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The Harmful Effect of Ammonium Ion on the Mineralization and Accumulation of Organic Matter in Soil

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Concentration of ammonium ion supplied to soil microbes, i.e., concentration of ammonium ion in soil solution, is one of the important factors affecting decomposition of plant residues and soil organic matters in soil. When it is more than 200 ppm, the growth of soil microorganisms concerned with decomposition of plant residues or soil organic matters is prevented and the decomposition of them is remarkably retarded. Accumulation of the organic matter becoming decomposable through the effect of drying a soil which is directly concerned with the supply source of carbon dioxide to atmosphere of fields and the naturally supplied nitrogen to crops is also hindered due to such a concentration of ammonium ion as more than 200 ppm nitrogen.

INTRODUCTION

In order to increase the yield of crops per unit area, it is necessary to apply nitrogen fertilizer in quantities and make crops absorb it in safety. For this purpose it is essential to understand the varied functions of the nitrogen fertilizer for the crops and soils in full detail.

It is well-known in low land cultivation that about 80 percent of nitrogen absorbed by rice crop is derived from soil organic nitrogen and that the mineralization of soil organic nitrogen is considerably accelerated through various pretreatments of soil such as air-drying, changing pH, raising temperature, additions of bulky organic matter, salts including fertilizers and so on (Harada, 1959, Miyaguchi *et al.*, 1969). From a view-point of soil fertility, it is interesting and important as a practical problem in agricultural cultivation to make clear the effects of the pretreatments of soil on the behaviours of organic matter in soil, because a large part of carbon, nitrogen and other mineral nutrients taken up by crops may come from the organic matter in soil through the mineralization process.

In the previous paper (Yoshida *et al.*, 1972), it was shown that the addition of mineral nitrogen with plant residues to soil led to a increase of the organic matter becoming decomposable through the effect of drying a soil and that the accumulation amounts of this organic matter was in linear proportion to the amounts of the mineral nitrogen added. Actually, this organic fraction of soil organic matter is directly concerned with the supply source of carbon dioxide to atmosphere of the fields and the naturally supplied nitrogen to crops and ac-

cumulated through the mineralization process of organic residue added to soil.

The study presented here was carried out to obtain information about the effect of ammonium ion on the behaviours of organic matter in soil. In this study, the following five experiments are involved; the effect of addition of ammonium sulfate on the mineralization of soil organic matter (Experiment 1), the effect of addition of ammonium sulfate on the mineralization of plant residue and the accumulation of organic matter becoming decomposable through the drying effect (Experiment 2), the effect of addition of ammonium sulfate on the mineralization of plant residue in water culture under shaking condition (Experiment 3), the effect of ammonium ion on the growth of fungi (Experiment 4) and the effects of applications of ammonium nitrate and ammonium sulfate on the mineralization of plant residue and the accumulation of the organic matter becoming decomposable through the drying effect (Experiment 5).

MATERIALS AND METHODS

Experiment 1

Soil sample

Soil sample used in this experiment was taken from furrow slice of the upland field at the Agricultural Farm of Kyushu University at the middle of December in 1969, to which farmyard manure has been applied with an annual dressing of about 1200 kg per 10 a for 46 years since 1923 and sweet potato crop has been cultivated. It was allowed to make dry just until soil crumbs no longer adhered under slight pressure, passed through a 2-mm wire sieve and stored at room temperature in polyethylen bags under dark condition until the beginning of next April. It contained 16.8 % water, which corresponded to **32.3 %** of its maximum water holding capacity, **1.20 %** carbon and 0.106 % nitrogen. Cation exchange capacity of the soil sample was 11.3 me per 100 g dry soil.

Incubation methods

In order to determine CO₂ evolved during incubation period at intervals, 20 g of the stored soil was weighed into 570 ml incubation jars in the same manner as shown in the previous paper (Yoshida *et al.*, 1972). The soil was treated with different levels of nitrogen. The levels of nitrogen were four as follows ; 0, 15, 30 and 60 mg N per 100 g dry soil as ammonium sulfate. They were moistened with the distilled water enough to bring the moisture content to 60 % of the maximum water holding capacity and then incubated at 30°C during the periods of 2, 5, 10 and 17 days. The oven-dried soil was also used in this experiment to obtain information about the effect of ammonium ion added to soil on the mineralization of the organic matter becoming decomposable through the drying effect which has been accumulated in the soil. In the case of the oven-dried soil, the soils treated with the different levels of nitrogen were dried at 30°C for 3 hrs in thermostat. After drying, they were added with 0.4 ml soil suspension (1: 5) per 20 g soil as an inoculum and water enough to bring moisture content to 60 % of the maximum water holding capacity and

then incubated at 30°C during the period of the same days as shown in the stored soil.

Analytical procedures

The amounts of carbon evolved as CO₂ during the incubation period and the effect of drying a soil on the mineralization of soil organic matter were determined by the method as described in the previous paper (Yoshida *et al.*, 1972). Mineral nitrogen in soil was extracted with N KC1 (pH 7.0) and determined by the Conway's micro-diffusion method using Devarda's alloy as a reducing agent (Kai *et al.*, 1972). Total nitrogen was determined by the micro-Kjeldahl method using the reduced iron as a reducing agent (Yamasaki, 1940). On the basis of the determination of total nitrogen, it was ascertained that loss of nitrogen did not occur through the denitrification and volatilization during the incubation period under the experimental conditions.

Experiment 2

Preparation of media and plant residue

Two kinds of media were prepared in this experiment. One is sea sand ground to fine sand in a mortar and another is M-soil which was made by mixing soil clay with the sea sand at the rate of **40** %. The soil clay was obtained from Ariake soil treated with H₂O₂ by usual method, dried at about 80°C under hot drier and then ground to 100 meshes. Main clay mineral contained within the soil clay was detected as montmorillonite from the diffraction patterns of X-ray. Cation exchange capacity of the soil clay was determined as 67me per 100 g dry soil clay by the Schollen-berger's method.

