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ABSTRACT

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The dynamic behavior of soil microorganisms related to the nitrogen cycle in the paddy and upland farm soils of Thailand was investigated and was compared with that of Japanese soils. Microflora of tropical soils was found to be different from that of temperate soils.

Microflora showed marked variations among great soil groups, and was found to show seasonal variations. Relationships between populations of microorganisms, and soil chemical and physical properties were discussed. The tropical upland farm soils contained predominantly chromogenic actinomycetes with strong protease and cellulase activities and this trend was particularly evident in soils with low carbon content. In the field experiments, the populations of ammonia oxidizer and denitrifier showed distinctly lower values in Brackish Water Alluvial Soils than in Low Humic Gley Soils during rice growing season. Methane gas was hardly detected in the control plot of the former soils. The application of phosphate or rice straw (6 tons/ha) with and without N-fertilizers affected variously the population of microorganisms related to the change of mineral nitrogen. Following either phosphate or rice straw application, the amount of molecular nitrogen and methane in soil gas increased in both soil groups.

Index words: microflora of tropical soils, actinomycete flora, composition of soil gas

CONTENTS

INTRODUCTION

Among the factors contributing to soil fertility and hence to crop production, an important one is generally considered to be the nitrogen content either in paddy or in upland farm soils, irrespective of climatic zones. The amount and form of available soil nitrogen which are regulated by the metabolism of numerous microbial groups related to the nitrogen cycle are known to show variations depending on soil groups, soil layers and seasons.[44, 65] In order to understand the behavior of soil nitrogen, it is important to study the chemical and physical properties of the soil which condition the behavior of soil nitrogen and it is also necessary to clarify the dynamic behavior of soil microorganisms related to the nitrogen cycle. In tropical soils, however, only a few studies on this aspect have been made so far.[48, 59] Therefore, the authors investigated microflora related to the nitrogen cycle in the tropical paddy and upland farm soils.

The nitrogen contents in the tropical upland farm soils are generally low compared with those in Japanese soils. This fact has been thought to be due mainly to the high temperatures prevailing in the tropics.[42] The decomposition of organic matter which is enhanced by high temperature, proceeds at a much faster pace in the tropical than in the temperate region.[39] However, it is also considered that microflora related to the decomposition of organic matter might be different between tropical and temperate soils. On the other hand, actinomycetes are the most important agents responsible for the decomposition of organic matter against microbial attack, in upland soils. Therefore, the composition of actinomycetes in soils of the two regions was compared.

Recently, various soil management procedures such as the use of rice straw and fertilizer etc. have been applied to increase rice yield, but even in the tropical areas where soils are low in nutrient content, the effect of rice straw application on the yield of rice has not yet been clarified.[20, 83, 89] The reason why rice straw is not used as a substitute for fertilizer in tropical areas remains unknown. Therefore, the effects of rice straw on the composition of volatile soil gas and microflora related to the change of mineral nitrogen in tropical paddy soils were investigated, too.

I Microflora related to the nitrogen cycle in the tropical paddy soils

Microflora was examined immediately after collecting soil samples from farmers' paddy fields located in Thailand. The following aspects were investigated: 1. the number of microorganisms present in the submerged top (0-1 cm) and subsurface (1-10 cm) soils under rice cultivation; 2. the change of microbial populations depending on the season; 3. the variations of populations depending on the great soil groups; 4. the difference in populations between soils in Thailand and in Japan.

Materials and methods

Soil samples. Paddy soil samples were collected from top (0-1 cm) and subsurface (1-10 cm) layers at 103 sites of Thailand, as shown in Fig. 1. Collections were made twice, in the middle of the rainy season and in the middle of the dry season. In the former case, rice plants were growing under submerged condition, while in the latter case, no crops were cultivated. A total of about 2 Kg of each soil sample was gathered from 3 to 4 areas in each large field to eliminate fluctuations.

Microbial analysis. Prior to microbial analysis each soil sample was thoroughly mixed so as to become homogeneous. Counts of aerobic bacteria, actinomycetes, anaerobic bacteria, denitrifier and cellulose decomposer were made in using the method reported in the literature.[97] Ammonifier was counted using Nessler's reagent after two weeks' incubation in a medium containing 20.0 g

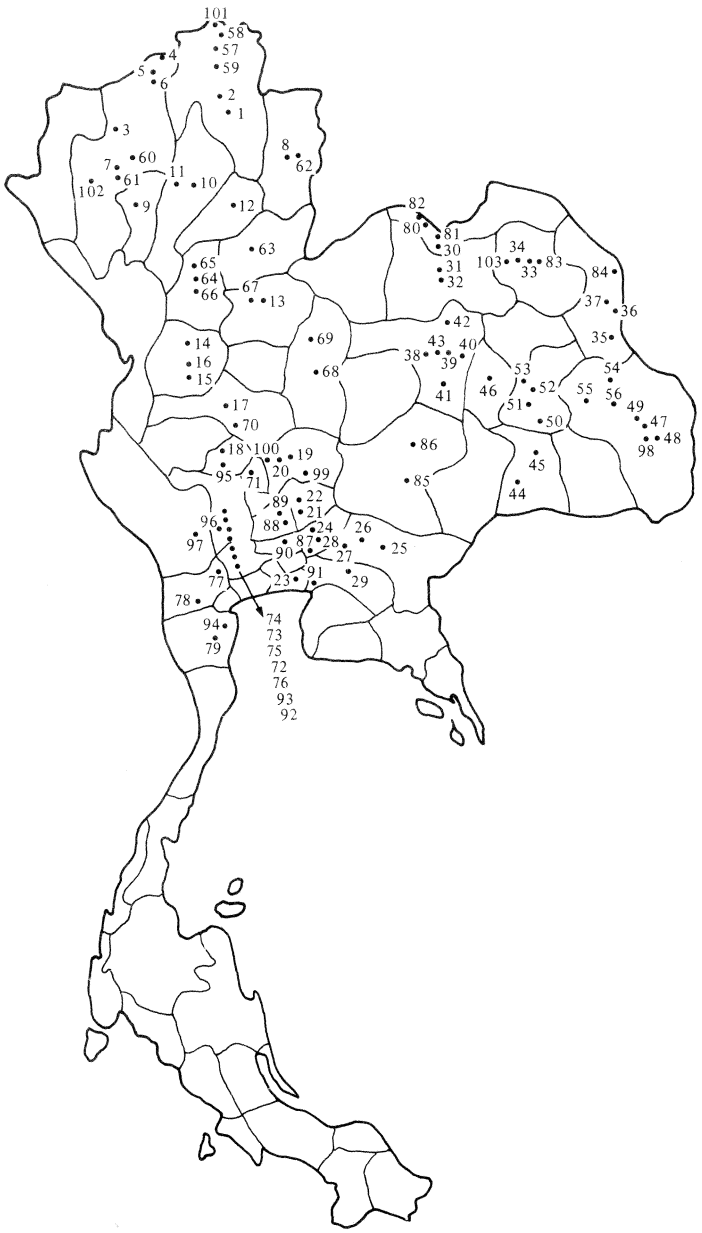

Fig. 1. Sites of soil samples taken in Thailand.

urea, 5.0 g meat extract, 1.0 g K_2HPO_4, 0.1 g $CaCl_2$, 0.3 g $MgCl_2 \cdot 6H_2O$, 0.1 g NaCl and trace of $FeCl_3 \cdot 6H_2O$ in 1,000 ml of distilled water. Ammonia oxidizer; A solution—1.0 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 2.0 g NaCl, Each 0.5 ml of 0.1% solution of $FeSO_4 \cdot 7H_2O$, $Na_2MoO_4 \cdot 2H_2O$ and of $CuSO_4$, 0.1 g $NaClO_3$, 10.0 g $CaCO_3$, Dist. Water 900 ml. B solution—1.0 g $(NH_4)_2SO_4$, Dist. Water 100 ml. Mix A and B solutions just before incubation. The formation of nitrite was determined using α-naphthylaminesulfanilic reagent after incubation at room temperature for 4 weeks. The formation of purple color indicates the presence of ammonia oxidizer. Nitrite oxidizer; A solution is the same as A solution of ammonia oxidizer except for the absence of $NaClO_3$. B solution 6 mg $NaNO_2$, Dist. Water 100 ml. Mix A and B solutions just before incubation. Judgement is the same as that for ammonia oxidizer but the formation of purple color indicates the absence of nitrite oxidizer. Nitrogen-fixing blue green algae, *Clostridium*, purple nonsulfur bacteria and *Azotobacter* were counted each using KRATZ and MYERS' liquid medium,[51] ROSS' agar medium,[72] KOBAHASHI's liquid medium[47] and BROWN's agar medium,[13] respectively.

Results

1 Populations of each group of microorganisms in top and subsurface soils (rice growing period in the rainy season)

As shown in Table 1, among aerobic bacteria, anaerobic bacteria, actinomycetes and cellulose decomposer, the population level of aerobic bacteria was highest, amounting to 10^7 to 10^6 per 1 g of dry soil, and was followed by actinomycetes (10^6 to 10^5) and anaerobic bacteria (10^5), while that of cellulose decomposer was lowest (10^4).

Among the ammonifier, ammonia oxidizer, nitrite oxidizer and denitrifier populations related to the nitrogen metabolism, denitrifiers showed the largest population (10^5) followed by ammonifier (10^5 to 10^4) and nitrite oxidizer (10^4), while ammonia oxidizer showed the lowest level, namely 10^3.

Among nitrogen-fixing blue green algae, *Azotobacter*, purple nonsulfur bacteria and *Clostridium* which are non-symbiotic nitrogen fixers, *Clostridium* showed the largest population (10^5 to 10^4), while in the other three groups a level of 10^3 was recorded.

Populations of all twelve groups examined were largest in the oxidized top soil which contains a larger amount of nutrients than that in the subsurface soil. Especially, the populations of nitrite oxidizer and purple nonsulfur bacteria in the top soil were more than 4 times higher than those in the subsurface soil. The populations of aerobic bacteria and nitrogen-fixing blue green algae in the top were also twice as high as those in the subsurface soil.

2 Comparison of the population level of microorganisms between the rainy and dry season

As shown in Table 1, among the twelve groups of microorganisms investigated, only the population of cellulose decomposer significantly increased in the dry season. The population of the other microorganisms decreased in the dry season though the range of the decrease showed some variations among microbial groups.

The largest decrease was recorded in the population of nitrite oxidizer which dropped to the level of 1/26 especially in the top soil, but only to 1/5 in the subsurface soil, and was followed by the population of purple nonsulfur bacteria (1/15 in the top and 1/11 in the subsurface soil). The population of ammonifier largely decreased also in the dry season to the level of 1/10 in the top soil and 1/9 in the subsurface soil, and populations of both denitrifier and ammonia oxidizer noticeably decreased in the dry season. Populations of aerobic bacteria and actinomycetes decreased slightly in the dry season, while those of anaerobic bacteria decreased significantly in the dry season.

Table 1. Comparison of population of microorganisms in paddy soils between rainy (water logged-condition, rice growth) and dry season (dry condition, no crop)

(Counts per gram of dry soil)

Microorganisms	Sampling depth (cm)	Rainy season Soil samples	Rainy season Average	Rainy season S.D.	Dry season Soil samples	Dry season Average	Dry season S.D.	Difference	Rainy / Dry
Aerobic bacteria	0— 1	101	127	113	103	119	218	7.9	1.07
(× 10^5)	1—10	100	50.4	50.8	103	43.8	78.3	6.6	1.16
Anaerobic bacteria	0— 1	100	4.68	5.11	103	2.81	4.92	1.87***	1.67
(× 10^5)	1—10	99	3.78	4.40	103	2.40	4.15	1.38**	1.58
Actinomycetes	0— 1	101	47.0	31.7	103	45.8	74.1	1.2	1.03
(× 10^5)	1—10	100	36.0	60.1	103	27.7	36.1	8.3	1.30
Cellulose decomposer	0— 1	100	27.7	89.4	103	49.8	62.2	22.1*	0.56
(× 10^3)	1—10	100	19.3	35.0	103	34.0	53.1	14.7*	0.57
Ammonifier	0— 1	101	112	224	103	10.9	16.4	101*****	10.3
(× 10^3)	1—10	101	72.5	95.5	103	8.20	12.8	64.3*****	8.84
Ammonia oxidizer	0— 1	101	2.95	6.30	103	0.748	1.98	2.20****	3.94
(× 10^3)	1—10	101	2.68	5.77	103	0.879	3.20	1.80***	3.05
Nitrite oxidizer	0— 1	100	53.3	135	102	2.08	4.08	51.2*****	25.6
(× 10^3)	1—10	101	13.4	43.6	102	2.60	5.28	10.8**	5.15
Denitrifier	0— 1	91	917	1910	103	238	807	711****	4.45
(× 10^3)	1—10	91	573	1310	103	129	730	435***	4.37
Nitrogen-fixing blue	0— 1	100	8.63	12.3	103	5.45	6.37	3.18**	1.58
green algae (× 10^3)	1—10	73	3.58	6.41	103	2.75	4.22	0.83	1.30
Azotobacter	0— 1	101	1.58	8.96	103	0.202	1.01	1.38	7.82
(× 10^3)	1—10	74	1.26	7.51	103	0.372	1.92	0.89	3.39
Purple nonsulfur	0— 1	100	4.05	7.93	103	0.261	1.19	3.79*****	15.1
bacteria (× 10^3)	1—10	73	1.18	3.88	103	0.100	0.303	1.08**	11.8
Clostridium	0— 1	101	178	165	103	120	191	58**	1.48
(× 10^3)	1—10	74	95.4	140	103	97.2	144	1.8	0.98

*, **, ***, **** and ***** mean significant at 5%, 2.5%, 1%, 0.5%, 0.1% level, respectively.

Populations of both nitrogen-fixing blue green algae and *Clostridium* significantly decreased in the top soil. The population of *Azotobacter* also decreased in the dry season, though the decrease was not significant.

3 Variations in the populations of microorganisms depending on the great soil groups

As shown in Table 2, the populations of twelve groups of microorganisms except those of anaerobic bacteria, in the top, nitrite oxidizer and purple nonsulfur bacteria recorded the largest value in Fresh Water Alluvial Soils followed by Low Humic Gley Soils, while the smallest value was generally recorded in Brackish Water Alluvial Soils. The population of *Azotobacter*, which is a typical example of the above fact, showed very small values in Brackish Water Alluvial Soils. The populations of aerobic nitrite oxidizer were largest in Low Humic Gley Soils with low organic matter content, followed by Fresh Water Alluvial Soils.

On the other hand, the population of purple nonsulfur bacteria showed the largest value in Brackish Water Alluvial Soils where the populations of other microorganisms were small. The population of ammonifier noticeably recorded a high level in the subsurface soils of Brackish Water Alluvial Soils with high organic matter content compared with those in other great soil groups. The populations of aerobic bacteria, anaerobic bacteria and actinomycetes showed small variations among great soil groups.

As described above, the populations of each microorganism showed different levels among the great soil groups owing to variations in soil properties. Each great soil group displayed a characteristic microflora both in the rainy and dry season.

Table 2. Population of each microorganism group in great soil groups of paddy fields in Thailand in the rainy season

(Counts per gram of dry soil)

	0—1 cm			1—10 cm		
	Sample number	Average	S.D.	Sample number	Average	S.D.
Aerobic bacteria ($\times 10^5$)						
A) L.H.G.	56	102.0	80.2	55	35.9	37.6
B) F.W.A.	30	167.0	144.8	30	71.0	63.8***
C) B.W.A.	4	88.3	77.8	4	32.2	34.2
Anaerobic bacteria ($\times 10^5$)						
A) L.H.G.	55	4.79	5.86	54	3.09	4.02
B) F.W.A.	30	4.75	3.80	30	4.90	5.05
C) B.W.A.	4	3.23	3.92	4	2.88	2.44
Actinomycetes ($\times 10^5$)						
A) L.H.G.	56	42.5	27.9	55	23.9	19.9
B) F.W.A.	30	57.9	38.4	30	57.3	99.9
C) B.W.A.	4	37.0	19.1	4	30.5	12.9
Cellulose decomposer ($\times 10^3$)						
A) L.H.G.	56	19.4	43.7	55	18.0	35.4
B) F.W.A.	30	50.7	149.3	30	24.8	40.6
C) B.W.A.	4	10.4	9.14	4	8.30	5.32

	0—1 cm			1—10 cm		
	Sample number	Average	S.D.	Sample number	Average	S.D.
Ammonifier ($\times 10^3$)						
A) L.H.G.	56	108.8	238.1	56	62.3	79.7
B) F.W.A.	30	151.1	240.7	30	72.6	82.9
C) B.W.A.	4	32.0	30.6	4	307	156
Ammonia oxidizer ($\times 10^3$)						
A) L.H.G.	56	2.41	6.81	56	1.65	4.09
B) F.W.A.	30	3.86	5.11	30	4.19	6.74
C) B.W.A.	4	1.55	2.68	4	0.12	0.202
Nitrite oxidizer ($\times 10^3$)						
A) L.H.G.	56	74.8	162	56	13.9	42.5
B) F.W.A.	29	19.7	65.7*	30	11.6	40.9
C) B.W.A.	4	2.68	2.47	4	0.337	0.387
Denitrifier ($\times 10^3$)						
A) L.H.G.	50	941	2290	50	564	1600
B) F.W.A.	27	1077	1377	27	559	759
C) B.W.A.	4	134	88.2	4	132	125
Nitrogen-fixing blue green algae ($\times 10^3$)						
A) L.H.G.	55	6.45	10.3	43	1.51	2.24
B) F.W.A.	30	12.8	15.0*	20	7.57	8.79***
C) B.W.A.	4	7.52	13.0	4	0.267	0.219
Azotobacter ($\times 10^3$)						
A) L.H.G.	56	0.12	0.298	44	0.025	0.097
B) F.W.A.	30	4.06	15.2	20	3.81	13.7
C) B.W.A.	4	0.005	0	4	0.004	0
Purple nonsulfur bacteria ($\times 10^3$)						
A) L.H.G.	55	4.84	9.58	43	0.648	3.00
B) F.W.A.	30	2.50	4.33	20	1.40	2.81
C) B.W.A.	4	8.68	6.21	4	6.14	10.3
Clostridium ($\times 10^3$)						
A) L.H.G.	56	151	151	44	63.0	71.7
B) F.W.A.	30	240	161**	20	169	207*
C) B.W.A.	4	35.5	54.7	4	26.0	9.67

*, **, *** and **** mean significant between populations in Fresh Water Alluvial Soils and Low Humic Gley Soils, at 5%, 2.5%, 1%, 0.5% level, respectively.

L.H.G., Low Humic Gley Soils; F.W.A., Fresh Water Alluvial Soils; B.W.A., Brackish Water Alluvial Soils.

Table 3. Comparison of microflora in paddy soils between Thailand and Japan

(Counts per gram of dry soil)

Soil condition at site	Thailand[a] (A) Water-logged condition (Rainy season)		(B) Dry condition (Dry season)		Japan[b] (C) — After drainage in autumn)		(D) — (Before irrigation in spring)	
Rice plant	Growing		No		No		No	
Microorganisms	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
Aerobic bacteria ($\times 10^5$)	59.3	53.6	52.2	87.2	292	151	318	168
Anaerobic bacteria ($\times 10^5$)	3.86	4.26	2.47	4.16	21.5	11.1	12.1	7.48
Actinomycetes ($\times 10^5$)	37.1	55.0	29.4	37.2	26.6	25.6	32.9	35.5
Denitrifier ($\times 10^3$)	606	1350	142	664	286	334	200	327
Azotobacter ($\times 10^3$)	1.34	7.82	0.364	1.89	0.389	1.16	0.047	0.089

Number of soil samples were as follows; (A) Aerobic bacteria and Actinomycetes: 100 each, Anaerobic bacteria: 98, Denitrifier: 91, *Azotobacter*: 74, (B) All groups: 103 each, (C) All groups: 21 each, (D) All groups: 10 each.

a) Each microbial count shows the value per gram of dry soil, adjusted to a soil depth ranging from 0 to 10 cm.

b) ISHIZAWA, S. and TOYODA, H. (1964c)

Table 4. Difference in the ratios of aerobic bacteria to actinomycetes among paddy and upland soils in Thailand and Japan under different soil conditions

	Thailand		Japan[a]	
	Water-logged condition (Rainy season)	Dry condition (Dry season)	— (After drainage in autumn)	— (Before irrigation in spring)
Number of soil samples	100	103	21	10
Paddy soils (Aerobic bac./Act.)	1.60	1.78	11.0	9.7
	Autumn (Rainy season)		Autumn	
Number of soil samples	28		26	
Upland farm soils (Aerobic bac./Act.)	1.70		4.9	

a) ISHIZAWA, S. and TOYODA, H. (1964b, 1964c)

4 Comparison of microflora in paddy soils between tropical and temperate zone

As shown in Table 3, microorganisms were counted after drainage and before irrigation in the case of the Japanese soils, while in Thailand they were counted both in the rainy season under submerged conditions and in the dry season. Therefore, it should be emphasized that the soil conditions at sampling time were different in the two countries.

Comparing the data in the dry condition, namely between (B) and (D), the population of *Azotobacter* was higher in the tropical than in Japanese soils, while the populations of aerobic and anaerobic bacteria were smaller in the tropical soils, each of them being less than 1/5 of that in Japanese soils. Therefore, as shown in Table 4, the ratio of aerobic bacteria to actinomycetes in the tropical soils showed a small value, namely 1.78 in the dry season, while that of Japanese paddy soils was about 9.7.

Discussion

Since the number of each microbial group is generally known to show variation during the growing period of rice plants as shown in part VII, paddy soil samples reported in this paper were taken from the middle to the later stages of rice growth. As the sampling time was not identical, it would not be important to discuss small differences in microbial populations. On the other hand, in the dry season soil samples were taken from the middle to the late period of the season and few variations would occur, because the dry season lasted six months. Therefore variations resulting from the sampling time are usually negligible in the dry season.

As the tropical paddy soils contain low organic matter and furthermore are cultivated without application of fertilizer, the nutrient content is generally low. Therefore, the population level of most microorganisms is low, especially that of anaerobic bacteria.

As the organic matter content in tropical paddy soil is low, reduced condition does not markedly develop during the rice growing period. Therefore the growth of anaerobic bacteria is estimated to be depressed. MOTOMURA[65] reported that the Eh value of tropical paddy soil (0-5 cm depth) during the later period of rice growth ranged from +157 to +145 in sandy soils with low organic matter content, and from +88 to +44 even in the heavy clay soils with rather high organic matter content.

On the other hand, the population level of nitrite oxidizer was high in tropical paddy soil and that in the top soil was 4 times as high as that in the subsurface soil. Furthermore, if one considers the variations among the great soil groups, the number of nitrite oxidizer showed the highest value in Low Humic Gley Soils which are characterized by the highest Eh value and the thickest oxidized-layer among the three great soil groups. In contrast, a small number of nitrite oxidizer was obtained in Fresh Water Alluvial Soils with high organic matter content, heavy clay, rather low Eh value and thin oxidized layer, while the populations of nitrite oxidizer in Brackish Water Alluvial Soils with low pH value and low available phosphorus content were the smallest. From the above phenomena it appears that the number of nitrite oxidizers corresponded to the development of an oxidized layer.

Among nitrogen-fixing groups, the population of *Clostridium* showed a high level. MATSUGUCHI *et al.*[59] reported also that the number of *Clostridium* was high in tropical paddy soils and contributed largely to the non-symbiotic nitrogen fixation in these soils. Compared with the value recorded in Japanese paddy soil,[35] the number of *Clostridium* in tropical paddy soil was high. The count of *Clostridium* as in the case of anaerobic bacteria, was highest in Fresh Water Alluvial Soils where reduced conditions developed and was followed by Low Humic Gley Soils in which oxidative conditions tended to prevail. The count of *Clostridium* was smallest in Brackish Water Alluvial Soils with strong acid and low phosphorus content. The reasons for a large population of *Clostridium* in tropical paddy soil, contrasting with the comparatively small number of anaerobic bacteria, were

interpreted as follows; i) *Clostridium* survives the dry season which lasts six months because it is capable of forming spores enabling it to display a worldwide distribution.[41] Therefore, though the number of *Clostridium* was found to be large, it is doubtful whether *Clostridium* is actually active in the soil or not. ii) *Clostridium* may have an advantage over other soil microorganisms which are in soils with low nitrogen content.

HIURA *et al.*[23] reported that the mineralization of organic matter is mostly achieved by anaerobic bacteria whose number is parallel to the existence of a reduced condition and the correlation coefficient between ammonium nitrogen content and the number of anaerobic bacteria in the soils was highly significant. TAKEDA and FURUSAKA[82] reported that *Clostridium* isolated from paddy soils, except for *C. tentium*, could easily assimilate organic nitrogen and produce ammonia and fatty acid from two amino acid through the STRICKLAND reaction. As a high level of population of nitrogen-fixing *Clostridium* which can easily assimilate carbohydrates was found in tropical paddy soils, particularly in those soils with reduced condition, it would be interesting to know the population level of *Clostridium* which can assimilate organic nitrogen and their role in the mineralization of soil organic nitrogen in the tropical paddy soils. In this connection, the count of ammonifier paralleled the organic matter content in soils and was highest in Brackish Water Alluvial Soils followed by Fresh Water Alluvial Soils. It was very low in Low Humic Gley Soils. The population level of ammonifier was several times as high as that of *Clostridium* in Brackish Water Alluvial Soils and was about 1/2 of that of *Clostridium* in Fresh Water Alluvial Soils, while the count of ammonifier in Low Humic Gley Soils was similar to that of *Clostridium*. These results suggest that the composition of ammonifier was different among great soil groups. The results reported in this paper are in agreement with the findings of KARPOVA[42] who demonstrated that the counts of *Clostridium pasteurianum* and ammonifier relating to the decomposition of organic matter increased by the application of organic matter.

Actinomycete flora differed between upland farm soils of Thailand and Japan as shown in part V. There was also a difference between the species of *Clostridium*[61] present in upland soils of the cold and of the warm areas of USSR, and between those found in upland soils of low and high elevations. Furthermore, isolates of the same species of *Clostridium* have been reported[61] to show differences in physiological properties depending on soils where they were sampled, and thus their physiological properties appear to reflect the soil properties.

The number of *Azotobacter* was higher in the top soil than in the subsurface soil like in the case of aerobic bacteria, but in Brackish Water Alluvial Soils their population level was very low. JENSEN[41] reported that the population of *Azotobacter* was very small in the soils with a pH value lower than 6.0. The low pH value (average, 4.3) of Brackish Water Alluvial Soils is thought to be responsible for the small number of *Azotobacter* in these soils. Furthermore, the growth of *Azotobacter* which requires a fairly large amount of phosphorus is delayed in soils where the available phosphorus content is less than 10 ppm.[41] As the value of available phosphorus content in Brackish Water Alluvial Soils averages 18.9 ppm only,[65] such low content of phosphorus, in addition to low pH, is thought to be responsible for the small number of *Azotobacter* observed in such soils.

Although the count of each microbial group was generally small in Brackish Water Alluvial Soils because of their low pH value and low available phosphorus content, that of purple nonsulfur bacteria was high. As purple nonsulfur bacteria can utilize simple fatty acids as hydrogen donor, and can use butyric acid as the carbon source for the constitution of the cell,[49] the formation of organic acid in Brackish Water Alluvial Soils is worth noticing.

The accumulation of acetic acid and propionic acid was also reported in Brackish Water Alluvial Soils.[9] As shown in part VIII, the formation of organic acid was also assumed to take place in tropical Brackish Water Alluvial Soils because methane gas formation was not observed though a

fairly large amount of organic matter was detected in these soils. The high population level of photosynthetic purple nonsulfur bacteria in Brackish Water Alluvial Soils is considered to be due to an adequate supply of light, because the water in the fields of Brackish Water Alluvial Soils with low pH value is clear and transparent. Furthermore, the development of an anaerobic condition due to the depth of the water in such fields could be conducive to the proliferation of anaerobic purple nonsulfur bacteria. The existence of a large population of purple nonsulfur bacteria in the subsurface soil can be explained as follows: purple nonsulfur bacteria with flagella usually move to the subsurface soil where they can live in using simple organic acid as energy source under dark conditions, and they also are supposed to move downwards with the percolation of water. As shown in Table 1, the count of nonspore-forming nitrite oxidizer decreased in the dry season. MOTOMURA[65] reported that the moisture content of field soils (0-15 cm) in the middle of the dry season averaged only 15.9% in 74 samples of Low Humic Gley Soils and 18.3% in 28 samples of Fresh Water Alluvial Soils, while that of Brackish Water Alluvial Soils with heavy clay content was rather high, namely 28.7%, on the average for 9 soil samples. The count of nitrite oxidizer in the dry season paralleled the moisture content of soils. Its maximum decrease in the dry season was observed in samples of Low Humic Gley Soils and was followed by that in Fresh Water Alluvial Soils and Brackish Water Alluvial Soils.[4] As shown in Table 1, the sharp decrease in the count of nitrite oxidizer in the top soils in the dry season can be attributed to the fact that drought condition developed more severely in the top than in the subsurface soil. Next to the number of nitrite oxidizers, the populations of purple nonsulfur bacteria decreased largely in the dry season and during the preservation of soils, KOBAYASHI *et al.*[48] suggesting that purple nonsulfur bacteria were sensitive to the drought in the dry season.

On the contrary, only the number of cellulose decomposer increased in the dry season, corresponding to the increase in the amount of substrate originating from the crop residues and soil organic matter. Furthermore, one of the factors affecting the increase in the number of aerobic cellulose decomposer could be the fact that aerobic soil condition tended to prevail in the dry season. The count of cellulose decomposer was reported[47] to increase by the addition of organic matter. The decomposition of cellulose in soils is generally performed by aerobic microorganisms but under anaerobic conditions, sporogenous bacteria whose number was small in the soil are generally responsible for such function[25]. In the case of paddy soils in Thailand, yellow-pigment producing bacteria were found to be widely distributed, and were considered to belong to *Sporocytophaga* and *Cytophaga*[25]. As for cellulolytic actinomycetes, a large number of *Micromonospora* which is usually found in paddy soils[32] was detected in tropical paddy soils.

