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Effects of different levels of compost application on amounts and distribution of organic nitrogen forms in soil particle size fractions subjected mainly to double cropping

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ABSTRACT

Effects of different levels of compost application on the amounts and percentage distribution of organic N forms in whole soils and particle size fractions were investigated. Soil samples were collected from three plots: a) F, only chemical fertilizers; b) F + LC, chemical fertilizers plus low level of compost; (c) F + HC, chemical fertilizers plus high level of compost. Each soil sample 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. The sand fractions were subdivided into decayed plants (DP) and mineral particles (MP). The amounts of total N and different organic N forms in the whole soils as well as size fractions generally increased with increasing the amount of compost. In the whole soils, percentage distribution of non-hydrolysable-N and amino sugar-N increased by compost application while the distribution values of the hydrolysable ammonium-N and unidentified-N decreased. The application did not affect the distribution degree of amino acid-N. In the size fractions, the distribution values of most organic N forms increased in the CSA-DP, MSA-DP and FSA-DP fractions by compost application. In the CLA fractions, the amounts and percentage distribution of organic N forms were the highest, although the application caused decreases in their distribution values. These findings indicate that the CLA fraction merit close attention as an important reservoir of various organic N.

Keywords: Compost Application; Upland and Paddy Fields; Soil Organic N Forms; Size Fractions

1. INTRODUCTION

In the surface layer of most soils, over 90% of N occurs in organic forms [1]. The forms of soil organic N can be divided into two broad categories: 1) organic residues and 2) soil organic matter or humus [2]. 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.

Application of organic fertilizer has received great attention from agriculturists and environmentalists because of directly and indirectly effects on crop growth and yield as well as soil properties. Organic amendments may affect the amounts and percentage distribution of organic N forms in whole soils and their particle size fractions. Results of ^{15}N studies clearly demonstrated that from 20% to 40% of the fertilizer N (inorganic N) added to agricultural soils was incorporated into organic forms during the first growing season [2]. Hassink [3] suggested that N associated with particle size fraction of less than 20 μm were better protected against decomposition. A number of authors have studied the influence of different types of organic fertilizer on the amounts and properties of N in the particle size fractions. For example, Schulten and Leinweber [4] 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. Leinweber and Reuter [5] pointed out that N concentration was generally highest in the clay fraction, followed by the silt and sand fractions. The N content of the soil particles from 250 to 2000 μm was reported to be greater with increased management intensity [6]. According to Xu *et* *al.* [7], 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. Tanaka and Shindo [8] found that long-term compost application increased the amount of N to a larger degree in the silt-sized aggregate fraction than in the other size fractions.

On the other hand, several authors have observed the effects of different levels of organic amendments on soil properties and fertilities. For example, soil organic matter content, total N content and soil microbial population increased with increasing the rate of compost application [9-11]. Angers and N'Dayegamiye [12] presented evidences that bi-annual application of 40 and 80 Mg·ha^{-1} increased N contents of whole soil and all particle size fractions studied. However, the effects of amendments on the contents and distribution of forms of organic N in particle size fractions of soils and the relationship between the amounts of amendment and organic N forms have received little attention. The objective of present study was to assess the influences of different levels of compost application on the amounts and distribution of several organic N forms in whole soils and their particle size fractions of mainly double cropped fields (paddy rice and barley). This is the first report on the relationships among the organic N groups, soil particle size fractions of double cropped fields and levels of compost applied.

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, Yamaguchi, Japan. The soil at this site was classified as Gray Lowland soil (FAO-UNESCO: Eutric Fluvisol). For the field experiments, we selected three plots (200 m^2 each): a) F plot, only chemical fertilizers containing N, P and K were applied; b) F + LC plot, chemical fertilizers plus a low level of compost (5 Mg·ha^{-1}) were applied; c) F + HC plot, chemical fertilizers plus a high level of compost (15 Mg·ha^{-1}) 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 as described above. 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 three 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.

2.2. Particle Size Fractions

The size fractionation of soil samples was conducted by physical fractionation described in Tanaka and Shindo [8], 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 - 2000 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 then 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.

All the fractions including DP and MP were freeze-dried and weighed.

2.3. Organic N Forms

Organic N composition was analysed according to the method described in Yonebayashi and Hattori [13], who partly modified the N fractionation method proposed by Bremner [14]. It was considered that the improved method is suitable for establishing the origin of soil organic N fractions, especially in case of tropical soils.

Soil sample containing about 10 mg N was hydrolysed with 20 mL of 6 mol·L^{-1} HCl and octyl alcohol for 24 hours 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 **Figure 1.**

The amount of hydrolysable ammonium-N (HAN) (a) was estimated by distilling the hydrolysate with MgO.