Sun-dried rice straw was used as plant residue. It was dried at 80°C under a hot-air drier and then ground to **32** meshes in a mill made of iron. Chemical composition of the rice straw was shown in Table 1.

Table 1. Chemical composition of rice straw.

H ₂ O	17.4 (%)	CaO	0.31
Total C	33.8	MgO	0.13
Total N	0.554	Mn ₂ O ₃	0.02
C/N ratio	61.0	Organic	
Inorganic		Cellulose	33.73
SiO ₂	9.82	Lignin	21.08
P ₂ O ₅	0.24	Crude protein	3.46
K ₂ O	2.42	Crude fat	0.80

Incubation methods

The M-soil and sand mentioned above were treated with different levels of mineral nitrogen. The levels of nitrogen added to the M-soil and sand were four as follows ; 0, 20, 60 and 120 mg N per 100 g soil and sand as ammonium sulfate. In order to determine CO₂ evolved during the incubation period at

intervals, the same incubation jars as used in Experiment 1 were used. Ten grams of each soil and sand treated with different amounts of mineral nitrogen were weighed into the incubation jar, mixed with 400 mg the rice straw, moistened with the distilled water enough to bring moisture content to 60 % of the maximum water holding capacity, added with mineral nutrients (P, K, Ca and trace elements) as shown in the previous paper (Yoshida *et al.*, 1972) and 0.2 ml of soil suspension (1: 5) as an inoculum, and then incubated at 30°C for 85 days. The M-soil contained ammonium nitrogen corresponding to 4.9 mg N per 100 g dry soil, because exchangeable ammonium ion remained in the clay.

Analytical procedures

A part of each sample incubated for 70 days was previously oven-dried at 80°C for 3 hrs and further incubated for 15 days under the same conditions as controlled before oven-drying. Accumulation amounts of the organic matter becoming decomposable through the drying effect was estimated from the difference between the amounts of carbon mineralized in the non-dried sample for 15 days and those mineralized in the oven-dried sample for the same period. The other procedures were the same as used in Experiment. 1.

Experiment 3

In order to determine CO₂ evolved from rice straw during incubation period at intervals, incubation bottle as described in Figure 1 was used in this experiment. One hundred milliliters of water solutions of ammonium sulfate corresponding to 50, 100, 200, 400 and 800 ppm N were placed respectively in 200 ml incubation bottles. The rice straw corresponding to 135.2 mg carbon was added to them. Then, they were added with mineral nutrients (P, K, Ca and trace elements) and soil suspension as an inoculum, and then incubated at 30°C for 85 days under shaking condition on the shaking machine adjusted with temperature.

Analytical procedures of the total and mineral nitrogen and the CO₂ evolved

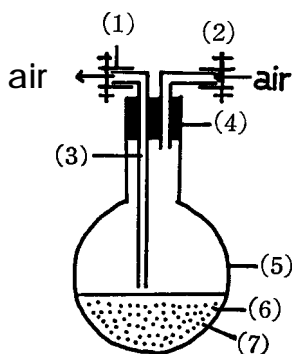


Fig. 1. Incubation bottle.

- (1) Rubber tube, (2) Pinch-cock, (3) Glass tube, (4) Rubber stopper,
- (5) Glass bottle, (6) Straw, (7) Nutrient solution.

from the rice straw during the incubation period were the same as used in Experiment 1.

Experiment 4

Preparation of fungal spores suspension

Spores of three different sorts of fungi, i.e., *Aspergillus*, *Penicillium* and *Rhizopus* which were taken separately from the fungi grown in the M-soils and sands treated with different amounts of ammonium nitrogen at the early stage of incubation period in Experiment 2 (see Table 7), were brought respectively into suspensions by using the sterilized water. The number of each fungal spore was made to 20-30 per 1 ml suspension by dilution with the sterilized water. This was confirmed by counting fungal colonies grown on the plate of standard medium containing rose bengal (Tanabe *et al.*, 1966).

Preparation of culture medium

Nutrient agar solution containing rose bengal (Tanabe *et al.*, 1966) was made to be acidic to prevent the growth of bacteria. This solution contains glucose as carbon source and is free from nitrogen source. To this solution ammonium sulfate or potassium nitrate was added at the rate of from 10 to 1600 ppm nitrogen.

Incubation methods

Thirty milliliters of each medium treated with mineral nitrogen, which was mixed with 1 ml of each fungal spores suspension, were poured to petri dishes under 40°C. They were incubated at 30°C and the growth of each fungus in the medium was observed. Under the coexistence of three kinds of fungus the growth of each fungus was also examined by the same method using medium treated with ammonium sulfate.

Experiment 5

Preparation of soils

Three kinds of soil treated with H₂O₂, i.e., M-Nagano soil, H-Choyo soil and A-Choyo soil and sand were used in this experiment. The soils were washed with 0.1 N CaCl₂ solution (pH 6.0) to remove extractable ammonium ion which remained in the soils. Main clay mineral in the M-Nagano soil was montmorillonite, that in the H-Choyo soil halloysite and that in the A-Choyo soil allophane. Cation exchange capacities of the M-Nagano soil, the H-Choyo soil and the A-Choyo soil were 20.6, 9.1 and 11.5 me per 100 g dry soil, respectively.

