Plant and Soil 111, 151-154 (1988)
© Kluwer Academic Publishers
PLSO 7568

Application of N-15 dilution for simultaneous estimation of nitrification and nitrate reduction in soil-water columns

C.W. LINDAU[1], R.D. DeLAUNE[2], M.L. WILLIAMS[1] and W.H. PATRICK, JR.[2]
[1]Nuclear Science Center and Laboratory for Wetland Soils and Sediments Louisiana State University, Baton Rouge, LA 70803, USA and [2]Laboratory for Wetland Soils and Sediments Louisiana State University, Baton Rouge, LA 70803, USA

Received 29 October 1987. Revised April 1988

Key words: denitrification, mineralization, N-cycling, rice soils, stable N isotopes

Abstract

Nitrification and denitrification rates were estimated simultaneously in soil-floodwater columns of a Crowley silt loam (Typic Albaqualfs) rice soil by an ^{15}N isotopic dilution technique. Labeled NO_3^- was added to the floodwater of soil-water columns, half were treated with urea fertilizer. The (NO_3^- + NO_2^-)-N and (NO_3^- + NO_2^-)-N concentrations in the floodwater were measured over time and production and reduction rates for NO_3^- calculated. Nitrate reduction in the urea amended columns averaged 515 μmol N $m^{-2}h^{-1}$ and nitrification averaged 395 μmol N $m^{-2}h^{-1}$ over the 35–153 d incubation. The nitrification rate for 4–19 d sampling period (1,560 μmol N $m^{-2}h^{-1}$) in the urea amended columns was almost 9 times greater than the reduction rate (175 μmol N $m^{-2}h^{-1}$) over the same period. Without the addition of urea the NO_3^- production rate averaged 32 μmol N $m^{-2}h^{-1}$ and reduction 101 μmol N $m^{-2}h^{-1}$.

Introduction

Recovery of applied nitrogen to flooded rice (*Oryza sativa* L.) rarely exceeds 40% and denitrification is thought to be a major N loss mechanism (DeDatta, 1981; Patrick, 1982). Nitrogen balance studies and other indirect methods (isotope dilution) are used to estimate denitrification in flooded rice soils. Direct field measurement of evolved N_2 is severely restricted due to accuracy of field measurements and cost and availability of high precision isotope ratio mass spectrometers (Parkin, 1984).

Nitrification-denitrification reactions are significant processes known to occur simultaneous in flooded rice systems where both aerobic and anaerobic zones exist (Reddy and Patrick, 1984). In flooded rice these reactions can occur in (1) the surface oxidized soil layer and the underlying reduced soil zone, and (2) the rhizosphere. The significance of nitrification-denitrification reactions in waterlogged soils as a N loss mechanism has been previously reported for rice soils (Fillery and Vlek, 1982; Reddy and Patrick, 1984).

The objective of this study was to determine the simultaneous formation (nitrification) and reduction of NO_3^- in rice soil water columns by isotope dilution of added labeled N.

Materials and methods

A Crowley silt loam soil (Typic Albaqualfs) was used for the study. It contained 7.0 g total C kg^{-1}, 0.8 g total N kg^{-1}, a cation exchange capacity (CEC) of 9.4 cmol (+) kg^{-1} of soil and a pH of 5.8 (1:1 soil/water). The soil contained 10.8% clay and 70.7% silt.

In the laboratory, the air-dried and ground soil was placed into four polyvinyl chloride (PVC) containers (36-cm length by 27-cm i.d.). The soil-water columns consisted of a 10-cm layer of soil and 20-cm floodwater depths and were preincubated at

25°C for 30 d for development of a thin soil surface-oxidized layer.

After equilibration, 40 atom %^{15}N labeled NO_3^- was added to the floodwater of each container at a rate of 1.5 mg N l^{-1}. In addition, urea was added to two microcosms (12 g N m^{-2}). The soil systems were incubated in the dark at 25°C for 153 d. At selected intervals 30 ml aliquots of floodwater were filtered (0.45 μm) and stored at 2°C prior to analysis. Dissolved inorganic NO_2^- + NO_3^- concentrations were determined by steam distillation procedures with Devardas alloy. The distillates were acidified and evaporated to dryness and stored in glass vials prior to N-15 analysis (Hauck, 1982; Keeney and Nelson, 1982). A Dupont 21-621 mass spectrometer was used to determine N-15 concentration.

The rates of production (nitrification) and consumption (reduction) of NO_3^- were determined over time by measuring the floodwater concentration of NO_2^- + NO_3^- and the corresponding N isotopic composition. If only NO_3^- reduction and nitrification are responsible for the changes in the soil-water column NO_3^- pool, the rates of production and reduction can be calculated using equations outlined by Koike and Hattori (1978).

