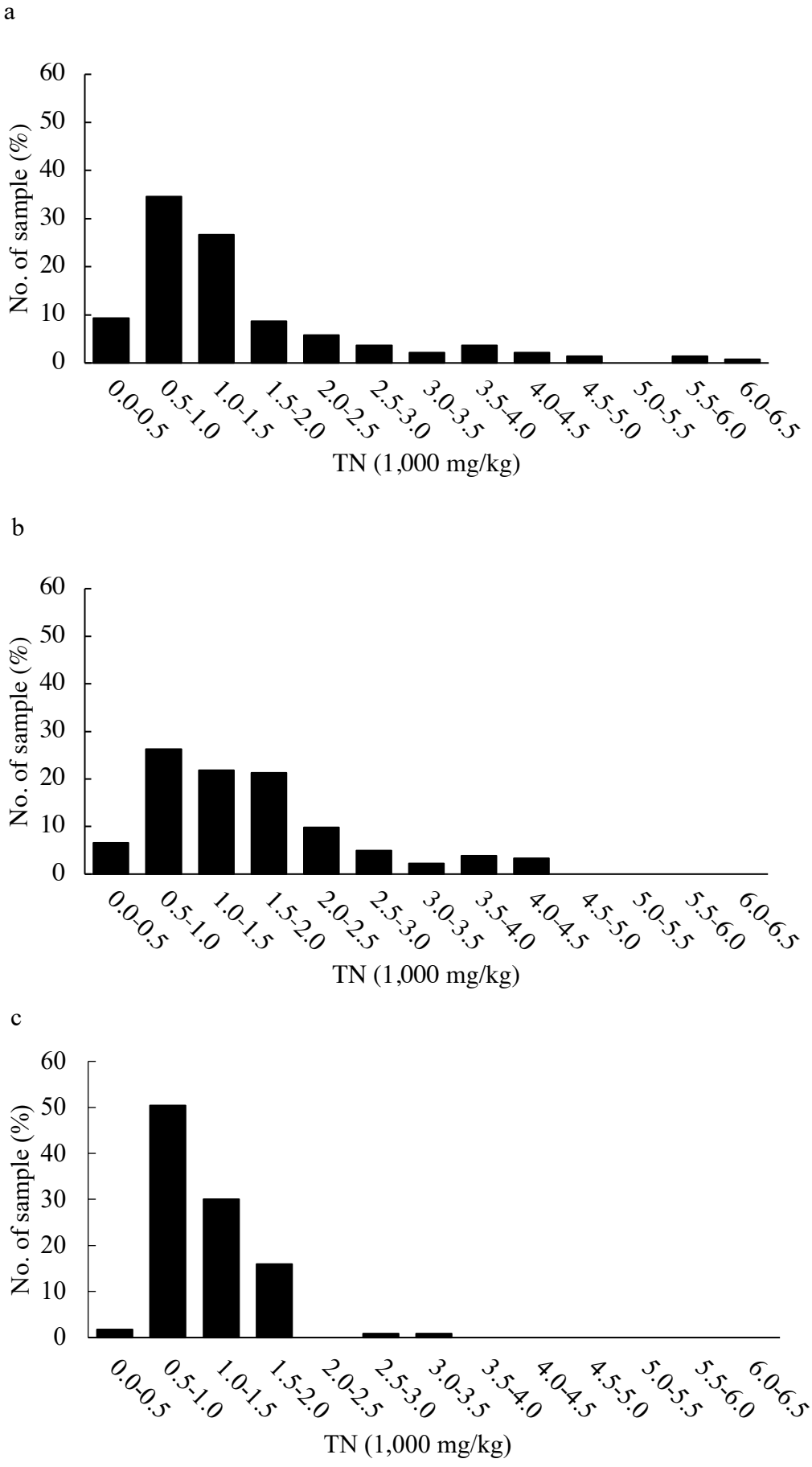

Figure 9. Frequency distribution of TN in the orchard (a), upland (b), and paddy (c) fields.

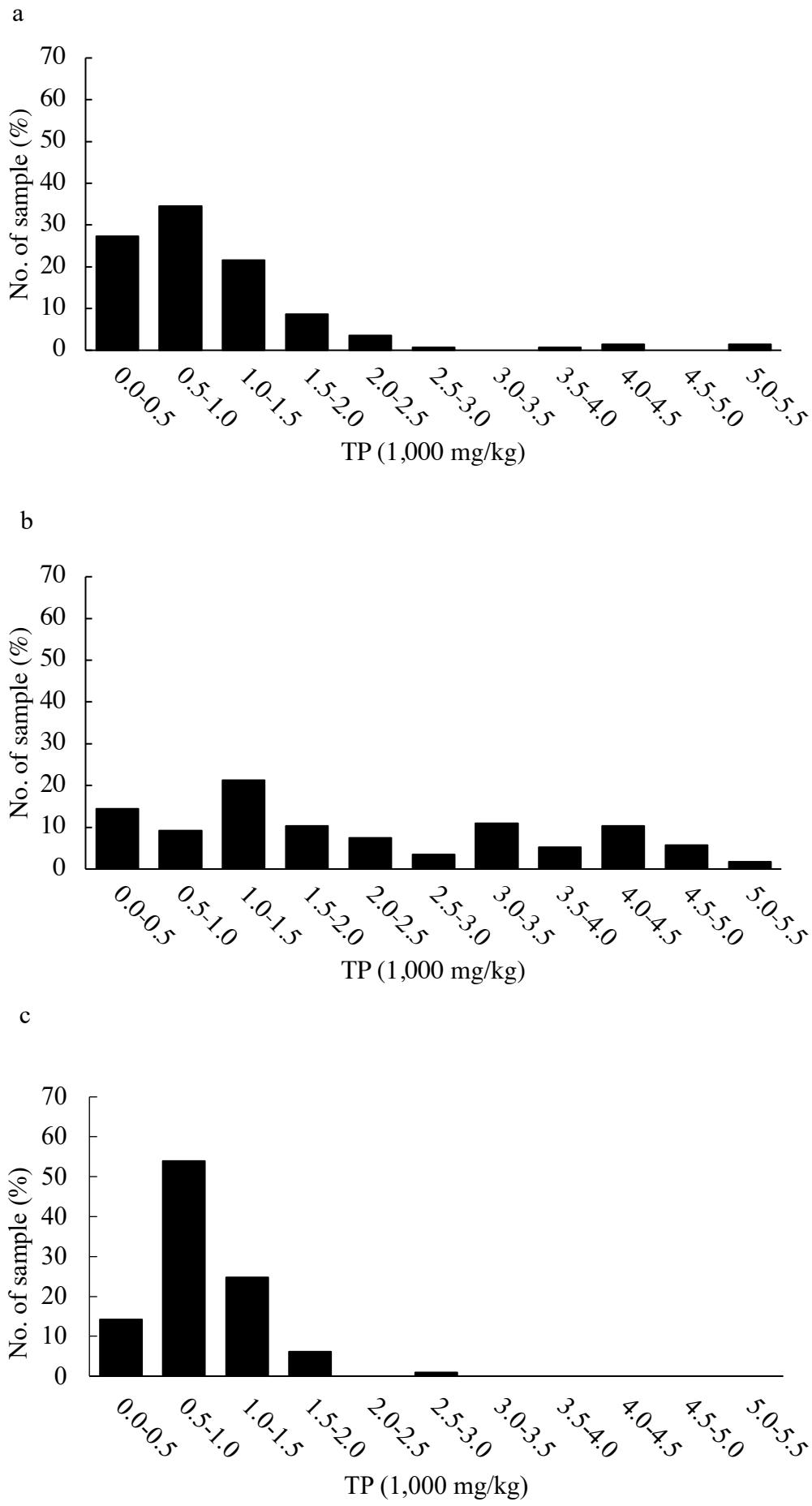

Figure 10. Frequency distribution of TP in the orchard (a), upland (b), and paddy (c) fields.

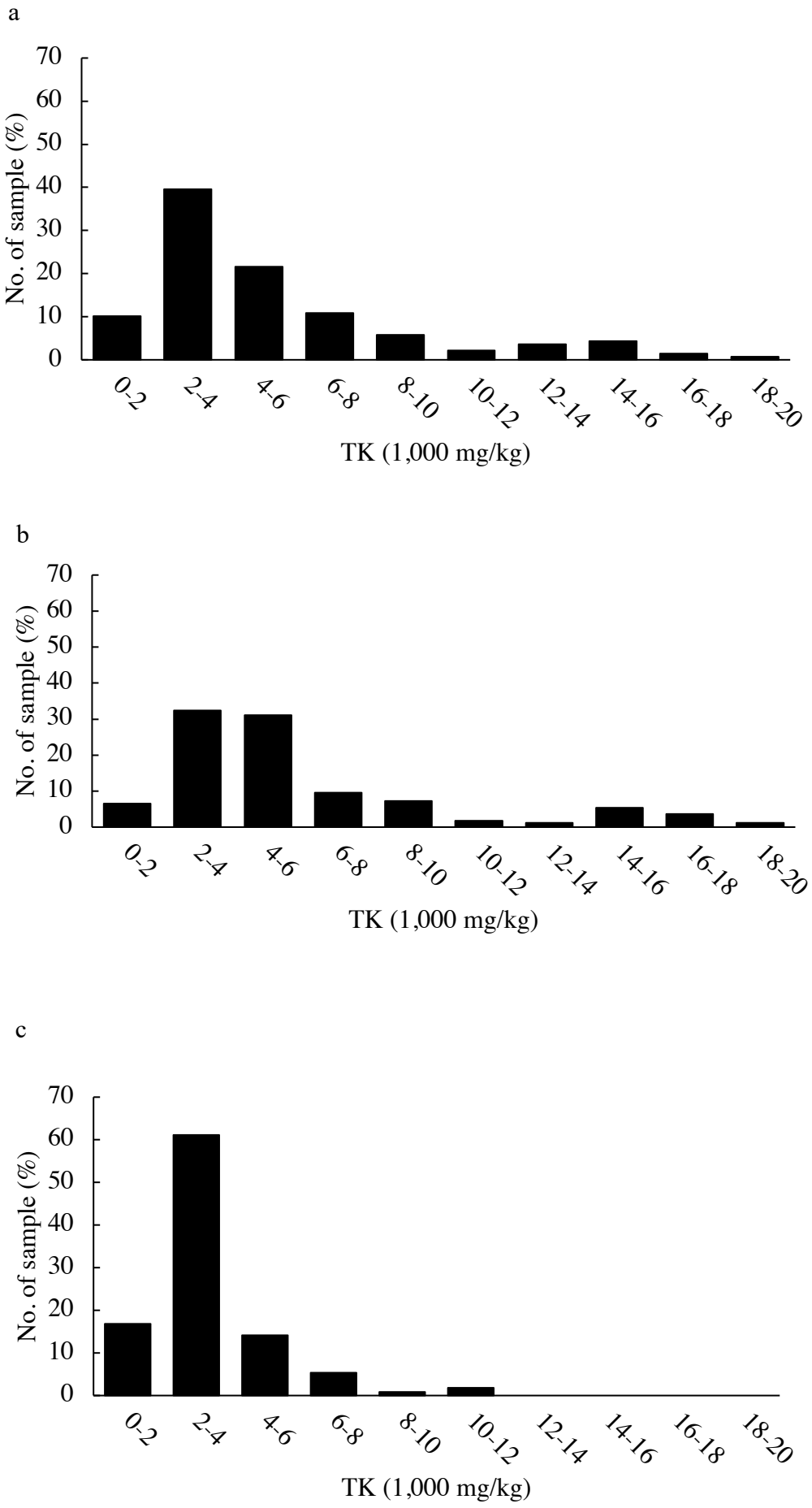

Figure 11. Frequency distribution of TK in the orchard (a), upland (b), and paddy (c) fields.

