

## 7. Denitrification in rice soils

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### 1. Introduction

Denitrification is defined as the reduction of nitrate or nitrite to gaseous nitrogen (usually  $N_2O$  and  $N_2$ ) and is usually catalyzed by bacteria. Though other organisms are known to produce  $N_2O$  [119], denitrifiers are thought to be those in which the reduction of nitrogenous oxides is coupled to electron transport phosphorylation thereby providing energy to the cell. Most of the nitrate used by denitrifiers is reduced to gas which is not the case for other  $N_2O$  producing organisms [111, 118]. The pathway of denitrification is thought to be  $NO_3^- \rightarrow NO_2^- \rightarrow [Enz-NO] \rightarrow N_2O \rightarrow N_2$ . Whether free NO is an intermediate is still in dispute, but evidence is now stronger for at least an enzyme bound NO intermediate [32, 50]. Denitrifiers often excrete the intermediates  $NO_2^-$  and  $N_2O$ , but significant production of NO is rarely reported. Perhaps owing to the renewed interest in denitrification a number of recent reviews have been prepared on the microbiological, biochemical, physiological, and ecological aspects of denitrification [25, 26, 30, 34, 35, 37, 43, 63, 67, 79, 80, 81, 87]. This text is focused only on those aspects of denitrification of relevance to rice culture.\*

Denitrification is of interest in paddy rice culture because nitrogen-fertilizer losses from this cropping system are well known. Abichandani and Patnaik [1] estimated these losses to be 20 to 40% in India, while losses of 30 to 50% were reported by Mitsui [74] in Japan. From a recent review of a number of  $^{15}N$  balance studies by Craswell and Vlek [20], it appears that N losses in these ranges are typical. In the past it was reasoned that denitrification was the most significant loss mechanism, but ammonia volatilization and leaching losses have recently been shown to be more significant in some situations. Even in the non-fertilized rice paddy, which still is the major practice in the world, denitrification losses apparently occur. Wetselaar [132], in his summary and evaluation of N budget of traditional Asian wet season culture, estimated denitrification losses of 3 to 34 kg N ha<sup>-1</sup> yr<sup>-1</sup> with an average of 18.5 kg N lost ha<sup>-1</sup> yr<sup>-1</sup>\*\* . When all nitrogen inputs were maximum and outputs were minimum (except denitrification), the maximum estimated denitrification loss became 129 kg N ha<sup>-1</sup> yr<sup>-1</sup>. This latter value is unlikely because high inputs would be from nitrogen fixation which would

\* The literature review for this chapter was completed in February, 1981.

\*\* In his estimates  $NH_3$  volatilization, leaching, predatory harvest, overflows, plant harvest, and wind erosion were separately evaluated, and thus not lumped with the denitrification loss.

not likely lead to high denitrification because of the slow transfer of biologically fixed nitrogen to nitrate. In these same estimates he calculated a net gain of  $50 \text{ kg N ha}^{-1} \text{ crop}^{-1}$  in these steady-state agro-systems. His average denitrification loss is equivalent to 37% of the nitrogen accretion. Thus even in the N-stressed, non-fertilized system, denitrification losses appear to be significant and should translate into a loss in productivity.

## 2. Environmental factors affecting denitrification

The major environmental factors that control denitrification are *oxygen*, adequate *energy sources* (i.e., available organic matter), and a supply of *nitrate* [30]. Oxygen is the preferred electron acceptor for denitrifiers; its presence both inhibits the activity and prevents synthesis of the denitrifying enzymes [79, 86]. Increasing available carbon [49, 97] and nitrate both favor denitrification. However, high organic matter can also cause immobilization [19] thereby reducing denitrification.

Rice soils generally have two of these major environmental factors controlling denitrification – limited oxygen due to flooding, and available organic matter released from the decomposing straw. Thus the major limiting factor is the supply of nitrate. Nitrate fertilizers are no longer used for this obvious reason. Ammonium produced by mineralization or from fertilizers must be oxidized by nitrification before nitrate is available to denitrification. Nitrification is an obligately oxygen requiring process so it can only occur in aerobic environments. In rice culture, these are: (1) the aerobic layer at the surface of the soil, (2) the aerobic zone surrounding the oxygen excreting rice root, and (3) the aerobic period caused by intermittent flooding. The first two environments and the reactions which describe the rice paddy nitrogen cycle are diagrammed in Fig. 1.

For a better understanding of denitrification and other nitrogen losses from paddy soils, it is essential to understand this unique habitat. Figure 1 is meant to aid this understanding. Paddy soils are characterized by two distinct layers [2, 74, 85, 122, 137]: an oxidized (aerobic) surface layer generally a few millimeters to 1 to 2 cm deep. The depth depends on the amount of organic matter, since this results in oxygen consumption by respiring microorganisms, and by the structure of the soil which influences the rate of diffusion of oxygen into the mud. Below the aerobic layer is a reduced (anaerobic) layer. If ammonium fertilizer is applied to the surface layer, it is first nitrified ( $k_3$ ) and the nitrate or nitrite produced then can diffuse down ( $k_4$ ) where it is reduced by denitrification ( $k_7$ ) to  $\text{N}_2\text{O}$  and  $\text{N}_2$ . A similar aerobic–anaerobic interface exists around the rice roots (Fig. 1, inset) which can also result in denitrification as is discussed later. The alternate wetting and drying conditions create an ideal environment for denitrification. The nitrate formed during the dry period is rapidly lost through denitrification when the soil is reflooded and a stimulation of decomposition occurs [83, 94, 102].