The markedly low ratio of aerobic bacteria to actinomycetes in the tropical paddy soils, as compared with that in Japanese paddy soils may be attributed to the low number of nonspore-forming bacteria which are unable to tolerate the conditions prevalent in the dry season, while spore-forming actinomycetes are able to survive.

Summary

Paddy soil samples were collected from top (0-1cm) and subsurface (1-10cm) soils in 103 sites of Thailand. Collections were made twice, in the middle of the rainy season (rice cultivation under submerged condition) and in the middle of the dry season (no cultivation, dry condition). The counts of twelve groups of microbes related to the nitrogen cycle were determined.

In the soils sampled in the rainy season the population level of aerobic bacteria per gram of dry soil ranged from 10^7 to 10^6. The levels of other microorganisms, were as follows: actinomycetes-(10^6 to 10^5), anaerobic bacteria-(10^5), cellulose decomposer-(10^4), denitrifier-(10^5), ammonifier-(10^5 to

10^4), nitrite oxidizer-(10^4), ammonia oxidizer-(10^3), *Clostridium*-(10^5 to 10^4), nitrogen-fixing blue green algae, *Azotobacter* and purple nonsulfur bacteria-(10^3), respectively. Populations of all twelve groups examined were larger in the oxidized top soil than in the subsurface soil and, in particular, the populations of aerobic nitrite oxidizer and photosynthetic purple nonsulfur bacteria were more than 4 times higher than those in the subsurface soil.

The populations of microorganisms, except for cellulose decomposer, decreased in the dry season compared with those in the rainy season. The largest decrease was recorded in the population of nitrite oxidizer which dropped to the level of 1/26, especially in the top soil, but only to 1/5 in the subsurface soil, and was followed by the population of purple nonsulfur bacteria.

The populations of twelve groups of microorganisms, except those of nitrite oxidizer and purple nonsulfur bacteria, recorded the largest value in Fresh Water Alluvial Soils with rather high organic matter content and heavy clay followed by Low Humic Gley Soils with low organic matter content, while the smallest value was generally recorded in Brackish Water Alluvial Soils with low available phosphorus content and low pH value. The population of nitrite oxidizer was largest in Low Humic Gley Soils where the oxidized layer developed, while that of purple nonsulfur bacteria showed the largest value in Brackish Water Alluvial Soils.

The ratio of aerobic bacteria to actinomycetes in the tropical soils showed a small value, namely 1.78 in the dry season, while that of Japanese soils was about 9.7 before irrigation in spring.

II Vertical change of microbial populations in the tropical paddy soils

It is well known that the counts of each group of soil microorganisms decrease with depth in different ways.[3, 30, 50] The vertical change of microflora can be ascribed to the difference in chemical, physical and biological properties of soils in each layer. The microbiological properties of the lower layer exert some effects on the properties of surface soil, for example on the decrease in the nitrogen content through denitrification. On the other hand, as the population of microorganisms in the surface soil of the tropical paddy soils showed characteristic properties described in the previous part, it was deemed important to study the vertical change in the populations of microorganisms in the tropical paddy soils.

In this part, microorganisms related to the nitrogen cycle were examined in the soil samples collected from each layer of paddy soils in Thailand during the dry season.

Materials and methods

Soil samples were collected from 12 sites of paddy fields. Three sites representing each of the major great soil groups namely, Fresh Water Alluvial Soils, Low Humic Gley Soils, Marine Alluvial Soils and Brackish Water Alluvial Soils, were selected for this study. Soil samples were collected in the dry season when crops were not cultivated. A total of 43 soil samples were collected from each layer at 12 sites. After passing soil samples through a 2 mm sieve, microorganisms were analyzed by the same methods as those described in part I. As shown in Table 5, some chemical and physical soil properties of each layer were reported by MOTOMURA.[65]

Table 5. Some chemical and physical properties of each soil layer in each location

Location	Depth (cm)	Texture	Total-C (%)	Total-N (%)	Available-P_2O_5 (ppm)
1. Low Humic Gley Soils					
1) Banphot Phisai	0—46<	CL—HC	0.82—0.32	0.07 —0.04	20.9
2) Songkhla Rattaphum	0—37<	SCL—LiC	0.70—0.39	0.062—0.030	11.0
3) Lampang Ngao	0—65	SiC—SiC	1.25—0.45	0.12 —0.044	20.1—10.5
2. Fresh Water Alluvial Soils					
4) Phetchabun, Muang	0—57<	LiC—HC	1.71—0.80	0.15 —0.06	65.3—51.0
5) Lom Sak	0—52<	HC—HC	1.81—0.55	0.15 —0.04	46.1—39.1
6) Payuhakiri	0—60<	HC—HC	1.38—0.34	0.11 —0.04	39.4—18.5
3. Brackish Water Alluvial Soils					
7) Ongkarak	0—90<	HC—HC	1.90—0.47	0.17 —0.05	13.5—14.8
8) Wang Noi	0—52	HC—HC	1.65—1.01	0.15 —0.09	18.0—20.7
9) Songkhla, Muang	0—33<	HC—HC	3.16—0.40	0.20 —0.038	19.5
4. Marine Alluvial Soils					
10) Nakhon Chaisi	0—60	HC—HC	1.64—0.34	0.15 —0.04	19.6—27.2
11) Phetchaburi, Muang	0—30<	HC—HC	1.14—0.30	0.09 —0.03	28.5—28.8
12) Thon Buri, Nong Khaem	0—55<	HC—HC	1.21—0.27	0.13 —0.04	15.5—37.6

MOTOMURA, S. (1973)

Results and discussion

Aerobic bacteria: It is well known that the number of aerobic bacteria in soil decreases with depth, although in fertile Phetchabun and Lomsak soils, values as high as 10^7 were recorded even in the lower layer as shown in Fig. 2, suggesting a high activity of microorganisms in this layer. In Marine Alluvial Soils, the number increased slightly in the lower layer. The number of aerobic bacteria in Brackish Water Alluvial Soils was as low as 10^4 to 10^5 in the top soil. In Ongkarak soil the number did not decrease even at a depth of 60 cm and increased again at a depth of about 80 cm. Aerobic bacteria in Low Humic Gley Soils with sandy texture except Lampang, showed a rapid decrease with depth as compared with other great soil groups.

Anaerobic bacteria: The number of anaerobic bacteria decreased with depth, though in a somewhat different manner than in the case of aerobic bacteria.

Actinomycetes: Their counts decreased also with depth, though the trends were not always similar to those of aerobic bacteria. In the case of Brackish Water Alluvial Soils, the number of actinomycetes was conspicuously higher than that of aerobic bacteria whose number was extremely low.

As shown in Fig. 3, ratios of aerobic bacteria to actinomycetes (Bac./Act.) in Low Humic Gley Soils and Fresh Water Alluvial Soils were smaller in the second than in the first layer and became slightly larger again in the third layer. In the case of Brackish Water Alluvial Soils, the ratios were slightly larger in the second than in the first layer. On the other hand, in the case of Marine Alluvial Soils, vertical change was not evident.

As shown in Fig. 3, the ratios of aerobic bacteria to actinomycetes in paddy soils of Thailand were smaller in all layers as compared with those in Japanese paddy soils which were sampled in spring.[30] The ratios did not show marked changes in the soils of Thailand, but those in Japanese soils exhibited large variations.

Ammonifier and denitrifier: Ammonifier tended to decrease with depth, whereas denitrifier increased slightly or remained at the same level and hence, the number of denitrifier was fairly higher than that of ammonifier in the lower layer in many cases. Counts of ammonifier and denitrifier in the first layer did not follow any specific pattern.

Ammonia oxidizer and nitrite oxidizer: The counts of ammonia oxidizer in each layer were lower than those of ammonifier. The vertical change of ammonia oxidizer showed variations among soils.

In general, the number of nitrite oxidizer was rather higher or similar to that of ammonia oxidizer. The number of nitrite oxidizer showed often unexpectedly a rather high level in the lower layer. In such cases, it may be suggested that nitrite oxidizer was severely affected by dryness in the surface soil.

Cellulose decomposer and its activity: As a rule, the number and activity of cellulose decomposer decreased with depth. In the case of Brackish Water Alluvial Soils, however, a rather high peak was found at a depth of about 30 cm. Furthermore, the high microbial count which was similar to that in the surface soil was also observed even in the deep layer, whereas the activity in Low Humic Gley Soils decreased sharply in the second layer.

Summary

The change of microflora related to the nitrogen cycle was examined using 43 soil samples collected from each layer of paddy soils belonging to the major great soil groups during the dry season (no cultivation) in Thailand.

In general, population of aerobic bacteria, anaerobic bacteria, actinomycetes, ammonia oxidizer and cellulose decomposer decreased with depth. The counts of denitrifier in the lower layer showed a slightly higher or similar value to those in the first layer. Nitrite oxidizer counts did not follow any specific pattern.

In comparison with other great soil groups, the numbers of aerobic bacteria and cellulose decomposer of Low Humic Gley Soils generally dropped sharply in the second layer. In the case of Brackish Water Alluvial Soils, it was noted that the highest level of cellulose decomposer was observed at a depth of about 30 cm and that the number of actinomycetes was high in all layers as compared with other microbial groups. The fertile soils showed high levels of aerobic bacteria in all layers.

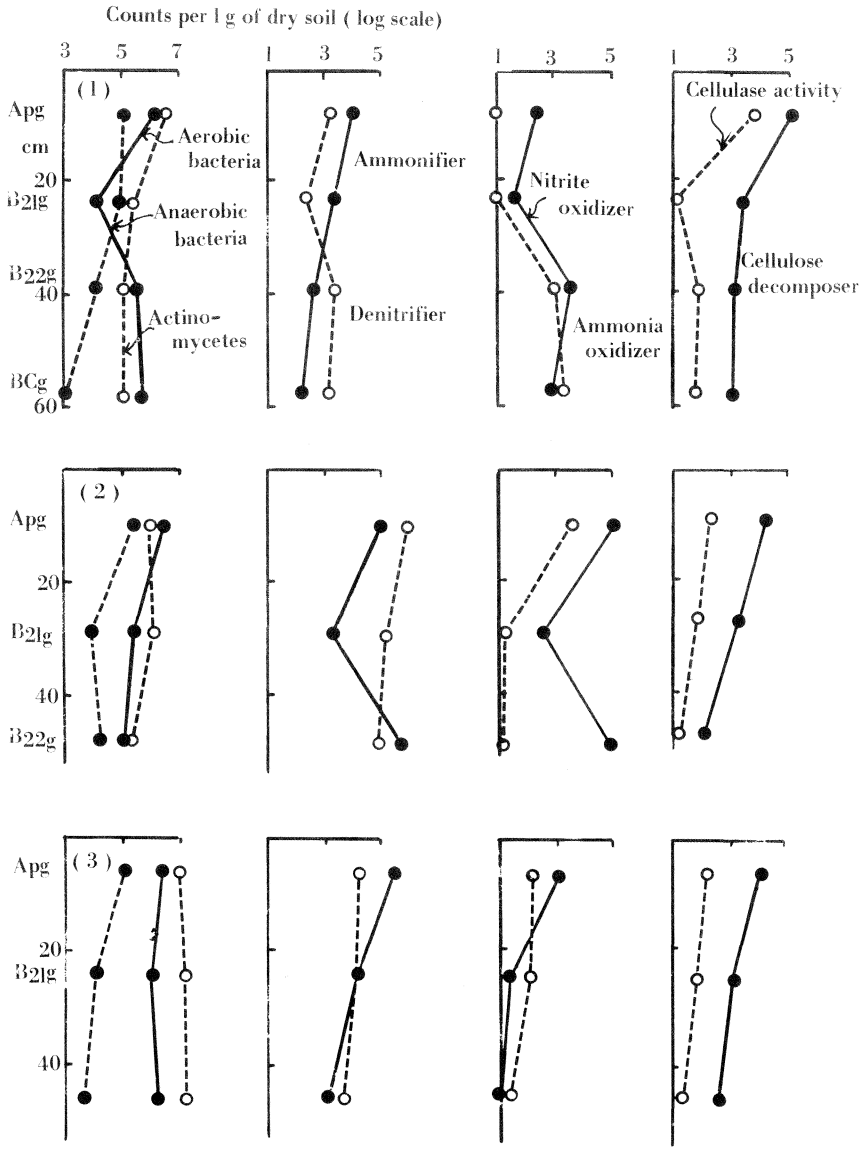

Fig. 2-a. Population of microorganisms in each layer of Low Humic Gley Soils (Number shows the location in Table 5).

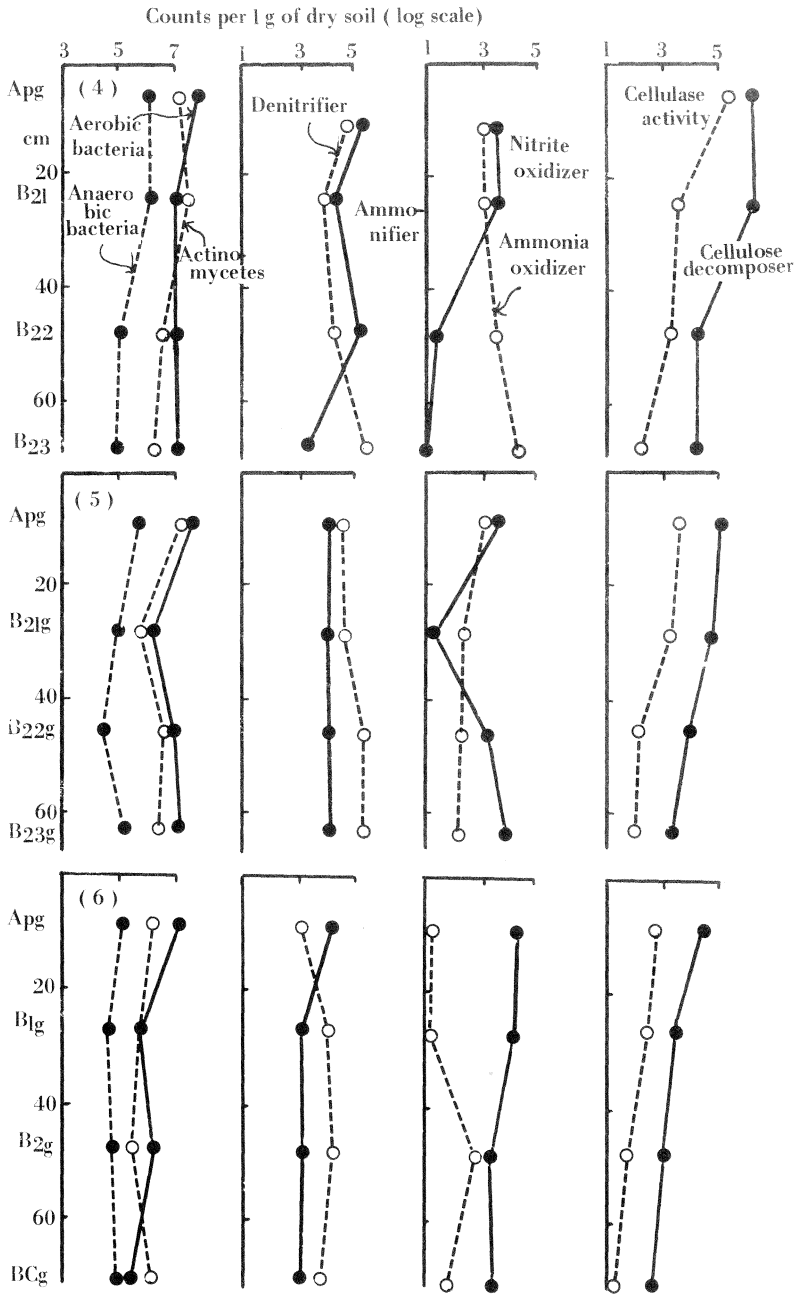

Fig. 2-b. Population of microorganisms in each layer of Fresh Water Alluvial Soils (Number shows the location in Table 5).

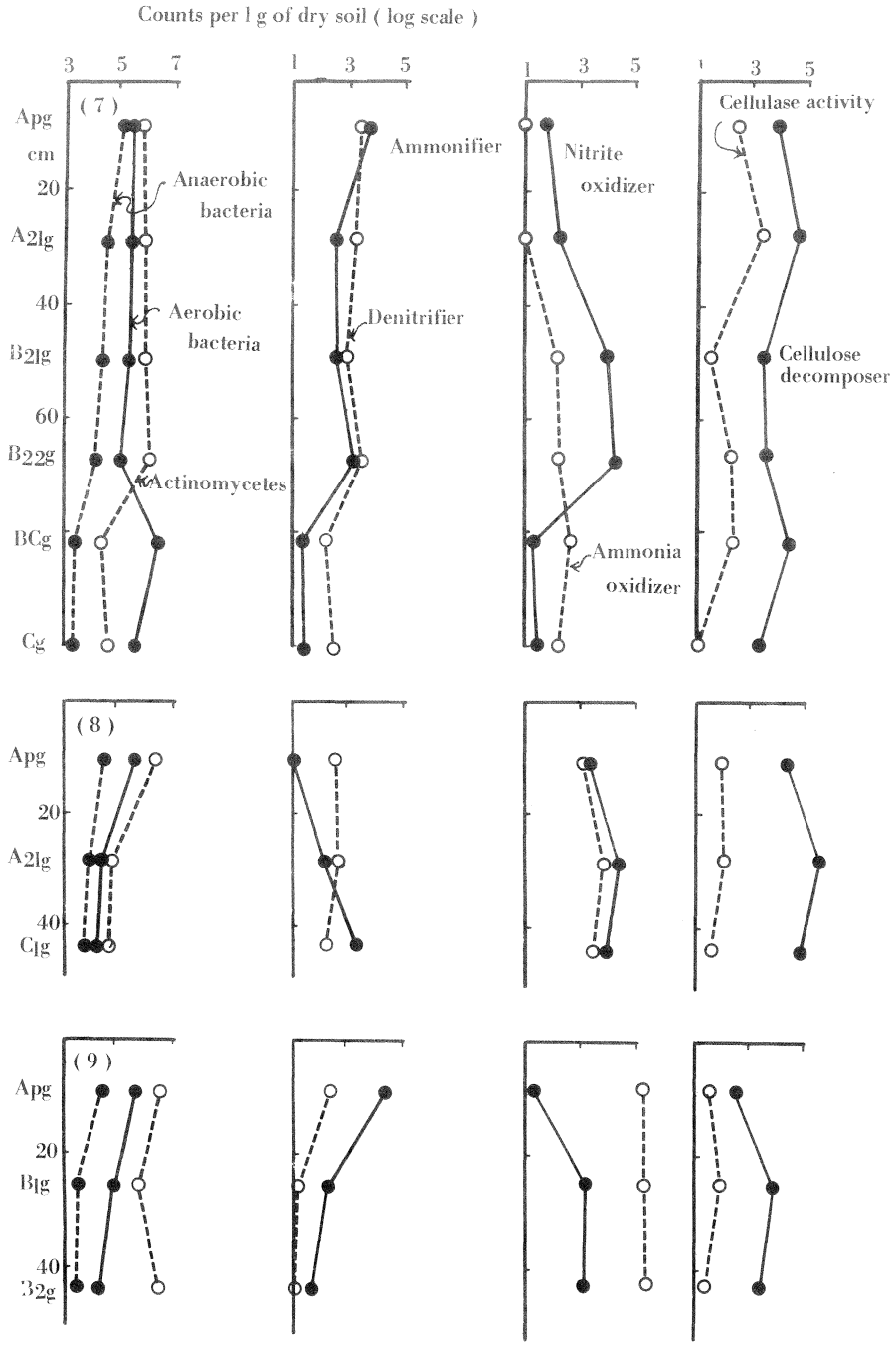

Fig. 2-c. Population of microorganisms in each layer of Brackish Water Alluvial Soils (Number shows the location in Table 5).

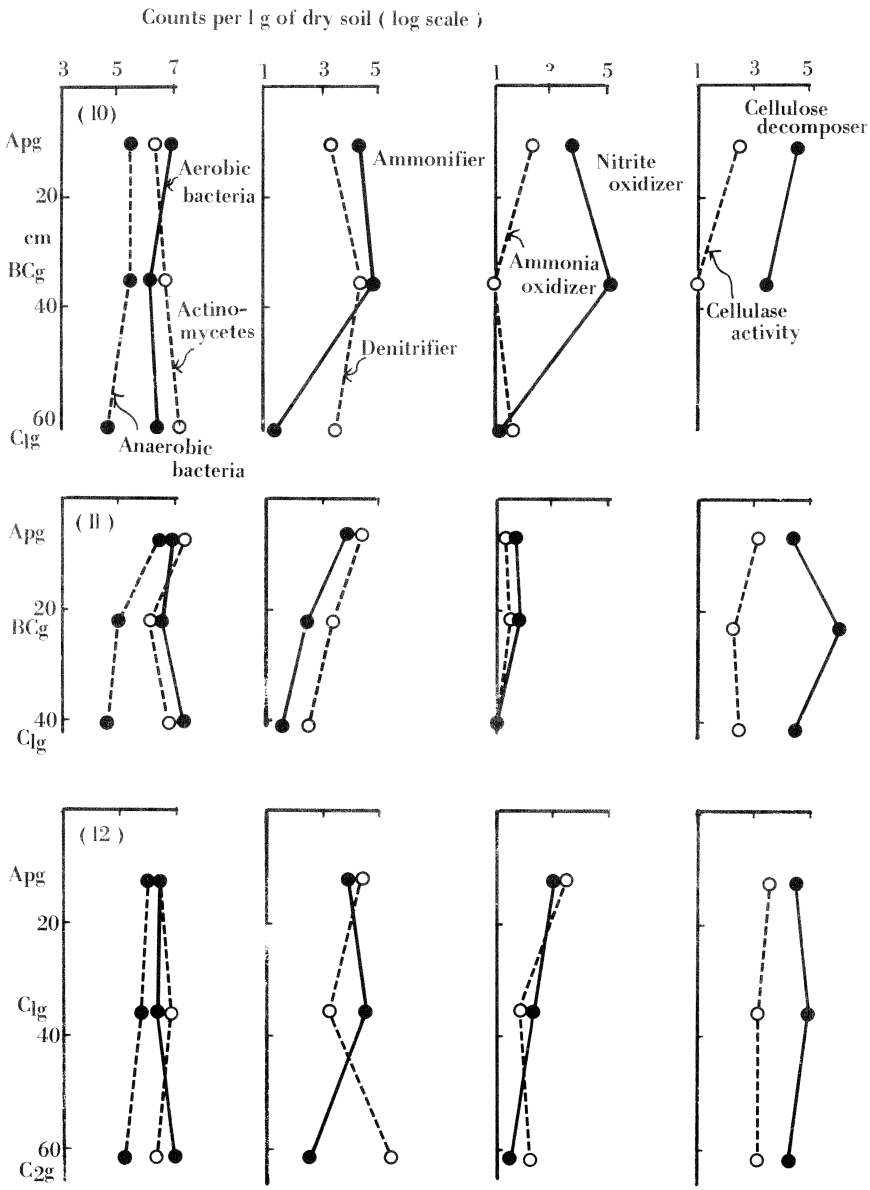

Fig. 2-d. Population of microorganisms in each layer of Marine Alluvial Soils (Number shows the location in Table 5).

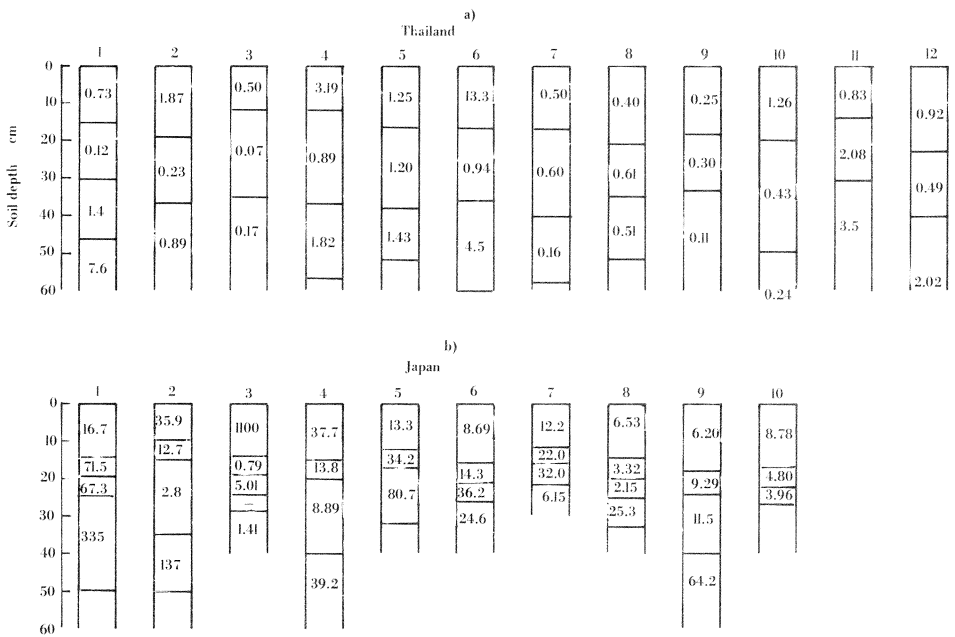

Fig. 3. Vertical change of the ratios of aerobic bacteria to actinomycetes in paddy soils of Thailand and Japan.
a) Soil profiles are the same as those in Fig. 2.
b) ISHIZAWA S. and TOYODA H. (1964c)

III Mineral nitrogen content in tropical paddy soils in the dry season, and relationships between mineral nitrogen content and populations of microorganisms

In the first part of this investigation, the characteristics of microflora related to the nitrogen cycle in the tropical paddy soils during the rainy season were described.

In this part, the ammonium and nitrate nitrogen content in the tropical paddy soils during the dry season is examined along with the relationships between the mineral nitrogen content and the population of related microorganisms. Although some investigations on the mineral nitrogen contents in the tropical soils in the dry season have been reported,[45, 96] so far only a few microbiological studies have been published on the subject.

Materials and methods

The soil samples used and the method of microbial analysis were the same as those in part I.

The ammonium nitrogen and nitrate nitrogen contents were analyzed by CONWAY's method[96] after extracting 40 g of soil with 400 ml of N KC1 (pH 7.0) for 30 min. In the analysis of nitrate nitrogen content, Devarda's alloy was used.

Results and discussion

1 Ammonium nitrogen and nitrate nitrogen content in the paddy soils in the dry season

As shown in Table 6, the ammonium nitrogen content amounted to 3.04 mg per 100 g of dry soil on the average in the top soils (0-1 cm) and 1.35 mg in the subsurface soils (1-10 cm), respectively. On the other hand, the nitrate nitrogen content was 5.12 mg per 100 g of dry soil on the average in the top soils and 2.46 mg in the subsurface soils, respectively. Therefore, the ratio of nitrate nitrogen to ammonium nitrogen was 2.31 on the average in the top soils and 2.69 in the subsurface soils.

From the above results, both the ammonium and nitrate nitrogen contents were higher in the top soils than in the subsurface soils, and the nitrate nitrogen content was more than twice as high as the ammonium nitrogen content both in the top and subsurface soils. This difference in ammonium and nitrate nitrogen content, and the ratio of nitrate nitrogen to ammonium nitrogen content between the top and subsurface soils, were significant at the level of 0.1%, respectively.

Many reports have been published so far on the transformation of mineral nitrogen in the soil, but rather few reports[7, 45, 58, 97] have been concerned with tropical paddy soils under field conditions. The production of mineral nitrogen is affected by such many factors as the quantity and quality of soil organic matter, soil temperature, pH, oxidation-reduction potential, soil moisture, nutrient for related microorganisms[2, 73] as well as population of microorganisms related to the nitrogen cycle. Therefore, the amount of ammonium and nitrate nitrogen shows variations depending on the differences in climate, crop, soil type, soil management etc. and is known to change daily[66] and seasonally.[74] As a matter of course, the vertical change in the amount of ammonium and nitrate nitrogen has also been reported.[96]

The amount of ammonium nitrogen in soils, in case of absence of crop, is mostly affected by the ammonification of soil organic matter and is also negatively correlated to the rate of nitrification.[73] The amount of nitrate nitrogen is also largely affected by the ammonification of soil organic matter,

Table 6. Ammonium and nitrate nitrogen content and rate of nitrate nitrogen to ammonium nitrogen content in the great soil groups of paddy soils in the dry season

Great soil group	Soil depth (cm)	Number of soil samples	NH_4^+—N + NO_3^-—N		NH_4^+—N		NO_3^-—N		NO_3^-—N/NH_4^+—N	
			Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
A) Low Humic Gley Soils	0— 1	52	7.69*	7.22	3.07*	3.38	4.57*	4.15	1.99	1.93
	1—10	52	3.55	5.29	1.40	2.43	2.15	2.96	2.14	1.84
B) Fresh Water Alluvial Soils	0— 1	24	10.22	11.92	3.55	5.27	6.67	7.49	2.27	1.90
	1—10	24	4.65	3.05	1.52	1.13	3.13	2.31	3.71	5.91
C) Brackish Water Alluvial Soils	0— 1	4	5.19	2.66	1.54	0.95	3.65	2.06	2.87	2.21
	1—10	3	1.07	0.21	0.38	0.13	0.69	0.22	2.23	1.20
D) Marine Alluvial Soils	0— 1	3	5.11	1.89	2.34	0.95	2.78	0.96	1.26	0.24
	1—10	3	2.09	0.50	0.77	0.32	1.31	0.18	1.92	0.57
E) Hydromorphic Non-calcic Brown Soils	0— 1	3	5.51	3.46	2.37	1.83	3.14	1.65	1.59	0.45
	1—10	3	2.82	0.43	0.72	0.12	2.10	0.44	3.01	0.85
F) Other soil groups	0— 1	3	10.08	6.27	1.58	0.59	8.50	6.86	9.28	10.53
	1—10	3	6.90	1.65	1.26	0.29	5.64	1.92	5.00	2.39
Total	0— 1	89	8.18	8.56	3.04	3.84	5.12	5.36	2.31	2.98
	1—10	88	3.76	4.48	1.35	1.98	2.46	2.73	2.69	3.54

*mg per 100 g dry soil

because ammonium is an important substrate for nitrite formation and hence the rate of ammonium formation is the most important factor for the nitrate formation.[73]

The reason why the total amount of mineral nitrogen was larger in the top than in the subsurface soils can be ascribed to the following factors: (A) the organic matter content is higher in the top soil because the amount of weed, algae and small animals which proliferated in the rainy season are restored mostly to the top soil in the dry season when no crops are cultivated, and (B) the soil temperature is higher in the top soil in the daytime. This is favourable for ammonification which increases with temperature at a maximum of approximately 50°C, with a decline at 60°C.[66]

In addition, the higher amount of nitrate nitrogen in the top than in the subsurface soils can be explained as follows; (A) ammonification is more extensive in the top soil than in the subsurface soil, and (B) nitrate is accumulated in the top soil because in the rainy season nitrate was leached down but moved upwards with the evaporation of paddy water from the lower layer in the dry season.[71,77,88]

Furthermore, the reason why nitrate nitrogen content was higher than ammonium nitrogen content is considered partly to be due to the development of nitrification in the early period of the dry season, when the soil became more aerobic. The high amount of nitrate nitrogen is feared to be lost by leaching or denitrification in the next rainy season.

