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Annual Changes in Soil Nitrogen and Microflora in Paddy Fields not Fertilized Long-term and Fertilized Paddy Fields*

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Synopsis

From 1987 to 1989, we measured changes in soil nitrogen and microflora in two kinds of paddy fields, one not fertilized for 40 years and one fertilized regularly.

1) The soil total-N content was significantly higher in the fertilized field (F-field) than in the non-fertilized field (NF-field) ; in both fielis, the content increased from starting in the maximum-tillering stage until the heading and flowering stage, then temporarily decreased in the middle growing stage, and then returned to the initial level. The soil total-C reached a maximum in both F-and NF-fields at the ripening stage ; its variation was smaller than that of total-N. Thus, the C/N ratios ranged from 7.0 to 8.0 over the growing period of the rice plants.

2) Fungal populations were significantly larger in the NF-field than in the F-field while the rice plants were growing. Actinomycetes were much more abundant in the F-field than in the NF-field. The total number of bacteria increased in the NF-field at the middle growing stage of the rice plants. Consequently, the B/F value (bacterial number/fungal number) in the upper plow layer was high in the NF-field.

The numbers of anaerobic bacteria in the NF-field were far below those in the F-field through the 2-year period, which reflected the extent of the oxidative state in the whole soil horizon of the NF-field because of its continuous supply of irrigation water.

3) The genus *Paecilomyces* was abundant in the NF-field, and the genera *Paecilomyces* and *Trichoderma* were both abundant in the F-field. The fungal florae in the F-field were less abundant than those in the NF-field. Of the actinomycetous florae, the genus *Strepotomyces* was most numerous in both fields, followed by the genus *Nocardia*.

Introduction

In recent years, a renewed interest in farming techniques based on the energy conversion and the recycling of nutrients in the soil ecosystem has appeared in Japan. Under these circumstances, it may be necessary to establish guidelines about rice cropping practices and management in harmony with nature.

In our laboratory, joint studies on naturalized agricultural techniques have been carried out since 1975, centering on "Comparative Agronomic Studies on Crop Production Practice"[2], but research on microflora in paddy soil has not yet been done in detail.

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* A part of this study was presented in the 14th International Congress of Soil Science, August, 1990, Kyoto, Japan[1].

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Analysis of soil nitrogen and microflora in paddy fields in which fertilizers, manures, and pesticides have not been applied will give useful information needed for reassess the conventional agricultural techniques in Japan, which rely primarily on the heavy application of fertilizers and pesticides. Such studies will give suggestions about the efficient use of natural resources and energy, soil conservation, and more stable rice crop production.

Materials and Methods

1) Overview of the paddy fields investigated

The non-fertilized paddy (NF) field (owned by Mrs. TANAKA) is in Ritto-cho, Shiga Prefecture, Japan. In this field, farming without any fertilizers, manures, and pesticides has been strictly done under the direction of the Reimei Kyokai, Kyoto Prefecture, since 1951. The NF-field has a constant and adequate supply of continuous irrigation water over the 120-day period from transplanting (early May) until about 20 days before the harvest (mid-October) of the rice plants. The menagements of this field are summerized in Table 1. Organic matter returned to the soil was restricted to a small amount of weeds and rice stubbles in the paddy field when it was plowed.

Table 1. Overview of management and analysis (1989) of paddy field not fertilized long-term*

Date	Field management**
Late May	Soil puddling/transplanting
16～26 June	Weeding by hand or by rotary weeder
15 July	Soil sampling for microbial analysis
18 Aug.	Soil sampling for microbial analysis
9 Sept.	Soil sampling for microbial analysis
Early Oct.	Drainage stage
Mid-Oct.	Harvesting stage
Nov. ～ Mar.	Rice stubbles and some weed residues incorporated into the soil by plowing

*Located in Ritto-cho, Shiga-ken ; no fertilizers, manures, and pesticides have been applied since 1951.

**During the growth of rice plants, the field has a constant and adequate supply of continuous irrigation water.

Table 2. Annual amounts of fertilizer applied to the paddy field being cultivated by conventional practices*

Element	Basal	Supplement	Topdressing		Total amounts (kg/ha/year)
	Late Apr.	Late May	July		
			15	30	
N	56	28	28	28	140
P_2O_5	32	16	16	16	80
K_2O	56	28	28	28	140

*Located in Ritto-cho, Shiga-ken, where transplanting was done in early May, application of herbicides in mid-May, and harvesting in mid-September, 1989.

For comparison, a fertilized (F) paddy field being clutivated by the usual practice (owned by Mr. SHIMADA), adjacent to the west side of the NF-field, was analyzed for its total-N and total C content in the soil in 1987, and used in 1988 for its microbial analysis. In 1989, another F-field (owened by Mr. TANAKA), adjacent to the south side of the F-field, was used to study changes in the soil microflora during the growth of rice plants. The amounts of fertilizer applied to this field are shown in Table 2.