Factors other than oxygen, energy, and nitrate which influence denitrification rates are temperature, pH, salinity, and perhaps iron concentration. Denitrifying

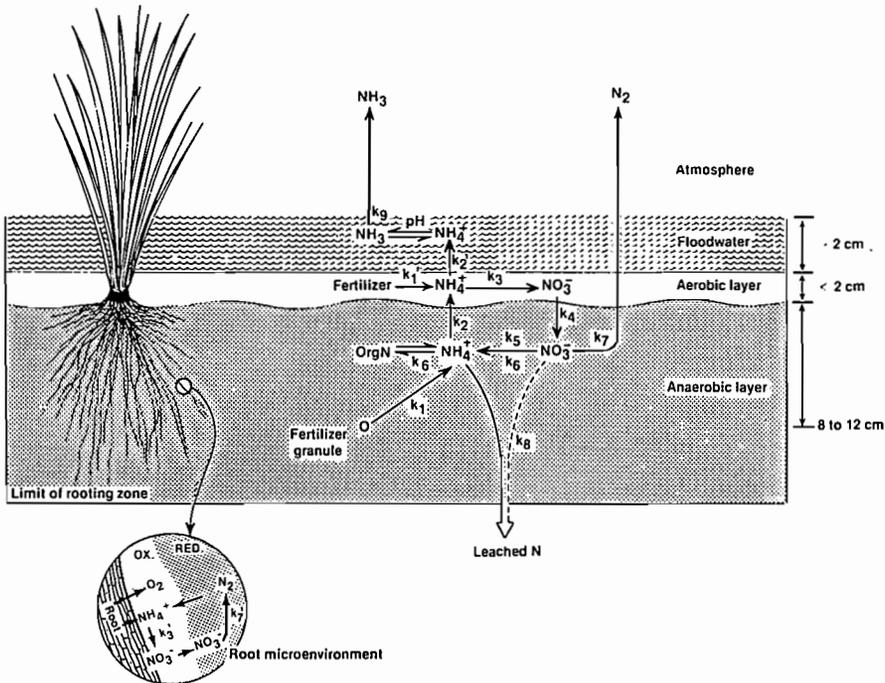


Fig. 1. Diagram of paddy rice environment which emphasizes the three major losses of nitrogen ( $N_2$ ,  $NH_3$  and leaching), the two sites of denitrification – the rhizosphere and just below the aerobic layer – and the preceding mechanisms which control these losses. The rates depicted are:  $k_1$ , release of  $NH_4^+$  from fertilizer;  $k_2$ , diffusion of  $NH_4^+$  to aerobic layer;  $k_3$ , nitrification;  $k_4$ , diffusion of  $NO_3^-$  to anaerobic layer;  $k_5$ , dissimilatory reduction of  $NO_3^-$  to  $NH_4^+$ ;  $k_6$ , immobilization reactions. The loss mechanisms are:  $k_7$ , denitrification;  $k_8$ , percolation rate of N;  $k_9$ , volatilization rate of  $NH_3$ .  $k_n$  refers to the same reaction but in a different micro-environment.

activity is strongly correlated with temperature in soil. It is negligible at 3 to 10°C but the rate of nitrate reduction rises with temperature to reach a maximum at 65°C and ceases at 85°C [78]. In rice soil Garcia [41] showed the existence of two maxima during  $N_2O$  reduction which were 37 and 65°C; the higher maxima is no doubt due to the existence of thermophilic *Bacillus* species [46].

The rate of denitrification is low in acidic conditions and rises with pH to reach an optimum activity in the neutral range [78]. In the acid sulfate rice soils, which are prevalent in certain areas of the world, denitrification has been shown to be slow with 70% of 100 ppm  $NO_3^-$ -N still remaining after one week of anaerobic incubation [10]. Salinity appears to depress denitrification [49]. In these studies a low correlation was found between the denitrifying activity and the initial number of denitrifying bacteria in Senegal rice soils. In preliminary studies by Sexstone and Tiedje (unpublished data), ferrous iron in concentrations as low as  $10^{-4}$  M inhibited

the overall denitrification sequence by *Pseudomonas fluorescens*, a common denitrifier in nature.

Environmental factors have also been shown to have a large influence on the N gases produced. Increasing concentrations of  $\text{NO}_3^-$  [9, 33, 78],  $\text{NO}_2^-$  [33],  $\text{O}_2$  [8, 33, 78],  $\text{S}^{2-}$  [115, 117] increase the proportion of  $\text{N}_2\text{O}$  produced. Decreasing pH [33, 39, 70, 78, 133] and temperature [65, 78] also increase the  $\text{N}_2\text{O}$  production. However a decrease in  $\text{N}_2\text{O}$  is caused by more available carbon [78] and a longer anaerobic period [31]. In fact, most soils become sinks for  $\text{N}_2\text{O}$  if they are anaerobic for more than 1 to 2 days [31]. This is particularly significant for rice since it suggests that rice paddies should not evolve much  $\text{N}_2\text{O}$ . This was confirmed in a recent field study where a maximum of only 1.4% of the  $\text{NO}_3^-$ -N was emitted as  $\text{N}_2\text{O}$  [24].

NO is rarely found in nature except under acid conditions where it is produced from chemical decomposition of nitrite [77, 125]. It is known, however, that sulfide causes some increase in NO which originates from biological reduction [115]. Furthermore, at the aerobic-anaerobic interface in marine sediments (which are high in sulfide), substantial NO has been detected [113]. In rice soil where there is sulfate intrusion or ammonium sulfate fertilizers are heavily used, one might expect some NO to be produced. Garcia [45] has reported some NO to be biologically produced by rice soils but the role of sulfide in its production was not investigated. Because of the chemical reactivity of NO, however, its life in the paddy soil should not be long.

