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Assessment of Long-Term Compost Application on Physical, Chemical, and Biological Properties, as Well as Fertility, of Soil in a Field Subjected to Double Cropping

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Abstract

The aim of this article was to assess the influence of long-term application of compost on the physical, chemical, and biological properties, as well as the fertility, of soil in a field subjected to double cropping (paddy rice and barley), mainly by integrating previous studies of the effects of compost and manure on soil qualities. Continuous compost application, especially at a high level $(30 \ Mg \cdot ha^{-1} \cdot y^{-1})$, into the double cropping soils increased the activities of organic C-, N-, and P-decomposing enzymes and the contents of organic C, total N, and microbial biomass N, as well as the cation exchange capacity, thereby contributing to the enhancement of soil fertility. Also, the compost application increased the degree of water-stable soil macroaggregation (>0.25 mm), which was correlated significantly ($r > 0.950$, $p < 0.05$) with the contents of hydrolyzable carbohydrates (with negative charge) and active Al (with positive charge), and resulted in the modification of soil physical properties. Furthermore, the application increased the amount of soil organic matter, including humic acid with a low degree of darkening and fulvic acid, and contributed to C sequestration and storage. Physical fractionation of soil indicated that about 60% of soil organic C was distributed in the silt-sized (2 - 20 μm) aggregate and clay-sized (<2 μm) aggregate fractions, while about 30% existed in the decayed plant fractions (53 - 2000 μm). The results obtained unambiguously indicate that long-term application of compost can improve soil qualities in the field subjected to double cropping, depending on the amount applied.

Keywords

Beneficial Effect, Double Cropping Soil, Long-Term Compost Application, Soil Quality

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1. Introduction

Organic amendments applied into soil include compost, farmyard manure, plant residues, food processing wastes, and sewage sludge. A great number of studies have shown the beneficial effects of these amendments on various properties of soils and crop yields in upland and paddy fields (e.g., [1]-[5]), although the degree of benefit is influenced by many factors such as amendment and soil types, soil managements, and environmental conditions. Double cropping (paddy and upland crops) system is a useful practice for an efficient land use. However, the effects of organic amendments on soil qualities in a field subjected to double cropping have received little attention. Furthermore, the distribution and quality of the amendment-derived organic matter (OM) in the particle size fraction of double cropping soil have not been studied, although the amendments incorporated into the soils become small in size with the progression of transformation and decomposition by abiotic and biotic agents. Thus, Shindo and his co-authors initiated a series of studies on the influence of compost application into a field subjected to double cropping (paddy rice and barley) from the perspective of soil science. In their studies, the changes of enzyme activities [6], formation of microbial biomass [6], water-stable macroaggregation [7], N composition [6] [8] [9], and the humus composition in the double cropping soils [8] [10] [11] as influenced by long-term compost application were investigated, using whole soils or their particle size fractions as analytical samples.

The aim of this article was to assess the influence of long-term application of compost on the physical, chemical, and biological properties, as well as the fertility, of soil in a field subjected to double cropping, mainly by integrating previous studies of the influence of continuous compost or manure application on soil qualities.

2. Materials and Methods

2.1. Field Experiment

The field experiments with different types of management were established in 1975 at Yamaguchi Prefecture Agricultural Experimental Station, Japan (34˚14'N, 131˚4'E). Yamaguchi is most west Prefecture of Honshu island. Yamaguchi City has a humid subtropical climate: mean annual temperature is ca. 15˚C and mean annual precipitation ca. 1800 mm. The soil at this field site was classified as Gray Lowland soil (Fluvisols). Each plot of the field experiment was 200 m^2, and the treatment of plot was carried out without replications. For the comparison of potential role of compost application, three plots were selected: (i) plot F, only chemical fertilizers containing N, P, and K were applied; (ii) plot F + LC, chemical fertilizers plus a low level of compost were applied; (iii) plot F + HC, chemical fertilizers plus a high level of compost were applied. The same plots were used as paddy fields for Japanese rice (e.g., Koshihikari) in summer and as upland fields for barley in winter until June 2001. The application rate of N, P_2O_5, and K_2O for each crop was 100 kg·ha^{-1}, respectively (N: 200 kg·ha^{-1}·y^{-1}; P_2O_5: 200 kg·ha^{-1}·y^{-1}; K_2O: 200 kg·ha^{-1}·y^{-1}). After harvest (June and November) of each crop, rice straw-cow dung compost (the mixture of rice straw and cow dung in the ratio of 7 to 3 was stacked in a composting room for 6 months with mixing intermittently) was applied at the rates of 5 Mg·ha^{-1} (10 Mg·ha^{-1}·y^{-1}) for the low level and 15 Mg·ha^{-1} (30 Mg·ha^{-1}·y^{-1}) for the high level. However, since June 2001 (26 years after the start of field experiment), these plots were used only as paddy fields and consequently the amounts of chemical fertilizers and compost applied were reduced by half. Water irrigation and weed control were performed conventionally.

To obtain an average soil sample in each plot, soils were taken from the plow layer (0 - 15 cm) of five sites across each of the three plots and mixed well. In many experiments, the soils were air-dried, gently crushed, passed through a 2-mm mesh sieve, and then employed for analytical determinations.

As described later, the long-term application of chemical fertilizer and compost into the plots increased organic C (OC) and total N (TN) contents of whole soil. The OC and TN contents (g·kg^{-1}) increased from 13.1 and 1.20 before the start of the field experiment to 17.6 and 1.56 for plot F, 22.1 and 2.18 for plot F + LC, and 30.5 and 2.84 for F + HC, respectively, in April 2007 (32 years after the start of the field experiment) [12].