Table 12. Average TC, TN, TP, and TK in each bacterial biomass ranging.

Bacterial biomass ($\times 10^8$ cells/g-soil)	TC (mg/kg)	TN (mg/kg)	TP (mg/kg)	TK (mg/kg)
< 2.0	14,840	1,070	710	6,110
2.0 ~ 6.0	20,300	1,210	830	5,370
> 6.0	36,580	2,100	1,530	4,650

Table 13. Average TC, TN, TP, and TK in each N circulation activity ranging.

N circulation activity (point)	TC (mg/kg)	TN (mg/kg)	TP (mg/kg)	TK (mg/kg)
< 20	17,240	1,080	830	5,640
≥ 20	32,400	1,940	1,270	5,040

Table 14. Average TC, TN, TP, and TK in each P circulation activity ranging.

P circulation activity (point)	TC (mg/kg)	TN (mg/kg)	TP (mg/kg)	TK (mg/kg)
< 20	25,380	1,530	1,050	5,910
20 ~ 80	21,580	1,390	920	3,880
> 80	15,650	980	1,100	3,480

Table 15. The minimum requirement and recommended values of the orchard, upland, and paddy fields.

Field type	Value	Bacterial biomass (cells/g-soil)	TC (mg/kg)	TN (mg/kg)	TP (mg/kg)	TK (mg/kg)
Orchard	Minimum	2.0×10^8	≥ 12,000	≥ 1,000	≥ 800	≥ 1,500
	Recommended	6.0×10^8	≥ 25,000	≥ 1,500	≥ 900	2,500-10,000
Upland	Minimum	2.0×10^8	≥ 12,000	≥ 1,000	≥ 1,000	≥ 1,500
	Recommended	6.0×10^8	≥ 25,000	≥ 1,500	≥ 1,100	2,500-10,000
Paddy	Minimum	4.5×10^8	≥ 13,000	650-1,500	≥ 650	-
	Recommended	6.0×10^8	≥ 20,000	≥ 800	≥ 650	2,500-10,000

The recommended values in the orchard fields are TC: ≥ 25,000 mg/kg, TN: ≥ 1,500 mg/kg, and TK: 2,500 to 10,000 mg/kg. The minimum and recommended values of TP are: ≥ 800 and ≥ 900 mg/kg, respectively. Table 15 summarizes the TC, TN, TP, and TK minimum requirement and recommended values. These values helped determine the minimum and recommended values of the orchard fields.

1.4 Discussion

Orchard trees are cultivated as a monoculture growing for many years on flat land or in mountainous areas. Plowing is an agricultural practice done several times per year after crop rotation in the upland and paddy fields [15][16]. The successive crops of the agricultural rotation are not typically carried out in orchard fields [14]. Plowing in the orchard fields is practiced before permanent planting to avoid damage to root systems [17].

TC, TN, TP, TK, bacterial biomass, and their activities, which are all SOFIX in parameters, are the most critical factors contributing to soil fertility. These parameters showed a similar tendency in all 4 orchard fields except for the lower pH of the soil (around pH 4.0) was found in the tea fields. Generally, tea grows efficiently in an acidic soil environment, and tea is an Al accumulator [49]. Al biogeochemical cycling in tea leaves and fertilization the fields over the long term leads to soil acidification in tea fields [49][50][51]. Additionally, tea trees prefer ammonium as a nutrient, and more N fertilizers are added to tea fields to increase the plant quality and yield [52][53][54]. Soil acidification can result from the release of H^+ during the process of NH_4^+-N uptake from soil [54][55].

Soil conditions in the orchard and upland fields were almost the same except for the accumulation of TP. The primary sources of TC and TN in the orchard fields were organic materials such as fallen leaves, wood, and organic fertilizer [56][57]. Accumulation of TC and TN were directly proportional to each other in the orchard and the upland fields. At the same time, levels of TC and TP did not correspond, suggesting that TP-rich organic materials exist in nature (e.g., rice bran and bone meal) [25][58]. Generally, phosphorus is a major nutrient limiting element as a result of immobilization by high levels of Al and Fe [59].

The orchard field database was constructed using 139 fields with the aims of better understanding the orchard soil features and determining suitable soil conditions. The fields used to build the database included apple, grape, tea, and other field types. The soil of the orchard fields resembles that of the upland fields; therefore, the minimum and recommended values of TC, TN, and TK were similar for both field types.

1.5 Summary

Orchard soil database was construed by studying soil fertility of 139 orchard fields including apple, grape, tea, and other fields and comparing with upland and paddy fields. Among 4 types of orchard field, apple field showed the highest bacterial biomass and N circulation activity, while that in the tea fields was the lowest in bacterial biomass and nitrogen circulation activity. These findings indicate that bacterial biomass and N circulation activity and were related to each other. Soil fertility of 422 orchard, upland, and paddy fields were studied the relationships between TC and bacterial biomass, TC and TN, and TC and TP to understand the differences in soil properties between 3 agricultural fields. Features of the orchard soil show that bacterial biomass, TC, and TN are related to each other. Among agricultural fields, bacterial biomass, TC, TN, and TK in orchard field was similar as in upland field but different from paddy field because environmental conditions such as accumulation of TC and TN by fallen leaves in orchard and upland fields. Therefore, the minimum and recommended values of TC, TN, and TK in the orchard and upland fields should be similar but TP is difference. The minimum required values in the orchard fields are TC: ≥12,000 mg/kg, TN: ≥1,000 mg/kg, TP: ≥ 800 mg/kg, and TK: ≥ 1,500 mg/kg. The recommended values in the orchard fields are TC: ≥ 25,000 mg/kg, TN: ≥ 1,500 mg/kg, TP: ≥ 900 mg/kg, and TK: 2,500 to 10,000 mg/kg

Chapter 2
Investigation on soil fertility of upland soil at different soil types

2.1 Introduction

In Japan, there are many soil types, which have been classified at several previous works [60][61][62][63]. Soil provides nutrients to plant and a habitat for microorganism. Properties of soil have formed under both weathering and biological activities, in which microorganism plays a vital role [64]. Soil characteristics, especially chemical properties are different among Japanese soil types. Chemical properties of agricultural soils in Japan are characterized for soil types due to both parent materials and degree of weathering of the soils [65].

However, soil properties in arable fields seem to be influenced by agricultural practices [18][19][20][21][22]. The widespread use of chemical fertilizers and agrochemicals had started in Japan when the Agricultural Chemicals Regulation Law was established by the government since 1948 [66]. Nevertheless, the enactment of the Three Acts on Agri-Environment in 1999 planed the reduction of chemical fertilizers and agrochemicals and improvement of soil quality by application of organic fertilizers [67]. Therefore, the different long-term agricultural management practices have influenced soil properties.

The effects of soil types and agricultural management practices on soil fertility, especially microbial biomass have not been investigated. Microbial biomass plays important roles in nutrient cycles and organic degradation rates but is relatively sensitive to agricultural practices [68][69][70]. In Japan, upland fields under different agricultural management practices, would be investigated in this study. There has been little information on biological properties of upland soils at different soil types in Japan. Only the chemical properties at different soil types but did not investigated biological properties [65]. With the analysis of Soil Fertility Index (SOFIX), soil bacterial biomass and chemical properties were measured in soil [10]. To exam reproducibility of soil, SOFIX has been developed to measure bacterial biomass, material circulation, and physicochemical properties. In this study, soil bacteria were commonly limited due to availability of carbon and nitrogen [18][71][72][73][74]. Therefore, bacterial biomass,

TC, and TN were selected to evaluate soil fertility. This study aims to observe relationships between soil types and soil fertility in upland fields in Japan.

2.2 Materials and methods

2.2.1 Soil samples

One thousand soil samples were sampled from upland fields located in 8 regions (36 prefectures) from March 2014 to March 2018. The soil samples included 106 from Hokkaido, 36 Tohoku, 158 Kanto, 140 Chubu, 456 Kinki, 36 Chugoku, 2 Shikoku, 66 Kuyshu and Okinawa. The soil samples were collected from 0 to 15 cm depth of surface layer from agricultural upland fields where vegetables, flowers, and cereal crops were cultivated. The soils were sieved through 2 mm sieve and finally kept at 4^{o}C for analysis.

2.2.2 Analytical methods

The following biological and chemical properties of soil were analyzed: bacterial biomass, TC, and TN. Bacterial biomass was estimated by quantification of environmental DNA (eDNA) using the low stirring method followed by chapter 1, topic 1.2.2.1. The TC was analyzed using a total organic carbon analyzer (SSM- 5000A, Shimadzu, Kyoto, Japan). The TN was analyzed by extracting soil samples using the Kjeldahl digestion method followed by chapter 1, topic 1.2.3.2. Then, TN of soil was measured by using the indophenol blue method followed by chapter 1, topic 1.2.3.3.

2.2.3 Data analysis

Soil types were classified following the Comprehensive Soil Classification System of Japan-First Approximation (Table 16). Soil types were determined by searching the sample locations on the soil inventory map provided by Japanese National Agriculture and Food Organization [75]. The data of bacterial biomass, TC, and TN were grouped basing on the optimal values according to SOFIX [10]. For instance, the optimal values of bacterial biomass, TC, and TN are greater than or equal to 6.0×10^8 cells/g-soil, 2,500 mg/kg, and 1,500 mg/kg, respectively. Out of those, the optimal value of TN has been adjusted to 1.5 mg/kg due to the recent observations (has not been published yet). The recommended value of C/N ratio is from 8 to 25. In addition, bacterial biomass is lower than 2.0×10^8 cells/g-soil categorized low level, between 2.0×10^8 cells/g-soil and 6.0

× 10^8 cells/g-soil categorized medium level, and greater than 6.0 × 10^8 cells/g-soil categorized high level [76]. The TC is lower than 1,200 mg/kg categorized low level. There were 12 categories of soil samples based on bacterial biomass and TC. Statistical analysis (Kruskal-Wallis H test, $p < 0.01$) of bacterial biomass, TC, and TN values and correlation (Pearson analysis) between parameters at each soil type were determined with SPSS software 16.0 (Chicago, IL, America).

Table 16. Soil classification between Japanese system and World base system.