Incubation methods

Each soil and the sand were treated with ammonium sulfate or ammonium nitrate corresponding to 22.6 mg nitrogen per 100 g soil. Ten grams of the soils and sand treated with mineral nitrogen were placed in 570 ml incubation jars as same as in Experiment 1, mixed with 400 mg rice straw, added with mineral nutrients (P, K, Ca and trace elements) and soil suspension as an inoculum

and then incubated at 30°C for 84 days. A part of the soils and sand incubated for 70 days were previously oven-dried at 80°C for 3 hrs and further incubated for 14 days under the same conditions as controlled before oven-drying to determine the accumulation amounts of organic matter becoming decomposable through the effect of drying a soil in the soils and the sand.

RESULTS AND DISCUSSION

Experiment 1

In Experiment 1, studies on the effect of addition of ammonium sulfate on the mineralization of the organic carbon and nitrogen in soil were carried out using the non-dried and oven-dried soils. The results obtained on the organic carbon are shown in Table 2 and Figure 2. As seen in Table 2 and Figure 2, when the amounts of ammonium nitrogen added were less than 30 mg N per 100 g soil, the larger the amounts of ammonium nitrogen added, the larger the amounts of carbon evolved as CO₂ from soil organic matter. When the amounts of ammonium nitrogen added was more than 30 mg N per 100 g soil, the mineralization of soil organic carbon was repressed. The amounts of carbon evolved

Table 2. The amounts of carbon evolved as CO₂ from soil organic matter in soils treated with different amounts of NH₄-N.
(mg C/200g Fresh soil)

Amounts of NH ₄ -N added	Incubation period (days)		
	2	5	17
Fresh soil			
0 mg/100 g soil	5.6	13.7	40.8
15	9.4	14.9	45.3
30	10.1	20.1	60.5
60	10.5	16.8	50.6
Oven-dried soil			
0	18.7	33.8	63.4
15			72.6
30	25.0 33.1	37.1 47.7	84.8
60	19.9	31.6	61.3

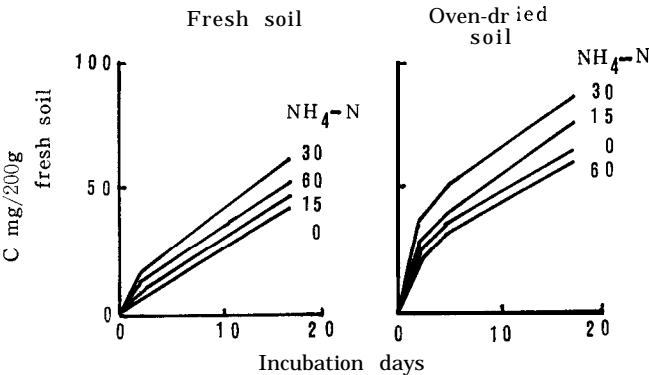


Fig. 2. Mineralization of soil organic carbon in soil treated with NH₄-N.

as CO_2 increased in the following order; $30 > 60 > 15 > 0$ mg ammonium nitrogen per 100 g dry soil for the fresh soil and $30 > 15 > 0 > 60$ mg ammonium nitrogen per 100 g dry soil for the oven-dried soil (see Figure 2).

The drying effect on the mineralization of soil organic carbon was calculated by the way indicated in the previous paper (Yoshida *et al.*, 1972) and shown in Table 3. As seen in Table 3, the drying effect was the largest in the soil treated with 15 mg ammonium nitrogen per 100 g dry soil.

Next, the results obtained on the organic nitrogen are shown in Table 4. As seen in Table 4, the mineralization of soil organic nitrogen in the oven-dried soils was delayed by application of ammonium sulfate. But in the fresh soil, the amounts of nitrogen mineralized were increased to some extent by application of ammonium sulfate. Broadbent (1965) also showed that addition of ammonium fertilizer accelerated the mineralization of soil organic nitrogen. It may safely be said that this acceleration occurs due to the same effect as have been called the effect of addition of neutral salt, i. e., Tohlen-effect, and appears on account of the same action as the drying effect made clear by Harada (1959).

In this experiment, it was shown that though when ammonium nitrogen was added to soil with suitable amount (less than 30 mg N per 100 g dry soil) the mineralization of soil organic matter was accelerated, when it was added in excess (more than 30 mg N per 100 g soil) the mineralization of soil organic matter was retarded. This retardation was more severe in the oven-dried soil than

Table 3. The drying effect on the mineralization of organic carbon in soils treated with different amounts of $\text{NH}_4\text{-N}$.

Amounts of $\text{NH}_4\text{-N}$ added	(mg C/200g Fresh soil)		
	Incubation period (days)		
	2	5	17
0 mg/100 g soil	12.8	20.1	22.6
15	15.6	22.2	27.3
30			
60	23.9 4.0	27.6 14.8	24.3 10.7

* mg C of <oven-dried> - mg C of <fresh>.

Table 4. The amounts of nitrogen mineralized from soil organic matter in soils treated with different amounts of $\text{NH}_4\text{-N}$.

Amounts of $\text{NH}_4\text{-N}$ added	(mg N/200 g Fresh soil)			
	Incubation period (days)			
	2	5	10	17
Fresh soil				
0 mg/100 g soil			2.4	3.2
		0.6		4.6
30	tr. 6.4	1.0	3.2	4.2
60	0.4	0.4	0.4	4.0
Oven-dried soil				
0	3.8	4.2	7.2	7.8
15	2.8	4.0	5.6	6.2
30	1.6	2.2	5.0	6.0
60	0.2	1.2	5.0 1.6	5.4

in the fresh soil (see Table 2). These results suggest that ammonium ion may be harmful to the mineralization of soil organic matter at high concentration, and this harmful effect may occur due to an interference of the growth of soil microorganisms by ammonium ion. Further, this interference may be found on account of disturbance of metabolism of soil microorganisms by toxicity of ammonium ion accumulated in their cells as made clear with plant by Harada *et al.* (1968). Kai *et al.* (1969) found out that nitrification was repressed at high concentration of ammonium ion in soil solution and showed that this occurs due to an interference of the growth of nitrobacter by ammonium ion.