2.2. Enzyme Activity and Chemical Property of Soil

Soils were taken from plots F, F + LC, and F + HC in October 1994 (19 years after the start of field experiment) [6]. Moist soil samples (<2 mm, sieved without air-drying) were used for the determination of enzyme activities and microbial biomass N. The activities of organic C-decomposing enzymes (α-glucosidase, β-glucosidase,

$α$-galactosidase, and $β$-galactosidase), organic N-decomposing enzymes (protease, $β$-acetylglucosaminidase, and adenosine deaminase), and organic P-decomposing enzymes (phosphomonoesterase [pH 6.5], phosphomonoesterase [pH 11], and phosphodiesterase) were determined according to the methods described previously [13]-[17]. On the other hand, OC, TN, and cation exchange capacity (CEC) were analyzed according to the method described elsewhere [18], using air-dried soil sample (<2 mm). The relationships between the enzyme activities and the chemical properties of soils were examined by statistics analysis (correlation coefficient and t-test).

2.3. Organic N Analysis

The amount of microbial biomass N in the moist soils described above was determined according to the chloroform fumigation-extraction method reported by Brookes *et al.* [19].

The amount of phosphate-extractable organic N (PEON) in the soils, which were taken from plots F and F + HC in June 2000 (25 years after the start of field experiment), was determined [8]. This extraction method, using neutral 1/15 mol·L^{-1} phosphate buffer as an extractant, was proposed as a useful index for estimating the amount of available N in Japanese paddy soils [20].

The amount of hydrolyzable amino acid-N in the soils, which were taken from plots F and F + LC, and F + HC in October 2008 (33 years after the start of field experiment), was determined as described elsewhere (e.g., [9]).

2.4. Water-Stable Soil Aggregation

Soils were taken from plots F, F + LC, and F + HC in November 1996 (21 years after the start of field experiment) [7]. Part of moist soil was used to determine the hyphal length after sieving with a 2-mm mesh sieve. The remaining part was air-dried, gently crushed, and then sieved with a 4-mm mesh sieve, followed by a 2-mm mesh sieve. These sieved samples (2-4 mm in diameter) were used for the determination of soil aggregation and other analytical determinations.

In this study [7], the aggregate stability index was represented by the degree of aggregation. The soil samples were sieved by the wet sieving method [21] [22], using the apparatus designed by Daiki Rika Kogyo Co. Ltd., Tokyo, Japan. The details of the sieving procedure were described previously by Ibrahim *et al.* [23]. The degree of aggregation is an expression of the degree to which small particles are aggregated. The aggregation degree was estimated from the differences of mass weights of aggregate fractions obtained before and after the digestion of OM with H_2O_2 [23], Furthermore, relationships between the degree of macroaggregation (>0.25 mm) and the contents of OC, TN, hydrolyzable carbohydrates, and dithionite-citrate-bicarbonate (DCB)-soluble Al and Fe, or hyphal length were examined by statistics analysis (correlation coefficient and t-test).

2.5. Humus Composition

2.5.1. Whole Soil

Soils were taken from plots F, F + LC, or F + HC in November 1996 (21 years after the start of the field experiment), June 2000 (25 years), and April 2007 (32 years) [8] [10] [11]. Air-dried fine soil samples (<70 mesh) were used for humus composition analysis (as described later).

2.5.2. Particle Size Fraction

Physical fractionation of soil (collected in April 2007) was carried out as described elsewhere (e.g., [11] [12]). After mechanically shaking the mixture of soil, glass beads, and water, the suspension obtained was divided into five size fractions: *i.e.*, coarse sand-sized aggregate (CSA, 212 - 2000 μm) and medium sand-sized aggregate (MSA, 53 - 212 μm) fractions were separated successively by sieving, and then clay sized-aggregate (CLA, <2 μm), silt-sized aggregate (SIA, 2 - 20 μm), and fine sand-sized aggregate (FSA, 20-53 μm) fractions, in this order, were recovered by sedimentation. Subsequently, the CSA and MSA fractions were subdivided into "mineral particles" (MP) and "decayed plants" (DP) by a density fractionation (decantation) in water. These 7 fractions obtained were freeze-dried and used for the analysis of humus composition. The reason why the fraction obtained was referred to as aggregate fraction is based on the following finding: the differences in the distribution patterns of particle size fractions obtained before (this study) and after digestion of OM with H_2O_2 [11] [12] in-

dicated that the soils used contained organic-inorganic associations which are resistant to physical disruption under study. This finding indicates that the distribution pattern of soil fraction is influenced by fractionation procedure. We employed a mild dispersion method (shaking, wet sieving using 53- and 212-μm mesh sieves, and sedimentation).

2.5.3. Humus Composition Analysis

Traditionally, humus has been divided into humic acid (HA, alkali soluble and acid insoluble), fulvic acid (FA, alkali soluble and acid soluble), and humin (alkali insoluble). Humus composition in whole soil and its particle size fraction was principally analyzed according to the method described in Kumada [24]. The OM in whole soil was extracted with 0.1 mol·L^{-1} NaOH at 100°C for 30 min. After NaOH extraction, the OM remaining in the soil residues was extracted with 0.1 mol·L^{-1} Na$_4$P$_2$O$_7$. The NaOH and Na$_4$P$_2$O$_7$ extracts were divided into HA and FA by acidification. Since the amounts of HA and FA in the Na$_4$P2O7 extract were much smaller than those in the NaOH extract, the OM in the particle size fraction was extracted with the mixture of 0.1 mol·L^{-1} NaOH + 0.05 mol·L^{-1} Na$_4$P$_2$O$_7$ (1:1) at 100°C for 30 min, and then the extract was divided into HA and FA [11].