Soil Classification System of Japan First Approximation (2011)	World Reference Base for Soil Resources (2006)
1. Man-made soil (Soil type A)	Technosol, Regosol
2. Organic soil (Soil type B)	Histosol
3. Podzol (Soil type C)	Podzol
4. Andosol (Soil type D)	Andosol
5. Dark Red soil (Soil type E)	Alosol, Acrisol, Cambisol
6. Lowland soil (Soil type F)	Fluvic Hydragric Anthrosol, Gleyic Fluvisol
7. Red-Yellow soil (Soil type G)	Alisol, Acrisol, Cambisol
8. Stagnic soil (Soil type H)	Gleysol, Stagnosol, Anthrosol
9. Brown Forest soil (Soil type I)	Cambisol, Stagnosol
10. Regosol (Soil type J)	Regosol, Arenosol, Leptosol, Phaeozem

2.3 Results

2.3.1 Soil type classification and soil fertility

Japanese soils have been classified based on diagnosing horizons, properties, and materials. There were 10 soil types: Man-made soil (soil type A), Organic soil (soil type B), Podzol (soil type C), Andosol (soil type D), Dark Red soil (soil type E), Lowland soil (soil type F), Red-yellow soil (soil type G), Stagnic soil (soil type H), Brown Forest soil (soil type I), and Regosol (soil type J). The equivalence between soil classification in Japan and in the world is shown in Table 16. Six soil types (excluded the soil type A, C, E, and J) were found in this study. The 6 selected soil types (soil type B, D, F, G, H, and I) occupies the majority in whole upland field area and thus representative of upland soils

in Japan [77]. From the database, 633 type F soil samples occupied the majority, followed by the 176 type D soil samples and 73 soil type G soil samples as depicted at Figure 12. The sample numbers of the soil type H, I, and B were 44, 41, and 29, respectively. These soils were also the common types in upland fields in Japan, particularly the soil type D and F [63][77].

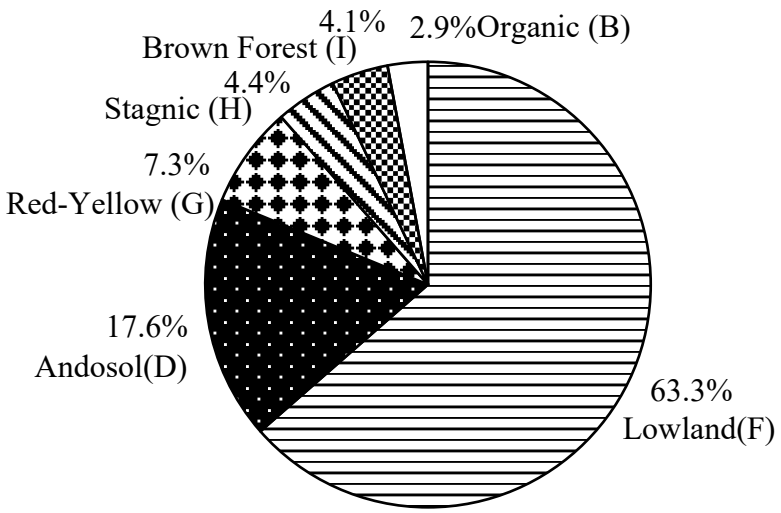

Figure 12. The percentage of investigates sample at 6 soil types.

Average values of bacterial biomass, TC, and TN were varied among the soil types (Figure 13). Total bacterial biomass was the highest at the soil type I (9.4×10^8 cells/g-soil), followed by the soil type F and G (6.6×10^8 cells/g-soil). Meanwhile, the lowest bacterial biomass was seen at the soil type D (3.7×10^8 cells/g-soil). The TC was the highest at the soil type B with 31,400 mg/kg, while that was the lowest at the soil type F (20,400 mg/kg) among the soil types. The TN was the highest at the soil G with 2,100 mg/kg whereas only 1,000 mg/kg of TN was seen at the soil H. According to Kruskal-Wallis H test in Figure 13, there were significant differences of bacterial biomass, TC, and TN between the soil types. At most of the soil types, there were large variation of the data of bacterial biomass, TC, and TN.

2.3.2 The variation of TC and bacterial biomass in different soil types of upland field

2.3.2.1 Correlation between TC and bacterial biomass in different soil types of upland field

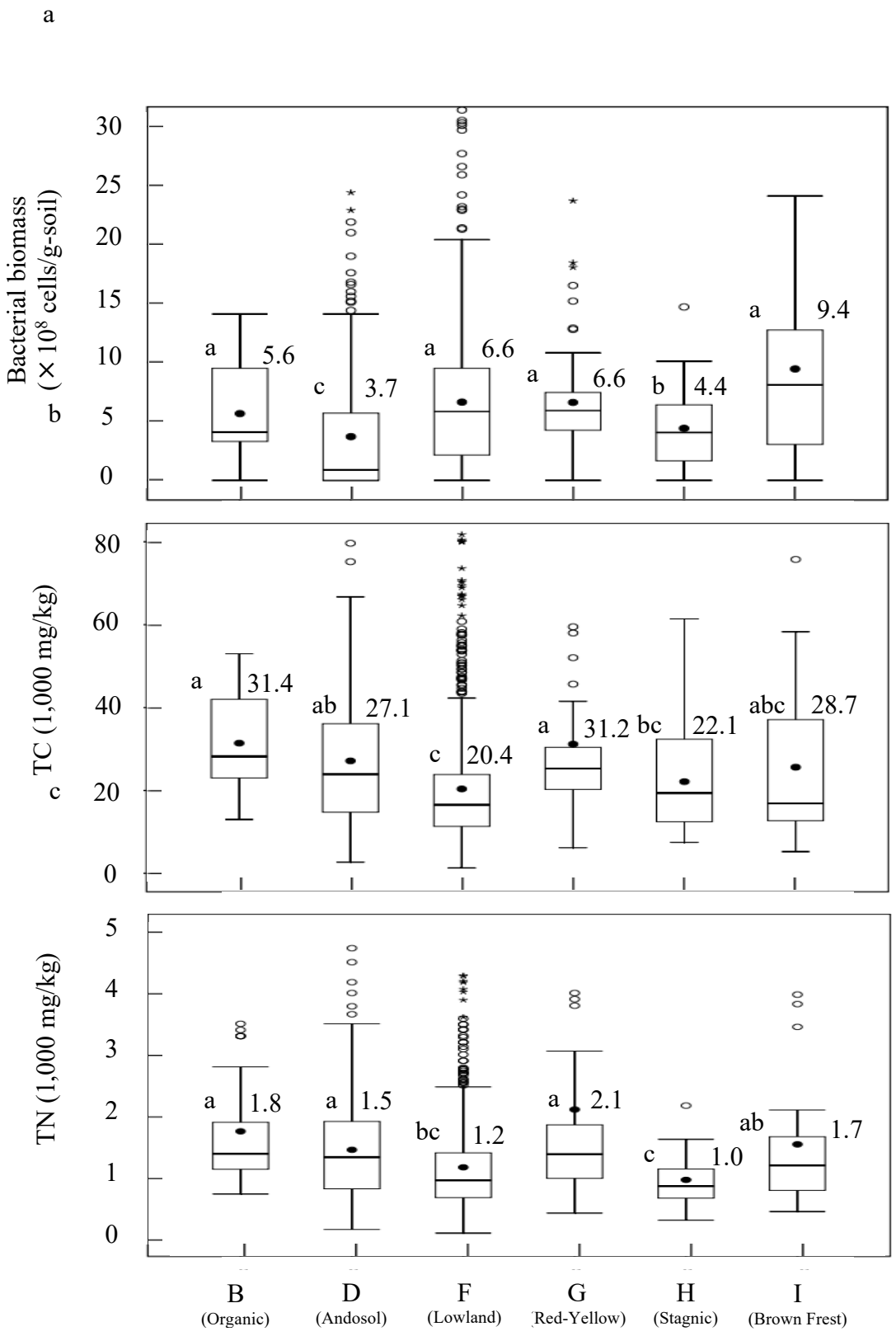

Figure 13. Variation of bacterial biomass (a), TC (b), and TN (c) at different soil types. Boxplots show median values (solid horizontal line) and mean values (solid dots and biomass). Letters represent the results of Kruskal-Wallis H test, $p < 0.01$.

The relationships between TC and bacterial biomass at the different soil types are shown in Figures 14 and 15. Generally, the data of bacterial biomass and TC of upland field were dispersed at all soil types and showed weak correlation (R^2= 0.05). Pearson analysis showed moderate correlations between bacterial biomass and TC at the soil type B (R^2= 0.36) and I (R^2= 0.38), while that did not show the significant correlations at the soil type G (R^2= 0.00) and H (R^2= 0.02). A weak correlation was found at the soil type D (R^2= 0.05) and F (R^2= 0.07).

2.3.2.2 The category of TC and bacterial biomass in upland soils

The variation of soil TC and bacterial biomass was plotted into the 12 categories (Table 17). A large variation of bacterial biomass and TC was also seen at all soil types. Table 18 shows the distribution of samples in 12 categories at each soil type. The number of soil samples type B, F, G, H, and I in the categories of high and medium bacterial biomass and TC (the categories 1, 2, 4, and 5) was higher than that in the categories of low or not detected bacterial biomass and TC (the categories 3, 6, 9, 10, 11, and 12). The categories 1 and 2, which are considered suitable organic management, were experienced high distribution of samples at the soil type B, F, G, and I. In the categories 4 and 5, the number of samples at the soil type B, G, and H was high. In contrast, the number of samples distributed in the categories 7, 8, 9, 10, 11, and 12 was overwhelming at the soil type D. Out of those, the samples in the categories 10 and 11 were occupied the majority at the soil type D.

Table 19 shows the distribution of sample in 4 categories of bacterial biomass. The pattern was similar to that at Table 18. At the soil type B, F, G, H, and I, the sample with high and medium bacterial biomass accounted for the majority. In this study, 809 soil samples type D and F were mainly collected in agricultural upland fields. At the soil type D, the percentage of samples with the not detected and low bacterial biomass levels were up to 40% and 17%, respectively. The figure of high bacterial biomass was only 24%. At the soil type F, the samples with high bacterial biomass level occupied to 49%, followed by the medium bacterial biomass level (27%). Soil samples with not detected bacterial biomass level and low bacterial biomass level were 7% and 17% respectively.

2.3.3 Correlation between TN and bacterial biomass in different soil types of upland field

The correlations between bacterial biomass and TN are presented at Figures 16 and 17. Basically, the correlations were relatively weak between bacterial biomass and TN. For instance, while the weak correlations were seen at the soil type B (R^2=0.06), D (R^2=0.07), F (R^2=0.04), and I (R^2=0.03) there is likely no correlation at the soil G (R^2=0.00) and H (R^2=0.00). Thus, the variation of bacterial biomass cannot be explained the content of TN in upland soil.