### 3. Denitrifying bacteria

A number of methods for enumerating denitrifying bacteria have been reported and are summarized elsewhere [119]. Most of these are based on the most probable number (MPN) technique [36, 40, 124, 129]. Recent reevaluation of the MPN technique in Tiedje's laboratory has shown that some false positive tubes can occur due to nitrate and nitrite removal by ammonium producing organisms and that false negative tubes also occur due to minute bubbles in Durham tubes and/or failure to totally remove the nitrate and nitrite. To avoid these problems a modified method has been recommended in the Revised Methods of Soil Analysis [119]. This method recommends using a medium of 5.0 mM  $\text{NO}_3^-$  (instead of 9.9) in nutrient broth to reduce the number of false negatives. To avoid false positives, a confirmatory procedure is suggested in which acetylene is added and tubes with  $\text{N}_2\text{O}$  concentrations over 20% are judged as positive for denitrifiers. The latter procedure requires more time and equipment and is probably not necessary when comparative results within the same experimental design are intended.

Comprehensive lists of genera of denitrifying bacteria are compiled in recent reviews [30, 63, 80]. According to Gamble *et al.* [38], *Pseudomonas fluorescens* biotype II and the 'alcaligenes-like' group are the most commonly encountered numerically dominant denitrifiers. Virtually all recognized denitrifying bacteria are

strict aerobes and are incapable of growing anaerobically by fermentative means. Recently, however, many denitrifying strains of the genus *Bacillus* have been isolated from rice soils in Senegal and are able to ferment carbohydrates [46]. All of these organisms tolerate high concentrations of nitrite during growth and some can use NO as the electron acceptor for growth [46, 88]. They were isolated in a complex medium with  $5 \text{ gl}^{-1}$  yeast extract and  $10 \text{ gl}^{-1}$  trypticase. A thermophilic denitrifying *Bacillus* was also isolated [46]. The taxonomic study of these *Bacillus* isolates demonstrates that they are a new and unique group of this genus [88, 89]. Perhaps there are other fermentative denitrifiers. Indirect evidence suggests that *Propionibacterium pentosaceum* produces N gas from nitrite [123]. Furthermore, Kaspar *et al.* have noted denitrifying activity in deep lake sediments, anaerobic sludge with long retention times and in a continuous culture of a methanogen growing on acetate [64]. In these habitats devoid of  $\text{O}_2$  and  $\text{NO}_3^-$  survival could only be by fermentative growth.

#### 4. Methods of analysis of denitrification in soils

Early studies of denitrification depended on manometric assays [71] since this was the only means to measure the gaseous products. But soil samples had to be dried before introduction into respirometer vessels which produced biological modifications, and the method was not sensitive enough to measure natural denitrification rates.

Mass spectrometry has also been employed to trace the fate of  $^{15}\text{N}$  fertilizer [20]. The classical approach has been to establish a  $^{15}\text{N}$  balance for the soil and to assume the losses are due to denitrification [53]. A more recent approach by Rolston *et al.* [99] was to measure denitrified gas flux directly from a field soil. Following addition of  $^{15}\text{NO}_3^-$  to microfield plots  $^{15}\text{N}_2$  and  $\text{N}_2\text{O}$  flux were measured to establish a field denitrification rate. This is the only reported direct measurement of denitrification in the field; unfortunately it is rather insensitive as rates below  $1 \text{ kg N ha}^{-1} \text{ day}^{-1}$  cannot be measured. Thus it is doubtful that this method is directly applicable to denitrification studies on rice. The disadvantage of  $^{15}\text{N}$  for many researchers working on rice is that the equipment and isotope are expensive and the technology rather complicated. Nonetheless,  $^{15}\text{N}$  balance studies remain the best method for field assessment of nitrogen losses.

Gas chromatography was quickly recognized to be a key method for studies on denitrification since it is simple, rather inexpensive and precise, and repetitive analyses can be made. The key development was by Hollis [55] in 1966 when he introduced the porous polyaromatic beads for chromatographic separation of nitrogen, oxygen, nitric oxide, nitrous oxide, and carbon dioxide. Helium is usually used as the carrier gas and thermoconductivity wires or microthermistors for detectors [67]. Barbaree and Payne [7] devised methods for using columns packed with these beads in their assays for products of denitrification. Subsequently, gas chromatographs equipped with helium ionization [27, 108]  $^{63}\text{Ni}$  electron capture

[100, 107], and ultrasonic detectors [8a] have been shown to measure  $N_2O$  concentrations of 1 ppm (v/v) or less. These methods were made even more useful with the discovery in 1973 of Fedorova *et al.* [29] who showed that acetylene blocked the reduction of  $N_2O$  to  $N_2$ . Balderston *et al.* [6] and Yoshinari and Knowles [138] used this method to inhibit denitrification at the  $N_2O$  stage and showed that it could be used as an assay for rate of denitrification [66, 112, 139].

During a study of the sequential products of denitrification *in vitro* in Senegal rice soils, Garcia [39] noted that the rate of formation and reduction of  $N_2O$  was directly related to the rate of nitrate reduction and to the soil organic carbon content. This led the author to develop a new method to estimate the rate of denitrification in soil based on the rate of reduction of a supplied quantity of  $N_2O$  [91]. This gaseous substrate can be introduced into a water-saturated soil without previous drying of soil. A battery-operated portable chamber was devised for use in the field for incubations of soil samples at  $37^\circ C$  in 250 ml serum flasks immediately after collecting the sample. At regular intervals, gas samples are withdrawn into evacuated gas vials. These samples are returned to the laboratory for analysis of the remaining  $N_2O$  by gas chromatography. Calculations are based on the  $N_2O$ : Kr ratio [44].

