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Assessment of Long-term Compost Application on Organic Nitrogen Composition of Whole Soils and Their Particle Size Fractions

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ABSTRACT

We assessed the effects of long-term compost application on organic N composition of whole soils and their particle size fractions in a field subjected mainly to double cropping. Soil samples were collected from two plots: (a) F plot, only chemical fertilizers; (b) F+C plot, chemical fertilizers plus compost. Soil was divided into five fractions, coarse sand-sized aggregate (CSA), medium sand-sized aggregate (MSA), fine sand-sized aggregate (FSA), silt-sized aggregate (SIA) and clay-sized aggregate (CLA) fractions. Then CSA, MSA and FSA fractions were subdivided into “decayed plants” (DP) and “mineral particles” (MP). The amounts of total N and different forms of organic N in the whole soil and size fractions were much larger in the F+C plot than in the F plot. In the whole soil, the percentage distribution of non-hydrolysable N to total N was markedly increased by compost application while the other forms of N were generally maintained or reduced. In the size fractions, the percentage distribution of total N and different organic forms of N in the CSA-DP, MSA-DP and FSA-DP fractions were increased by compost application while their distribution degree in the CSA-MP, MSA-MP, FSA-MP were less affected. The application induced an increase in distribution values of total N and amino sugar-N in the SIA fraction while the other forms of organic N tended to maintain or decline. In the CLA fraction, the distribution values of total N and forms of organic N were decreased by the application. However, the amounts and percentage distribution of total N and organic N forms were the highest in the CLA fraction. The findings indicate that in the F and F+C plots, CLA fraction merit close attention as an important reservoir of various organic N.

Keywords: forms of organic N, fractionation, paddy and upland fields, total N

Abbreviations: CLA, clay-sized aggregate; **CSA**, coarse sand-sized aggregate; **DP**, decayed plants; **FSA**, fine sand-sized aggregate; **MP**, mineral particles; **MSA**, medium sand-sized aggregate; **PB**, phosphate-borate; **SIA**, silt-sized aggregate

INTRODUCTION

In agricultural soils, organic amendments (including compost, farmyard manure, plant residues, food processing wastes and sewage sludge) is one of the most important sources of soil organic matter. Many researchers have reported beneficial effects of these amendments on the physical, chemical and biological properties and fertility of soils in upland or paddy fields. Furthermore, the effect of continuous compost application on several properties of soils in a field subjected to long-term double cropping (paddy rice and barley) was investigated: i.e., enzyme activities and microbiomass content (Shindo and Shojaku 1999), aggregation (Ibrahim and Shindo 1999) and humus composition (Shindo *et al.* 2006).

After compost application, the components of compost may be distributed into soil fractions with different particle size under the soil conditions. Physical fractionation of soil according to particle size, therefore, has been adopted to gain better understanding of the organic matter stabilization and turnover. For example, Tanaka and Shindo (2009) presented evidence that long-term compost application increased the amounts of C and N to larger degrees in the silt-sized aggregate fraction than in the other particle size fractions. Leinweber and Reuter (1992) found that compost had clearly greater effects than fresh farmyard manure and straw plus chemical fertilizers for the enrichment of organic C and N in the particle size fractions. Both C and N concentrations in the soil particles from 250 to 2,000 μm were reported to be greater with increasing management intensity (Dubeux *et al.* 2006). Hassink (1995) and Quiroga *et al.* (1996) concluded that input organic residues had greater effect on the amount of N in coarser fractions than that in finer fractions.

In the surface layer of most soils, over 90% of the N occurs in organic forms and most of the remainder is present as NH_4^+ which is held by clay minerals (Stevenson 1982). The forms of soil organic N can be divided into two broad categories: 1) organic residues and 2) soil organic matter or humus. The former consists of un-decayed plant and animal residues and partial decomposition products. The latter includes humic substances and non-humic substances such as amino acids, carbohydrates, nucleic acids, etc. All these materials play key roles in terms of maintaining or improving soil fertility and plant nutrition through the direct and indirect effects on microbial activity and nutrient availability (Kelley and Stevenson 1995).

