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Analysis of Nitrogen Dynamics and Fertilizer Use Efficiency in Rice Using the Nitrogen-15 Isotope Dilution Method Following the Application of Biogas Slurry or Chemical Fertilizer

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Abstract: The fates of nitrogen-15 (^{15}N)-labeled biogas slurry (BS) and chemical fertilizer (CF) applied to soil with low fertility cropped with rice were investigated. The ^{15}N dilution method was used to estimate N uptake and recovery; potted soil was labeled with 1.0 atom % excess [^{15}N] ammonium chloride ([^{15}N]NH_4Cl). Certain select soil characteristics were also measured in soil amended with the slurry to explain N losses. The values of the % N derived by fertilizer application to the plant exhibited significant differences among different plant parts. N uptake from the CF applied to rice grain, straw and the roots was significantly ($p<0.05$) higher than that from BS, but the highest proportion of N uptake by rice was from the soil. At harvest, an average of 6.2 and 13.2% of applied N remained in the soil treated with the CF and BS, respectively. Fertilizer use efficiency calculated by the ^{15}N dilution method tended to be higher for CF than BS. A significant amount of N fertilizer (average: 30%) was apparently lost from the soil-plant system by ammonia (NH_3) volatilization. Following BS application, the pH increased by 1 to 1.2 units in the top 5 cm of the soil, resulting in high NH_3 volatilization in the first 2 days of the experiment. The NH_3 volatilization accounted for the decrease in soil ammonium-N (NH_4^+-N) content.

Key words: ^{15}N dilution, N uptake, biogas slurry, rice, chemical fertilizer, N recovery

INTRODUCTION

Biogas is a by-product of the biological breakdown of organic wastes such as plants, crop residues, food waste and human and animal manure under anaerobic conditions. Biogas generators or digesters yield 2 products: the biogas itself and a semisolid by-product called effluent or slurry that can be used as a high-quality fertilizer. The digestion process converts the nitrogen (N) in the organic materials to ammonium (NH_4^+), a form that becomes more stable when incorporated into soil. NH_4^+ is readily fixed (bonded) in soil and is absorbed by plants (Chantigny *et al*., 2004). In contrast, the N in raw manure is oxidized to nitrates and nitrites, which do not fix well in soil and are readily washed away. Biogas slurry (BS) is a valuable source of crop nutrients and organic matter and improves soil physical conditions. However, as inorganic fertilizers have become available at relatively low cost, slurry is now considered more of a waste than a resource. Fertilizers have a guaranteed nutrient content and are readily available, while BS varies widely in composition. Further, the nutrients in the organic fraction must be mineralized to become available to plants. The difficulty in accurately predicting the availability of BS nutrients to crops renders it rather inadequate as a definite crop nutrient source.

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Farmers often acknowledge the beneficial effects of BS on soil quality and nutrient levels; however, despite the economic benefits, BS is not the preferred nutrient source (Nowak *et al*., 1998). The Fertilizer equivalence (FE) approach and measuring the apparent N recovery by the difference method (Diff Meth) are 2 methods commonly used to determine N availability to crops. The results of these indirect methods are often highly variable. For example, using the FE approach, Motavalli *et al*. (1989) recorded 12 to 63% of dairy manure N as plant available during the first season after application. Other estimates for dairy manure N availability have ranged from 10 to 57% (Paul and Beauchamp, 1993). A direct assessment of manure N recovery can be attained by labeling the manure with ^{15}N and then measuring the amount of ^{15}N in the crop (Sørensen *et al*., 1994). However, estimating availability also requires that the N recovery from ^{15}N-enriched chemical fertilizer (CF) be considered. It is frequently assumed that the inorganic portion of manure N is available as fertilizer N. However, Paul and Beauchamp (1995) estimated the amount of fertilizer N to be approximately 59% due to large losses through NH_3 volatilization, denitrification and immobilization. Although the general mechanisms of slurry N transformations and losses are known, the actual proportions of the N from BS that is lost to the environment, recovered by the crop, or left in the soil remain to be elucidated. Moreover, the rates at which immobilized and clay-fixed N are released and the influence of clay fixation on slurry N availability has not been well studied.

