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TRANSFORMATION OF ^{15}N-ENRICHED FERTILIZER NITROGEN DURING RICE STRAW DECOMPOSITION IN SUBMERGED SOIL*

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Abstract

The effect of rice straw on the immobilization and mineralization of fertilizer nitrogen (N) in submerged soil incubated under different temperature regimes and sequences was assessed using the ^{15}N-isotope tracer technique. In the absence of straw, most of the fertilizer N remained in the mineral forms. Straw enhanced N immobilization only moderately. It was observed that under the incubation conditions of this study, the environment within the incubation containers quickly became anaerobic and microbial activities were slowed down. The gradual decrease in the proportion of fertilizer N in the mineral forms was accompanied with a steady increase of fertilizer N in the amino acid fraction of organic N. Little accumulation of fertilizer N in the amino sugar or the insoluble humin fractions was found. No distinct trend could be discerned in relation to temperature variations, although the temperature range of 25-30°C tended to favor N transformations activities.

Introduction

Although farmers in Taiwan commonly apply nitrogen (N) fertilizers to paddy soils for rice production, much still needs to be learned about how N fertilization would increase the rice productivity. Among the causes of low rice yields are inadequate management of N fertilization (Hsieh and Liu, 1979) and possible influence of phytotoxicity produced during decomposition of rice

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straw residues in the soil (Chou *et al.*, 1976, 1977, 1979, 1981). Chou *et al.* (1977) have shown that the higher the amount of rice stubble left in the paddy soil, the higher would be the amount of phytotoxic phenolic compounds produced, and the less the amount of leachable N available. Oyama (1975) has also pointed out that after three years of continuous addition of straw, rice plants could take up more N from soil and increase rice yield. However, rice plants grown without the addition of N fertilizers generally yielded less than 75% of those grown in the presence of a complete NPK fertilizer (Dei, 1975). Harada (1974) and Houng (1976) have both emphasized the role of organic matter in N immobilization following various pretreatment of soil with residues. Since crop yield is closely related to the amount of N available in soil during the growing season and N availability is in turn governed by the rates of N immobilization and mineralization, an understanding of the factors affecting the N transformation processes in soil is essential.

Nitrogen transformation processes in soil are affected by soil properties and environmental parameters such as soil organic matter content, texture, pH, cation exchange capacity, nutrient status, microbial biomass, as well as available moisture and temperature (Russell, 1973; Harada, 1975; Houng, 1976; Ponnamperuma, 1976; Hsieh and Liu, 1979). The processes of N immobilization in paddy soils are particularly complex. The complexity is further accentuated under the intensive culture practices for rice production, including growing two crops of rice from the same field during one growing season with little time between the two crops for straw residues in the field to decompose and dissipate. The objective of this study was to elucidate the effect of rice straw residues on N transformations in submerged soil under different temperature regimes and sequences. The ^{15}N-isotope tracer technique was used to follow the conversion of fertilizer N into various soil nitrogen fractions.

Materials and Methods

Materials

For the incubation experiment, soil and rice straw samples were taken from the experimental farm of Academia Sinica, Nankang, Taiwan in July 1978. The soil, which contained 2.5% C and 0.11% N, was air-dried and screened to pass through a 1 mm sieve to remove visible plant residues. Straw (35.7% C, 0.42% N, C/N=85) was air-dried and cut into pieces about 3 mm long. Reagent-grade $(NH_4)_2SO_4$ (ammonium sulfate) was used as fertilizer and was enriched to contain 30% $(^{15}NH_4)_2SO_4$, which was purchased from Wako Chemical Ltd., Japan.

Experimental setup

Six combinations of soil, straw, and fertilizer treatments were compared in the incubation experiment. For each treatment, 40 g of air-dried soil was placed in a 250-ml, wide mouth plastic centrifuge tube with cap and mixed thoroughly with or without 0.4 g of rice straw and with or without ^{15}N-enriched $(NH_4)_2SO_4$ fertilizer according to the following designations: (1) soil alone (C/N=23); (2) S+S: soil+straw (C/N=25); (3) AF: soil+0.054 g $(NH_4)_2SO_4$ (C/N=18); (4) AFS: AF+straw (C/N=20); (5) BF: soil+0.145 g $(NH_4)_2SO_4$ (C/N=13); and (6) BFS: BF+straw (C/N=15). The soil or soil mixture in each tube was submerged in 40 ml of distilled water before incubation began. All treatments were replicated 4 times, and sets of tubes containing the treated soil were placed in environment-controlled chambers maintained at initial temperatures of 15, 20, 25, or 30°C (±1°C) in a phytotron at the National Taiwan University, Taipei. At three-week intervals, four tubes from each set were removed for analysis and the remaining tubes were shifted to the next temperature chamber according to the following sequences: I, 15°C→20°C→25°C→30°C; II, 20°C→25°C→30°C→25°C; III, 25°C→30°C→25°C→20°C; and IV, 30°C→25°C→20°C→15°C.

