Nitrification-denitrification at the plant root–sediment interface in wetlands

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Abstract

Oxygen transport through the air spaces (aerenchyma tissue) of the stem and roots of aquatic macrophytes into the root zone supports nitrification of NH_4^+, with the NO_3^- formed diffusing into the adjacent anaerobic zone where it undergoes denitrification. To test this hypothesis, we conducted a growth chamber study to determine the transformation of applied $^{15}NH_4^+$-N to $^{15}N_2$ in the root zone of three aquatic macrophytes: rice (*Oryza sativa* L.), pickerel weed (*Pontederia cordata* L.), and soft rush (*Juncus effusus* L.). Detection of gaseous $^{15}N_2$ in the air above the floodwater of the soil column with aquatic plants provided direct evidence of nitrification-denitrification in the root zone, while such losses were not measurable for soil columns without plants. Air spaces in aquatic plants can also function as conduits for denitrified gases from anaerobic sediments to the atmosphere. Maximal $^{15}N_2$ flux due to this process was 102, 113, and 122 mg N m^{-2} d^{-1} for soft rush, rice, and pickerel weed. This N loss mechanism has important agronomic and ecological consequences.

Denitrification is the major pathway for N loss from aquatic sediments to the atmosphere, primarily through a series of biochemical processes: mineralization of organic nitrogen and nitrification of NH_4^+-N, followed by denitrification (Reddy and Patrick 1984). Nitrate concentrations in these systems are generally low, primarily due to rapid denitrification and slow rates of nitrification. Nitrification in littoral sediments of lakes and wetlands occurs primarily in two zones: the water column and oxidized sediment–water interface, and the oxidized rhizosphere. In both zones, there are two basic requirements, oxygen supply from the atmosphere into the zone where nitrification occurs and NH_4^+ supply through mineralization of organic N or external inputs of inorganic N.

In littoral sediments of lakes and wetlands, N cycling is regulated by transport of O_2 through aerenchymous tissue of stems and roots into the root zone. The movement of air in plants is by both diffusion and advection (Dacey 1980). CO_2 formed during root respiration can also diffuse through the same O_2 transport system, but in the opposite direction for eventual release into the atmosphere (Conway 1937; Teal and Kanwisher 1966; Grosse and Mevi-Schutz 1987; Mevi-Schutz and Grosse 1988).

Oxygen transported into the root zone is involved in several biogeochemical processes. They include oxidation of reduced compounds such as ferrous iron to the insoluble ferric form, of sulfides to elemental sulfur (Ponnamperuma 1972; Engler and Patrick 1975), and of NH_4^+ to NO_3^-. In many mineral soils, reddish-brown precipitates on the root surface of wetland plants are due to the oxidation of ferrous iron (Bacha and Hossner 1977; Chen et al. 1980).

Among the more poorly understood N reactions in aquatic soils and sediments
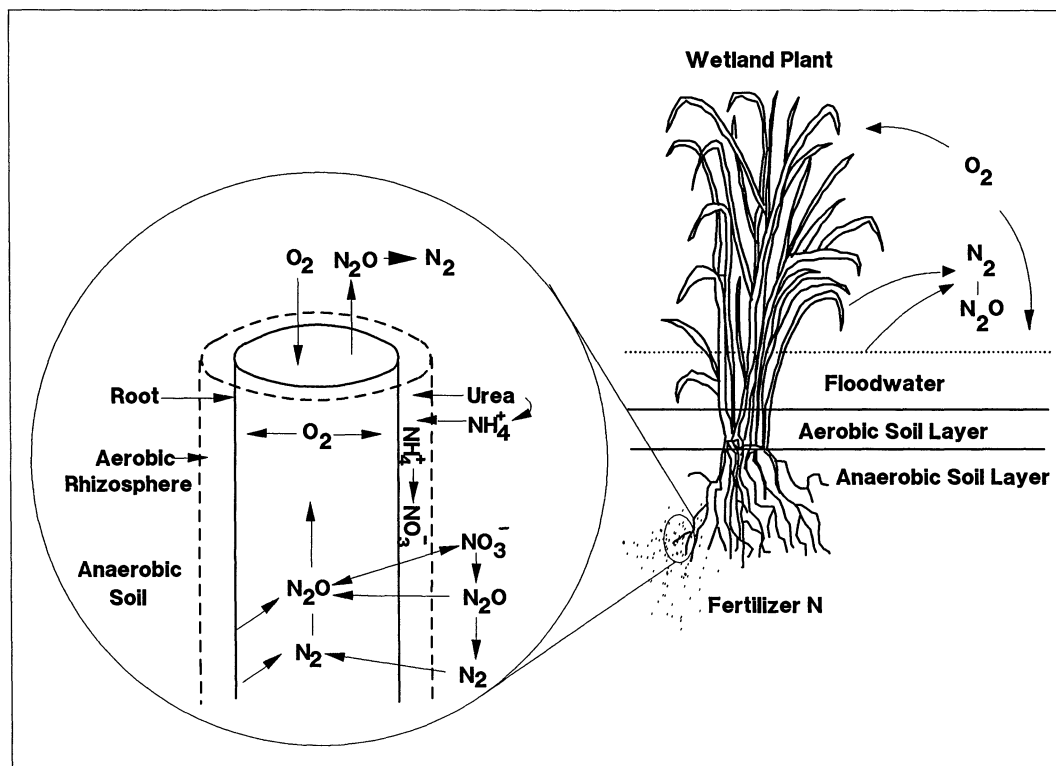
Fig. 1. Schematic presentation of nitrification-denitrification in the root zone of rice and other aquatic macrophytes.