Comparing the value of ammonium nitrogen, nitrate nitrogen content and the ratio of the latter to the former among great soil groups, ammonium nitrogen content showed the highest value in the Fresh Water Alluvial Soils both in the top and subsurface soils. This result is explained by their high organic matter and high clay mineral content and moreover, by better preservation of soil moisture in the dry season.[96] Conversely, the lowest value of ammonium nitrogen content in the Brackish Water Alluvial Soils is mostly due to the low pH value (about 4.0) which is not favourable for the growth of ammonifier.

In case of the nitrate nitrogen content, the highest value was found in other soil groups followed by Fresh Water Alluvial Soils, whereas lower values were found in Brackish Water Alluvial Soils and Marine Alluvial Soils. The optimum pH value of ammonia oxidizer ranges from 7 to 9, and that of nitrite oxidizer approximates neutrality to slightly alkaline reaction.[2] Therefore, the low nitrate nitrogen content may be ascribed to the low pH value in Brackish Water Alluvial Soils. Furthermore, the adaptation of nitrite oxidizer to the low pH value,[11] the presence of heterotrophic nitrite oxidizer[26] and non-biological oxidation of nitrite to nitrate in the top soil may play a role in the formation of nitrate. The low value of nitrate content in Marine Alluvial Soils, suggests that chloride exerts an inhibitory effects on the nitrification process.[73]

The correlation coefficients of nitrate nitrogen to ammonium nitrogen contents were positively significant at the level of 0.1% both in the top ($r=0.776^{***}$) and subsurface soils ($r=0.808^{***}$). Comparing the correlation coefficient of nitrate nitrogen to ammonium nitrogen contents among great soil groups, those of Low Humic Gley Soils and Fresh Water Alluvial Soils were both significant at the level of 0.1% ($r=0.841^{***}$, $r=0.736^{***}$) in the top soil, and at the level of 0.1% and 5% ($r=0.921^{***}$, $r=0.505^{*}$) in the subsurface soils. The fact that the correlation coefficient was higher in Low Humic Gley Soils than in Fresh Water Alluvial Soils, could be ascribed to better homogeneity of the former soils compared with that of the latter ones.

2 Relationships between the mineral nitrogen content and the populations of related microorganisms

The relationship between ammonium nitrogen content and the population of ammonifier was examined. In the top soils, a significant correlation coefficient was obtained at the level of 5% ($r=0.261^{*}$), unlike in the subsurface soils.

As shown in Table 6, a large part of ammonia is changed to nitrate by nitrification. And hence, the relationship between the total amount of ammonium and nitrate nitrogen and the population of ammonifier was examined and the correlation coefficient in the top soils was significant at the level of 5% ($r=0.263^*$), while that in the subsurface soils, was not significant. The same findings applied to the correlation coefficient between the ammonium nitrogen content and the population of ammonifier.

The correlation coefficient between the nitrate nitrogen content and population of nitrite oxidizer was examined, but no correlation was obtained either in the top or in the subsurface soils. The nitrate nitrogen content in the soil showed a close correlation with the amount of ammonium nitrogen as described before. Therefore, the correlation coefficient between the ratios of nitrate nitrogen to ammonium nitrogen contents, and the population of nitrite oxidizer was calculated and that in the top soil was significant at the level of 5% ($r=0.215^*$), whereas that in the subsurface soil was not significant.

Summary

The relationships between the amount of mineral nitrogen and the populations of the related microorganisms, was examined in the tropical paddy soils collected from 89 sites of paddy fields in Thailand during the dry season when crops were not cultivated. The average amounts of ammonium nitrogen were 3.04 mg per 100 g of dry soil in the top soils (0-1 cm) and 1.35 mg in the subsurface soils (1-10 cm), respectively, whereas those of nitrate nitrogen were 5.12 and 2.46 mg, respectively. The ratio of nitrate nitrogen to ammonium nitrogen was 2.31 on the average in the top soils and 2.69 in the subsurface soils. Accumulation of nitrate nitrogen was conspicuous in the surface soils (0-10 cm) of tropical paddy soils in the dry season. Significant correlation coefficients were obtained at the level of 5% between ammonium nitrogen contents and the population of ammonifier, between the content of mineral nitrogen and the population of ammonifier, and between the ratios of nitrate nitrogen to ammonium nitrogen contents, and the population of nitrite oxidizer, only in the top soils.

IV Microflora related to the nitrogen cycle in the tropical upland farm soils

Crop cultivation in the upland soils of the tropics has been mostly practiced under the natural supply of nutrients or with the application of a small amount of fertilizer. The maintenance of soil fertility, especially that of soil nitrogen, is one of the most important factors enabling to sustain high yields.

In this part, the microflora related to the nitrogen cycle will be described in the upland soils of great soil groups in Thailand and relationships between the population of microorganisms and some soil properties will be examined. Furthermore, the comparison with the microflora of upland farm soils of Japan was made in order to clarify the characteristics of microflora in tropical upland farm soils.

Materials and methods

Chemical and physical analysis. Maximum water-holding capacity was determined by HILGARD's method.[43] Organic carbon was determined by means of the WALKLEY-BLACK method[87] and total nitrogen by KJELDAHL digestion method. Available phosphorus was determined colorimetrically by means of the molybdophosphoric blue method after extraction with BRAY No.2 solution, while ammonia content in soils was determined by CONWAY's method.[96] In the case of the determination of nitrate nitrogen, CONWAY's method was used after reduction by Devarda's alloy.[96] Exchangeable potassium was determined by flame photometry. Chemical and physical properties of soil samples used are shown in Table. 7.

Microbial analysis. The counts of fungi were made in using the methods reported in the literature.[97] Populations of other microorganisms were determined by the methods described in part I.

Soil samples. Surface soils (0-10 cm in depth) were collected from 28 upland farm soils belonging to various great soil groups in Thailand in September during the rainy season. Soil samples were passed through a 2 mm sieve and microbial analysis was made as soon as possible after sampling.

Results and discussion

1 Microflora in upland farm soils in Thailand (Rainy season)

As shown in Table 8, the population of aerobic bacteria except that in Regosols, was the largest as compared with that of fungi and actinomycetes, showing a level of 10^7 to 10^6 per 1 g of dry soil. It was followed by that of actinomycetes, namely about half the number of aerobic bacteria, while that of fungi totaled 10^5 to 10^4.

Among the microorganisms related to the transformation of nitrogen, such as ammonifier, ammonia oxidizer, nitrite oxidizer and denitrifier, the count of denitrifier showed maximum levels of 10^6 to 10^5, despite the existence of large variations among the great soil groups, followed by ammonifier.

In the case of nitrogen-fixing groups, the number of *Azotobacter* showed variations among the great soil groups and ranged from 10^3 to zero in Rendzinas. The population level of nitrogen-fixing blue green algae was high, namely 10^3 to 10^1. Compared with the count of nitrogen-fixing blue green algae in paddy soil of the tropics in the rainy season[3], which amounted to 8.6×10^3 in the top and 3.58×10^3 in the subsurface soil, that in upland farm soils belonging to Alluvial Soils was unexpectedly high.

Table 7. Some chemical and physical properties of upland farm soils in Thailand

Great soil groups	Number of soil samples	Maximum Water-holding capacity (Dry weight %)	pH	Organic carbon (%)	Total nitrogen (%)	NH_4^+—N (mg/100 g dry soil)	NO_3^-—N (mg/100 g dry soil)	Available P (ppm)	Exchangeable K (ppm)
1) Red-Yellow Podzolic Soils	7	40.3	6.4	0.83	0.060	0.52	1.46	6.5	89
2) Regosols	5	36.1	6.0	0.56	0.042	0.31	0.93	3.0	53
3) Gray Podzolic Soils	4	37.1	6.8	0.64	0.045	0.33	1.11	6.5	86
4) Brown Forest Soils	3	78.6	7.3	2.05	0.122	0.40	1.68	23.3	272
5) Non-calcic Brown Soils	2	53.1	6.1	1.48	0.091	0.49	2.15	45.5	193
6) Reddish Brown Lateritic Soils	2	63.5	5.9	1.12	0.089	1.00	1.07	24.3	83
7) Grumusols	2	94.3	6.9	2.55	0.146	1.00	1.83	17.2	268
8) Alluvial Soils	1	40.5	6.6	0.49	0.031	0.25	1.13	21.3	29
9) Rendzinas	1	72.2	7.3	2.19	0.155	0.54	2.04	20.5	180
10) Low Humic Gley Soils	1	35.8	6.8	0.68	0.044	0.80	1.63	4.5	31

Table 8. Populations of microorganisms in each great soil group (Rainy season)

(Counts per gram of dry soil)

Microorganisms / Great soil group	Fungi (× 10⁵)	Actinomycetes (× 10⁵)	Aerobic bacteria (× 10⁵)	Ammonifier (× 10³)	Ammonia oxidizer (× 10³)	Nitrite oxidizer (× 10³)	Denitrifier (× 10³)	*Azotobacter* (× 10³)	Nitrogen-fixing blue green algae (× 10³)
1) Red-Yellow Podzolic Soils	1.7	87	124	150	1.1	0.41	3400	1.7	4.0
2) Regosols	1.8	41	38	50	0.62	0.099	280	0.011	0.73
3) Gray Podzolic Soils	1.8	64	90	73	2.2	1.5	480	0.36	3.1
4) Brown Forest Soils	4.0	108	320	75	6.0	2.0	1070	4.9	0.13
5) Non-calcic Brown Soils	1.7	56	110	144	6.9	0.63	270	5.5	2.4
6) Reddish Brown Lateritic Soils	0.70	73	87	21	3.2	0.15	560	6.5	2.1
7) Grumusols	2.8	49	150	34	2.1	0.34	770	9.9	1.1
8) Alluvial Soils	1.8	54	61	37	2.5	0.26	710	1.8	10
9) Rendzinas	5.6	210	270	53	0.26	0.25	1200	0	0.048
10) Low Humic Gley Soils	1.4	28	81	44	0.39	0.37	600	0.036	0.88
Average	2.1	73	124	84	1.9	0.68	1280	2.83	3.7
Standard deviation	1.6	54	125	87	2.3	1.2	3090	4.90	3.7

Numbers of soil samples of each great soil group were the same as those in Table 7.

As shown in Table 8, the population of each of the 9 groups of microorganisms showed variations which reflected the differences in the chemical and physical properties among the great soil groups. Soil samples used in this paper could be classified into 6 groups depending on the soil properties. i) group with high content of organic matter, available phosphorus and exchangeable potassium—Brown Forest Soils, Rendzinas and Grumusols. ii) group with intermediate values of organic matter and high content of available phosphorus and available potassium—Non-calcic Brown Soils. iii) group with intermediate value of organic matter, high content of available phosphorus and intermediate content of exchangeable potassium—Reddish Brown Lateritic Soils. iv) group with low content of organic matter and exchangeable potassium, and high content of available phosphorus—Alluvial Soils. v) groups with low content of organic matter and available phosphorus, and intermediate values of exchangeable potassium—Red-Yellow Podzolic Soils, Gray Podzolic Soils. vi) groups with low content of organic matter, available phosphorus and exchangeable potassium—Low Humic Gley Soils and Regosols.

The soil aggregates of group (i) with high organic matter content developed well and furthermore this group was comparatively rich in nutrient contents. Therefore the counts of microorganisms in this soil group generally tended to be high especially those in Brown Forest Soils, except for nitrogen-fixing blue green algae. In the case of Rendzinas, the counts of ammonia oxidizer, nitrite oxidizer and nitrogen-fixing blue green algae were low. *Azotobacter* was not detected presumably because of high nitrogen content. On the other hand in the case of Grumusols, the counts of microorganisms were generally high except those of actinomycetes, ammonifier and nitrite oxidizer.

The great soil groups belonging to the groups (ii), (iii), (iv) and (v) which lack in some nutrients showed an intermediate number of microorganisms. In the case of Non-calcic Brown Soils with intermediate values of organic matter and high content of nitrogen, a large number of both ammonifier and ammonia oxidizer and a small number of denitrifier were observed. In the case of Reddish Brown Lateritic Soils, the counts of fungi, ammonifier and nitrite oxidizer were low, while the count of nitrogen-fixing blue green algae in Alluvial Soils was high presumably due to flooding. SUZUKI and KAWAI[78] reported that the count of nitrogen-fixing blue green algae in the upland soils of Cambodia (Alluvial Soils) was high, namely 10^6 to 10^5. In the case of Red-Yellow Podzolic Soils, the counts of denitrifier and ammonifier were high, and the number of nitrite oxidizer was large in Gray Podzolic Soils. On the other hand, microbial populations in Low Humic Gley Soils and Regosols with low content of organic matter, available phosphorus and available potassium, were generally small.

As described above, each microbial population showed large variations among the great soil groups and hence the soil function related to the nitrogen cycle differed, too, in each soil group. Therefore, the amounts of total and mineral nitrogen varied also depending on the great soil groups as seen in Table 7.

2 Relationships between populations of microorganisms and soil chemical and physical properties

As the abundance of microbes is affected by many chemical and physical properties of soils[62] it is difficult to define the relationships between the microbial population and each soil property. The positive correlation between the content of organic matter in soils and the microbial population was significant at the level of 1% only in the case of fungal population ($r = 0.551$). Such findings may be related to the important role played by fungi in the initial stages of organic matter decomposition as they live mainly on the surface of organic matter rather than on soil particles.[73]

On the other hand, the positive correlation between the amount of available phosphorus in the

soil and the microbial population was significant at the level of 0.1% only in the case of *Azotobacter* ($r=0.682$). It was reported[41] that in soils of temperate regions *Azotobacter* are seldom observed below a pH level of 6.0, according to their behavior in vitro. Below pH 6, they are usually few in number and are not displaying active growth even when suitable energy material and mineral nutrients are provided. In tropical and subtropical soils there is some indication of a more widespread representation in acid soils.[5] As the pH value of soil samples in this report was above 6.0, its effect on the number of *Azotobacter* would be negligible. JENSEN[41] reported that the growth of *Azotobacter* which requires a fairly large amount of phosphorus is retarded in soils where the available phosphorus content is less than 10 ppm. Therefore, phosphorus could be the major factor affecting the abundance of *Azotobacter* in these soils.

The relationships between the total nitrogen and the microbial population, and between the exchangeable potassium and the microbial population were not significant in any microbial groups. In addition, the relationships between the amount of NH_4^+–N and the count of ammonifier, between the amount of NH_4^+–N + NO_3^- and the count of ammonifier, and between the amount of NO_3^-–N and the count of denitrifier showed no significance. SORIANO and WALKER[75] reported that the soil conditions that were favourable to NH_3 oxidizing autotrophic bacteria reflect their nutritional requirements, namely a supply of CO_2, NH_3, O_2, traces of certain inorganic salts including Fe and pH value of 7 to 8.5. Furthermore, in Barnfield and Broadbalk soils of Rothamsted with pH values ranging between 7 and 8, the highest counts were found in plots that had received farmyard manure or some other organic fertilizer.

In this experiment, a significant correlation between the amount of NH_4^+–N and the population of ammonia oxidizer was not obtained, presumably due to many factors affecting the count of ammonia oxidizer in soils. The count of nitrite oxidizer was high in such soil groups with high organic matter contents as Brown Forest Soils and Non-calcic Brown Soils, while in Rendzinas in spite of the high organic matter content, it was low. Among the soil groups with low organic matter content such as Alluvial Soils, Gray Podzolic Soils, Regosols, Low Humic Gley Soils and Red-Yellow Podzolic Soils, Gray Podzolic Soils showed high population level of nitrite oxidizer. Therefore population levels showed no significant correlation with the organic matter content in soils.

As NH_4^+–N and NO_3^-–N in soils are partly absorbed by the plant and partly lost through denitrification and leaching, the correlation between the amount of NH_4^+–N or NO_3^-–N in soils and population related to the nitrogen cycle is not considered to be significant.

Maximum water-holding capacity is considered as an index of the moisture condition of soils. A significant positive correlation ($r=0.905$) was obtained between the maximum water-holding capacity and the organic matter content, while a significant positive correlation was found only between the maximum water-holding capacity and the fungal population at the level of 5% ($r=0.426$). The reasons for the significant correlation between the maximum water-holding capacity and the fungal population were interpreted as follows; i) the population of fungi tends to increase under high moisture conditions, as compared with that of other microorganisms.[90] ii) as demonstrated before, a high significant positive correlation was observed between the maximum water-holding capacity and the organic matter content, and on the other hand, as a significant positive correlation was also observed between the organic matter content and the fungal population, the organic matter content of soil could be indirectly involved. iii) apart from the above consideration, it is also conceivable that a difference in fungal flora may exist among soil groups.

3 Comparison of microflora in upland farm soils between Thailand and Japan

All of upland-farm soil samples used in this report were non-volcanic soils. Therefore as shown in Table 9, in contrast with the population of microbes found in the non-volcanic upland farm soils of

Japan,[29] the population of actinomycetes observed in Thailand was larger, but that of aerobic bacteria was about half, while each count of fungi, actinomycetes and aerobic bacteria in Thailand was similar to those in upland farm soils of volcanic ash origin in Japan.[28] As volcanic ash soils are smaller in volume weight than non-volcanic soils, upland farm soils in the tropics usually harbour a larger number of microbes per volume of soil than volcanic upland farm soils in Japan.

On the other hand, the counts of microbes relating to the nitrogen cycle such as denitrifier and *Azotobacter* showed striking differences between the soils in Thailand and those in Japan. The count of denitrifier in upland farm soils of Thailand was 9 times as high as that in non-volcanic upland farm soils of Japan and was 23 times that in volcanic ones, though the level of significance of difference between the soils of the two countries was low because of the large variations among great soil groups.

In the case of nitrogen-fixing *Azotobacter*, the count recorded in upland farm soils of Thailand averaged 2,800 per 1 g of dry soil, while that in non-volcanic upland farm soils of Japan was 77 on the average. Azotobacters were not detected in volcanic upland farm soils.[28] The occurrence of *Azotobacter* in the upland farm soils was more frequent in Thailand (61% of the samples) than in the non-volcanic ash soils of Japan (27% of the samples). The percentage of *Azotobacter* detected in Japanese soils[29] was smaller than the average, if compared with the data obtained in other countries,[41] while that in Thailand was similar to the average in the world. In the case of non-volcanic ash soils of Japan, *Azotobacter* was reported to be found in the soils of high pH (above 6), though not without exception.[29]

Table 9. Comparison of microflora in upland farm soils between Thailand (Rainy season) and Japan

(Counts per gram of dry soil)

	Soils					
	Thailand		Japan[a)]			
	Non-volcanic (n=28)		Non-volcanic (n=26)		Volcanic (n=29)	
Microorganisms	Average	S.D.	Average	S.D.	Average	S.D.
Fungi ($\times 10^5$)	2.1	1.6	2.5	1.5	2.4	0.98
Actinomycetes ($\times 10^5$)	73	124	47*	33	85	55
Aerobic bacteria ($\times 10^5$)	124	125	233****	136	165	85
Denitrifier ($\times 10^3$)	1280	3090	144	140	55*	69
Azotobacter ($\times 10^3$)	2.8	4.9	0.077***	0.30	0*****	—

*, ***, **** and *****: Significant at 5%, 1%, 0.5% and 0.1% level, respectively.

a) ISHIZAWA, S. and TOYODA, H. (1964a, 1964b)

As shown in Table 10, each ratio of aerobic bacteria to actinomycetes, aerobic bacteria to fungi, and actinomycetes to fungi in upland farm soils of Thailand was similar to that recorded in volcanic upland farm soils of Japan. The ratio of aerobic bacteria to actinomycetes in upland farm soils of Thailand was 2.31, while that of non-volcanic ash soils of Japan was comparatively larger averaging 7.28. On the other hand, the ratio of actinomycetes to fungi in the former soils was 51.8, while that in the latter soils was 22.0. The difference in the ratios was significant at the level of 0.1%. Therefore the microflora in tropical upland-farm soils consists mainly of actinomycetes as compared with that in temperate upland-farm soils.

MISHUSTIN[60] who studied the microflora of the great soil groups in USSR from the northern to southern part of the country showed that the ratio of aerobic bacteria to actinomycetes became lower southwards. Such observation is in agreement with our findings recording a ratio of aerobic bacteria to actinomycetes lower in the tropical areas of the south than in the temperate areas of the north. The conditions conducive to the predominance of actinomycetes seem to be related to high temperature and low moisture.[27] WILLIAMS, S.T. *et al.*[90] reported also that the number of actinomycetes increased relatively in soil at suctions above pF 2.3 while at suctions of pF 5.6, the number of actinomycetes exceeded that of bacteria and fungi. Growth of all actinomycete strains tested was severely limited or absent at tensions higher than pF 4.0 but spores of streptomyces survived for long periods in the dry soil.

The factors affecting the relatively high ratio of actinomycetes to aerobic bacteria or fungi in tropical upland farm soils compared with that in temperate ones, could be found in low moisture content as tropical soils generally show a low water-holding capacity.[67] The influence exerted by the dry season which lasts six months is also considerable.

Table 10. Comparison of the ratios of aerobic bacteria to actinomycetes, aerobic bacteria to fungi, and actinomycetes to fungi among non-volcanic upland farm soils in Thailand (Rainy season), non-volcanic soils in Japan and volcanic soils in Japan

	Soils					
	Thailand		Japan[a]			
	Non-volcanic (n=28)		Non-volcanic (n=26)		Volcanic (n=29)	
Microorganisms	Average	S.D.	Average	S.D.	Average	S.D.
Aerobic bacteria/Actinomycetes	2.31	2.74	7.28**	9.68	2.21	1.19
Aerobic bacteria/Fungi	82.9	72.2	112	78.2	76.7	34.8
Actinomycetes/Fungi	51.8	39.7	22.0*****	14.3	39.6	20.1

** and *****; Significant at 2.5% and 0.1% level, respectively.

a) ISHIZAWA, S. and TOYODA, H. (1964a, 1964b)

Summary

Samples of upland-farm surface soils (0-10 cm) belonging to various great soil groups were collected at 28 upland sites in Thailand during the rainy season.

Among the microbes related to the transformation of nitrogen, namely ammonifier, ammonia oxidizer, nitrite oxidizer and denitrifier, the count of denitrifier showed the maximum value amounting to 10^6 to 10^5 per 1 g of dry soil, followed by ammonifier. The population level of nitrogen-fixing blue green algae was unexpectedly high, namely 10^3 to 10^1.

The microbial counts in Brown Forest Soils, Rendzinas and Grumusols with high content of organic matter, available phosphorus and exchangeable potassium tended to be high.

Non-calcic Brown Soils, Reddish Brown Lateritic Soils, Alluvial Soils, Red-Yellow Podzolic Soils and Gray Podzolic Soils which lack in some nutrients showed intermediate levels of microbial populations, while the counts of nitrogen-fixing blue green algae in Alluvial Soils and those of denitrifier in Red-Yellow Podzolic Soils were markedly high. In the case of Low Humic Gley Soils

and Regosols with low content of organic matter, available phosphorus and available potassium, the population of microbes was generally small.

The relationship between the organic matter content and the microbial population of soils was positively significant at the level of 1% only in the case of fungal population ($r = 0.551$), while the relationship between the available phosphorus content and the microbial population was positively significant at the level of 0.1% only in the case of *Azotobacter* ($r = 0.682$).

The relationships between the total nitrogen, the exchangeable potassium, the amount of NH_4^+–N, the amount of NO_3^-–N, or the amount of NH_4^+–N + NO_3^-–N and each microbial population were not significant in any microbial group.

The count of denitrifier in upland farm soils of Thailand was 9 times as high as that in non-volcanic upland-farm soils of Japan and was 23 times that in volcanic ones. In the case of *Azotobacter*, the count in upland farm soils of Thailand averaged 2,800 per 1 g of dry soil, while that in non-volcanic upland farm soils of Japan was 77 on the average.

The ratio of aerobic bacteria to actinomycetes in upland farm soils of Thailand was 2.31, while that of non-volcanic ash soils of Japan was 7.28.

V Comparison of actinomycete flora between tropical and temperate upland farm soils on the basis of physiological properties

Actinomycetes have been considered to play an important role in the decomposition of organic matter, especially in upland soils. Actinomycete flora is known to show variations in its composition depending on soil types, reflecting soil physical and chemical porperties.[3, 19, 37, 55] Furthermore actinomycete flora peculiar to each soil type is reported to be maintained throughout the seasons, though some small variations can be observed.[3]

As there is a difference between temperate and tropical soils, actinomycete flora is estimated to be different in the two regions. On the other hand as shown in part IV, the ratios of the number of actinomycetes to that of bacteria in tropical upland farm soils were very high compared with those in temperate ones. Therefore, the composition of actinomycetes in soils of the two regions is considered to be an important topic of investigation.

So far comparison of actinomycete flora between tropical and temperate soils has been not reported in the literature. Therefore, the actinomycete flora in Thailand upland farm soils belonging to various great soil groups was compared with that in Japanese soils on the basis of physiological characteristics of actinomycetes.

Materials and methods

Among the main upland soil types in Thailand, 28 soil samples were selected and collected from the plow-sole during the period of crop cultivation in the rainy season. A total of about 2 Kg of each soil sample was gathered from 3 to 4 areas in the same field covering a large surface to eliminate fluctuations. Soil samples were passed through a 2 mm sieve.

Some chemical and physical properties of soil samples used were analyzed by the same methods as those described in part IV. Exchangeable calcium was determined by the atomic absorption spectrophotometer method after extraction with ammonium acetate solution (pH 7.0). Results are shown in Table 11. After two weeks' incubation at 28°C on albumin agar,[98] fifty isolates of actinomycetes were obtained by random selection from each soil sample. Their physiological properties were analyzed by the same methods as those described in the previous paper.[3]

Results and discussion

1 Comparison of actinomycete flora on the basis of physiological characteristics

Since all of the soil samples from the tropics belonged to the non-volcanic ash group, data on non-volcanic ash soils from Japan,[3] were used for comparison.

Physiological properties to produce chromogenic-pigment and protease are used as criteria for identification of actinomycetes.[54,86] Ratios of chromogenic or protease-positive actinomycetes to the total number of actinomycetes isolated from each soil sample were plotted in Fig. 4. From this figure, a significant difference in the composition of actinomycete flora between the tropical and Japanese soils, and moreover marked differences among major tropical great soil groups can be recognized.