Fractionation of nitrogen in treated soil

The soil or soil mixture in the incubation tubes was fractionated according to the scheme outlined in Fig. 1. The contents of the tubes were first centrifuged to separate the supernatant from the soil residue, which was then

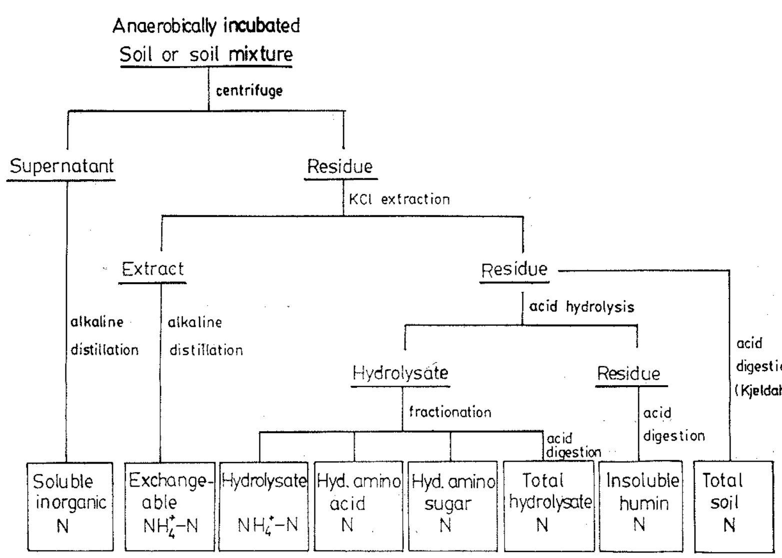

Fig. 1. Soil nitrogen fractionation scheme.

extracted with 2 N KCl before being hydrolyzed with 6 N HCl for 16 hours. The hydrolysate was further fractionated using the methods described by Cheng and Kurtz (1963). The inorganic N in the supernatant was determined by the alkaline distillation method in the presence of Devarda's alloy (Bremner, 1965b); the exchangeable NH_4^+-N in the 2 N KCl extract was determined by the steam distillation method; and the total soil N, total hydrolysate N, and insoluble humin N were determined by the Kjeldahl method (Bremner, 1965a).

Determination of ^{15}N in soil nitrogen fractions

Following conversion of nitrogen in various soil fractions to NH_4^+ for quantitative determination, aliquots of the resultant solutions were dried in conversion vials. The ^{15}N contents in the vials were determined at the Soil Biochemistry Laboratory, Washington State University, Pullman, Washington, U. S. A., using a Consolidated Electrodynamics Corporation model 21–620 mass spectrometer equipped with a Keithley 410A picoammeter for the isotope ratio measurements. A Rockwell R-6500 advanced interactive microcomputer (AIM 65) was used for peak integration and data reduction. The evacuated system for conversion of sample NH_4^+ to N_2 gas was adopted from Porter and O'Deen (1977), and the alkaline LiOBr oxidant was prepared by the procedure of Ross and Martin (1970). Errors in analysis were either minimized or corrected according to Bremner *et al.* (1966).

Results and Discussion

Soluble inorganic N

A considerable amount of inorganic N was found in the supernatant solution of soil, which had been treated with fertilizer N in the presence or absence of rice straw, throughout the incubation period (Table 1). The presence of straw, however, decreased the soluble inorganic N contents with time. Practically all inorganic N found was in the form of NH_3^+, as little NO_3^- was present under the anaerobic incubation conditions. The AFS/AF and BFS/BF ratios are useful indicators of the relative changes in N status in soil with time as affected by the presence of straw. For soluble inorganic N, these ratios decreased to below 1, indicating net N immobilization in the presence of straw. The immobilization process was most evident in the temperature sequences II and III, when the temperature fluctuated between 20 and 30°C. The contribution of fertilizer N to the soluble inorganic pool is readily seen from the ^{15}N data (Fig. 2). The presence of fertilizer N in soil solution decreased with time from approximately 10% of initially added ^{15}N to less than 3% in the presence of straw at the end of the incubation period.