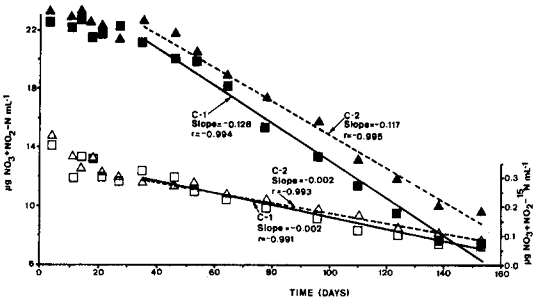

Fig. 1. Reduction of (NO_3^- + NO_2^-)-N in rice soil-water columns (μg NO_3 + NO_2 − N ml^{-1}) Changes *with time* (days) in floodwater (NO_3^- + NO_2^-)-N (■, ▲) and (NO_3^- + NO_2^-)-^{15}N (□, △) for duplicate columns.

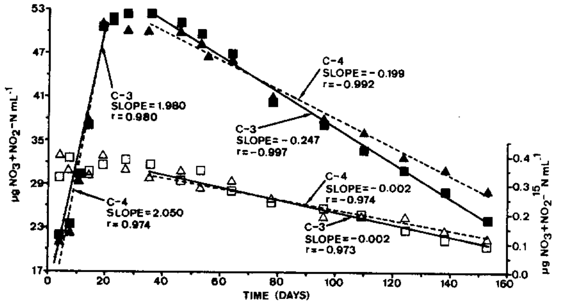

Fig. 2. Reduction and production of (NO_3^- + NO_2^-)-N (μg NO_3 + NO_2 − N ml^{-1}) in urea amended rice soil-water columns. Changes *with time* (days) in floodwater (NO_3^- + NO_2^-)-N (■, ▲) and (NO_3^- + NO_2^-)-^{15}N (□, △) for duplicate columns.

Results and discussion

Inorganic (NO_3^- + NO_2^-)-N and (NO_3^- + NO_2^-)-^{15}N concentrations in the floodwater of the soil-water microcosms with incubation time are shown in Fig. 1 and Fig. 2. The decrease in (NO_3^- + NO_2^-)-N and (NO_3^- + NO_2^-)-^{15}N for both sets of columns (35–153d) and the increase in (NO_3^- + NO_2^-)-N content for columns 3 and 4 (4–19 d) were linear over time. Correlation coefficients ranged from 0.97 to 0.99 and were highly significant ($P < 0.01$). No significant correlations were calculated between the (NO_3^- − NO_2^-)-N and 0–35 d (columns 1 and 2) and 19–35 d (columns 3 and 4) sampling intervals. For columns 1 and 2 (no urea added) the rates of total N decrease were 0.128 and 0.117 μg N $ml^{-1}d^{-1}$, respectively and labeled inorganic N decreased at an average rate of 0.002 μg N $ml^{-1}d^{-1}$ over the 35–153 d incubation period. The mean rate of total NO_3^- + NO_2^- removal was about 60 times greater than ^{15}N removal from the floodwater of columns 1 and 2. In the urea amended columns (3 and 4) the decrease in total N rates were 0.247 and 0.199 μg N $ml^{-1}d^{-1}$, respectively and ^{15}N-(NO_3^- + NO_2^-) decreased at the same rate as columns 1 and 2. In addition to the linear decreases in total and labeled NO_3^- + NO_2^- a linear increase in (NO_3^- + NO_2^-)-N was observed for columns 3 and 4 over the 4–19 d incubation (Fig. 2). The rates of increase were 1.980 and 2.050 μg N $ml^{-1}d^{-1}$, respectively.

The mean rate of nitrification in columns 1 and 2 was 3.8 μmol N $l^{-1}d^{-1}$ and the mean rate of NO_3^- reduction was 11.9 μmol N $l^{-1}d^{-1}$ over the 35–153 d incubation (Table 1). When urea was applied the mean nitrification rate was 46.7 μmol N $l^{-1}d^{-1}$ and reduction was 60.9 μmol N $l^{-1}d^{-1}$. Over the 4–19 d incubation of columns 3 and 4 mean nitrification (184.7 μmol N $l^{-1}d^{-1}$) was over eight fold greater than NO_3^- reduction (20.8 μmol N $l^{-1}d^{-1}$). The columns amended with urea showed increases of twelvefold and fivefold in production and reduction of NO_3^-, respectively compared to the unamended columns. Urea addition accounted for an

Table 1. Nitrification and nitrate reduction (μmol N $l^{-1}d^{-1}$) in soil-water columns of a Crowley silt loam rice soil