Schnitzer and Ivarson (1982) found that the amounts of different forms of N varied with particle size fractions in two soils. Schulten and Leinweber (1991) reported that the amounts of N compounds increased with decreasing particle size and that the fine- and medium-clay and the fine- and medium-silt fractions of farmyard manure soil were enriched in amino-N and amide-N. According to Xu *et al.* (2003), in a plot where only mineral fertilizer was applied, most of N remaining in soils distributed in the particle size fraction of less than 2 μm, while in the plot received mineral fertilizer plus organic manure, most of the N remaining was transferred into amino sugar-N in every size fractions studied and amino acid-N in the size fraction of larger than 2 μm. However, the effects of compost application on the organic N composition in various particle size fractions of soils need to be further studied. The objective of this present study was to assess the effects of long-term compost ap-

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Table 1 Percentage distribution of mass weight in particle size fractions.

Plot	CSA[c]			MSA[d]			FSA[e]			SIA[f]	CLA[g]	Total
	MP[h]	DP[i]	Sum	MP	DP	Sum	MP	DP	Sum			
							(%)					
F[a]	30.9	0.77	31.7	20.0	0.95	21.0	16.3	1.14	17.4	18.7	11.2	100
F+C[b]	32.6	1.73	34.3	20.4	2.40	22.8	14.2	3.24	17.4	17.0	8.49	100

[a] Only chemical fertilizers were applied
[b] Chemical fertilizers plus compost were applied
[c] Coarse sand-sized aggregate fraction
[d] Medium sand-sized aggregate fraction
[e] Fine sand-sized aggregate fraction
[f] Silt-sized aggregate fraction
[g] Clay-sized aggregate fraction
[h] Mineral particles
[i] Decayed plants

plication on the contents and distribution of different forms of organic N in whole soils and their particle size fractions in a field subjected mainly to double cropping (paddy rice and barley).

MATERIALS AND METHODS

Field experiment

The field experiments with different types of management were established in 1975 at Yamaguchi Prefecture Agricultural Experimental Station, Yamaguchi, Japan. The soil at this site was classified as Gray Lowland soil (FAO-UNESCO: Eutric Fluvisol). From the field experiments, we selected two plots (200 m^2 each): (a) F plot, only chemical fertilizers containing N, P and K were applied; (b) F+C plot, chemical fertilizers plus compost were applied. The same plots were used as paddy fields for rice 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}. After harvest (June and November), rice straw-cow dung compost was applied at the level of 15 Mg ha^{-1}. However, since June 2001, these plots were used only as paddy fields and the amounts of chemical fertilizers and compost applied were reduced by half. In October 2008, 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 two plots and mixed well. The soils were air-dried, gently crushed, and then passed through a 2-mm mesh sieve. These sieved samples were used for analytical determinations and physical fractionation.

Particle size fractionation

The size fractionation of soil samples was conducted by physical fractionation described in Tanaka and Shindo (2009), except that the particle size fraction of 20-53 μm was recovered by sieving. Firstly, the samples were divided into five particle size fractions, namely coarse sand-sized aggregate (212-2,000 μm, CSA), medium sand-sized aggregate (53-212 μm, MSA), fine sand-sized aggregate (20-53 μm, FSA), silt-sized aggregate (2-20 μm, SIA) and clay-sized aggregate (< 2 μm, CLA) fractions. Three sand-sized fractions were separated by sieving and silt-sized and clay-sized fractions were separated by sedimentation. Secondly, the CSA, MSA and FSA fractions were subdivided into "mineral particles" (MP) and "decayed plants" (DP) by a density fractionation (decantation) in water. Each sand-sized fraction on the sieves was transferred into a beaker by adding de-ionized water and then gently mixed. After standing for 1 min, the DP fraction was separated.

All the fractions including DP and MP were freeze-dried (TAITEC, FREEZE DRYER VD-15) and weighed.

Forms of organic N

Organic N composition was analysed according to the method described in Yonebayashi and Hattori (1980), who partly modified the N fractionation method proposed by Bremner (1965). Soil sample containing about 10 mg N was hydrolysed with 20 mL of 6 mol L^{-1} HCl and octyl alcohol for 24 h at 150°C. The hydrolysate obtained was made up to a volume of 100 mL after filtering under suction and neutralizing (pH from 6.4 to 6.6) by NaOH. A scheme for the fractional determination of soil organic N is shown in **Fig. 1**.