The use of ^{15}N a tracer is the most powerful tool to distinguish between the fates of a particular N source and background soil N (Fillery and Recous, 2001; Ghoneim *et al*., 2006). The feeding of poultry (Kirchmann, 1990), hogs (Chantigny *et al*., 2004) and sheep (Jensen *et al*., 1999) with a ^{15}N-enriched diet has been used to obtain animal manure with ^{15}N-labeled mineral and organic fractions to study the fate of manure N in the soil-plant system. The main objective of this study was to determine the impacts of ^{15}N-enriched BS and CF on N uptake and to determine N distribution and fertilizer use efficiency.

MATERIALS AND METHODS

Soil, Biogas Slurry Properties and Treatment

A pot experiment was set up at the Ehime University farm in southwestern Japan (33°57' N, 132°47' E at 20 m above sea level). The soil used was gray lowland paddy soil (eutric fluvisol according to FAO/UNESCO) that exhibited the following properties in the top 0-20 cm: cation exchange capacity (CEC), 9.24 cmol (+) kg^{-1}; pH, 6.7 (H_2O); electrical conductivity (EC), 0.37 dS m^{-1}; total carbon (C), 14.6 g kg^{-1}; total N, 1.5 g kg^{-1}; C/N ratio, 9.7; available phosphorus (P), 1.89 g kg^{-1}; exchangeable potassium (K), 0.63 g kg^{-1}; exchangeable calcium (Ca), 1.45 g kg^{-1}; exchangeable magnesium (Mg), 0.34 g kg^{-1}; NH_4^+-N, 2.0 mg kg^{-1}; sand, 585 g kg^{-1}; silt, 281 g kg^{-1}; and clay, 134 g kg^{-1}. The mean ambient temperature during the study period was 35/10.1°C max/min (mean daily temperature: 25.7°C) and the soil temperature in the pots during the cultivation period was 31/12.7°C (max/min day/night; average temperature, 22.7°C). Rainfall was typically not uniformly distributed throughout the rice cultivation period. BS (mainly cattle manure and dairy waste) was obtained from Yagi town in Kyoto, Japan; its chemical properties are shown in Table 1.

The following 2 N fertilization treatments were set up using a completely randomized design: (1) CF at a concentration of 80 kg N ha^{-1} labeled with 1.0 atom % N, (2) soil amended with BS application at a concentration of 80 kg N ha^{-1} was applied at the different stages of rice growth. The ^{15}N tracer [^{15}N]NH_4Cl with 99.7 atom % ^{15}N was thoroughly applied at a concentration of 0.1 g m^2 to the BS-treated soil and P was applied at a concentration of 80 kg P ha^{-1} to only the dry BS-treated soil prior to transplantation. The N fertilizer was applied in 3 splits (40 kg N ha^{-1} during transplantation, 20 kg N ha^{-1} at 30 days after transplantation and 20 kg N ha^{-1} at 60 days after transplantation). Chemical fertilizer was amended with PK at the same rate of 80 kg PK ha^{-1} applied to dry soil. Wagner

Table 1: Chemical characteristics of the biogas slurry at the time of application

Characteristic	Mean
pH	7.60*
EC (dS m^{-1})	1.70
Total C (g kg^{-1})	14.00
Organic N (g kg^{-1})	1.10
Total VFA-C (%)†	70.60
NH_4^+-N (mg L^{-1})	1800.00
NO_2-N (mg L^{-1})	0.02
NO_3^--N (mg L^{-1})	15.00
C/ N ratio	4.80
Phosphate (mg L^{-1})	180.00
Exchangeable K (mg L^{-1})	0.57
SO_4^{-2} (mg L^{-1})	380.00
Total Fe (mg L^{-1})	560.00

†: Volatile fatty acids; *: Values are the mean of triplicate measurements

pots (0.05 m^2) were filled with 3.30 kg of dry soil mixed with equivalent amounts of CF or BS. On June10, the pots were flooded with tap water before transplanting 3 seedlings per pot of 4 week-old rice (*Oryza sativa* L. cv. Hinohikari).