The higher the C/N ratio in soil (e.g., AFS vs. BFS), the less fertilizer N remained in the soil solution.

Exchangeable ammonium N

Addition of fertilizer N greatly increased the total exchangeable NH_4^+ level in soil which persisted throughout the incubation period at a level 3 fold higher than the inorganic N in soil solution (Table 2). Relative changes in AFS/AF and BFS/BF ratios are not as evident as in soluble inorganic N, partly due to higher NH_4^+ concentrations. The ^{15}N data show that most of

Table 1. *Soluble inorganic N contents of supernatants of soils variously treated with rice straw and nitrogen fertilizer*(1) *and anaerobically incubated under different temperature regimes and sequences* Temperature changes and sampling were conducted at three-week interval. Data are averages of three replicates.

Temperature sequence	Soluble inorganic N (μg/ml supernatant)						
	S+S	AF	AFS	AFS/AF ratio	BF	BFS	BFS/BF ratio
I 15°C ↓	3	71	71	1.00	232	214	.92
20°C ↓	5	71	68	.96	247	242	.98
25°C ↓	3	72	53	.74	262	217	.83
30°C	1	69	37	.53	237	178	.75
II 20°C ↓	3	65	47	.72	220	194	.88
25°C ↓	3	75	62	.83	251	218	.87
30°C ↓	5	71	43	.61	260	185	.71
25°C	1	59	22	.37	253	140	.55
III 25°C ↓	4	73	71	.96	238	235	.99
30°C ↓	3	75	46	.61	236	209	.89
25°C ↓	2	66	34	.51	258	170	.66
20°C	1	75	32	.43	248	184	.74
IV 30°C ↓	2	77	64	.83	241	223	.92
25°C ↓	3	73	51	.70	260	200	.77
20°C ↓	2	69	49	.71	237	208	.88
15°C	1	62	51	.82	228	203	.89

(1) Soil=40 g soil+40 ml H_2O.
S+S=40 g soil+0.4 g rice straw+40 ml H_2O.
AF=40 g soil+0.054 g $(^{15}NH_4)_2SO_4$+40 ml H_2O; AFS=AF+0.4 g rice straw
BF=40 g soil+0.145 g $(^{15}NH_4)_2SO_4$+40 ml H_2O; BFS=BF+0.4 g rice straw
The $(^{15}NH_4)_2SO_4$ was enriched with 30% ^{15}N.

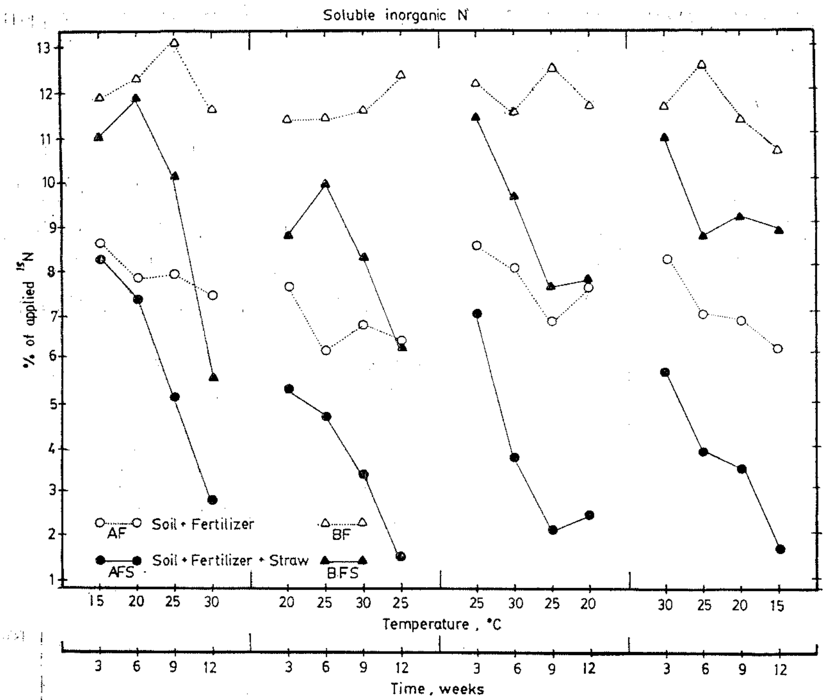

Fig. 2. Changes in % of applied ^{15}N in the soluble inorganic N fraction of soil incubated in the presence or absence of rice straw under different temperature regimes and sequences.