(Kemp et al. 1982; Reddy and Patrick 1984, 1986a; Seitzinger 1988) are the processes that take place in the vicinity of the roots of plants (Fig. 1) in littoral sediments or wetlands. Aquatic soils and sediments are characterized by the accumulation of NH_4^+ (Ponnamperuma 1972). Ammonium in the anaerobic zone of the sediment diffuses either into the root zone or into the overlying water column as a result of concentration gradients. Some of the NH_4^+ is taken up directly by aquatic macrophytes, and part is oxidized to NO_3^-. The NO_3^- formed is either taken up by the plant or diffused into adjacent anaerobic zones where it is denitrified.

To test this hypothesis, we have conducted a series of experiments, of which the first was reported by Reddy and Patrick (1986b) with rice (*Oryza sativa* L.) as a model aquatic plant. In that study, we reported N loss during an experiment designed to maximize nitrification-denitrification reac-

tions in the root zone of rice. Nitrogen losses were measured with the mass balance of added N in the rhizosphere (soil column with rice plants) and nonrhizosphere (soil column without rice plants) soil systems. In the rhizosphere system, 18% of the applied N was unaccounted for, while <5% of the applied N was unaccounted for in the non-rhizosphere system. Although we attribute the N loss to nitrification-denitrification reactions in the root zone, quantitative documentation can be provided only when gaseous end products derived from the applied NH_4^+-N are measured and accounted for.

For nitrification to take place in anaerobic layers of the litoral sediment, O_2 must enter the sediment by means other than direct diffusion through the water column. It has been known for some time (Armstrong 1964, 1967, 1979) that some O_2 can enter a wetland soil by diffusing through the stems and roots of aquatic plants. Denitrifying activity measured as reduction of N_2O to N_2 was

found to be greater in the rice rhizosphere than nonrhizosphere treatments (Raimbault et al. 1977). Oxygen transport into the root zone and subsequent nitrification in the rhizosphere of eelgrass (*Zostera maxima*) in littoral sediments has been demonstrated by Iizumi and Hattori (1980). More recently, Christensen and Sorensen (1986) measured higher denitrification rates in lake sediments covered with *Littorella* sp. than in nonvegetated areas. They concluded that higher denitrification rates in vegetated sediments were due to O_2 transport into the root zone that stimulated nitrification. Under certain conditions, however, such as in algal mats covering rocks in streams, denitrification rates are inhibited by O_2 production as evidenced by lower rates in the light than in the dark (Triska and Oremland 1981).

The objectives of our studies are to demonstrate the possibility of nitrification-denitrification reactions in the root zone of selected aquatic plants and to determine the escape route of denitrification gases from the root zone into the atmosphere above the water column.