The constant rate of  $N_2O$  reduction observed during the first few hours represents the initial denitrifying activity present in soil at the time of sampling. After 4 to 6 hours the rate increases sharply to a new constant rate and is indicative of the final denitrification capacity [41, 47]. A similar result was obtained by Smith and Tiedje [110] using the acetylene inhibition method for the assay of denitrification. The existence of the two distinct phases of denitrification was confirmed in the two laboratories by the use of chloramphenicol. The activity measured by these methods after long incubation represents only a small fraction of the potential activity in the soil because the electron acceptor and sometimes the electron donor becomes rate limiting [49]. The limitation of the  $N_2O$ -reduction method is that nitrate must not be present as a competing electron acceptor [9]. But in waterlogged rice soils the level of nitrate is generally very low. This method also suffers from the classical limitations of requiring complete anoxia and high levels of electron acceptor, but it has the advantage over the nitrate disappearance method in that it reflects only dissimilatory nitrogen transformations.

The acetylene inhibition method at least initially appeared to offer more promise for field measurements of denitrification in rice paddy soils. A gasing apparatus such as the one developed by Ryden *et al.* and used to measure denitrification rates in irrigated California soils looked promising for field studies [100]. The most serious limitation to the acetylene approach is the finding that acetylene also inhibits nitrification [56, 130] which prevents further nitrate formation and thus accurate measurements of denitrification. A lack of complete inhibition of  $N_2O$  reduction by acetylene has also been noted on a few occasions [136, 117]. This seems to be a function of the intensity of microbial activity and can often be overcome by adding more acetylene to be in proportion to microbial activity [64]. As a further caution, it has been shown that high sulfide can relieve the

acetylene inhibition [117], and that acetylene can be decomposed in anaerobic soils at fairly high rates (up to 100  $\mu$ mole decomposed by 20 g soil in 4 days) [131]. Anaerobic oxidation of acetylene has recently been confirmed in sediments and was shown to be metabolized to acetate and then converted to methane [23]. These findings make it clear that adequate inhibition of  $N_2O$  reduction by acetylene must be carefully demonstrated for each habitat.

Field methods using acetylene which are more appropriate for the rice paddy appear to be those developed for aquatic studies. Chan and Knowles [15] devised a chamber which could be inserted into sediment and enclosed the overlying water. The enclosed water was circulated by pump. Acetylene (to yield 5 to 10% final concentration) and ethane (1%, internal standard) were injected into the pumped waterline to distribute the hydrocarbons. Water samples were withdrawn periodically to measure the change in N gases and ions. When nitrate was added  $N_2O$  accumulation could be measured, but in the absence of this amendment  $N_2O$  accumulation did not occur except to a limited extent in one case where there was a natural measurable nitrate concentration. The acetylene inhibition may not have been complete since only 14 and 6% of the added nitrate was recovered as  $N_2O-N$ . At first thought this type of approach would seem applicable to measurement of denitrification in rice paddies. However, as Chan and Knowles found,  $N_2O$  accumulation was undetectable or very low. The likely problem is that in habitats with extremely low nitrate pools, any denitrification requires prior nitrification which would be inhibited by the acetylene treatment.

Another approach which reduces this problem by being a more short-term *in situ* measurement is the direct injection technique used by Sørensen *et al.* [114] in marine sediments. They injected 100  $\mu$ l of acetylene-saturated water at 1 cm intervals in small sediment cores. The cores were incubated and then extracted and the accumulated  $N_2O$  measured by gas chromatography. They were able to measure denitrification rates, but this process was a significant fraction of the total electron flow only in the winter when nitrate concentrations were higher in the interstitial water. This technique should also be applicable to rice soils, but it is likely to work only if denitrification is not intimately linked to the nitrate supply from nitrification.

The last method is the use of the radioactive isotope of nitrogen,  $^{13}N$ , which has a half-life of 10 min. Gersberg *et al.* [52] and Tiedje *et al.* [120, 121] have used  $^{13}N$  in short-term denitrification studies. The  $^{13}N$  is usually generated by irradiation of water with 12 to 15 meV proton beams from a cyclotron. A gas chromatograph-proportional counter was developed to separate and measure  $^{13}N-N_2$ ,  $^{13}N-N_2O$  and  $^{13}NO$ , and a radio-high pressure liquid chromatograph was developed to separate and measure  $^{13}NO_3^-$ ,  $^{13}NO_2^-$  and  $^{13}NH_4^+$  [120].  $^{13}N$  has the advantage in denitrification studies of allowing direct measurement of  $N_2$ , is very sensitive, and allows isotope exchange experiments [32]. One need only add  $^{13}N$  at femtogram quantities (68 fg = 0.1 mCi) for an experiment; this should not alter the indigenous nitrate concentration even in anaerobic habitats with extremely low nitrate concentrations. Gersberg used this technique with slurries of rice soils [52] and found denitrifi-

cation rates of 1 to  $6 \mu\text{g N liter}^{-1} \text{ hour}^{-1}$  which was about 10 times higher than the rates he reported for oligotrophic lake sediments [51]. The need for highly sophisticated equipment and for production of the isotope at the site of its use due to its short half-life, limits the general application of this method. Despite these limitations,  $^{13}\text{N}$  holds some promise for denitrification studies in habitats such as rice where the nitrate pool is low and its turnover rapid. A recent summary of all  $^{13}\text{N}$  denitrification work and methods has been prepared by Tiedje *et al.* [121].