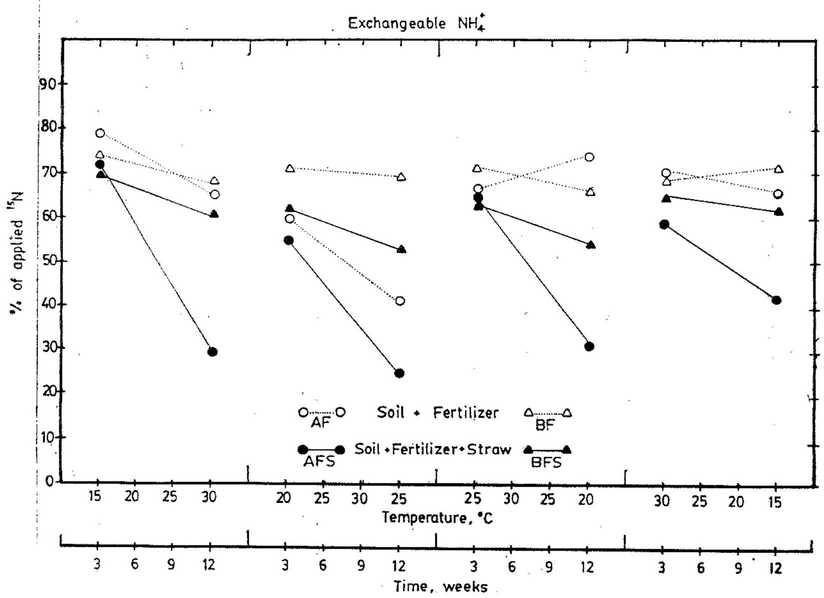

Fig. 3. Changes in % of applied ^{15}N in the exchangeable NH_4^+ fraction of soil incubated in the presence or absence of rice straw under different temperature regimes and sequences.

Table 2. *Exchangeable ammonium N contents of soils variously treated with rice straw and nitrogen fertilizer*[1] *and anaerobically incubated under different temperature regimes and sequences* Temperature changes and sampling were conducted at three-week interval. Data are averages of three replicates.

Temperature sequence		Exchangeable NH_4^+-N ($\mu g/g$ soil)						
		S+S	AF	AFS	AFS/AF ratio	BF	BFS	BFS/BF ratio
I	15°C	26	307	289	.94	724	696	.96
	↓ 20°C	46	305	283	.93	731	673	.92
	↓ 25°C	30	301	248	.82	677	657	.97
	↓ 30°C	47	328	276	.84	725	694	.96
II	20°C	38	308	272	.88	711	674	.95
	↓ 25°C	47	312	289	.93	708	679	.96
	↓ 30°C	36	313	276	.88	711	667	.94
	↓ 25°C	55	278	211	.76	721	636	.88
III	25°C	45	316	288	.91	705	687	.97
	↓ 30°C	43	312	281	.90	719	677	.94
	↓ 25°C	69	325	299	.92	707	659	.93
	↓ 20°C	48	345	253	.73	710	668	.94
IV	30°C	43	317	296	.93	696	693	.99
	↓ 25°C	40	320	286	.89	759	666	.88
	↓ 20°C	43	313	285	.91	717	692	.96
	↓ 15°C	40	345	253	.73	710	668	.94

(1) See Table 1.

the fertilizer NH_4^+ added to soil was initially in the exchangeable from (Fig. 3). In the absence of straw, the level of fertilizer N in the exchangeable form decreased little, whereas in the presence of straw, the fertilizer N in exchange form decreased from around 80% to near the 40% level. The N immobilization process appeared to be enhanced by ascending rather than descending temperature sequence during incubation.