2) **Soil sampling and preparation**

Soil samples were taken from the three plots at their water inlets, centers, and water outlets in both NF- and F-fields with stainless two-core samplers, comprising of the two cylindrical samplers (5 cm inside diameter×5 cm long) mutually interconnected. Soil samples collected from the upper plow layer were of two kinds, those from the depth of 0-10 cm and those from the depth of 0-1 cm, both of which were prepared separately by the method of reduction[3]. Air-dried soil samples from the depth of 0-10 cm were passed through a 2-mm sieve, mixed thoroughly, and used for the analyses of total-N and total-C contents. For the microbial analyses, fresh pasty soils from the depth of 0 1 cm were used as soon as possible.

3) **Determination of the total-N and total-C contents in soil**

Air-dried soil samples were analyzed for total-N and total C contents by the method of Kjeldahl digestion and the method of Tyurin, respectively[4]. Samples were taken in 1987, or April 26 (post-plowing/drainage stage), May 15 (post-transplanting/rooting), June 24 (panicle initiation), July 15 (active-tillering), August 3 (maximum tillering or heading/flowering), August 22 (ripening), September 17 (drainage and post-harvesting in the F-field), October 1, October 14 (drainage and post-harvesting in the NF-field), and November 27.

4) **Enumeration of microorganisms in soil**

Seasonal changes in microflora in the soil were studied for two successive years from July to the harvesting stage of rice plants. In 1988, fresh soil samples were collected on August 6 and October 8 (Experiment 1), and in 1989, they were collected on July 15, August 18, and September 9 (Experiment 2).

The numbers of individual microorganisms were counted with use of the following media and procedures[3,5] :

① Fungi : Rose bengal agar (Smith-Dawson's medium), dilution agar plate method.

② Anaerobic bacteria : peptone plus meat-extract agar medium, dilution agar plate method (using commercially available O_2 absorbents).

Four replicate plates prepared at suitable dilutions were incubated at 28°C in the dark for about 10 days, and the colonies that grew were counted under a stereomicroscope (magnification, 20×).

Of the individual microorganisms, fungi and actinomycetes were used in the observation of florae. Their morphological features were examined under a photomicroscope (magnification, 400×), and their relative abundance among the genera was estimated.

Results and Discussion

1) **Seasonal changes in total-N and total-C contents in soil**

The seasonal changes in the total-N and total-C contents in both kinds of fields are shown in Figs. 1 and 2. The increases in the total-N content were greater in the F-field than in the NF-field during the entire period of growth, although the pattern of change was similar. The mean total-N content in the center plot of the F-field was about 270 mg N/100 g of dry soil ; in the NF-field at the inlet, center, and outlet, the means were about 210, 190, and 200 mg N/100 g of dry soil, respectively.

In the NF-field, the soil total-N increased gradually from early May to late June and reached

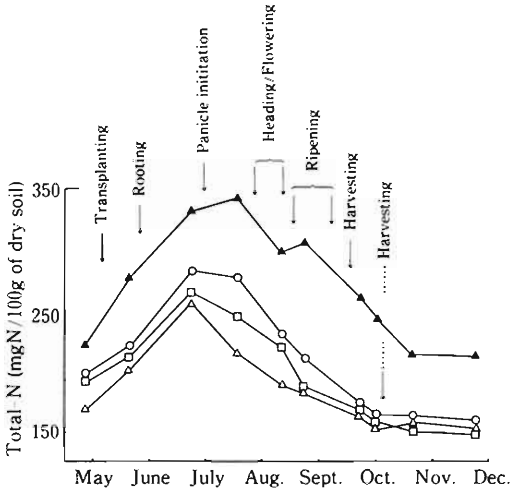

Fig. 1. Seasonal changes in total-N content in paddy soils (0-10 cm)* in the paddy fields in Ritto-cho, Shiga-ken (1987)
* Non-fertilized plot:
○——○Inlet, △——△Center,
□——□Outlet
* Fertilized plot:
▲——▲Center

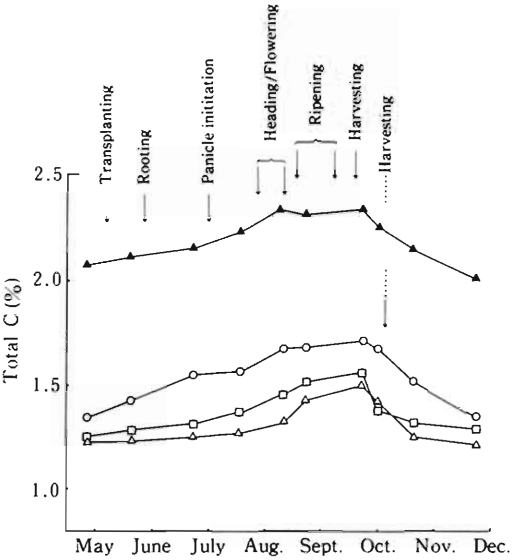

Fig. 2. Seasonal changes in total-C content in paddy soils (0-10 cm)* in the paddy fields in Ritto-cho, Shiga-ken (1987)
* Non-fertilized plot:
○——○Inlet, △——△Center,
□——□Outlet
* Fertilized plot:
▲——▲Center

a maximum when the rice plants were in the panicle initiation stage, then decreasing until it stabilized at an almost costant level. The total-N in the inlet was higher than that in the other locations from late June to early September.