In connection with the decomposition of organic matter, ratios of cellulase-positive or protease-positive actinomycetes to the total number of actinomycetes were plotted in Fig. 5. From this figure, marked differences in ratios of cellulase-positive actinomycetes to the total isolates were found between the tropical and Japanese soils. The ratios of cellulase-positive actinomycetes in tropical upland farm soils were obviously higher than those in Japanese upland farm soils. But there

Table 11. Some chemical and physical properties of soil samples used

Soils	Moisture (Fresh soil %)	pH (H_2O)	Organic -C (%)	Total -N (%)	NH_4^+—N plus NO_3^-—N mg/100 g dry soil	Available -P (ppm)	Exchangeable K (ppm)	Exchangeable Ca (ppm)
1. Red-Yellow Podzolic Soils								
1) Phetchaburi, Thayang	4.9	7.2	1.62	0.087	1.42	6.3	164	1200
2) Prachuapkirikhan, Pranburi	8.8	6.3	0.54	0.032	2.26	2.2	160	310
3) Prachuapkirikhan, Muang	7.6	6.0	1.49	0.141	3.63	2.4	100	820
4) Lopburi, Phattananikom	7.6	6.2	0.62	0.057	1.82	4.3	30	780
5) Nakhonratchasima, Sikhiew (i)	7.3	6.1	0.48	0.041	1.68	20.0	97	2930
6) Nakhonratchasima, Sikhiew (ii)	8.7	6.3	0.34	0.022	1.71	4.4	25	4600
7) Chaiyaphum, Bamnet Narong	9.9	6.5	0.75	0.042	1.36	6.1	49	266
2. Regosols								
8) Prachuapkirikhan, Hua-Hin (i)	5.8	4.9	0.54	0.055	1.08	2.4	70	74
9) Prachuapkirikhan, Hua-Hin (ii)	4.1	5.7	0.32	0.029	1.05	2.1	58	100
10) Prachuapkirikhan, Hua-Hin (iii)	7.5	6.4	0.77	0.040	0.85	7.0	43	370
11) Prachuapkirikhan, Muang (i)	5.7	6.8	0.56	0.030	2.14	1.1	13	730
12) Prachuapkirikhan, Muang (ii)	12.4	6.3	0.62	0.055	1.07	2.5	80	780
3. Gray Podzolic Soils								
13) Phetchaburi, Thayang	5.2	7.8	0.78	0.078	1.11	10.5	120	1410
14) Phetchaburi, Cha-Am (i)	2.4	6.2	0.76	0.037	1.02	4.6	51	614
15) Phetchaburi, Cha-Am (ii)	2.5	6.4	0.55	0.039	1.38	6.7	94	615
16) Prachuapkirikhan, Pranburi	4.9	6.8	0.47	0.027	1.24	4.2	78	1220
4. Brown Forest Soils								
17) Lopburi, Chaibadan (i)	10.2	7.4	1.98	0.071	2.56	48.4	445	7350
18) Lopburi, Chaibadan (ii)	12.9	7.4	2.31	0.172	2.69	15.5	253	5510
19) Lopburi, Chaibadan (iii)	7.9	7.2	1.85	0.122	0.98	6.0	119	2600

Soils	Moisture (Fresh soil %)	pH (H_2O)	Organic -C (%)	Total -N (%)	NH_4^+—N plus NO_3^-—N mg/100 g dry soil	Available -P (ppm)	Exchangeable K (ppm)	Exchangeable Ca (ppm)
5. Non-calcic Brown Soils								
20) Prachuapkirikhan, Kuiburi	4.3	5.9	0.81	0.061	2.91	3.7	102	1230
21) Suphanburi, U-Thong	15.1	6.2	2.14	0.121	2.36	87.2	283	1390
6. Reddish Brown Lateritic Soils								
22) Lopburi, Muang	4.1	7.1	0.70	0.058	2.34	45.9	68	1230
23) Nakhonratchasima, Pak-chong	19.3	4.7	1.54	0.120	1.80	2.7	97	1190
7. Grumusols								
24) Lopburi, Phattananikhom	17.2	7.2	2.65	0.169	2.97	15.5	140	6180
25) Lopburi, Chaibadan	12.2	6.6	2.44	0.123	2.68	18.8	396	4470
8. Alluvial Soils								
26) Phetchaburi, Thayang	10.3	6.6	0.49	0.031	1.38	21.3	29	780
9. Rendzinas								
27) Lopburi, Phattananikom	6.9	7.3	2.19	0.155	2.58	20.5	180	5070
10. Low Humic Gley Soils								
28) Chaiyaphum, Chatturat	10.5	6.8	0.68	0.044	2.43	4.5	31	715

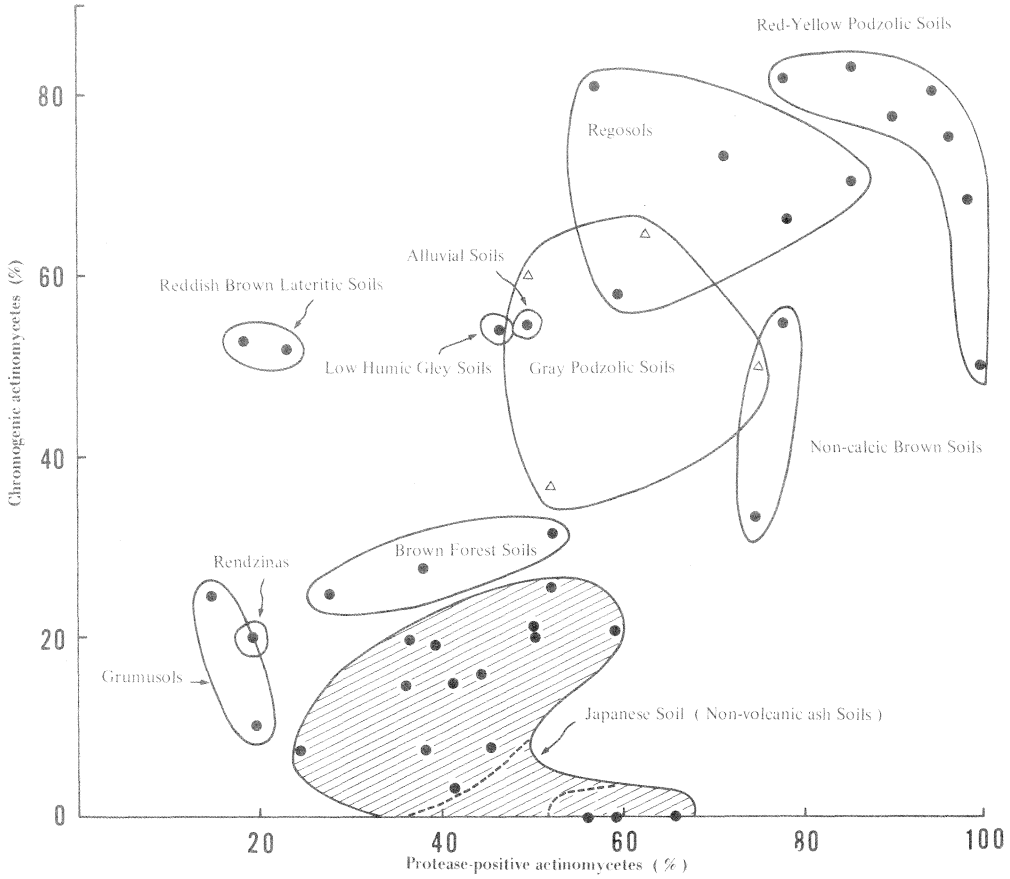

Fig. 4. Actinomycete flora in the tropical and Japanese upland farm soils, on the basis of chromogenic pigment and protease production.

was no difference in the ratios of cellulase-positive actinomycetes to the total isolates among the great soil groups of the tropics.

On the other hand, the ratios of protease-positive actinomycetes to the total isolates showed large variations among great soil groups of the tropics. Their ratios showed high values in such great soil groups as Red-Yellow Podzolic Soils and Regosols. A significant negative correlation ($r = -0.5089$) between the percentage of protease-positive actinomycetes to the total actinomycetes and the exchangeable calcium content in soils was obtained. Likewise, significant negative correlations between the percentage of protease-positive actinomycetes to the total actinomycetes and the total nitrogen content in soils, and between the former and the potassium content in soils were found, respectively ($r = -0.4546, -0.4025$).

As shown in Table 12, the percentage of chromogenesis-positive actinomycetes to the total isolates was 52.4% on the average in the soils of Thailand and 11.9% in the soils of Japan. The

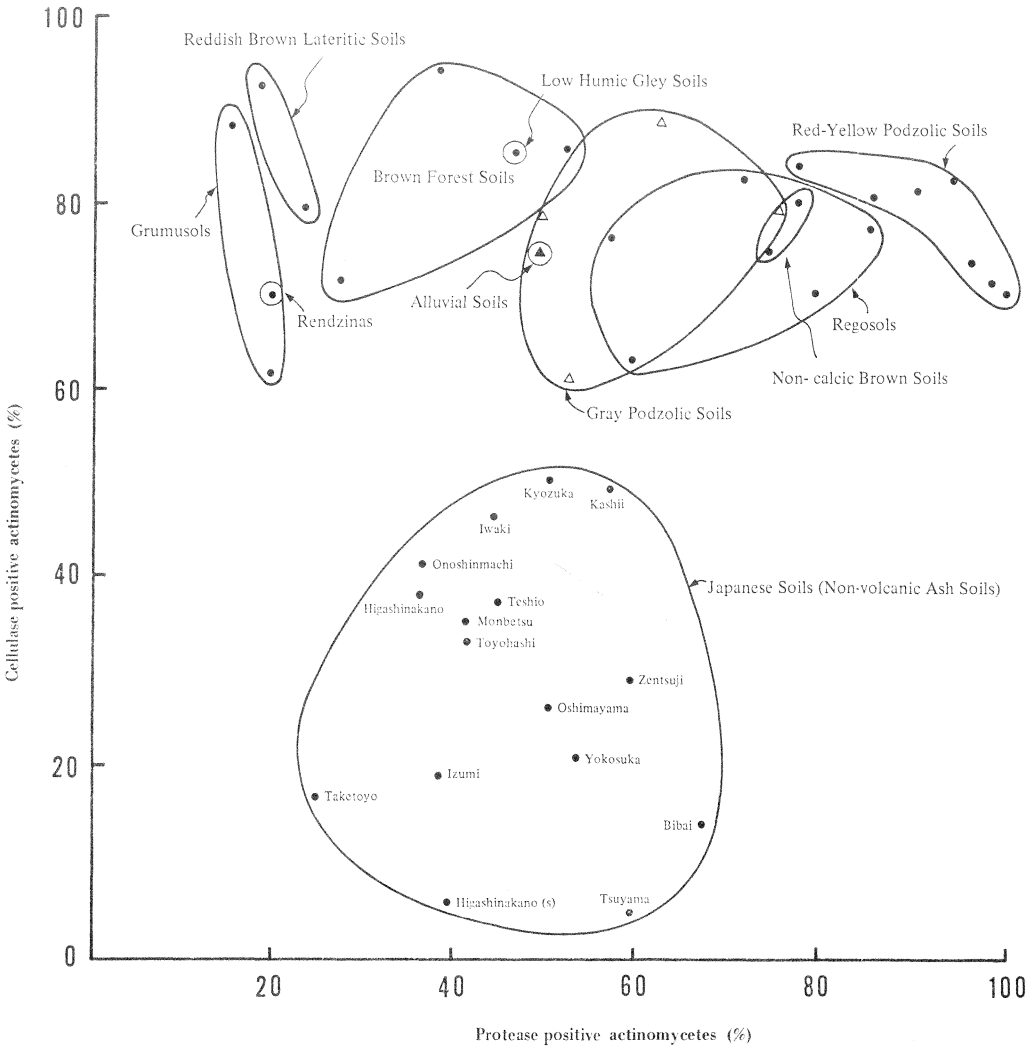

Fig. 5. Actinomycete flora in the tropical and Japanese upland farm soils, on the basis of cellulase and protease production.

difference was significant at the level of 0.1%. In the case of Japanese volcanic-upland farm soils with higher organic matter content, the percentage of chromogenic actinomycetes was 20.9%, whereas that in Australian upland soils was 22.6%.[36] On the basis of these data, the percentage in tropical upland farm soils was considered to be very high. A high proportion of chromogenic streptomyces has been reported in grassland but their proportion was found to be much lower in compost samples and soils to which manure had been applied.[17]

The percentage of protease-positive actinomycetes to the total isolates in the soils of Thailand was 60.1%, while that in the soils of Japan was 46.1%. The difference in the percentage was significant at the level of 2.5%. In the case of Japanese volcanic-upland farm soils, the percentage of protease-positive actinomycetes was 44.7% and was similar to that in non-volcanic ash soils, while

Table 12. Percentage of isolates positive for each physiological criterion in upland farm soils of Thailand (Rainy season) and Japan

Physiological criterion	Soils				
	Thailand (n=28)		Japan (n=16)		
	Average	Standard deviation	Average	Standard deviation	Difference between Thailand and Japan
	%		%		%
Chromogenesis	52.4	20.1	11.9	8.0	40.5*****
Protease	60.1	26.5	46.1	10.6	14.0**
Cellulase	77.3	8.3	29.1	13.9	48.2*****

***** and **: Significant at 0.1% and 2.5% level, respectively.

that in Australian upland soils was 32.1%.[36] The value observed in tropical upland farm soils was the highest ever recorded.

On the other hand, the percentage of cellulase-positive actinomycetes to the total isolates in the soils of Thailand was very high, amounting to 77.3%, while that in the soils of Japan was 29.1%. The percentage in each soil of Thailand was higher than that in any soil of Japan. The percentage of cellulase-positive actinomycetes in Japanese volcanic-upland farm soils was 24.6% and in Australian soils[36], 37.5%. Soils with low organic matter content generally showed higher percentage of cellulase-positive actinomycetes to the total isolates.

Among the factors contributing to soil fertility and hence to crop production, an important one is generally considered to be the nitrogen content either in upland farm soils or in paddy soils, irrespective of climatic zones.[95] There is also a parallelism between nitrogen and carbon contents of soils.

The nitrogen content in tropical upland farm soils is generally low compared with that in temperate soils, though there are some variations among the great soil groups. It was reported[40] that the nitrogen content in the soils of Canada and of the USA decreased from the north to the south, corresponding to the increase in the yearly mean temperature in these areas. Furthermore, as the amount of organic matter supplied to the soils was conspicuously affected by the yearly mean temperature, the variations in soil nitrogen contents have been reported to be mainly caused by the difference in the effect of temperature on microbial activity. The decomposition of organic matter which is enhanced by high temperature, proceeds at a much faster pace in the tropical than in the temperate region.[39]

However, it is also considered that the microflora related to the decomposition of organic matter may be different in tropical and temperate soils. In the case of upland soils, actinomycetes is an important agent responsible for the decomposition of organic matter. As shown in Fig. 5, a difference in the ratio of cellulase-positive actinomycetes to the total isolates was clearly demonstrated between tropical and temperate upland farm soils. Moreover the ratio of protease-positive actinomycetes to the total isolates was also higher in tropical soils than in temperate soils though some variations were found among the great soil groups. Actinomycete flora in tropical upland farm soils was found to be constituted mainly by isolates with cellulase and protease activities.

2 Relationship between carbon content of soils and chromogenic actinomycetes

As shown in Fig. 4, high ratios of chromogenic actinomycetes to the total isolates were found in great soil groups with low organic matter contents, while low ratios were observed in great soil

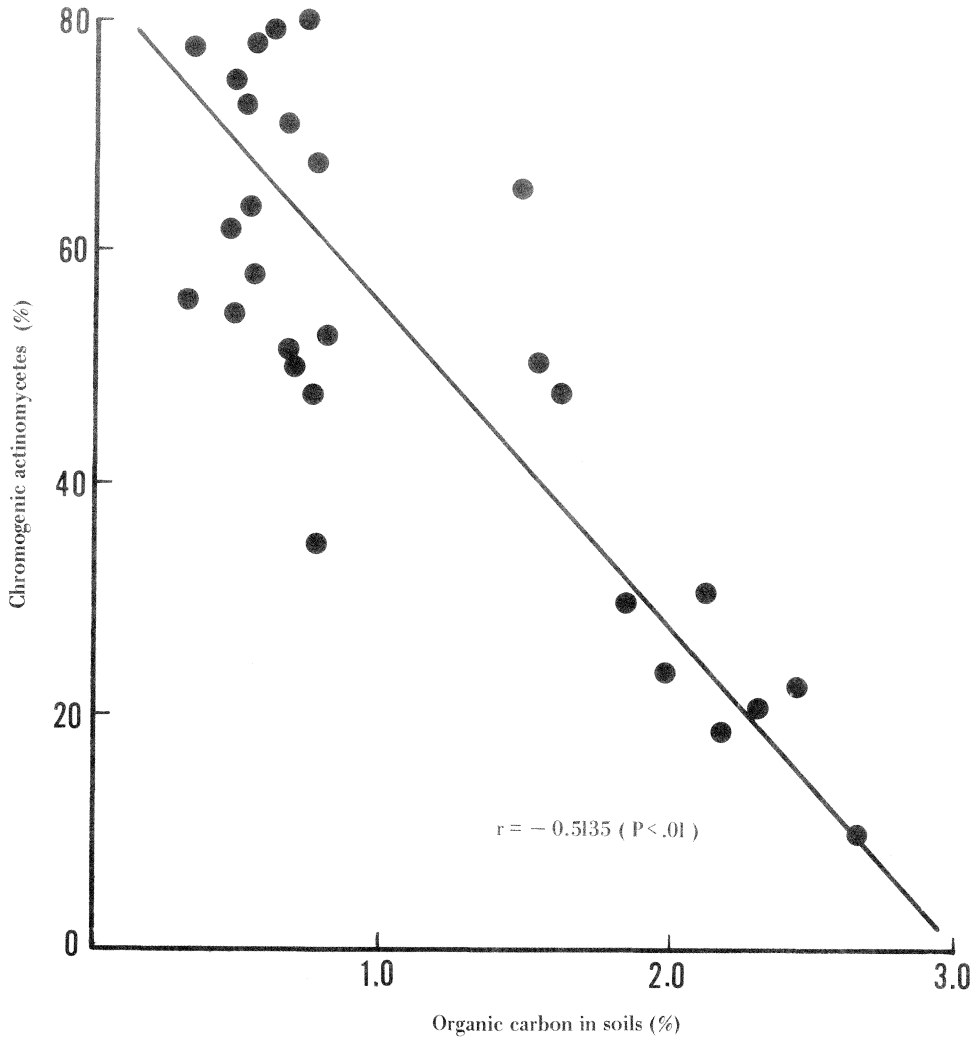

Fig. 6. Correlation between carbon contents and the ratio of chromogenic actinomycetes to the total number of actinomycetes in tropical upland farm soils.

groups with high organic matter content. Accordingly, the correlation between carbon contents in soils and the ratios of chromogenic actinomycetes to the total ones was examined as shown in Fig. 6. A correlation significant at 1% level was obtained, showing that the chromogenic actinomycetes were a dominant group especially in soils with low carbon content.

From Fig. 6, it was assumed that the chromogenic actinomycetes might possess a strong ability for decomposing organic matter. Subsequently, the physiological properties to produce protease and cellulase in chromogenic and non-chromogenic actinomycetes are shown in Table 13. Ratios of each of protease- and cellulase-positive isolates to the total ones were higher in the chromogenic group than in the non-chromogenic group. It was also noticed that the chromogenic group contained high percentage (70%) of isolates positive to both protease and cellulase, in contrast to the non-chromogenic group (24%).

Table 13. Percentage of colonies producing protease, cellulase, or both protease and cellulase in chromogenic and non-chromogenic actinomycetes

	Total of colonies	Protease positive	Cellulase positive	Both protease and cellulase positive
Chromogenic actinomycetes	674	516 (76.5%)	596 (88.4%)	475 (70.4%)
Non-chromogenic actinomycetes	606	257 (42.4%)	404 (66.5%)	148 (24.4%)

These results indicate that tropical soils contain predominantly chromogenic actinomycetes with strong protease and cellulase activities and this trend is evident in soils with low carbon content, as shown in Fig. 6.

In the case of Japanese upland farm soils, the ratio of chromogenic actinomycetes to the total ones was larger in volcanic ash soils with high organic matter content than in non-volcanic soils. The ratio was 20.9% in the former ones and 11.9% in the latter, respectively. This result was not in accord with that recorded in Thailand. The factors causing this discrepancy are considered as follows; i) the difference in the quality of organic matter between volcanic ash soils and non-volcanic ones in Japan[52] ii) the difference in soil physical properties between them.[63]

On the other hand, melanin (chromogenic substance) fulfills a protective function against digestion by lytic enzymes, e.g., chitinase, ß-glucanase from bacteria and streptomycetes.[8,53] Cell wall and cytoplasm of an unnamed melanin fungus were more resistant to decomposition than those of non-melanin hyaline fungus.[24] The protective effect is thought to be due either to the polyaromatic compounds, precursors of melanin formation, which cover the biodegradable polysaccharides, or to the melanin itself which inhibits the function and lytic activity of the enzymes involved.[54]

The dominance of chromogenic actinomycetes in tropical upland soils where competition among microorganisms is severe may be ascribed partly to the inhibitory role of melanin.

Summary

Actinomycete flora in upland farm soils was compared between tropical (Thailand) and temperate (Japan) areas. The percentage of chromogenic actinomycetes to the total isolates was 52.4% on the average in the soils of Thailand and 11.9% in the soils of Japan with a significant level of 0.1%, while that of protease-positive actinomycetes was 60.1% in the former soils and 46.1% in the latter with a significant level of 2.5%. The percentage of cellulase-positive actinomycetes to all isolates of actinomycetes in the soils of Thailand was very high, amounting to 77.3%, while that in the soils of Japan was 29.1%. The percentage in each soil of Thailand was higher than that in any soils of Japan. A significant difference in the composition of actinomycete flora between the tropical and temperate soils, and moreover marked differences among major tropical great soil groups were recognized.

A correlation between carbon contents in soils and the ratios of chromogenic actinomycetes to the total isolates was obtained with a significance at 1% level. It was also noticed that the chromogenic group contained high percentage (70%) of isolates with both protease and cellulase, in contrast to the non-chromogenic group (24%). These results indicate that soils in Thailand (Rainy season) contain predominantly chromogenic actinomycetes with strong protease and cellulase activities and this trend is particularly evident in soils with low carbon content.

VI Comparison of actinomycete flora between tropical and temperate upland farm soils on the basis of genus composition and antagonistic property

In part V, the flora of actinomycetes which is a major component of soil microorganisms in upland soils was compared on the basis of physiological properties between tropical (Thailand) and temperate (Japan) upland farm soils. And it was demonstrated that the actinomycete flora showed marked differences between the two regions, and also among the great soil groups of the tropics.

In this part the genus composition of actinomycetes was investigated to clarify the composition of actinomycete flora of tropical upland farm soils in detail. Moreover the antibiotic production of actinomycetes, which has been considered to be one of the factors regulating soil microflora,[86] was studied in the upland farm soils of major great soil groups in Thailand, and was compared with that in Japanese soils. Antibiotic-spectrum of actinomycetes was analyzed in relation to soil chemical and physical properties. Furthermore the relationship between the percentage of actinomycetes antagonistic to gram-negative bacteria to all actinomycete isolates and population of gram-negative bacteria was investigated in relation to soil properties.

Materials and methods

1 Genus composition and antibiotic production of actinomycetes

Actinomycete isolates, numbering 1240, were the same as those used in part V. The method of identification of genus was the same as that reported in the literature.[98] An indication of the antagonistic property of isolates was obtained by using the agar cylinder method as shown in the previous paper.[31]

Test organisms used for antibiotic production of actinomycetes were as follows: *Rhizoctonia solani Pi-63, Fusarium oxysporum F. cucumerinum* Owen, *Bacillus cereus var mycoides* IAM 1190, *Bacillus megaterium* IAM 1030, *Bacillus subtilis* IAM 1069 (ATCC 6633), *Sarcina lutea* IAM 1099, *Enterobacter aerogenes* IAM 1063 (ATCC 8303), *Erwinia aroidea* (Townsend) Holland, *Pseudomonas marginalis* (Brown) *Steunes, Escherichia coli najjar strain* IAM 1239.

2 Experiment on a relationship between population of Gram-negative bacteria and antibiotic production of actinomycetes

Red-Yellow Podzolic Soils (No.6), Regosols (No.12) and Gray Podzolic Soils (No.16) were selected as representative soils in which actinomycetes antagonistic to Gram-negative bacteria among all actinomycete isolates showed a low percentage, while Brown Forest Soils (No.18), Grumusols (No.24) and Rendzinas (No.27) were selected as representative soils containing a high percentage of such actinomycetes. The soils in parentheses are the same as those in Table 17, and their maximum water-holding capacity, organic carbon contents and percentages of actinomycetes antagonistic to Gram-negative bacteria among all actinomycete isolates, are shown in this table. The determinations of maximum water-holding capacity and organic matter content in soils were made in using the same methods as those described in part IV. Some other chemical soil properties were outlined in part V.

Each soil was incubated at 28°C for one week after adjusting the moisture content so as to reach a level of 20, 40 and 60% for maximum water-holding capacity. Population of Gram-negative bacteria was counted after growth on crystal violet medium,[98] while that of Gram-positive bacteria was obtained by subtracting the number of Gram-negative bacteria on crystal violet medium from the bacterial number on albumin agar medium.[98] The population of actinomycetes was counted after growth on albumin agar medium.

Results and discussion

1 Genus composition

As shown in Table 14, genus composition of actinomycetes in the upland farm soils of Thailand located in the tropical savanna zone was, as a whole, as follows: 75.4% of total isolates belonged to *Streptomyces*, followed by *Nocardia* (4.8%), *Streptosporangium* (3.2%) and *Micromonospora* (1.5%), respectively. On the other hand, actinomycetes producing non-aerial mycelium (without spores) accounted for 13.0% of all isolates. In the case of non-volcanic ash soils of Japan located in the humid temperate zone[3], the percentage of actinomycetes producing non-aerial mycelium (without spores) was markedly higher than that in Thailand, and that of *Micromonospora*, slightly higher in Japan. In contrast, in Thailand, the percentage of *Streptomyces* was higher and that of *Streptosporangium*, slightly higher in Thailand. In the case of non-volcanic ash soils of Australia located in the dry zone, *Streptomyces* showed a high percentage, namely 80%, and *Streptosporangium* (5.1%), *Micromonospora* (4%) and *Nocardia* (0%) were also reported.[36] Compared with the data in Australian soils, the percentages of *Streptomyces*, *Streptosporangium* and *Micromonospora* in the soils of Thailand were lower to some extent, while that of *Nocardia* was higher. Moreover in the case of tropical desert soils of Saudi Arabia (17), *Streptomyces* accounted for 94% of all isolates, followed by *Nocardia* (2.5%) and *Micromonospora* (2.5%). Compared with such soils, the soils of Thailand which are located in a more humid climate, though in the tropics, contained a slightly lower percentage of *Streptomyces*, and were found to harbour *Micromonospora* which was frequently observed in paddy soils[32] and peat soils.[33]

It could be concluded that the more severe the dryness of soils, the higher the value of the percentage of *Streptomyces* and the lower that of actinomycetes producing non-aerial mycelium (without spores), though some variations in the composition of actinomycetes were observed among great soil groups. Szabo *et al.*,[79] who found that sterile types predominate in deeper layers (B-horizon), while sporulating types occur more frequently in the A-horizon, suggested that this phenomenon was caused by better aeration and dryness of the upper layers compared with the wet B-horizon. Similar result was reported by the author.[3] The low percentage of actinomycetes producing non-aerial mycelium (without spores) in the soils of tropical savanna and desert may be ascribed to dryness of soils. Williams *et al.* also reported[90] that the growth of all actinomycete strains tested was severely limited or absent at tensions greater than pF 4.0 but that spores of *Streptomyces* survived for a long period in the dry season and furthermore that their tolerance of high moisture tensions was greater than that of their own vegetative hyphae.

On the other hand as shown in Table 14, spiral forms of *Streptomyces* amounted to 38.8% in the soils of Thailand and showed low value, namely 11.5%, in the non-volcanic ash soils of Japan.[31] The difference was significant at the level of 0.1%. Differences in the morphology of sporophore enabled to distinguish species composition of *Streptomyces* in the two regions.

The species composition of *Streptomyces* has been reported to vary depending on soil types.[3, 19, 38, 55] Küster found that *Streptomyces malachiticus* was detected only in tropical soils,[55] while in the tropical desert soils, *S. aburabiensis* and *S. pseudogriseus* were reported to be the dominant species.[16] The dominant species of *Streptomyces* in non-volcanic ash upland farm soils of Japan were *S. lavendulae* and *S. griseus*.[3]

As shown in part V, actinomycete flora in such soils with high organic matter contents as Grumusols, Rendzinas and Brown Forest Soils was similar to that of Japanese non-volcanic ash soils on the basis of chromogenic-pigment and protease production. But as seen in Table 14, the genus composition of actinomycete flora of the above three great soil groups differed from that of Japanese soils. In the case of Grumusols, the percentage of *Streptomyces* was lower, and that of

Table 14. Percentage distribution of actinomycete genus in upland farm soils of Thailand (Rainy season)

Great soil groups	Number of isolates	*Streptomyces*		*Streptosporangium*	*Nocardia*	*Micromonospora*	Unidentified	
		spiral	straight				(A)*	(B)**
1) Red-Yellow Podzolic Soils	312	54.5%	29.8%	2.2%	2.2%	0.3%	9.0%	1.9%
2) Regosols	204	48.0	38.2	1.4	2.9	0	7.4	2.0
3) Gray Podzolic Soils	181	42.0	30.4	2.2	12.7	0	8.8	3.3
4) Brown Forest Soils	138	26.8	51.5	0.7	2.2	0	18.8	0
5) Non-calcic Brown Soils	81	23.5	48.1	3.7	2.5	1.2	19.8	1.2
6) Reddish Brown Lateritic Soils	97	29.9	47.4	5.2	2.1	0	8.2	7.2
7) Grumusols	91	15.4	20.8	14.3	7.7	16.5	23.1	2.2
8) Alluvial Soils	46	32.6	32.6	2.2	6.5	0	26.1	0
9) Rendzinas	41	19.5	41.5	2.4	14.6	2.4	19.5	0
10) Low Humic Gley Soils	49	30.6	42.9	2.0	0	2.0	22.4	0
Average		38.8	36.6	3.2	4.8	1.5	13.0	2.1

* Actinomycetes producing non-aerial mycelium (without spores)

**Actinomycetes producing aerial mycelium (without spores)

Streptosporangium (14%) and *Micromonospora* (17%) was higher than in the Japanese soils. It was conspicuous that Grumusols with high maximum water-holding capacity contained a fairly high percentage of *Micromonospora* which was generally found in such soils with high moisture content as paddy and peat soils.[32, 33]

On the other hand, Rendzinas and Gray Podzolic Soils showed high percentages of *Nocardia*. In relation to this, it was reported[15] that the nocardia forms increased where there was active decomposition of humus. Numbers of nocardia forms were lower in peat bog and chernozem soils where there was humus accumulation. Unfertilized soil of Timiryazev near Moscow, which had remained fallow for over 50 years, contained a higher percentage of nocardia forms than the soil from adjacent plots which had been continuously planted with rye. However, in the case of tropical upland farm soils, the organic matter content in Rendzinas was high, and great soil groups with low organic matter content showed low percentages of *Nocardia* as shown in Tables 14 and 17.