Soil Sampling and Analyses

Soil samples were collected to a 5 cm in depth at 0, 2, 4, 6 and 8 days after BS application. The samples were categorized as 0-2 and 2-5 cm fractions and soil pH was measured in a 1:2.5 soil/water mixture. Soil mineral N was extracted by mixing 20 g of field-moist soil with 40 mL of 2 M potassium chloride (KCl) for 30 min in a 250 mL polypropylene bottle. The samples were centrifuged and the extracts filtered through Whatman No. 42 filter papers prewashed with 2 M KCl to eliminate possible NH_4^+ contamination. Blank samples were included during the extraction to correct for possible NH_4^+ background contamination. The NH_4^+-N content of the KCl extracts was quantified by steam distillation (Bremner, 1965).

Rice Sampling and Chemical Analysis

Rice growth parameters (plant height, number of tillers and leaf chlorophyll content) were measured at 0, 30, 36, 43, 54, 66 and 82 days after transplantation (DAT). Chlorophyll content was measured with a chlorophyll meter (SPAD-502; Minolta Co. Ltd., Japan). The rice plants were harvested by cutting above the soil surface. They were thoroughly washed, first under running tap water and finally with distilled water to remove soil particles and plant debris. The plants were then separated into grain, straw and roots and oven dried at 70°C to a constant weight. The dried samples were weighted and then ground into a fine powder with an electric mill in preparation for chemical analysis. The total N content and ^{15}N abundance in the plant samples was determined by automatic combustion in tin (Sn) capsules and analyzed using a stable isotope mass spectrometer (ANCA-SL; Europa Scientific Ltd.).

Nitrogen Dynamics and ^{15}N-recovery Calculations

The atom % of ^{15}N excess was calculated as the difference between the ^{15}N atom % in plants and its natural abundance in the atmosphere (0.3663%). Rice N uptake was determined by multiplying the dry matter yield by the N concentration in the corresponding tissues. The contribution of BS to rice N was calculated by the A-value method (Stevenson *et al*., 1998). It is assumed that the available N, as determined by the A-value of the soil, implies that when CF and BS applied as sources of N are present in the soil, the N absorbed by a plant from each source is proportional to their respective availabilities (Fried and Dean, 1952).

Fertilizer N use Efficiency

Fertilizer use Efficiency (FUE) was calculated as follows;

$$FUE_{CF} = (\text{atom \% } ^{15}N \text{ excess}_{plant})/(\text{atom \% } ^{15}N \text{ excess}_{CF}) \times (N_{plant}/N_{CF}) \times 100$$

Where,

atom % ^{15}N excess $_{plant}$ = atom % ^{15}N excess (above background level) in the plant, atom % ^{15}N excess $_{CF}$ = atom % ^{15}N excess in the labeled chemical fertilizer N, N_{plant} = total plant N (kg ha^{-1}) and N_{CF} = applied CF (kg ha^{-1}).

$$FUE_{BS} = N_{plant\ form\ BS}/N_{BS}$$

Where,

$N_{plant\ form\ BS}$ is the plant N uptake from BS calculated from the *A* value and N_{BS} = BS applied (kg ha^{-1}). The relative N use efficiency of the BS as compared to that of the CF was calculated using the following equation as described by Nishida *et al*. (2004).

$$\text{Relative efficiency (\%)} = FUE_{BS}/FUE_{CF} \times 100$$

Statistical Analyses

Data were subjected to analysis of variance (ANOVA). Subsequently, the significance of differences between the amendments was determined by a multiple comparison test using the Tukey-Kramer test ($p<0.05$) that was performed using the software KyPlot (KyensLab Inc., Tokyo, Japan).