From these results, it is assumed that though when ammonium nitrogen is added to soil with suitable amount, this form of nitrogen is preferable nutrient source for soil microorganisms and then promotes the mineralization of soil organic matter, when ammonium nitrogen is added to soil in excess, this form of nitrogen disturbs the metabolism of soil microorganisms and then represses the mineralization of soil organic matter.

Experiment 2

In Experiment 2, the following studies were carried out : (1) the effect of addition of ammonium nitrogen on the mineralization of rice straw added to soil, (2) the same effect on the accumulations of total organic matter and such a organic matter as becoming decomposable through the drying effect during the mineralization of the rice straw added to soil with different levels of ammonium nitrogen.

(1) *Studies on the mineralization of carbon of the rice straw added to both sand and M-soil with different levels of ammonium nitrogen*

The amounts of carbon evolved as CO₂ from the rice straw during the incubation period at various time intervals are shown in Table 5. The rates of mineralization of carbon at each interval are calculated and shown in Table 6.

As seen in Table 6, the rate of decomposition of the rice straw was the largest at the earliest stage of the incubation period and became small in both the soil and sand as incubation time went on. At the earlier stage, in the case of the sand, the rate of decomposition of the rice straw was the largest in that

Table 5. The amounts of carbon evolved as CO₂ from rice straw in soils and sands treated with the different amounts of NH₄-N.

Amounts of NH ₄ -N added	Incubation period (days)						(C mg/100g soil)		
	2	6	10	20	30	50	70	85	Rate of min- eralization
N mg/100 g	Sand						%		
Gil									
0	90	157	210	308	408	547	640	690	51.3
20	105	220	306	413	474	571	630	670	49.5
60	80	180							
120	60	160	251	312	385	476	482	534	612
							400	400	
							706	457	757
								483	56.0
									35.7
M-soil									
4.9	100	185	260	362	431	499	560	605	44.7
20	115	230	335	446	503	588	647	686	50.7
60	118	242	323	431	480	584	665	711	52.6
120	90	230	308	400	476	590	680	725	53.6

treated with 20 mg of ammonium nitrogen per 100 g sand, next in that with 60 mg ammonium nitrogen and the smallest in that with 120 mg ammonium nitrogen. In the case of the M-soil, it increased in the following order ; 60>20>120) 4.9 mg ammonium nitrogen per 100 g soil. At the later stage of the incubation period, it increased in the following order ; 60>0>20>120 mg ammonium nitrogen per 100 g sand in the case of sand, and 120>60>20> 4.9 mg ammonium nitrogen per 100 g soil in the case of the M-soil.

Table 6. The rate of mineralization of carbon from rice straw, at each interval of the incubation period.

Amounts of NH ₄ -N added N mg/100 g soil	(C mg/100 g soil/day)							
	Incubation period (days)							
	0-2	2-6	6-10	10-20	20-30	30-50	50-70	70-85
	Sand							
0				9.8		6.9	4.7	3.3
20	45.0 32.5	28.7 16.7	21.5 13.2	10.7	10.0 6.1	4.9	3.0	2.7
60	40.0	25.0	17.7	13.4	9.7	6.5	4.7	3.4
120	30.0	25.0	13.0	6.4	4.8	3.8	2.9	1.7
	M-soil							
4.9	50.0	21.2	18.7	10.2	6.9	3.4	3.1	3.0
20	57.5	28.7	26.2	11.1		4.2	3.0	
60					5.7	5.2	4.1	2.6
120	50.0 45.0	31.0 35.0	20.2 19.3	10.8 9.2	4.9 6.6	5.7	4.0	3.1 3.0

At the earlier stage of the incubation period, the rate of decomposition of the rice straw was larger in the M-soil than in the sand in all cases. At the later stage it was larger in the sand than in the M-soil, except the sand and soil treated with 120 mg ammonium nitrogen per 100 g soil or sand.

From these results, the following matters are pointed out ; the decomposition of plant residue like rice straw in soil is accelerated by addition of ammonium nitrogen, but when its concentration is high, the decomposition is repressed. The repression of the decomposition by ammonium ion is more severe at the early stage of the incubation period, at which soil organisms develop rapidly, than at the later stage of the incubation period. When the application amounts of ammonium nitrogen are comparatively large, soil clay reduces the concentration of ammonium ion in soil solution and consequently the repression of decomposition of plant residue by toxicity of ammonium ion is avoided.

Next, the growth of fungi at the early stage of the incubation period was observed with the naked eye and the following results were obtained ; kinds of fungi grown at the early stage of the incubation period varied with the addition amounts of ammonium nitrogen and kinds of medium. These results are shown in Table 7. These results also suggest that the growth of each fungus is affected by the concentration of ammonium ion in soil solution. Particularly, in such a condition that the concentration of ammonium ion in soil solution may be high the growth of fungi is repressed.

(2) *Studies on the accumulation of total organic matter and such a organic matter as becoming decomposable through the drying effect during the mineralization of rice straw added to soil with different levels of ammonium nitrogen*

a) Accumulation of total organic matter

After 85-day incubation, the amounts of carbon and nitrogen accumulated in organic form in the M-soils and sands were determined. The results obtained are shown in Table 8. Carbon/nitrogen ratios of organic matters accumulated in the soils and sands is calculated and also shown in the same Table.

Table 7. The kinds of fungi growing at the early stage of decomposition of rice straw in the soils and sands treated with the different amounts of $\text{NH}_4\text{-N}$.