2 Difference in antibiotic production of actinomycetes among great soil groups

As shown in Table 15, of all actinomycete isolates, the percentages of those antagonistic to any of the 9 test organisms used showed similar high values, namely 58 to 74%, among the great soil groups. On the other hand, those antagonistic to each test organism showed variations among great soil groups, reflecting the differences in the composition of actinomycetes as follows: high percentage of isolates antagonistic to Gram-negative bacteria (Rendzinas), high percentage antagonistic to both Gram-positive and Gram-negative bacteria (Brown Forest Soils), high percentage antagonistic to both *R. solani* and Gram-positive bacteria (Gray Podzolic Soils), high percentage antagonistic to *R. solani* and low percentage of Gram-positive bacteria (Red-Yellow Podzolic Soils), high percentage antagonistic to both fungi and Gram-positive bacteria (Low Humic Gley Soils). The differences in percentage of antagonistic actinomycetes to all actinomycete isolates may be mainly ascribed to the difference in the composition of actinomycetes, and also partly to the difference in the antagonistic properties of strains of the same species, reflecting soil properties.[3, 86] Antibiotic spectrum of soil actinomycetes varied depending on soil groups. VALYI-NAGY *et al.*[85] reported that the percentage incidence of antagonistic streptomyces isolates was higher in soils rich in organic matter than in poorer soils, whereas in the case of tropical upland farm soils as shown in Tables 17 and 18, such correlation was observed only for Gram-negative bacteria.The positive correlation coefficients between the organic carbon content in soil and the percentage of actinomycetes antagonistic to *Ent. aerogenes, Erw. aroidea, Ps. marginalis* or *E. coli* were 0.6689, 0.6532, 0.6393, 0.5657, respectively. The former three were significant at 0.1% level, while the last one was significant at 1% level.

3 Difference in antibiotic production between tropical and temperate upland farm soils

As all tropical upland farm soils used belonged to non-volcanic ash groups, the comparison with Japanese upland farm soils was also made in using soils of non-volcanic ash groups. As shown in Table 16, significant differences in the percentage of antagonistic actinomycetes among all actinomycete isolates were observed in the cases of *R. solani* and all four Gram-positive bacteria. All the percentage of antagonistic actinomycetes in tropical soils showing significant differences with Japanese soils were higher than those of Japanese soils, and in particular the difference in *B. cereus* showed the highest significant level, namely 0.1%. In the case of other test organisms, except for *Ps. marginalis*, the percentage was slightly higher in the tropical soils, though not significantly.

Compared with each percentage of actinomycetes antagonistic to *R. solani* or Gram-positive bacteria in Japanese upland farm soils of volcanic ash,[31] or peat group,[33] the percentage in tropical

Table 15. Difference in percentage of actinomycetes antagonistic to each test organism among great soil groups of upland farm soils in Thailand (% to all isolates)

Antagonistic to Great soil groups	*Rhizoctonia solani*	*Fusarium oxysporum*	*Bacillus cereus*	*Bacillus subtilis*	*Erwinia aroidea*	*Escherichia coli*	Any of test organisms
Red-Yellow Podzolic Solis	47.9	16.9	12.6	23.9	6.0	1.5	69.2
Regosols	39.1	15.0	24.4	24.4	6.9	2.6	66.6
Gray Podzolic Soils	53.0	15.3	43.2	39.8	2.8	8.5	72.7
Brown Forest Soils	34.4	12.5	46.0	49.0	19.2	22.9	70.0
Non-calcic Brown Soils	36.3	17.5	32.8	37.0	6.7	8.9	69.7
Reddish Brown Lateritic Soils	34.0	18.7	25.1	26.9	2.1	5.3	60.8
Grumusols	26.1	28.1	28.3	32.7	11.8	10.7	57.6
Alluvial Soils	42.9	16.7	31.0	31.0	16.7	11.9	71.4
Rendzinas	40.4	23.4	31.9	40.4	25.5	17.0	66.0
Low Humic Gley Soils	40.8	40.8	42.9	38.8	8.2	10.2	73.5

upland farm soils was higher. Therefore, as chromogenic actinomycetes were found predominantly in tropical soils as described in part V, the antagonistic property was investigated in the chromogenic and non-chromogenic group, respectively. The percentages of actinomycetes antagonistic to fungi, Gram-positive bacteria and Gram-negative ones were 46.7, 46.1 and 9.7% respectively in the chromogenic group, while 39.2, 40.4 and 14.8% in the non-chromogenic group. The chromogenic group showed a slightly higher percentage of actinomycetes antagonistic to fungi and Gram-positive bacteria than the non-chromogenic one.

The percentage of actinomycetes antagonistic to *F. oxysporum* is very low compared with that to *R. solani* and it has been reported that among eight plant pathogens used as test organisms, *F. oxysporum* showed the highest degree of tolerance.[69]

Table 16. Percentage of actinomycetes antagonistic to each test organism in upland farm soils of Thailand (Rainy season) and Japan (% to all actinomycete isolates)

Soil	Thailand (n=28)		Japan (n=15)		
Test organisms	Average	S.D.	Average	S.D.	Difference
1) *Rhizoctonia solani Pi-63*	41.5	15.0	28.9	16.4	12.6**
2) *Fusarium oxysporum f. cucumerinum* Owen	17.9	9.2	13.6	7.7	4.3
3) *Bacillus cereus var mycoides* IAM 1190	28.5	15.3	14.0	4.6	14.5*****
4) *Bacillus megaterium* IAM 1030	31.4	15.2	18.0	9.2	13.4****
5) *Bacillus subtilis* IAM 1069 (ATCC 6633)	32.0	13.3	20.8	9.0	11.2****
6) *Sarcina lutea* IAM 1099	31.0	14.3	22.2	5.6	8.8***
7) *Enterobacter aerogenes* IAM 1063 (ATCC 8303)	4.6	5.9	2.2	2.3	2.4
8) *Erwinia aroidea* (Townsend) Holland	8.5	7.4	6.1	6.1	2.4
9) *Pseudomonas marginalis* (Brown) *Steunes*	3.4	4.3	4.7	3.3	−1.3
10) *Escherichia coli najjar strain* IAM 1239	7.7	7.7	4.5	4.3	3.2

, *, **** and *****: Significant at 2.5%, 1%, 0.5% and 0.1% levels, respectively.

4 Relationship between populations of Gram-negative bacteria and antibiotic production of actinomycetes

Of all actinomycete isolates, the percentage of those antagonistic to Gram-negative bacteria showed low values as reported by other investigators.[15, 56] As shown in Table 17, however, the percentage differed largely among great soil groups. Brown Forest Soil, Rendzinas and Grumusols with high organic matter content and maximum water-holding capacity, showed high percentage of actinomycetes antagonistic to Gram-negative bacteria, while great soil groups with low organic matter content and maximum water-holding capacity showed low percentage. Soil aggregates in the former three soils were well developed, unlike those in the latter ones. Gram-negative bacteria have been reported[22] to be found in abundance inside soil aggregates because their population density is

mostly regulated by moisture content. On the other hand, paddy soil with low population of fungi is reported to show a low percentage of actinomycetes antagonistic to fungi among all actinomycete isolates.[3] The difference in population of Gram-negative bacteria as well as Gram-positive ones among these great soil groups was deemed interesting to investigate. On the other hand, as indicated in part IV, a significant positive correlation ($r = 0.905$) was obtained between the maximum water-holding capacity and the organic matter content in these soil samples. Therefore, as shown in Table 18, the relationship between the percentage of actinomycetes antagonistic to Gram-negative bacteria among all actinomycete isolates and maximum water-holding capacity which is considered as an index of moisture condition of soils, was examined. The positive correlation coefficients were 0.5979 (*Ent. aerogenes*), 0.5108 (*Erw. aroidea*), 0.5725 (*Ps. marginalis*), 0.5388 (*E. coli*), respectively. The former one was significant at 0.1% level, while the latter three were significant at 1% level. The soils with high percentage of actinomycetes antagonistic to Gram-negative bacteria were significantly high in organic matter content and maximum water-holding capacity.

Therefore, from the data in Table 17, two sets of three soils with high and low percentage of actinomycetes antagonistic to Gram-negative bacteria were selected as indicated previously and incubated at three moisture levels (20, 40, 60% of maximum water-holding capacity, respectively), for one week. In the field conditions, the moisture content of soils varies daily, and so this experiment was carried out at three moisture levels. As shown in Table 19, populations of Gram-negative bacteria in Red-Yellow Podzolic Soils, Regosols and Gray Podzolic Soils were lower than those in Brown Forest Soils, Grumusols and Rendzinas. The ratio of Gram-negative bacteria to Gram-positive ones, and the ratio of Gram-negative bacteria to actinomycetes also showed lower values in the former than in the latter. These data seem to show that actinomycetes in soils with high percentage of actinomycetes antagonistic to Gram-negative bacteria may live to meet more intense competition with Gram-negative bacteria than actinomycetes in soils with lower percentage. These data also suggest that antibiotic production of actinomycetes may function as one of the factors regulating competition among microorganisms.

Table 17. Organic matter content and maximum water-holding capacity of upland farm soils in Thailand (Rainy season) and percentage of actinomycetes antagonistic to Gram-negative bacteria in each soil

Soils	Organic carbon (%)	Maximum water-holding capacity (dry soil %)	Test organisms			
			Enterobacter aerogenes	*Erwinia aroidea*	*Pseudomonas marginalis*	*Escherichia coli*
Red-Yellow Podzolic Soils						
(1)	1.62	45.2	4.3%*	14.9%*	0%*	4.3%*
(2)	0.54	41.2	0	2.2	0	0
(3)	1.49	52.1	2.1	12.8	4.3	2.1
(4)	0.62	38.9	2.0	4.0	0	2.0
(5)	0.48	41.1	0	4.3	2.1	0
(6)	0.34	27.0	0	2.0	0	0
(7)	0.75	36.7	4.1	2.0	2.0	2.0
Regosols						
(8)	0.54	37.6	0	2.2	2.2	0
(9)	0.32	28.3	2.0	8.2	2.0	2.0

Soils	Organic carbon (%)	Maximum water-holding capacity (dry soil %)	Test organisms			
			Enterobacter aerogenes	*Erwinia aroidea*	*Pseudomonas marginalis*	*Escherichia coli*
(10)	0.77	39.0	2.3	13.6	2.3	4.6
(11)	0.56	39.3	0	6.4	0	4.3
(12)	0.62	36.4	0	4.3	0	2.1
Gray Podzolic Soils						
(13)	0.78	36.2	4.4	4.4	4.4	8.7
(14)	0.76	34.2	0	0	2.4	9.5
(15)	0.55	31.7	6.7	6.7	2.2	13.3
(16)	0.47	46.4	0	0	0	2.3
Brown Forest Soils						
(17)	1.98	86.9	20.5	13.6	9.1	25.0
(18)	2.31	81.3	20.4	30.6	20.4	32.7
(19)	1.85	67.6	8.9	13.3	8.9	11.1
Non-calcic Brown Soils						
(20)	0.81	46.5	2.9	2.9	2.9	11.4
(21)	2.14	59.6	6.3	10.4	4.2	6.3
Reddish Brown Lateritic Soils						
(22)	0.70	40.5	4.3	2.1	2.1	8.5
(23)	1.54	86.5	0	2.0	0	2.0
Grumusols						
(24)	2.65	98.4	7.5	15.0	7.5	15.0
(25)	2.44	90.1	4.3	8.5	2.1	6.4
Alluvial Soils						
(26)	0.49	40.5	4.8	16.7	2.4	11.9
Rendzinas						
(27)	2.19	72.5	19.2	25.5	8.5	17.0
Low Humic Gley Soils						
(28)	0.68	35.8	2.0	8.2	2.0	10.2

*Percentage to all actinomycete isolates

Table 18. Relationship between percentage of actinomycetes antagonistic to Gram-negative bacteria and organic matter content of soils as well as maximum water-holding capacity of soils (% to all actinomycete isolates)

Soil properties / Test organisms	Organic matter content	Maximum water-holding capacity
Enterobacter aerogenes IAM 1063 (ATCC 8303)	r = 0.6689**	r = 0.5979**
Erwinia aroidea (TOWNSEND) HOLLAND	0.6532**	0.5108*
Pseudomonas marginalis (BROWN) *Steunes*	0.6393**	0.5725*
Escherichia coli najjar strain IAM 1239	0.5657*	0.5388*

* and **: Significant at 1% and 0.1% levels, respectively

Table 19. Effect of soil moisture on the populations of Gram-negative bacteria, Gram-positive bacteria and actinomycetes in upland farm soils of Thailand

Soils	Moisture level	Gram-negative bacteria ($\times 10^4$)	Gram-positive bacteria ($\times 10^4$)	Actinomycetes ($\times 10^4$)	Gram(−) bacteria /Gram(+) bacteria	Gram(−) bacteria /Actinomycetes
Red-Yellow Podzolic Soils (No.6)	20*	8.3**	169**	398**	0.049	0.021
	40	18.0	215	285	0.085	0.063
	60	24.3	273	353	0.089	0.069
Regosols (No.12)	20	27.3	217	268	0.126	0.104
	40	32.5	208	300	0.156	0.108
	60	38.5	212	278	0.182	0.139
Gray Podzolic Soils (No.16)	20	44.3	368	1143	0.120	0.039
	40	53.5	292	1040	0.183	0.051
	60	80.3	220	1090	0.365	0.074
Brown Forest Soils (No.18)	20	88.8	472	388	0.188	0.228
	40	234	216	465	1.08	0.503
	60	172	198	308	0.869	0.559
Grumusols (No.24)	20	302	283	420	1.07	0.720
	40	121	494	175	0.245	0.690
	60	217	146	570	1.49	0.380
Rendzinas (No.27)	20	91.3	196	273	0.466	0.335
	40	267	128	425	2.09	0.628
	60	134	364	308	0.368	0.434

* Moisture level refers to 20, 40 and 60% of moisture content for maximum water-holding capacity.

**Counts per gram of dry soil.

Summary

The genus composition and antibiotic production of actinomycete isolates (1240), sampled from major great soil groups of tropical upland farm soils in Thailand (Rainy season) were investigated. Of these 75.4% of total isolates belonged to *Streptomyces* followed by *Nocardia* (4.8%), *Streptosporangium* (3.2%) and *Micromonospora* (1.5%). Actinomycetes producing non-aerial mycelium (without spores) accounted for 13.0% of all isolates. A high percentage of *Streptomyces* and a low one of actinomycetes producing non-aerial mycelium (without spores) were found and were ascribed to dryness of soils. Spiral forms of *Streptomyces* were more abundant in the soils of Thailand (38.8%) than in the soils of Japan (11.5%).

The composition of actinomycete flora in the soils of Thailand, as a whole, was found to be different from that of Japan.

The genus composition of actinomycete flora varied among great soil groups. It was conspicuous that Grumusols with high maximum water-holding capacity contained a fairly high percentage of *Micromonospora* (17%), which was generally found in such soils with high moisture content as paddy and peat soils. Rendzinas and Gray Podzolic Soils showed a rather higher percentage of *Nocardia* (13 to 14%).

Percentages of actinomycetes antagonistic to *Rhizoctonia solani* and four test organisms of Gram-positive bacteria were significantly higher in tropical soils than in Japanese soils, while percentages of actinomycetes antagonistic to *Fusarium oxysporum* and to four test organisms of Gram-negative bacteria were not significantly higher.

Percentage of actinomycetes antagonistic to each test organism showed variations among great soil groups. The soils with high percentage of actinomycetes antagonistic to Gram-negative bacteria showed significantly high maximum water-holding capacity and high organic matter content. Furthermore such soils with high percentage of actinomycetes antagonistic to Gram-negative bacteria as Brown Forest Soils, Rendzinas and Grumusols contained higher population of Gram-negative bacteria, higher ratios of population of Gram-negative bacteria compared with Gram-positive ones, and also higher ratios of Gram-negative bacteria to actinomycetes than the soils with low percentages of them.

VII Effect of rice straw on the composition of volatile soil gas and microflora in the tropical paddy field

Even in the tropical areas where soils are low in nutrient content, the effect of rice straw application on rice production has not yet been clarified.[20, 83, 89] Actually, in Thailand, Burma, the Philippines, Indonesia and Malaysia, rice straw is mostly burned out before starting the next rice cultivation. But in some areas of the latter three countries, the incorporation of rice straw into the soil has been practiced by farmers.[83] The reason why rice straw is not used as a substitute for fertilizer in the tropical areas remains unknown.

Many reports on the effect of rice straw application on rice yield in tropical soils have been published, but the analysis of its effect on soil conditions is very complex.

In this part, the effects of rice straw with and without fertilizer application on the composition of volatile soil gas released into the atmosphere and microflora related to the nitrogen cycle during the growing period of rice plants were studied to get more information about microorganisms following the application of rice straw in the tropical paddy fields.

Materials and methods

Field experiment was carried out at the Khok Samrong Rice Experimental Station in Thailand. The design of field experiment was as follows; **a)** Variety: RD1 **b)** Plot size: 5 × 5 m **c)** Replication: 3 **d)** Spacing: 25 × 25 cm **e)** Transplanting: Aug. 15, '72 **f)** Harvesting: Dec. 9, '72 **g)** Soil: Low Humic Gley Soils, Texture CL, Total-C 0.43%, Total-N 0.03%, Total P_2O_5 0.032%, Available P_2O_5 21.1 ppm, Total K_2O 0.0327%, Available K_2O 316 ppm, pH(H_2O) 5.45, as shown by Motomura.[65] **h)** Soil gas collection method: Bottles whose bottoms (6.4 cm in diameter) were cut off, were painted black in order to inhibit the algal growth inside the bottle. Six bottles were set per plot. The top of the bottle, filled up with water, was covered with a rubber-stopper. The bottles, whose bottoms were placed 1 cm above the soil surface were hung inside the surface water. Gas samples were taken periodically from the top of the bottle with a syringe and transferred into a large gas syringe. **i)** Analysis of soil gas: HITACHI 063 TCD gas chromatography was used. (1) N_2, CH_4, O_2: Molecular sieve 5A, 30-60 mesh, was packed in the 3 mm × 200 cm stainless steel column. (2) N_2O, CO_2: Activated charcoal washed with 0.1N H_2SO_4 and rinsed again in water, was packed in the 3 mm × 150 cm stainless steel column. **j)** Microorganisms investigated: ammonifier, ammonia oxidizer, nitrite oxidizer, denitrifier, purple nonsulfur bacteria, *Azotobacter*, *Clostridium*, cellulose decomposer. Medium composition and incubation method used are the same as those described in part I. **k)** Treatment of field experiment: 9 treatments were designed as follows;

Treatment			Kg/ha
Without rice straw	N	P_2O_5	Rice straw
(A) Control	—	—	—
(B) Phosphate	—	40	—
(C) Ammonium sulfate + Phosphate	32	40	—
(D) Ammonium chloride + Phosphate	32	40	—
(E) Ammonium phosphate	32	40	—
With rice straw			
(F) Phosphate + Rice straw	—	40	6,000
(G) Ammonium sulfate + Phosphate + Rice straw	32	40	6,000
(H) Ammonium chloride + Phosphate + Rice straw	32	40	6,000
(I) Ammonium phosphate + Rice straw	32	40	6,000

All plots received 32 Kg-K_2O/ha in the form of KCL. Phosphate was applied in the form of superphosphate except for the plots **(E)** and **(I)**. Fertilizers were applied just before transplanting. On the other hand, rice straw which was cut 5 cm in length was applied on the surface of soils two weeks before transplanting and was kept under submerged condition in order to decrease its inhibitory effect on the rice plant.

Results and discussion

1 Amount and composition of volatile soil gas

As shown in Fig. 7, there were marked variations in the amount of volatile soil gas* between plots with and without rice straw. The amount of volatile soil gas increased nearly 10 times following the application of rice straw amounting to 6 tons per hectare.

Either phosphate alone or phosphate with N-fertilizer application increased also the amount of volatile soil gas. In the case of the former, the amount of volatile soil gas increased about 50% more than that in the control plot. In the latter case the amount of gas varied depending on the nature of N-fertilizers applied and the differences were enhanced by rice straw application. The largest amount of volatile soil gas was obtained in the plot with ammonium sulfate, phosphate and rice straw.

The rate of gas formation in the plots without rice straw showed the maximum value one to three weeks after transplanting (3 to 5 weeks after submergence), whereas that in the plots with rice straw remained very high until 7 weeks after transplanting (9 weeks after application of rice straw).

As shown in Table 20, among the components of volatile soil gas in the control plot, the highest value was recorded for molecular nitrogen followed by molecular oxygen, other gases, carbon dioxide and methane, respectively until two weeks after transplanting, whereas 35 to 65 days after transplanting, their order was changed to molecular nitrogen, other gases, molecular oxygen, carbon dioxide and methane. Percentage distribution of molecular nitrogen gas in the control plot ranged from 41 to 62% until 65 days after transplanting. BELL[6] reported that more than 92% of the gas found in untreated soil was molecular nitrogen until 98 days after submergence and 49% between 98 and 126 days after submergence. HARRISON *et al.*[21] reported that molecular nitrogen made up more than 70% of the gas phase in the planted soil at the later growth stage of rice plant. Percentage of molecular nitrogen in volatile soil gas of the sandy soil with low organic nitrogen content studied in the present investigation was lower than values reported before.

As shown in Table 20, methane gas formation in the control plot was low and was found only during the middle stage of rice growth in sandy soil with low organic matter content. Following phosphate application, the percentage of methane gas increased, especially until 5 weeks after transplanting due to the enhancement of microbial growth by phosphate application in sandy soil with low phosphate content. In the case of plots with rice straw, methane was the gas most abundantly found until 7 weeks after transplanting. Molecular nitrogen was found to be the most abundant gas during the later stage of rice growth. It was reported that when peptone or cellulose was added to the soil, most of the gas present in the soil was methane and carbon dioxide about 20 to 40 days after submergence with only a small percentage of nitrogen gas.[6]

Unexpectedly high percentage of molecular oxygen was supposed to be transported by rice plant,[94] and to originate from the activity of algae and weeds, because the bottom of the bottle was near the surface of paddy soils.

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* Volatile soil gas in this report means soil gas released into the atmosphere.

As shown in Table 20, the percentage of other gases, except molecular oxygen, molecular nitrogen, methane and carbon dioxide was fairly high and increased at the later stage of rice growth. ADAMSON *et al.*[1] reported that less than 10% of added carbon was recovered as organic compound

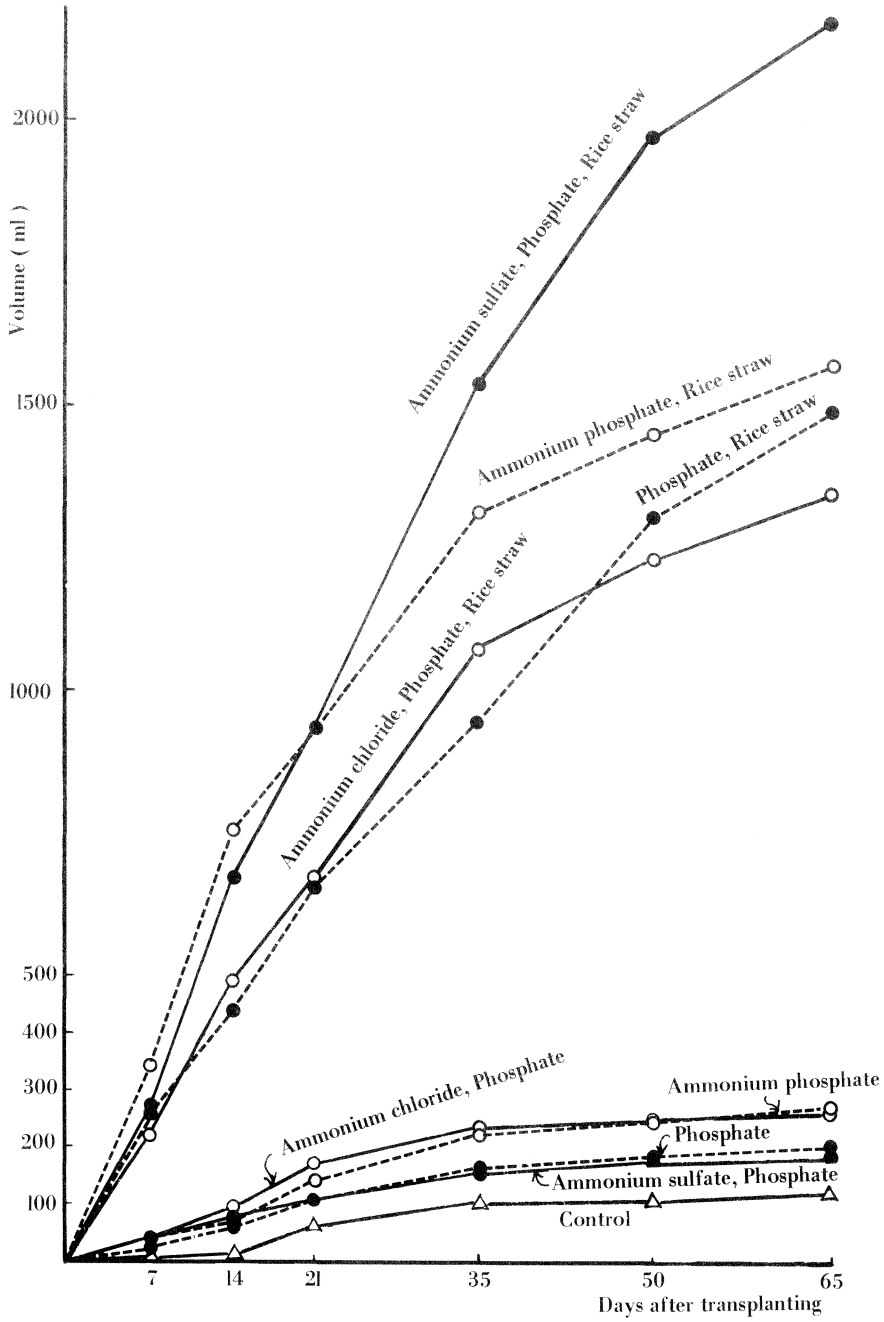

Fig. 7. Volume (1 atm, 25°C) of volatile soil gas released into atmosphere from a surface area of 193 cm² in each plot during the growing period of rice plants.

Table 20. Percentage distribution of volatile soil gas components in each plot during the growing period of rice plants

Treatment	Gas	Days after transplanting				
		0—7	7—14	21—35	35—50	50—65
Without rice straw						
(A) Control	N_2	56.0%	62.3%	41.0%	47.8%	46.3%
	CH_4	trace	trace	5.9	0.4	trace
	CO_2	0.7	2.3	3.5	1.0	1.0
	O_2	35.5	31.1	27.1	22.8	25.5
	others	7.8	4.3	22.5	28.0	27.0
(B) Phosphate	N_2	38.5	46.4	27.0	48.8	45.8
	CH_4	11.6	9.5	9.6	0.8	1.3
	CO_2	2.1	1.7	3.4	0.7	4.4
	O_2	28.1	19.0	44.3	21.9	21.2
	others	19.7	23.4	15.7	27.8	27.3
(C) Ammonium sulfate, Phosphate	N_2	27.6	31.0	18.4	50.7	45.8
	CH_4	trace	trace	3.0	0.8	1.7
	CO_2	0.7	0.2	1.8	2.6	2.5
	O_2	60.0	51.3	58.0	25.8	19.4
	others	11.7	17.5	18.8	20.1	30.6
With rice straw						
(F) Phosphate, Rice straw	N_2	17.9	16.5	24.3	42.3	41.3
	CH_4	45.8	45.1	41.7	23.6	15.6
	CO_2	6.5	8.0	10.0	0.5	2.5
	O_2	10.1	6.9	3.8	15.4	16.6
	others	19.7	23.5	20.2	18.4	24.6
(G) Ammonium sulfate, Phosphate, Rice straw	N_2	20.1	21.6	14.4	43.0	35.4
	CH_4	44.6	44.7	45.4	26.3	15.4
	CO_2	6.3	9.3	8.9	2.7	8.8
	O_2	5.8	6.0	1.4	18.8	13.6
	others	23.2	18.3	29.9	9.2	26.8

(D), (E), (H) and (I) were abbreviated.

under anaerobiosis. Other gases are supposed to be hydrogen gas,[91] carbonyl compounds, alcohols, esters and sulfur compounds.[18] Nitrous oxide was not detected in any samples.

2 Amount of molecular nitrogen and methane in volatile soil gas

1) Molecular nitrogen

As shown in Table 21, 42.1 mg of molecular nitrogen was volatilized into the air from a surface of 193 cm^2 in the case of control plot, and phosphate application (P_2O_5 - 40 Kg/ha) clearly increased the amount of molecular nitrogen. As shown in Fig. 8-h, application of phosphate led to the increase of the population of microorganisms, especially cellulose decomposer which was thought to play a role in the mineralization of soil organic matter.

Table 21. Amount of molecular nitrogen in volatile soil gas during the growing period of rice plants

Treatment	Days after transplanting					
	0—7	7—14	21—35	35—50	50—65	Total
Without rice straw						
(A) Control	0.702*	0.535*	1.37*	0.279*	0.671*	42.1**
(B) Phosphate	1.31	3.30	1.28	1.05	0.591	74.8
(C) Ammonium sulfate, Phosphate	2.19	1.69	0.863	0.486	0.668	56.6
(D) Ammonium chloride, Phosphate	2.20	2.78	1.37	0.684	0.347	69.5
(E) Ammonium phosphate	2.13	2.10	1.45	0.91	0.86	76.5
With rice straw						
(F) Phosphate	8.38	5.07	6.25	12.8	6.41	470
(G) Ammonium sulfate, Phosphate, Rice straw	9.59	15.4	7.77	15.5	5.97	606
(H) Ammonium chloride, Phosphate, Rice straw	10.2	12.9	2.17	6.36	4.42	354
(I) Ammonium phosphate, Rice straw	12.5	15.9	3.63	5.99	2.49	377

* N mg/day evolved from 193 cm^2

**Total N mg evolved from 193 cm^2 during 58 days

On the other hand, in the case of plots with phosphate plus N-fertilizer, the amount of molecular nitrogen was insignificant compared with that in the phosphate plot, and in the case of plots with ammonium sulfate or ammonium chloride, the amount of molecular nitrogen rather decreased.