In the F-field, the total-N content reached a maximum at the active-tillering stage, and after the drainage/harvesting stage, it tended to diminish gradually. KAWAMURA *et al.*[6] reported that the total-N in the inlet of this field was higher than that in the other locations. The total-N content in both fields decreased gradually from the middle to the late cropping period, but it did not fall below its initial levels in either field. Such a shift pattern seems to reflect the mineralization of organic nitrogen in the soil, which proceeds in proportion to the growth in rice plants, and matches well with the changes in the nutrient needs of the rice plants.

KAWAMURA *et al.*[6] also showed that the total-N content in the NF-field fluctuated annually for four successive years over a smaller range than that in the F-field; its level was approximately the same during the entire growth period of the rice plants. In connection with this, OKUMURA[7] has suggested that of the mineralized nitrogen in the soil, the nitrogen that has not been utilized by the rice plants might be to great extent reimmobilized in the soil. TAKEUCHI *et al.*[8] concluded that rice productivity in the NF-field depends heavily upon the quantities of nutrient sources, primarily supplied by irrigation water.

The seasonal changes in the total-C content in the soil are shown in Fig. 2. The range of variation was smaller than that in total-N content. In both fields, the total-C content slightly increased from the early to middle stage of the growth of rice plants, then reached a maximum at the ripening stage. However, finally the total-C content declined to the initial level. The mean value of the total-C content during the whole rice cropping period was 2.22% in the center of the

F-field, while that in the NF-field decreased in the order of the inlet (1.54%) >the outlet (1.37%) >the center (1.30%). Consequently, the C/N ratios in both fields were in the range of 7.0 to 8.0. Such a difference in the total-C content between the two fields seemed to arise mainly from the difference in the total amount of weeds and rice plant residues incorporated into the soil as supplied sources of organic matter at the time of plowing[9]. According to research on weed growth in the Ritto's paddy fields before plowing on May 7, 1976, the amounts of weed biomass were 2,332 kg dry wt./ha for the F-field and 972 kg dry wt./ha for the NF-field[10].

These results suggest that the amount of organic matter returned to paddy soil differed so extremely that the soil microflorae in both fields were affected quantitatively and qualitatively.

2) Test of significance between the numbers of colonies of microorganisms in the different plots of the fields

Table 3 shows the average values, standard deviation, and coefficient of variation for the numbers of colonies per dilution agar plate of fungi, actinomycetes, total bacteria, and anaerobic bacteria in the soils, taken from the inlet, center, and outlet. The coefficient variation varied somewhat with the different microorganisms ; it ranged generally from 25 to 45% in the NF-field, and from 5 to 30% in the F-field, of which the former showed its wider range of variation than the latter. The differences in the numbers of colonies by t-test were significant in both fields at the 10% level, generally accepted as being in common use[11].

Table 3. Fluctuations in microbial numbers in paddy soils (0-1 cm)* in the paddy fields in Ritto-cho, Shiga-ken (1988)

Microorganisms	Microbial numbers per gram of dry soil**											
	6 August						8 October					
	Non-fertilized			Fertilized			Non-fertilized			Fertilized		
	A	σ	CV	A	σ	CV	A	σ	CV	A	σ	CV
Fungi ($\times 10^4$)	3.33	1.08	32.4	7.18	1.61	22.4	17.3	4.89	28.3	15.5	1.44	9.29
Actinomycetes ($\times 10^5$)	16.6	4.15	25.0	39.5	11.7	29.6	13.2	3.71	28.1	23.1	3.30	14.3
Total bacteria ($\times 10^7$)	280.8	72.5	25.8	270.0	14.1	5.22	142.4	64.5	45.3	134.8	20.4	15.1
Anaerobic bacteria ($\times 10^5$)	9.72	4.05	41.7	22.6	2.47	10.9	10.8	2.45	22.7	17.0	3.39	19.9
B/F value***	84,300			37,600			8,230			8,697		

*Soils were taken from each plot of inlet, center, and outlet of both non-fertilized and fertilized fields.
**Microbial numbers represent the average values (A) of the number of colonies per agar plate (4 replications), the standard deviations (σ), and the coefficient of variation ($CV = \sigma/A \times 100\%$).
***Ratio of total bacterial number to fungal number

3) Seasonal changes in soil microflora (Experiment 1)

A preliminary investigation of the soil microflora in the upper plow layers of both F- and NF-fields was done in 1988. As shown in Table 1, fungal populations in the F-field were somewhat larger than those in the NF-field in August (heading/flowering stage of rice plants), then increased slightly in the NF-field in October (post-harvesting time in the F-field, pre-harvesting time in the NF-field).

