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DIVISION S-3—SOIL MICROBIOLOGY AND BIOCHEMISTRY

Nitrogen Immobilization in Flooded Soils[1]

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ABSTRACT

Nitrogen immobilization in flooded soils amended with straw was measured by following incorporation of tagged inorganic N into the soil organic fraction. Increases in tagged organic N were found to be highly correlated with net immobilization as measured by decrease in mineral nitrogen in the soil. Since most of the soils used contained clay minerals capable of ammonium fixation, apparent immobilization figures were corrected by subtraction of clay-fixed ammonium.

In comparisons of nitrogen immobilized under different soil conditions it was found that values obtained in flooded soils were intermediate between those obtained under aerobic and anaerobic conditions. The nitrogen factor, or additional N immobilized, per unit weight of straw, was found to vary with quantity of straw added, nitrogen content of the straw, and nature of the soluble nitrogen supplied, but in no case was it negligible. Rice straw (*Oryza sativa* L.) containing 0.47% N immobilized an additional 0.51% N in a flooded soil, and another sample of rice straw containing 1.17% immobilized 0.43% N.

Additional Key Words for Indexing: **nitrogen factor, anaerobic, decomposition.**

THE CONVERSION of inorganic nitrogen to organic forms through assimilation by micro-organisms during the decomposition of organic residues is an important aspect of the nitrogen cycle in soil. In the most dramatic cases severe nitrogen deficiency may be manifested by a crop growing on a soil in which the decomposition of residues low in nitrogen favors rapid immobilization of all available nitrogen. Tracer studies have shown (7) that even in the absence of added residues some immobilization takes place.

The quantity of nitrogen required to meet the needs of the microbial population depends on the nature of the organic amendment (14), kind of nitrogen supplied to the micro-organisms (4), moisture (M. H. Kuo. 1955. Factors influencing the immobilization of nitrogen during the decomposition of plant residues. *M.S. Thesis, Iowa State University, Ames.*), temperature (4, 12), pH (7, 13), aeration (2), and possibly other factors. Previous work, mostly under aerobic conditions, has been reviewed recently by Bartholomew (3). In the ususal aerobic situation net immobilization of nitrogen by mature plant residues reaches a maximum corresponding to 1.2–1.7% N in the original material within a few days or weeks. Inorganic N decreases rapidly at first and changes very slowly thereafter (6).

On the other hand, the limited data available indicate that decomposition under anaerobic conditions results in much less nitrogen immobilization than in the presence of oxygen. According to Acharya (1, 2) the amount of available N required in anaerobic decomposition of rice (*Oryza sativa* L.) is relatively insignificant. He found that the nitrogen factor, a value defined by Hutchinson and Richards (9) as the weight in grams of nitrogen immobilized per 100 g original weight of decomposing residue, to be 0.54 under aerobic conditions compared with 0.07 for anaerobic decomposition. When the rice straw was waterlogged by submerging under about 2.5 cm water, the nitrogen factor was 0.39, not greatly different from the aerobic situation. Karim (10) investigated nitrogen immobilization in the decomposition of water hyacinth and found that under anaerobic conditions added ammonium sulfate was assimilated until the nitrogen content of the decomposing organic materials was about 1.4% over a 6-month period, whereas the corresponding figure for aerobic decomposition was 2.8%.

It should be noted that most of the experiments cited previously were performed in the absence of soil. Williams et al. (15) studied the nitrogen supplying power of rice straw for flooded rice production in field experiments and found that "net nitrogen immobilization" was 0.54% of the original weight of the straw, as determined by grain yield response in a single season. Straws with higher nitrogen concentrations increased grain yield on nitrogen deficient soils and straws with lower nitrogen concentrations depressed yield.

This paper reports an investigation in which nitrogen immobilization was measured directly by use of tagged nitrogen fertilizers added to flooded soils. For purposes of comparison, some experiments were aerobic and some completely anaerobic.