Materials and methods

The three aquatic macrophytes used in this study were rice (*O. sativa* L.), a common agronomic crop, and pickerel weed (*Pontederia cordata* L.) and soft rush (*Juncus effusus* L.), two freshwater emergent macrophytes in Florida. The soil used to culture the rice was Crowley silt loam (Typic Albaqualfs) collected from the Rice Experiment Station, Crowley, Louisiana. The sediment used to culture the two freshwater wetland plants was obtained from wetlands located north of Lake Okeechobee in south Florida.

Fate of ammonium and nitrate N in the root zone of rice—Air-dried Crowley soil was ground to pass through a 0.84-mm sieve and placed in Plexiglas cylinders (30 cm long and 10-cm i.d.) (Reddy and Patrick 1986*b*). Six rubber septa (three on each side of the cylinder, spaced 2 cm apart) had been previously installed in the bottom of the cylinder. Before planting rice, the soil was treated with 50 mg of N kg^{-1} (as NH_4Cl), 25 mg of P kg^{-1} (as KH_2PO_4), and 50 mg

of K kg^{-1} (as KCl), and flooded with 3 cm of water. Soil column depth at this stage was 13 cm. In one set of columns, two healthy 20-d-old rice seedlings (var. Labonett) were transplanted; the second set of columns was maintained without plants. The soil portion of the columns was wrapped in aluminum foil to prevent direct exposure of the soil to light. The surface of the floodwater was exposed to lights (250 μEinst cm^{-2} s^{-1}) operated on a 14:10 L/D cycle. Temperature during the growth period was 25°C. After the plants were grown for 15 d, additional soil was added to increase the soil depth to 20 cm.

To obtain direct evidence for nitrification-denitrification in the root zone of rice, we applied labeled $^{15}NH_4Cl$ (50 atom% ^{15}N) fertilizer (150 mg N per soil column) in two equal applications at 28 and 35 d after planting by injecting the fertilizer solution through rubber septa located at the 15–20-cm soil depth. There were eight columns for each rhizosphere and nonrhizosphere soil. After the second application of $^{15}NH_4^+$-N, plants in four columns were allowed to grow for an additional 11 d; those in the second set of four columns were grown for 18 d before they were killed to measure gas and mass balance. After 11 d, shoots of the plants in one set of columns (four replicates per treatment) were harvested (with 3 cm of the shoot left above the floodwater) and a gas-collection chamber was placed over the column and sealed with silicone to prevent gas leakage. Gas samples (N_2 and N_2O) were obtained after 24-h equilibration and analyzed for ^{15}N content with an isotope-ratio mass spectrometer. N_2O was reduced to N_2 by injecting the gas samples into heated copper columns before their entry into the mass spectrometer. Plant samples were dried at 70°C and analyzed for total N and labeled N. Soil samples were extracted with 2 M KCl, and both the extracted solutions and the residual soil samples were analyzed for total and labeled N (Bremner and Mulvaney 1982; Hauck 1982). This procedure was repeated on the second set of soil columns, 18 d after the second application of $^{15}NH_4^+$-N.

A second experiment was conducted to determine the escape route of gases formed

during denitrification in the root zone of rice; labeled $K^{15}NO_3$ (50 atom% excess; 150 mg N per soil column) was injected into rhizosphere and nonrhizosphere soil columns between the 15- and 20-cm depths. Rice plants at the time of NO_3^- application were 35-d old. After 2 d, plant shoots were harvested and a gas-collection chamber was placed over the column. After a 12-h equilibration, N_2O and N_2 gas samples were obtained and analyzed for ^{15}N content via mass spectrometer. The gas-collection chamber was removed at the end of each sampling period. This procedure was repeated at 4, 7, and 10 d after NO_3^- application. Plant and soil samples were analyzed for total and labeled N contents with the procedures described above.

Fate of ammonium N in the root zone of freshwater aquatic macrophytes — The experimental setup was similar to the one described for rice. Wet sediment was placed in Plexiglas cylinders (30 cm long and 10-cm i.d.) to a depth of 20 cm. Sediment was flooded with 3 cm of distilled water. One seedling of pickerel weed or two seedlings of soft rush were transplanted into the sediment columns, and the plants were allowed to grow for a period of 28 d under lights (250 μEinst cm^{-1} s^{-1}). There were four replicates for each plant type. Similarly, four additional columns containing the same sediment with no plants were established to function as a control.