## 5. Rice rhizosphere effect on denitrification

In non-submerged soil it is well known that denitrification activity in the rhizosphere is stimulated by plant roots. Woldendorp [134, 135] obtained evidence that rates of organic carbon excretion and consumption of oxygen by roots and microorganisms could be sufficient to create microenvironments in which denitrification would occur. Smith and Tiedje [109] later confirmed that denitrification enzyme concentration increased with distance to roots. Interestingly, however, they showed that plants could decrease denitrification compared to unplanted soil *if* nitrate was limiting. If nitrate was high, however, plants stimulated denitrification as had been shown before. This suggests that plants can compete well with denitrifiers for limiting nitrate.

In the case of rice, however, it is not known how this different environment of anaerobic soil and an oxygen-excreting root (Fig. 1, inset) affects denitrification. Another marked difference in the rice rhizosphere is the very high concentration of ferrous iron which precipitates as  $\text{FeOOH}$  forming iron coatings representing up to 14% of the root dry weight [16, 17]. Since  $\text{Fe}^{3+}$  and  $\text{Fe}^{2+}$  both show some inhibitory effect on denitrification and  $\text{N}_2\text{O}$  reduction in lab studies [68, 105], it is conceivable these ions could also have an inhibitory effect on denitrification in the rice rhizosphere. The question of denitrification in the rhizosphere was approached by examining the potential denitrifying activity and the number of denitrifiers in paddy soils from Senegal. In these studies Garcia [40] showed that rice has a stimulatory effect on these parameters. This stimulation is restricted to the thin layer of soil adhering to the roots and is especially evident in soils low in organic carbon. Using the  $\text{N}_2\text{O}$  reduction method a positive rhizosphere effect was demonstrated for 3-week old rice seedlings which had been grown in 15 g waterlogged soil in glass tubes [42]. The study was made during the period of active photosynthesis and presumably oxygen exudation by comparing induced activities of  $\text{N}_2\text{O}$  reductase in planted and nonplanted soils. This effect may be attributed to the development of anaerobic zones, the presence of root exudates and the large number of denitrifiers in the rhizosphere. These results were confirmed by use of a simple device developed to study metabolic gases in the rice rhizosphere [92].

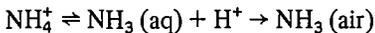
The positive effect of the rice rhizosphere on denitrification was also confirmed with experiments made in rice fields in Senegal [44] and in pot cultures [44, 48]. The rhizosphere effect on potential denitrifying activity was maximum in the first stages of growth and diminished progressively with age of plant.

In the rice rhizosphere the nature of fertilizers or the manner of application have a lesser effect than for nonplanted soil [48] as was previously shown by De and Digar [28] and by Broadbent and Tusneem [11]. They demonstrated that rice culture diminished the losses of nitrogen compared to nonplanted soil. This could result from  $\text{NH}_4^+$  absorption by rice plants before it was oxidized and lost through denitrification.

## 6. Description of the components of the nitrogen cycle in paddy rice (Fig. 1).

### 6.1. Ammonium volatilization ( $k_9$ )

This loss is considered in this chapter on denitrification because some of the nitrogen loss reported in the past has likely been due to ammonia volatilization. This loss has only recently been quantified. Ammonium volatilization has been shown to be first order ( $k_9$ ) with respect to  $\text{NH}_3(\text{aq})$  (ammonia concentration in the flood water) [127]. Basically  $\text{NH}_3$  will diffuse into an atmosphere as long as  $\text{NH}_3(\text{aq})$  is present in flood water. The rate of volatilization increases with wind as well as with any factor which increases the ammonia in solution such as fertilizer input ( $k_1$ , and  $k_2$ ), temperature and pH [10]. pH acts according to the equation [127]



The floodwater is largely buffered by the bicarbonate system and usually does not closely reflect the soil pH [126]. The greatest effect on the pH is due to algal photosynthesis and respiration [73],  $n\text{CO}_2 + n\text{H}_2\text{O} \rightleftharpoons (\text{CH}_2\text{O})_n + n\text{O}_2$ . During active photosynthesis,  $\text{CO}_2(\text{aq})$  is depleted driving the pH values up to 8.5 to 10. At night respiration produces an excess of  $\text{CO}_2$  forcing the pH down to 6.5 to 7.5. It is not uncommon for pH values to change two to three pH units per daily cycle. The consequence of this is that during high pH periods the  $\text{NH}_3(\text{aq})$  concentration greatly increases thereby causing a proportional increase in loss by ammonium volatilization [73, 126]. Losses as high as 20 to 50% of fertilizer N have been reported due to volatilization. Factors such as water chemistry, form and location of fertilizer, and plant cover which affects wind directly above the water surface and light reaching the algae are the major parameters affecting this loss [10, 126, 127].

In some nitrogen-fertilizer experiments in the past algal growth has not been controlled and thus it is difficult to determine how important ammonia losses were to the poor N recovery. One pot experiment where ammonia was trapped, a significant amount of the N loss was accounted for by the trapped ammonia [126]. Since denitrification losses still cannot be measured directly, it is necessary that future N-budget experiments accurately control or assess ammonia volatilization if N losses are to be equated to denitrification losses.

### 6.2. Leaching losses ( $k_8$ )

As for ammonia volatilization, leaching losses are sometimes not adequately assessed and can contribute to the N loss in budget studies. In two recent studies these losses were evaluated. Vlek *et al.* [128] reported that leaching losses in lighter texture soils for deep placed  $^{15}\text{N}$ -labeled fertilizer were substantial if percolation rates were high. The losses were particularly high for the supergranules (10% loss for 4.4 mm water/day to 88% loss for 18.3 mm water/day). The leach form was ammonium. These authors recommended not to use deep placement of supergranules if percolation rates exceeded 5 mm/day particularly if the cation-exchange capacity of the soil is low. In another study, Rao and Prasad [90] found less than 20% of the N leached from several fertilizers placed in experimental pots and subjected to the same leaching rate as found in the field for the Delhi region. About half of the N leached from ammonium fertilizers in this study was in the nitrate form. By use of nitrification inhibitors they achieved a modest reduction in the amount of N leached.