MATERIALS AND METHODS

Soils—The experiments with flooded soils were conducted with Sacramento clay, an imperfectly drained basin soil on which rice had been grown in the previous season, and Mormon clay, a member of the same series-group but containing a moderate concentration of alkali. Experiments under aerobic conditions included these and three other soils, but most comparisons are based on data from the two soils normally used in rice production.

Plant Materials—Ground barley straw (*Hordeum vulgare* L.) containing 0.33% N, rice straw containing 0.47% N, and rice straw containing 1.17% N were used.

Procedures

1) *Nitrogen Immobilization in Absence of Straw*—Fifty-gram samples of dried, sieved soil were weighed into 178.8 g-bottles and 2 ml of solution containing either ammonium sul-

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fate or urea enriched with ^{15}N was pipetted onto the soil. Additions corresponded to 30 and 60 ppm N on the soil basis. An additional 50 g of soil was then placed in the bottle and 125 ml water added carefully to avoid disturbing the soil sample. This produced a layer of water about 5 cm deep above the soil sample. The bottles were incubated at 25C and three replicate samples were removed for analysis at the end of suitable time intervals. Solid KCl was weighed into the bottles in sufficient quantity to make the soil extract 1 *N* in this salt. After 30 minutes shaking the suspension was transferred to Buchner funnels and soil on the filter was washed with an additional 50 ml of 1 *N* KCl added in small increments. The filtrates were transferred to 250-ml volumetric flasks and the soil placed in a drying oven for subsequent analysis. Ammonium nitrogen was determined in the filtrate by steam distillation of an aliquot with magnesium oxide. Initially, nitrate was also determined in the filtrate, but since this was found to be negligible no further nitrate analyses were conducted. Total nitrogen in the extract was determined by Kjeldahl digestion of an aliquot and organic nitrogen calculated by substracting the value for ammonium nitrogen. Total nitrogen in the extracted soil sample was determined by a Kjeldahl digestion procedure, and clay-fixed ammonium in the soil was determined by the procedure of Dhariwal and Stevenson (8). Organic nitrogen in the soil was obtained by substracting clay-fixed nitrogen from the total. All fractions were analyzed for ^{15}N by mass spectrometry. Evidence of adequacy of the methods is provided by the fact that recovery of tracer N in all forms totaled 95–100% at the beginning of the experiments. Fertilizer enrichments were in the range 5–15% ^{15}N excess.

2) *Nitrogen Immobilization in the Presence of Straw*—The first experiment with straw was conducted in much the same manner as described previously, except that prior to addition of the fertilizer solution, 1 g of ground barley straw was added to 100 g of Sacramento clay and mixed thoroughly in the dry condition. Then 10 mg N as tagged ammonium sulfate was added, and the soil was waterlogged. Although the water layer was about 5 cm deep initially, because of copious gas evolution, soil and water formed a more-or-less uniform suspension, the surface of which was in contact with the atmosphere by the end of 30 days. Analyses were as described.

To provide a deeper water layer only 50 g soil was used in subsequent experiments. After the soil was mixed with straw 5 mg N as tagged ammonium sulfate was pipetted in and distilled water added to provide a layer about 7 cm in depth over the soil surface.

In some experiments a series of samples was weighed into 200-ml round bottom flasks fitted with high-vacuum stopcocks. After addition of straw and ammonium sulfate the samples were wetted with sufficient water to bring them to field capacity, then were evacuated and filled with argon. Another series of samples was weighed into small erlenmeyer flasks, wetted to field capacity, and incubated with a slow stream of air passing over the soil surface. In this series, both ammonium and nitrate in the KCl extract were determined.

In one experiment air was bubbled through the supernatant water of flooded samples to measure the effect of oxygen in the soil solution.

In one experiment tagged glycine was used as the nitrogen source in order to evaluate the effect of uniform distribution of the fertilizer throughout the soil sample as compared with the localized distribution obtained by application of an ammonium salt.