The application of rice straw increased largely the amount of volatile molecular nitrogen, irrespective of the application of N-fertilizer. In plots with rice straw and phosphate, an amount of molecular nitrogen as high as 470 mg was volatilized. Even in the plots with N-fertilizer, application of rice straw increased the amount of molecular nitrogen. Compared with the rice straw plot without N-fertilizer, the amount of molecular nitrogen in the rice straw plots with N-fertilizers did not show a significant difference.

Volatile nitrogen gas may originate partly from denitrification, but as suggested by YOSHIDA *et al.*,[94] rice plants supply gaseous nitrogen to the rhizosphere. Unexpectedly large amounts of molecular nitrogen in gas sample seem to suggest the transportation of molecular nitrogen by rice plant.

2) Methane

As shown in Table 22, even in the control plot, a small amount of methane gas was found 21 to 50 days after transplanting. When phosphate was applied, the total amount of methane gas was 5 fold as high as that of the control plot, owing to the increase of oxygen consumption by enhancement of microbial growth. N-fertilizers except ammonium phosphate inhibited the formation of methane gas.

This was in agreement with the experimental results obtained by several authors[10, 80, 92] who could demonstrate that nitrate prevented the formation of methane.

Rice straw application with or without N-fertilizer increased the total amount of methane gas 27 to 63 times as compared with the phosphate plot. The highest value was obtained in the rice straw plot to which ammonium sulfate had been added. It is conceivable that sulfide of reducing agent for Methanobacteriaceae[76] might be formed from sulfate.

The rate of methane formation in the control plot showed the maximum value 3 to 5 weeks after transplanting, whereas that in the phosphate plot was seen one to two weeks after transplanting. On the other hand, the plots with N-fertilizer except the ammonium phosphate plot, showed the maximum rate of methane gas formation 3 to 5 weeks after transplanting. In the rice straw plot, the peak was reached during the first week after transplanting or 2 to 3 weeks after rice straw application. In plots with both rice straw and N-fertilizer, the appearance of the peak was delayed and seen one to two weeks after transplanting.

In general, the peak of the rate of nitrogen gas formation appeared earlier than that of methane gas. The formation of methane gas was reported to begin,[10] when no further denitrification could be observed and nitrogen gas concentration remained constant.

As mentioned above, the quantitative and qualitative changes of volatile soil gas in various treatments gave information about the activity of microorganisms in the tropical paddy soils.

Table 22. Amount of methane in volatile soil gas during the growing period of rice plant

Treatment	Days after transplanting					
	0—7	7—14	21—35	35—50	50—65	Total
Without rice straw						
(A) Control	trace*	trace*	0.085*	0.001*	trace*	1.21**
(B) Phosphate	0.168	0.288	0.195	0.006	0.007	6.13
(C) Ammonium sulfate, Phosphate	trace	trace	0.061	0.003	0.011	1.06
(D) Ammonium chloride, Phosphate	trace	0.129	0.104	0.033	0.009	2.99
(E) Ammonium phosphate	0.070	0.106	0.44	trace	0.036	7.93
With rice straw						
(F) Phosphate	9.18	5.93	4.60	3.09	1.00	232
(G) Ammonium sulfate, Phosphate, Rice straw	9.10	13.7	10.5	4.07	1.11	384
(H) Ammonium chloride, Phosphate, Rice straw	7.34	8.49	2.75	0.79	0.46	168
(I) Ammonium phosphate, Rice straw	12.0	14.8	3.14	0.89	1.09	261

* CH_4—C mg/day evolved from 193 cm^2

**Total CH_4—C mg evolved from 193 cm^2 during 58 days

3 Characteristics of change in populations of microorganisms in the soil during the growing period of rice plants (Fig. 8)

1) Population change in the control plot

Populations of ammonia oxidizer and denitrifier in the subsurface soil (1-10 cm) mostly did not show any change during the growing period of rice plants. On the other hand, population of *Azotobacter* in the top (0-1 cm) and subsurface soils showed two peaks both at the early and middle stages of rice growth.

Populations of purple nonsulfur bacteria and *Clostridium* of anaerobic group in the top and subsurface soils increased at the middle stage of rice growth when anaerobic condition

developed as seen in the formation of methane (Table 22). MATSUGUCHI *et al.*[59] reported that population of purple nonsulfur bacteria increased at the middle stage of rice growth in the tropical acid sulfate soils.

Population of nitrite oxidizer in the top soil showed a high value owing to puddling at transplanting time, but decreased sharply 5 weeks after transplanting when the field suffered from severe flooding. Although even the top soil was submitted to anaerobic conditions by flooding, a high level of population was recovered 7 weeks after transplanting. The population of nitrite oxidizer in the subsurface soil was noticeably lower than that in the top soil. The population of nitrite oxidizer in tropical sandy soils showed large fluctuations as compared with that in Japanese paddy soils.[34]

2) Effect of phosphate

The application of phosphate resulted in a sharp increase in the population of *Clostridium* in the top soil until 3 weeks after transplanting which was nearly 10 times as high as that of the control plot. Population of cellulose decomposer increased throughout rice growing period and that of *Azotobacter*, until 3 weeks after transplanting. In the case of acid sulfate soils, application of phosphate did not clearly lead to an increase in the population of N-fixer, but application of phosphate with lime gave rise to an increase in the population of heterotrophic group and photosynthetic bacteria as well as N-fixing blue green algae.[59]

On the other hand, such application led to a decrease in the population of nitrite oxidizer in the top soil except that of 3 weeks after transplanting and the populations were nearly 1/10 that of the control plot. This trend to a decrease was also found in the population of *Azotobacter* and nitrite oxidizer both in the subsurface soils until 3 weeks after transplanting and the population of the latter dropped to 1/10 that of the control plot. Other microorganisms were not conspicuously affected by phosphate application.

Determination of available phosphorus content and grain yield showed that this field lacked in phosphorus. Although the application of phosphate was supposed to lead to an increase of the population of microorganisms, the number of some groups of microorganisms decreased following phosphate application. The reason for the decrease in the population of *Azotobacter* and nitrite oxidizer was attributed to the development of anaerobic conditions due to enhancement of microbial activity. Furthermore, it is possible to consider that phosphate application changed qualitatively the competition among microorganisms and hence, some groups eventually proliferated while the number of other groups actually decreased.

3) Effect of N-fertilizer in the presence of phosphate

Application of 32 Kg-N per hectare led to a decrease in the population of *Clostridium* 1 to 3 weeks after transplanting as compared with that in the phosphate plot. Several investigators[64] could demonstrate high concentrations of mineral nitrogen inhibited nitrogen fixation. LUND[57] also reported that a mixture of phosphate and potassium was especially effective for N-fixing blue green algae while ammonium nitrate was ineffective.

On the other hand, the population of ammonia oxidizer in the top soil increased 3 to 16 weeks after transplanting while that in the subsurface soil increased at the middle stages. Ammonium phosphate inhibited the growth of populations of both denitrifier and cellulose decomposer.

4) Effect of rice straw in the presence of phosphate but in the absence of N-fertilizer

As shown in Fig. 8, comparison between plots **(B)** and **(F)** illustrates the effect of rice straw

application on the growth of microorganisms in the presence of phosphate. Such application did not exert a significant influence on the populations of nitrite oxidizer and purple nonsulfur bacteria.

The population of *Azotobacter* in the top soils increased mostly ten times that of phosphate plot until 5 weeks after transplanting and was still higher until 11 weeks, whereas that in the subsurface soil became more than ten times higher than that of phosphate plot throughout the rice growing period. Incorporation of organic matter was repeatedly seen to increase the number of *Azotobacter* in upland soils.[9] Even in the paddy soils MATSUGUCHI *et al.*[59] also reported the same results. The population of cellulose decomposer (Fig. 8-h) in the top soil was larger in the phosphate plot to which rice straw had been added than that in the phosphate plot, 3 to 5 weeks after transplanting. The population of ammonifier in the top soil was larger 5 to 11 weeks after transplanting and that of denitrifier markedly increased 16 weeks after transplanting both in the top and subsurface soils (Fig. 8-d). In the case of ammonia oxidizer, the sharp increase in population was observed 3 to 7 weeks after transplanting in the top soil, and 1 to 3 weeks in the subsurface soil. It was reported that application of rice straw increased the number of aerobic bacteria, anaerobic bacteria, *Clostridium* and purple nonsulfur bacteria in the same great soil group.[59]

5) Effect of rice straw in the presence of phosphate and N-fertilizer

As shown in Fig. 8, the application of rice straw in the presence of phosphate and N-fertilizer either increased the number of microorganisms or did not affect it greatly as compared with that in the corresponding plots with phosphate and N-fertilizers. The increase was most conspicuous in the case of *Azotobacter* population 1 to 7 weeks after transplanting. The number of denitrifier in the top and subsurface soils, also increased 16 weeks after transplanting. As shown in Fig. 8, the effect of rice straw with fertilizers, depended on the nature of N-fertilizer. Application of ammonium sulfate with rice straw increased the number of nitrite oxidizer in the top soil and also that of cellulose decomposer in the top and subsurface soils, at the later stage of rice growth, whereas, ammonium phosphate application decreased the number of ammonia oxidizer in both layers and increased the number of cellulose decomposer in the top soil at the middle to later stages of rice growth.

Among the populations of each microorganism in the plots with N-fertilizer, phosphate and rice straw, the number of ammonia oxidizer in the top soils of the plot **(G)** with ammonium sulfate, phosphate and rice straw showed a high value in the later stages of rice growth. On the other hand, the number of denitrifier in the subsurface soil of the plot **(I)** with ammonium phosphate and rice straw decreased 16 weeks after transplanting, but that of purple nonsulfur bacteria in the top soil of this plot increased at this stage.

As described above, the application of rice straw variously affected the population of each microorganism, and the effect of N-fertilizer differed also.

Summary

The effect of rice straw (6 tons/ha) on the composition of volatile soil gas released into the atmosphere and microflora in the tropical paddy field was studied with and without fertilizer application.

The volatile soil gas most abundantly found in plots with rice straw was methane during the early stage of rice growth, while molecular nitrogen predominated in the later stages.

The amount of molecular nitrogen in the soil gas increased following phosphate application as

well as rice straw application as compared with that in the control plot.

Rice straw application with or without N-fertilizer in the presence of phosphate increased methane gas formation by 27 to 63 times as compared with the phosphate plot and the peak of its formation was found 5 to 7 weeks after rice straw application. However methane formation in the control plot was very low and was found only 5 to 9 weeks after flooding.

Rice straw application variously affected each microbial group. But the stimulating effect was chiefly observed in the population of *Azotobacter*.

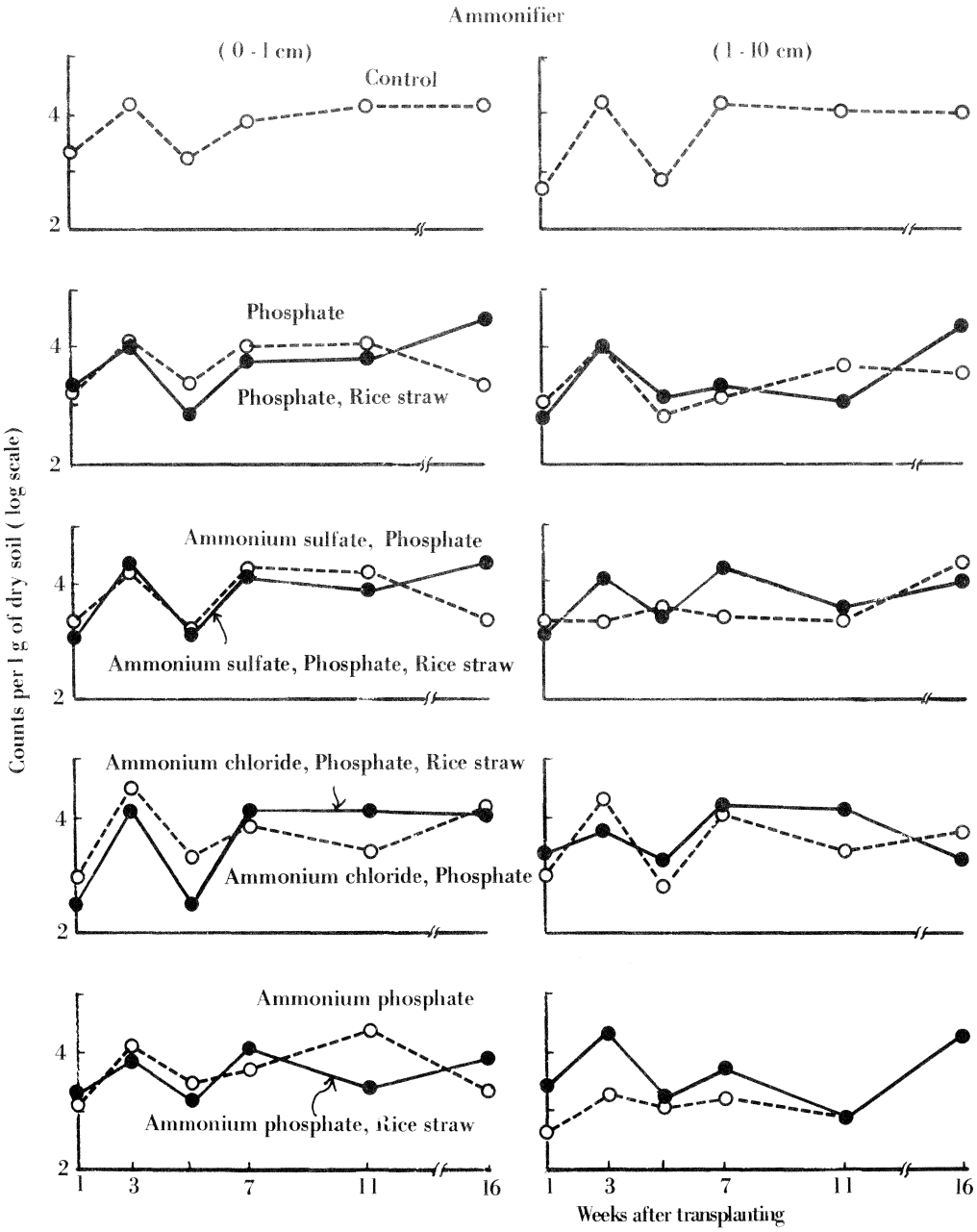

Fig. 8-a. Population change of ammonifier in the paddy soils during the growing period of rice plants.

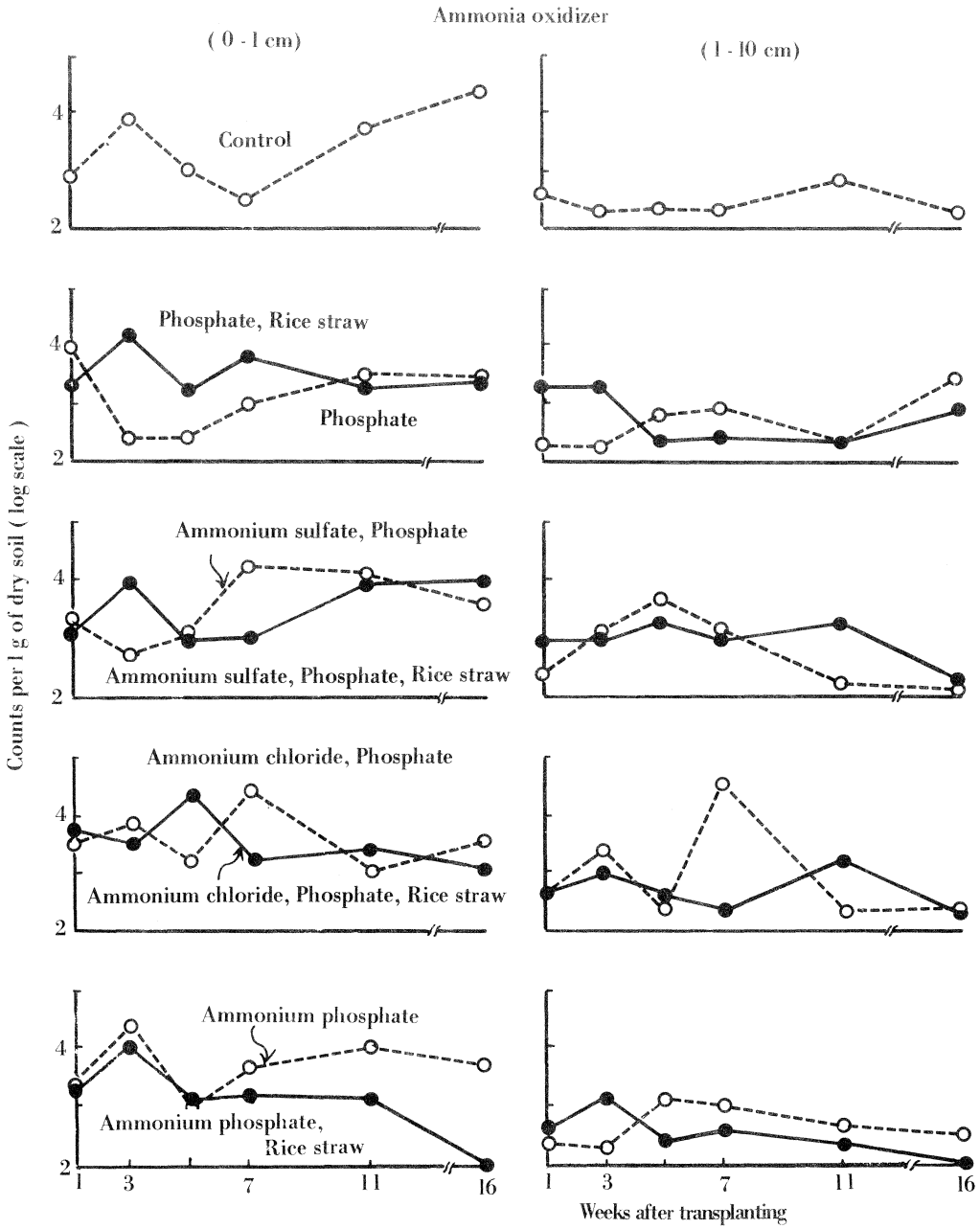

Fig. 8-b. P\ ·lation change of ammonia oxidizer in the paddy soils during the growing period of rice plants.

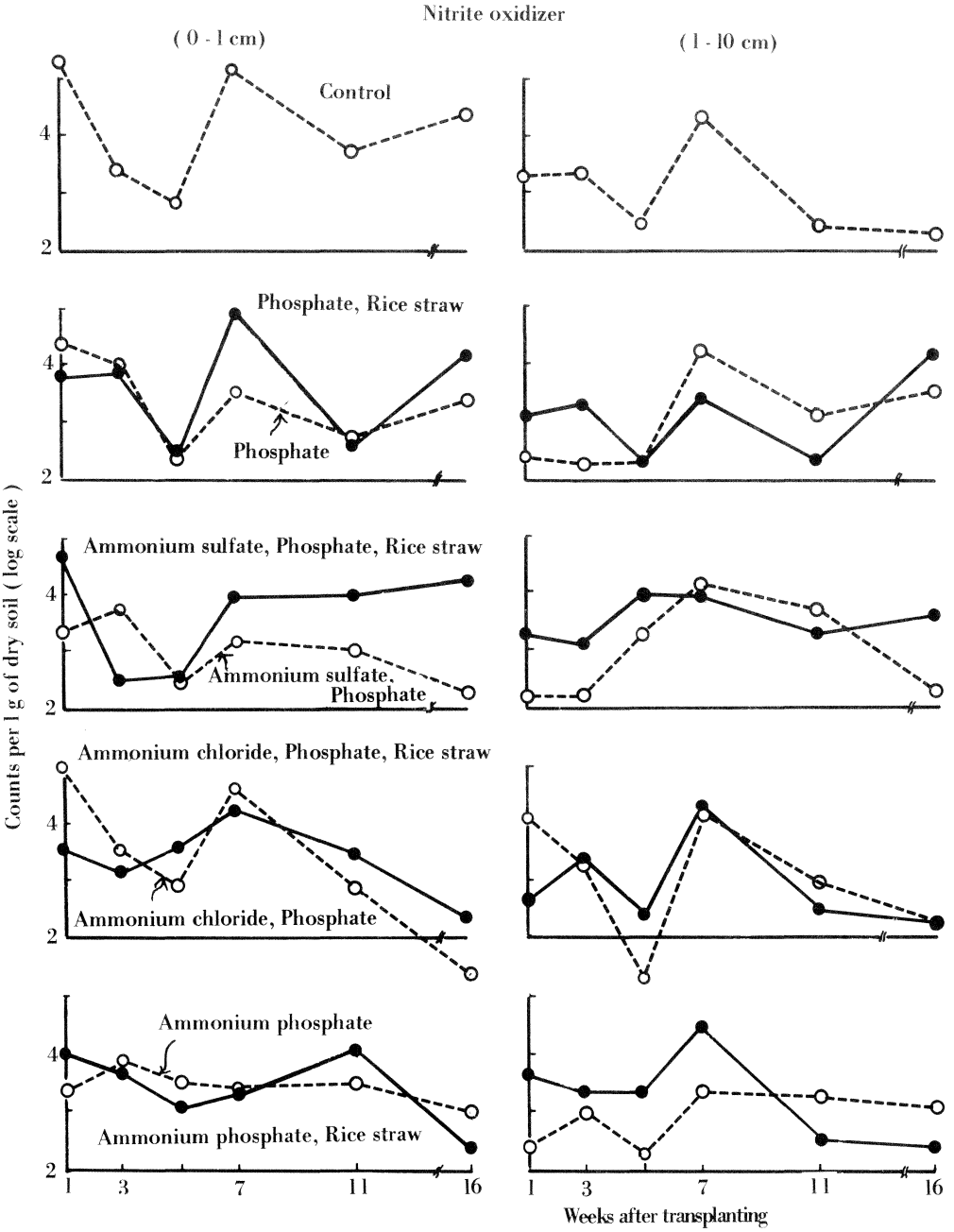

Fig. 8-c. Population change of nitrite oxidizer in the paddy soils during the growing period of rice plants.

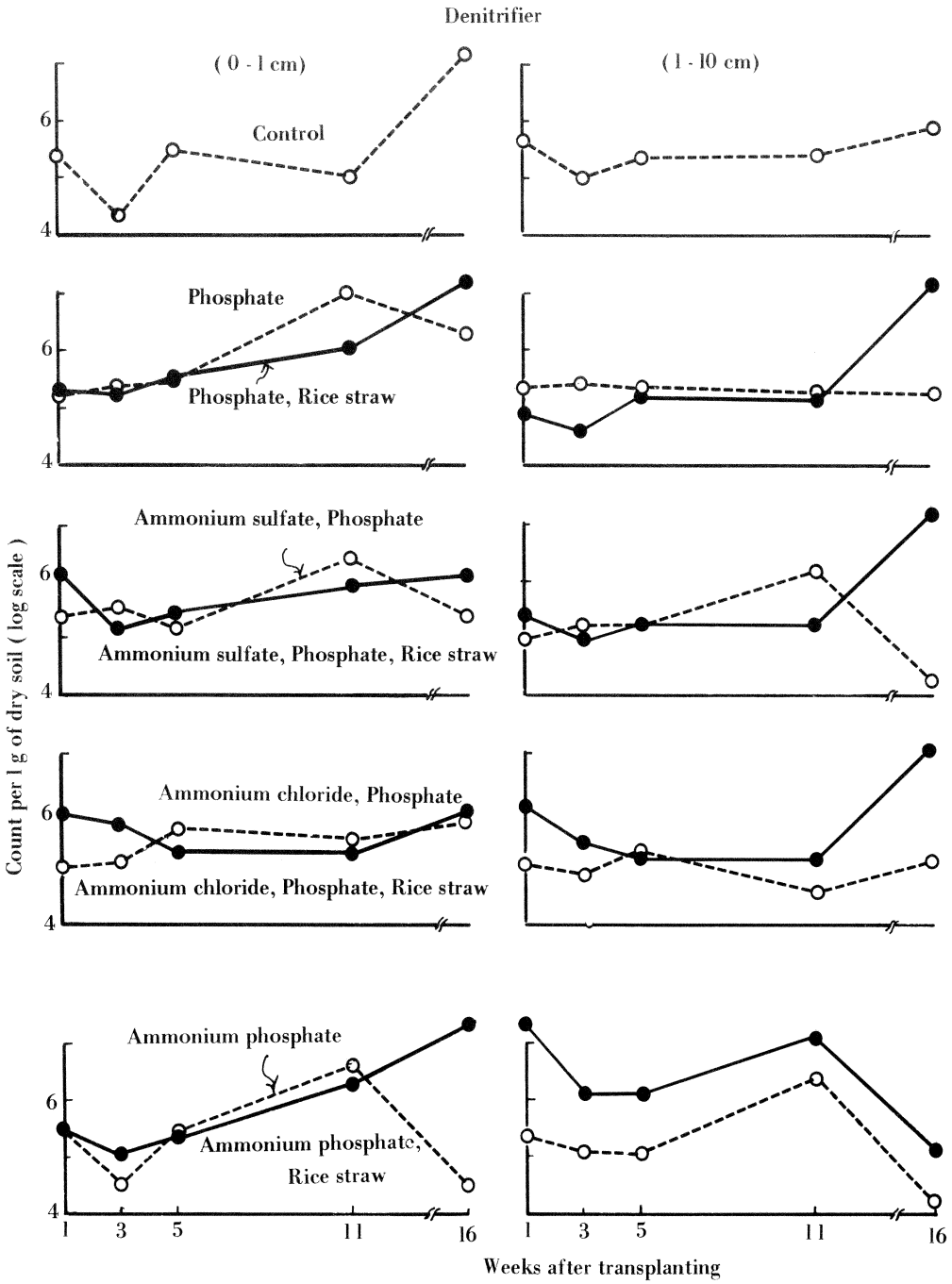

Fig. 8-d. Population change of denitrifier in the paddy soils during the growing period of rice plants.

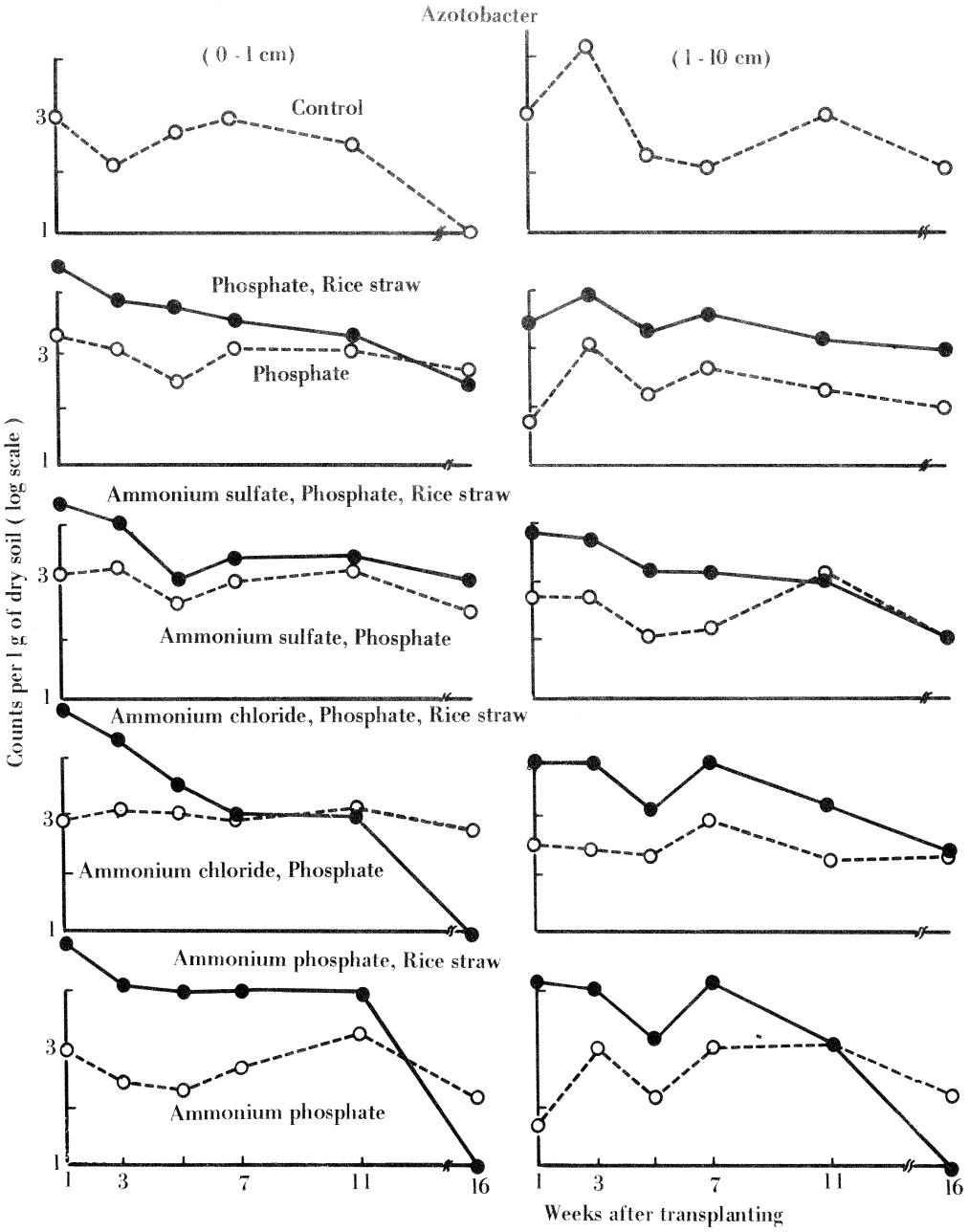

Fig. 8-e. Population change of *Azotobacter* in the paddy soils during the growing period of rice plants.