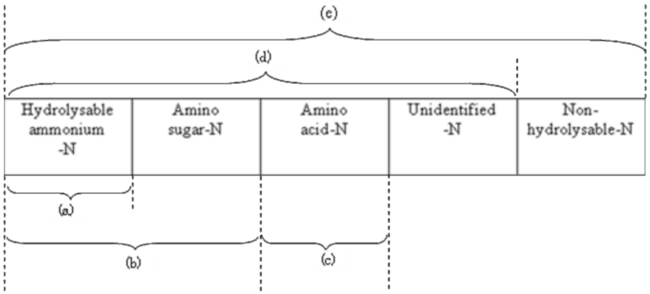

Fig. 1 Scheme for fractional determination of soil organic N.

The amount of hydrolysable ammonium-N (a) was estimated by distilling hydrolysate with MgO. The sum amounts (b) of hydrolysable ammonium-N and amino sugar-N were determined by distilling hydrolysate with phosphate-borate (PB) buffer. Amino sugar-N was calculated by subtraction of (a) from (b).

In the determination of amino acid-N (c), the hydrolysate was heated at 100°C in the presence of 0.5 mol L^{-1} NaOH and ninhydrin powder and then was distilled with PB buffer.

Total N (e) and total hydrolysable-N (d) were determined by Kjeldahl procedure recommended by Bremner and Mulvaney (1982). The unidentified-N and non-hydrolysable-N contents were calculated as following formulations:

Unidentified-N = (d) – (c) – (b).
Non-hydrolysable-N = (e) – (d).

The amounts of different forms of organic N were analysed in duplicate at least and the average values obtained were given in tables.

RESULTS AND DISCUSSION

Percentage distribution of mass weight in particle size fractions

The recovery of mass weight by physical fractionation was 99.5% for the F+C plot and 101% for the F plot. Thus, the percentage distribution of mass weight in the particle size fractions was corrected to a total of 100% (**Table 1**). The distribution of mass weight in the CSA, MSA, FSA, SIA and CLA fractions ranged from 11.2 to 31.7% in the F plot and from 8.49 to 34.3% in the F+C plot. The distribution of mass weight in the particle size fractions was not greatly affected by compost application. As expected, in the sand fractions of both plots, the distribution values of the MP were much larger than those of the DP.

It is well known that the digestion of soil samples by hot H_2O_2 can decompose soil organic matter. Comparison of percentage distribution of mass weight in the particle size fractions before and after H_2O_2 treatment revealed that the distribution values in the 2-20 μm and 210-2,000 μm fractions were higher in the former system than in the latter system, and the reverse was true for the fractions of less than 2 μm and 20-210 μm (data was not shown). These findings

Table 2 Amounts of total N and different forms of organic N in whole soils.

Plot[a]	Total N	Hydrolysable ammonium-N	Amino acid-N	Amino sugar-N	Unidentified-N	Non-hydrolysable-N
			(mg kg^{-1})			
F	1556	237	352	191	588	188
F+C	2836	318	628	368	659	863

[a] See **Table 1**

Table 3 Percentage distribution of different forms of organic N to total N of whole soils.

Plot[a]	Total N	Hydrolysable ammonium-N	Amino acid-N	Amino sugar-N	Unidentified-N	Non-hydrolysable-N
			(%)			
F	100	15.2	22.6	12.3	37.8	12.1
F+C	100	11.2	22.1	13.0	23.2	30.4

[a] See **Table 1**

indicate that highly recalcitrant organic-inorganic complexes, which are resistant to water-sieving, exist in the soils studied. Thus, in the present study, particle size fractions obtained were designated as aggregate fractions. The formation of the complexes might be promoted, when the plots were changed from paddy field to upland field, due to the directly and indirectly effects of wet-dry cycles on aggregate stabilization as well as soil organism's activities.

