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Dynamics of ^{15}N-labeled ammonium sulfate in various inorganic and organic soil fractions of wetland rice soils

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Summary. The dynamics of basally applied ^{15}N-labeled ammonium sulfate in inorganic and organic soil fractions of five wetland rice soils of the Philippines was studied in a greenhouse experiment. Soil and plant samples were collected and analyzed for ^{15}N at various growth stages. Exchangeable NH_4^+ depletion continued after 40 days after transplanting (DAT) and corresponded with increased nitrogen uptake by rice plants. Part of the applied fertilizer was fixed by 2:1 clay minerals, especially in Maligaya silty clay loam, which contained beidellite as the dominant clay mineral. After the initial fixation, nonexchangeable ^{15}N was released from 20 DAT in Maligaya silty clay loam, but fixation delayed fertilizer N uptake from the soil. Part of the applied N was immobilized into the organic fraction. In Guadalupe clay and Maligaya silty clay loam, immobilization increased with time while the three other soils showed significant release of fertilizer N from the organic fraction during crop growth. Most of the immobilized fertilizer N was recovered in the nondistillable acid soluble (alpha-amino acid + hydrolyzable unknown-N) fraction at crop maturity. Between 61% and 66% of applied N was recovered from the plant in four soils while 52% of fertilizer N was recovered from the plant in Maligaya silty loam. Only 20%–30% of the total N uptake at maturity was derived from fertilizer N. Nmin (mineral N) content of the soil before transplanting significantly correlated with N uptake. Twenty-two to 34% of applied N was unaccounted for possibly due to denitrification and ammonia volatilization.

Key words: ^{15}N balance – Ammonium fixation – Fertilizer N transformation – Soil organic nitrogen – Plant N uptake

Since plant uptake of applied nitrogen is a prime concern in crop production, it is important to determine the magnitude of reactions which utilize added N in other ways, such as ammonium fixation, NH_3 volatilization, denitrification, and biological immobilization. Although immobilization does not present N loss from soil, it competes with plant uptake. It is of great interest then to determine the rate at which it occurs. Almost no information is available indicating how long fertilizer N, once immobilized, remains unavailable to the rice crop.

Nitrogen transformations in wetland rice soils were reviewed recently (Savant and de Datta 1982). However, mainly the single N transformation process was discussed sequentially. Most studies conducted on N fertilizer efficiency were restricted to balance sheets at 30 days after transplanting (DAT) and at crop maturity.

In this study, N fertilizer dynamics in various soil fractions during the entire rice growth period is emphasized.

Materials and methods

A pot experiment was conducted in the greenhouse with five representative wetland rice soils from the Philippines, namely, Pili loam (Typic Pelludert), Guadalupe clay (Typic Pellustert), Maahas clay (Andaqueptic Haplaquoll), Sta. Rita clay (Typic Pelludert), and Maligaya silty clay loam (Vertic Tropaquept). Table 1 shows the characteristics of these soils.

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Table 1. Chemical and physical properties of wetland soils from five experimental sites in the Philippines

Soil property	Pili loam	Guadalupe clay	Sta. Rita clay	Maahas clay	Maligaya silty clay loam
pH (1:1, H_2O)	5.9	7.0	6.7	7.1	6.2
pH (0.01M $CaCl_2$)	4.5	6.5	6.0	6.5	5.4
Organic C (%)	1.6	3.1	2.4	1.5	1.4
Total N (%)	0.16	0.27	0.20	0.16	0.11
CEC (mEq × 100 g^{-1})	34	44	53	47	30
Exchangeable bases (mEq × 100 g^{-1})					
K	0.31	1.42	0.62	1.52	0.15
Na	0.30	1.00	0.60	1.45	0.46
Mg	6	10	19	16	9
Ca	18	30	31	28	19
Nonexchangeable NH_4^+ (mg kg^{-1})	78	82	185	72	55
P (Olsen) mg (kg^{-1})	5	60	8	11	4
Clay (%)	56.9	59.2	71.4	66.8	45.2
Silt (%)	31.8	36.4	25.9	27.7	50.0
Sand (%)	11.3	4.4	2.7	5.6	4.8
Clay mineralogy	Montmorillonite	Montmorillonite	Montmorillonite Kaolinite	Montmorillonite	Beidellite Vermiculite
Soil series	Pili	Guadalupe	Sta. Rita	Maahas	Maligaya
Soil orders	Vertisol	Vertisol	Vertisol	Mollisol	Inceptisol