At both stages, the numbers of actinomycetes and total bacteria were large in both the F- and NF-fields, of which the numbers of total bacteria increased in the NF-field at the heading/flowering stage. Thus, the B/F value, accepted broadly as a criterion of microbial characteristics in soil[12], in the NF-field was twice that in the F-field. From this value, it seemed that soil microflorae in the F-field might undergo a succession from bacterial type to fungal type, mostly because of the application of fertilizers and pesticides. These results are compatible with observations made in the experimental paddy field under the repeated applications of herbicides[13].

The numbers of anaerobic bacteria were smaller in the NF-field than in the F-field in both August and October ; the difference between the two fields was significant in August (when the rice plants were heading and flowering). This result may show that in the F-field, the inner parts of soil horizon had more differentiated oxidized and reduced layer[14], and that in the F-field, they had not always clearly differentiated layers. This means by and large that the whole layers in the NF-field became uniformly oxidative, probably because of the small amount of organic matter and the abundant oxygen supplied by the continuous flow of irrigation water.

4) **Seasonal changes in soil microflora (Experiment 2)**

In 1989, the seasonal changes in soil microflora were as shown in Fig. 3. Fungal populations in the NF-field were larger than those in the F-field in mid-July (maximum-tillering), and their subsequent chages gave rise to a small difference between the two fields.

The numbers of actinomycetes in the F-field greatly exceeded those in the NF-field over much of the growth stage of the rice plants. The total numbers of bacteria in the NF-field (especially at the inlet) were very high in the maximum-tillering and heading/flowering stages. This difference in each stage was greater in the NF-field than in the F-field ; the numbers declined in the ripening stage, and the difference was no longer great. OKUMURA[7] reported earlier that the numbers of aerobic bacteria in the NF-field were temporarily large in July and after that the numbers declined significantly in both fields, which are compatible with the results described here. The B/F value in the NF-field (about 4,210) higher than that in the F-field (about 3,900). This result by and large was similar to that obtained in the preceding year. The numbers of fungi and of total bacteria in both fields were lower than in the preceding year. The reason may be some difference in the amount of organic matter or other environmental factors.

The numbers of anaerobic bacteria in the F-field were larger than those in the NF-field throughout the growth of rice plants. This result, as mentioned before, seemed to reflect the oxidative state of the soil horizon, especially in the NF-field. However, the numbers of such bacteria in the inlet of the NF-field were slightly smaller than those in the two other plots. In addition, the differences between the numbers of anaerobic bacteria in the NF- and F-fields gradually decreased as the rice plants grew, which might be responsible for the appearance of facultative anaerobes after submergence[15].

5) **Composition of the florae of fungi and/or actinomycetes**

The relative abundance of each genus to the total microbial numbers was represented as the frequency (%) of the genus of fungi and/or actinomycetes. Miscellaneous groups of fungi and/or actinomycetes, comprising of only a few unidetified genera, are shown comprehensively as "others" in Figs. 4 and 5. Some morphological features of certain representative genera seen by photomicroscope are illustrated in Plate I[16,17] and Plate II[18].

The frequency of fungal flora in the NF-field was 10 to 30% for the genus *Paecilomyces* ; in the F-field, it was 20 to 40% and 15 to 30% for *Paecilomyces* and *Trichoderma*, respectively. These genera were observed as the fungi predominating in the fields. WAKSMAN[19] has reported that the Trichodermae are extensively distributed in acid and in waterlogged soil. Besides these predominant genera, in the NF-field in particular, the genera *Mucor*, *Chrysosporium*, and *Mortierella* were observed at a lower frequency.

The relative abundance of the genera (fungi) in the NF-field to that in the F-field, with the exception of "others", was about 2 : 1. The composition of fungal flora was much more abundant in the NF-field than in the F-field. The most simplified composition of fungal flora in the F-field seemed to be caused by the different field maintenance. Another reason could be that the genus