The amounts of total OM (TOM, sum of HA, FA, and humin), HA, and FA in whole soil and its particle size fraction were determined using the KMnO$_4$ oxidation method [24]. In this paper, one ml of 0.02 mol·L^{-1} KMnO$_4$ consumed was calculated as corresponding to 0.48 mg C [25]. The degree of humification (darkening) of HA was determined using optical properties: *i.e.*, color coefficient (Δ log K) and relative color intensity (*RF*) values, where the Δ log K is the logarithm of the ratio of the absorbance of HA solution at 400 nm to that at 600 nm; the *RF* represents the absorbance of HA solution at 600 nm multiplied by 1000 and then divided by the number of milliliters of 0.02 mol·L^{-1} KMnO$_4$ consumed by 30 mL of HA solution.

2.5.4. ^{13}C-Nuclear Magnetic Resonance (NMR) Analysis

Soils were collected from plots F and F + HC in June 2000 (25 years after the start of field experiment) [8]. Soil HA and FA were extracted at 25°C for 48 h in 0.1 mol·L^{-1} NaOH + 0.1 mol·L^{-1} Na$_4$P$_2$O$_7$ (1:1) and then purified using a modified procedure of the preparation method described in Ikeya and Watanabe [25].

^{13}C-NMR spectra of HA and FA were obtained at a ^{13}C resonance frequency of 75.45 MHZ on a JNM-alpha 300 solid NMR system (JEOL, Tokyo, Japan) using solid-state cross-polarization magic-angle spinning (CP-MAS) and total suppression of spinning side bands (TOSS) techniques for eliminating sidebands [8]. The other operating conditions were described previously [8]. In this study, the C species were divided into four groups, namely, alkyl-C (0 - 45 ppm), *O*-alkyl-C (45 - 108 ppm), aromatic-C (108 - 163 ppm), and carbonyl-C (163 - 220 ppm).

3. Results and Discussion

3.1. Enzyme Activity

Soil enzymes are mainly microbial origins, and they play an important role in the transformation and decomposition of organic components involving C, N, or P in soils (e.g., [15]). A number of studies have shown that the application of organic amendments can increase the activities of enzymes in soils. For example, according to Kanazawa [5] [26] continuous application of manure or compost into various paddy soils in Japan increased the enzyme activities such as *β*-glucosidase, cellulase, protease, *β*-glucosaminidase, L-glutaminase, and L-asparaginase. Sato and Omura [27] found that continuous compost application increased the activities of protease, adenosine deaminase, and *β*-acetylglucosaminidase in the soil of an Andosol paddy field. On the other hand, Omura *et al.* [28] reported that continuous compost application into a greenhouse field increased the activities of hydrolytic enzymes such as protease, L-glutaminase, and L-asparaginase. Chang *et al.* [29] described that compost application increased enzyme activities, such as protease and *β*-glucosidase, in greenhouse cultivation soils, although the degrees of increase were influenced by the quantity of compost applied. Shindo [30] found that continuous compost application increased the activities of protease, *β*-acetylglucosaminidase, and adenosine deaminase in the upland soils differing in parent materials and that protease and *β*-acetylglucosaminidase activities were correlated significantly with the amount of N mineralized during the incubation of their soils. However, enzyme activities in double cropping soils have received little attention. Thus, Shindo and Shojaku [6] investigated the effect of compost application on the activities of organic C-, N-, and P-decomposing enzymes of soil

in a field subjected to double cropping (19 years after the start of field experiment). Furthermore, the relationships between the enzyme activities and the chemical properties or microbial biomass of soil were examined.

The activities of organic C-decomposing enzymes (α-glucosidase, β-glucosidase, α-galactosidase, and β-galactosidase) in the soils of plots F, F + LC, and F + HC ranged from 2.4 to 14.9 U (μmol·min^{-1}·kg^{-1} dried soil) (**Table 1**). On the other hand, the activities of organic N-decomposing enzymes (protease, β-acetylglucosaminidase, and adenosine deaminase) and organic P-decomposing enzymes (phosphomonoesterase [pH 6.5], phosphomonoesterase [pH 11], and phosphodiesterase) varied from 3.4 to 21.7 U and from 4.6 to 61.8 U, respectively (**Table 1**). All the activities of organic C-, N-, and P-decomposing enzymes increased remarkably in the order: plots F < F + LC < F + HC. Furthermore, in most enzymes, their activities were correlated significantly ($r \geq 0.950$, $p < 0.05$) with the contents of OC, TN, and microbial biomass N or CEC value (**Table 2**). These findings unambiguously indicate that the degrees of increase in those enzyme activities are influenced by the amount of compost applied as well as the enzyme types. Also, it appears that the compost application contributes to the improvement of soil qualities in the double cropping field.

