

DENITRIFICATION AS A COMPONENT OF NITROGEN BUDGET IN A TROPICAL PADDY FIELD

B.K. PATHAK^{1,} * T. IIDA² F. KAZAMA¹ ¹Center of Excellence Integrated River Basin Management for Asian Monsoon Region Interdisciplinary Graduate School of Medicine and Engineering University of Yamanashi 4-3-11, Takeda, Kofu, Yamanashi, 400-8511, Japan ²Department of Bio-production Faculty of Agriculture, Yamagata University 1-23 Wakaba, Tsuruoka, Yamagata 997-8555, Japan

Received: 21/11/05 Accepted: 15/02/06 *to whom all correspondence should be addressed: e-mail: pathakbipin@gmail.com

ABSTRACT

The direct assessment of denitrification suffers from a number of problems. A mass balance model is simple and widely accepted to calculate nitrogen (N) loss as a denitrification component. The objective of study was to estimate the potential N loss a denitrification from a tropical paddy in the central region of Thailand. N inputs to and outputs from field were measured by direct method. Inputs of N to the site were commercial fertilizer, precipitation, irrigation water, seeds and pre-cultivation soils. Outputs of nitrogen from the site were leaching to groundwater, harvested crops, loads in surface runoff, post harvest soils and loss from the field as denitrification. Biological N fixation, groundwater contribution, ammonium volatilization and contribution to weed growth were ignored. Based on the three month observation, the total amount of N load in irrigation, precipitation, fertilizer, drainage, percolation and plant uptake were 9.22, 10.85, 100, 4.65, 18.20 and 80.57 kg ha⁻¹ respectively. The difference of total N in the soil before and after cultivation was not significant and taken as constant value. Sum of N loss calculated as denitrification component from the nitrogen mass balance model was 16.7 kg ha⁻¹ and rate of loss was 0.19 kg ha⁻¹ d⁻¹. It means that the contribution of applied N fertilizer to the atmosphere was 13.6% of total N input; indicating one of the major source pollutants.

KEYWORDS: Denitrification, Mass balance, Nitrogen leaching and Vacuum lysimeter.

1. INTRODUCTION

The process of climate change through increased green house effect gases concentrations is thought to lead a rise in average global temperature, changes in frequency and distribution of precipitation and variations in pattern and occurrence of droughts and floods. The greenhouse gas indicator measures the gross agricultural emissions of four gases: nitrous oxide (N₂O), nitric oxide (NO), carbon dioxide (CO₂) and methane (CH₄). Use of inorganic fertilizer is the one of the key source of N₂O gas through denitrification, nitrification and ammonia volatilization (OECD, 2001). Moreover, N loss is economically and environmentally undesirable. Nitrogen loss as denitrification represents the loss of a valuable plant nutrient and hence an economic cost to agriculture. Unless, they are managed properly; their positive contribution to agricultural productivity could be negated by their adverse impact on the environment.

Thailand remains as the largest rice exporter in the world (Ministry of Agriculture and Cooperatives, Thailand, 1991) in terms of both by volume and value. About one third of Thailand's gross area (51million ha) is arable and 52.8% of arable area is used for rice production while 98.3 % of rice field is occupied by paddy field. In these intensively cultivated

rice cropping systems, an in-depth understanding of these processes is therefore important. Irrigated rice paddy soils have a specific zone due to the constant flooding of the fields during rice growth (Reddy and Patric, 1984). Microbial soil processes, e.g. mineralization, nitrification and denitrification, greatly affect nitrogen dynamics in soil and govern the supply of nitrogen to the plants. The overall regulation of this ammonification-nitrification-denitrification pathway is relatively complex due to the involvement of both aerobic and anaerobic microbial processes (Seitzinger ,1998), and consequently, the relative importance of N_2 as a product depends strongly on the environmental conditions. Coupled nitrification–denitrification has been suggested as a significant source of N loss in rice paddy soils, and earlier studies on rice fields have reported coupled nitrification denitrification losses up to 40% of the applied nitrogen (Buresh, 1991).

Different methods for measuring denitrification have been developed: the acetylene inhibition technique (Sørensen, 1978), direct measurement of N_2 production (Seitzinger, 1980), the nitrogen mass balance approach (Ahlgren, 1967), and the ¹⁵N isotope pairing technique (IPT) (Nielsen, 1992). However, each method has numerous technical difficulties and limitations. The acetylene block method is sensitive to relatively low rates of denitrification, and is the source of most existing information on denitrification rates (Seitzinger, 1993). Direct measurement of N_2 evolution from nitrate is possible, but has been used less frequently because analysis of N_2 accumulation in an N_2 -rich medium is difficult (Seitzinger, 1990). Nitrate disappearance over cores or tracer methods involving ¹⁵N also can be used, but present various interpretational difficulties (Seitzinger, 1988).