It is not clear how important leaching losses might have been in past field and pot nitrogen-budget studies. Since these losses are more easily recognized and tend to be smaller under the typical study conditions than for ammonia volatilization, they probably have not been major contributors to the lack of nitrogen recovery.

### 6.3. Immobilization ( $k_6$ ) and dissimilatory ( $k_5$ ) reduction of nitrate to ammonium

These reactions are of interest because they compete for nitrate with the two nitrate loss mechanisms and thereby conserve nitrogen. Assimilatory and dissimilatory nitrate reduction both produce ammonium but the latter occurs at a faster rate since it is linked to electron transport while the former is slower since it parallels growth. The assimilatory process is inhibited by ammonium and the dissimilatory one by oxygen [18]. In flooded rice soils the ammonium concentration often exceeds 50 ppm which is usually considered sufficient to shut off the assimilatory process [18]. Furthermore, many organisms capable of the dissimilatory process are common in soil [14, 111, 118]. Therefore, it is Tiedje's assumption that the dissimilatory mechanism is the dominant one responsible for any nitrate reduced to ammonium in the rice habitat.

The reduction of  $^{15}\text{NO}_3$  to  $^{15}\text{NH}_4^+$  + organic-N (org-N) in rice soils has been studied by several authors and the percentages of  $^{15}\text{N}$  found in the reduced form range from a few percent to 39% in the absence of a carbon amendment [12, 19, 69]. Additions of available carbon do substantially enhance the reduction but rice straw, which is more slowly degraded, resulted in only 6% reduction to ammonium [12]. However, non-amended rice soil preincubated anaerobically 20 days, which should be similar to the natural rice habitat resulted in 24% reduced N of which 20.5% was free ammonium [12]. Higher amounts of conversion to reduce nitrogen

have been achieved by increasing the density of clostridia (83%) [14], a prevalent soil organism capable of this process, by preincubating with glucose and adding extra glucose (70%) [12], or by maintaining the redox at  $-200$  mv (42%) [13]. Typically during the reduction, free ammonium accumulates and then drops to lower levels as the ammonium is converted to organic nitrogen. However in the studies above where high yields were obtained, much of the reduced nitrogen remained as free ammonium throughout the experiment.

All of the above studies have been made with anaerobic soil slurries to which nitrate was added; this does not reflect the way in which nitrate would naturally enter the anaerobic zone (Fig. 1) since the gradient from high to low redox was avoided. In a very recent study Buresh and Patrick [13] added nitrate periodically to aerobic water overlying estuarine sediment. In this way nitrate was allowed to diffuse into the anaerobic zone much like it should in nature. They found 15% of the  $^{15}\text{NO}_3^-$  recovered as  $\text{NH}_4^+$  whereas 28% was recovered as  $\text{NH}_4^+$  if the  $\text{NO}_3^-$  was directly added to the sediment. Since stronger reducing conditions favor the reduction to ammonium, these results suggest that as nitrate enters the transition zone to the anaerobic layer, the ammonium-producing reaction is less favored compared to denitrification. The generally low recoveries of nitrate in the ammonium form in rice soils and the probably inhibitory effect of the transition zone causes us to conclude that this fate of nitrate is generally minor. It does remain an intriguing process, however, since one could perhaps manage this competition to better favor the conserving reaction over denitrification.

#### 6.4. Other transfers of nitrogen ( $k_1, k_2, k_3, k_4$ )

$k_1$  refers to the rate of release of N from the fertilizer form applied. Most commercial forms have a rapid release but there is special interest for rice culture in use of coatings or materials with low dissolution rates to slow this reaction. These fertilizers will be discussed in the last section. Rates  $k_2, k_3, k_4$  can be discussed as a group since they are integrated precursor steps for denitrification. Patrick, Reddy and Phillips first emphasized the importance of considering each of the sequential steps as a unit to evaluate controls on denitrification losses. Very recently the same authors have been able to obtain experimental values for each of these constants for several rice soils in the Louisiana and Mississippi rice-growing regions [96]. Their rates were:  $\text{NH}_4^+$  diffusion ( $k_2$ )  $0.059$  to  $0.216 \text{ cm}^2 \text{ day}^{-1}$ ,  $\text{NH}_4^+$  oxidation ( $k_3$ )  $1.2$  to  $3.5 \mu\text{g g}^{-1} \text{ day}^{-1}$ ,  $\text{NO}_3^-$  diffusion ( $k_4$ )  $0.96$  to  $1.91 \text{ cm}^2 \text{ day}^{-1}$ , and  $\text{NO}_3^-$  reduction (first order,  $k_{5,6,7}$ )  $0.315$  to  $0.520 \text{ day}^{-1}$ . A quantitative study focused on mechanisms such as this one allows one to evaluate which steps are rate limiting so that management strategies can be soundly developed. In this case the rate-limiting steps were ammonium diffusion into the aerobic layer and nitrification. Nitrate diffusion and reduction were both more than 10 times faster than the above.