At the end of 28 d, labeled $^{15}NH_4^+$-N was applied (100 mg N per column) at the 15–20-cm depth. Adding $^{15}NH_4^+$-N increased the overall NH_4^+ content of the sediment ~10%. All columns were incubated under lights for an additional period of 13 d. At that time, shoots of the plants were harvested (with 3 cm of the shoot left above the floodwater) and a gas-collection chamber was placed over the column and sealed with silicone to prevent gas leakage. Gas samples were obtained after equilibration for 24 h and anlyzed for labeled $N_2O + N_2$ on an isotope-ratio mass spectrometer as described for the earlier experiments. This procedure was repeated at 14, 15, 19, 21, and 26 d after $^{15}NH_4^+$-N application. Plant samples were dried at 70°C and analyzed for total and labeled N. Sediment samples

were extracted with 2 M KCl, and both the extracted solutions and the residual sediments were analyzed for labeled and nonlabeled N fractions, as described previously.

Results and discussion

Fate of ammonium N and nitrate N in the root zone of rice — In the soil columns treated with $^{15}NH_4Cl$, a significant increase in ^{15}N enrichment of air above the floodwater of the rhizosphere soil column was recorded as compared to the nonrhizosphere treatments (Table 1). Atom% ^{15}N in the air above the floodwater of the soil column with plants was increased from a background level of 0.369 to 0.425 in 11 d; the increase for the soil column without plants was only from 0.369 to 0.378 (Table 1). Since very little or no N_2O was detected, we will refer to the gaseous end products formed during denitrification as N_2 only. The flux of $^{15}N_2$ was found to be sixfold to ninefold higher for the rhizosphere soil column than for the nonrhizosphere soil column. For the rhizosphere soil column, the flux of $^{15}N_2$ was 11.3 μg N cm^{-2} d^{-1} 11 d after fertilizer application and 7.8 after 18 d. For the nonrhizosphere soil column, corresponding fluxes were 1.7 and 0.8 μg N cm^{-2} d^{-1}. It should be noted that these flux measurements were based on one time measurement, and it was assumed that gaseous losses during the 18 d after application were linear. In this experiment it was not possible to make continous flux measurements, since we had to kill the shoots to measure the gases and obtain ^{15}N mass balance for soil-water-plant systems. Mass balance of ^{15}N (Table 2) indicated that 34% of the added NH_4^+-N was not accounted for in the rhizosphere treatment, and only 11% was lost from the nonrhizosphere treatment. ^{15}N recovery in the gases amounted to only 4–14% of the N lost from the system, indicating that a substantial portion of the gases may be trapped in the root zone. Low recovery of ^{15}N in gaseous forms was also due to errors associated with N_2 flux measurements, which probably underestimated overall N_2 production.

Transport of a portion of the denitrification gases (N_2) from soil to the atmosphere above the floodwater was rapid for

Table 1. Transport of $N_2O + N_2$ gases formed during nitrification-denitrification in a flooded Crowley silt loam soil with and without rice plants. A gas-collecting chamber was placed over the soil column for a period of 24 h before gas samples were obtained. Age of the seedlings at the time of NH_4^+-N application was 35 d. Error bounds represent ±1 SD.

Time after $^{15}NH_4^+$-N application (d)	Rhizosphere	Nonrhizosphere
	(atom% $^{15}N_2$ in air above the floodwater*)	
11	0.425±0.013	0.378±0.006
18	0.408±0.001	0.374±0.002

* Atom% ^{15}N in the air sample outside the chamber was 0.369±0.001.

Table 3. Transport of $N_2 + N_2O$ gases formed during denitrification in a flooded Crowley silt loam soil with and without rice plants. A gas-collecting chamber was placed over the system for a period of 16 h on days 2, 4, and 10, and for 12 h on day 7. Error bounds represent ±1 SD.