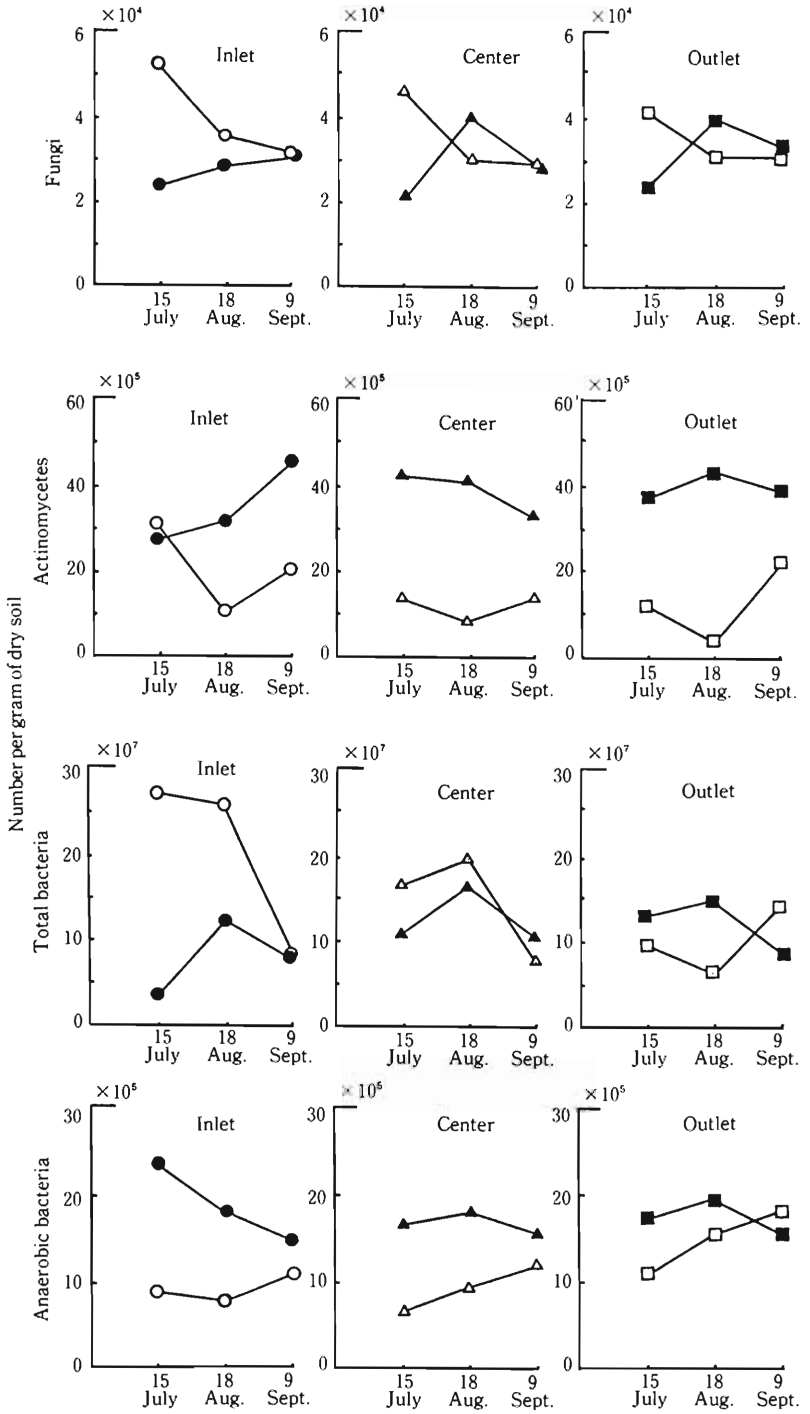

Fig. 3. Seasonal changes in microflora in paddy soils in the paddy fields in Ritto-cho, Shiga-ken (1989)
○—○, △—△, □—□ Non-fertilized soil (0-1 cm)
●—●, ▲—▲, ■—■ Fertilized soil (0-1 cm)