Hydrolysate ammonium N

The acid hydrolysis process chemically breaks down complex N forms in soil organic matter into simpler amino compounds and eventually to NH_4^+. The NH_4^+ in acid hydrolysate is derived from decomposition of amino acids and amino sugars as well as release of clay-fixed NH_4^+ (Cheng and Kurtz, 1963). The fertilizer treatments and incubation temperature sequences had little effect on the hydrolysate NH_4^+ contents in treated soils over the incubation period (Table 3). Both the AFS/AF and BFS/BF ratios increased slightly with time. The ^{15}N data revealed that the proportion of fertilizer N in hydrolysate NH_4^+ was higher (~20%) in the absence of straw at early stages of incubation but decreased with time, whereas the ^{15}N contents of hydrolysate NH_4^+ in the presence of straw were between 6 to 9% of added fertilizer (Fig. 4). This would indicate that in the presence of straw, fertilizer N was rapidly immobilized into more complex forms of organic N and was less susceptible to decomposition during acid hydrolysis; in the absence of straw, the immobilization process was slower and the organic N formed was more susceptible to decomposition.

Table 3. *Ammonium N contents of acid hydrolysates of soils variously treated with rice straw and nitrogen fertilizer*[1] *and anaerobically incubated under different temperature regimes and sequences* Temperature changes and sampling were conducted at three-week interval. Data are averages of three replicates.

Temperature sequence		Hydrolysate NH_4^+-N ($\mu g/g$ soil)						
		S+S	AF	AFS	AFS/AF ratio	BF	BFS	BFS/BF ratio
I	15°C ↓	279	292	276	.95	289	274	.95
	20°C ↓	294	324	333	1.03	314	337	1.07
	25°C ↓	322	354	376	1.06	371	402	1.08
	30°C	327	317	336	1.06	359	396	1.10
II	20°C ↓	284	305	327	1.07	290	342	1.21
	25°C ↓	313	321	336	1.05	333	327(?)	.98
	30°C ↓	312	318	348	1.09	310	392	1.26
	25°C	332	355	378	1.06	345	394	1.14
III	25°C ↓	295	320	273	.85	332	321	.97
	30°C ↓	320	319	334	1.05	323	369	1.14
	25°C ↓	313	348	365	1.05	370	383	1.04
	20°C	313	312	312	1.19	361	406	1.12
IV	30°C ↓	315	316	308	.97	330	310	.94
	25°C ↓	311	326	345	1.06	316	365	1.16
	20°C ↓	318	324	365	1.13	368	374	1.02
	15°C	337	329	335	1.02	343	308	.90

(1) See Table 1.

Table 4. *Amino sugar N contents of acid hydrolysates of soils variously treated with rice straw and nitrogen fertilizer*[1] *and anaerobically incubated under different temperature regimes and sequences* Temperature changes and sampling were conducted at three-week interval. Date are averages of three replicates.

Temperature sequence		Hydrolyzed Amino Sugar N (μg/g soil)						
		S+S	AF	AFS	AFS/AF ratio	BF	BFS	BFS/BF ratio
I	15°C	44	39	34	0.87	57	50	0.88
	↓ 20°C	45	43	50	1.16	45	45	1.00
	↓ 25°C	43	42	45	1.08	50	46	0.92
	↓ 30°C	59	50	59	1.18	61	50	0.82
II	20°C	42	46	44	0.96	33	27	0.82
	↓ 25°C	38	40	37	0.93	47	49	1.04
	↓ 30°C	42	54	41	0.76	58	50	0.86
	↓ 25°C	44	58	47	0.81	47	46	0.98
III	25°C	42	32	55	1.72	53	49	0.92
	↓ 30°C	43	51	62	1.22	32	35	1.09
	↓ 25°C	53	42	42	1.00	42	45	1.07
	↓ 20°C	49	59	45	0.76	36	54	1.50
IV	30°C	38	38	62	1.63	36	38	1.06
	↓ 25°C	47	40	38	0.95	41	45	1.10
	↓ 20°C	53	54	48	0.89	34	58	1.71
	↓ 15°C	53	40	44	1.10	32	90	2.81

(1) See Table 1.