A mass-balance approach to the analysis of denitrification often is simple, practical and widely used method. For flooded rice where the fluxes of water and nitrogen can be measured, may be the reliable way of measuring denitrification. This paper reports the results of a mass-balance study in which nitrogen loss through potential denitrification are estimated for acid sulfate soils in tropical paddy field.

2. MATERIALS AND METHODS

Site description

This study was conducted in an experimental field in 2001 at Asian Institute of Technology, Pathumthani Province, Thailand. The elevation of the proposed site is 2 to 2.5 m above the mean sea level. The region has a humid climate and the mean annual rainfall is 1300 to1400 mm of which more than 80% of precipitation falls in the rainy season. The average monthly temperature ranges from 19 to 35°C and relative humidity fluctuates from 70-80%. Groundwater table is usually high and varied from 0.40 to 1.5 m during the crop period. The area of the experimental field is about 1600 m² with irrigation canal on its three sides and meteorological station located at about 60 m from the experimental plot.

Experimental field arrangement

To evaluate the quantities of nitrogen input and output in the field, major components of the flow and the storage of water and nitrogen were measured at the experimental field. Triangular weirs with pressure sensor were installed at the inlet and the outlet of the experimental field to measure the water level and flow rate. Two rain gauges were installed diagonally to the field to catch the precipitation. Lysimeter was installed inside the plot to measure the evapotranspiration from the field and plants. Eight PVC tube wells having 10 cm inside diameter and 2m length were drilled around the plot at a distance of 4m while four PVC wells were drilled at 80m from the field to observe the groundwater level and water quality. Daily groundwater table was measured by the measuring probe. The depth of water in the plot was recorded daily by vernier scale installed at two corner of plot and converted into volume of water stored in the field.

Soil water existing in the soil pores were collected by soil water samplers having diameter of 4.8 cm (Model 1900, Soil moisture Equipment Co.), in order to get more precise estimation of the amount of N leaching into subsurface flow. To avoid possible contamination, ceramic cups of the soil water samplers were washed with dilute acid before using (Litaor, 1988). Vertical holes of 76 mm in diameter were dug with a hand auger up to the depths of 20, 40 and 60 cm from the soil surface at two points in the experimental field on September 23, just after the emergence. To get good contact between the ceramic cups and the soil, slurry of the soil

material was put in the bottom of the hole before inserting the soil water sampler and in the side after inserting the soil water sampler (Grossmann and Udluft, 1991).

Rice seeds were directly sown on the ground on September 14. Major components of nitrogen flow had been measured until rice was harvested (December 24). The local practice in field management and cropping systems were applied. Three different does of inorganic fertilizer (Table 1) was supplied at the time of seeding and during the cultivation as per local practice in the Thailand. As urea is quickly hydrolyzed to NH₄-N and subsequently nitrified to NO₃-N, the amount of nitrogen input through fertilizer during the cultivation period was calculated to be about 100 kg N ha⁻¹. Weeds were controlled manually and pesticide was applied occasionally.

Samples collection and analysis

Water samples

Irrigation water, drainage water, and precipitation samples were collected at every event in polyethylene bottles. Ground water samples and ponding water samples were collected twice in a week. Soil-water samples were collected by suction pump at twice in a week from each sampler during whole cultivation period. Vacuum of 30 KPa (1/3 bar) was applied to each sampler 24 hour before the sampling. The extracted sample volume were generally be as small as possible to minimize the disturbances of the system and sampling period was short to avoid alteration of the sample in the sampling system. The first sample was rejected in each case and water samples were analyzed for NH_4 -N, NO_3 -N, and TN concentrations by cadmium reduction method and digestion followed by Phenate method respectively (APHA, 1998).

Date of Fertilizer applied	Fertilizer Dose	Type of Inorganic Fertilizer	Amount of Fertilizer Kg- N/ha	Frequency of water sampling	Sampling depth (cm)
September 14	I	Metrophos	30	*Every weeks	20
October 13	II	Urea	43	after the soil	40
November 20	III	Urea	27	installed	60

Table 1. Summary of fertilizer application and water sampling date

* Rice seeding was sown on September 14 and the soil water samplers were installed about 2 weeks later.