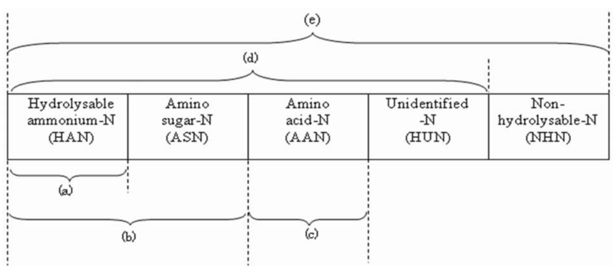

Figure 1. Scheme for the fractional determination of soil organic N.

The sum amount (b) of the HAN and amino sugar-N (ASN) was determined by distilling the hydrolysate with phosphate-borate (PB) buffer. The amount of the ASN was calculated by subtraction of (a) from (b).

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

Total hydrolysable-N (d) and total N (e) were determined by Kjeldahl procedure recommended by Bremner and Mulvaney [15]. The unidentified-N (HUN) and non-hydrolysable-N (NHN) contents were calculated as following formulations:

$$\text{HUN} = (\text{d}) - (\text{c}) - (\text{b}).$$

$$\text{NHN} = (\text{e}) - (\text{d}).$$

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

3. RESULTS AND DISCUSSION

3.1. Distribution of Mass Weight in Particle Size Fractions

In the physical fractionation of soil samples, the recoveries of mass weight ranged from 99.5% to 101% in all plots. The percentage distribution of mass weight in the particle size fractions was corrected to a total of 100% (**Table 1**). In all the plots, the distribution of mass weight in the particle size fractions was generally in the order of CLA < FSA < SIA < MSA < CSA. As expected, in the CSA, MSA and FSA fractions of all plots, the distribution values of MP were much larger than those of DP. The particle size fractions obtained were designated as aggregate fractions because percentage distribution of mass weight in particle size fractions differed before and after H_2O_2 treatment of soils [16].

3.2. Total N

The amounts and percentage distribution of total N in the particle size fractions are presented in **Figures 2** and **3**. In all size fractions, the amount of total N (mg·kg^{-1} soil) was higher in the F + LC and particularly F + HC plot than in the F plot (**Figure 2**). These results were in line with findings obtained by some authors that N concentration in the particle size fractions increased with increasing levels of organic amendments application [8,11,17,18].

Xu *et al.* [7] described that organic amendments application did not change the order of N content of particle size fractions studied. The same results were obtained in this study. In the F+HC plot, the amount of total N was in order of CSA-MP, MSA-MP < FSA-MP <

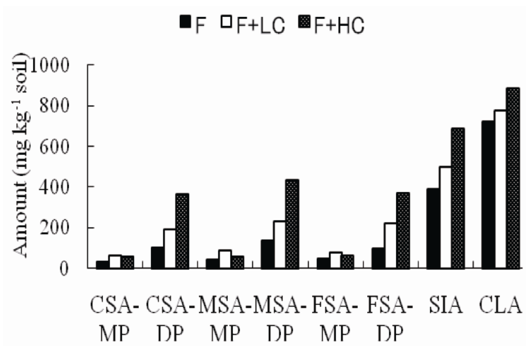

Figure 2. Amounts of total N in particle size fractions. See **Table 1** for F, F + LC and F + HC. See **Figure 1** for CSA, MSA, FSA, SIA, CLA, MP and DP.

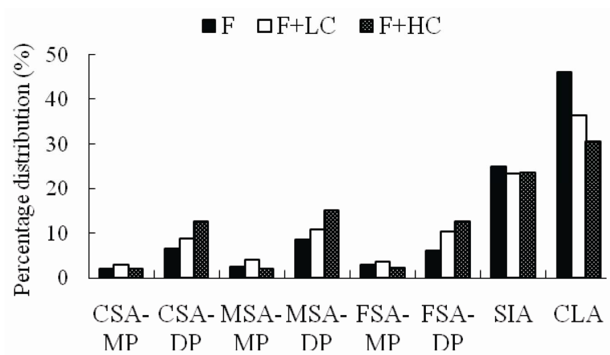

Figure 3. Percentage distribution of total N in particle size fractions. See **Table 1** for F, F + LC and F + HC. See **Figure 1** for CSA, MSA, FSA, SIA, CLA, MP and DP.

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

Plot	CSA[d]			MSA[e]			FSA[f]			SIA[g]	CLA[h]	Total
	MP[i]	DP[j]	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+LC[b]	30.1	1.28	31.4	20.4	1.56	22.0	16.1	2.05	18.2	18.8	9.60	100
F+HC[c]	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 low level of compost (5 Mg·ha^{-1}) were applied; c. chemical fertilizers plus high level of compost (15 Mg·ha^{-1}) were applied; d. coarse sand-sized aggregate fraction; e. medium sand-sized aggregate fraction; f. fine sand-sized aggregate fraction; g. silt-sized aggregate fraction; h. clay-sized aggregate fraction; i. mineral particles; j. decayed plants.