Whole soils

1. Total N of whole soils

Table 2 shows the amounts of total N in the whole soils. The amount of total N in the F+C plot was 1.8 times larger than that in the F plot. This result confirms the changes reported by Tanaka and Shindo (2009) that long-term compost application induced an increase in the amount of total N of soil subjected mainly to double cropping. This is also in accordance with the results obtained by Xu *et al.* (2003), who found that chemical plus organic manure application increased total N content in the upland soil.

As described above, long-term compost application increased the amount of total N. The reasons are proposed as follows: (1) the growth of paddy rice and barley is accelerated to a larger extent in the F+C plot than in the F plot due to continuous application. As a result, larger amounts of plant materials such as root and stubble remain in the F+C plot than in the F plot. (2) The compost application supplies larger amount of organic N in F+C plot than in the F plot. (3) In the F+C plot, a slow release of N from compost and plant residues results in smaller losses of N as suggested by Bhandari *et al.* (2002).

2. Forms of organic N of whole soils

In the present study, in order to investigate the effects of compost application on soil organic N, different forms of organic N, including hydrolysable ammonium-N, amino acid-N, amino sugar-N, unidentified-N and non-hydrolysable-N, were analysed. In the reviews on soil organic N, Kelley and Stevenson (1995) and Schulten and Schnitzer (1998) summarized that hydrolysable ammonium-N originated from indigenous fixed NH_4^+ plus some fractions from the breakdown of hydroxyl amino acids and other amino acids, amino sugars, amides and the deamination of purines and pyrimidines. Amino acids occur in soils in the form of proteins and peptides closely associated with and protected by humic materials and inorganic soil constituents such as clay minerals and hydrous oxides of Fe and Al (Schulten and Schnitzer 1998). Amino sugars appear as structural components of a broad group of substances called macro-polysacharides. Some amino sugars exist as chitin and others are polysaccharides that are not chitin (Stevenson 1982). One-fourth to one-half of unidentified-N in soils has been reported to occur as the non-α-amino acid-N and such amino acids as arginine, tryptophan, lysine and proline (Goh and Edmeades 1979). Kelley and Stevenson (1995) described that part of the non-hydrolysable-N might occur in the form of N-phenyl amino acids resulting from the bonding between amino groups and aromatic rings.

The amounts of different forms of organic N in the whole soils and their percentage distribution are presented in **Table 2** and **3**, respectively. In the F+C plot compared to the F plot, the amounts of hydrolysable ammonium-N and unidentified-N were slightly larger while their distribution values were smaller. The amount of amino acid-N in the F+C plot was 1.8 times larger than that in the F plot and the distribution values were similar between these plots. The amount of amino sugar-N increased by compost application while the distribution value was less affected. Both amount and distribution degree of non-hydrolysable-N increased by compost application. These findings were similar to the results reported by Xu *et al.* (2003), except that the amount of hydrolysable ammonium-N decreased by organic amendments. The increase of non-hydrolysable-N may be because N derived from compost, chemical fertilizers and plant residues were transferred into resistant organic forms as suggested by Olson and Swallow (1984) and Kelley and Stevenson (1987).

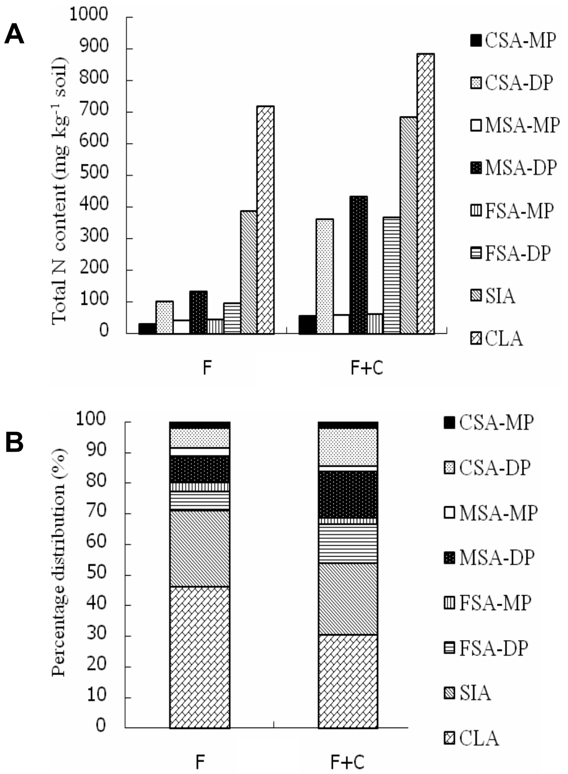

Fig. 2 Amount (A) and percentage distribution (B) of N in particle size fractions. See **Table 1** for F, F+C, CSA, MSA, FSA, SIA, CLA, MP and DP.