RESULTS AND DISCUSSION

Rice Growth, Yield and Yield Components

There was no significant difference in the mean plant height between the CF and BS amendments (Fig. 1a). However, there was a significant difference in the number of tillers between the amendments (Fig.1b). The highest mean chlorophyll value was recorded at 40 DAT and was followed by a rapid decline in the chlorophyll content with both CF and BS amendments with no significant difference observed (Fig. 1c). The values for grain yield, panicle number and 1000-grain weight were rather higher with CF amendment than BS amendment, although the differences were not significant (Table 2).

Biomass Yield and N Uptake from the CF, BS and Soil

Rice straw accounted for the highest dry weight in both the CF and BS treatments (Table 3). However, there was no significant difference between the dry weights of the grain, straw and roots in the case of both CF and BS treatments at harvest. N uptake varied between the grain, straw and roots in the case of both CF and BS treatments. As compared to CF treatment, N uptake by the grain was significantly higher ($p<0.05$) with BS treatment. N uptake from the CF by the grain, straw and roots were significantly ($p<0.05$) higher than that from the BS. The total N derived from the CF and BS was 133.0 and 68.9 mg N pot^{-1}, respectively (Table 3). It is clear from the data that most of the N uptake by rice was from the soil, ranging from 83.2 to 91.1% and not from the CF or BS. No significant difference was observed in the amount of N derived from the soil by the different parts of the plant treated with either the CF or BS (Table 3). Some papers have reported a higher N use efficiency in soil amended with inorganic fertilizers as compared to when organic amendments with low mineralization potential were used (Ebid *et al*., 2007; Asagi *et al*., 2007). The combined use of compost and CF

Table 2: Yield and yield components of rice (*Oryza sativa* L. cv. Hinohikari)

Treatment	Yield (g pot^{-1})	No. of panicle pot^{-1}	1000-grain wt. (g)
CF	24.6	18.5	23.2
BS	23.1	16.5	22.1
	NS	NS	NS

CF: Chemical Fertilizer; BS: Biogas Slurry; NS = Not Significant

Table 3: Dry weight and N uptake from the chemical fertilizer, biogas slurry and soil at harvest

			N uptake (mg pot^{-1})		
Amendment	Rice part	DW (g pot^{-1})	Ndf^a	Ndfs	Total (mg pot^{-1})
CF	Grain	25.9a$^{\#}$	63.9 (16.8)a	316.9 (83.2)a	380.8a
BS		28.5a	26.9 (8.93)b	274.2 (91.1)a	303.1a
CF	Straw	37.6a	48.3 (14.7)a	280.9 (85.3)a	329.9a
BS		34.7a	27.3 (9.74)b	257.6 (90.2)a	285.9a
CF	Root	14.9a	20.8 (14.1)a	127.2 (85.9)a	148.0a
BS		10.8a	14.7 (11.4)b	113.9 (88.6)a	128.6a
CF	Whole-plant	78.4a	133.0 (15.5)a	725.0 (84.5)a	858.0a
BS		74.0a	68.9 (9.64)b	645.7 (90.4)a	714.6a

DW = dry matter; Ndf^a, N derived from applied chemical fertilizer (CF) or biogas slurry (BS); Ndfs, N derived from soil; Numbers in parentheses are N uptake % derived from CF, BS and soil; $^{\#}$: Means in a column followed by the same letter were not significantly different (Tukey-Kramer test: p = 0.05)