Chang *et al.* [29] reported that compost application increased microbial (bacteria, actinomycetes, and fungi) populations and enzyme activities, such as protease and β-glucosidase, in soils of greenhouse cultivation, although the populations and activities were influenced by the amount of compost applied. As described above, the compost application at the high level (plot F + HC) increased largely the activities of organic C-, N-, and P-decomposing enzymes, contents of OC, TN, and microbial biomass N, and the CEC value. The reasons are proposed as follows: (i) the growth of paddy rice and barley is accelerated to a larger extent in plot F + HC than in plots F and F + LC, due to the larger amount of compost application; (ii) as a result, larger amounts of plant remains such as root and stubble are left in plot F + HC than in the other plots; (iii) those remains and compost serve as energy and nutrient of soil microorganisms and, simultaneously, contribute to the maintenance and improvement of soil qualities, thereby increasing the amount, population, and species of microorganisms, and thus (iv) larger amounts of organic C-, N-, and P-decomposing enzymes are produced and released in plot F + HC, compared with the other plots.

Table 1. Enzyme activities of soils.

Enzyme	Plot F[a]	Plot F + LC[b]	Plot F + HC[c]
	U (μmol·min^{-1}·kg^{-1} dried soil)		
Organic C-decomposing enzyme			
α-Glucosidase	2.39 (0.12)[*]	2.44 (0.44)	3.07 (0.08)
β-Glucosidase	10.7 (0.55)	11.6 (0.40)	14.9 (0.25)
α-Galactosidase	3.31 (0.09)	3.85 (0.03)	4.27 (0.26)
β-Galactosidase	4.49 (0.13)	5.16 (0.04)	6.47 (0.44)
Organic N-decomposing enzyme			
Protease	5.87 (1.35)	9.48 (2.00)	15.0 (1.70)
β-Acetylglucosaminidase	3.44 (0.14)	4.07 (0.22)	6.38 (0.68)
Adenosine deaminase	11.1 (0.49)	14.9 (0.55)	21.7 (1.62)
Organic P-decomposing enzyme			
Phosphomonoesterase (pH 6.5)	43.3 (2.70)	48.6 (2.12)	61.8 (5.21)
Phosphomonoesterase (pH 11)	6.88 (0.31)	9.99 (0.47)	15.3 (0.24)
Phosphodiesterase	4.56 (0.11)	6.05 (0.31)	12.2 (0.39)

a. Plot F: Only chemical fertilizers containing N, P, and K were applied; b. Plot F + LC: Chemical fertilizers plus compost at low level (10 Mg·ha^{-1}·y^{-1}) were applied; c. Plot F + HC: Chemical fertilizers plus compost at high level (30 Mg·ha^{-1}·y^{-1}) were applied. *Standard deviation (n = 4).

3.2. Nitrogen Fertility

Nitrogen is an essential element for plants. In the surface layer of most soils, over 90% of N occurs in organic forms. It is well known that hydrolyzable N is the major source of mineralizable N in soils, and this hydrolyzable N contains protein-like N compounds (e.g., [31]). Continuous manure application could increase significantly the amount of amino acid-N in the hydrolyzable fractions of soils (e.g., [32]). Similar results were obtained for the double cropping soils (33 years after the start of field experiment) studied (**Table 3**) [9]. The amount of PEON, containing protein-like N compounds, is employed as a useful index for estimating the amount of available N in Japanese paddy soils [20]. According to Shindo *et al.* [8], in the soils taken from plots F and F + HC (25 years after the start of field experiment), the amounts of PEON and TN in plot F + HC were 1.2 and 1.7 times larger than those in plot F, respectively (**Table 3**).

It is accepted that microorganisms are a transformation agent of OM as well as a labile reservoir of nutrients such as N, P, S, and C in soils (e.g., [33] [34]). Microbial bodies are formed and then died may accumulate in soils. Predation upon soil microorganisms may release most of the immobilized C, N and P, which would otherwise be unavailable to crop plants. Matsumoto *et al.* [35] suggested that protein-like N compounds in the PEON fraction, derived from the remains of microorganisms, were adsorbed on surfaces of soil colloids and became a source of mineralizable N in soils. Mineralization of organic N in soils proceeds with the mediation of organic N-decomposing enzymes. As described earlier, compost application increased the amount of microbial biomass N as well as the activities of organic N-decomposing enzymes in the soils of plot F + LC and, especially, plot F + HC (**Table 1** and **Table 3**). Based on these findings, it is concluded that the compost application, especially at the high level, is effective for the maintenance and enhancement of N fertility in the double cropping soils studied.

Table 2. Correlation matrix of enzyme activities, contents of organic C, total N, and microbial biomass N, and cation exchange capacity (CEC)[a].

	Phospho-diesterase	Phosphomonoesterase		Adenosinedea-minase	β-Acetylglucosam inidase	Protease	Galactosidase	
		(pH11)	(pH6.5)				β-	α-
Organic C	0.966	0.997	0.986	0.996	0.970	0.999	0.994	0.989
Total N	0.988	0.999	0.998	1.000	0.991	0.998	1.000	0.967
Microbial biomass N	0.967	0.998	0.987	0.997	0.972	0.999	0.995	0.988
CEC	0.970	0.999	0.989	0.998	0.975	1.000	0.996	0.986

	Glucosidase		CEC	Microbial biomass N	Total N	Organic C
	β-	α-				
Organic C	0.970	0.928	1.000	1.000	0.994	1.000
Total N	0.991	0.963	0.996	0.995	1.000	
Microbial biomass N	0.972	0.930	1.000	1.000		
CEC	0.975	0.935	1.000			

a. Correlation coefficients of 0.950 or higher indicate $p < 0.05$.

Table 3. N composition of soils.