Time after $^{15}NO_3^-$-N added (d)	Rhizosphere	Nonrhizosphere
	(atom% $^{15}N_2$ in air above the floodwater*)	
0	0.369±0.001	0.369±0.001
2	0.688±0.025	0.369±0.001
4	0.608±0.029	0.369±0.001
7	0.459±0.028	0.373±0.006
10	0.467±0.023	0.375±0.007

* Atom% ^{15}N in the air sample outside the chamber was 0.368±0.0001.

the soil containing rice plants. Only trace concentrations of N_2O were detected, indicating that most N_2O had been further reduced to N_2. Little or no denitrified N was recovered in the atmosphere above the floodwater for the soil column with no plants (Table 3). Atom% $^{15}N_2$ in the headspace gases increased from a background level of 0.369 to 0.688 in 2 d for the soil column with rice plants. This increase in $^{15}N_2$ content was due to reduction of the $^{15}NO_3^-$ added to the root zone of rice (15–20-cm

Table 2. Mass balance of added $^{15}NH_4^+$-N in flooded Crowley silt loam planted to rice; 150 mg of $^{15}NH_4^+$-N added per core at 15–20-cm depth in two equal applications at 28 and 35 d after planting. ^{15}N balance was determined at 46 and 53 d after planting or 11 and 18 d after the second application of $^{15}NH_4^+$-N. Rhizosphere—with rice plants; nonrhizosphere—without rice plants. Values shown in parentheses are percent of applied fertilizer N.

N fraction	Rhizosphere	Nonrhizosphere
	[mg ^{15}N (soil column)$^{-1}$]	
11 d after second $^{15}NH_4^+$-N application		
Soil		
Inorganic	70.2(46.8)	132.6(88.4)
Organic	14.3(9.5)	8.8(5.9)
Plant	40.2(26.8)	—
$N_2O + N_2$	6.3(4.2)	0.9(0.6)
Total	130.9(87.2)	142.3(94.9)
Unaccounted	19.2(12.8)	7.7(5.1)
18 d after second $^{15}NH_4^+$-N application		
Soil		
Inorganic	37.2(24.8)	123.7(82.4)
Organic	10.7(7.1)	9.6(6.4)
Plant	51.9(34.1)	—
$N_2O + N_2$	7.1(4.7)	0.7(0.5)
Total	106.1(70.7)	134.0(89.3)
Unaccounted	43.9(29.3)	16.0(10.7)

depth) and subsequent movement of N_2 to the atmosphere. During the first 2 d for the soil columns with no plants, however, little or no change in ^{15}N content of the air above the floodwater had occurred. Even after 10 d, only a small amount of denitrification gases had escaped from the soil columns without plants.

The flux of $^{15}N_2$ from the soil columns with rice plants was in the range of 29.4–95.9 μg N cm^{-2} d^{-1}, while the corresponding fluxes from columns without plants were 0.09–1.77 μg N cm^{-2} d^{-1}. These results strongly suggest that cortical intercellular space in the stems and roots of rice plants was the primary means of gaseous transport from the root zone to the atmosphere (Armstrong and Beckett 1987).

Data on the mass balance of added $^{15}NO_3^-$-N (Table 4) indicate that, for both rhizosphere and nonrhizosphere soil columns, 88–94% of the added $^{15}NO_3^-$-N was unaccounted for and was assumed to be lost through denitrification. During the first 4 d, however, $^{15}N_2$ gas was detected in the headspace only for the rhizosphere treatment, while virtually all gases formed in the nonrhizosphere treatment remained trapped in the soil. In this experiment, only 24% of the denitrified gases escaped as N_2 through the plant, and the remaining 76% of denitrified gases probably stayed trapped in the soil. Under laboratory conditions, 28% of the [^{15}N]urea and 40% of the K$^{15}NO_3$ remained trapped as N_2 in the soil 33 d after application (Lindau et al. 1988). Buresh and Aus-

Table 4. Mass balance of added $^{15}NO_3^-$-N in flooded Crowley silt loam planted to rice; 150 mg of $^{15}NO_3^-$-N added per core at 15–20-cm depth at 35 d after planting. N balance was measured 10 d after $^{15}NO_3^-$-N application (45 d after planting). Rhizosphere—with rice plants; nonrhizosphere—without rice plants. Values shown in parentheses are percent of applied fertilizer N.