(After 6 days)				
Amounts of $\text{NH}_4\text{-N}$ added	Sand		M-soil	
0	Pen	†	Rhi	±
4.9				
20	Pen	††	Rhi	±
60	Asp Pen	†† (Fus)	Rhi	±
120	Asp pen	†† (Fus)	Rhi	††

Pen : **Penicillium.**

Asp : **Aspergillus.**

Rhi : **Rhizopus.**

Fus : **Fusarium.**

From these results, the following matters are pointed out ; the larger the amount of ammonium nitrogen added, the larger the amount of nitrogen accumulated in organic form, and the lower the rate of accumulation of nitrogen in both the soil and sand. The amount of nitrogen accumulated in organic form was larger in the M-soil than in the sand and the C/N ratio of organic matter accumulated was narrower in the M-soil than in the sand in all cases.

b) Accumulation of such a organic matter as decomposable through the effect of drying a soil

Table 8. The amounts of carbon and nitrogen accumulated in organic form and C/N ratios of organic matter accumulated in the soils and sands treated with different amounts of $\text{NH}_4\text{-N}$.

Amounts of $\text{NH}_4\text{-N}$ added N mg/100 g soil	Carbon accumulated C or N mg/100 g soil	Nitrogen accumulated	C/N
Sand			
0	669	21.5	30.8
20	682	34.8	39.5
60			
120	809.795	40.3 52.4	14.8 36.6
M-soil			
4.9	747	26.8	27.8
20	666	40.5	16.4
60			
120	641.627	742.58.3	11.0 8.5

The amounts of carbon evolved as CO₂ from the oven-dried and fresh soils including the sands during the period of 15 days after 70-day incubation are shown in Table 9. The amounts of the organic matter becoming decomposable through the drying effect which were accumulated in the soil and sand were calculated and shown in the same Table.

Table 9. The amounts of such a organic matter as becoming decomposable through the effect of drying a soil accumulated in the soils and sands treated with different amounts of NH₄-N.

Amounts of NH ₄ -N added N mg/100 g soil	Carbon ac- cumulated (A)	(C mg/100 g soil)			
		Amounts of Carbon miner- alized for 15-days incubation		(B)-(C)*	(B)-(C)** (A)
		Oven-dried	Fresh		
		(B)	(C)		
Sand					
0	712	61	50	11	1.5
20	722	47	40	7	1.0
60	646	55	51	3	0.5
120	895	25	26	-1	-0.1
M-soil					
4.9	792	52	45	7	0.9
20	705	55	39	16	2.3
60	687	64	46	18	2.6
120	672	67	45	22	3.3

* The amounts of the organic matter becoming decomposable through the drying effect.

** The percentage of total organic matter accumulated in the soils and sands.

As seen in Table 8, the larger the amounts of ammonium nitrogen added, the smaller the magnitude of flush decomposition due to the drying effect in the case of the sand, but the larger it in the case of the M-soil. The magnitude of flush decomposition in the sand treated with 120 mg ammonium nitrogen was negative. In this connection, the amount of carbon evolved as CO₂ from the sand which was treated with 120 mg ammonium nitrogen, incubated for 70 days and dialyzed against the distilled water was compared with that non-dialyzed. The result obtained is shown in Table 10. The amount of carbon evolved as CO₂ increased with the treatment of dialysis and the magnitude of flush decomposition of carbon became positive. But this findings were not com-

Table 10. The amounts of carbon mineralized in the sand treated with 120 mg NH₄-N and dialyzed against the distilled water after 70-days incubation.

	(C mg/100g soil)	
	Amounts of carbon mineralized for 15 days incubation	
	Oven-dried	Fresh
Non-dialyzed	25	26
Dialyzed	41	38

parative with that treated with small amounts of ammonium nitrogen.

Relation-ship between the organic matter which is shown as the percentage of the total organic matter accumulated in the soils and sands and the amounts of nitrogen accumulated in organic form is shown in Figure 3. In the case of the M-soil, the larger the amounts of nitrogen accumulated in organic form, the higher the organic matter percentage of the total organic matter accumulated in the soil as same as shown in the previous paper (Yoshida *et al.*, 1972). But in the case of the sand, the opposite tendency was observed. These results suggest that the high concentration of ammonium ion in soil solution may be harmful to the decomposition of the organic matter accumulated during the mineralization of the rice straw and to the accumulation of such a organic matter as becoming decomposable through the drying.

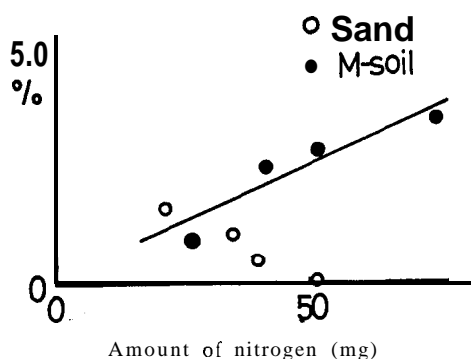


Fig. 3. Relation-ship between the organic matter which is shown as the percentage of the total organic matter accumulated in the soils and sands and the amounts of nitrogen accumulated in organic form.

From the results obtained by chemical determination on the source of flush decomposition due to the drying effect, Hayashi *et al.* (1969) presume that the main origin of organic matter becoming decomposable through the drying effect is kinds of peptide complex substances and the structural proteins which may have been originated from the cell walls of soil microorganisms and the plant and animal residues. Salter (1931) shows that the amounts of cell residues and metabolic products of soil microorganisms which are accumulated in soil increases with the increasing amounts of nitrogen added to soil. These suggest that the amounts of the organic matter becoming decomposable through the drying effect increase with the increasing amounts of nitrogen added to soil. Aomine *et al.* (1946) showed plactically that the drying effect was smaller in the soils of paddy fields applied with only P and K fertilizers, i. e., without N fertilizer, than in the soil applied with N, P and K fertilizers, and stated that application of nitrogen fertilizer to the fields not only gave nitrogen nutrient to the plants, but also increased soil fertility of the fields. We also obtained the same result in the experiment in which the M-soil was used as shown in Figure 3. But in the experiment in which the sand was used, the tendency was opposite. This means that the high concentration of ammonium ion in soil solution hinders

the accumulation of the organic matter becoming decomposable through the drying effect.