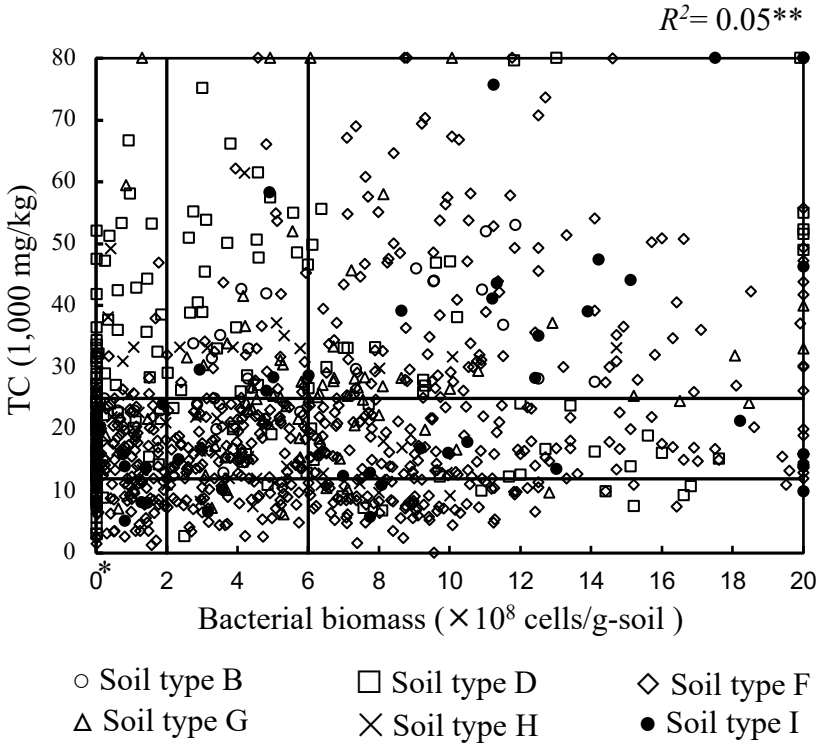

Figure 14. Relationship between TC and bacterial biomass at the different soil types. The Figure was divided into 12 categories. * indicates not detected bacteria number (< 6.6 ×10^6 cells/g-soil). ** indicates significant correlation at p < 0.01. Lines show the minimum and optimum values of TC and bacterial biomass.

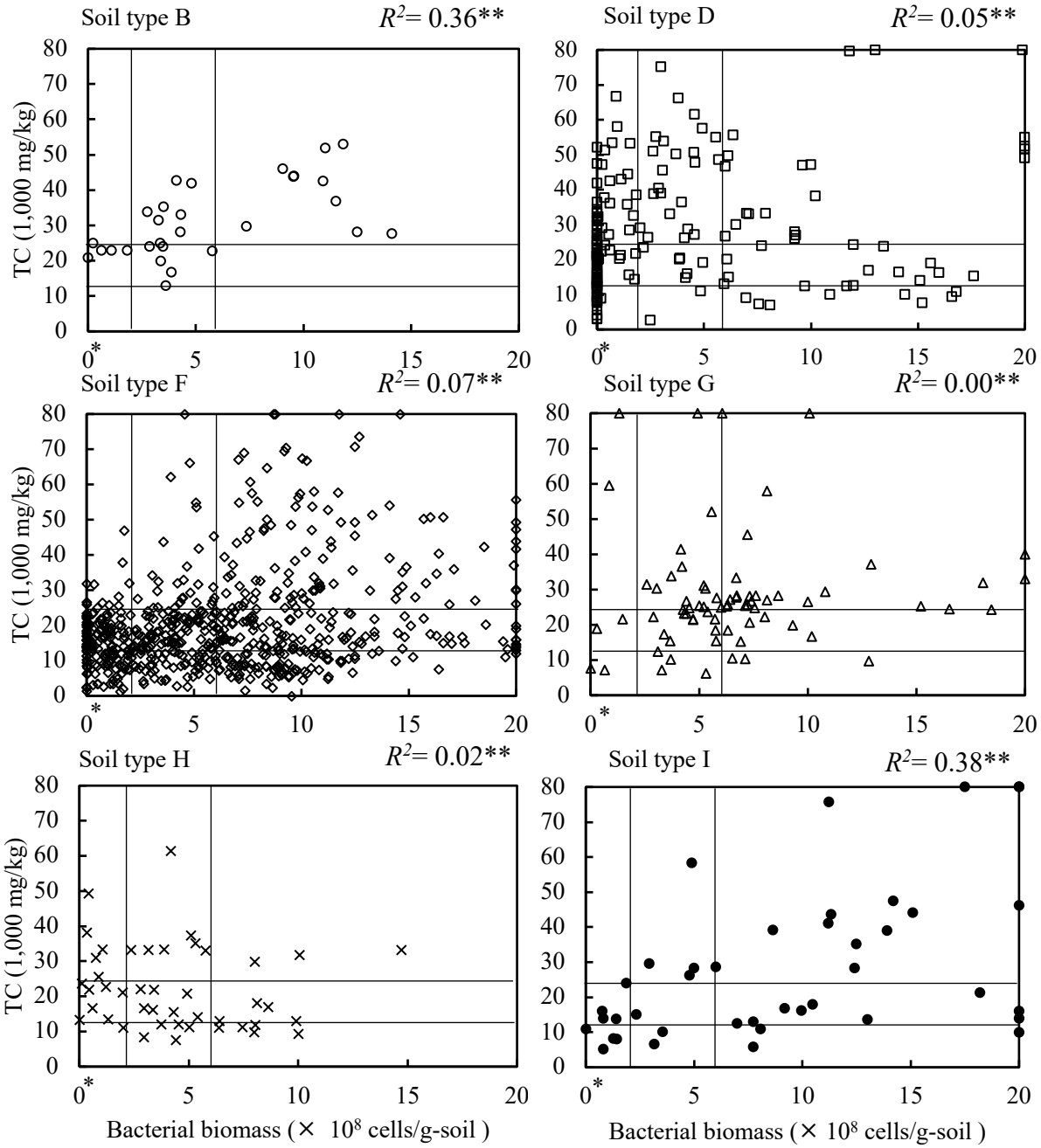

Figure 15. Relationship between TC and bacterial biomass at the different soil types. * indicates not detected bacterial biomass (< 6.6 $\times 10^6$ cells/g-soil). ** indicates significant correlation at $p < 0.01$. Lines show the minimum and optimum values of TC and bacterial biomass.

Table 17. Categories of soil TC and bacterial biomass.

Category	Bacterial biomass ($\times 10^8$ cells/g-soil)	TC ($\times$ 1,000 mg/kg)	Evaluation of soil
1	≥ 6	≥ 25	High bacterial biomass and high TC
2	≥ 6	12-25	High bacterial biomass and medium TC
3	≥ 6	< 12	High bacterial biomass and low TC
4	2-6	≥ 25	Medium bacterial biomass and high TC
5	2-6	12-25	Medium bacterial biomass and medium TC
6	2-6	< 12	Medium bacterial biomass and low TC
7	< 2	≥ 25	Low bacterial biomass and high TC
8	< 2	12-25	Low bacterial biomass and medium TC
9	< 2	< 12	Low bacterial biomass and low TC
10	ND	≥ 25	Not-detected bacterial biomass and high TC
11	ND	12-25	Not-detected bacterial biomass and medium TC
12	ND	< 12	Not-detected bacterial biomass and low TC

ND: not detected bacterial biomass (< 6.6×10^6 cells/g-soil)

Table 18. The sample distributed in each group at the different soil types.

Category	Soil type (soil number %)					
	B (Organic)	D (Andosol)	F (Lowland)	G (Red-Yellow)	H (Stagnic)	I (Brown Forest)
1	35	12	18	34	7	34
2	0	8	19	12	9	22
3	0	5	12	4	11	7
4	28	14	4	18	16	10
5	21	4	16	19	21	2
6	0	1	7	4	9	5
7	3	10	1	3	11	0
8	10	6	10	3	14	10
9	0	1	7	1	0	7
10	0	11	1	0	0	0
11	3	21	4	0	2	0
12	0	9	2	1	0	2

Table 19. The variation of bacterial biomass at the different soil types.

Soil type	Soil type B	Soil type D	Soil type F	Soil type G	Soil type H	Soil type I
High bacterial biomass (%) ($\geq 6 \times 10^8$ cells/g-soil)	34	24	49	50	27	63
Medium bacterial biomass (%) ($2 \times 10^8 \sim 6\,6 \times 10^8$ cells/g-soil)	49	19	27	41	45	17
Low bacterial biomass (%) ($6.6 \times 10^6 \sim 2 \times 10^8$ cells/g-soil)	13	16	18	7	26	17
Not-detected bacteria biomass (%) ($<6.6 \times 10^6$ cells/g-soil)	4	41	6	2	2	3

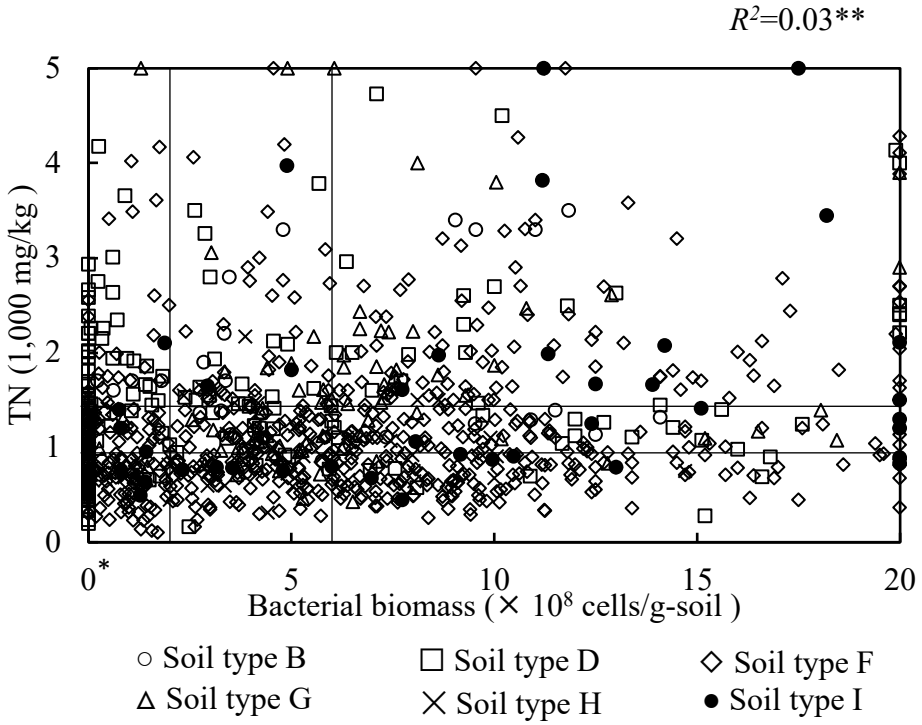

Figure 16. Relationship between TN and bacterial biomass at the different soil types. * indicates not detected bacteria number (< 6.6 ×10^6 cells/g-soil). ** indicates significant correlation at $p < 0.01$. Lines show the minimum and optimum values of TN and bacterial biomass.

