Soil Sci. Plant Nutr., **23** (1), 1–8, 1977

RELATIONSHIP BETWEEN AN ACCUMULATION OF SOIL ORGANIC MATTER BECOMING DECOMPOSABLE DUE TO DRYING OF SOIL AND MICROBIAL CELLS

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Received May 17, 1974

The purpose of this experiment is to make clear the relationship between an accumulation of soil organic matter becoming decomposable due to drying of soil and microbial cells. The results are summarized as follows:

1) The accumulation of soil organic matter becoming decomposable due to drying occurred in the decomposition process of organic matter applied to soil, and its quantity clearly increased with an increase of microbial cells. Further, the accumulation increased in company with an increase of reimmobilization during the decomposition process of organic matter applied to soil.

2) The accumulation of the decomposable soil organic matter was clearly recognized during the decomposition process of microbial cells in soil. The accumulation rate was higher in newly immobilized organic matter of soil than in native soil organic matter.

3) It was suggested that microbial cells and their cell walls considerably contribute as a source of the decomposable soil organic matter.

Many research papers (*1–4*, *6*) on the decomposition of microbial cells in soil have been reported. However, no reports on the contribution of microbial cells to an accumulation of soil organic matter becoming decomposable due to drying (hereinafter referred to as the decomposable soil organic matter) has been published to date.

In the previous paper (*7*), it was reported that major amino acids in soil were similar to those existing in microbial cell walls, and that amino sugar compounds were newly synthesized by soil microorganisms during the decomposition process of the uniformly ^{14}C-labeled rye-grass applied to soil. The amino sugar compounds might be accumulated in soil probably as an organic-mineral complex showing resistance to microbial decomposition. In this paper, therefore, two experiments were carried out on the relationship between an accumulation of the decomposable soil organic matter and microbial cells.

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EXPERIMENT 1 ACCUMULATION OF THE DECOMPOSABLE SOIL ORGANIC MATTER DURING THE DECOMPOSITION PROCESS OF GLUCOSE AND MICROBIAL CELLS

Materials and methods

Twenty g of sea sand (diameter: 0.50 to 0.25 mm) was weighed in a 50 ml Erlenmeyer flask. Four mg of nitrogen as KNO_3 and 80 mg or 160 mg of carbon as glucose were added to the sand to make C/N ratio 20 and 40, respectively. The samples were mixed thoroughly. Then, the mineral nutrition shown in Table 1 and inoculum were added to each sample. The inoculum was prepared as follows: 50 ml of distilled water was added to 10 g of paddy soil taken at the farm of Kyushu University. It was shaken for 10 min and allowed to stand for 5 min. Then 1.0 ml of its supernatant solution was added to each sample. Next, the samples were adjusted with distilled water to bring the moisture content to 60 per cent of the maximum water holding capacity and to pH 6.5 with dilute HCl or NaOH solution. The flasks were covered with polyethylene film and incubated at 30°C. The decrease of water by evaporation during the incubation period was corrected by the addition of distilled water.

The total carbon and organic carbon becoming decomposable due to drying (hereinafter referred to as the decomposable organic carbon) was determined by the following method: after 1, 2, and 3 weeks' incubation, part of samples were removed, dried at 100°C for 2 hr, and reincubated for 2 weeks under the same conditions mentioned above. Before and after reincubation, the total carbon was determined (see Total C (1) and Oven-dried (3) in Table 2, respectively). In addition, the total carbon of the non-heat treatment samples after reincubation was also determined (see Control (2) in Table 2). The decomposable organic carbon was computed from the difference between the total carbon of the non-heat treatment samples and that of the heat treatment samples after reincubation (see Organic-C becoming decomposable due to drying (2) − (3) in Table 2).

Table 1. Mineral nutrition.