Equivalent amounts of 6 kg dry soil (oven-dry basis) were incubated in plastic pots with a total of 18 pots per soil sample. Soils were preflooded for 6 weeks before conducting the experiment.

Nitrogen fertilizer as ^{15}N-labeled (5% atom excess) ammonium sulfate solution was added to each pot at 100 mg N kg^{-1} soil, P fertilizer as $Ca(H_2PO_4)_2$ at 20–25 mg available P kg^{-1}, and K as KCl at 150 mg available K kg^{-1}. Fertilizers were thoroughly mixed with wet soil. For each soil sample, each of the 15 pots was planted with three IR36 rice seedlings dipped in 2% zinc oxide suspension.

After transplanting, the pots were flooded with deionized water to a depth of 7.5 cm, then covered with black cotton cloth, leaving a center hole for the plants, to prevent algae growth on the floodwater surface. Soils of the remaining three pots were also incubated with the same fertilizer rate and served as a zero-time check for ^{15}N recovery.

Soil and plant (shoot) samples were taken at 10, 20, 40, and 60 DAT and at crop maturity. Plant samples were analyzed for total N and ^{15}N content. Soil samples were analyzed for exchangeable NH_4^+, nonexchangeable NH_4^+, hydrolyzable organic N (and subfractions), and nonhydrolyzable organic N. Floodwater samples were taken at 2, 7, 10, and 20 DAT then analyzed for total NH_4^+ and ^{15}N.

Analytical procedure. All soil samples were analyzed for inorganic and organic N fractions according to a scheme assessing all fractions from a soil sample. KCl extraction (Bremner 1965) was done on fresh soil samples immediately after sampling. Nonexchangeable NH_4^+-N was determined according to the method of Silva and Bremner (1966). Fractionation and determination of organic N fractions were carried out according to the method of Fleige et al. (1971a). Floodwater ammonium N was determined by steam distillation. Plant N was analyzed by the semi-micro-Kjeldahl method.

Nitrogen-isotope ratio analysis. To determine ^{15}N, the distillates after acid filtration were collected, acidified with 1 $N H_2SO_4$, and evaporated to dryness in glass vials. Plant and soil ^{15}N were determined by mass spectrometry using a VG micromass M 622 after converting (NH_4^+) to molecular N_2 with lithium.

Results

Exchangeable ammonium

The exchangeable $^{15}NH_4^+$ concentration in the soil decreased rapidly during the first 40 days. It became less than 10% of the initial value at transplanting and decreased only slightly until maturity. Unlike in other soils, unlabeled exchangeable NH_4^+ in Pili loam and Guadalupe clay pronouncedly increased between 10 and 20 DAT (Fig. 1).

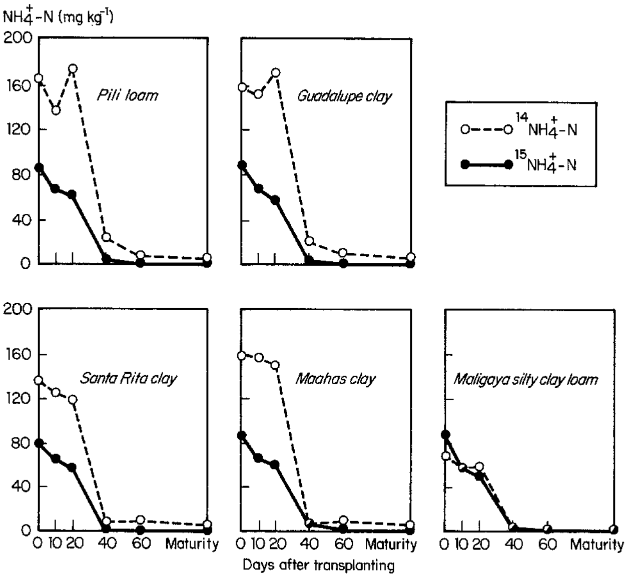

Fig. 1. Labeled (^{15}N) and nonlabeled (^{14}N) exchangeable NH_4^+ at various growth stages of IR36 in five Philippine soils