Soil samples

Soil sampling was carried out pre-cultivation and post harvesting from four different layers specified in results and discussion to identify the physical and chemical properties of soil. Soil samples were taken from four layers with a typical double-cylinder core having 5.6 cm in diameter and 4.0 cm in height to obtain bulk density of the soil sample. Particle density was measured by pycnometer method. Soil samples for saturated hydraulic conductivity were also taken with a typical double-cylinder core having 9.8 cm in diameter and 9.5 cm in height and Falling head method was adopted to measure the saturated hydraulic conductivity.

For chemical analysis, soil samples were also taken to plastic bags at four different depths. Soil-water pH, particle size and organic matter were determined by glass electrode method, hydrometer method and dry ash method respectively. Extraction method (2.0M potassium chloride) was used to measure the NO₃-N in soil. The difference in amount of N in soil samples before cultivation and after harvesting was considered as the amount of N in the soil stored in the soil.

Water and nitrogen mass balance

Mass balance approach was used to determine the deep percolation of water as follows:

DPR= I+P -ET-R

Where DRP is the daily water percolation in mm, I is irrigation water applied during the day in mm, P is precipitation in mm, ET is the evapotranspiration in mm and R is runoff from the plot in mm.

(1)

In this experimental field, the water loss from the field in terms of lateral seepage was assumed to be zero, as polyethylene sheets were set on the levees to prevent lateral seepage. Leaching at the experimental field was considered above the groundwater surface; therefore the groundwater outflow was not taken into account.

Total amount of nitrogen in input and outs terms were calculated by multiplying the nitrate concentration in each term mentioned in Euqation 1 with the volume of water. The nitrogen mass balance in the experimental field can be expressed as

$N_{loss} = N_{in} - N_{out} - N_{diff \ soil}$

(2)

where, $N_{in} = [Nf + Ni + Np]$, $N_{out} = [Nd + NI + Nu + Nloss]$ and $N_{diff soil} = [Nsf - Nsi]$. Suffixes f, i, p, d, u, l, and loss represent fertilizer, irrigation water, precipitation, drained water, leaching to groundwater, uptake by plants and the loss from the experimental field as denitrifcation and anaerobic oxidation of ammonia (anammamox) respectively. Nsi is the amount of nitrogen stored in pre-cultivation soil and Nsf is the amount of nitrogen stored in post-cultivation soil. The other minor component such as biological nitrogen fixation groundwater contribution, ammonium volatilzation were and weeds productions were ignored.

3. RESULTS AND DISCUSSION

Properties of the soil

Four different color of soil in the vertical profile (Fig. 1) was found until total depth of 120 cm. and has been separated into four different soil layer (Table 2a). The pore size distribution of the soil profiles was assessed by visual observation and estimated as meso-pores (30 to 100 μ m diameter).



Figure 1. Soil profile at experimental field before cultivativation

Average saturated hydraulic conductivity of the soil before cultivation ranged between 2.951E-06 to 4.478E-04 cm/s while the after cultivation was varied from 9.259E-07 to 3.741E-04 cm/s (Table 2a). Saturated hydraulic conductivity at the second layer (20-40 cm) from the soil surface before and after cultivation presented quite different values, suggesting the effect of rice cultivation activities. The criteria set for saturated hydraulic conductivity of clay is generally less than 2.0E-03 m d⁻¹ (2.31E-06 cm s⁻¹) supports that the soil in this experimental field is basically classified as clay (Smedema and Rycroft, 1983). Average bulk density was varied from 1.18 and 1.35 g cm⁻³ while average particle density was between 2.65 and 2.71

g cm⁻³. The porosity was between 0.47 and 0.62. The bulk density decreased during cultivation by average 18 % and this decrease in bulk density means the increase of pore space in the soil during cultivation which is supported by the increase in porosity (Table 2a).

Table 2a. Physical properties of soli in the experimental heid								
Depth (cm)	Color	Particle distribution		size	Saturatec conductiv	Porosity		
(0)		Clay (%)	Silt (%)	Sand (%)	В	А	В	А
0-20	Black	76.80	11.25	11.95	2.951E-06	9.259E-07	0.47	0.51
20-40	Red.brown	70.55	15.00	14.75	1.742E-05	4.630E-07	0.49	0.57
40-60	Yello.green	80.55	13.75	5.70	1.852E-05	1.470E-05	0.55	0.57
60-120	Brown gray	79.30	16.25	4.45	4.478E-04	3.741E-04	0.55	0.62