Experiment 3

In Experiment 3, studies on the effect of addition of ammonium sulfate on the mineralization of rice straw added to liquid solution were carried out by using water culture under shaking condition. Besides, similar to Experiment 2, accumulations of total organic matter during the mineralization of rice straw added to the culture solution with different levels of ammonium nitrogen.

(1) *Mineralization of rice straw carbon in water culture*

The amounts of carbon evolved as CO₂ at various time intervals are shown in Table 11. The carbon mineralized during 85-day incubation which is shown as the percentages of rice straw carbon added is also shown in this Table. The rates of mineralization of carbon at each stage of the incubation period are calculated and shown in Table 12.

Table 11. The amounts of carbon evolved as CO₂ from rice straw in water cultures treated with different amounts of NH₄-N.

Concentration of NH ₄ -N	(C mg, 4.00 g straw)							
	Incubation period (days)					Rate of mineralization		
	3	6	10	30	50	70	85	
0 ppm	43	85	140	340	468	565	637	47.0 %
50	106	149	200	396	500	579	64%	47.4
100	98	190	227	432	540	610	650	48.0
200	54	57	147	320	443	535	590	33.6
400	26	-	93	267	381	493	572	42.3
800	12	26	60	210	345	460	541	40.0

Table 12. The rate of mineralization of rice straw carbon at each stage of incubation period.

Concentration of NH ₄ -N	(Cmg/4.00 g straw, day)						
	Incubation period (days)						
	0-3	3-6	6-10	10-30	30-50	50-70	70-85
50 ppm	14.3	14.0	13.8	9.8	6.4	4.9	4.8
100	35.3	32.7	23.7	14.3	12.8	10.3	4.2
			17.0	8.7	5.4	4.6	2.7
200	18.0	12.0	14.3	8.7	6.2	5.6	3.7
400	8.7	10.3	9.0		5.7		
800	4.0	4.7	8.7	7.5	6.8	5.8	53.4

The rate of mineralization of carbon was the largest in the cases treated with 100 and 50 ppm of ammonium nitrogen and became smaller when the concentration of ammonium ion in the solution became higher. This tendency was especially remarkable at the early stage of the incubation period. The mineralization carbon percentage after 85-day incubation increased in the same order as seen in the rates of mineralization of carbon at the early stages.

From these results, the following matters are pointed out; if ammonium ion

is supplied to microbe with the low concentration below 200 ppm nitrogen, it accelerates the decomposition of the rice straw, but the decomposition of the rice straw is repressed by such a supply of ammonium ion that the concentration of ammonium nitrogen in the solution is more than 200 ppm and the degree of the repression is very marked at the early stage of the incubation period at which the microbe develop rapidly.

(2) *Accumulation of carbon and nitrogen in organic form*

The amounts of carbon and nitrogen accumulated in organic form and the C/N ratios of organic matter accumulated after 85-day incubation are shown in Table 13.

Table 13. The amounts of carbon and nitrogen accumulated in organic form and C/N ratios of organic matter accumulated.

Concentration of NH ₄ -N ppm	Carbon accumulated mg/1000 ml water	Nitrogen accumulated		C/N
		Total	water soluble	
0	715	22.2	trace	32.2
50	710	63.0	4.2 ^a	11.3
100	702	94.6	23.3	7.4
200	762	352.8		5.0
400	780	141	60.7	5.5
800	811	157	94.1	5.2

The amounts of nitrogen accumulated in organic form varied according to the concentrations of ammonium nitrogen in the solution and the higher the concentrations of ammonium ion, the larger the amounts of nitrogen accumulated in the organic form. But when the concentration of ammonium ion in the solution was more than 200 ppm, a large part of nitrogen was accumulated as the organic nitrogen soluble in water. This result suggests that the nitrogen metabolism of soil microbe becomes abnormal at such a high concentration of ammonium nitrogen as more than 200 ppm.

The higher the concentrations of ammonium ion in the solution within 200 ppm, the narrower the C/N ratios of organic matter accumulated after 85-day incubation. But there was no significant difference among the C/N ratios of the organic matters accumulated at such a high concentration of ammonium nitrogen as more than 200 ppm.

Experiment 4

In Experiment 4, three kinds of fungus, which appeared in Experiment 2, were grown separately or coexistently in the rose bengal agar media with from 10 to 1600 ppm N of both ammonium nitrogen and nitrate nitrogen and the effects of ammonium ion and nitrate ion on the growth of the fungi were compared.

Relative values of the growth of each fungus in the medium treated with different levels of ammonium nitrogen are shown in Table 14. The word of

“Separateness” written in the Table means that each fungus examined were inoculated separately into the medium. The word of “Coexistence” means that all the fungus examined were inoculated together.

Table 14. Growth of fungi in medium treated with different amount of ammonium sulfate.

Concentration of $\text{NH}_4\text{-N}$	<i>Aspergillus</i>	<i>Penicillium</i>	<i>Rhizopus</i>
Separateness			
10 ppm	+	+	++
50	++	+++	+++
100	+++	+++	++
200	+++	+++	+
400	+++	+++	+
800	+++	+++	+
1600	+	+	+
Coexistence			
50	—	—	+++
10	—	—	+++
200	+++	+++	—
800	+++	+++	—

As seen in the Table 14, in the case of “Separateness” the optimum concentration of ammonium ion to the growth of fungus varied with the variety of fungus examined. The higher the concentration of ammonium ion in the medium up to **200** ppm, the larger the growth of *Aspergillus*. The optimum concentration of ammonium ion to the growth of *Penicillium* was **100** ppm and that of ammonium ion to the growth of *Rhizopus* was **50** ppm. In the case of “Coexistence”, at such a low concentration of ammonium ion as less than **50** ppm, only *Rhizopus* grew and both *Aspergillus* and *Penicillium* could not grow. Whereas, at such a high concentration of ammonium as over **200** ppm, both *Aspergillus* and *Penicillium* grew and only *Rhizopus* could not grow.