RESULTS AND DISCUSSION

Immobilization without Addition of Organic Matter

Fig. 1 gives an accounting of the tagged N added at the 60 ppm rate to unamended Sacramento clay. Data obtained with the 30 ppm rate, when expressed as percent of added N, were virtually identical. In these experiments

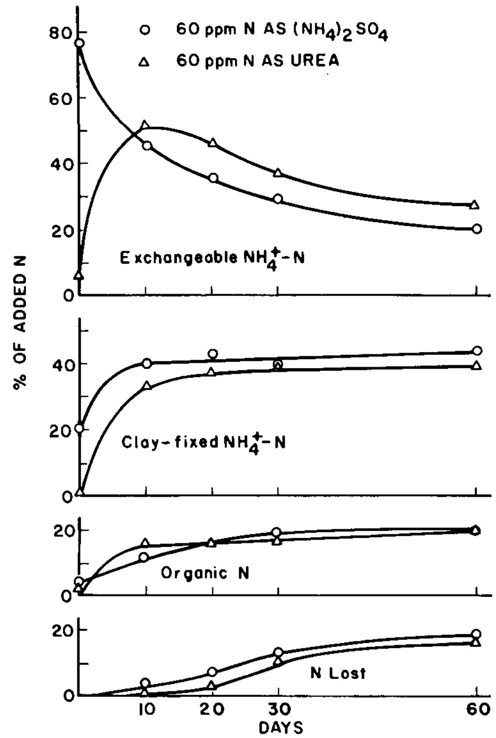

Fig. 1—Fate of ammonium sulfate and urea N added to unamended Sacramento clay at the rate of 60 ppm.

more than 20% of the added N was immobilized as indicated by the occurrence of tracer nitrogen in the organic fraction. However, the level of ammonium nitrogen in the soil did not decrease by a corresponding amount (data not shown) indicating that net immobilization did not necessarily occur. As much as 40% of the added N was fixed in the crystal lattice of clay minerals.

Immobilization with Added Organic Matter

a) *Tagged Organic N in Relation to Net Immobilization*—Fig. 2 shows increases of tagged organic N compared with decreases of tagged exchangeable ammonium in flooded Sacramento clay amended with 1% barley straw. The two curves are almost mirror images. Tagged nitrogen in the soil organic fraction provides clear-cut evidence of nitrogen immobilization, but it may not provide a quantitative measure of net immobilization unless it is highly correlated with changes in the level of total inorganic N. Where nitrogen losses from soil are small and ammonium fixation does not occur, net immobilization can be measured by the decrease in inorganic N. In many soils ammonium fixation is significant, and initial changes in the level of inorganic N are not a true reflection of nitrogen immobilization. By calculating changes in total inorganic N and in tagged organic N which occurred after the first day of incubation, the effect of ammonium fixation can be minimized, since the fixation reaction is rapid and in most soils is relatively complete within a few hours. The correlation between decrease in exchangeable ammonium-N and the increase in tagged organic N calculated in this way from data of several experiments with two flooded soils is shown

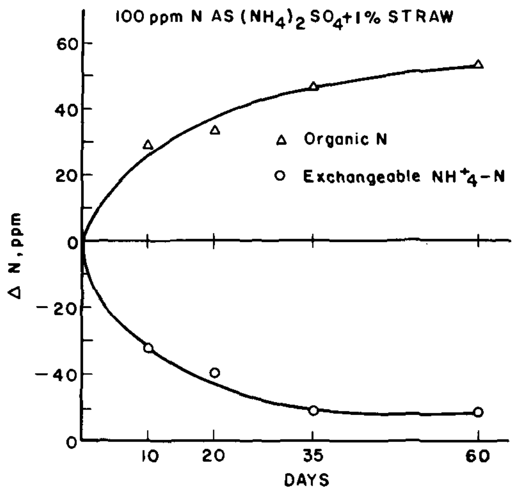

Fig. 2—Changes in tagged organic N and tagged exchangeable ammonium in Sacramento clay receiving 1% barley straw and 100 ppm tagged N as ammonium sulfate.