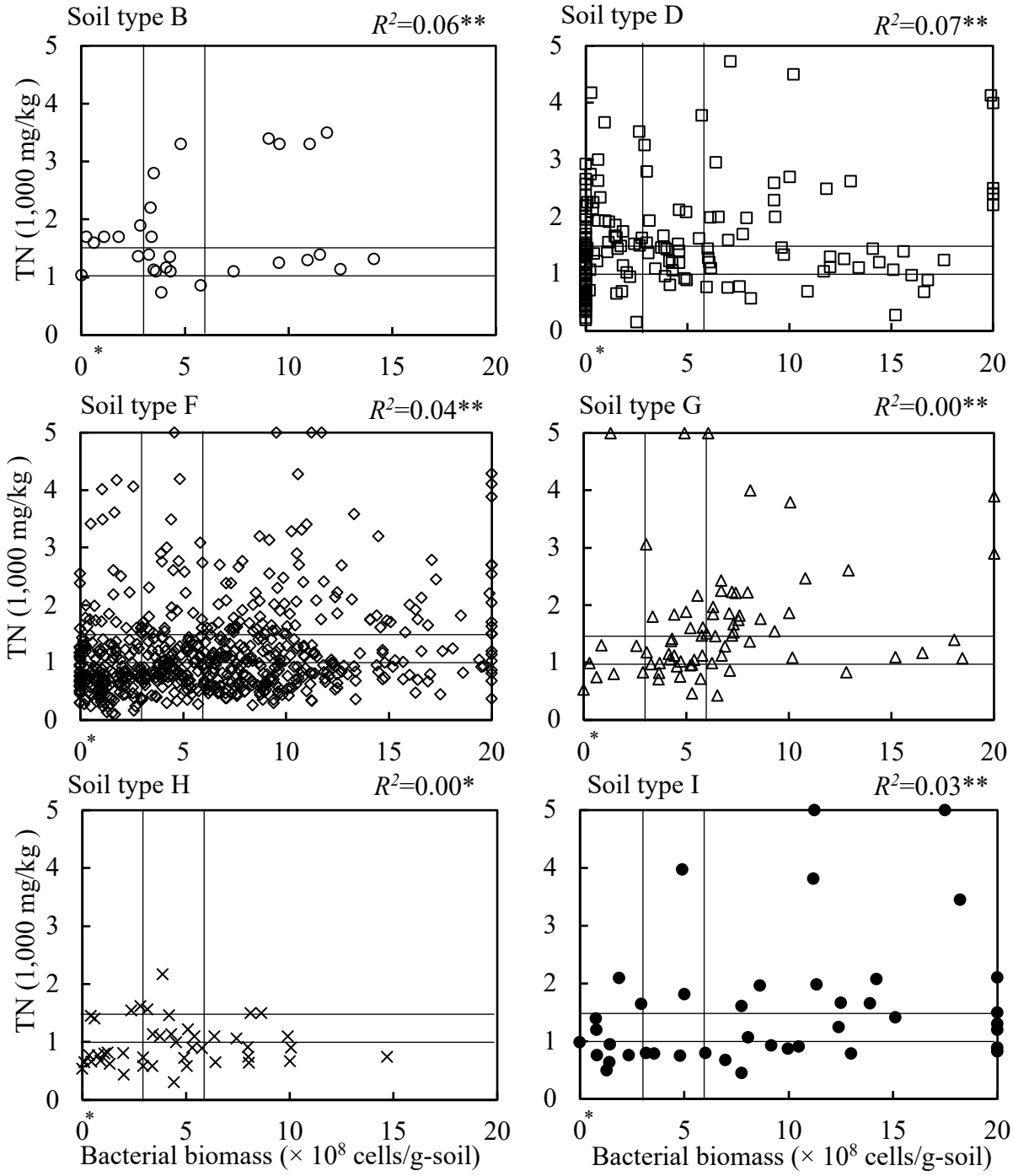

Figure 17. Relationship between TN and bacterial biomass at the different soil types. * indicates not detected bacteria number (< 6.6×10^6 cells/g-soil). ** indicates significant correlation at $p < 0.01$. Lines show the minimum and optimum values of TN and bacterial biomass.

2.3.4 Correlation between TC and TN in different soil types of upland field

Generally, the correlation between TC and TN in upland soil was significantly moderate with R^2=0.46 (Figure 18). Particularly, the correlation between TC and TN was strong only at the soil G (R^2=0.81) and weak at the soil H (R^2=0.16) (Figure 19). The values of correlation at the soil B, D, F, and I showed the moderate correlations. According to SOFIX, the C/N ratio is important to the activity of biological process and ranging from 8 to 25 [10]. Majority of samples at all soil types was located in the range of C/N ratio 8-25.

In summary, the significant differences of bacterial biomass, TC, and TN were seen between the soil types. However, the large variations of data were seen at most of the soil types and not indicated particular characteristics of the soil fertility at each soil type. Bacterial biomass is weakly correlated with TC and TN at all soil types, while the moderate correlation was experienced between TC and TN in upland soil. The percentage of sample having high and medium bacterial biomass levels was high at most of the soil types (except for the soil type D). Therefore, the soil fertility is not characterized by the soil types in upland fields.

2.4 Discussion

Soil type is a determinant factor of soil fertility due to effects of parent material [78]. Particularly, soil type has a great influence on microbial populations and chemical properties in agricultural land [65][79][80][81]. However, this study shows the wide variations of bacterial biomass, TC, and TN in upland fields regardless of the soil type and the weak correlation values between bacterial biomass and TC or TN. Although there was a significant difference of bacterial biomass between the soil types, the tendency of the category of bacterial biomass was same at most of soil types. As a result, the soil fertility (bacterial biomass, TC, and TN) was not characterized by the soil types in upland soil. The large variation of soil fertility can be explained by the effects of agricultural management practices.

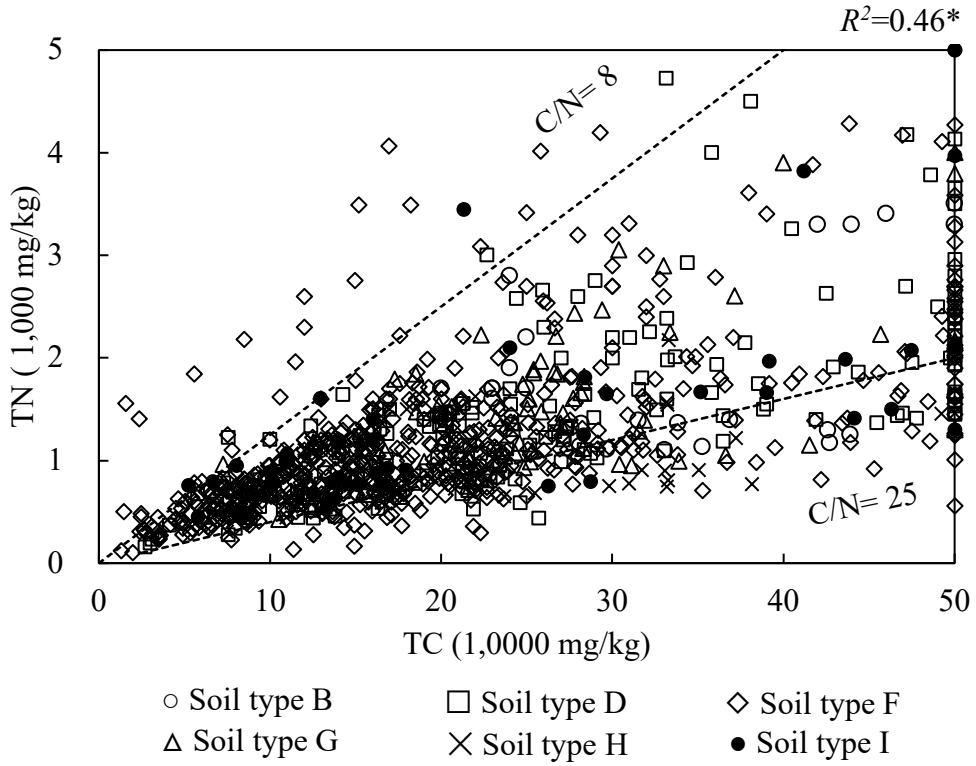

Figure 18. Relationship between TN and TC at the different soil types. * indicates significant correlation at $p < 0.01$. Dashed lines show C/N ratio range from 8 to 25 according to SOFIX.

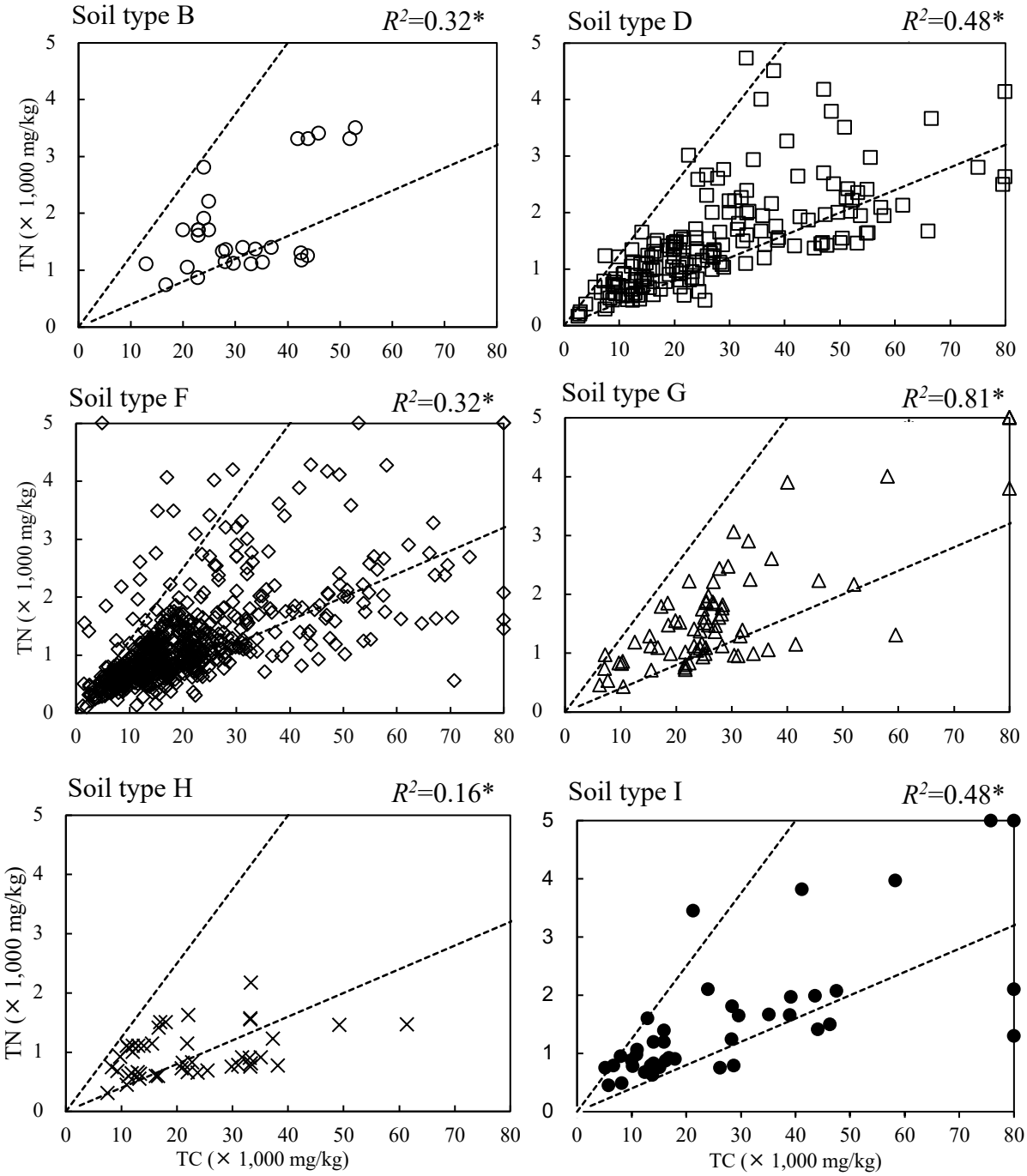

Figure 19. Relationship between TN and TC at the different soil types. * indicates significant correlation at $p < 0.01$. Dashed lines show C/N ratio range from 8 to 25 according to SOFIX.