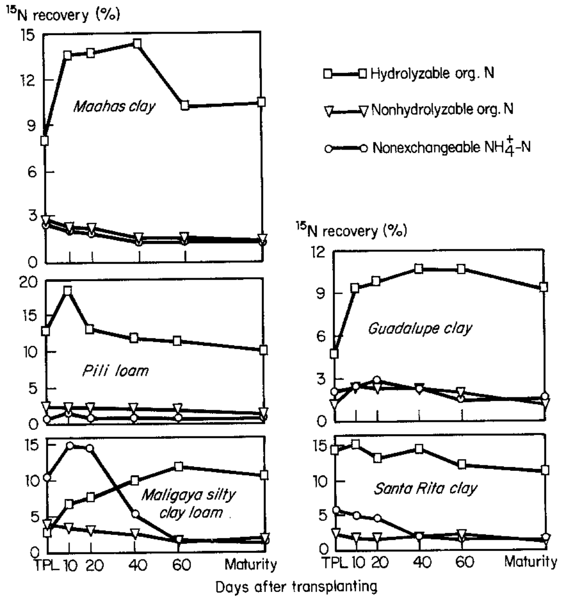

Fig. 2. ^{15}N fertilizer recovery from three soil N fractions at various growth stages of IR36 in five Philippine soils

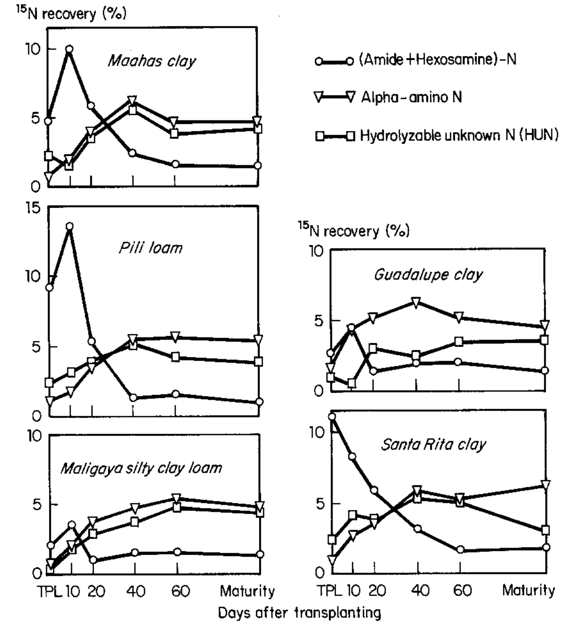

Fig. 3. ^{15}N fertilizer recovery from three organic soil N fractions at various growth stages of IR36 in five Philippine soils

Nonexchangeable ammonium

Immediately after applying the fertilizer, ^{15}N was fixed in the nonexchangeable fraction only up to 6% in all soils, except in Maligaya soil, which fixed up to 15% during the first 10 DAT (Fig. 2). Fixed $^{15}NH_4^+$ was steadily released during the growth period (Fig. 2). This was particularly evident in Maligaya soil at 20 – 60 DAT. In Sta. Rita clay and Maligaya silty clay loam, the nonexchangeable fraction released a considerable amount of untagged ammonium, i.e., 35 mg N kg^{-1} soil in Maligaya and 20 mg N kg^{-1} soil in Sta. Rita clay.