N fraction	Rhizosphere	Nonrhizosphere
	[mg ^{15}N (soil column)$^{-1}$]	
Soil		
Nitrate	0.4(0.3)	12.5(8.4)
Ammonium	2.1(1.4)	1.1(0.7)
Organic	6.4(4.3)	4.3(2.9)
Plant	0.9(0.6)	
$N_2O + N_2$	26.7(17.8)	0.5(0.3)
Total	36.6(24.4)	18.5(12.3)
Unaccounted	113.5(75.6)	131.5(87.8)

tin (1988) observed that $(N_2O + N_2)$-^{15}N flux was much smaller (1.1% of applied N) than total gaseous ^{15}N loss (40% of applied N) as measured from ^{15}N unaccounted for in the ^{15}N mass balance. Our experiment revealed that denitrification gases which had formed in the root zone of rice plants es-

Table 5. Transport of $N_2O + N_2$ gases formed during nitrification-denitrification in a wetland soil with and without plants. A gas-collecting chamber was placed over the sediment for a period of 24 h at each sampling period. $^{15}NH_4^+$-N applied—100 mg N (column)$^{-1}$. Error bounds represent ±1 SD.

Days after $^{15}NH_4^+$-N added	Atom% $^{15}N_2$ in the air above floodwater	Increase in atom% ^{15}N over natural standard
	Pontederia cordata	
14	0.439±0.009	0.067
15	0.450±0.009	0.082
19	0.430±0.022	0.061
21	0.407±0.041	0.038
26	0.378±0.006	0.010
	Juncus effusus	
14	0.412±0.040	0.044
15	0.413±0.015	0.045
19	0.443±0.014	0.074
21	0.431±0.013	0.062
26	0.412±0.012	0.043
	Control (no plants)	
14	0.379±0.010	0.010
15	0.380±0.011	0.012
19	0.377±0.006	0.008
21	0.379±0.009	0.010
26	0.376±0.005	0.007

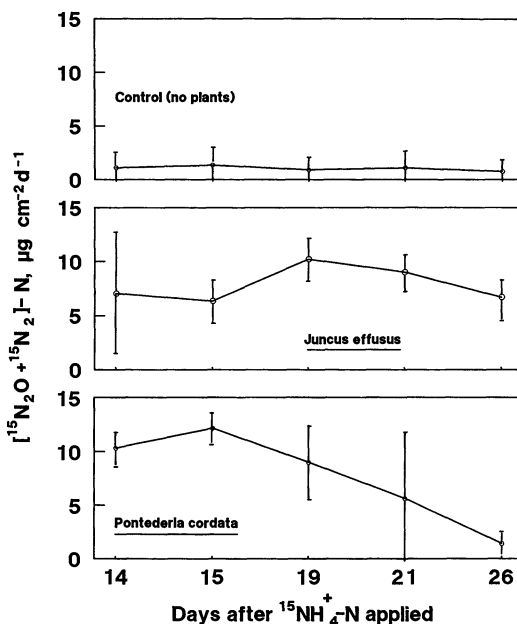
Fig. 2. Flux of N_2 through selected aquatic macrophytes. Error bars represent ±1 SD.

caped into the atmosphere through the plant. Diffusion of N_2 gases through the saturated soil column without rice plants was very slow. Studies at Duck Lake, Michigan, have also shown that shoots of the yellow water lily (*Nuphar luteum*) function as conduits for the transport of CH_4 from anaerobic sediments to the atmosphere above overlying floodwaters (Dacey and Klug 1979; Cicerone and Shetter 1981).

Fate of ammonium N in the root zone of freshwater wetland plants—Atom% $^{15}N_2$ in the air above the floodwater of the sediment columns containing pickerel weed and soft rush was higher than for the columns with no plants, indicating the occurrence of nitrification-denitrification in the root zone (Table 5). Negligible concentrations of $^{15}N_2O$ were detected in the air above the floodwater for the sediment columns with and without plants. The flux of ^{15}N from pickerel weed sediment columns was in the range of 1.43–12.18 μg ^{15}N cm^{-2} d^{-1}, with a maximal rate of N loss measured at 15 d after $^{15}NH_4^+$-N application (Fig. 2). For soft rush, $^{15}N_2$ fluxes were in the range of 6.7–10.2 μg ^{15}N cm^{-2} d^{-1}; N_2 flux from sediment columns with no plants was in the range of

Table 6. Mass balance of $^{15}NH_4^+$-N in soil-water-plant systems. Wetland soils and plants obtained from Lake Okeechobee drainage basin (south Florida); 100 mg $^{15}NH_4^+$-N applied into the root zone. Mass balance was measured 26 d after $^{15}NH_4^+$-N application for *Pontederia cordata* and no-plant treatments, and 27 d for *Juncus effusus*.