A	KH_2PO_4		0.95 g
	K_2HPO_4		1.24 g
	Distilled water		500 ml
B	$MgSO_4 \cdot 7H_2O$		0.5 g
	$CaCl_2 \cdot 2H_2O$	1% solution	2.0 ml
	$FeSO_4 \cdot 7H_2O$	"	1.0 ml
	$CuSO_4 \cdot 5H_2O$	"	1.0 ml
	$ZnSO_4 \cdot 7H_2O$	"	1.0 ml
	$MnSO_4 \cdot 4H_2O$	"	0.5 ml
	$Na_2MoO_4 \cdot 2H_2O$	"	0.5 ml
	Distilled water		500 ml

A and B solutions were mixed just before the examination, and 0.8 ml was added to 20 g of sand.

A block diagram of the determination of the decomposable organic carbon and nitrogen of soil is shown in Fig. 1.

Microbial population in the sample was measured by the plate method. The media for bacteria and fungi were the egg-albumin agar and the rose-bengal agar, respectively (*9*). The residual glucose in the sample was determined by SOMOGYI method (*8*).

Table 2. Accumulation of organic carbon becoming decomposable due to drying.

C/N ratio	Incubation period (weeks)	Total C (1)	Total-C after 2 weeks of incubation		Organic-C becoming decomposable due to drying (2)−(3)	Accumulation rate $\frac{(2)-(3)}{(1)}\times 100$
			Control (2)	Oven-dried (3)		
		(mg C / 100 g sand)				(%)
20	1	86.6	61.9	57.4	4.5	5.2
	2	64.7	59.0	50.7	8.3	12.8
	3	61.9	58.0	47.0	11.0	17.8
40	1	356.2	120.4	—	—	—
	2	185.8	116.0	101.1	14.9	8.0
	3	120.4	112.0	88.2	23.8	19.8

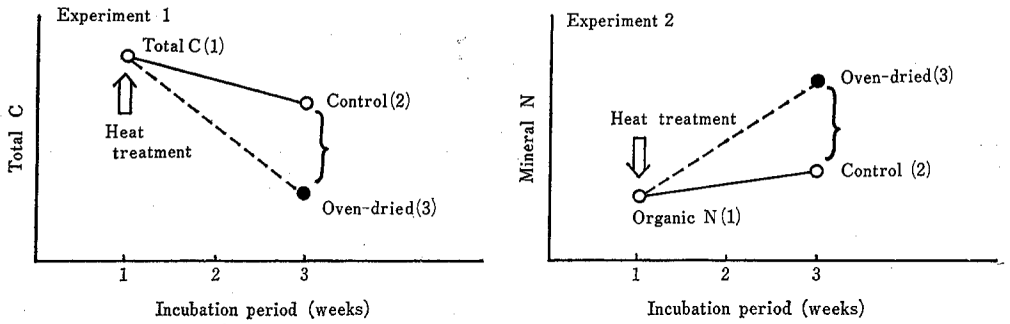

Fig. 1. Block diagram of the determination of organic carbon and organic nitrogen becoming decomposable due to drying of soil.

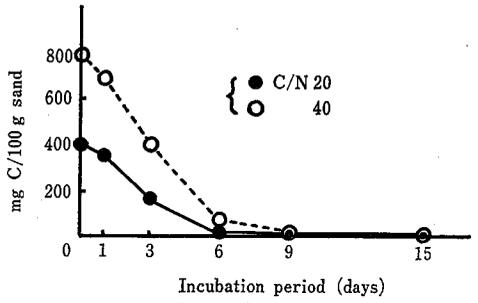

Fig. 2. Tendency of the decomposition process of glucose-C.

Table 3. Microbial population (number/100 g sand).