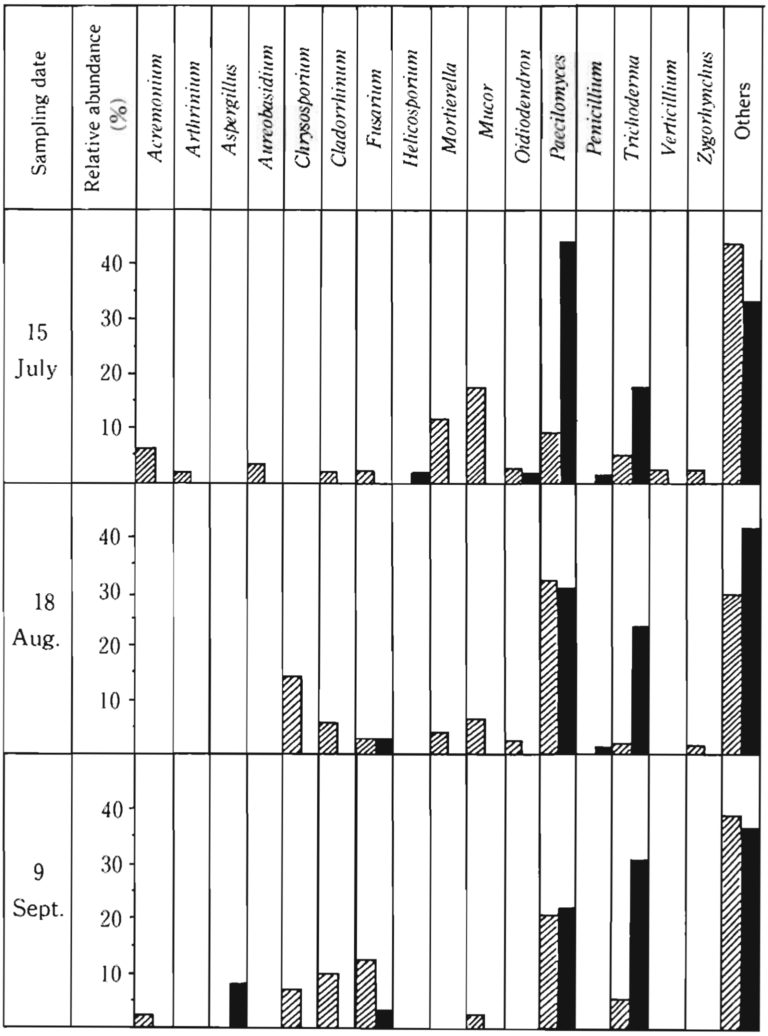

Fig. 4. Relative abundance* of fungal flora (genus) in paddy soils in the paddy fields in Ritto-cho, Shiga-ken (1989)
*Average percentage of the numbers of colonies (genus) to the total fungal numbers per agar plate, obtained from soil at the inlet, center, and outlet of the fields
▨ Non-fertilized soil (0-1 cm)
■ Fertilized soil (0-1 cm)

Paecilomyces has a sort of "antagonistic effect" on the genus *Trichoderma*, and vice versa, as shown in Plate I. These results seemed to support the information that in the NF-field, the annual changes in soil total-N content tend to be maintained at an approximately same level[6].

The genera (actinomycetes) were classified according to Bergey's manual[18]. The frequency of the genera (actinomycetes) in the soil was about the smae in both kinds of fields. During the entire growing period, *Streptomyces* was the most frequently encountered genus (about 30%) in both fields, followed by the genus *Nocardia* (5 to 15%). The genera *Streptoverticillium* and *Micromonospora* were observed at very low frequencies. However, the relative abundance of the unidentified genera, or "others" was high in both fields (Plate II). Miscellaneous groups should

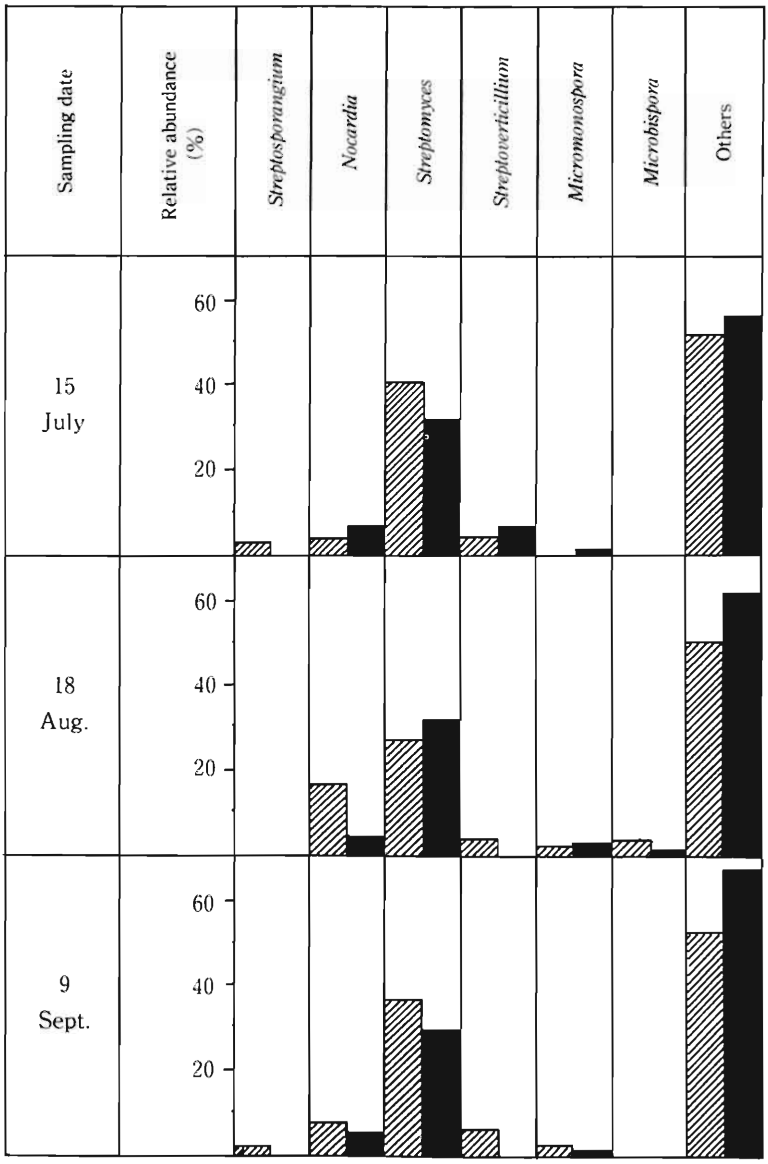

Fig. 5. Relative abundance* of actinomycetous flora (genus) in paddy soils in the paddy fields in Ritto-cho, Shiga-ken (1989)
*Average percentage of the numbers of colonies (genus) to the total actinomycetous numbers per agar plate, obtained from soil at the inlet, center, and outlet of the fields
▨ Non-fertilized soil (0-1 cm)
■ Fertilized soil (0-1 cm)

be broadly classified and identified in detail from the aspect of their biochemical and physiological characteristics in the future.