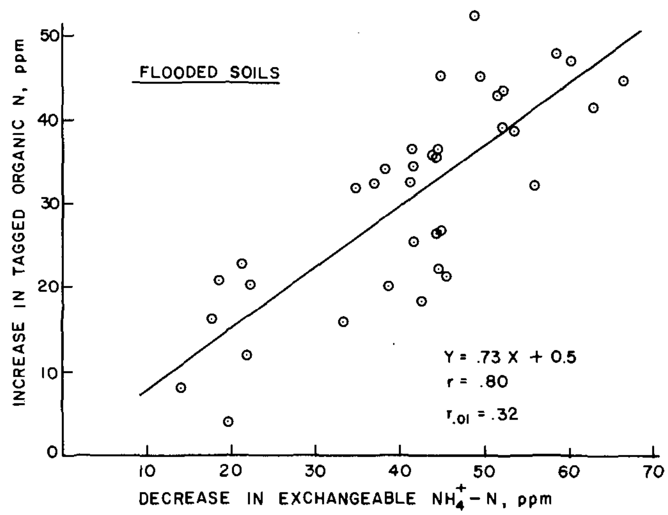

Fig. 3—Correlation of increases in tagged organic N with decreases in exchangeable ammonium in flooded soils.

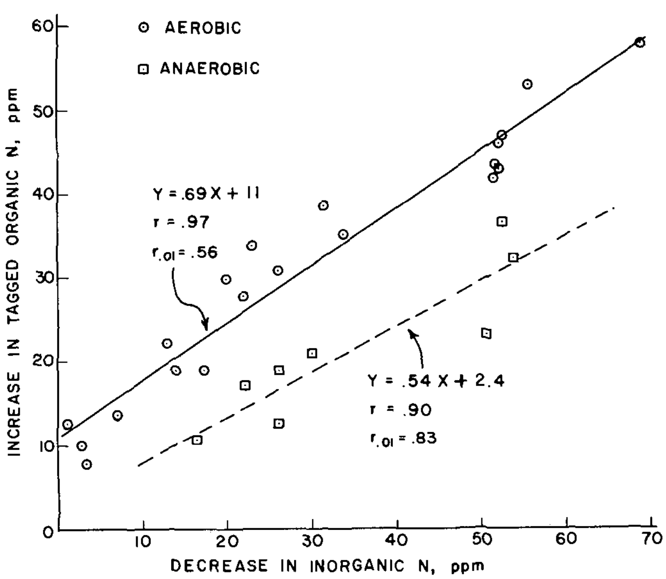

Fig. 4—Correlation of increases in tagged organic N with decreases in mineral N in aerobic and anaerobic soils.

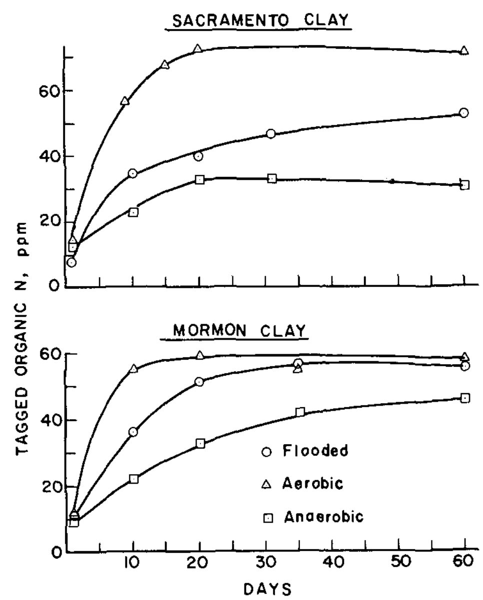

Fig. 5—Tagged organic N in two soils incubated under aerobic, flooded, and anaerobic conditions.