Incubation period (weeks)	C/N ratio 20		C/N ratio 40	
	Bacteria	Fungi	Bacteria	Fungi
0	1.2×10^{6}	3.0×10^{4}	1.2×10^{6}	3.0×10^{4}
1	1.1×10^{12}	5.8×10^{9}	1.3×10^{14}	1.2×10^{11}
3	3.3×10^{10}	1.6×10^{12}	1.9×10^{12}	2.1×10^{12}

Results and discussion

The tendency of the decomposition process of glucose-C is shown in Fig. 2. About 50 per cent of the glucose applied decomposed in 3 days irrespective of the C/N ratio, and about 98 per cent in 9 days. From these results, it is sure that the organic carbon accumulated in the sample after 1 week of incubation originated in the organic matter newly immobilized during the decomposition process of glucose applied, namely, microbial cells, their residues, and their metabolic products.

The accumulation of the decomposable organic carbon is shown in Table 2. In the case of the C/N ratio 40, its accumulation was about twice that of the C/N ratio 20. This shows that the more organic carbon was newly immobilized, the more the decomposable soil organic carbon was accumulated. Further, the quantity of the decomposable organic carbon increased with an increasing decomposition of glucose in both C/N ratios. Its accumulation rates, however, was about equal in both the C/N ratios. Those of the C/N ratio 20 and 40 after 3 weeks of incubation were 17.8 and 19.8 per cent respectively.

Microbial population in the sample is shown in Table 3. The number of microbial cells was larger in the C/N ratio 40 than in the C/N ratio 20, but it was indicated that tendency of the number to increase in both the C/N ratios was almost equal. Bacteria increased remarkably with an increasing decomposition of glucose and their number became largest at 1 week of incubation. Fungi also increased remarkably and their number became largest at 3 weeks of incubation.

From these results, it was shown that the accumulation of the decomposable organic carbon increased with an increase of organic carbon newly immobilized, namely an increase of microbial cells. So, it may be concluded that the microbial cells and their residues considerably contribute as a source of the decomposable soil organic matter.

EXPERIMENT 2 DECOMPOSITION OF *Aspergillus niger* IN SOIL AND ITS CONTRIBUTION TO AN ACCUMULATION OF THE DECOMPOSABLE SOIL ORGANIC MATTER

Materials and methods

Properties of the three paddy soils employed are shown in Table 4. Moist soil was weighed in a 50 ml Elrenmeyer flask in amount corresponding to 20 g dry soil. Next, 100 mg of *A.niger* corresponding to 4 mg of nitrogen was added to the soil. *A.niger* was prepared as follows: *A.niger* was inoculated in 100 ml of the culture medium (glu-

cose: 30.0g, $(NH_4)_2SO_4$: 1.55g, KH_2PO_4: 0.088g, K_2HPO_4: 1.168g, $MgSO_4 \cdot 7H_2O$: 0.5g, $FeSO_4 \cdot 7H_2O$: 0.01g, distilled water: 1 liter, pH 6.5) in the Fern-bach flask (diameter: 18 cm), and placed at 30°C for 45 hr. After *A.niger* was collected on the glass filter and washed with distilled water, it was dried at 100°C for 1 hr or at 70°C overnight. *A.niger* thus dried was ground in a mortar (<60 mesh).

The samples were then mixed thoroughly, the mineral nutrition shown in Table 1 was added to the soil, and the soil was adjusted with distilled water to bring the moisture content on 60 per cent of the maximum water holding capacity and to pH 6.5 with dilute HCl or NaOH solution. The flasks were covered with polyethylene film and incubated at 30°C. The decrease of water by evaporation during the incubation period was corrected as shown in Experiment 1. After 12 weeks of incubation, the decomposable organic nitrogen was determined after the method described in Experi-

Table 4. Properties of soils.

Soil	Texture	Major clay mineral	Clay content	Total C	Total N	CEC
			(per cent/dry soil)			(me/100 g dry soil)
Toyama	SL	Halloysite	9.6	2.34	0.18	7.3
Isahaya	LiC	Montmorillonite	44.2	1.51	0.16	32.6
Handa	CL	Allophane	24.5	7.31	0.61	29.4

Table 5. Nitrogen mineralization of *A. niger*.