Hydrolyzed amino sugar and insoluble humin N

The levels of amino sugar N in the acid hydrolysate of soil under all treatments were relatively low (Table 4). Addition of fertilizer N did not increase the total amino sugar N levels in soil appreciably. Since the amino sugar fraction in soil is associated with the extent of microbial population and microbial activities, low amino sugar N levels indicated comparatively low microbial activities under the anaerobic conditions of incubation. Less than 5% of fertilizer N was converted into amino sugar N (Fig. 5). Similarly only a small fraction of fertilizer N could be found in the humin fraction (Fig. 5), indicating that humification of fertilizer N was low, an observation which agreed with the findings that N immobilization under anaerobic

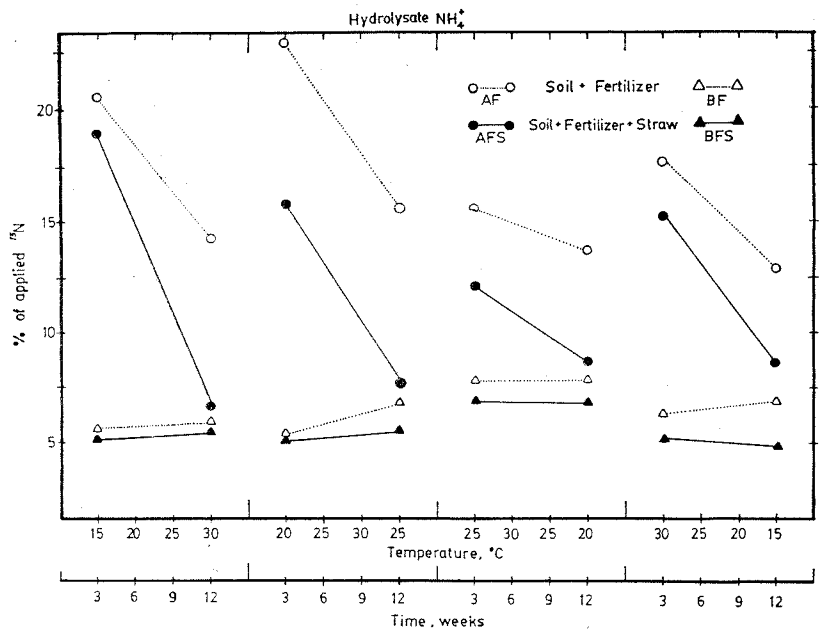

Fig. 4. Changes in % of applied ^{15}N in the hydrolysate NH_4^+ fraction of soil incubated in the presence or absence of rice straw under different temperature regimes and sequences.

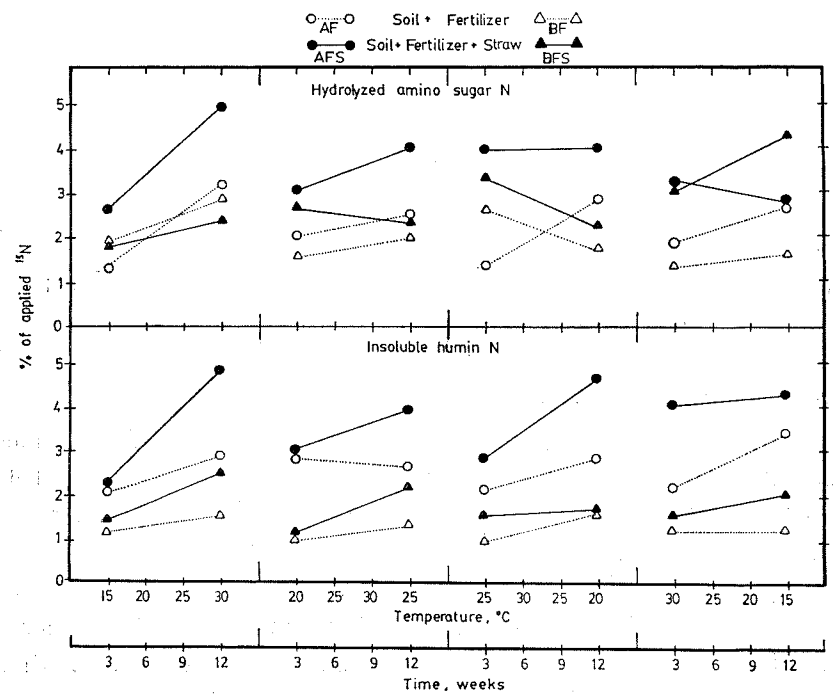

Fig. 5. Changes in % of applied ^{15}N in the hydrolyzed amino sugar and insoluble humin fractions of soil incubated in the presence or absence of rice straw under different temperature regimes and sequences.

conditions was slow and that most of the fertilizer N remained in the exchangeable NH_4^+ form.