Organic soil nitrogen

The immobilization and remineralization patterns of fertilizer N of the hydrolyzable organic fraction were not similar (Fig. 2). In Maligaya silty clay loam and Guadalupe clay, ammonium immobilization into organic fraction increased with time; whereas in Pili loam and Maahas clay, initial immobilization was followed by a significant fertilizer N release after 20 and 40 DAT, respectively. Remineralized fertilizer in Maahas clay was 23%, and in Pili loam, 46% of the initially immobilized nitrogen. In all soils, about 10% of applied fertilizer remained in the hydrolyzable organic fraction at maturity.

Hexosamine nitrogen was not separated from amide N because its portion of hydrolyzate is comparatively small and does not show considerable variations (Fleige and Capelle 1975).

In Pili loam, up to 14% ^{15}N was found in the amide + hexosamine N fraction shortly after applying fertilizer. Its rapid release followed. Only 0.40% – 2.0% ^{15}N remained in this fraction at maturity (Fig. 3).

In the alpha-amino N fraction, ^{15}N recovery was up to 6.3% and it increased until 40 or 60 DAT. This fraction accounted for up to 55% of the total hydrolyzable organic N at maturity. Only in Maahas clay and Guadalupe clay were small amounts of ^{15}N released (Fig. 3).

Recovery of ^{15}N in the hydrolyzable unknown N (HUN) fraction was generally less but followed a similar pattern as that in the alpha-amino fraction (Fig. 3). It accounted for 27% – 42% of total hydrolyzable organic N at maturity.

Fertilizer N recovered in the nonhydrolyzable organic fraction varied between 2.3% and 4.0%, although a significant ^{15}N release after initial fixation was observed in all soils (Fig. 2).

Floodwater nitrogen

^{15}Nitrogen in floodwater was highest in Maligaya soil with 7% of applied fertilizer at 2 DAT. It decreased to undetectable concentrations in all soils, 20 DAT. Table 2 shows the highest N losses (largest decrease in recovery percentage) during the first 10 DAT, where concentration of fertilizer N in the soil solution was high and the plant-root system has not yet developed a large sink strength for N.

Table 2. Percentage ^{15}N recovery from plant and soil by IR36 at various growth stages in five wetland soils

Soil	Sampling date	Total soil N	Total plant N	Total soil + plant N
Pili loam	Transplanting	103 a	–	103 a
	10 DAT	89 b	0.4 d	89 ab
	20 DAT	79 c	8.5 c	88 b
	40 DAT	19 d	58.8 b	78 c
	60 DAT	14 e	61.4 ba	75 c
	Maturity	12 e	64.5 a	77 c
Guadalupe clay	Transplanting	97 a	–	97 a
	10 DAT	82 b	0.4 b	82 b
	20 DAT	73 c	6.3 b	79 b
	40 DAT	19 d	59.8 a	79 b
	60 DAT	15 de	62.2 a	77 b
	Maturity	12 e	65.8 a	78 b
Maahas clay	Transplanting	99 a	–	99 a
	10 DAT	84 b	0.5 c	85 b
	20 DAT	78 c	8.7 b	87 b
	40 DAT	24 d	57.6 a	82 b
	60 DAT	13 e	61.8 a	75 b
	Maturity	13 e	63.8 a	77 b
Sta. Rita clay	Transplating	103 a	–	103 a
	10 DAT	88 b	0.6 c	89 b
	20 DAT	78 b	8.3 b	86 b
	40 DAT	20 c	58.0 a	78 b
	60 DAT	16 c	60.2 a	76 b
	Maturity	14 c	60.7 a	75 b
Maligaya silty clay loam	Transplanting	102 a	–	102 a
	10 DAT	83 b	0.4 b	83 b
	20 DAT	74 c	6.9 b	81 b
	40 DAT	21 d	44.3 a	65 c
	60 DAT	15 de	49.4 a	64 c
	Maturity	14 e	52.7	67 c

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Table 3. Dry matter yield (g pot^{-1}) of IR36 at various growth stages in five different soils. IRRI, 1983