N fraction	P. cordata	J. effusus	No plants
	(% of applied$^{15}NH_4^+$-N)		
Floodwater	ND	0.2	0.1
Sediment			
Inorganic	24.5	40.4	73.9
Organic	11.3	10.2	16.7
Plant	33.4	19.3	—
$(N_2O + N_2)$*	7.3	6.6	1.3
Total	76.5	76.8	92.0
Unaccounted	23.5	23.4	8.0

* Calculated value based on gaseous fluxes measured 14, 15, 19, 22, and 26 d after $^{15}NH_4^+$-N application.

0.76–1.36 μg ^{15}N cm^{-2} d^{-1}. These results show a severalfold higher loss of N from the sediment column with plants compared to the columns without plants.

Data on mass balance of added $^{15}NH_4^+$-N (Table 6) indicate that, for both freshwater emergent aquatic macrophytes, 77% of the applied ^{15}N was recovered. Nitrogen loss measured by direct measurement of gaseous $^{15}N_2$ flux was 6.6 and 7.3% of applied N for soft rush and pickerel weed, and 23% of applied N loss was unaccounted for by both plants, indicating that about 16% of the denitrified gas was trapped in the sediment. About 8% of the applied ^{15}N was lost (1.3% of the applied N loss was measured by gaseous flux) from the sediment column with no plants, indicating that some of the applied $^{15}NH_4^+$-N at 15–20-cm depth had diffused into the floodwater–sediment interface where it was oxidized to nitrate and subsequently denitrified.

In aquatic ecosystems used for rice production, nitrification-denitrification at the plant root–sediment interface may have significant implications with respect to N management in the soil column. Loss of N applied to soil with plants as a result of the nitrification-denitrification sequence (as determined by gaseous fluxes) was 6.3 mg N (soil column)$^{-1}$ at 11 d after fertilization. It accounts for only 25% of the total N lost from the system [totaling 25.5 mg N (soil column)$^{-1}$]. Eighteen days after planting, total loss of N increased to 51.0 mg N (soil column)$^{-1}$ for the rhizosphere treatment. These losses amount to 17 and 34% of the applied N at 11 and 17 d after fertilization. During the same period, N losses from the nonrhizosphere treatment were 5.7 and 11.2% of the applied N, respectively. Results of pot and field experiments from 76 studies (Reddy and Patrick 1986a) with ^{15}N indicate that about 35% of the applied N (ammonium or urea) was recovered by the plant and 26% remained unaccounted for. Losses of N from these systems were attributed to NH_3 volatilization, nitrification-denitrification, and leaching. Ammonia volatilization was found to be dominant in soils where fertilizer N was surface applied (Mikkelsen et al. 1978), although losses due to this process were significantly reduced when fertilizer N was applied directly to the root zone. The efficiency of N use by rice did not improve, however, and the portion of N unaccounted for remained high.

In a field study (Patrick and Reddy 1976), 30% of the applied fertilizer N had been lost 4 weeks after fertilizer was added to the root zone (7.5-cm depth). Similarly, in other studies, 45% of the deep-placed fertilizer N was not accounted for and was assumed to be lost from the system (R. Wetselaar *cited by* Savant and DeDatta 1982). Ammonia volatilization in this study accounted for only 0.7% of the applied fertilizer N. These field studies and results of our study strongly suggest that such N losses were due to nitrification-denitrification in the root zone of rice. Several other studies (Reddy and Patrick 1980, 1986b; Fillery and Vlek 1982; Smith and DeLaune 1984) have attempted to evaluate the effect of rice plants on N loss from flooded soils, but none has provided definitive conclusions on the direct effects of plants on N loss. Under field conditions, it is also likely that NO_3^- may become limiting to overall N loss due to nitrification-denitrification because of the competition for NO_3^- between denitrifying bacteria and rice roots (Reddy and Patrick 1986b). Under field conditions, it is also likely that NO_3^- may become limiting to overall N loss due to nitrification-denitrification because of the competition for NO_3^- between denitrifying

bacteria and rice roots (Reddy and Patrick 1986*b*). Because of high electron pressure in the root zone and preferential uptake of NH_4^+ over NO_3^- by the rice plant, denitrifying bacteria may outcompete plants for NO_3^-.