## 7. Fertilization and management of paddy rice

In a recent historical review on denitrification Mitsui [75] noted that there has been an enormous number of field studies to minimize this loss since the time when losses attributed to denitrification in paddy soils were first recognized by Shioiri. In earlier periods the recommendation was to apply nitrogen to the dry field and plow it under or mix it with the furrow slice. The irrigation water was introduced soon after; loss of urea by leaching was less than the loss due to nitrification (which occurred before irrigation) and subsequent denitrification. Subsequently it was recognized that deep placement of ammonium fertilizers in flooded soils further improved nitrogen efficiency [1, 3, 54, 60, 72, 74, 82, 95, 116]. The reason for this should be apparent from Fig. 1; with deep placement losses are restricted to leaching or by diffusion of ammonium to the surface ( $k_2$ ). Though the rate constants were not known then, intuition suggested that this rate should be slow in puddled soils, as has now been proved. This strategy was feasible because rice plants have long been recognized to assimilate ammonium forms of nitrogen. A second approach to controlling losses has been to reduce the rate of release of ammonium from the applied material ( $k_1$  or  $k_1$ ) [103]. This has been attempted by use of coatings and low solubility materials. A third approach has been to incorporate nitrification inhibitors with the fertilizer in order to reduce rate  $k_3$ . The fertilizer materials that have been evaluated most recently are summarized in Table 1;

Table 1. Most common fertilizers recently evaluated for paddy rice culture

Readily available
AS (ammonium sulfate)
GU (granular urea)
Large granule for deep placement
USG (urea supergranule, 1 to 3g)
MBU (mudball urea)
Slow release
SCU (sulfur-coated urea) <sup>a</sup>
SPCU (silicate polymer-coated urea)
SC-USG (sulfur-coated urea supergranule)
IBDU (isobutylene diurea) <sup>b</sup>
Nitrification inhibitors incorporated with fertilizer
NSU (N-Serve blended urea) <sup>c</sup>
NCU (Neem cake-coated urea) <sup>d</sup>

<sup>a</sup> Other less successful or inexpensive coatings which have been investigated are plastics, waxes, resins

<sup>b</sup> Other materials with low solubility products are ureaform and oxamide

<sup>c</sup> Other commercial nitrification inhibitors exist [54], principally thiazoles, triazines and thioureas, but none seem as promising as N-Serve

<sup>d</sup> Neem cake is the most effective natural nitrification inhibitor; it is obtained from the kernels of the neem tree (*Azadirachta indica*) which is a native, widely distributed tree in India and other semi-arid tropical regions [90]. Neem is coated on urea by use of coaltar

they are grouped according to the strategies which are employed to reduce the rate constants and thus the losses. There can also be combinations of these strategies to further reduce losses, e.g. deep placement of SC-USG [21]. The results from testing of some of these strategies are further described below.

The initial difficulty with deep placement was how to accomplish it in a practical manner. An early approach was to pack urea in a peat or mudball which then could be inserted by hand to the desired depth. Experiments with  $^{15}\text{N}$ -labeled peat ball fertilizer showed that deep placement of nitrogen at the panicle formation stage resulted in 86% of the nitrogen being taken up by the rice plant; surface broadcasting gave only 50% nitrogen uptake [75]. A second approach was mechanized deep placement of liquid ammonia or liquid fertilizers as was used in Japan. Also, fertilizer applicators attached to engine-driven cultivators, and deep-point applicators of paste fertilizer attached to engine-driven transplanters have been evaluated [75]. In 1974 IRRI [60] recommended enclosing concentrated urea in small mudballs and placing them about 10 cm deep in the soil beside newly transplanted seedlings. This process produced a yield of  $8 \text{ t ha}^{-1}$  of rice with 60 kg of nitrogen applied per hectare compared with  $6.6 \text{ t ha}^{-1}$  using  $100 \text{ kg N ha}^{-1}$  when applied as a topdressing. However, so many mudballs are needed ( $62,500 \text{ ha}^{-1}$ ) that the technique is probably feasible only in areas where land holdings are small and labor is plentiful [61].

IRRI [60] has also developed inexpensive, manually-operated machines to apply both fertilizer and insecticides. The chemicals are applied in a band below the soil surface between the new seedlings. To eliminate the process of making mudballs the International Fertilizer Development Center (IFDC), at Muscle Shoals, Alabama, USA, developed supergranules and briquets of varying quantities of urea. When tested at IRRI briquets equalled mudballs in efficiency of fertilizer recovery by the plant [62]. Another N-application method is mixing of urea with moist soil and incubating the mixture for 24 hours before application so losses can be limited by converting the released nitrogen to  $\text{NH}_4^+$  [91]. At IRRI, foliar application of urea in split doses was also tried but the results were not favorable [91].

A slow release nitrogen fertilizer developed by the Tennessee Valley Authority, sulfur-coated urea (SCU), has been tested in rice culture and is a promising fertilizer for the tropical regions. The advantages of SCU have been more obvious in soils with intermittent flooding and drying [91]. Anjaneya [4] obtained equivalent yields with  $100 \text{ kg SCU-N ha}^{-1}$  and  $150 \text{ kg urea-N ha}^{-1}$ . Similar results were obtained at the IRRI Center [62] with SCU at  $84 \text{ kg N ha}^{-1}$  compared to split applications of urea at  $120 \text{ kg N ha}^{-1}$  as part of the study of the International Network on Fertilizer Efficiency for Rice (INFER). From 1975 to 1977, 84 trials were conducted in 11 countries to obtain an insight into the relationship between N source, N management, and N efficiency under a variety of environmental conditions. In a recent summary of INSFFER (International Network on Soil Fertility and Fertilizer Efficiency in Rice), and other comparative studies, Craswell and Vlek [22] report that use of supergranules resulted in a significant increase in rice yield over that with broadcast prilled urea in 42% of the studies and was slightly better or equal in most of the rest.