Soil	Sampling date				
	10 DAT	20 DAT	40 DAT	60 DAT	Harvest
Pili loam	0.15	3.0	37	124	175
Guadalupe clay	0.17	3.2	39	139	217
Sta. Rita clay	0.19	2.7	35	107	163
Maahas clay	0.19	3.9	42	149	202
Maligaya silty clay loam	0.13	1.7	31	81	123

Plant nitrogen

Dry matter yields of rice plants are presented in Table 3. Total N uptake of plants was positively correlated with Nmin (exchangeable NH_4^+) content of soils before transplanting ($R^2 = 0.87$).

Figure 4 shows the N uptake patterns of the rice plant. Highest uptake rate occurred between 20 and 40 DAT, after which fertilizer N uptake was no longer significant. Eighty to ninety percent of fertilizer N in the plant was taken up until 40 DAT. Only 20% – 30% of fertilizer contributed to total N uptake of mature plants.

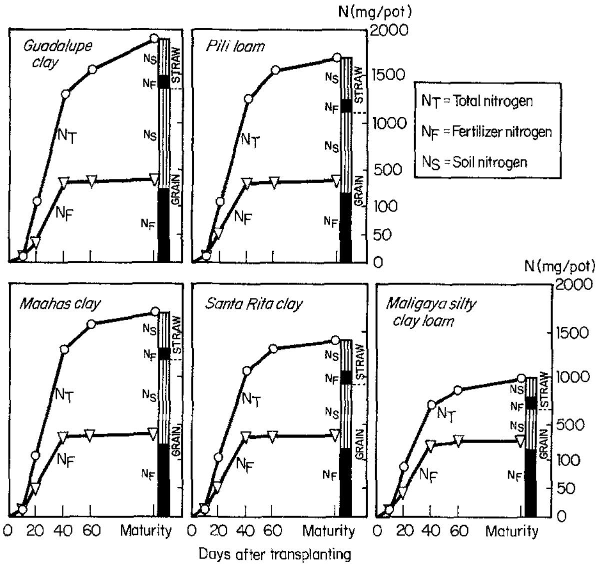

Fig. 4. Fertilizer and total N uptake at various growth stages of IR36 in five Philippine soils

Discussion

Nonexchangeable ammonium

Fixation and release of nonexchangeable NH_4^+ were not related to either the total amount of nonexchangeable NH_4^+ or the cation exchange capacities of soils (Table 1). Sta. Rita clay soil had about threefold more nonexchangeable NH_4^+ than Maligaya silty clay loam but fixation and release of nonexchangeable NH_4^+ in this soil were much lower than in Maligaya (Table 1) in line with results obtained by Keerthisinghe et al. (1984). They presumed that the fixing capacity of Maligaya silty clay loam was related to the vermiculites present as its major clay mineral in the soil. On the other hand, montmorillonite was presumed to be the dominant clay of other soils. Reexamination of clay minerals by Ca + glycerol diagrams showed that the major clay mineral in all soils was the smectites (peak at 18 Å). The assumption about vermiculites being a major clay mineral (peak at 14 Å) was not confirmed. But since vermiculites in the clay fraction (0.1 µm) were also able to expand to 17.6 Å upon Ca + glycerol saturation, occurrence of vermiculites in Maligaya soil is still possible.

The contraction test, upon K saturation to 10 Å, proved that smectites with high layer charge were present only in Maligaya silty clay loam. According to Lagaly and Weiss (1971), smectites with a high layer charge are considered as beidellites.

Bajwa (1984) found higher fixation in wetland rice soils containing vermiculites or beidellites than in those containing montmorillonites.