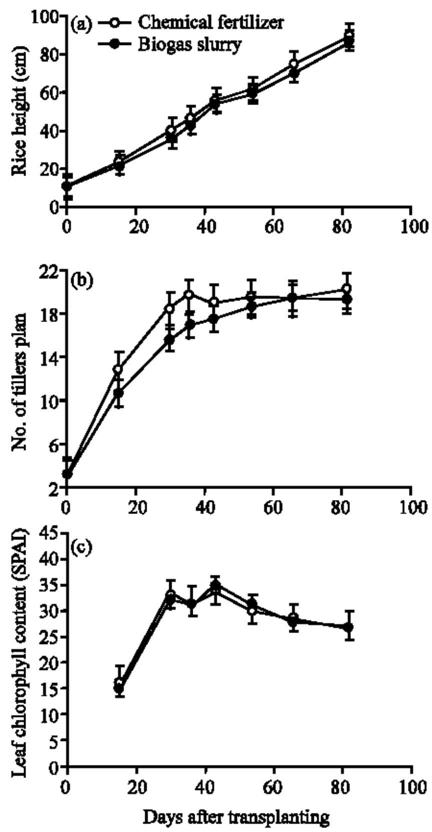

Fig. 1: Changes in height (a), tiller number (b) and leaf chlorophyll © at different growth stages in rice plants grown in soil amended with ^{15}N chemical fertilizer or biogas slurry. Bars represent standard deviation of the means

Table 4: Nitrogen use efficiency, relative efficiency and N distribution as affected by the application of Chemical Fertilizer (CF) and Biogas Slurry (BS)

Amendment	N use efficiency (%)	Relative efficiency# (%)	N distribution (%) Soil	Uptake	Loss
CF	70.3a	100.0	6.2a	70.3a	23.5a
BS	50.5b	71.8	13.2b	50.5b	50.5b

#: Relative uptake rate of biogas slurry N to chemical fertilizer N; Means in a column followed by the same letter were not significantly different (Tukey-Kramer test: $p = 0.05$)

(Ghoneim *et al*., 2006), is one of the reasons why most organic amendments supply low amounts of available N due to immobilization after organic matter decomposition and N mineralization. In addition, the N from organic matter is also involved in other soil processes such as nitrification and denitrification. On the basis of the results, the higher N uptake with CF treatment as compared to BS treatment can be attributed to the higher amounts of available N (Ghoneim *et al*., 2006). The proportional rate of N uptake by rice from the CF and BS is shown in Table 3. Generally, a higher N uptake (%) was observed in the rice amended with the CF than in that amended with the BS. Notably, the percentage of N uptake from the CF in rice grain (16.8%) was higher than that from the CF in straw and roots. The relationship between fertilizer N uptake and total N uptake during the growing season depends on the time at which the fertilizer N is applied (Guindo *et al*., 1994) and the amount of available fertilizer N (Bufogle *et al*., 1997). However, there was no significant difference ($p<0.05$) in the uptake of N derived from the soil between CF and BS treatments. The % of N derived from applied CF or BS (Ndf) was significantly higher with CF (45.6%) treatment than BS (30.1%) treatment. The low uptake of N from BS can be attributed mainly to the rapid immobilization of N due to microbial activity, leading to a significantly lower amount of available N relative to that with CF treatment, as observed in previous studies (Ghoneim *et al*., 2006). However, by using the ^{15}N isotope dilution method, it is difficult to directly measure the N uptake from the BS since immobilization and mineralization occur simultaneously. Further studies on gross N mineralization, denitrification and immobilization from BS should be carried out to completely explain the N dynamics in soil.

N Fertilizer Use Efficiency and Distribution

The FUE-^{15}N values measured in this study (Table 4) are comparable to those reported (43.3 to 68.3%) for soil with low fertility (Asagi *et al*., 2007). These values are higher than those reported for upland crops (Ghoneim *et al*., 2006) and may vary depending on the crop and soil types. The FUE of the CF and BS treatments was 50.5 and 70.5% of the applied N, respectively. In addition, the relative N use efficiency of BS as compared to CF was 71.8%. The relative efficiency of organic fertilizer used in paddy rice and estimated by the ^{15}N dilution method varies widely; for example, it is 18% for cattle manure, 70% for rape cake (Uenosono *et al*., 2004), 81% for poultry manure compost and 71% for swine feces (Nishida *et al*., 2004). The higher N use efficiency in this study can be attributed to the considerably low soil fertility. Table 4 shows the N distribution in the plants and soil and the loss measured at harvest. Whole-plant recovery with CF treatment (70.3%) was significantly higher ($p<0.05$) than that with BS treatment (50.5%). N retained in the soil after harvest was significantly lower when CF was applied (6.2%) than when BS was applied (13.2%). The N component in the BS that is organically bound (NH_4^+) can evaporate as NH_3. In addition, if a BS with a pH of 7.6 was used, it could have resulted in a higher rate of NH_3 volatilization (Ghoneim *et al*., 2006). The amount of unaccounted N from the BS (36.3%) was higher than that from the CF (23.5%). The unaccounted portion of the inorganic ^{15}N was potentially lost through NH_3