Hydrolyzed amino acid N

The amino acids present in the acid hydrolysate of soil represent a significant portion of organic N (Bremner, 1965b). Slightly higher amounts of hydrolysate amino acid N were found in soil treated with a higher application of N fertilizer (B vs. A) (Table 5). Both AFS/AF and BFS/BF ratios were greater than 1, indicating an increase in amino acid N in soil in the presence of straw. The amount of fertilizer N converted to the amino acid forms increased continually with time of incubation, with a greater N conversion in the presence than in the absence of straw (Fig. 6). As much as

Table 5. *Amino acid N contents of acid hydrolysates of soils variously treated with rice straw and nitrogen fertilizer*[1] *and anaerobically incubated under different temperature regimes and sequences* Temperature changes and sampling were conducted at three-week interval. Data are averages of three replicates.

Temperature sequence		Hydrolyzed Amino Acid N (μg/g soil)						
		S+S	AF	AFS	AFS/AF ratio	BF	BFS	BFS/BF ratio
I	15°C	337	334	361	1.08	460	392	0.85
	↓ 20°C	304	312	340	1.09	379	334	0.88
	↓ 25°C	410	375	527	1.41	456	502	1.10
	↓ 30°C	465	422	434	1.03	430	524	1.22
II	20°C	328	388	345	0.89	341	457	1.34
	↓ 25°C	395	297	399	1.34	340	442	1.30
	↓ 30°C	369	385	446	1.16	434	407	0.94
	↓ 25°C	427	394	446	1.13	451	555	1.23
III	25°C	322	349	401	1.15	394	397	1.01
	↓ 30°C	347	308	384	1.25	356	425	1.19
	↓ 25°C	419	428	512	1.20	468	535	1.14
	↓ 20°C	397	411	489	1.19	531	611	1.15
IV	30°C	338	396	308	0.78	442	498	1.13
	↓ 25°C	347	310	364	1.17	321	383	1.19
	↓ 20°C	431	407	513	1.26	461	653	1.42
	↓ 15°C	437	392	441	1.13	519	590	1.14

(1) See Table 1.

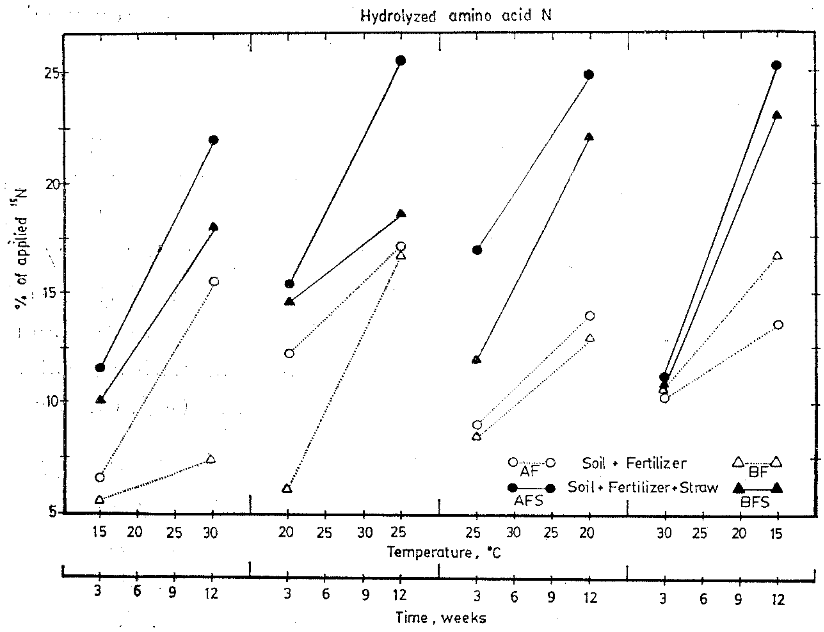

Fig. 6. Changes in % of applied ^{15}N in the hydrolyzed amino acid fraction of soil incubated in the presence or absence of rice straw under different temperature regimes and sequences.