With measurement of rates of  $N_2O$  reduction used for estimating the denitrifying capacity of potted rice-growing soils enriched with urea applied on the surface or at depth, or with SCU applied one time on the surface before transplanting rice seedlings, Garcia [48] showed that the best grain yield was obtained with deep placement of urea; with SCU the yield was equivalent to that obtained with non-coated urea spread in greater quantities and at two stages of growth. Similarly, other slow release nitrogen fertilizers like oxamide and IBDU were also found to increase the yield of rice by preventing gaseous losses [62, 93].

Losses of nitrogen by denitrification are also reduced if the nitrification of ammonium is prevented. A number of chemicals are known to inhibit nitrification and the two most popular are N-Serve [2-chloro-6-(trichloromethyl) pyridine] and AN (2-amino-4-chloro-6-methyl pyrimidine). Increase yields of rice by the use of N-Serve and AN have been reported [84, 93, 137]. Other studies have shown slight to moderate reduction in N loss by use of N-Serve [90, 102, 106] or neem-coated fertilizer [106]. The effectiveness of inhibitors in the field is not well established, however. A 22% increase in rice grain yield due to treatment of urea with an acetone extract of neem kernels was obtained in India [91]. The cake, seed, bark, and leaf of Karanj (*Pongamia glabra*) also possess nitrification-inhibiting properties [19]. Some chemicals have been reported to specifically inhibit denitrification, such as pesticides [5, 76], the azides and some aromatic ketonitriles [58, 59] but these products are expensive and not particularly promising for agricultural use.

Very recent studies on the most promising N-fertilizer materials have provided quantitative data on yield and nitrogen recoveries and losses. Some of these results are summarized in Table 2. The trends are clear and consistent. Yields and nitrogen

Table 2. Summary of recent studies done on the effectiveness of different fertilizer materials

Fertilizer (Ref)	Grain yield			Fertilizer-N recovered in plant			$NH_3$ volatilized [126]	N leached [90]	N lost [90] <sup>c</sup>
	[21]	[90]	[106] <sup>a</sup>	[21] <sup>b</sup>	[90]	[106] <sup>b</sup>			
	$g\ pot^{-1}$	$g\ ha^{-1}$		% of applied			%	%	%
Basal urea (BU)	38	19	44	28	25	27	48	11.5	43
Split urea	47	—	—	48	—	—	—	—	—
Basal $(NH_4)_2SO_4$	47	21	—	54	31	—	17	13.7	31
Basal-SCU	40	24	46	56	40	32	<3	7.1	15
USG (deep)	45	30	—	69	60	—	3	12.6	18
SC-USG (deep)	52	—	—	84	—	—	—	—	—
BU + N-Serve	—	26	49	—	41	38	—	9.1	20
BU + Neem	—	23	49	—	38	40	—	9.8	25

<sup>a</sup> Field experiment

<sup>b</sup> Recovery is of  $^{15}N$  label

<sup>c</sup> Since label was not used losses are sum of denitrification,  $NH_3$  volatilization, and any net immobilization; leaching was accounted for in previous column

recovery by plants go up and losses go down for materials employing slow release, deep placement, and/or inhibitors. The best material seems to be the combination of deep placement and slow release, the sulfur-coated urea supergranule (SC-USG). In other studies Savant and De Datta [103, 104] have shown that the release of ammonium from sulfur-coated materials was much slower than from USG or prilled urea. The apparent diffusion coefficient of ammonia from USG was  $2.2 \times 10^{-2} \text{ cm}^2 \text{ day}^{-1}$  versus  $1.6 \times 10^{-3} \text{ cm}^2 \text{ day}^{-1}$  for SCU [104]. As a result soil ammonium concentrations increased for four weeks for the sulfur-coated materials where other materials showed an ammonium maximum immediately and then declined. From this study it appears that the reduction of  $k_1$  results in yield improvement (Table 2). The second benefit of the SC-USG fertilization method is that it did not suppress growth of blue-green algae nor their acetylene reducing activity [98]. Fertilizers that release ammonium into the floodwater favor green algae which do not fix  $\text{N}_2$ .

Though denitrification activity of paddy soils has been widely demonstrated by gas chromatographic methods, quantitation of actual N losses by this mechanism has not been reported. In Table 2, the last column on N losses indirectly suggest denitrification is important since N-Serve reduced losses from urea-N by 23%; this value may be taken as an estimate of denitrification loss since in this study leaching and volatilization have been separately accounted for.

## 8. Conclusion

To minimize N losses slow-release nitrogen fertilizers are promising for the tropical regions. The objective of these fertilizers is to make the amount of N released coincide with the nitrogen requirement of growing plants, especially the tillering and heading stages, and thereby reducing N losses. There are two ways to achieve this objective: (1) use of chemicals with slow rates of dissolution such as IBDU. Field experiments in Japan showed that IBDU produced 20% more rice than ammonium sulfate applied in two split doses [91], (2) coating of conventional fertilizers to reduce their dissolution rates; of these products SCU has been the most widely tested for rice. The response to SCU was generally superior to the response to urea and to split applications of N [22].

Before new fertilizers can be recommended, the economics must show that the new technique is worth the extra cost. Average data for INFER trials showed that the extra yield resulting from use of SCU more than covered the 60% greater cost of this material. Most recent results suggest that the combination of controlled release especially by sulfur-coating and deep placement should substantially improve the efficiency of fertilizer nitrogen. For readers interested in the more agronomic aspects of efficient fertilizer use in rice crops, they are referred to the recent and comprehensive reviews by Craswell and Vlek [20, 22].

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