Soil	Incubation period (weeks)	Control (A)		Addition (B)		Based on *A. niger* (B)−(A)		
		Org-N	Min-N	Org-N	Min-N	Org-N	Min-N	Mineralization rate
		(mg N/100 g dry soil)						(%)
Toyama	0	176.3	3.7	196.3	3.7	20.0	0	—
	1	176.0	4.0	190.5	9.5	15.5	4.5	27.5
	3	175.0	5.0	187.0	13.0	12.0	8.0	40.0
	5	173.7	6.3	184.7	15.3	11.0	9.0	45.0
	7	173.2	6.8	184.5	15.5	11.2	8.8	43.5
	12	171.8	8.2	182.2	17.8	10.4	9.6	48.0
Isahaya	0	159.1	0.9	179.1	0.9	20.0	0	—
	1	157.1	2.9	176.0	4.0	18.9	1.1	5.5
	3	155.5	4.5	169.4	10.6	13.9	6.1	30.5
	5	154.0	6.0	167.3	12.7	13.3	6.7	33.5
	7	153.2	6.8	164.8	15.2	11.6	8.4	42.0
	12	150.4	9.6	162.3	17.7	11.9	8.1	40.5
Handa	0	609.6	0.4	629.6	0.4	20.0	0	—
	1	608.6	1.4	626.0	4.0	17.4	2.6	13.0
	3	606.0	4.0	620.0	10.0	14.0	6.0	30.0
	5	603.6	6.4	616.5	13.5	12.9	7.1	35.5
	7	601.8	8.2	613.5	16.5	11.7	8.3	41.5
	12	598.3	11.7	610.3	19.7	13.0	8.0	40.0

ment 1. Mineral nitrogen in the soil was extracted with N KCl and determined by Conway's microdiffusion method using Devarda's alloy as a reducing agent (*5*).

Results and discussion

Nitrogen mineralization of *A.niger* added to soil is shown in Table 5. The nitrogen mineralized in soils was clearly greater where *A.niger* was added (Addition (B)) than in those where no *A.niger* was added (Control (A)). The nitrogen mineralized in "Addition" cases includes that from native soil organic nitrogen as well as *A.niger* added to soil. In Table 5, nitrogen mineralized from *A.niger* added to soil was computed from the difference between "Addition" and "Control" groups (Based on *A.niger* added to soil (B)—(A)). These figures show the apparent amounts of nitrogen mineralized based on *A.niger* added to soil. It is assumed that the mineralization of native soil organic nitrogen occurring through the addition of *A.niger*—namely, the priming effect —was included in the nitrogen mineralized in the "Addition" groups. In this experiment, however, it is asserted that the priming effect is very low.

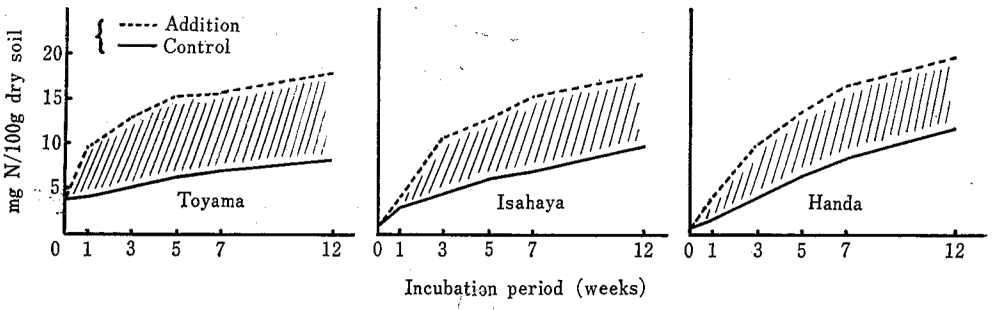

Fig. 3. Tendency of the decomposition process of *A. niger*. The oblique lines show an increase of nitrogen mineralization based on *A. niger*.