Incubation interval, days	$NO_3 + NO_2$ production		$NO_3 + NO_2$ reduction	
	columns 1 and 2	columns[a] 3 and 4	columns 1 and 2	columns 3 and 4
35–46	2.5[b]	28.3	9.6	35.4
46–53	6.1	32.2	12.1	48.6
53–64	3.2	29.9	14.9	34.9
64–78	3.6	29.5	13.8	59.6
78–96	4.6	49.7	11.9	61.0
96–110	6.5	13.3	17.6	27.3
110–124	0.7	52.1	9.4	68.4
124–138	5.7	72.8	15.1	84.3
138–153	1.9	112.8	3.0	128.6
Mean ± SE	3.8 ± 0.7	46.7 ± 10.0	11.9 ± 1.4	60.9 ± 10.4
Mean (μmol N $m^{-2}h^{-1}$)	32	395	101	515
4–7		20.0		0.0
7–11		90.9		30.5
11–14		447.7		52.5
14–19		180.0		0.0
Mean ± SE		184.7 ± 93.6		20.8 ± 12.8
Mean (μmol N $m^{-2}h^{-1}$)		1,560		175

[a] urea applied (12 g N m^{-2})
[b] mean value of two columns

estimated 92% of NO_3^- production and 80% of reduction in the floodwater of microcosms 3 and 4.

Nitrate production and reduction rates over the 4–19 d incubation for columns 3 and 4 were approximately fourfold and one-third, respectively of the mean nitrification and reduction rates for days 35–153. Urea is rapidly hydrolyzed and nitrified creating a large available soil-water NO_3^- pool that can undergo denitrification (Reddy an Patrick, 1984).

The isotope dilution technique is a measure of the total processes responsible for NO_3^- reduction and is an indirect measure of denitrification. Denitrification would best be quantified by direct measurement of N_2O and N_2 production. Direct measurement has been achieved in nonflooded soils using gas chromatography (N_2O) and mass spectrometry to measure the $^{28}N_2$,$^{29}N_2$, and $^{30}N_2$ intensities simultaneously (Mosier *et al.*, 1986; Mulvaney and Boast, 1986). Direct field measurements of N_2 from flooded rice is severely lacking and is attributed to the flooded soil condition which may be entrapping significant quantities of denitrification N_2 (Lindau *et al.*, 1988).

The nitrification and NO_3^- reduction rates in the amended and unamended columns (Table 1) were similar to published values for other flooded systems. Nitrification and denitrification rates of approximately 80 to 240 μmol N $m^{-2}h^{-1}$ were reported by Jenkins and Kemp (1984) in estuarine sediments. Reddy and Patrick (1986) reported N loss rates of about 100 μmol N $m^{-2}h^{-1}$ in flooded rice soil columns without plants and potential N loss rates up to 570 μmol N $m^{-2}h^{-1}$ with rice plants when elevated nitrogen concentrations were injected into the rhizosphere. Smith and DeLaune (1984) reported denitrification rates (acetylene reduction) following urea addition to the surface of a flooded rice soil of 10 to 15 μmol N $m^{-2}h^{-1}$ during the first week under greenhouse conditions.

The results presented demonstrated that the ^{15}N isotope dilution technique can be used to understand the fate and transformations of N in soil-floodwater systems. Nitrification and NO_3^- reduction can occur simultaneous where aerobic-anaerobic soil zones exist. The results also point out the contribution of the soil NH_4^+ pool to floodwater NO_3^- content.

Acknowledgement

Funding for this research was provided by the United States Agency for International Development Grant No. DAN-1406-G-SS-4091-00.

References

DeDatta S K 1981 Principles and Practices of Rice Production. John Wiley and Sons, New York. p. 515.

Fillery I R P and Vlek P L G 1982 Plant and Soil 65, 153–169.

Hauck R D 1982 Methods of Soil Analysis. Eds. A L Page *et al.* pp 735–779.

Jenkins M C and Kemp W M 1984 Limnol. Oceanogr. 29, 609–619.

Keeney D R and Nelson D W 1982 Methods of Soil Analysis. Eds. A L Page *et al.* pp 643–698.

Koike I and Hattori A 1978 Appl Environ. Microbiol. 35, 853–857.

Lindau C W *et al.* 1988 Soil Sci. Soc. Am. J. 52, 538–540.

Mosier A R *et al.*, 1986 Soil Sci. Soc. Am. J. 50, 344–348.

Mulvaney R W and Boast C W 1986 Soil Sci. Soc. Am. J. 50, 360–363.

Parkin T B *et al.*, 1984 Soil Biol. Biochem. 16, 323–330.

Patrick W H Jr 1982 Nitrogen in Agricultural Soils. Ed. F J Stevenson. pp 449–465.

Reddy K R and Patrick W H Jr 1984 CRC Crit. Rev. Environ. Control 13, 273–309.

Reddy K R and Patrick W H Jr 1986 Soil Sci. Soc. Am. J. 50, 649–651.

Smith C J and DeLaune R D 1984 Plant and Soil 79, 287–290.