The soil fertility at ploughed layer (0-15 cm depth) was greatly influenced by agricultural management practices regardless of the soil types. Chemical properties at different soil types are changed by agricultural management practices [82]. For example, the application of inorganic fertilizers (only N, P, and K) declines bacterial biomass in soil due to the reduction of soil organic carbon over decades [83][84][85][86]. The soil organic carbon in the most soil types decreased between 1970 and 2006 in Japan [87]. Overwhelmingly, the application of agrochemicals to prevent pathogens also lowers bacterial biomass in soil [88][89]. For instance, methyl bromide, chloropicrin, and metam sodium, which are widely used as pre-cultivation fumigant in Japan, remarkably led to inhibit the bacterial biomass in soil [90][91]. Up to 90% of bacterial population has been eliminated from Japanese soil due to the fumigation of meta sodium [90]. In addition, bacteria in chloropicrin-treated soil are severely changed and permanently destroyed by repeated application [92]. In this study, the correlation between TC and TN was moderate or strong, while those between bacterial biomass and TC or TN were relatively weak. This indicates the overwhelming effects of agrochemicals on prohibiting bacterial biomass in upland soil.

The soil type D (Andosols) covers more than 50% of whole upland fields in Japan and having excellent physical properties and high natural supply of nutrients for plant growth [77][93]. However, the status of bacterial biomass in Andosols in upland fields has been widely not known in Japan. Previous studies only described microbial biomass activities in laboratories [94][95]. In this study, Andosol collected in upland fields had the majority of samples characterizing not detected and low bacterial biomass (Table 19). It can be probably explained by effects of agricultural management practices.

A large content of carbon is one of characteristics of Andosol [96]. A strong correlation between microbial biomass and organic carbon in plough layer of Andosol [95]. However, bacterial biomass was relatively low and weakly correlated with TC in this study. It can be explained by the deficiency of organic carbon in soil. According to previous studies, the huge amount of carbon stabilized with aluminum in Andosol is recalcitrant to microbial decomposition [97][98][99]. Consequently, bacterial biomass is probably inhibited by the limited turnover or addition of organic carbon. Bacterial population is greater in Andosol soil amended with organic manures than with only chemical fertilizer [100]. Only Andosol and Gray lowland soil in Japan, which are most

common in cultivated soil, are experienced a decrease in carbon content during long-term agriculture practices [101]. Therefore, the lack of organic carbon from organic fertilizer addition might have caused low bacterial biomass in Andosol. In addition, the effects of agrochemicals significantly decrease bacterial biomass in Andosol. The agrochemical absorption of Andosol was higher than that of other soil types due to high aromatic carbon [102]. More than 50% of upland fields in Japan are Andosol fields, which showed trend degradation of bacterial biomass due to the possibility of application of chemical fertilizer and agrochemicals.

2.5 Summary

This study aims to observe the relationships between soil types and soil fertility of upland fields in Japan. The results show that the soil type is not a determinant factor of the soil fertility in upland soil. The weak correlation between bacterial biomass and TC or TN is probably explained by effects of management practices, especially agrochemical application. In comparison with the other soil types, Andosol had a degradation trend of bacterial numbers. Improvement methods of bacterial numbers in Andosol are suggested for further studies. Finally, management practices can either degrades or enhance the soil fertility regardless of the soil types.

Chapter 3
Construction of new organic soil based on soil fertility

3.1 Introduction

Agrochemicals including pesticides and chemical fertilizers use to enhance agricultural activities [32]. The application of chemical fertilizers easier to apply nutrients according to the crop requires [103]. The excessive use of agrochemicals causes serious problems to soil and microorganisms [34][35][104][105].

To protect soil microorganisms from negatively 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 [39][106]. Organic agriculture systems as an alternative system to prohibit use of chemical fertilizers and pesticides [107]. Soil microorganisms represent one of the most important indicators for stable organic agriculture. Microorganisms play important roles in the decomposition of organic materials and cycle nutrients such as carbon, nitrogen, phosphorus through the production of enzymes to inorganic molecules [108][109][110][111][112][113].

The soil fertility index (SOFIX) was developed considering the importance of physical, chemical, and biological soil characteristics [10]. More than 8,000 agricultural soil samples have been analyzed by the SOFIX. The optimum conditions for organic agriculture soil for upland field based on the SOFIX database are total carbon (TC) ≥ 25,000 mg/kg, total nitrogen (TN) ≥ 1,500 mg/kg, total phosphorus (TP) ≥ 1,100, total potassium (TK) 2,500 to 10,000 mg/kg, and bacterial biomass ≥ 6.0×10^8 cells/g-soil.

The standard organic soil has not been constructed because reproducible and stable organic soils with abundant microbial number and diversity are especially difficult to create. Farmers are lack of knowledge and understanding of the decision-making processes of organic fertilizers [23]. In this study, woodchips are the main material for producing new organic soils. Normally, woodchips are applied to reduce moisture loss, increase soil porosity, and water holding capacity [113][114][115]. In addition, woodchips have high nutrients such as carbon and nitrogen [116]. The previous experiments showed that wood chip leads to the increase of bacterial biomass and effect on plant growth [117][118]. This study aimed to construct a reproducible and stable new organic soil based on the SOFIX database through testing a range of base soils and additive materials. This chapter describes the process of control of the base soil and additive materials, the plant growth, and the bacterial analysis of the organic standard soil.

3.2 Materials and Methods

3.2.1 Selection of 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.

3.2.2 Analysis of soil and material biological properties

The following biological properties of soils and materials were analyzed: bacterial biomass, nitrogen (N) circulation activity, and phosphorus (P) circulation activity. Bacterial biomass was estimated by quantification of environmental DNA (eDNA) using the low stirring method followed by chapter 1, topic 1.2.2.1. Analysis of nitrogen (N) circulation activity was analyzed based on the bacterial biomass, ammonium oxidation rate and nitrite oxidation rate in the soil followed by chapter 1, topic 1.2.2.2. Estimate of phosphorous (P) circulation activity followed by chapter 1, topic 1.2.2.3.

3.2.3 Analysis of soil and material chemical properties

The following chemical properties of soils and materials were analyzed: TC, TN, TP, and TK. The TC was analyzed using a total organic carbon analyzer (SSM- 5000A, Shimadzu, Kyoto, Japan). The TN, TP, and TK contents were analyzed by extracting soil samples using the Kjeldahl digestion method followed by chapter 1, topic 1.2.3.2. The TN was measured by using the indophenol blue method followed by chapter 1, topic 1.2.3.3. The TP was measured by using the molybdenum blue method followed by chapter 1, topic 1.2.3.4. The TK was measured by performed using Z-2300 atomic absorption spectrophotometer (Hitachi High-technologies Corporation, Tokyo, Japan) followed by chapter 1, topic 1.2.3.5.

3.2.4 Analysis of soil and material physical properties

The following physical properties of soils and materials were analyzed: water content, water holding capacity, and bulk density. Water holding capacity and water content were measure followed by chapter 1, topic 1.2.4.1 and 1.2.4.2, respectively. Bulk density was measured by the standard methods by volumetric cylinder. Bulk density values of soils and materials from volumetric cylinder were calculated from the mass of a unit volume of dry soil and materials [119].

3.2.5 Preparation and analysis of the soil

The base soils and additive materials were dried at 37℃ for 1 week and then these materials were sieved through a 2-mm sieve. Seven organic soils were prepared by mixing the base soils and additive materials. A 200 g of each organic soil was preincubated in a 400 ml pot and maintained 30% of water content for 1 week to activate the microbial activities. The soil sample of each treatment was collected for analyzed TC, TN, TP, TK, water content, and water holding capacity. The bacterial biomass was analyzed on days 0, 3, 5, and 7, while N and P circulation activities 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℃ throughout the experimental period.

3.2.6 Plant cultivation

Chemical soil (control) and 7 organic soils were compared for plant growth. 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 4 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 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℃). The plant growth parameters were determined using one-way analysis of variance (ANOVA).

3.2.7 PCR-DGGE analysis

A best organic standard soil (among 7 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') [120]. 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℃ for 1 min, followed by 35 cycles of denaturation at 95℃ for 1 min, primer annealing at 55℃ for 30 s, and extension at 72℃ for 1 min and then a final extension at 72℃ 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℃ 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).

3.2.8 Plant growth of organic soil using other materials

For improvement plant growth (*B. rapa*) of organic soil, this organic soil was mixed with the new materials (slag, DSP, and wood vinegar) at different ratio (Table 28). 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. Seeds of *B. rapa* were sown in a nursery tray for 1 week, and 4 seedlings were then transplanted to each Wagner pot. After 4 weeks of cultivation, *B. rapa* of each treatment were harvested and measured followed by topic 3.2.7.

3.3 Results

3.3.1 Analysis and 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 20 and Table 21). 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 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 6.0×10^8 cells/g. Among the 3 types of manure, cow manure was selected because of a well-balanced nutrient content and high bacterial biomass.

3.3.2 Construction and characterization of new prepare organic soils

The candidates of a standard soil based on SOFIX recommended values (Table 22) 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 23 and Table 24). 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 7 prepared organic standard soils are shown in Table 25. The TC, TN, TP, and TK contents, and the C/N and C/P ratios of the 7 candidate standard soils were 24,000 - 34,740 mg/kg, 1,580 – 1,840 mg/kg, 1,040 – 1,160 mg/kg, and 6,450 – 9,660 mg/kg, and 14 - 20 and 22 - 31, respectively. The bulk density and the WHC of the 7 organic standard soils were above 0.5 g/cm^3 and 1,200 ml/kg, respectively. The chemical and physical properties of the 7 organic soils were around SOFIX recommended values. Among 7 organic soils, T7 was showed the lowest bulk density but the highest WHC.

The biological properties of the 7 candidate organic soils after controlling the water (30% of water content) for 1 week are shown in Table 26 and Figure 20. The bacterial biomass of all candidate organic soils exceeded 6.0×10^8 cells/g-soil on day 3, and the bacterial biomass

Table 20. 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	1,770	2,070	4,000	39	34
	Mountain soil	300	90	410	8,000	3	1
	Vermiculite	400	180	300	33,000	2	1
	Peat moss	412,200	2,070	310	1,300	199	1,330
	Wood chip 1	445,100	700	270	2,500	636	1,649
	Wood chip 2	356,000	470	270	2,600	757	1,319
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	3,600	5	3
	Chicken manure	194,000	34,600	17,500	24,400	6	11
	Horse manure	113,600	4,729	3,350	4,330	24	34
	Cow manure	330,000	21,000	10,000	26,000	16	33

Table 21. 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	ND	980	0.84	1.2
	Mountain soil	ND	550	1.39	29.6
	Vermiculite	ND	3,000	0.22	0.2
	Peat moss	ND	300	0.14	3.6
	Wood chip 1	2.7	1,150	0.15	12.3
	Wood chip 2	8.8	1,120	0.10	9.2
Additive material	Oil cake	ND	-	-	-
	Soybean meal	ND	-	-	-
	Bone meal	ND	-	-	-
	Chicken manure	7.8	-	-	-
	Horse manure	71	-	-	-
	Cow manure	132.4	-	-	-

ND: Not detected

Table 22. The SOFIX recommended value.