These results suggest that both *Aspergillus* and *Penicillium* prefer to the relatively high concentration of ammonium ion and *Rhizopus* does to the relatively low concentration of ammonium ion and that when ammonium ion is such a high concentration as more than **200** ppm, it becomes harmful to the growth of all the fungi examined. From the results obtained in this experiment, it might be assumed that in the case of Experiment 2 the ammonium ion concentration in the M-soil treated with **120** mg ammonium nitrogen per **100** g soil corresponded to below **50** ppm N in the medium used in this experiment and that in the sand treated without ammonium nitrogen it corresponded to over **50** ppm, because in the Experiment 2 the fungi which appeared in the M-soil treated with **120** mg ammonium nitrogen and the sand treated without mineral nitrogen were *Rhizopus* and *Penicillium*, respectively (see Table 7).

Relative value of the growth of each fungus in medium treated with nitrate nitrogen is shown in Table 15. In the case of medium treated with nitrate nitrogen differing from the case of medium treated with ammonium nitrogen, the higher the concentration of nitrate ion in the medium up to **1600** ppm, the larger the growth of all the fungi examined.

Table 15. Growth of fungi in medium treated with different amount of potassium nitrate.

Concentration of NO ₃ -N	Asp.	Pen	Rhi.
10 ppm	+	+	+
50	++	++	++
200	+++	+++	+++
400	++++	++++	++++
800	++++	++++	++++
1600	++++	++++	++++

Experiment 5

In Experiment 5, the following studies were carried out, comparing ammonium sulfate with ammonium nitrate ; (1) the effects of applications of ammonium nitrate and ammonium sulfate on the mineralization of rice straw added to different soils including sand, (2) the same effects on the accumulation of the total organic matter and such a organic matter as becoming decomposable through the drying effect during the mineralization of rice straw added to the soils with ammonium nitrate or ammonium sulfate.

(1) *Studies on the mineralization of carbon of rice straw added to different soils including M-soil, H-soil, A-soil and sand treated with ammonium nitrate or ammonium sulfate*

The amounts of carbon evolved as CO₂ from rice straw are shown in Table 16 and Figure 4. The rates of mineralization of carbon at each stage of the incubation period were calculated and shown in Table 17.

As seen in Table 16 and Figure 4, in the case of the sand, the H-soil and the M-soil, the decomposition of rice straw was larger in the trials treated with ammonium nitrate than in those treated with ammonium sulfate, but in the case of the A-soil, ammonium sulfate was superior to ammonium nitrate for the straw decomposition. The difference in the straw decomposition between the ammonium nitrate and the ammonium sulfate was the largest in the sand, next in the H-soil and the smallest in the M-soil. As seen in Table 17, the difference in the rates of the straw decomposition between these two treatments was the

Table 16. The amounts of carbon evolved as CO₂ from rice straw in the soils and sand treated with ammonium or nitrate nitrogen.

		(C mg/100g soil)						
Soils	C	Incubation period (days)						Rate of min- eralization (%)
	6	10	20	40	70	84		
NH ₄ -N								
Sand	106	258	330	424	489	628	660	48.8
H-soil	31	161	253	359	481	601	636	47.0
A-soil	86	322	419	560	683	776	776	57.3
M-soil	102	232	310	390	470	753	5%	41.0
NO ₃ -N								
Sand	78	183	291	588	724	796	832	61.5
H-soil	35	180	329	500	593	670	709	52.4
A-soil	16	161	301	533	607	637	637	50.4
M-soil	90	240	312	420	500	550	602	43.3

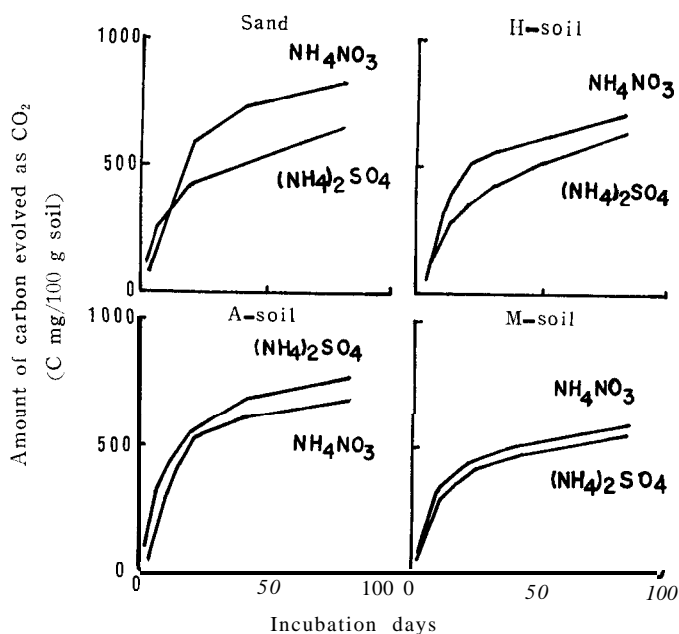


Fig. 4. Mineralization of straw carbon in soil treated with $(\text{NH}_4)_2\text{SO}_4$ or NH_4NO_3 .

Table 17. The rate of mineralization of carbon as CO_2 from rice straw at each interval of the incubation period.