in Fig. 3. For purposes of comparison, correlations were also calculated for two soils incubated in a completely anaerobic argon atmosphere and for five aerated soils (Fig. 4). It is clear that in straw-amended soils the quantity of tagged nitrogen found in the organic form can be used as a measure of net immobilization, although the relationship between the two values varies quantitatively with different soil conditions.

b) *Quantities of Nitrogen Immobilized*—The effect of flooding on amount of inorganic N immobilized as cpmpared with aerobic and anaerobic conditons is shown in Table 1. These values are expressed as percent of the weight of straw initially added and correspond to the nitrogen factor as defined by Hutchinson and Richards (9). The small difference in the flooded vs. aerobic condition in the Mormon clay is particularly surprising. Even in a completely anaerobic atmosphere the quantities of nitrogen immobilized were considerable. These experiments do not support the longheld view that nitrogen immobilization is negligible under anerobic conditions.

In this connection the curves of Fig. 5 are instructive. The initial rate of nitrogen immobilization in the aerated samples was higher than the others, but immobilization in the flooded and anaerobic samples continued over a longer period of time. This may be due in part to the maintenance of ammonium-N, the preferred form of nitrogen for soil micro-organisms, at an appreciable level, whereas in the aerated soils this was converted rapidly to nitrate.

Table 1—Nitrogen factor values for barley straw containing 0.33% N in two soils under three conditions of aeration

	Aerobic	Flooded	Anaerobic
Sacramento clay	0.72	0.52	0.31
Mormon clay	0.59	0.56	0.46

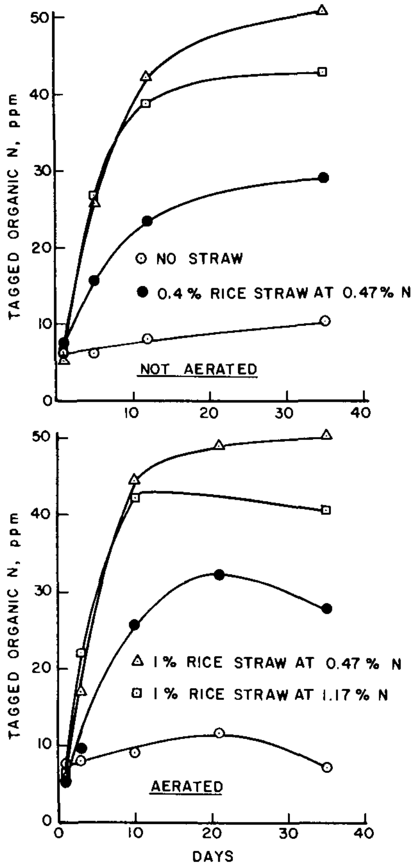

Fig. 6—Comparison of N immobilization in Sacramento clay with aerated, and nonaerated flood water.

c) *Effect of Aerating Supernatant Water.* Results shown in Fig. 6 show that bubbling air through the water layer above the soil had little effect on nitrogen immobilization. Values of the nitrogen factor (Table 2) were almost identical in soils with or without aeration of the supernatant. Of particular interest in these data is the finding that nitrogen immobilized in samples amended with rice straw containing 1.17% N was almost as great as in rice straw with 0.47% N content. Also given in Table 2 are nitrogen factor values obtained with glycine as the source of N. This source was immobilized in larger quantity than was ammonium sulfate.

The quantities of nitrogen immobilized as measured by increase in tagged organic N in these experiments are probably lower than actual net immobilization. The relationship for flooded soils between decrease in exchangeable ammonium (X) and increase in tagged organic N(Y), is $Y = 0.734\ X + 0.54$, which suggests that some untagged nitrogen is also immobilized since the slope of the regression line is less than 1.0, but it is not possible to calculate how much with the information at hand. The fact that the isotopic enrichment of the exchangeable ammonium fraction invariably decreased with time indicates continued dilution of the added N by untagged ammonium derived from the soil. Calculation of actual mineralization and immobilization rates by the equations of Kirkham and Bartholomew (11) was not possible since these data do not fit the boundary conditions required by either of their two solutions of the basic differential equations. However, the conclusion is inescapable that nitrogen immobilization is a process of significant magnitude in flooded soils. This is reinforced by work reported elsewhere (5) that more than half the tagged fertilizer applied in a greenhouse pot experiment remained in the soil after growth of a crop of rice.