Plot[a]	Total N[b]	Phosphate-extractable organic N[b]	Microbial biomass N[c]	Hydrolyzable amino acid-N[d]
	(mg N kg^{-1} dried soil)			
F	1514	37.8	49.4	406
F + HC	2529	44.9	79.0	759

a. See **Table 1**; b. Data from Shindo *et al.* [8]; c. Data from Shindo and Shojaku [6]; d. Data from Nguyen and Shindo [9].

3.3. Water-Stable Soil Aggregation

Water-stable aggregates are essential for the maintenance of a good structure for plant growth. These aggregates are divided into microaggregates (<0.25 mm) and macroaggregates (>0.25 mm). Microaggregates show a relatively high stability against physical disruption [36], whereas macroaggregates are sensitive to soil management [37].

The long-term incorporation of organic amendments such as straw increased the amount of 1 - 20 mm aggregates as well as the OM content in a loamy sandy soil [38]. Angers and N'Dayegamiye [39] showed that long-term application of manure increased the contents of C, N, and total carbohydrates of the whole soil and of the particle size fractions. Furthermore, it was reported that OC, TN, carbohydrates, DCB-soluble Al, and hyphae affect the stabilization of aggregates mainly based on the correlation with soil aggregation [23] [40]-[43]. In most of these studies, however, the role of only a few aggregate-forming factors was compared at the same time. Furthermore, the effect of continuous compost application on soil aggregation and its related factors in the fields subjected to double cropping remains to be studied. Thus, the influence of compost application on soil macroaggregation and the factors involved in the macroaggregation were investigated using the soil samples taken from plots F, F + LC, and F + HC (21 years after the start of field experiment) [7].

The average recovery of water-stable aggregates (>2.0 mm, 1.0 - 2.0 mm, 0.5 - 1.0 mm, 0.25 - 0.5 mm, 0.1 - 0.25 mm, and <0.1 mm) by duplicate wet sieving ranged from 99 to 100% in all the soils of plots F, F + LC, and F + HC. Thus, the degree of aggregation in five size fractions (>2.0 mm, >1.0 mm, >0.5 mm, >0.25 mm, and >0.1 mm) was determined. In all the size fractions, the degree of aggregation increased in the order: plots F < F + LC < F + HC. The degree was 1.8 to 13 times higher in plot F + HC, compared with plot F. The degree of macroaggregation (>0.25 mm) was 35% for plot F, 43% for plot F + LC, and 62% for F + HC, indicating that the compost application at the high level promoted largely macroaggregation.

It is assumed that a complex mechanism may be involved in the formation as well as the increase of the amount of water-stable macroaggregates. To gain a fundamental understanding about the mechanism in the double cropping soils, the relationships between the degrees of macroaggregation and several properties of soils were examined. The degree of macroaggregation showed significant correlations ($r \geq 0.950$, $p < 0.05$) with the contents of OC, TN, hydrolyzable carbohydrates, and DCB-soluble Al (Table 4). Furthermore, these contents were significantly intercorrelated ($r \geq 0.950$, $p < 0.05$) among themselves. As described later, continuous compost application largely increased the amounts of fulvic acids (FAs) and, especially, humic acids (HAs) in the soils of experimental plots. The increase in the amounts of negatively charged organic constituents such as hydrolyzable carbohydrates (involving polysaccharides), HAs, and FAs and of positively charged DCB-soluble (active) Al which are capable of binding soil particles into secondary organomineral complexes, *i.e.*, water-stable aggregates, may promote macroaggregation. On the other hand, Oades [44] reported that a higher degree of macroaggregation was provided by fungal hyphae through the physical enmeshment of soil particles. N'Dayegamiye and Angers [45] observed that the application of manure to soil increased the fungal population. Ibrahim and Shindo [46] found that when the soil amended with rice straw was incubated at 30℃ under the moist conditions, the

Table 4. Correlation matrix of degree of macroaggregation, contents of organic C, total N, dithionite-citrate-bicarbonate (DCB)-soluble Al and Fe, and hydrolyzable carbohydrates, and hyphal lengtha.

	Degree of macroaggregation	Organic C	Total N	DCB-soluble		Hydrolyzable carbohydrate	Hyphal length
				Al₂O₃	Fe₂O₃		
Hyphal length	0.934	0.953	0.931	0.978	−0.801	0.935	1.000
Hydrolyzable carbohydrate	1.000	0.998	1.000	0.988	−0.962	1.000	
DCB-soluble Fe₂O₃	−0.962	−0.944	−0.965	−0.909	1.000		
DCB-soluble Al₂O₃	0.989	0.995	0.986	1.000			
Total N	1.000	0.998	1.000				
Organic C	0.998	1.000					

a. Correlation coefficients of 0.950 or higher indicate $p < 0.05$.

amount of the >2.0 mm macroaggregates remarkably increased, accompanying with the increases of hyphal length and of the amount of microbial biomass C. Furthermore, the compost application at the high level in our studies (plot F + HC) markedly increased the hyphal length (264 km kg^{-1} dried soil), compared with plots F (197 km) and F + LC (192 km) [7]. Based on the results obtained, in the fields subjected to long-term double cropping under study, it appears that non-humic (polysaccharides) and humic substances (HAs and FAs), active Al, and hyphae contribute together to the formation as well as the increase of the amount of water-stable macroaggregates. Continuous application of compost, especially at the high level, enhanced the role of those factors in the improvement of the soil structure.