The tendency of the decomposition process of *A.niger* added to soil is shown in Fig. 3. The oblique lines show an apparent increase of nitrogen mineralization based on *A.niger* added. As seen in Fig. 3, the mineralization rate of *A.niger* added during the early incubation period (from 0 to 3 weeks) varied among three soils. The rates at 3 weeks of incubation were 40.0, 30.5, and 30.0 per cent in Toyama, Isahaya and Handa soils, respectively. Its mineralization reached a stable level after about 5 weeks of incubation in Toyama and 7 weeks of incubation in Isahaya and Handa. These results show that the decomposition of organic matter contained in *A.niger* was almost finished from 7 to 12 weeks of incubation. The rates at 7 weeks of incubation were 44.0 per cent in Toyama, 42.0 per cent in Isahaya and 41.5 per cent in Handa. On the average, 42.5 per cent was decomposed and 57.5 per cent was accumulated.

At 12 weeks of incubation when the decomposition of organic matter in soils added

Table 6. Soil organic nitrogen becoming decomposable due to drying at 12 weeks of incubation.

Soil	Organic N	N mineralized for 2 weeks		Organic N becoming decomposable due to drying	Accumulation rate
		Control	Oven-dried		
	(1)	(2)	(3)	(3)−(2)	$\frac{(3)-(2)}{(1)}\times 100$
		(mg N/100 g dry soil)			(%)
Control (A)					
Toyama	171.8	0.1	3.6	3.5	2.0
Isahaya	150.4	0	5.4	5.4	3.6
Handa	598.3	1.2	6.0	4.8	0.8
Addition (B)					
Toyama	182.2	1.4	5.8	4.4	2.4
Isahaya	162.3	1.5	7.6	6.1	3.8
Handa	610.3	2.5	7.7	5.2	0.9
Based on *A. niger* (B)−(A)					
Toyama	10.4	1.3	2.2	0.9	8.7
Isahaya	11.9	1.5	2.2	0.7	5.9
Handa	13.0	1.3	1.7	0.4	3.1

with *A.niger* was assumed to have ceased, the soil was dried, remoistened, and reincubated as indicated in Experiment 1, in order to estimate the accumulation amount of the decomposable soil organic matter during the decomposition process of *A.niger* applied to soil. The result is shown in Table 6. From the result obtained, the quantity of the decomposable soil organic nitrogen was greater in "Addition" than in "Control" groups. It is shown that the addition of *A.niger* increased its accumulation as seen in "Based on *A.niger*" groups. These results clearly indicate that the decomposable soil organic matter was newly accumulated during the decomposition process of *A.niger* applied to soil. Its accumulation rate were 0.8 to 2.0 per cent in "Control (A)", 0.9 to 3.8 per cent in "Addition (B)" and 3.1 to 8.7 per cent in "Based on *A.niger* (B)−(A)", respectively. Thus, it is clear that its rate was higher in organic matter newly produced through the addition of *A.niger* than in native soil organic matter.

From these results, it is assumed that the residue of *A.niger* added, other microbial cells related to its decomposition and their residues, *etc.*, contribute considerably as sources of the decomposable soil organic matter.

Further, with regard to the accumulation rates of the decomposable organic nitrogen in the three soils (see Table 6), those in the "Control" decreased in the following order: Isahaya>Toyama>Handa, but those in "Based on *A.niger*": Toyama> Isahaya>Handa. As mentioned above, the rate of organic matter newly produced through the addition of *A.niger* was greater than that of native soil organic matter. The magnitude of its rate, however, was not proportional to the quantity of organic matter newly produced, but varied considerably among the three soils. From the results in this experiment, its rate in soil containing allophane (:Handa) was the least. As to

soils containing crystalling clay minerals, its rate was greater in soil containing halloysite (:Toyama) than in that containing montmorillonite (:Isahaya). The reason for a considerable variation in the rate of organic matter newly produced among various clay minerals contained in soil is a subject for further study.

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