Particle size fractions

1. N of size fractions

Fig. 2 shows the amounts (mg kg^{-1} soil) of N in the particle size fractions and their percentage distribution. In all particle size fractions of the F+C plot, the amount of N substantially increased. Similar results were obtained in the previous studies by Leinweber and Reuter (1992) and Tanaka and Shindo (2009). Angers and N'Dayegamiye (1991) reported that in all size fractions studied, the N content was, on average, 25% larger in the manured treatments than in the unmanured treatment.

In the F+C plot, the amount of N was in the order of CSA-MP, MSA-MP < FSA-MP < CSA-DP < FSA-DP < MSA-DP < SIA < CLA fractions. A similar order was found in the F plot. In the size fractions of both plots, the percentage distribution of N was greater in the SIA and CLA fractions, ranging from 23.5 to 46.1%, than in the other fractions, ranging from 1.96 to 12.6%. The distribution values of N in the CLA fraction reduced from 46.1% for F plot to 30.4% for F+C plot. In the CSA-MP, MSA-MP, FSA-MP and SIA fractions, the quantitative distribution of N was similar between the F and F+C plots. In the CSA-DP, MSA-DP and FSA-DP fractions, the distribution values increased from 6.20-8.60% for the F plot to 12.5-14.9% for the F+C plot. As in the case with the present study, Christensen (1988), Leinweber and Reuter (1992) and Xu *et al.* (2003) found that the clay fraction contained the highest N across the size fractions.

2. Forms of organic N of size fractions

The amounts (mg kg^{-1} soil) of different forms of organic N in the particle size fractions and their percentage distribution are **Table 4** and **5**, respectively. In the F plot, both amounts and percentage distribution of all forms of organic N were the highest in the CLA fraction, generally followed by the SIA fraction. Their amounts and distribution values in the CSA-DP, MSA-DP and FSA-DP fractions tended to be much higher than those in the CSA-MP, MSA-MP and FSA-MP. Similar results were obtained for the F+C plot, except that the amount and distribution degree of non-hydrolysable-N in the SIA fraction were lower than those in the CSA-DP, MSA-DP and FSA-DP fractions. It is well known that N content of fine texture soils is larger than that of coarse texture soils. Clays can protect proteins and other nitrogenous compounds from decomposition by both adsorption and entrapment (Stevenson 1982). Hassink (1997) found that N associated with the fraction of less than 20 μm is resistant to decomposition.

The amounts of hydrolysable ammonium-N, amino acid-N, amino sugar-N and unidentified-N increased in all particle size factions by long-term compost application. No relationship was found between the amount of non-hydrolysable-N and the particle size fractions. The effects of com-

Table 4 Amounts of different forms of organic N in particle size fractions.

Plot[a]	Particle size fraction[b]		Hydrolysable ammonium-N	Amino acid-N	Amino sugar-N	Unidentified-N	Non-hydrolysable-N
			(mg kg^{-1} soil)				
F	CSA	MP	10.8	9.62	1.52	7.83	1.45
		DP	19.7	37.4	4.76	38.0	3.66
	MSA	MP	9.30	11.3	3.08	7.20	9.88
		DP	23.6	39.4	9.38	48.4	14.0
	FSA	MP	10.7	15.3	2.51	5.74	13.2
		DP	26.0	33.8	2.87	28.5	6.37
	SIA		111	106	7.93	155	12.4
	CLA		152	153	55.1	158	202
F+C	CSA	MP	21.3	18.2	3.91	9.86	4.43
		DP	77.2	72.3	15.9	130	68.5
	MSA	MP	16.0	14.8	4.64	7.50	15.0
		DP	80.5	116	18.6	136	83.5
	FSA	MP	20.1	18.4	3.97	14.5	6.53
		DP	95.4	98.2	15.9	136	25.0
	SIA		195	197	25.5	249	19.4
	CLA		212	224	59.8	266	126

[a] and [b] See **Table 1**

Table 5 Percentage distribution of different forms of organic N in particle size fractions.