Total nonexchangeable NH_4^+ released from Maligaya silty clay loam and Sta. Rita clay was higher than the labeled nonexchangeable NH_4^+, indicating that a substantial amount of unlabeled NH_4^+ was also released. The other soils showed no apparent release of unlabeled nonexchangeable NH_4^+ while labeled nonexchangeable NH_4^+ content significantly decreased. The fixation and release of nonexchangeable NH_4^+ was also influenced by the K status of the soil. Nommik (1957) observed that simultaneous or previous K^+ fixation reduces the ability of the soil to fix NH_4^+. Similarly, nonexchangeable NH_4^+ release is reduced if K^+ fertilizer is applied after NH_4^+ fertilizer (Atanasiu et al. 1968). Net release of nonexchangeable NH_4^+ occurs when plant uptake progressively reduces both NH_4^+ and K^+ in the soil solution.

NH^+ refixation may occur especially in soils with high mineralization rate (Mengel and Scherer 1981). Refixation likely took place in Pili, Guadalupe, and Maahas soils.

Organic soil nitrogen

Organic soil nitrogen supplied 70% – 80% of N taken up by the crop. Considerable amounts of added N were rapidly immobilized and incorporated into the hydrolyzable organic N fractions of the soil. However, immobilization and mineralization patterns differed markedly among soils.

Fertilizer N immobilized into the organic fraction in Maligaya silty clay loam and Guadalupe clay increased with time. In Pili loam and Maahas clays, significant amounts of initial immobilized N were remobilized. Increase in unlabeled exchangeable NH_4^+ in Pili loam between 10 and 20 DAT, despite plant uptake, indicates high mineralized native soil organic N. These results are similar to Schoen's (1982), which showed that exchangeable NH_4^+ remained at a constant level for a longer period after transplanting in Pili loam than in other soils. Its relationship to soil biomass is also discussed in this paper.

Fertilizer nitrogen was remobilized only at the early growth stages (Pili loam and Maahas clay). At the late growth stages, remineralization was negligible. Tyler and Broadbent (1958) noted that immobilized N seems progressively less available because the nitrogenous compounds are constantly converted into more biologically stable compounds. Kai and Kawaguchi (1977) observed that remineralization of immobilized fertilizer N after submergence proceeds rapidly and almost ceases at an early incubation stage. In contrast, soil organic N mineralization may continue at a steady rate for a long period.

Immobilization is regulated by the amount and type of carbonaceous materials in the soil (Broadbent and Nakashima 1965). Studies by Kai et al. (1973) showed that the highest ^{15}N net immobilization may be reached in 3 days in the presence of glucose, and in 2 months with mature crop residues. Though no organic substrate was added in this experiment, net immobilization was highest in Pili loam at 10 DAT and in Maligaya silty clay loam and Guadalupe clay at 60 DAT. This suggests differences in the properties of the soil organic matter.

For immobilization and mineralization processes, the biomass holds an unique position among soil organic matter fractions. It functions as both sink and transformation station for nutrients and energy in the soil (Haider and Azam 1983). Freytag and Rausch (1982) observed that N present in living cells of biomass seems to be less important for N availability than N turnover, which is significant in alternating synthesis-autolysis cycles. Further, Marumoto et al. (1982) reported the N turnover from dead microbial cells to be about 5 times faster than that from native soil organic N. Beck (1983) found mineralization rate to be well correlated to the biomass of the soils ($r = 0.96$). The different immobilization-mineralization patterns of the soils may be closely correlated to soil biomass rather than to soil organic matter or other soil properties. In this respect, Pili loam was expected to have the highest soil biomass production.

Soil N fractionation supports this hypothesis because the major part of the immobilized fertilizer was incorporated in organic, microbially synthesized N compounds. According to Marumoto et al. (1982), organic N compounds of microorganisms are hydrolyzable to a greater extent than the native organic soil N. Therefore, microbially immobilized N enriches the hydrolyzable organic compounds. The hydrolyzable organic matter, especially the easily hydrolyzable fraction, plays a dominant role in N mineralization although this fraction contains only 7% of total C and 16% of total N (Campbell 1978).