In unmanaged natural wetlands and littoral lake sediments containing pickerel weed and soft rush, nitrification-denitrification in the root zone can play a significant role in transforming mineralized organic N to N_2 gas and in subsequent loss from the ecosystem. In our study, NH_4^+ concentrations in the root zone were higher than those observed under natural conditions. Under certain conditions, however, anthropogenic N inputs of urban- and agricultural-related wastewaters can result in significantly elevated levels of NH_4^+ in the root zone. Our results suggest that about 23% of the added NH_4^+-N was lost from the root zone within 27 d after application, and about a third of the denitrified N moved up through the aerenchymous structure of the plants into the atmosphere above the floodwater. Maximal potential N_2 flux was 122 and 102 mg N m^{-2} d^{-1} (1.22 and 1.02 kg N ha^{-1} d^{-1}) for pickerel weed and soft rush.

In littoral sediments, rhizosphere-associated denitrification can account for a major portion of mineralized organic-N loss. For example, in littoral lake sediments colonized by the perennial macrophyte *Littorella uniform,* the root zone accounted for as much as 50–70% of annual denitrification (Christensen and Sorensen 1986). Oxygen transport through the leaves of eelgrass into the rhizomes and roots and into the adjacent root zone stimulated nitrification. In sandy lake sediments covered with *Littorella* sp., however, O_2 transport into the root zone inhibited denitrification and stimulated nitrification (Christensen and Sorensen 1986). In a recent study, Reddy et al. (in prep.) have shown the rate of O_2 transport of pickerel weed to be 19 mg O_2 (g root)$^{-1}$ d^{-1}; similarly, DeBusk et al. (in prep.) have shown CH_4 from sediments containing pickerel weed to be 0.5 mg CH_4 m^{-2} h^{-1}. In both studies, 90% of O_2 transport into the sediment or 90% of CH_4 emission from sediment was through the plant.

Labeled $^{15}N_2$ production in the rhizo-sphere seems to be the result of two processes. The first involves the reaction NH_4^+ $\rightarrow NO_2^- \rightarrow NO_3^- \rightarrow N_2O \rightarrow N_2$, and thus is classical denitrification (Firestone et al. 1980). Nitrification potentially occurs in the aerobic rhizosphere, while denitrification of NO_3^- occurs in the adjacent anaerobic layer. Subsequently the N_2 gas formed appears to diffuse into roots as a result of pressure gradients and then to be transported into the atmosphere above the floodwater. The second process involves the reaction: $NH_4^+ \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$. In this process, the first step in nitrification occurs in the aerobic rhizosphere and, because of high concurrent electron pressure in the rhizosphere, NO_2^- can be used as an electron acceptor and reduced to N_2O. The N_2O is subsequently reduced to N_2 and transported through the plant into the air above the floodwater (Bremner and Blackmer 1978). In a recent study, NO_2^- formed during nitrification was shown to be reduced to N_2O by the nitrifying bacterium *Nitrosomonas europaea* under conditions of oxygen stress (Poth and Focht 1985).

In conclusion, our study has shown that 29% of NH_4^+-N was lost through nitrification-denitrification in 18 d after application in the root zone of rice. About 23% of the applied $^{15}NH_4^+$ was lost through nitrification-denitrification in the root zone of two freshwater aquatic macrophytes (pickerel weed and soft rush). Maximal $^{15}N_2$ flux was 102, 113, and 122 mg N m^{-2} d^{-1} for soft rush, rice, and pickerel weed. For all three species of aquatic macrophytes used, about a fourth of the added N was lost from the soil-plant system 3–4 weeks after application. Where no plants were grown, about a tenth of the N was lost. Detection of $^{15}N_2$ in the gas phase above the floodwater provided direct evidence of nitrification-denitrification in the root zone of aquatic macrophytes. A significant portion of the denitrified gas formed in the rhizosphere was trapped in the soil before escaping through the plant. Aquatic macrophytes functioned as a conduit for transport of denitrified gases from the root zone to the atmosphere. The processes responsible for this several-step conversion were diffusion of NH_4^+ from the anaerobic soil to the oxy-

genated rhizosphere, microbial oxidation of the NH_4^+ in the rhizosphere, diffusion of NO_3^- back to the anaerobic zone, microbial denitrification of NO_3^- to N_2 and N_2O, and possible diffusion of part of this N_2 and N_2O through aerenchymous channels to the atmosphere (Fig. 1).

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