3.4. Humus Composition in Whole soil and Its Particle Size Fraction

Humus is an integral part of soil and affects various soil properties as well as global C cycle. It is well known that continuous application of organic amendments can affect the humus composition of paddy and upland soils (e.g., [24] [47]). However, the effect of compost application on the humus composition of double cropping soils remains to be studied.

It is easy to assume that organic amendments such as compost, manure, and plant remains incorporated into soils become small in size and the amounts of mineral particles adhering and/or being associated with them increase, with the progression of transformation and degradation by abiotic and biotic agents. According to Roppongi and Mundie [48], OM of recent plant origin is considered to be preferentially recovered in the sand-size fraction, whereas more microbially processed material can be found in the silt- and clay-size fractions.

Thus, we investigated the qualitative and quantitative changes of humus in the double cropping soils as influenced by continuous compost application, using whole soils taken from plots F, F + LC, and F + HC and their particle size fractions [8] [10] [11].

Long-term compost application (21 years after the start of the field experiment) increased remarkably the contents of TOM, HA, and FA in the whole soils in the order: plots F < F + LC < F + HC (**Table 5**) [10]. Humic acid content exceeded largely FA content in all the plots and, especially, plot F + HC. On the other hand, the degree of darkening of HA increased in the order: F + HC < F + LC < F. In other words, the HA content was the highest in plot F + HC, but the degree of darkening of HA was the lowest. The reasons are proposed as follows: (i) the growth of paddy rice and barley is accelerated to a larger extent in plot F + HC than in the other plots, due to the continuous compost application at the high level; (ii) as a result, larger amounts of plant remains such as root and stubble, which can be transformed into HA and FA, are left in plot F + HC than in the other plots; (iii) the degrees of darkening of HAs in the compost used [10] and plant remains decayed and degraded in soils [24] are very low; and thus (iv) continuous compost application at the high level contribute to the accumulation of larger amounts of TOM, HAs with a low degree of darkening, and FAs, although part of indigenous humus may be decomposed by soil microorganisms, presumably due to priming effect.

The effects of organic amendments on humus composition have also been studied for the soils of upland fields. For example, Roppongi et al. [49] reported that the incorporation of compost into an upland field (Fulvisols) induced an increase in the amounts of TOM, HA, and FA and a decrease in the darkening degree of HA. Similar results were obtained even when cattle manures were applied into upland fields of an Andosol [50] and a Cambisol [51]. Hirahara [52] found that long-term (55 years) compost application into an Andosol upland field decreased the δ13C values of FA and, especially, HA, the reduction being a larger at a higher level of compost application. These findings indicate that the accumulation of plant material-derived HAs with a low degree of darkening in soils is accelerated by continuous application of compost and manure.

Table 5. Humus composition of soils[a].

Plot[b]	Total organic matter	Humic acid	Fulvic acid	Humic acid	
	(g C kg^{-1} dried soil)			Δlog K[c]	RF[c]
F	10.2	4.29	3.69	0.690	44
F + LC	13.6	6.67	4.11	0.750	35
F + HC	22.7	12.1	5.42	0.827	27

a. Data from Shindo and Shimada [10]; b. See **Table 1**; c. Δlog K and RF values stand for color coefficient and relative color intensity, respectively.

^{13}C-NMR analysis can provide valuable information on the C form in complex macromolecules. Thus, Shindo *et al.* [8] characterized the C species of HAs and FAs, which were isolated from the soils of plots F and F + HC (25 years after the start of the field experiment), by their ^{13}C-NMR analyses (TOSS method) (**Figure 1**). In the ^{13}C-NMR spectra of all the HAs, peaks appeared at 30, 55, 74, 130, 150, and 173 ppm. On the other hand, in the ^{13}C-NMR spectra of all the FAs, peaks appeared at approximately 20, 75, 100, and 175 ppm. In this study, individual C types were not assigned from ^{13}C-NMR spectra because both HAs and FAs are heterogeneous polymers. The peaks at 55 and 150 ppm in the NMR spectra of the HAs, which show the presence of lignin-like compounds [53], were more intense for the HA with a lower degree of darkening in plot F + HC, compared with plot F. This finding suggests that part of lignin is transformed into HA with a low degree of darkening. To gain a better understanding of the C species in the HAs and FAs, the contents of individual C species (alkyl-C, *O*-alkyl-C, aromatic-C, and carbonyl-C) and the quantitative contribution of individual C species relative to total C in the HAs or FAs were estimated from the ^{13}C-NMR spectra and C contents of HA and FA (**Table 6**). Although the compost application increased the contents of all C species in the HAs and FAs, the quantitative contribution of individual C species relative to total C in the HAs or FAs did not differ largely between plots F and F + HC. However, there were distinct differences in the distribution of C species between the HAs and FAs. The percentage contribution of individual C species in the HAs of plots F and F + HC was the highest for aromatic-C (37% and 38%, respectively), generally followed by carbonyl-C (24% and 24%), alkyl-C (22% and 19%), and *O*-alkyl-C (17% and 19%). On the other hand, in the FAs of plots F and F + HC, the distribution was the highest for *O*-alkyl-C (48% and 45%, respectively), generally followed by carbonyl-C (27% and 24%), alkyl-C (12 and 17%), and aromatic-C (13% and 14%). The HAs were characterized by presence of large amount of aromatic-C and small amount of *O*-alkyl-C, and the inverse relationship was found for the FAs. The aromatic-C species may involve the decaying and degradation products of lignin, while *O*-alkyl-C may include plant and microbial carbohydrate residues.