Putting all results together, the extent of recycling of soil nutrients such as nitrogen or carbon and the seasonal changes in the soil microflorae might be caused not only by a natural or artificial supply of nitrogen, but also by the characteristics of soil microorganisms, the amount of weeds and rice stubbles incorporated into the soils, and the supply of available irrigation water.

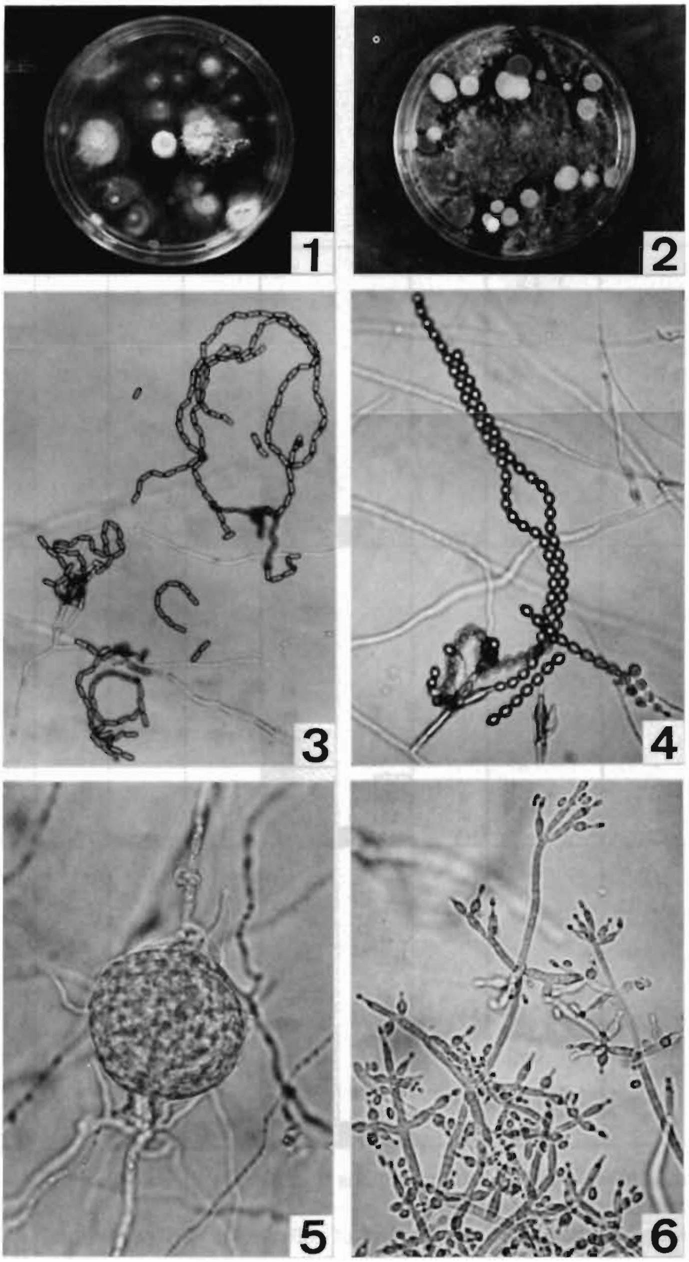

Plate I. Morphology of fungal colonies grown on a plate agar (Plate No. 3～6 ; Magnification 400×)

1. Fungal colonies in the non-fertilized field
2. Fungal colonies in the fertilized field
3. Morphology of the genus *Oidiodendron*. See the fragment of branched phialides.
4. Morphology of the genus *Paecilomyces*. See the conidiospores with long chains of phialospores.
5. Zygospores of Mucorales
6. Morphology of the gunus *Trichoderma*