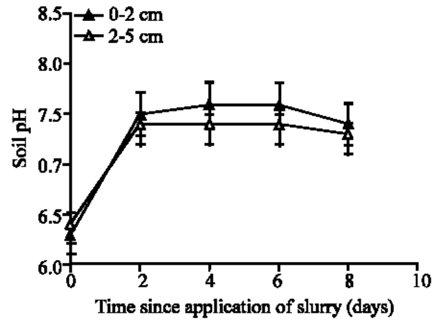

Fig. 2: Soil pH at depths of 0-2 and 2-5 cm over a period of 8 days in soil amended with biogas slurry. Bars represent standard deviation of the means

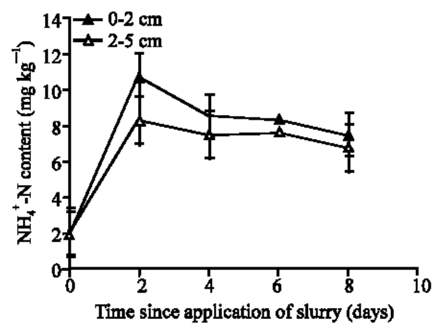

Fig. 3: Soil NH_4^+-N content at depths of 0-2 and 2-5 cm over a period of 8 days in soil amended with biogas slurry. Bars represent standard deviation of the means

volatilization. The proportion of slurry N recovered by the rice plants as well as the fate and form of the slurry N remaining in the soil at harvest would be influenced by the soil texture (Sørensen and Amato, 2002).

Soil pH

Soil pH increased by 1.20 and 1.0 units in the 0-2 and 2-5 cm soil layers, respectively, 2 days after BS application (Fig. 2). Increases of 1 to 2 units in soil pH have been previously reported in the first few centimeters of soil amended with animal slurry (Sommer and Hutchings, 2001). An increase in soil pH following BS application can be partially attributed to the higher slurry pH, decomposition of volatile fatty acids (Table 1) and the dissociation of slurry carbonates (Chantigny *et al*., 2001). From 2-6 days after the BS addition, the pH values remained stable in the top 5 cm of the soil. The slight decline in the soil pH after day 6 could be attributed to the acidifying effects of NH_3 volatilization and nitrification (Rochette *et al*., 2001). Sørensen and Amato (2002) found that most of the slurry-derived volatile fatty acids were decomposed 4 days after slurry application, indicating that these compounds are rapidly used by soil microbes.

Effect of Treatment on Soil Mineral N

The initial soil NH_4^+-N content was approximately 2.0 mg kg^{-1} but increased sharply following BS application (Fig. 3). At day 2 after BS addition, the soil NH_4^+ content was significantly ($p<0.05$) higher in the 0-2 cm soil layer due to the higher NH_4^+ content of the slurry (Table 1). From days 2 to

8 after BS application, the NH_4^+ concentration gradually decreased in the top 5 cm of the soil. Since the initial soil NH_4^+ content was low, the amount of soil NH_4^+ recovered from the top 5 cm after the BS application was assumed to represent the slurry-derived NH_4^+. The difference observed from days 2 to 8 suggested that biological processes such as immobilization and nitrification significantly contributed to slurry NH_4^+ transformation after the NH_3 volatilization rate returned to a lower level, as observed by Chantigny *et al*. (2001) and Ghoneim *et al*. (2006). NH_3 volatilization was mainly related to soil pH (Fig. 2) and the NH_4^+ concentration in the top 5 cm of soil.

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