The soils taken from the three plots (32 years after the start of field experiment) were divided physically into seven fractions as described earlier. In many particle size fractions, the amounts (g C kg^{-1} whole soil) of TOM, HA, and FA increased in the order: plots F < F + LC < F + HC [11]. This result indicates that the accumulation of TOM, HA, and FA in the particle size fractions of double cropping soils was influenced by the amounts of plant materials supplied, involving compost (as described earlier). Here, percentage distribution of the amounts of TOM, HA, and FA in each fraction relative to those in the whole soil was compared between plots F and F + HC (**Figure 2**). In both plots, 62% of TOM, 66% - 70% of HA, and 77% - 78% of FA was occupied in SIA (2 - 20 μm) and CLA (<2 μm) fractions. Most of the rest OM existed in CSA- and MSA-DP (212 - 2000 and 53 - 212 μm, respectively) fractions. The distribution values in CSA- and MSA-MP (212 - 2000 and 53 - 212 μm, respectively) and FSA (20 - 53 μm) fractions were low or very low.

Table 6. Contents and properties of individual C species in humic and fulvic acids[a].

Plot[b]	Total organic matter	humic acid	C species of humic acid			
			Alkyl-C	*O*-alkyl-C	Aromatic-C	Carbonyl-C
	– g C kg^{-1} dried soil –		g C kg^{-1} dried soil (% of humic acid)			
F	10.4	4.41	0.96 (21.7)	0.76 (17.3)	1.65 (37.4)	1.04 (23.6)
F + HC	25.6	12.5	2.33 (18.6)	2.34 (18.7)	4.84 (38.7)	3.00 (24.0)

Plot[b]	fulvic acid	C species of fulvic acid			
		Alkyl-C	*O*-alkyl-C	Aromatic-C	Carbonyl-C
	– g C kg^{-1} dried soil –	g C kg^{-1} dried soil (% of fulvic acid)			
F	4.22	0.51 (12.0)	2.01 (47.7)	0.56 (13.2)	1.14 (27.1)
F + HC	7.06	1.19 (16.9)	3.17 (44.9)	0.98 (13.9)	1.71 (24.2)

a. Data from Shindo *et al.* [8]; b. See **Table 1**.
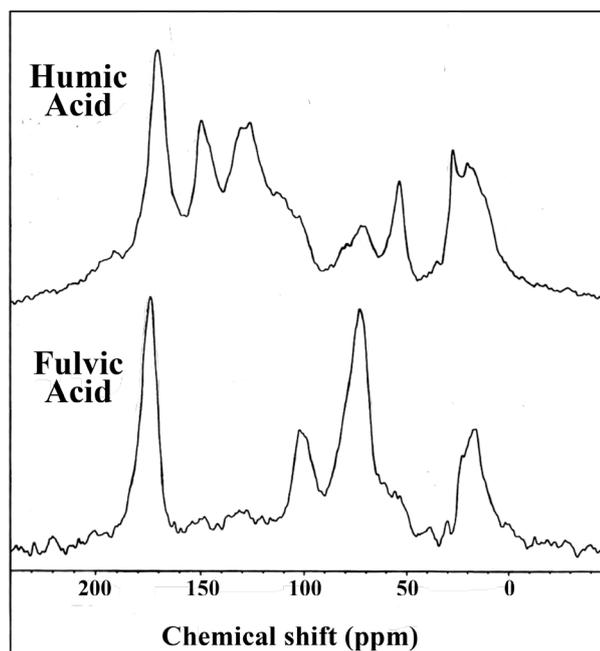
Figure 1. ^{13}C-NMR spectra of soil humic and fulvic acid in plot F + HC. The spectra were obtained by TOSS method. See **Table 1** for plot F + HC. Redrawn from the data of Shindo *et al.* [8].

Aoyama and Kumakura [50] reported that the application of large amounts of manure (up to 320 Mg·ha^{-1}·y^{-1}) into Kuriyagawa Andosol (upland field) for 20 years increased significantly the amount of particulate OM (>53 μm), but it did not affect the amount of mineral-associated OM (<53 μm). The increase of particulate OM was influenced largely by the amount of manure applied, and it was in the order of 80 < 160 < 320 Mg·ha^{-1}·y^{-1}. A similar result was also obtained for Hirosaki Andosol (upland field) [54]. However, in the double cropping fields described above, where compost was applied at the rates of 10 Mg·ha^{-1}·y^{-1} (plot F + LC) and 30 Mg·ha^{-1}·y^{-1} (plot F + HC) for about 30 years, the amount of TOM was much larger in the SIA and CIA fractions than in the other fractions. In Fujisaka Andosol (upland field) where compost was incorporated continuously at the rates of 11 and 34 Mg·ha^{-1}·y^{-1} for about 40 years [47], the distribution pattern of total OC in size fractions was similar to that of TOM in our studies. These findings may indicate that in the soils where organic amendments are continuously applied, the accumulation pattern of OM in the particle size fraction is influenced largely by the amounts of amendments applied.

The optical properties (Δ log *K* and *RF* values) of HAs in CSA-DP, MSA-DP, SIA, and CLA fractions were examined, since these fractions contained enough amounts of HAs to determine their properties. Kumada [24] proposed to classify soil HAs employing the optical properties. According to his classification system, the lower the Δ log *K* value and the higher the *RF* value, the higher the degree of darkening of HA. In both plots F and F + HC, the degree of darkening of HA was the highest in CLA, followed by SIA, MSA-DP, and CSA-DP fractions (**Figure 3**). Although the amounts of TOM and HA in the whole soil and SIA and CLA fractions were much larger in plot F + HC than in plot F [11], the degrees of darkening of HAs in both fractions were much lower in plot F + HC than in plot F (**Figure 3**). These results indicate that larger amounts of plant materials, which can be transformed into HA with a low degree of darkening, were supplied into plot F + HC than plot F.