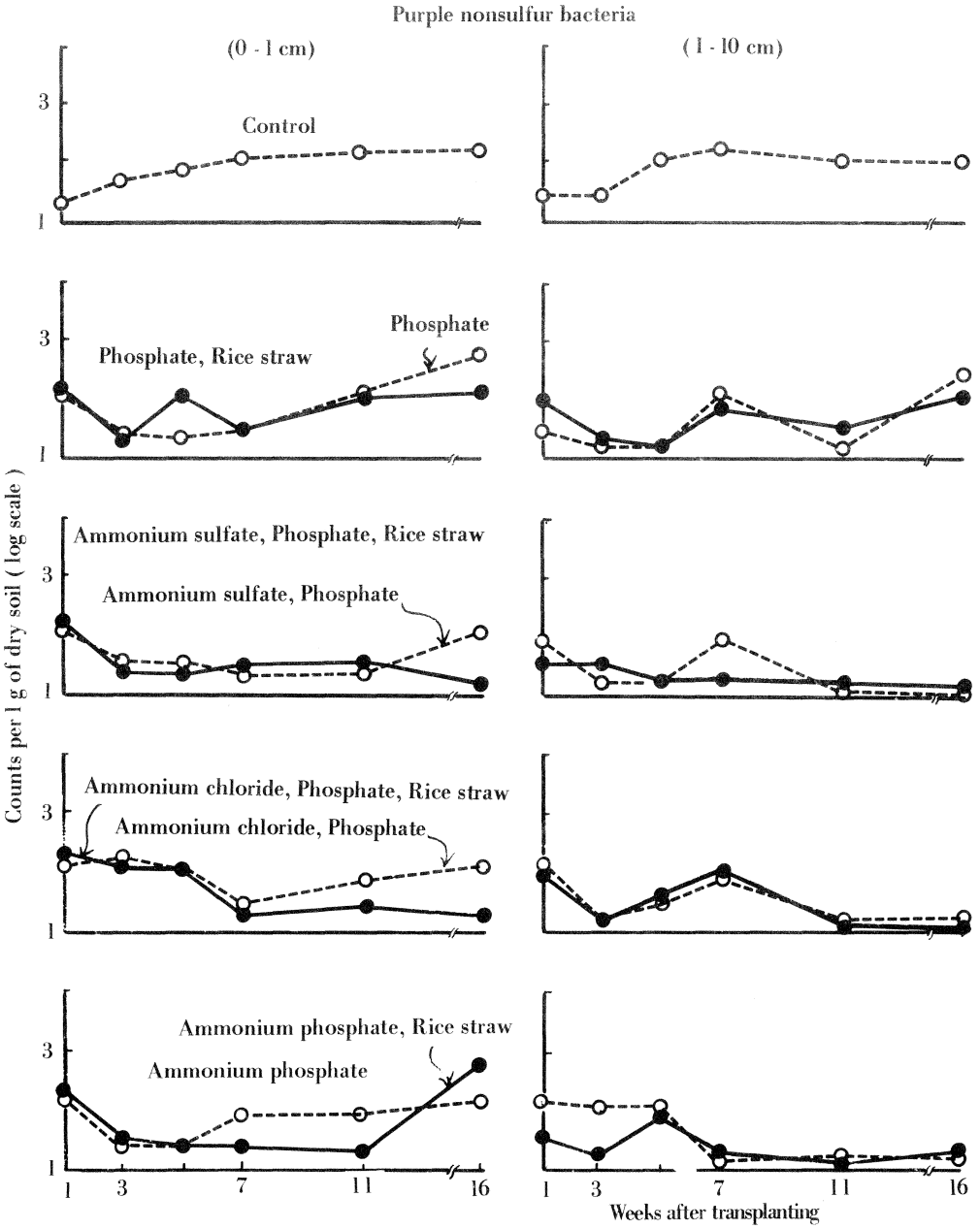

Fig. 8-f. Population change of purple nonsulfur bacteria in the paddy soils during the growing period of rice plants.

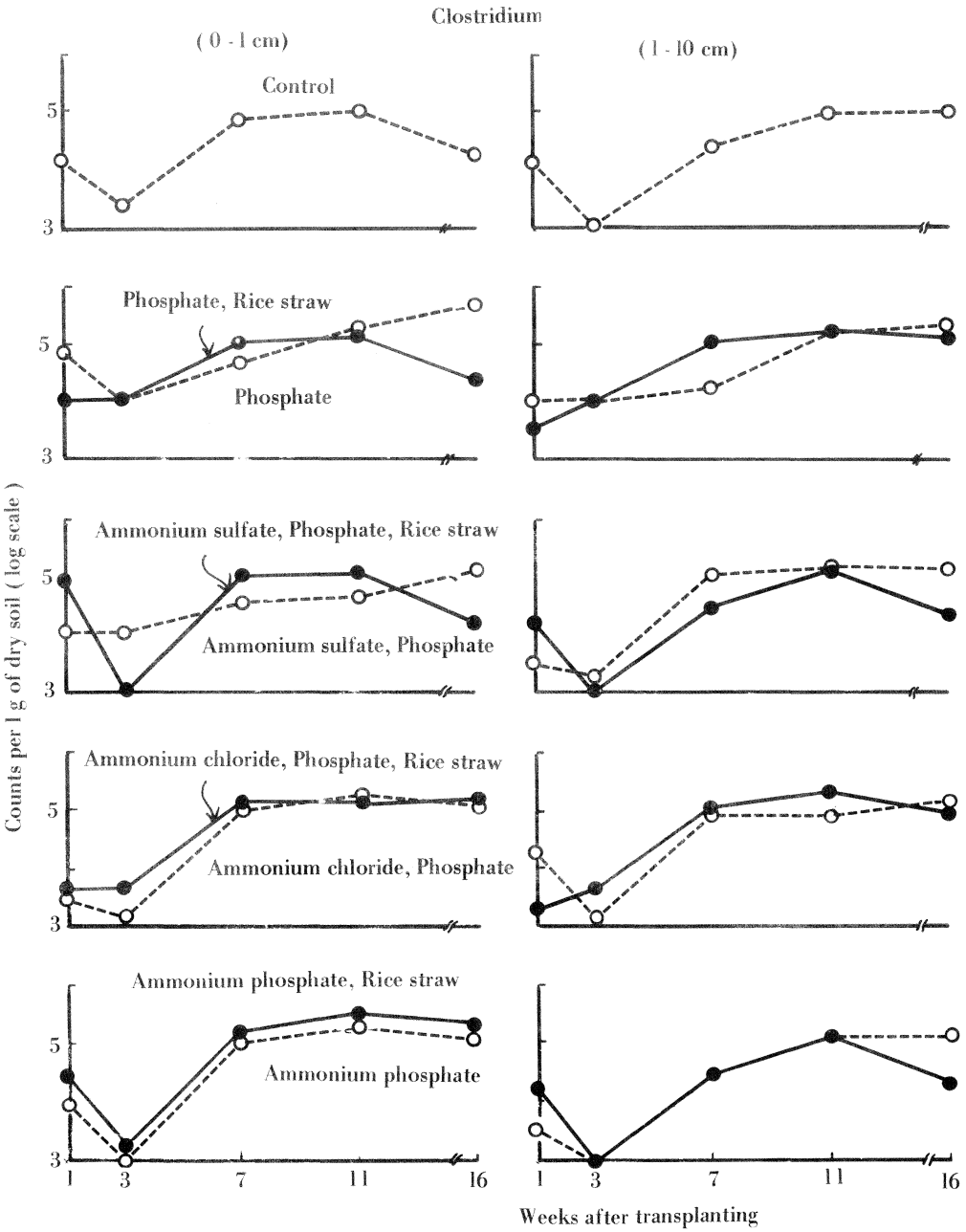

Fig. 8-g. Population change of *Clostridium* in the paddy soils during the growing period of rice plants.

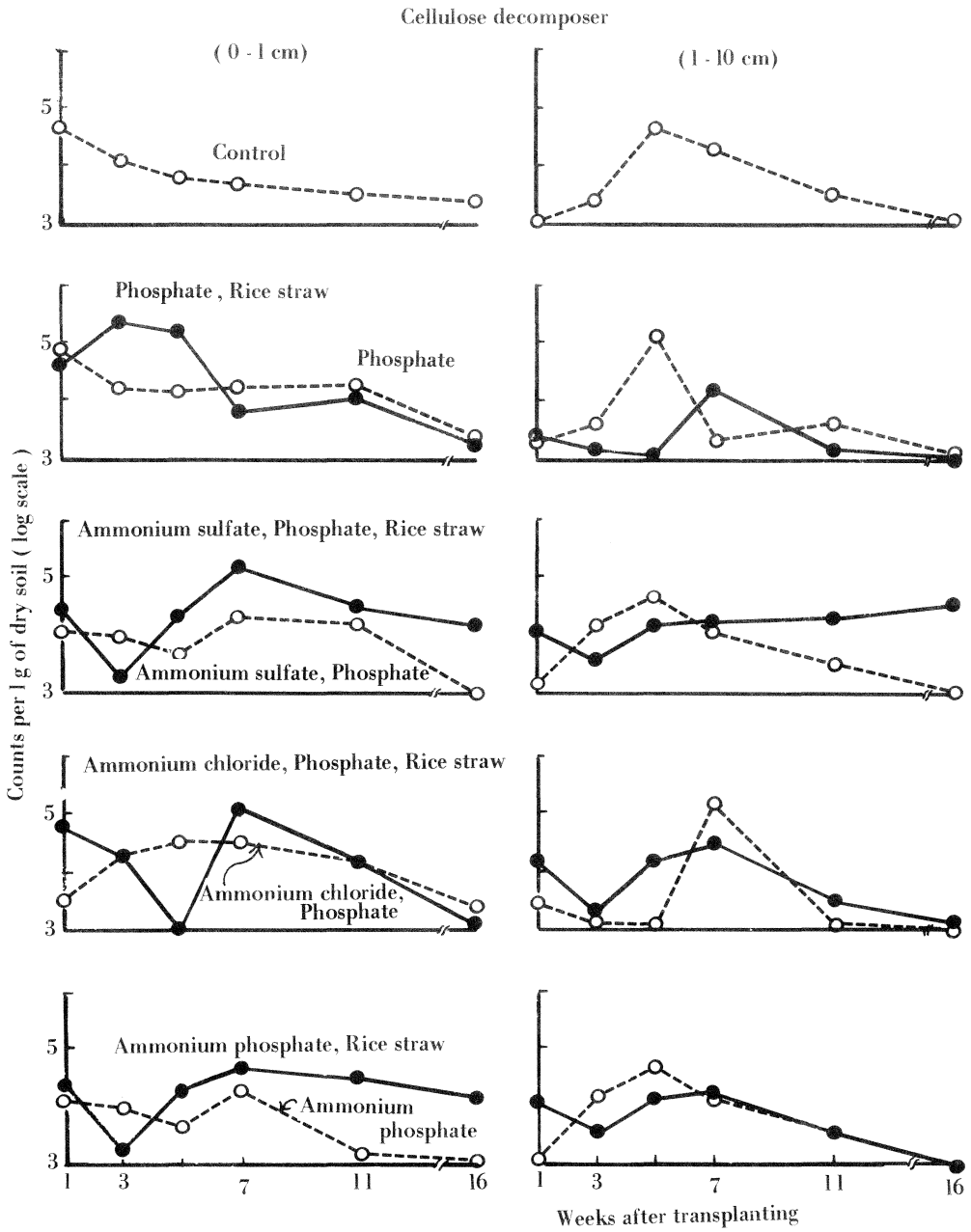

Fig. 8-h. Population change of cellulose decomposer in the paddy soils during the growing period of rice plants.

VIII Comparison of effects of rice straw on the composition of volatile soil gas and microflora related to the change of mineral nitrogen between Brackish Water Alluvial Soils and Low Humic Gley Soils

In part VII, the effect of rice straw on the composition of volatile soil gas and microflora in Low Humic Gley Soils was reported. On the other hand, as marked differences in chemical, physical and microbiological properties among great soil groups were observed,[65] it was assumed that the effect of rice straw on the composition of volatile soil gas released into the atmosphere and microflora would also show differences among great soil groups.

Although Brackish Water Alluvial Soils are widely distributed in South-east Asia,[46] their productivity is low and needs to be improved.

In this part, the effect of rice straw on the composition of volatile soil gas* and microflora related to the change of mineral nitrogen in Brackish Water Alluvial Soils with low pH value, high organic matter content and heavy clay was studied under field conditions and compared with that in Low Humic Gley Soils which were sandy and had a low organic matter content.

Materials and Methods

Field experiments were designed the same way as those described in part VII, and were carried out simultaneously both at Khok Samrong and Khlong Luang Rice Experimental Stations in Thailand. **a)** Soils: Khok Samrong--Low Humic Gley Soils, Texture CL, Total-C 0.43%, Total-N 0.03%, Total P_2O_5 0.032%, Available P_2O_5 21.1 ppm, Total K_2O 0.033% Available K_2O 316 ppm, pH(H_2O) 5.45, Khlong Luang—Brackish Water Alluvial Soils, Texture HC, Total-C 1.50%, Total-N 0.13%, Total P_2O_5 0.037%, Available P_2O_5 13.6 ppm, Total K_2O 1.29%, Available K_2O 193 ppm, pH(H_2O) 4.2, as shown by MOTOMURA.[65] **b)** Ammonium nitrogen was determined in using the method described in part III. **c)** Microorganisms investigated: ammonifier, ammonia oxidizer, nitrite oxidizer and denitrifier. Media composition and incubation methods used are the same as those described in part I. **d)** Soil gas collection method and analysis of soil gas were the same as those in part VII. **e)** Treatment of field experiments were designed as follows;

Treatment			Kg/ha
Without rice straw	N	P_2O_5	Rice straw
(A) Control	—	—	—
(B) Phosphate	—	40	—
(C) Ammonium sulfate + Phosphate	32	40	—
(D) Ammonium chloride + Phosphate	32	40	—
With rice straw			
(E) Phosphate + Rice straw	—	40	6,000
(F) Ammonium sulfate + Phosphate + Rice straw	32	40	6,000
(G) Ammonium chloride + Phosphate + Rice straw	32	40	6,000

* Volatile soil gas in this report means soil gas released into the atmosphere.

Results and discussion

1 Amount of volatile soil gas

As shown in Fig. 9, the amount of volatile soil gas in the plots of Brackish Water Alluvial Soils was markedly lower than that in the corresponding plots of Low Humic Gley Soils, though organic matter content of the former was higher than that of the latter. The application of rice straw amounting to 6 tons per hectare markedly increased the amount of volatile soil gas in the plots of Low Humic Gley Soils, while in the case of Brackish Water Alluvial Soils a marked increase in volatile soil gas was obtained only in the ammonium chloride, phosphate, rice straw plot **(G)**. The low activity of microbes in Brackish Water Alluvial Soils is considered to be due mainly to the low pH value of about 4.2. The application of rice straw made the pH value higher except for that of the

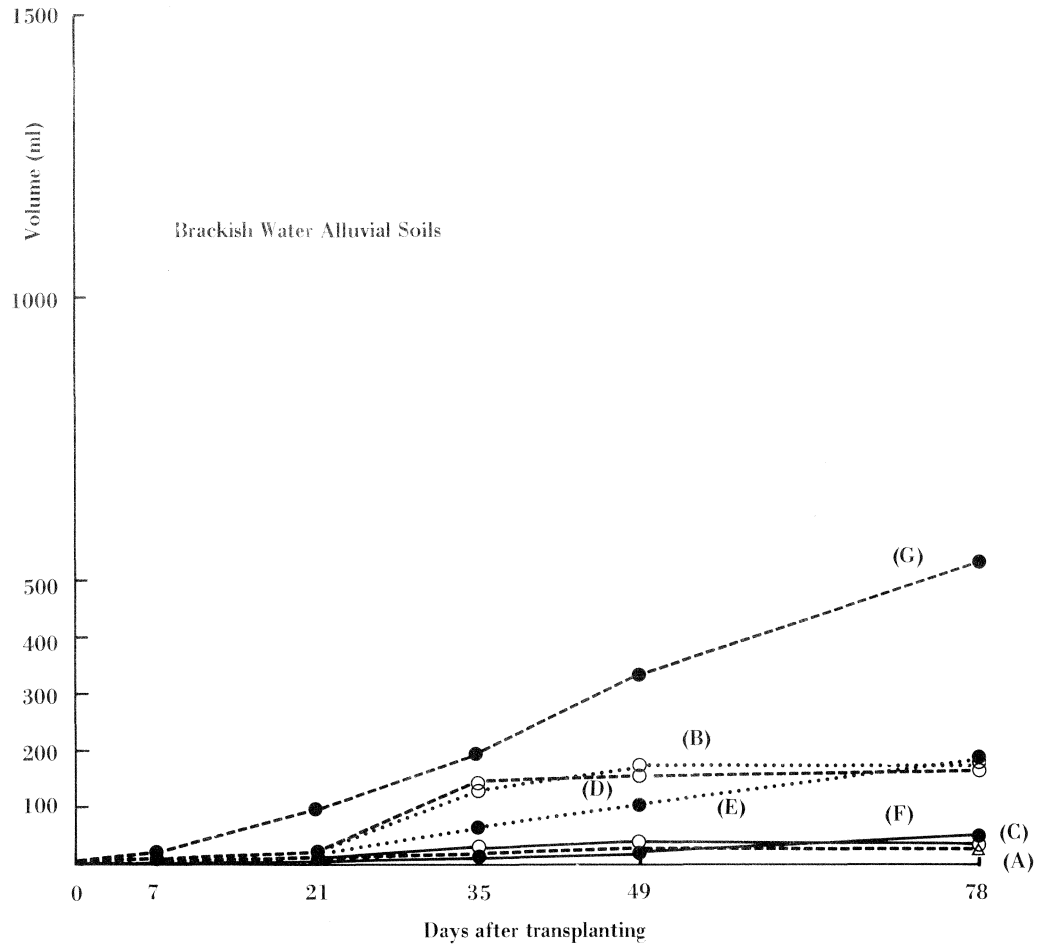

Fig. 9-a. Volume (1 atm., 25°C) of volatile soil gas released from a surface area of 193 cm² in each plot of Brackish Water Alluvial Soils during the growing period of rice plants.
(A), Control plot; (B), Phosphate plot; (C), Ammonium sulfate and phosphate plot; (D), Ammonium chloride and phosphate plot; (E), Phosphate and rice straw plot; (F), Ammonium sulfate, phosphate and rice straw plot; (G), Ammonium chloride, phosphate and rice straw plot.

subsurface soil (1-10 cm) of the ammonium sulfate plot with rice straw,[4] in which the amount of volatile soil gas was small. It was also reported that the application of Italian ryegrass[68] and farmyard manure[93] gradually increased the pH value. The increase in the amount of volatile soil gas following rice straw application is mainly due to the increase in the amount of easily utilizable organic matter, but the rise of the pH value may also be concerned partly with the increase in the amount of volatile soil gas.

The carbon content (0.43%) of Low Humic Gley Soils was lower than that (1.50%) of Brackish Water Alluvial Soils, suggesting that the decomposition of organic matter was probably quicker in the former than in the latter group under natural conditions, because nearly the same amount of organic matter was presumed to be supplied annually. As shown in Fig. 9, the decomposition of rice straw was quicker in Low Humic Gley Soils than in Brackish Water Alluvial Soils.

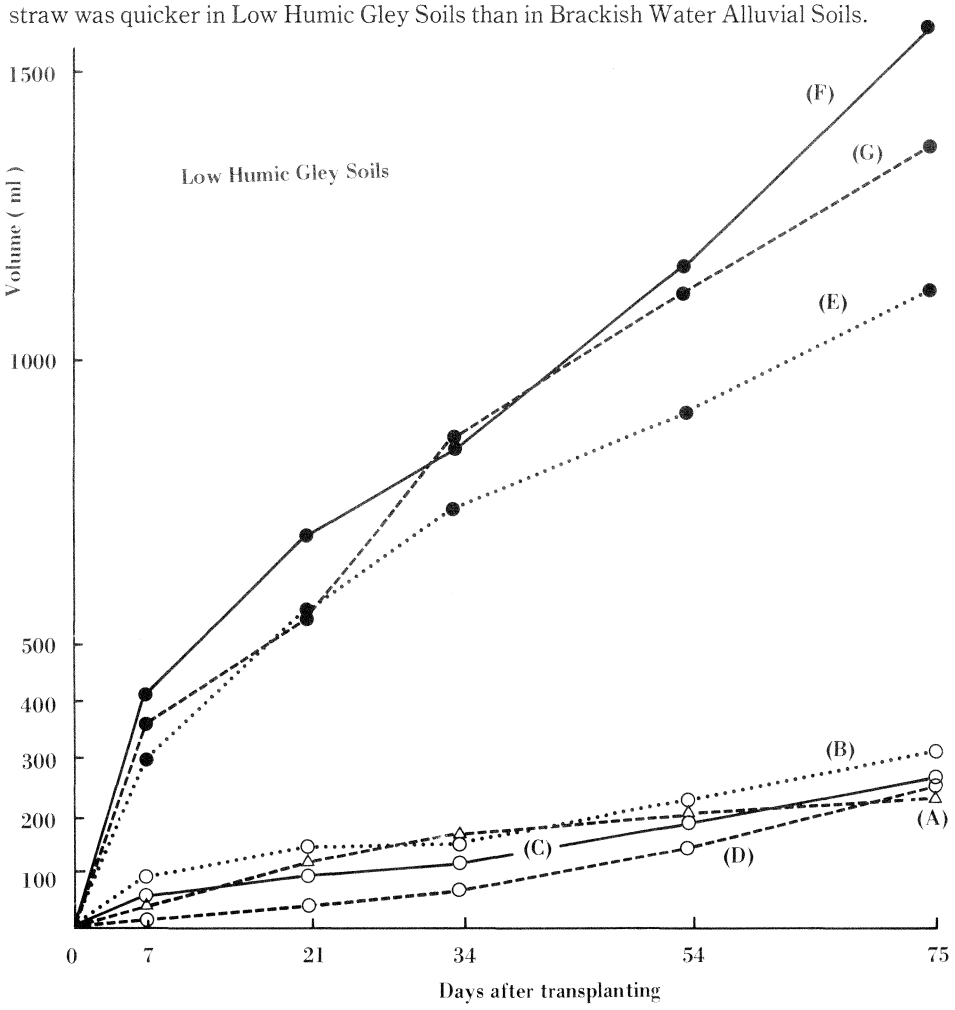

Fig. 9-b. Volume (1 atm., 25°C) of volatile soil gas released from a surface area of 193 cm² in each plot of Low Humic Gley Soils during the growing period of rice plants.
(A), Control plot; (B), Phosphate plot; (C), Ammonium sulfate and phosphate plot; (D), Ammonium chloride and phosphate plot; (E), Phosphate and rice straw plot; (F), Ammonium sulfate, phosphate and rice straw plot; (G), Ammonium chloride, phosphate and rice straw plot.

2 Amount of molecular nitrogen in volatile soil gas

As shown in Table 23, there was a marked difference in the amount of molecular nitrogen in volatile soil gas between Brackish Water Alluvial Soils and Low Humic Gley Soils. The amount of molecular nitrogen in the control plot of Low Humic Gley Soils was about 7 times as high as that of Brackish Water Alluvial Soils. Though the total nitrogen content was higher in Brackish Water Alluvial Soils than in Low Humic Gley Soils, the amount of molecular nitrogen in volatile soil gas was far less in the former.

The amount of molecular nitrogen in volatile soil gas increased in both great soil groups following phosphate application amounting to 40 Kg per hectare, and especially in the Brackish Water Alluvial Soils it increased more than 4 times as compared with that in the control plot. As indicated previously, the available phosphate content of both soils was low, hence phosphate was considered to be one of the limiting factors for microbial activity.

The amount of molecular nitrogen in volatile soil gas increased following the application of rice straw, especially when N-fertilizer was added, as compared with the values obtained in the corresponding plots without rice straw. As shown in Table 23, the amount of molecular nitrogen was least in the ammonium sulfate plots irrespective of rice straw application as compared with that in the corresponding ammonium chloride or ammonium phosphate plots. Sulfate ion seemed to have an inhibitory effect on the formation of molecular nitrogen in soil gas.

As shown in Table 23, the rate of formation of molecular nitrogen showed a peak 1 to 3 weeks after transplanting in the control plots of both soils. Following phosphate application, a peak appeared earlier in Low Humic Gley Soils than in Brackish Water Alluvial Soils, where it was observed only 3 to 5 weeks after transplanting. In the case of rice straw application, the peak of the rate of molecular nitrogen formation was observed in the first week after transplanting. The molecular nitrogen of the air kept inside the soil was supposed to be evolved and to form a part of molecular nitrogen in evolved soil gas. The application of rice straw and the management of water-logged condition were made 2 weeks before transplanting. Therefore a large part of the air inside the soil is considered to have evolved during the two weeks before transplanting. However, it is conceivable that the evolution of air was enhanced by the application of phosphate or phosphate plus rice straw as compared with that of the control plot.

3 Amount of methane in volatile soil gas

As shown in Table 24, methane gas was hardly detected in the plots of Brackish Water Alluvial Soils without rice straw, while in the case of the Low Humic Gley Soils, a small amount of methane gas was found.

Following rice straw application, the amount of methane gas increased largely in both great soil groups except the ammonium sulfate plot with rice straw in Brackish Water Alluvial Soils. MORAGHAN and AYOTADE[99] reported differences in the effect of organic matter between acid and alkaline soils and observed that the addition of organic matter (0.05 g maize straw/ 5 g soil) to an acid soil greatly reduced methane formation during anaerobic incubation, while in alkaline soils methane formation was increased. The increased methane formation observed even in acid Brackish Water Alluvial Soils following the application of rice straw may be related to the high rate of organic matter decomposition in tropical soils.

Under field conditions, formation of methane and molecular nitrogen gas was observed simultaneously, as shown in Tables 23 and 24. YAMANE and SATO[92] reported that methane formation from flooded paddy soils does not commence until Eh falls below −200 mV. BELL[6] reported that the Eh value dropped sharply from +200 mV, which was compatible with active denitrification, to −250 mV. After an interval, formation of methane gas was reported to be observed and then

Table 23. Amount of molecular nitrogen in volatile soil gas during the growing period of rice plants

Great soil groups	Low Humic Gley Soils						Brackish Water Alluvial Soils					
Days after transplating	0—7	7—20	20—34	34—54	54—75	Total	0—7	7—21	21—35	35—49	49—78	Total
Without rice straw												
(A) Control	1.80*	3.05*	1.92*	1.73*	0.36*	121**	0.09*	0.43*	0.22*	0.19*	0.19*	17.9***
(B) Phosphate	5.32	3.47	1.17	2.81	1.08	178	0.07	0.51	3.00	1.32	0.32	77.4
(C) Ammonium sulfate, Phosphate	4.29	1.58	1.11	2.70	0.06	121	0.14	0.90	0.30	0.28	0.22	28.1
(D) Ammonium chloride, Phosphate	0.73	1.75	1.61	2.95	1.41	139	0.17	0.36	3.40	1.02	0.47	81.7
With rice straw												
(E) Phosphate, Rice straw	15.4	13.8	9.29	7.00	3.43	212	2.44	0.45	0.94	1.06	2.29	118
(F) Ammonium sulfate, Phosphate, Rice straw	16.4	10.9	7.79	11.8	5.52	716	0.65	0.22	0.26	0.36	0.75	38.4
(G) Ammonium chloride, Phosphate, Rice straw	12.1	7.77	14.3	10.5	3.54	668	2.84	9.79	5.22	3.31	4.90	419

* N mg/day, 193 cm^2

** N mg/75 days, 193 cm^2

*** N mg/78 days, 193 cm^2

Table 24. Amount of methane in volatile soil gas during the growing period of rice plants

Great soil groups	Low Humic Gley Soils					Brackish Water Alluvial Soils					
Days after transplating	0—7	7—20	20—34	34—54	Total	0—7	7—21	21—35	35—49	49—78	Total
Without rice straw											
(A) Control	1.03*	1.13*	0.25*	0.07*	26.8**	0*	0*	0*	0*	0.01*	0.29***
(B) Phosphate	0.20	0.03	0.09	0.07	4.45	0	0	0	0	0	0
(C) Ammonium sulfate, Phosphate	0.051	0	0	0.19	4.16	0	0	0	0	0	0
(D) Ammonium chloride, Phosphate	0.21	0.26	0.12	0.19	10.3	0	0	0	0	0	0
With rice straw											
(E) Phosphate, Rice straw	7.34	4.55	2.49	0.73	160	0.23	0.05	0.71	0.26	0.16	20.5
(F) Ammonium sulfate, Phosphate, Rice straw	11.8	5.56	1.61	2.26	223	0.10	0	0	0	0	0.75
(G) Ammonium chloride,Phosphate, Rice straw	11.1	3.18	3.51	0.95	187	0	2.3	3.13	0.72	0.56	103

* CH_4—C mg/day, 193 cm^2

** CH_4—C mg/54 days, 193 cm^2

*** CH_4—C mg/78 days, 193 cm^2

denitrification became weak. Under field conditions, as oxygen is continuously supplied from the surface of paddy fields, heterogeneity of the Eh values from +200 mV to −250 mV may exist simultaneously in every part of the paddy soils. Hence the formation of molecular nitrogen through denitrification and methane was considered to be observed at the same time.

4 Change of ammonium nitrogen content of soil

As shown in Fig. 10, the peak of ammonium nitrogen content in the control plots was observed one week after transplanting in Low Humic Gley Soils where the decomposition of organic matter was rapid, while that in Brackish Water Alluvial Soils where the development of reduced condition was slow, was seen 5 to 7 weeks after transplanting. However the ammonium nitrogen content in both great soil groups, especially that in Low Humic Gley Soils decreased, presumably due to subsequent nitrification-denitrification process and partly due to absorption by rice plant.

The ammonium nitrogen content in the plots with N-fertilizer became similar to that in the phosphate plot 7 weeks after application of N-fertilizer, in the case of Low Humic Gley Soils.