Table 2—Nitrogen factor values for rice straws in flooded Sacramento clay as affected by level of addition, N content, and source of N

% straw added, soil basis	% N in straw	Source N	N-factor
0.4	0.47	$(NH_4)_2SO_4$	0.72
1.0	0.47	$(NH_4)_2SO_4$	0.51
1.0	0.47	Glycine	0.63
1.0	1.17	$(NH_4)_2SO_4$	0.43

It should be emphasized that in nearly all of the experiments with straw-amended soils it was possible to recover more than 90% of the fertilizer N added. This was present at the end of the experiments largely as clay-fixed ammonium and as organic N, with very little exchangeable ammonium remaining.

LITERATURE CITED

1. Acharya, C. N. 1935. Studies on the anaerobic decomposition of plant materials. I. The anaerobic decomposition of rice straw. Biochem. J. 29:528–541.
2. Acharya, C. N. 1935. Studies on the anaerobic decomposition of plant materials. III. Comparison of the course of decomposition of rice straw under anaerobic, aerobic, and partially aerobic conditions. Biochem. J. 29:1116–1120.
3. Bartholomew, W. V. 1965. Mineralization and immobilization of nitrogen in the decomposition of plant and animal residues. *In* W. V. Bartholomew and F. E. Clark (ed.) Soil nitrogen. Agronomy 10:285–306.
4. Broadbent, F. E. 1966. Interchange between inorganic and organic nitrogen in soils. Hilgardia 37:165–180.
5. Broadbent, F. E., and D. S. Mikkelsen. 1968. Influence of placement on uptake of tagged nitrogen by rice. Agron. J. 60:674–677.
6. Broadbent, F. E., and K. B. Tyler. 1962. Laboratory and greenhouse investigations of nitrogen immobilization. Soil Sci. Soc. Amer. Proc. 26:459–462.
7. Broadbent, F. E., and K. B. Tyler. 1965. Effect of pH on nitrogen immobilization in two California soils. Plant and Soil 23:314–322.
8. Dhariwal, A. P. S., and F. J. Stevenson. 1958. Determination of fixed ammonium in soils. Soil Sci. 86:343–349.
9. Hutchison, H. G., and E. H. Richards. 1921. Artificial farmyard manure. Ministry of Agr. 28:398–411.
10. Karim, A. 1948. Microbiological decomposition of water hyacinth. Soil Sci. 66:401–416.
11. Kirkham, D., and W. V. Bartholomew. 1955. Equations for following nutrient transformations in soil, utilizing tracer data: II. Soil Sci. Soc. Amer. Proc. 19:189–192.
12. Nommik, H. 1962. Mineral nitrogen immobilization and carbon dioxide production during decomposition of wheat straw in soil as influenced by temperature. Acta Agr. Scand. 12:81–94.
13. Norman, A. G. 1931. The biological decomposition of plant materials. VI. The effect of hydrogen-ion concentration on the rate of immobilization of nitrogen by straw. Biochem. J. 25:1779–1787.
14. Pinck, L. A., F. E. Allison, and V. L. Gaddy. 1948. Utilization of nitrogen in cropping systems with and without green manure in the greenhouse. Soil Sci. 66:39–52.
15. Williams, W. A., D. S. Mikkelsen, K. E. Mueller, and J. E. Ruckman. 1968. Nitrogen immobilization by rice straw incorporated in lowland rice production. Plant and Soil 28: 49–60.