Table 2a. Physical properties of soil in the experimental field

A-after cultivation, B-before cultivation,

Table 2b. Physical and chemical p	properties of soil in the experimental field

Depth (cm)	Bull ((Julk densityParticle density(g cm ⁻³)(g cm ⁻³)		Total organics (%)		рН		
	В	А	В	А	В	А	В	А
0-20	1.35	1.30	2.65	2.65	4.43	5.24	4.70	3.58
20-40	1.33	1.27	2.70	2.68	4.39	4.16	3.85	3.42
40-60	1.22	1.20	2.71	2.69	4.62	3.61	3.59	3.19
60-120	1.18	1.13	2.69	2.66	3.62	3.27	3.30	3.08

A-after cultivation, B-before cultivation

The pH value of pre-cultivated soil varied layer by layer and its values ranged from 4.70 to 3.3 (Table 2b). Since the pH in all layers was below five and has high percentage of clay contents, the soil was categorized as acid sulfate. Because of the acid sulfate clayey soil (with pH range from 4.70 to 3.58 on the first layer), NH₃ losses from these soils were less probable (Mountonnet and Fardeau, 1982). Average organic carbon was ranged from 3.45 to 4.84 % and found highest in first the layer.

Water and Nitrogen mass balance

There were 37 rainfall events during the experimental period and the total depth of precipitation was 541.1 mm. Irrigation was done 14 times during the cultivation period and ponded water depth was kept at 10 cm usually. The total volume of irrigation water supplied to the field was 1480.6 m³ (925.4 mm) in and in average, 79.5 mm irrigation water was taken into the plot by one irrigation event. Drainage occurred 15 times during the cultivation period and the total volume of drainage water from the plot was 550.7 m³ (344.2 mm). The total amount of evapotranspiration measured by a lysimeter during the cultivation period was 416.75 mm and the average daily evapotranspiration was 4.13 mm. The percolation rates calculated from water balance method during the cultivation period ranged between 0 and 13 mm d⁻¹ and the average daily percolation was 5.21 mm. At the end of the experiment, the percolation rate was slightly less than that in other periods.

The total amount of NO₃-N, NH₄-N and TKN leached to groundwater during whole cultivation period was calculated to be 3.63, 9.82 and 14.57 kg ha⁻¹ respectively. In the beginning of crop period, the rate of nitrate leaching was very less and maximum leached was found after the one week of first and second dose of urea fertilizer application. It suggests that groundwater is vulnerable due to increased level of nitrate right after the fertilizer application. However, the leaching duration and amount varied with soil type, crop type and water availability. Moreover,

the rate of increase and decrease was not consistent which may be due to the nitrification in flooding water in the field and others agricultural activities such as weeding, wilting.

 NO_3 -N, NH_4 -N and TKN concentration in the precipitation ranged from 0.08 to 1.30 , 0.55 to 15.64 and 0.11 to 5.03 mg l⁻¹ respectively. The total NO_3 -N, NH_4 -N and TKN in precipitation were 1.14, 3.68 and 9.72 kg ha⁻¹ respectively. The total amounts of NO_3 -N, NH_4 -N and TKN in irrigation water were 0.64 , 1.72 and 8.58 kg ha⁻¹ respectively. The difference of NO_3 -N, NH_4 -N and TKN in drainage water were 0.52, 1.52 and 4.13 kg ha⁻¹ respectively. The total amount of N load in drainage water was 4.65 kg ha⁻¹. The total amount of N fertilizer applied in three times during the cultivation period was 100 kg ha⁻¹.

Nitrogen contained in the seeds was 3.83 kg ha⁻¹. Average nitrogen concentrations in harvested grains, stems, leaves and roots were 1.23, 0.51, 0.75 and 0.63 % dried matter, respectively. The total amount of nitrogen stored in harvested plant tissues was 84.4 ± 1.7 kg ha⁻¹ and the amount of nitrogen uptake by plants was therefore 80.57 kg ha⁻¹.

The amount of nitrogen accumulated in the soil during the cultivation period was estimated by subtracting the amount of nitrogen contained in the soil at pre-cultivation to the post-cultivation. The sphere of one meter depth from the soil surface was assumed to consider the amount of nitrogen and N stores was calculated by multiplying the weight of dried soil by the average nitrogen content at each layer of soil. The total amount of nitrogen in pre-cultivation soil was 9,672.5 kg ha⁻¹ and that in post-cultivation soil was 9,673.3 kg ha⁻¹, and the difference was not significant and taken as constant value. However, the calculation of nitrogen amount in soil may contain considerable error due to the spatial variability of soil properties both horizontally and vertically.

The total amount of N in irrigation, precipitation, fertilizer, drainage, percolation and plant uptake were 9.22, 10.85, 100, 4.65, 18.20, and 80.57 kg ha⁻¹ respectively. The amount of total N in the soil before and after cultivation was not significant and taken as constant value. Sum of N loss calculated from the nitrogen mass balance model was 16.7 kg ha⁻¹ (Fig. 2) which corresponds to the 13.6% of total N input.