Subfractions of organic soil nitrogen

Fertilizer N was first incorporated mainly into the amide-hexosamine fraction until 10 DAT. High ^{15}N recovery in this fraction was found in Pili loam, Sta. Rita clay, and Maahas clay. Decrease in ^{15}N in the amide-hexosamine fraction was accompanied by an increase in the alpha-amino and HUN fractions, an agreement with results obtained by Fleige et al. (1971b).

Fleige et al. (1971b) also reported that amides asparagine and glutamine have a storage function in microorganisms. Similar to higher plants, they probably buffer an oversupply of inorganic N. Hexosamines are mainly located in microbial cell walls. It is possible that they are particularly prone to decomposition.

Distinctive net mineralization of immobilized fertilizer N in Pili loam between 10 and 20 DAT seems to be due to rapid decomposition of the amide-hexosamine fraction. However, Stewart et al. (1963) confirmed that the alpha-amino and HUN fractions likely decomposed later. Voelker and Asmus (1981) further noted that alpha-amino N plays a major role in the N supplying capacity of the soil.

Since the ^{15}N pattern in the HUN fraction is similar to that in the alpha-amino fraction, a close correlation to amino acid metabolism and microbial N turnover should exist as reported by Kai et al. (1973) and Marumoto et al. (1982). The nonhydrolyzable organic N fraction is considered to consist of heterocyclic N compounds (Fleige et al. 1971a), i.e., mainly the "nucleus-N" of the humic acids and, thus, regarded as being generally very stable.

The small but significant ^{15}N decrease in this fraction, however, is not consistent with the assumed stability. In a ^{15}N field experiment (without straw), Fleige and Capelle (1975) reported an initial ^{15}N increase and later on an almost complete disappearance of ^{15}N in this fraction. They associated this with a short and apparently weak chemical fertilizer NH_4 fixation by organic substances (Lindbeck and Young 1965). Laboratory experiments proved that heterocyclic N compounds are decomposed by fungi and bacteria (Ivarson and Schnitzer 1979). This might also be valid for wetland soil though it contains mainly bacteria.

Uptake of fertilizer and soil nitrogen by the rice plant

Fertilizer nitrogen recovery in the rice plant ranged from 61% to 66% in four soils. In Maligaya silty clay loam, however, plant recovery accounted for only 52% of applied fertilizer N. Uptake of basally applied N was almost completed at 40 DAT and derived mainly from the exchangeable NH_4^+ pool. This confirms the results of Keerthisinghe et al. (1985). Nitrogen taken up until the maximum tillering period critically influences yield potential (Vlek and Craswell 1979). This was apparent in Maligaya silty clay loam, where fertilizer N uptake at 40 DAT was significantly low. Low ^{15}N uptake from the soil was partly due to high ammonium fixation (Keerthisinghe et al. 1985) and ammonia volatilization losses during the first 20 days as suggested by the high percentage (7%) of floodwater ^{15}N at 2 DAT. Though fertilizer N in the nonexchangeable fraction was released during the later growth period, its uptake was delayed and it negatively affected plant growth. Atanasiu et al. (1968) similarly observed that ammonium fixation not only delayed N uptake but also increased total fertilizer N loss from the soil-plant system.

Unlike fertilizer N uptake, soil N uptake by rice continued until maturity in all soils, but the amounts taken up differed markedly among soils. These differences represent the various N supplying capacities of the soils as reflected by their Nmin contents at transplanting.

Nmin content and soil N uptake were neither related to total organic C content nor to total N content of the soils. Thus, soil N uptake in Pili loam and Maahas clay were much higher than in Sta. Rita clay even though the organic C content of Sta. Rita clay was higher (2.4%) than in Pili loam (1.6%) and Maahas clay (1.5%).

Poor correlation between total soil N and N uptake were also reported by Fox and Piekielek (1978). Total soil N and organic matter seem to be reliable parameters only for N uptake if biomass is proportional.

Results of this study suggest that parts of the soil N rather than the total N content are important for N-supplying capability. Although fertilizer N uptake was high (60% – 65% recovery), soil N accounted for 70% – 80% of the total N in the plants at maturity, indicating that the rice plant largely depends on available soil N.

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