CSA-DP < FSA-DP < MSA-DP < SIA < CLA fractions. Similar orders were found in the F and F + LC plots.

In all the plots, the percentage distribution of N was greater in the SIA and CLA fractions, ranging from 23.3% to 46.0%, than in the other fractions, ranging from 1.96% to 14.9% (**Figure 3**). As pointed out by Tanaka and Shindo [8], in the CLA fraction, distribution value of total N was in the order of F + HC < F + LC < F plots. Furthermore, in all the plots, the distribution of N was the highest in the CLA fractions. These findings were in agreement with results reported by Leinweber and Reuter [5] and Xu *et al.* [7] that clay-sized fraction contained highest N.

3.3. Organic N Forms in Whole Soils

In the reviews on soil organic N, Kelley and Stevenson [2] and Schulten and Schnitzer [19] summarized that the HAN 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 [19]. Amino sugars appear as structural components of a broad group of substances called macropolysacharides. Some amino sugars exist as chitin and others are polysaccharides that are not chitin [1]. One-fourth to one-half of the HUN in soils has been reported to occur as the non-α-amino acid-N such as arginine, tryptophan, lysine and proline [20]. Kelley and Stevenson [2] described that part of the NHN might occur as structural components of humic substances and in the form of N-phenyl amino acids resulting from the bonding between amino groups and aromatic rings.

The amounts and percentage distribution of organic N forms in the whole soils are given in **Figures 4** and **5**. The amount of the HAN was higher in the F + LC, especially F + HC plots than in the F plot. However, the distribution value of the HAN was in the order of F + HC < F + LC < F plot. Similar results were found for the HUN. The amounts of the AAN in the F + LC and F + HC plots were 1.3 and 1.8 times higher than those in the F plot, respectively and the distribution value was not influenced by compost application. The amounts and distribution degrees of the ASN and NHN substantially increased at high level of compost application. The increase of the NHN may be because N derived from chemical fertilizers, compost and plant residues were transferred into resistant organic forms as suggested by Olson and Swallow [21] and Kelley and Stevenson [22]. These findings were similar to the results reported by Xu *et al.* [7], except that the amount of the HAN decreased by organic amendments application.

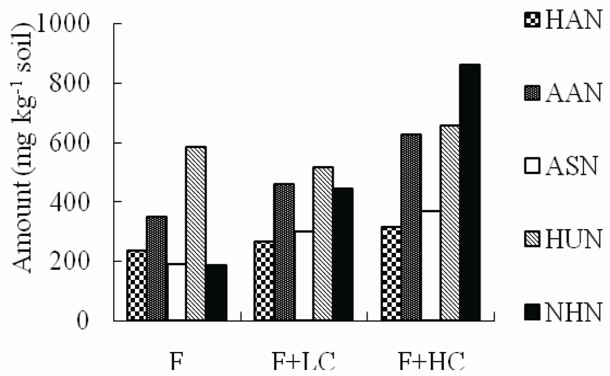

Figure 4. Amounts of organic N forms in whole soils. See **Table 1** for F, F + LC and F + HC. See **Figure 1** for HAN, AAN, ASN, HUN and NHN.

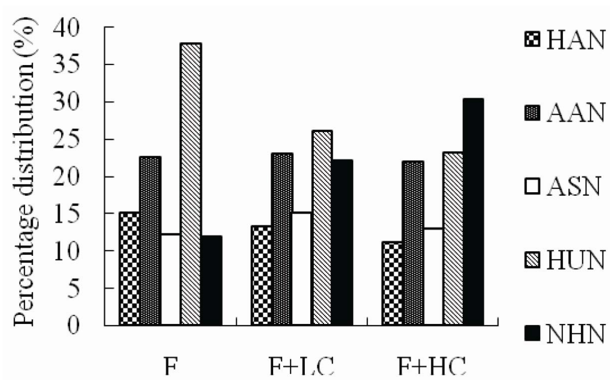

Figure 5. Percentage distribution of organic N forms in whole soils. See **Table 1** for F, F + LC and F + HC. See **Figure 1** for HAN, AAN, ASN, HUN and NHN.

3.4. Organic N Forms in Particle Size Fractions.

The amounts ($mg \cdot kg^{-1}$ soil) and percentage distribution of organic N forms in the particle size fractions are presented in **Tables 2** and **3**. It is noteworthy that in all three plots, the CLA and SIA fractions enriched most organic N forms. Their amounts and distribution values were the highest in the CLA fraction, generally followed by the SIA fraction. As expected, the content and distribution degree of organic N forms in the CSA-DP, MSA-DP and FSA-DP fractions were higher than those in the CSA-MP, MSA-MP and FSA-MP fractions. These results were in accordance with data published by several authors that finer fractions contain larger amounts of N than coarser fractions [1,3,5].