Since nitrogen loss was determined by the difference of nitrogen components with respect to the total, the quality of the result was influenced by the accuracy of all calculated/ assessed terms. In this study minor components such as groundwater input, mineralization and input from the atmosphere through biological fixation were ignored due to lack of data where it should be included. Under Thai conditions, however these results may be important particularly in finding better management practice on flooded paddy field.



Figure 2. Nitrogen flow in the experimental field for the whole cultivation period

4. CONCLUSIONS

This study aimed to assess the N loss to atmosphere from a tropical paddy field. Based on the three month observation, N loss calculated as denitrification component from the nitrogen mass balance model was 16.7 kg ha⁻¹ and rate of loss was 0.19 kg ha⁻¹ d⁻¹. The contribution of applied N fertilizer through denitrification to the atmosphere was found to be 13.6% of total N fertilizer; indicating one of the major source of pollutants.

Thus, agricultural activities may produce the adverse impact on the environment. Recently it was reported that anammox (anaerobic oxidation of ammoina) process exist every where in the nature and produce N₂ gas. It is recommended for further study to find the faction N₂ from denitrification and anammox. Finally, this study contributes to the still limited database in tropical climate about the quantification of the nitrogen loss.

5. ACKNOWLEDGEMENT

The authors would like to express sincere thank to Dr. Watehara Phuriwirokul Rice Research Institute, Kassetsart University, for his kind support in research. The authors are deeply grateful to the German Academic Exchange Service (DAAD) and Japanese Government through JICA and GMS-JG fellowship for providing financial support.

REFERENCES

- Ahlgren I., (1967), Limnological studies of Lake Norrviken, a eutrophicated Swedish lake, Water chemistry and nutrient budget, *Hydrologie*, **29**, 53–90.
- American Public Health Association (1998), Standards methods for the examination of water and wastewater, Twentieth edition, New York.
- Arth I. and Frenzel P., (2000), Nitrification and denitrification in the rhizosphere of rice: the detection of processes by a new multi channel electrode, *Biol. Fert. Soils*, **31**, 427–435.
- Buresh R.J., De-Datta S.K., Samson M.I., Phongpan S., Snitwongse P., Fagi A.M. and Tejasarwana R., (1991), Dinitrogen and nitrous oxide flux from urea basally applied to puddle rice soils, *Soil Sci. Soc. Am. J.*, **55**, 268–273.
- Grossmann J. and Udluft P., (1991), The extraction of soil water by suction cup method: a review, *Journal of soil science*, **42**, 3-93.
- Laegreid M., Bockman O.C., Kaarstad O., (1999), Agriculture fertilizers and Environment. CABI Publ., Wallingford and Norsk Hydro ASA, Oslo.
- Litor M., Iggy, (1988), Review of soil solution samplers, *Water Resources Research*, **24**(5), 727-733.
- Ministry of Agriculture and Cooperatives, Thailand, (1991), Agricultural statistics of Thailand, crop year 1990/1991, Bangkok., Thailand
- Moutonnet P. and Fardeau J.C., (1997), Inorganic nitrogen in soil solution collected with tensionic samples, *Soil Science Society of American Journal*, **61**, 822-825.
- Nielsen L.P., (1992), Denitrification in sediment determined from nitrogen isotope pairing, *FEMS Microbiol. Ecol.*, **86**, 357–362.
- OECD, (2001), Environmental indicators for agriculture development, Organization for Economic Cooperation and Development, Canada
- Reddy K.R. and Patrick W.H., (1984), Nitrogen transformations and loss in flooded soils and sediments, *Crit. Rev. Env. Contr.*, **13**, 273–309.
- Seitzinger S.P., (1988), Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance, *Limnol. Oceanogr.*, **33**, 702–724.
- Seitzinger S.P., Nielsen L.P., Caffrey J. and Christensen P.B., (1993), Denitrification measurements in aquatic sediments: a comparision of three methods, *Biogeochemistry*, 23, 147–167.
- Seitzinger S., Nixon S., Pilson M.E.Q. and Burke S., (1980), Denitrification and N₂O production in near-shore marine sediments, *Geochim. Cosmochim. Acta*, **44**, 1853–1860.
- Smedema L.K and Rycroft D.W., (1983), Land drainage: planning and design of agricultural drainage systems, Batsford, London
- Sorensen J., (1978), Denitrification rates in a marine sediment as measured by the acetylene inhibition technique, *Appl. Environ. Microbiol.*, **36**,139–143.