Nitrogen source	Incubation period (days)		
	0-20	20-40	40-84
Sand			
$\text{NH}_4\text{-N}$	21.2	2.3	3.6
$\text{NO}_3\text{-N}$	29.4	6.8	2.4
H-soil			
$\text{NH}_4\text{-N}$	18.0	6.1	3.5
$\text{NO}_3\text{-N}$	25.0	4.7	2.6
A-soil			
$\text{NH}_4\text{-N}$	28.0	6.3	2.1
$\text{NO}_3\text{-N}$	26.7	3.7	1.7
M-soil			
$\text{NH}_4\text{-N}$	19.5	4.0	1.9
$\text{NO}_3\text{-N}$	21.0	4.0	2.0

largest at the early stage of incubation period in all cases. The predominance of ammonium nitrate over ammonium sulfate for the straw decomposition in each soil may be explained on the basis of considering about toxicities of ammonium ion to the decomposition of the rice straw and the actions of clay to adjust ammonium ion to the right concentration to soil microorganisms. The inferiority of ammonium nitrate to ammonium sulfate in the A-soil occurs due to the adsorption of nitrate ion through the large AEC of allophane contained

(Singh *et al.*, 1969). The predominance of ammonium nitrate over ammonium sulfate in the M-soil having large CEC occurs on account of the higher nitrogen supplying concentration. The predominance of ammonium nitrate over ammonium sulfate in the sand and the H-soil having small CEC occurs due to the lower concentration of ammonium ion in the solution. The effects of the soil clays on the nitrogen supplying concentration to soil microorganisms will be reported in detail in other paper.

(2) *Accumulations of the total organic matter and such a organic matter as becoming decomposable through the effect of drying a soil*

The accumulation amounts of the total organic matter and such a organic matter as becoming decomposable through the drying effect in the soils and sands are shown in Table 18. As seen in Table 18, in the sand and the H-soil having small CEC, the accumulation amount of the organic matter becoming decomposable through the drying effect was larger in the ammonium nitrate than in the ammonium sulfate. In the M-soil having large CEC, it was also larger in the ammonium nitrate than in the ammonium sulfate. In the A-soil containing allophane, there was not very large difference in the accumulation amount of the organic matter between two treatments.

Table 18. The amounts of the organic matter becoming decomposable through the drying effect which were accumulated in the soils and sands treated with different amounts of $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$.

Soils	Carbon ac- cumulated (A)	Amounts of carbon miner- alized for 14-day incubation		(C mg/100g soil)	
		Oven-dried	Fresh	(B)-(C)*	(B)-(C)** (A) (%)
		(B)	(C)		
NH ₄ -N					
Sand	724	68	32	22	3.0
H-soil	762	92	35	53	4.3
A-soil	607		31	61	10.0
M-soil	824	116	27	99	12.0
NO ₃ -N					
Sand	556	72	36	36	6.5
H-soil	682	324	39	85	12.5
A-soil	695	90	25	63	9.4
M-soil	810	154	29	125	15.4

Since considerably old time, decomposition of plant residues in soil has been studied by numerous workers from various points of view. The studies show that decomposition of plant residues in soil is affected by temperature (Waksman *et al.*, 1931), moisture content (Greaves *et al.*, 1920, Waksman *et al.*, 1931), texture, type and content of clay (Allison *et al.*, 1949, Aomine *et al.*, 1956, Estermann *et al.*, 1959, Lynch *et al.*, 1956, Pinck *et al.*, 1951 and 1954), pH (Stotzky *et al.*, 1966), contents of available nitrogen and other nutrients for soil microorganisms (Allison *et al.*, 1949, Aomine *et al.*, 1956), chemical composition of plant residues added to soil (Broadbent *et al.*, 1948, Jenkinson, 1965) and so on. The results obtained by our experiments show that the concentration of ammonium

ion in soil solution is one of the important factors affecting decomposition of plant residues in soil. Namely, when the concentration of ammonium ion in soil solution is higher than 200 ppm, the growth of soil microbes concerned with decomposition of plant residues is prevented and the decomposition is remarkably retarded. Usually C/N ratio of organic matter accumulated under this condition is narrow (see Experiment 3). So, at first sight the decomposition of plant residues seems to be in progress, But in fact, the nitrogen metabolism of soil microbes is disturbed and the organic nitrogen soluble in water is accumulated in large quantities (see Experiment 3). Under such a condition the significance of the existence of clay in soil is to reduce concentration of ammonium ion in soil solution to a low concentration and weaken toxicity of ammonium ion to soil microbes.

Numerous investigators have pointed out that the main reason why clay accelerates the decomposition of organic matter applied to soil is that clay has a buffer action to pH change during the decomposition process of organic matter (Stotzky *et al.*, 1966). However, the rice straw used in our experiments contained bases in large quantities as shown in Table 1 which are released more and more with the increasing decomposition of the rice straw, and consequently differing from the decomposition of organic substance like glucose, a lowering of pH value due to both the organic acids produced by soil microbes and the sulfate ion remaining in soil solution after the ammonium ion is absorbed by soil microbes did not occur. As an example, changes of pH value during the decomposition process of the rice straw in the sand is shown in Table 19. Such a great change of pH value that is assumed to affect the growth of soil microbes was not observed as seen in Table 19.

Table 19. Changes of pH value during the decomposition process of rice straw in the sands treated with different amounts of ammonium nitrogen.

Amount of mg/100g	Incubation period (days)							
	0	2	5	7	14	21	25	60
0								
20	6.20 6.20	6.19 6.35	6.22 6.63	6.43 6.58	6.49 6.12	6.20 6.12	6.30 6.34	6.20 6.10
60	6.20	6.18	6.52	6.49	6.10	6.3.2	6.13	6.10

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