The amounts of the HAN, AAN, ASN and HUN in the particle size factions generally increased with increasing levels of compost application. Such a relationship was not found for the NHN. The effects of compost application on percentage distribution of organic N forms are summarized as follows (**Table 3**): 1) The distribution

Table 2. Amounts of organic N forms in particle size fractions.

Plot[a]	Particle size fractions[b]		Forms of organic N[c]				
			HAN	AAN	ASN	HUN	NHN
					(mg·kg^{-1} soil)		
F	CSA	MP	10.8	9.62	1.52	7.83	1.45
		DP	19.7	37.4	4.76	38.1	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 + LC	CSA	MP	15.0	14.3	2.01	11.4	18.6
		DP	30.9	40.8	11.3	80.3	26.8
	MSA	MP	14.9	16.5	3.67	36.1	14.7
		DP	41.6	65.7	12.9	83.5	25.8
	FSA	MP	14.8	22.5	2.57	33.5	4.67
		DP	48.0	62.1	8.62	82.4	19.0
	SIA		131	147	11.3	197	12.8
	CLA		196	192	49.8	261	79.1
F + HC	CSA	MP	21.3	18.2	3.91	9.86	4.43
		DP	77.2	72.3	15.9	130	68.6
	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**; c. see **Figure 1.**

Table 3. Percentage distribution of organic forms of N in particle size fractions.

Plot[a]	Particle size fractions[b]		Forms of organic N[c]				
			HAN	AAN	ASN	HUN	NHN
					(%)		
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	2.46	5.01
		DP	7.15	8.32	3.30	6.27	2.43
	SIA		30.5	26.0	9.10	34.2	4.74
	CLA		41.9	37.7	63.2	34.8	76.8
	Sum		100	100	100	100	100
F + LC	CSA	MP	3.04	2.55	1.97	1.45	9.25
		DP	6.28	7.27	11.0	10.2	13.3
	MSA	MP	3.03	2.94	3.60	4.60	7.29
		DP	8.45	11.7	12.6	10.6	12.8
	FSA	MP	3.01	4.01	2.52	4.27	2.32
		DP	9.74	11.0	8.44	10.5	9.43
	SIA		26.6	26.3	11.0	25.1	6.34
	CLA		39.9	34.2	48.8	33.2	39.3
	Sum		100	100	100	100	100
F + HC	CSA	MP	2.96	2.39	2.63	1.03	1.27
		DP	10.8	9.54	10.8	13.5	19.7
	MSA	MP	2.23	1.95	3.13	0.78	4.31
		DP	11.2	15.3	12.6	14.2	30.0
	FSA	MP	2.81	2.43	2.68	1.51	1.88
		DP	13.3	12.9	10.7	14.1	7.19
	SIA		27.2	26.0	17.2	26.2	5.57
	CLA		29.5	29.5	40.3	27.7	36.1
	Sum		100	100	100	100	100

a and b. see **Table 1**; c. see **Figure 1.**

degrees of organic N forms in the CSA-DP, MSA-DP and FSA-DP fractions tended to increase while those in the CSA-MP, MSA-MP and FSA-MP fractions were less affected. 2) In the SIA fraction, the distribution value of the ASN increased while that of the HAN or HUN declined. The values of the AAN and NHN in the SIA fraction were not greatly affected. 3) In the CLA fraction, the distribution of all organic N forms remarkably reduced. 4) These effects were markedly in the soil received high level of compost.

As presented in the **Tables 2** and **3**, all organic N forms dominated in the CLA fraction. It is likely that the adsorption of organic N compounds by clay minerals protects the proteins and other nitrogenous compounds from decomposition by microorganisms or by proteinase enzymes. Some organic N may be also entrapped within the lattice structures of clay minerals [1]. These processes resulted in larger amounts of organic N in the CLA fraction than in the other fractions.

Among the organic N forms, large proportions were recovered as the AAN and HAN in every particle size fractions. The proportion of the HUN was generally higher than that of the ASN or NHN. In all size fractions, no consistent relationship was found between the percentage distribution of organic N forms and the amount of compost applied.

4. CONCLUSIONS

In the whole soils and many particle size fractions, the amounts of total N and different organic N forms generally increased with increasing the amount of compost applied. In the whole soil, the percentage distribution of the NHN markedly increased by compost application, particularly in the plot received a high level of compost (F+HC). In the size fractions, the distribution values of most organic N forms increased in the CSA-DP, MSA-DP and FSA-DP fractions by compost application. The amounts and percentage distribution of organic N forms were the highest in the CLA fractions, although the compost application caused decreases in their distribution values. The findings obtained in the present study indicate that the CLA fraction merit close attention as an important reservoir of various organic N.

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