Parameter	Recommended value
Total carbon (TC) (mg/kg)	≥ 25,000
Total nitrogen (TN) (mg/kg)	≥ 1,500
Total phosphorus (TP) (mg/kg)	≥ 1,100
Total potassium (TK) (mg/kg)	2,500-10,000
C/N ratio	8-25
C/P ratio	23-46
N circulation activity (point)	≥ 38
P circulation activity (point)	30-70

Table 23. The blend method.

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 24. 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 25. 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/cm3)	Water holding capacity (ml/kg)
T1	31,550	1,650	1,040	9,660	19	30	0.69	1,328
T2	34,400	1,740	1,130	7,510	19	31	0.59	1,340
T3	24,000	1,580	1,090	7,120	15	23	0.63	1,297
T4	26,120	1,840	1,120	6,460	14	24	0.51	1,332
T5	34,740	1,690	1,130	7,550	20	31	0.58	1,362
T6	25,350	1,650	1,160	7,920	15	22	0.55	1,338
T7	26,350	1,690	1,120	6,450	15	24	0.50	1,407

Table 26. N and P circulation activities 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

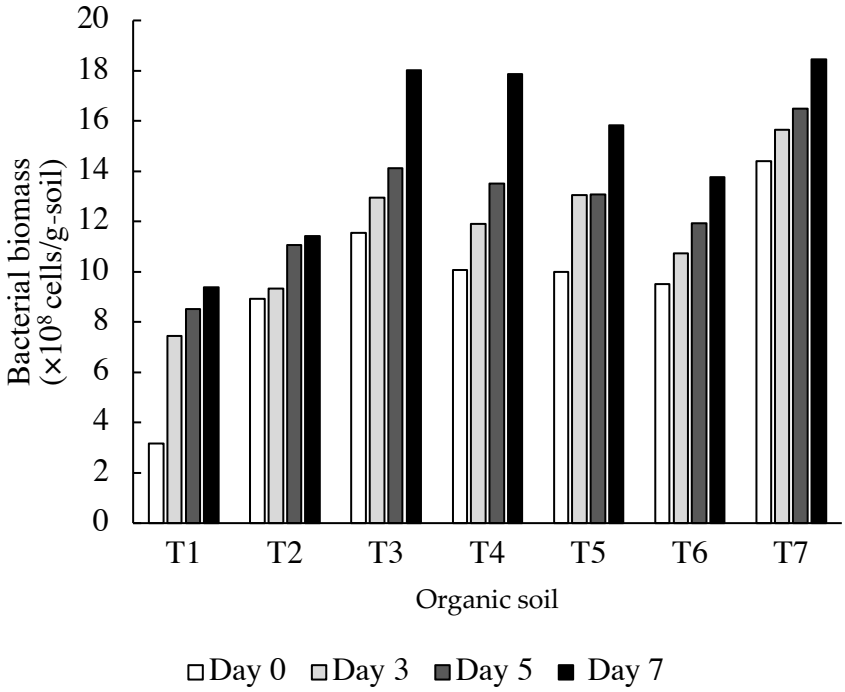

Figure 20. The bacterial biomass in the 7 organic soils (T1-T7) during 7 days.

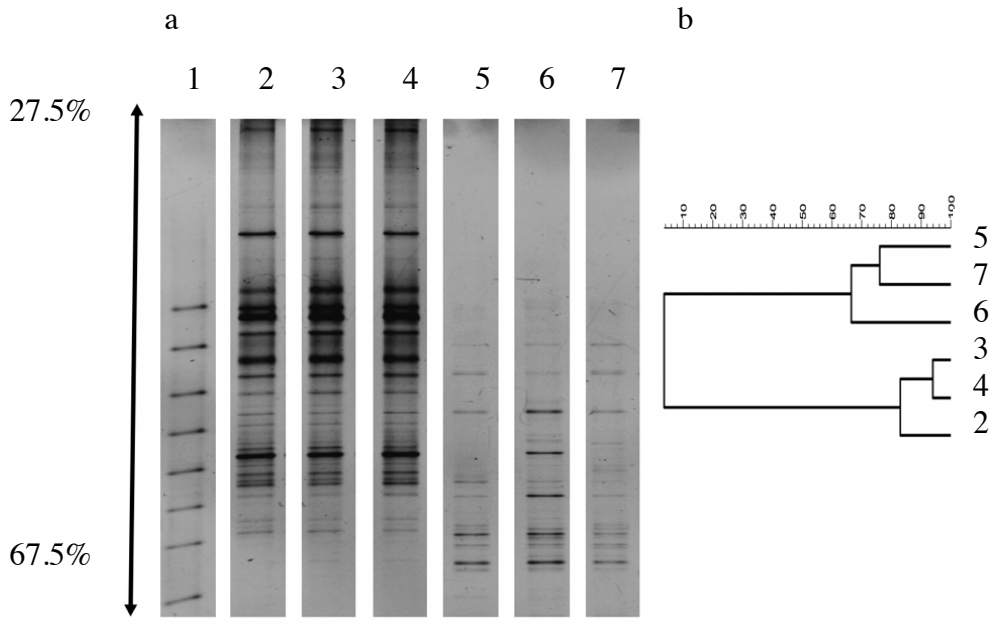

Figure 21. PCR DGGE analysis of 16S rRNA bacterial genes: image of electrophoresis (1: Marker, 2 - 4: Different lots of the organic standard soil, and 5 - 7: Different lots of the chemical soil) (a) and cluster analysis (b).

of T2, T3, T4, T5, T6, and T7 was greater than 11.0×10^8 cells/g-soil on day 7. This result indicates that the wood chips increase the bacterial biomass. Among the 7 organic soils, T7 showed the highest value of the bacterial biomass. The nitrogen and phosphorus circulation activities of the 7 candidates of the organic soil were close to the SOFIX recommended values.

3.3.3 Analysis of plant growth in new prepare organic soils

To compare the plant growth, *B. rapa* cultivation experiment was conducted (Table 27 and Figure 22). The performance of *Brassica rapa* in the 7 organic soils was similar 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.

3.3.4 Analysis of the bacterial diversity in the organic 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 21). 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.

3.3.5 Improvement plant growth of organic soil using other materials

For improvement plant growth (*B. rapa*), this organic soil was mixed with the new materials (slag, DSP, and wood vinegar) at different ratio (Table 28). Table 29 shows fresh weight, shoot length, root length, chlorophyll (SPAD reading), and number of leaves. The growth and height of shoot of *B. rapa* in 3 soils with adding slag, DSP, and wood vinegar (O2, O3, and O4) was better than that in the control soil (O1). The addition of slag, DSP, and wood vinegar led to a higher fresh weight (50-66%) and higher shoot length (7-13%) of *B. rapa* than that in the control soil. Especially, *B. rapa* growth in the organic soil O4 was the highest.

3.4 Discussion

Based on SOFIX database [10], the values of TC (≥ 25,000 mg/kg), TN (≥ 1,500 mg/kg), TP (≥ 1,100 mg/kg), TK (2,500 to 10,000 mg/kg), and C/N ratio (8 to 25) were controlled by base soils (vermiculite, mountain soil, black soil, peat moss, an, and wood chips) and additive materials. After controlling the water content to 30%, bacterial biomass of the organic soils with wood chips was higher than 6.0×10^8 cells/g-soil. 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 [121][122].

Table 27. Parameters of *B.rapa* growth in the organic soils and the chemical soil.

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

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

a

b

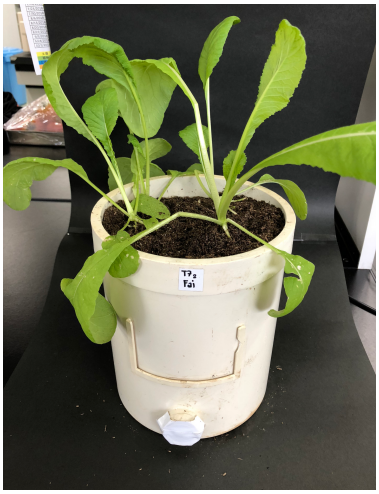

Figure 22. Growth of *B. rapa* treated with T1 (a) and T7 (b)

Table 28. The construction of 4 soil treatments.

Treatment	Soil	Slag (%w/w)	DSP (%w/w)	Wood vinegar (%w/w)
O1 (Control)	Standard organic soil	-	-	-
O2	Standard organic soil	0.1	1	-
O3	Standard organic soil	0.1	-	1
O4	Standard organic soil	0.1	1	1

Table 29. Parameters of *B. rapa* growth in the 4 soil treatments.

Treatment	Fresh weight (g/plant)	Shoot length (cm)	Root length (cm)	Chlorophyll (SPAD)	Number of leaves
O1	11.42 (± 6.00) (100%)	21.09 (± 6.46) (100%)	22.45 (± 4.52) (100%)	48.85 (± 13.29) (100%)	8 (± 1) (100%)
O2	17.93 (± 6.54) (157%)	23.92 (± 3.88) (113%)	23.33 (± 4.62) (104%)	41.37 (± 9.24) (85%)	10 (± 1) (125%)
O3	17.08 (± 5.08) (150%)	22.67 (± 4.90) (107%)	22.00 (± 2.31) (98%)	53.06 (±14.28) (109%)	9 (± 2) (113%)
O4	18.95 (± 9.26) (166%)	23.75 (± 5.10) (113%)	22.92 (± 5.92) (102%)	49.69 (± 12.71) (102%)	10 (± 1) (125%)

In fact, the bacterial biomass in the organic soils with wood chips 2 were obviously higher ($\geq 14.0 \times 10^8$ cells/g-soil) than that in vermiculite after 7 days. Wood chips have high carbon [116]. The previous study presented that the application of woodchips enhances fungal species abundance in orchard field and fungal communities related to carbon source [123][124].

The growth of *B. rapa* in the organic soil with woodchips was higher than that in the chemical soil. Soil microorganisms play an important role in soil nutrient cycling [125][126]. 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 [125] [126] [127][128]. Woodchips have ability to reduce moisture loss and increase water holding capacity [113][114]. Many studies showed that the application of woodchips had effect on plant growth [118][129]. The organic 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 [130][131][132]. 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 [133][132][135][136]. In addition, the bacterial diversities in the organic standard soil became almost the same within the PCR-DGGE experiment [137], 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.

3.5 Summary

In this study, new reproducible and stable 7 organic soils were prepared by using dried base soils and additive materials. Base soils including vermiculite, black soil, mountain soil, peat moss, and two types of wood chips (big- and small-sized) at 50%, 60%, and 70% (v/v). Additive materials including cow manure, soybean meal, oil cake, and bone meal were added

into 7 organic soils at the same amount. After organic materials were blended and controlled 30% water content for 1 week, 7 organic soil showed successfully achieved contents of TC ≥ 25,000 mg/kg, TN ≥ 1,500 mg/kg, TP ≥ 1,100, and TK of 2,500 to 10,000 mg/kg based on suitable values for soil. Moreover, these organic soils presented high bacterial biomass and nutrient circulation activities. Especially, organic soil prepared from 70% of small-sized wood chip had the highest bacterial biomass and stable bacterial diversity. In addition, 7 organic soils and the fertilizer-amended soil were compared plant cultivation. The plant cultivation experiment showed that fresh weight of *B. rapa* in the 7 organic soils were higher than that of the chemical soil. Especially, organic soil prepared from 70% of small- sized wood chip showed the highest fresh weight of *B. rapa*. These findings suggest that organic soil prepared from 70% small-sized wood chip, 20% mountain soil, and 10% black soil is the best suitable organic soil.