Plot[a]	Particle size fraction[b]		Hydrolysable ammonium-N	Amino acid-N	Amino sugar-N	Unidentified-N	Non-hydrolysable-N
			(%)				
F	CSA	MP	2.98	2.37	1.74	1.72	0.55
		DP	5.42	9.22	5.46	8.36	1.39
	MSA	MP	2.56	2.80	3.53	1.58	3.76
		DP	6.50	9.72	10.8	10.6	5.32
	FSA	MP	2.93	3.77	2.87	1.22	5.01
		DP	7.15	8.32	3.30	6.27	2.43
	SIA		30.5	26.1	9.10	34.2	4.74
	CLA		41.9	37.7	63.2	36.0	76.8
	Total		100	100	100	100	100
F+C	CSA	MP	2.96	2.39	2.63	1.04	1.27
		DP	10.8	9.54	10.8	13.7	19.7
	MSA	MP	2.23	1.95	3.13	0.79	4.31
		DP	11.2	15.3	12.6	14.4	24.0
	FSA	MP	2.81	2.43	2.68	1.53	1.88
		DP	13.3	12.9	10.7	14.3	7.19
	SIA		27.2	26.0	17.2	26.2	5.57
	CLA		29.5	29.5	40.3	28.0	36.1
	Total		100	100	100	100	100

[a] and [b] See **Table 1**

post application on percentage distribution of organic N forms are summarized as follows: (1) The application caused the increases in distribution degrees of forms of organic N in the CSA-DP, MSA-DP and FSA-DP fractions but did not clearly affect the degrees in the CSA-MP, MSA-MP and FSA-MP fractions. (2) In the SIA fraction, the percentage distribution of amino sugar-N increased while that of hydrolysable ammonium-N and unidentified-N slightly declined. The proportions of amino acid-N and non-hydrolysable-N in the SIA fraction were similar between the F and F+C plots. (3) In the CLA fraction, the percentage distribution of all organic forms of N remarkably reduced. These results suggest that compost application merit close attention in the accumulation and transfer of organic forms of N into the CSA-DP, MSA-DP and FSA-DP fractions of the F+C plot.

Compost application itself could provide various organic N forms for the soils and induced an increase in microbe-derived organic N due to the acceleration activities of microorganisms. The CSA-DP, MSA-DP, FSA-DP fractions may be enriched by compost- and/or plant-derived organic N substances while microbe-derived organic N components were accumulated into the CLA fractions as suggested by Cheshire and Mundie (1981).

Among the forms of organic N, a large proportion was recovered as amino acid-N in every particle size fractions. The proportions of hydrolysable ammonium-N and unidentified-N were generally higher than those of amino sugar-N and non-hydrolysable-N. In the CSA, FSA and MSA fractions (including DP and MP fractions), no relationship was found between the percentage distribution to total N of organic N forms and the compost application. In the SIA fraction, the distribution values of all organic forms of N were similar between the F and F+C plots. In the CLA fraction, the proportion of non-hydrolysable-N decreased while the other forms of N tended to maintain or increase by compost application.

CONCLUSION

In the present study, the increases in the amounts of total N and forms of organic N were obtained in both whole soil and particle size fractions of the F+C plot. In the whole soil, the percentage distribution of non-hydrolysable-N was markedly increased by compost application while the other forms of N were maintained or reduced. The application increased distribution degree of total N and organic forms of N in the CSA-DP, MSA-DP and FSA-DP fractions while their distribution degrees in the CLA fraction decreased. However, both amounts and percentage distribution of total N and organic N forms were the highest in the CLA fraction. The findings indicate that the CLA fraction merit close attention as an important reservoir of various organic N. These results can compliment current knowledge about dynamic of organic N in soils received long-term compost application. In order to use effectively and develop compost application programs, further studies will be requested.

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