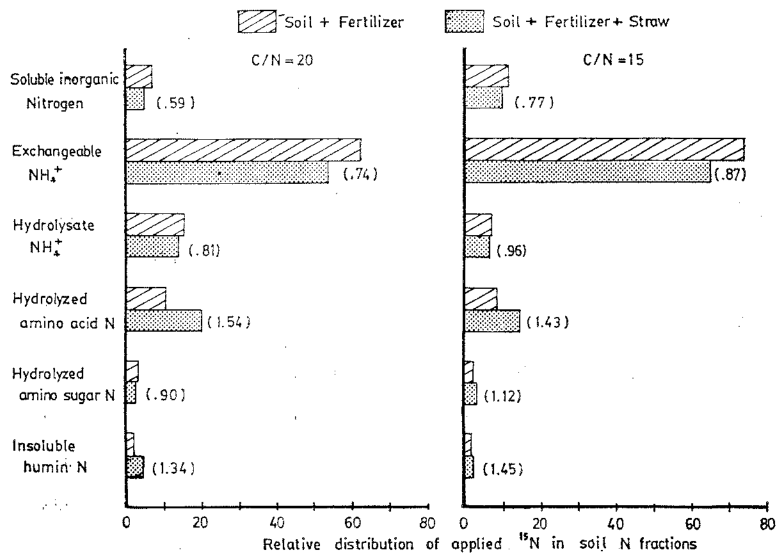

Fig. 7. Relative distribution of applied ^{15}N in soil nitrogen fractions. The number in parentheses indicates the ratios of AFS/AF (left figure) or BFS/BF (right figure).

30% of fertilizer N was converted in some AFS treatments by the end of the 12-week incubation period.

The relative distribution of fertilizer N as reflected by the ^{15}N contents of various soil fractions at the end of the incubation period is summarized in Fig. 7. The exchangeable NH_4^+ fraction contained the highest amount of fertilizer N, whereas the amino acid N fraction contained the highest amount of fertilizer N among the organic N fractions. Presence of straw in the soil tended to decrease the fertilizer N in the mineral fractions and increase it in the organic fractions. The effect of temperature regimes and sequences on N transformation was not obvious, although higher temperatures tended to favor higher microbial activities. The fact that the predominant products of fertilizer N immobilization were recovered in the amino acid fraction, with smaller amounts recovered in the amino sugar fraction, and little in the humin fraction indicates that under the incubation conditons of this experiment the N immobilization process was mainly microbial incorporation of available N. In comparison with N transformation processes under aerobic conditions, the rate of N immobilization reported in this study was relatively slow. It was observed that under the controlled incubation conditions in closed containers, the environment within the containers quickly became anaerobic and microbial activities were slowed down. In contrast with paddy soils under water, the overlying water in the open field would be better aerated and the submerged soil would not be kept under strictly anaerobic conditions. Thus the data reported here, although indicative of the transformation processes involved, must be assessed with caution and cannot be extrapolated directly to the field conditions. In a companion study (Chou *et al.*, 1981), it was found that during incubation in the presence of rice straw, phytotoxic substances released from straw decomposition reached the highest levels after 6 weeks and gradually disappeared after 12 weeks. Little relationship between phytotoxicity production and N transformations in soil could, however, be discerned.

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加強的氮-15肥料混合稻稈置土壤分解時氮之轉化

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將加强的氮-15肥料，切碎的稻稈，土壤及水分別的組合置於有蓋之離心塑膠瓶中混合均勻後，放在不同溫度變化的人工氣候室中，讓稻稈分解，測知 ^{15}N 在土壤中之變化以了解土壤中氮肥之固定化 (immobilization) 及礦物化 (mineralization) 速率。不加入稻稈的處理組中可看出大量的氮肥保持礦物化型態，而加入稻稈者僅適度地提高氮之固定化。在此分解過程中土壤迅速地變成無氧狀態，而礦物化型態的氮量因時間逐漸降低，但有機型態的氮如氨基酸量因施入氮肥之增加而增加，此期間僅少量的 ^{15}N 累積於胺基糖及腐植質部份。雖然此研究結果顯示在溫度達 25°C～30°C 時氮之轉化作用較爲顯著，但其轉化速率與溫度之變化並無明顯的趨勢。