Conclusion

Soil is important for crop cultivation. Conventional and organic agriculture systems are used to improve soil fertility and increase crop production. The soil fertility index (SOFIX) was developed considering the importance of soil physical, chemical, and biological characteristics and indicate soil health as a number through diagnosis and analysis the 3 indicators of microbial numbers, nitrogen activity, and phosphorous activity in the soil environment. Study on soil fertility of agriculture fields such as orchard and upland fields help to understand the features of soil. Among soil parameters, bacterial biomass, N circulation activity, P circulation activity, TC, TN, TP, and TK are important factors related to soil fertility. From agriculture fields information, new organic soil was constructed successfully attained sufficient contents of the nutrients (carbon, nitrogen, phosphorus, and potassium) and abundant microbial diversity.

Chapter 1,

Soil samples from 139 agricultural orchard fields (apple, grape, tea, and others) were analyzed using the soil fertility index. From these samples, an orchard field database was constructed and compared the soil properties between orchard, upland, and paddy fields. The average value of bacterial biomass in the orchard fields was 7.4×10^8 cells/g-soil, ranging from not detected (lower than 6.6×10^6 cells/g-soil) to 7.7×10^9 cells/g-soil. The average values of TC, TN, TP, and TK were 24,000 mg/kg (2,670 to 128,100 mg/kg), 1,460 mg/kg (133 to 6,400 mg/kg), 1,030 mg/kg (142 to 5,362 mg/kg), and 5,370 mg/kg (1,214 to 18,155 mg/kg), respectively. The C/N and C/P ratios were 19 (3 to 85) and 27 (2 to 101), respectively. Soil properties of the orchard fields were compared with those of the upland and the paddy fields. The average value of bacterial biomass in the orchard fields was almost the same as that in the upland fields (8.0×10^8 cells/g-soil), but the number was lower than that in the paddy fields (12.9×10^8 cells/g-soil). The average values of TC and TN in the orchard fields fell between those in the upland fields (TC: 33,120 mg/kg, TN: 2,010 mg/kg) and the paddy fields (TC: 15,420 mg/kg, TN: 1,080 mg/kg). The relationship between the bacterial biomass and TC in the orchard fields resembled that in the upland fields. A suitable soil condition for the orchard fields was determined as TC: ≥ 25,000 mg/kg, TN: ≥ 1,500 mg/kg, TP: ≥ 900 mg/kg and TK: 2,500 - 10,000 mg/kg. These recommended values will lead to improve the soil quality of the orchard fields by enhancing the number and activities of microorganisms.

Chapter 2,

Soil type is a vital determinant of soil fertility because of its characteristics of biological, chemical, and physical properties. However, the soil fertility of upland soil seems to be changed by different management practices regardless of soil type. This study was conducted to investigate the tendency of soil fertility (bacterial biomass, TC, and TN) and effect of soil types on the soil fertility in upland field in Japan. One thousand soil samples at different soil types were collected in upland fields located in 36 prefectures. The soil fertility was analyzed with Soil Fertility Index (SOFIX). The results show that there were 6 soil types such as Organic soil (B), Andosols (D), Lowland soils (F), Red-yellow soils (G), Stagnic soils (H), and Brown Forest soils (I) in this study. Out of those, the soil type D and F occupied the large percentage of whole investigated samples. The values of bacterial biomass, TC, and TN greatly varied regardless of the soil types. This indicates that the soil fertility was not characterized by the soil types in upland soil in Japan. The correlation between bacterial biomass and TC or TN was relatively weak, while that between TC and TN was moderate or strong. Regarding to the soil type D, there were up to 40% and 17% of samples having not detected and low bacterial biomass, respectively. In upland fields, the effect of the soil types is not a determinant factor on the soil fertility

Chapter 3,

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 ≥ 1,500 mg/kg, total phosphorus ≥ 1,100, and total potassium of 2,500 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 6.0×10^8 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 *B. rapa* 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 7 organic soils in this study.

Appendix

Tomato cultivation

The SOFIX garden soil was collected from the area near Techno complex. The SOFIX garden was analyze TC, TN, TP, and TK. Then, the 4 candidate soil treatments were constructed (Table 1 and Table 2). The materials were divided into 3 groups 1) Base soil (SOFIX garden soil), 2) additive materials (soybean meal, oilcake, cow manure, and bone meal), and 3) other materials (slag, DSP, and wood vinegar). The SOFIX garden soil was used as control and other 3 soil treatments were used the SOFIX garden soil mixing with new materials at different ratio. Slag, DSP, and wood vinegar were analyzed nutrients content (Table 3).

At the first step, tomato seeds were sown in nursery pots about 4 weeks. Then, each soil treatment was added 10% water content and mixed in plastic bag. All soil treatments were put into big pot size about 13.5 kg (3 pot/treatments) and placed in in green house. Soil treatments were incubated for 1 week. After incubation for 1 week, tomato was put in each treatment pot. Tomato plants were measured height of plant, chlorophyll (SPAD reading), number of leaves, the biggest leaf, number of flowers, and number of fruits after 1 and 2 mounts (Table 4 and Table 5).

Table 1. The construction of soil.

Treatment	Soil	Slag (%w/w)	DSP (%w/w)	Wood vinegar (%w/w)
F1 (Control)	SOFIX garden	-	-	-
F2	SOFIX garden	0.1	1	-
S3	SOFIX garden	0.1	-	1
F4	SOFIX garden	0.1	1	1

Table 2. The standard organic soil.

Additive material (% w/w)			
Cow manure	Soybean meal	Oilcake	Bone meal
5	0.25	0.25	0.05

Table 3. The chemical properties of materials.

Material	TC (mg/kg)	TN (mg/kg)	TP (mg/kg)	TK (mg/kg)
Slag	14,770	5,600	12,200	2,040
DSP	56	4,600	50	7,100
Wood vinegar	65,200	220	130	2,680

Table 4. Tomato growth at one month.

Treatment	Height of plant (cm)	Chlorophyll (SPAD)	Number of leaves	The biggest leaf (cm)
F1	85.3 (± 16.9) (100%)	37.1 (± 0.8) (100%)	303 (± 49) (100%)	11.9 (± 0.4) (100%)
F2	92.7 (± 14.3) (109%)	35.7(± 2.1) (96%)	317 (± 2) (104%)	13.7 (± 2.1) (115%)
F3	73.3 (± 25.6) (86%)	35.5 (± 3.5) (96%)	211 (± 22) (69%)	12 (± 2.2) (100%)
F4	82.0 (± 8.3) (96%)	34.8 (± 2.9) (94%)	282 (± 31) (93%)	11.8 (± 1.3) (99%)

Table 5. Tomato growth at two months.

Treatment	Height of plant (cm)	Chlorophyll (SPAD)	Number of leaves	The biggest leaf (cm)	Number of flowers	Number of fruits
F1	129.7 (± 6.5) (100%)	31.5 (± 1.0) (100%)	428 (± 97) (100%)	7.8 (± 3.0) (100%)	3 (± 1) (100%)	0
F2	136.5 (± 4.7) (105%)	32.6 (± 1.0) (103%)	323 (± 64) (75%)	9.7 (± 0.5) (124%)	4 (± 3) (133%)	3
F3	111.7 (± 13.9) (86%)	34.0 (± 0.4) (108%)	333 (± 109) (77%)	7.0 (± 0.7) (90%)	3 (± 2) (100%)	0
F4	122.7 (± 4.5) (95%)	30.1 (± 0.5) (96%)	303 (± 35) (70%)	7.0 (± 2.1) (90%)	2 (± 0) (0%)	1

Preparation method for new organic soil

In our previous study, the organic soil has been prepared from base soils by volume/volume (v/v) (S1 and S2) (Table 6). However, the measurement method (v/v) is unstable resulted in low nutrients content. To prepare stable and reproducible of organic soil, organic soil prepares by weight/weight (w/w) was constructed (S3) (Table 7). Base soils (mountain soil, black soil, peat moss, and wood chip) and additive materials (cow manure, soybean, oil cake, and bone meal) were dried (55 ˚C) until 0% water content and sieved. Bacterial biomass, TC, TN, TP, and TK contents in S3 was higher than those in S1 and S2 (Table 8). In additions, soil biological and chemical properties of S3 prepared by weight/weight were within the SOFIX recommended values. These results indicated that organic soil prepared by using base soils (mountain soil 700 g, black soil 150 g, peat moss 50 g, and wood chip 100 g) with additive materials (cow manure 5%, soybean 0.25%, oil cake 0.25%, and bone meal 0.10% w/w) is stable and successful of nutrient balance.

Table 6. The construction of S1 and S2 soil treatments (v/v).

Base soil (v/v)				Additive material (% w/w)			
Mountain soil	Black soil	Peat moss	Wood chip	Cow manure	Soybean meal	Oilcake	Bone meal
30	10	10	50	5	0.25	0.25	0.05

Table 7. The construction of S3 soil treatment (w/w).

Base soil (g)				Additive material (% w/w)				
Mountain soil	Black soil	Peat moss	Wood chip	Cow manure	Chicken manure	Soybean meal	Oilcake	Bone meal
700	150	50	100	5	1	0.25	0.25	0.1

Table 8. The biological and chemical properties of the organic soil treatments.

Treatment	Bacterial biomass (10^8cells/g-soil)	TC (mg/kg)	TN (mg/kg)	TP (mg/kg)	TK (mg/kg)
S1 (v/v)	10.3	48,200	370 (±15)	200 (±11)	3,350 (±123)
S2 (v/v)	10.5	49,300	400 (±59)	190 (±4)	3,880 (±75)
S3 (w/w)	11.7	69,820	2,050 (±35)	990 (±17)	1,590 (±50)

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List of publications

1. Pholkaw, P., Muraji, A., Maeda, K., Kawagoe, T., Kubota, K., Sanpa, S., Tran, Q. T. and Kubo, M. (2019) Utilization of Wood Biomass for Organic Soil Based on the Soil Fertility Index (SOFIX). *Journal of Agricultural Chemistry and Environment,* 8, 224–236.
2. Pholkaw, P., Tran, Q. T., Takamitsu K., K., Kawagoe, T., Kubota, K., Araki, S.K. and Kubo, M. (2020) Characterization of orchard fields based on Soil Fertility Index (SOFIX). *Journal of Agricultural Chemistry and Environment* (*in press*).
3. Construction of organic soil based on soil fertility index (SOFIX). Kubo M, Pholkaw P, Tran QT & Araki KS, Proceedings of International Workshop on Enabling Capacity· in Production and Application of Bio-pesticides and Bio-fertilizers for Soil-borne Disease Control and Organic Farming, 1-8, Hanoi, Vietnam, May 7-9, (2019).
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