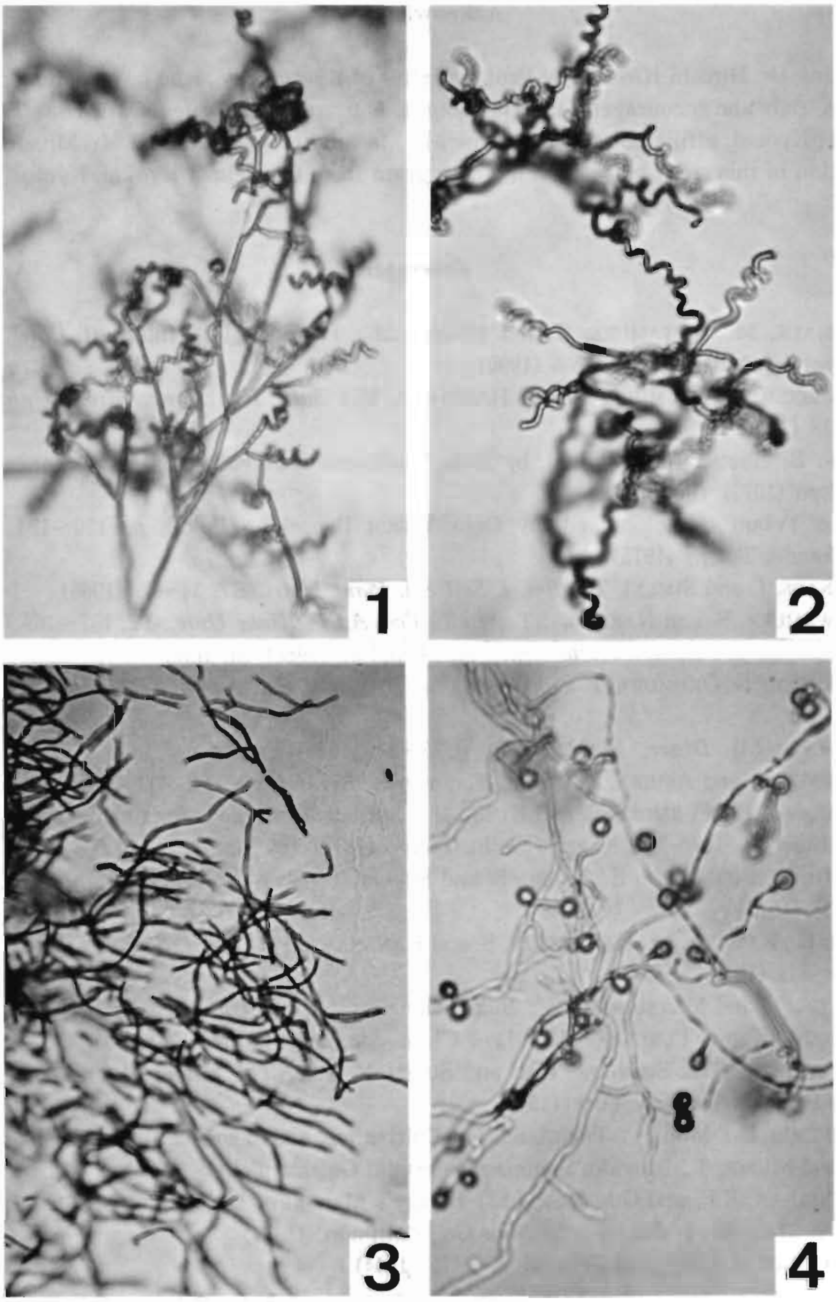

Plate II. Morphology of actinomycetous colonies grown on a plate agar (Plate No. 1~4 ; Magnification 400×)

1-2. Spiral chains of aerial hyphae of the genus *Streptomyces*

3. Fragments of aerial hyphae of the genus *Nocardia*

4. Aerial hyphae and spores of the genus *Micromonospora*

Acknowledments

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長期無施肥水田および施肥水田における土壌の窒素ならびに微生物相の周年変動

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摘　　要

長期無施肥田（40年間継続）ならびに施肥田土壌の窒素と微生物相の周年変動について，昭和62年度より３カ年にわたって比較検討した．

1)　作土表層の全窒素含量は，水稲生育の全期間を通じ明らかに施肥田の方が無施肥田よりも高く推移し，特に７月（最高分けつ期）から８月（出穂・開花期）にかけてかなり増加したが，水稲の生育後期には減少した．

一方，全炭素含量は，いずれの供試田においても９月（登熟期）に最大に達したが，その変動幅は全窒素含量のそれに比べて小さかった．

2)　作土表層の微生物相の時期別変動を検討した結果，糸状菌数は無施肥田で，放線菌数は施肥田でそれぞれ多かった．また，全細菌数は概して，無施肥田で多かったが，水稲の生育後期には両供試田ともにその有意差が認められなくなった．したがって，作土表層のB/F値（全細菌数/糸状菌数）は無施肥田の方がやや大であった．

嫌気性菌数は水稲の全生育期間を通じ，明らかに無施肥田の方が施肥田より下回った．この結果は，無施肥田が掛け流し式のかんがいシステムであることから，全層における土壌の酸化状態をよく反映していた．

3)　表層土壌における糸状菌フロラとその出現頻度は，無施肥田では *Paecilomyces* 属が，施肥田では *Paecilomyces*，または *Trichoderma* の各属がそれぞれ最も高く推移し，本供試田の優占的糸状菌であることが確認された．また，無施肥田と施肥田との糸状菌フロラの相対比は，ほぼ２：１の割合で推移したことから，明らかに無施肥田の本フロラ構成は豊富であった．

4)　同様にして，放線菌フロラとその相対比は，無施肥田と施肥田とでほぼ等しく，顕著な差異は認められなかった．両供試田ともに，*Streptomyces* 属が明らかに出現頻度が最も高く，次いで *Nocardia* 属であった．