In the physical fractionation method used, after CLA fraction was collected by sedimentation, SIA fraction was recovered. This means that free and fine decayed plant materials (a low density, not or hardly connected to mineral particles) may be recovered in CLA fraction. However, in plot F + HC, the amount of HA was much larger in SIA fraction than in CLA fraction (**Figure 2**), and the darkening degree of HA was much lower in SIA than in CIA (**Figure 3**). These results may indicate that in plot F + HC, larger amounts of decayed plant materials containing HA with a low degree of darkening were accumulated (presumably due to the complexation with mineral matrices) in SIA fraction than in CLA fraction. On the other hand, in plot F, the amount and dar-
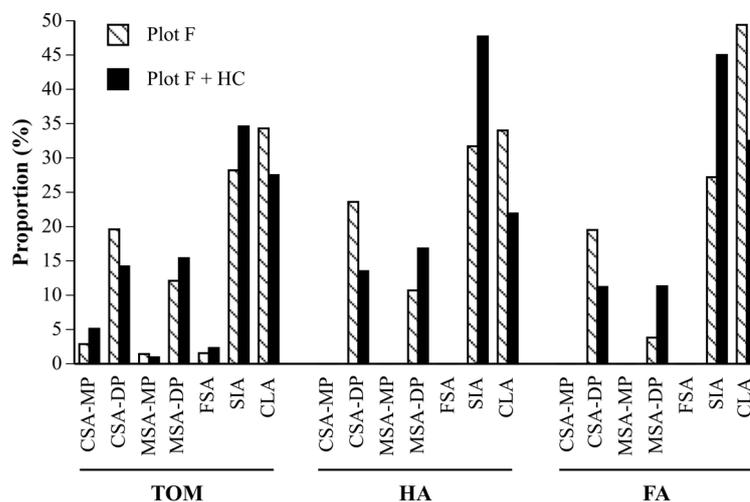
Figure 2. Proportions of total organic matter (TOM), humic acid (HA) and fulvic acid (FA) in particle size fractions relative to those in whole soils of plots F and F + HC. See Table 1 for plots F and F + HC. CSA, MSA, FSA, SIA, and CLA stand for coarse sand-sized (212 - 2000 μm) aggregate, medium-sand sized (53 - 212 μm) aggregate, fine-sand sized (20 - 53 μm) aggregate, silt-sized (2 - 20 μm) aggregate, and clay-sized (<2 μm) aggregate fractions, respectively. MP and DP stand for "mineral particles" and "decayed plants", respectively.
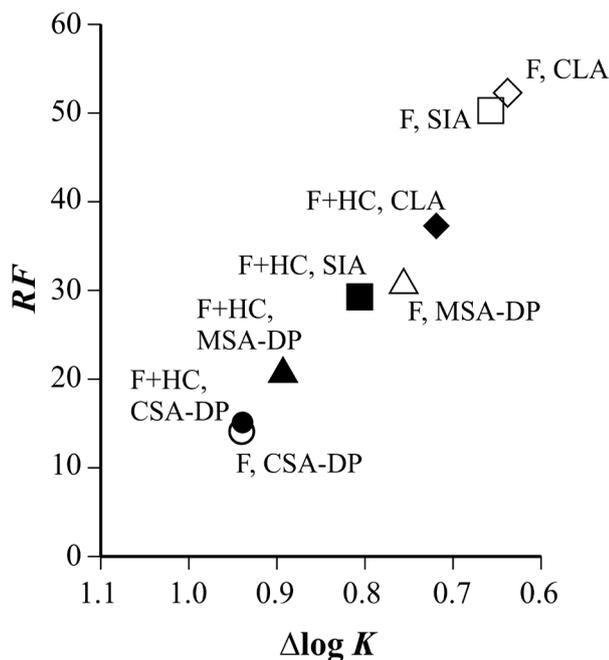
Figure 3. RF and $\Delta \log K$ values of humic acids in several particle size fractions. See Table 1 for plots F and F + HC and Figure 2 for CSA- and MSA-DP, SIA, and CLA, respectively.

kening degree of HA did not differ largely between SIA and CLA fractions (Figure 2 and Figure 3). This may be because smaller amounts of plant materials were supplied into plot F, compared with plot F + HC. The effects of continuous compost application on the quantitative and qualitative changes of humus were limited in plot F + LC, due to the low level of compost application.

4. Conclusion

Continuous compost application into the fields subjected to double cropping (paddy rice and barley) increased (i) the activities of organic C-, N-, and P-decomposing enzymes, (ii) the N fertility, (iii) the degree of water-stable soil macroaggregation, (iv) the contents of OC, TN, hydrolyzable carbohydrates and amino acid-N, microbial biomass N, and active Al as well as the hyphal length and CEC value. Furthermore, the application increased the amounts of TOM, HAs with a low darkening degree, and FAs, and affected the quantitative and qualitative changes of soil humus. Those beneficial effects on soil qualities, such as physical, chemical, and biological properties and fertility, were great in plot F + HC with compost application at the high level, depending on the amounts of compost applied.

Acknowledgements

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