The ammonium nitrogen content in the top soils of Low Humic Gley Soils increased following the application of rice straw and this increase might have contributed to the loss of molecular nitrogen into the atmosphere. In the case of the Brackish Water Alluvial Soils, the increase in ammonium nitrogen in the soils was not obvious, but in the top soils of the plot **(E)** with phosphate and rice straw and in that **(G)** with phosphate, ammonium chloride and rice straw, the ammonium nitrogen content increased at the late period of rice growth when the high rate of formation of molecular nitrogen was observed.

5 Change in population of microorganisms

Denitrification is known to be regulated by several factors such as the partial pressure of oxygen, organic matter content, pH, temperature, nitrate content, redox potential[12] and microorganisms related to denitrification, etc.

As shown in Table 23, there were important variations in the amount of molecular nitrogen in volatile soil gas between Brackish Water Alluvial and Low Humic Gley Soils. The reason for this low value in the former group is considered, as indicated previously, to be mainly due to the low pH value of this soil which is responsible for the slow development of a reduced condition. The fact that the redox potential did not show a low value can be explained by the absence or low values of methane gas formation, as seen in Table 24.

Furthermore, the change in populations of microorganisms related to denitrification was periodically compared between the two great soil groups.

1) Control plot

As shown in Fig. 11, the population of ammonifier which mineralizes organic nitrogen was slightly larger in Brackish Water Alluvial Soils with high organic matter content. On the other hand, the population of aerobic ammonia oxidizer in Brackish Water Alluvial Soils, whose oxidized layer was thinner than that in Low Humic Gley Soils, was smaller in the former and its marked increase in both great soil groups one week after transplanting was considered to be due to puddling at transplanting time. The population of ammonia oxidizer in the top soil of Brackish Water Alluvial Soils increased again 7 weeks after transplanting when the amount of ammonium nitrogen also showed a peak, and was at the same high level until 11 weeks, which corresponded with the decrease of ammonium nitrogen content in the top soil. The ammonium nitrogen was supposed to be nitrified during this period.

In the case of Low Humic Gley Soils, the population of ammonia oxidizer also increased 8 weeks after transplanting and showed a high level until 16 weeks as compared with that in Low

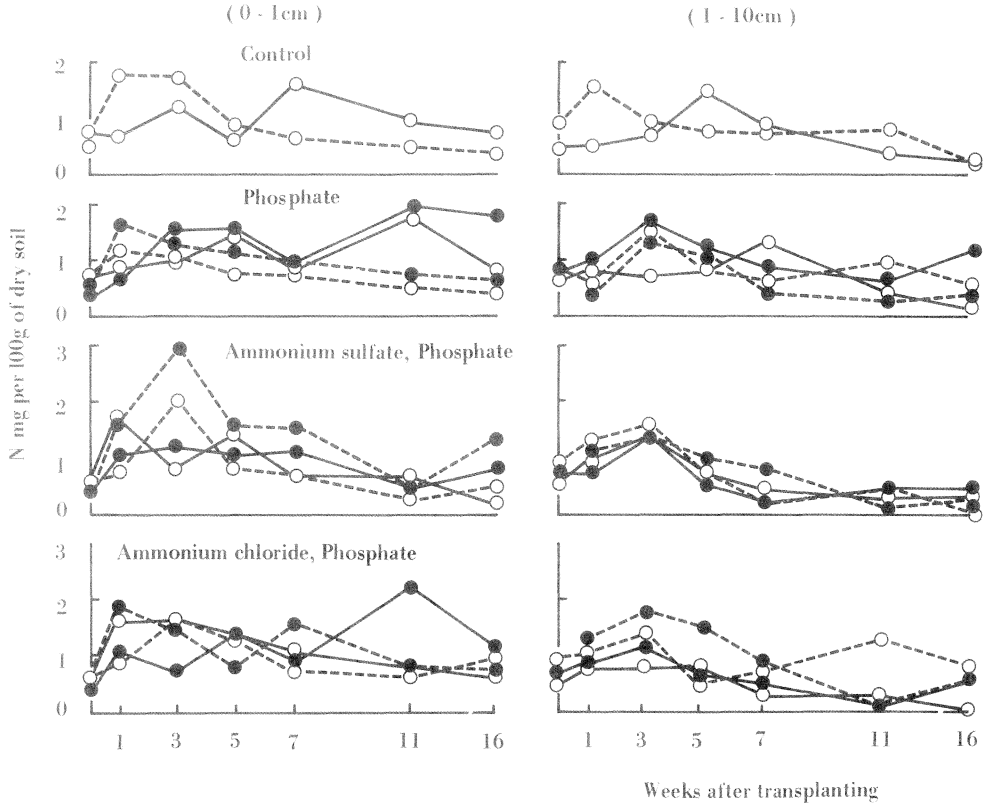

Fig. 10. Change of ammonium nitrogen content in the top (0-1 cm) and subsurface (1-10 cm) soils of each plot in both Brackish Water Alluvial Soils and Low Humic Gley Soils during the growing period of rice plants. Brackish Water Alluvial Soils: ●—● , with rice straw; ○—○ , without rice straw. Low Humic Gley Soils: ●--● , with rice straw; ○--○, without rice straw.

Humic Gley Soils. The amount of ammonium nitrogen did not show a peak 7 weeks after transplanting and showed a lower level as compared with that in Brackish Water Alluvial Soils. The nitrification was considered to take place more intensively in Low Humic Gley Soils than in Brackish Water Alluvial Soils in this later period of rice growth. The population of aerobic nitrite oxidizer in Brackish Water Alluvial Soils showed lower level 1 to 21 days after transplanting, but increased markedly after 3 weeks and became noticeably larger than that in Low Humic Gley Soils at the middle and later stages of rice growth. The peak of population of nitrite oxidizer in Low Humic Gley Soils was found 3 weeks after transplanting, and that in Brackish Water Alluvial Soils 5 weeks after transplanting. The reason for this phenomenon could be explained by the accumulation of ammonia and its subsequent decrease at this period, as seen in Fig. 10.

TAKAI and UEHARA[81] reported that at the middle stage of incubation period under submerged condition without planting, the rate of ammonification decreased, and Eh in the surface layer began to rise. Then the decrease of ammonium and the considerable increase of nitrifier in the surface layer occurred simultaneously.

The population of denitrifier in the control plot of Low Humic Gley Soils where the amount of volatile molecular nitrogen showed high value, was 10 times as large as that of Brackish Water Alluvial Soils throughout the growing period of rice plants.

AYANABA and OMAYULI[5] reported that in upland soils, the acidity significantly influenced microbial abundance. Soils with very high acidity (pH 4.2) contained the smallest number of populations of *Nitrosomonas, Nitrobacter* and denitrifier. In the case of tropical paddy soils, as indicated previously, the population of nitrite oxidizer at the early stage of rice growth, ammonia oxidizer and denitrifier showed lower levels in Brackish Water Alluvial Soils, while that of nitrite oxidizer during the middle and later stages was larger in Brackish Water Alluvial Soils than in Low Humic Gley Soils. The difference can be explained mainly by the difference in soil properties.

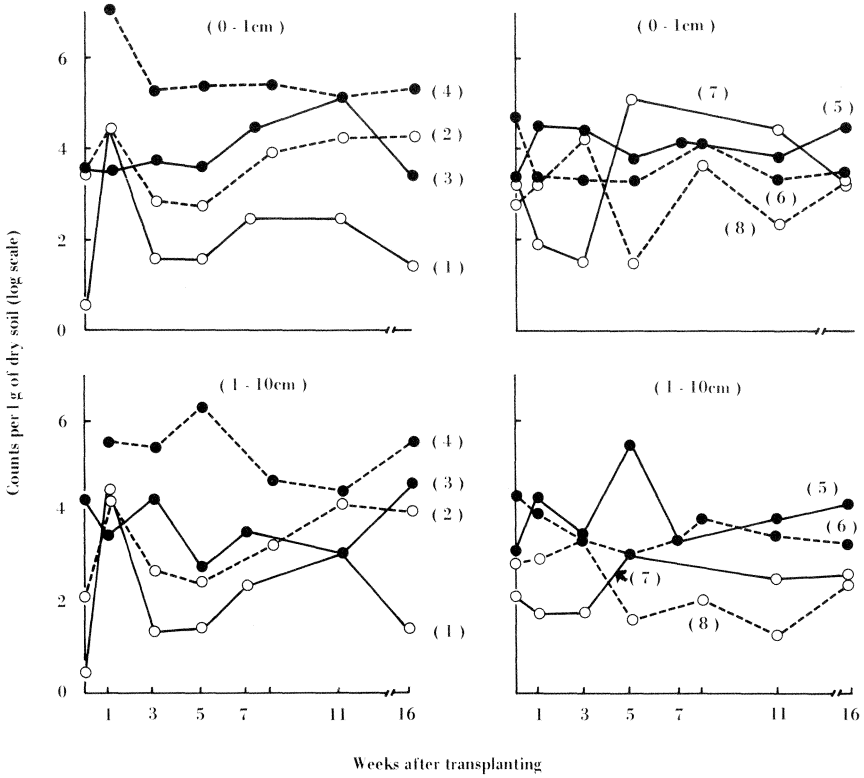

Fig. 11. Change in populations of microorganisms related to the change of mineral nitrogen in the control plot of both Brackish Water Alluvial Soils and Low Humic Gley Soils during the growing period of rice plants. Ammonia oxidizer: (1), in Brackish Water Alluvial Soils; (2), in Low Humic Gley Soils. Denitrifier: (3), in Brackish Water Alluvial Soils; (4), in Low Humic Gley Soils. Ammonifier; (5), in Brackish Water Alluvial Soils; (6), in Low Humic Gley Soils. Nitrite oxidizer: (7), in Brackish Water Alluvial Soils; (8), in Low Humic Gley Soils.

2) Effect of rice straw application without N-fertilizer

As shown in Fig. 12, the population of denitrifier increased in Brackish Water Alluvial Soils following the application of rice straw, while in the case of Low Humic Gley Soils such effect was not clear. The difference in population size between the two great soil groups became smaller until 11 weeks after transplanting.

On the other hand, even if rice straw was applied, the population of aerobic nitrite oxidizer kept the same level as compared with that in the phosphate plot except for the population in the top soil (0-1 cm) of Low Humic Gley Soils where decomposition of organic matter was more rapid.

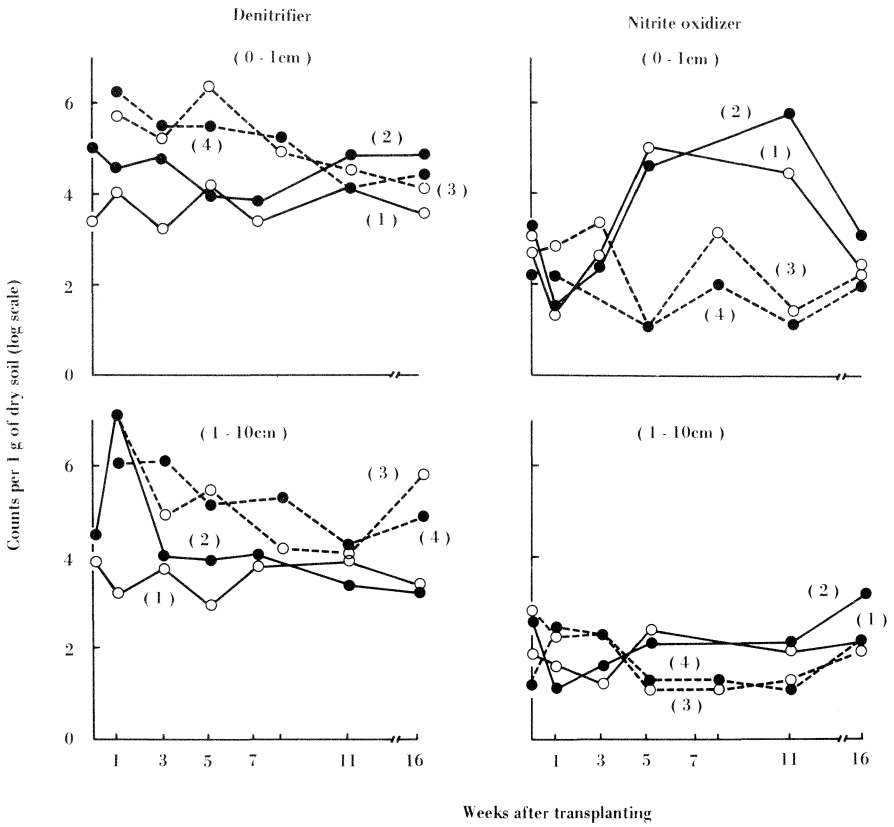

Fig. 12. Effect of rice straw application without N-fertilizers on the population change of denitrifier and nitrite oxidizer in both Brackish Water Alluvial Soils and Low Humic Gley Soils during the growing period of rice plants.
Brackish Water Alluvial Soils: (1), Phosphate plot; (2), Phosphate and rice straw plot. Low Humic Gley Soils: (3), Phosphate plot; (4), Phosphate and rice straw plot.

3) Effect of rice straw application with N-fertilizer

As indicated previously, the amount of molecular nitrogen in volatile soil gas increased following the application of rice straw compared with the plots with N-fertilizer and phosphate. As shown in Fig. 13-b, the population of denitrifier also mostly increased in both great soil groups following the application of rice straw. In the case of Low Humic Gley Soils, the population of denitrifier decreased largely at the later stage of rice growth, irrespective of rice straw application, presumably due to the decrease in easily utilizable organic matter content at this period.

UEHARA and TAKAI[84] reported that the denitrifiers increased rapidly at earlier stages of incubation under submerged conditions with the decomposition of easily utilizable organic matter, and that they maintained their large population throughout the middle stage of incubation. At later stages, however, their population decreased because easily utilizable organic matter and nitrate had been consumed.

As shown in Table 23, the amount of molecular nitrogen in the ammonium chloride, phosphate, rice straw plot **(G)** of Brackish Water Alluvial Soils was larger than that in the ammonium sulfate, phosphate, rice straw plot **(F)**. The population of denitrifier showed also a high level in the subsurface soil of the former plot until 7 weeks after transplanting.

Following the application of rice straw with N-fertilizer, the population of nitrite oxidizer in the top soil of Brackish Water Alluvial Soils showed a noticeably lower value 5 weeks after transplanting compared with that in the plot containing only N-fertilizer suggesting that the reduced condition developed at this stage in the rice straw plots. On the other hand, both in the top and subsurface soil of Low Humic Gley Soils where organic matter decomposition was rapid, the population of nitrite oxidizer in the rice straw plot with N-fertilizer showed mostly similar values to those in the N-fertilizer plot.

Summary

In order to compare the effects of rice straw on the composition of soil gas released into the atmosphere and microflora related to the change of mineral nitrogen between Brackish Water Alluvial Soils (B.W.A.) and Low Humic Gley Soils (L.H.G.), field experiments were carried out at two Rice Exp. Sta. in Thailand.

The amount of molecular nitrogen in soil gas released into the atmosphere from the control plot of L.H.G. was about 7 times as high as that of B.W.A. The amount of molecular nitrogen in soil gas increased following phosphorus application, especially in B.W.A. Following rice straw application (6 tons/ha) with N-fertilizer, the amount of molecular nitrogen in soil gas increased largely compared with that in the corresponding plots without rice straw.

Methane gas was hardly detected in the plots with B.W.A. prior to rice straw application. Following rice straw application, the amount of methane gas increased markedly in both great soil groups.

The population of ammonifier in the control plot was slightly larger in B.W.A. than in L.H.G., while that of aerobic ammonia oxidizer in B.W.A. which had a thinner oxidized layer was smaller than that in L.H.G. The population of aerobic nitrite oxidizer was also smaller in B.W.A. 1 to 21 days after transplanting, but increased markedly after 3 weeks. The change in population of nitrite oxidizer paralleled the accumulation of ammonia and its subsequent decrease at this period. The population of denitrifier in L.H.G. was 10 times as large as that of B.W.A. throughout the growing period of rice plants.

Following the application of rice straw with N-fertilizer, the population of denitrifier mostly increased in both great soil groups, while that of nitrite oxidizer in the top soil (0-1 cm) of B.W.A. showed a noticeably lower value 5 weeks after transplanting compared with that in the plot without rice straw.

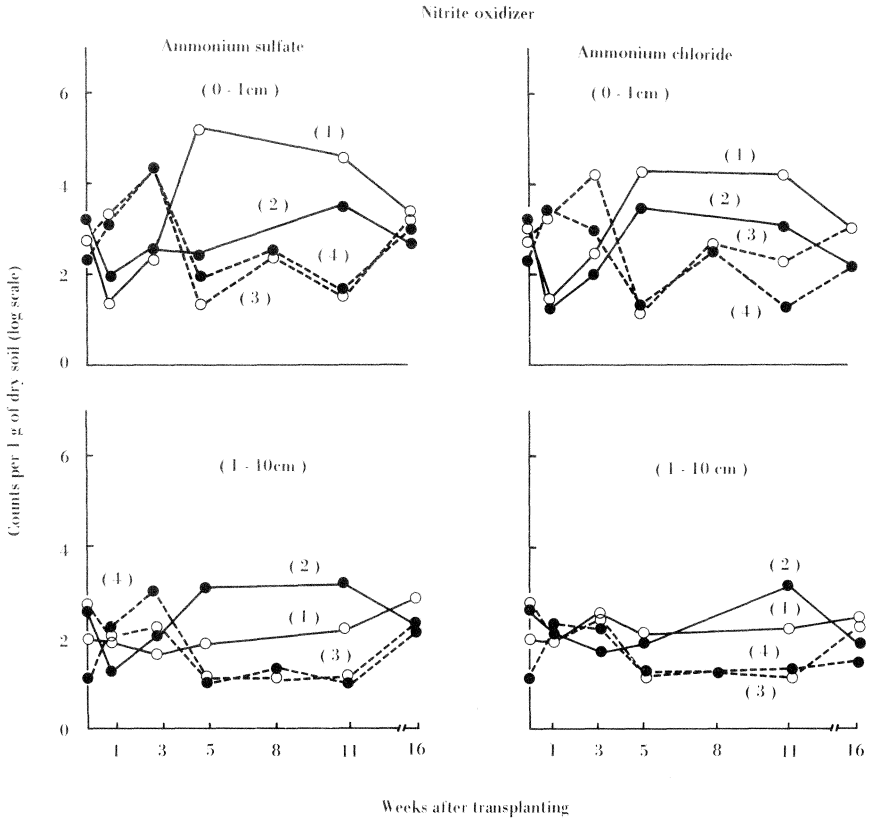

Fig. 13-a. Effect of rice straw application with N-fertilizers on the population change of nitrite oxidizer in both Brackish Water Alluvial Soils and Low Humic Gley Soils during the growing period of rice plants.
Brackish Water Alluvial Soils: (1), Nitrogen and phosphate plot; (2), Nitrogen, phosphate and rice straw plot. Low Humic Gley Soils: (3), Nitrogen and phosphate plot; (4), Nitrogen, phosphate and rice straw plot.

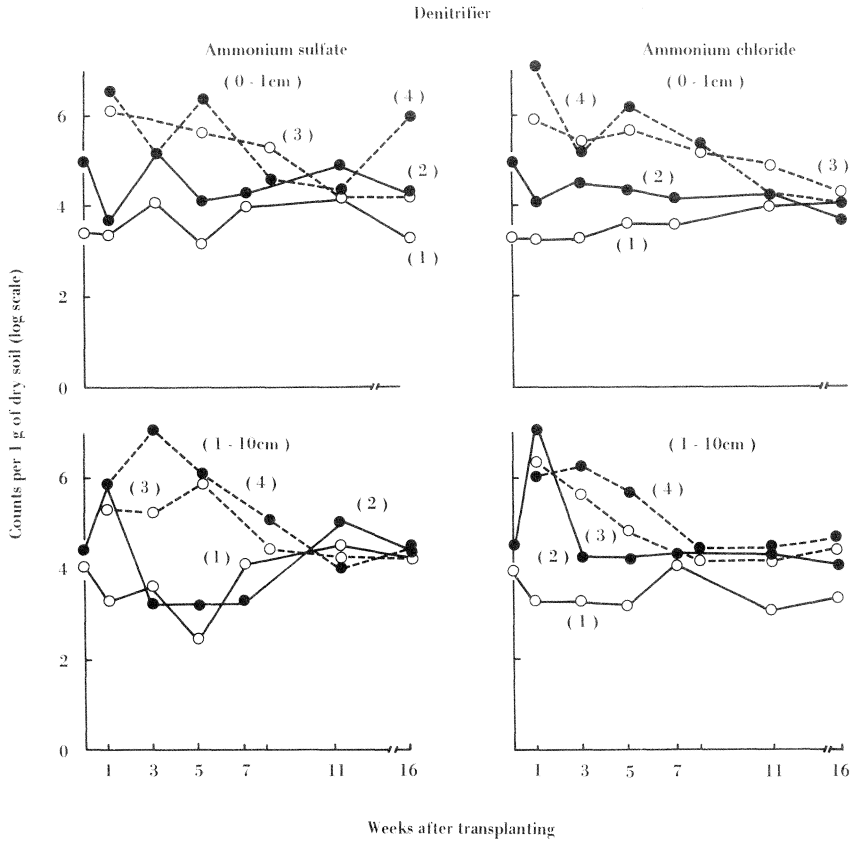

Fig. 13-b. Effect of rice straw application with N-fertilizers on the population change of denitrifier in both Brackish Water Alluvial Soils and Low Humic Gley Soils during the growing period of rice plants.
Brackish Water Alluvial Soils: (1), Nitrogen and phosphate plot; (2), Nitrogen, phosphate and rice straw plot. Low Humic Gley Soils: (3), Nitrogen and phosphate plot; (4), Nitrogen, phosphate and rice straw plot.

GENERAL SUMMARY AND CONCLUSION

1 Dynamic behavior of soil microorganisms related to the nitrogen cycle in the paddy soils of Thailand.

The populations of microorganisms, except for cellulose decomposer, decreased in the dry season (no cultivation) compared with those in the rainy season (rice cultivation under submerged condition). The largest decrease was recorded in the population of nonspore-forming nitrite oxidizer. The populations of twelve groups of microorganisms except those of nitrite oxidizer and purple nonsulfur bacteria recorded the largest value in Fresh Water Alluvial Soils (F.W.A.) with rather high organic matter content and heavy clay followed by Low Humic Gley Soils (L.H.G.) with low organic matter content, while the smallest value was generally recorded in Brackish Water Alluvial Soils (B.W.A.) with low available phosphorus content and low pH value.

The population level of nitrite oxidizer was high in tropical paddy soil and that in the top soil (0-1 cm) was 4 times as high as that in the subsurface soil (1-10 cm). The number of nitrite oxidizer showed the highest value in L.H.G. which are characterized by the highest Eh value and the thickest oxidized-layer among the three great soil groups. In contrast, a small number of nitrite oxidizer was obtained in F.W.A. with high organic matter content, heavy clay, rather low Eh value and thin oxidized layer, while the populations of nitrite oxidizer in B.W.A. with low pH value and low available phosphorus content were the least numerous. It appeared that the number of nitrite oxidizers corresponded to the development of an oxidized layer.

2 Dynamic behavior of soil microorganisms related to the nitrogen cycle in the upland farm soils of Thailand (Rainy season).

The microbial counts in Brown Forest Soils, Rendzinas and Grumusols with high content of organic matter, available phosphorus and exchangeable potassium tended to be high. In the case of Low Humic Gley Soils and Regosols with low content of organic matter, available phosphorus and available potassium, populations of microbes were generally small.

3 Actinomycete flora of upland farm soils of Thailand (Rainy season).

The genus composition and antibiotic production of actinomycete isolates (1240), sampled from major great soil groups of upland farm soils in Thailand were investigated, among which 75.4% of total isolates belonged to *Streptomyces* followed by *Nocardia* (4.8%), *Streptosporangium* (3.2%) and *Micromonospora* (1.5%). Sterile types accounted for 13.0% of all isolates. A high percentage of *Streptomyces* and a low one of sterile types were found and were ascribed to dryness of soils. The genus composition of actinomycete flora varied among great soil groups. It was conspicuous that Grumusols with high maximum water holding capacity contained a fairly high percentage of *Micromonospora* (17%), which was generally found in such soils with high moisture content as paddy and peat soils. Rendzinas and Gray Podzolic Soils showed a rather higher percentage of *Nocardia* (13 to 14%). Percentage of actinomycetes antagonistic to each test organism showed variations among great soil groups. The soils with high percentage of actinomycetes antagonistic to Gram-negative bacteria showed significantly high maximum water holding capa. ity and high organic matter content.

4 Comparison of microflora in paddy and upland farm soils between Thailand and Japan.

1) The largest difference in the general groups of microorganisms between tropical (Thailand) and temperate (Japan) paddy soils was found in the ratio of aerobic bacteria to actinomycetes.

The tropical soils showed a small value, namely 1.60 in the rainy season, while that of Japanese soils was about 9.7 before irrigation in spring. And also even in upland farm soils, the ratio of aerobic bacteria to actinomycetes was lower in the tropical soils, namely 2.3, while that of non-volcanic soils of Japan was 7.28. The major reason for such a phenomenon was ascribed to the dry season (no cultivation) which lasts six months, when spore forming actinomycetes are able to survive.

2) Though the population of physiological groups showed large variations with seasons and also with treatments, microorganisms related to the nitrogen cycle were compared. The count of denitrifier in upland farm soils of Thailand was 9 times as high as that in non-volcanic upland-farm soils of Japan and was 23 times that in volcanic ones. In the case of *Azotobacter*, the count in upland soils of Thailand averaged 2,800 per 1 g of dry soil, while that in non-volcanic upland farm soils of Japan was 77 on the average.

3) The percentage of chromogenic actinomycetes to the total isolates was 52.4% on the average in the soils of Thailand and 11.9% in the soils of Japan with a significant level of 0.1%. The percentage of cellulase-positive actinomycetes to all isolates of actinomycetes in the soils of Thailand was very high, amounting to 77.3%, while that in the soils of Japan was 29.1%. A significant difference in the composition of actinomycete flora between the tropical and temperate soils, and moreover marked difference among major tropical great soil groups were recognized.

5 Relationship between population of microorganisms and soil chemical and physical properties.

Accumulation of nitrate nitrogen was conspicuous in the surface soils (0-10 cm) of tropical paddy soils in the dry season. Significant correlation coefficients were obtained at the level of 5% between the ammonium nitrogen contents and the population of ammonifier, between the total content of mineral nitrogen and the population of ammonifier, and between the ratios of nitrate nitrogen to ammonium nitrogen contents and the population of nitrite oxidizer, only in the top soils.

The relationship between the organic matter content and the microbial population of upland farm soils was positively significant at the level of 0.1% only in the case of fungal population, while the relationship between the available phosphorus content and the microbial population was positively significant at the level of 0.1% only in the case of *Azotobacter*. The relationships between the total nitrogen, the exchangeable potassium, the amount of NH_4^+–N, the amount of NO_3^-–N, or the amount of NH_4^+–N + NO_3^-–N and each microbial population were not significant in any microbial groups.

A correlation between carbon contents in soils and the ratios of chromogenic actinomycetes to the total isolates was obtained with a significance at 1% level. It was also noticed that the chromogenic group contained high percentage (70%) of isolates with both protease and cellulase, in contrast to the non-chromogenic group (24%). These results indicate that tropical soils contain predominantly chromogenic actinomycetes with strong protease and cellulase activities and this trend was particularly evident in soils with low carbon content.

6 Effect of rice straw on the composition of volatile soil gas and microflora related to the nitrogen cycle in tropical paddy soils.

The effect of rice straw on the composition of volatile soil gas released into the atmosphere and microflora in the tropical paddy soils (L.H.G.) was periodically evaluated with and without fertilizer application.

The volatile soil gas most abundantly found in plots with rice straw was methane during the

early stage of rice growth, while nitrogen predominated in later stages. The amount of molecular nitrogen increased following phosphorus application as well as rice straw application as compared with that in the control plot. In the former case, the enhancement of decomposition of organic-N was assumed to be due to the increase in population of cellulose decomposer. Rice straw application with or without N-fertilizer increased methane gas formation by 27 to 63 times as compared with the phosphate plot and the peak of its formation was found 5 to 7 weeks after rice straw application. However methane formation in the control plot was very low and was found only 5 to 9 weeks after flooding. Rice straw application variously affected each microbial group. But the stimulating effect was chiefly observed in the population of *Azotobacter*.

7 Comparison of effect of rice straw on the composition of volatile soil gas and microflora related to the change of mineral nitrogen between B.W.A. and L.H.G.

The amount of molecular nitrogen released into the atmosphere from the control plot of L.H.G. was about 7 times as high as that of B.W.A. The amount of molecular nitrogen in soil gas increased following phosphate application, especially in B.W.A. Following the rice straw application (6 tons/ha) with N-fertilizer, the amount of molecular nitrogen in soil gas increased largely compared with that in the corresponding plots without rice straw.

Methane gas was hardly detected in the plots with B.W.A. prior to rice straw application. Following rice straw application, the amount of methane gas increased markedly in both great soil groups.

The population of ammonifier in the control plot was slightly larger in B.W.A. than in L.H.G., while that of aerobic ammonia oxidizer in B.W.A. which had a thinner oxidized layer was smaller than that in L.H.G. The population of aerobic nitrite oxidizer was also smaller in B.W.A. 1 to 21 days after transplanting, but increased markedly after 3 weeks. The change in population of nitrite oxidizer paralleled the accumulation of ammonia and its subsequent decrease at this period. The population of denitrifier in L.H.G. was 10 times as large as that of B.W.A. throughout the growing period of rice plants.

Following the application of rice straw with N-fertilizer, the population of denitrifier mostly increased in both great soil groups, while that of nitrite oxidizer in the top soil (0-1 cm) of B.W.A. showed noticeably lower value 5 weeks after transplanting compared with that in the plot without rice straw.

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