Concurrent Observation of Several Processes of Nitrogen Metabolism in Soil Amended with Organic Materials

V. Effects of Long-Term Application of Farmyard Manure and Nitrogen Fertilizer on N Cycling Processes in Upland Field Soil

Sutopo Ghani Nugroho[1], Shigekata Yoshida*, and Shozo Kuwatsuka

Laboratory of Soil Science, Faculty of Agriculture, Nagoya University, Nagoya, 464-01 Japan; and
**Laboratory of Land Cultivation, Nagoya University Farm, Togo, Aichi-gun, Aichi, 470-01 Japan*

Received January 29, 1991

In upland field experiments, concurrent ammonification of organic N and resultant nitrification processes were observed in farmyard manure (FYM)-treated plots. The applied N-fertilizer (urea) was hydrolyzed to NH_4-N and subsequently nitrified resulting in pulsed inputs of NH_4-N and NO_3-N in soil, which affected the denitrification and N_2 fixation processes.

The actual denitrification process was not substantial except for the short period following the simultaneous application of FYM and urea. At this time, the level of NO_3-N in soils was high together with the large number of denitrifiers. The denitrification rates increased ranging from 1.00 to 3.56 kg N_2O-N/ha/d by the application of FYM.

The positive effect of the long-term application of FYM on N_2 fixation was observed within the application rate of 20 t FYM/ha, although a larger number of N_2 fixers was observed with higher application rates. The maximum rates of N_2 fixation ranged from 13.0 to 21.0 g N_2-N/ha/d. In the control plot the fixation was less than 9.0 g N_2-N/ha/d. N_2 fixation was inhibited by the pulsed input of NH_4-N in soil originating from urea, as anticipated, but the inhibitory effect lasted for an unexpectedly long period of time, even when the NH_4-N level in soil decreased to the original low level. The number of N_2 fixers also decreased by the application of urea.

Key Words: **denitrification, farmyard manure, long-term application, N_2 fixation, upland field soil.**

In laboratory experiments, concurrent processes of ammonification, nitrification, denitrification, and N_2 fixation were apparently observed under soil moisture conditions

[1] Present address: Faculty of Agriculture, Lmpung University, Bandar Lampung 35145, Indonesia.

corresponding to 60% of the maximum water holding capacity (MWHC), and these processes were affected by the kinds and amounts of applied organic materials (OM) and the soil moisture contents. These processes were also regulated by the application of inorganic N (Ghani Nugroho and Kuwatsuka 1990, 1992a, b, c). The soil moisture contents at 60% MWHC or somewhat higher values depending on the soil types reflected the moisture conditions of upland field soils. These moisture conditions which occur after rainfall or irrigation, when the soil moisture level ranges from the field capacity to the saturation levels are conducive to the development of anaerobic microsites within the well-aerated soil matrix (Burford and Stefanson 1973). The anaerobic or partially anaerobic zones (pockets, microsites) also developed in the OM-treated plots due to the high level of microbial respiration (Scott Smith and Tiedje 1979).

Straw, farmyard manure (FYM), and compost from various sources are widely used in agricultural soils together with chemical fertilizers. These agronomic practices as well as the climatic conditions affect the major factors controlling the N metabolism in soil such as substrate availability, soil aeration, and inorganic N content. Soderstrom et al. (1983) reported that in general the microbial activity decreased due to N amendments. It may therefore be interesting to analyze in the field how the long-term application of OM and N-fertilizer affects the concurrent processes of ammonification, nitrification, denitrification, and N_2 fixation.

The objectives of this study were to (1) determine simultaneously the rates of denitrification and N_2 fixation *in situ* and the rates of associated NH_4-N and NO_3-N formation, and (2) enumerate the microbial populations related to N cycling, in particular the total bacteria, denitrifiers, and N_2 fixers. Several climatic and soil factors were analyzed to evaluate their impact on the concurrent processes of ammonification, nitrification, denitrification, and N_2 fixation.

MATERIALS AND METHODS

The experiment was conducted from April to October 1987 in the upland field of Nagoya University Farm, where the field experiment on long-term application of FYM had been conducted since 1975. The application rates of FYM were 0, 20, 50, and 100 t/ha/time (applied twice a year in late fall and late spring). The plots, 49 m^2, were arranged in a Latin Square Design. Although the composition of the FYM used slightly varied among the years, it was approximately the same as that of FYM used and described in our previous report (total C, 33.7%; total N, 2.4%; NH_4-N, 0.089%; and NO_3-N, 0.038%) (Ghani Nugroho and Kuwatsuka 1990). Table 1 shows some properties of the soil subjected to FYM amendment for 13 yr.

Table 1. Properties of the soil after 13 yr of continuous application of farmyard manure at different levels.

FYM[a] application levels (t/ha)	pH	Total C (%)	Total N (%)	MWHC (mL/100 g)
0	5.4	0.7	0.08	47
20	6.6	2.2	0.23	53
50	6.8	3.0	0.31	60
100	7.3	5.4	0.57	78

MWHC, maximum water holding capacity. [a] Farmyard manure was applied twice a year: in late fall and late spring.

Faba bean (*Vicia faba* L.) and sweet corn (*Zea mays* L.) were sequentially cultivated in 1987. The current experiment was conducted in the row-space zone between plants, to minimize the effects of plant roots. Table 2 shows the agronomic practices adopted during the experimental period.

For the *in situ* simultaneous measurement of denitrification and N_2 fixation, a device reported by Yoshida (1984) was used. The device consisted of a chamber with two ports sealed with rubber stoppers in the upper part. The chamber was a detopped cylinder made of refuse fire extinguisher, 16 cm in outer diameter and 35 cm in length. One port was connected to an electrode for monitoring the O_2 level and temperature, and the other for injecting and removing gases from inside the chamber.

The chamber was inversely inserted in the soil in the row-space zone between plants and driven to a depth of 15 cm. A sufficient amount of air was removed from the enclosed space above the ground with a syringe to allow replacement with C_2H_2. The C_2H_2 corresponding to a final concentration of about 10% v/v and 1 mL propane were injected into the chamber. The dilution ratio of the introduced propane was used to calculate the exact aerial volume of the reaction vessel. Immediately after gassing, and 1 and 3 h later, each gas sample was collected with a 10-mL syringe and transferred into a 10-mL vacuum blood collection tube (Roper 1983), and analyzed for N_2O and C_2H_4 with a gas chromatograph as described previously (Ghani Nugroho and Kuwatsuka 1990). Measurements were conducted at least every 2 weeks throughout the experimental period. The soil moisture content was determined at each sampling time. Each measurement was conducted with five replications. N_2 fixation was calculated based on a conversion factor of 3 (C_2H_2 reduced/N_2 fixed).

The soil samples were collected periodically from the upper 15 cm part of the plots for the determination of inorganic N contents and enumeration of microbial populations. The soil samples under field moist conditions were passed through a 2-mm mesh sieve and immediately analyzed or otherwise the soil samples were stored at −15°C (for inorganic N determination) or 4°C (for microbial enumeration) until analysis. The procedures for the determination of inorganic N and the enumeration of the microbial populations were previously described (Ghani Nugroho and Kuwatsuka 1990, 1992b). All the analyses were performed with duplication.

The climatic data were collected from the climatological station of the Nagoya University Farm located at a distance of 200 m from the experimental site. Weekly maximum and minimum air temperatures, and mean weekly soil temperatures were calculated on the

Table 2. Agronomic practices adopted during the experimental period.

Date	Practices	Application rate (per ha)
November 4, 1986	—Application of FYM	0, 20, 50, 100 t
	—Sowing of horse bean	
	—Application of urea[a]	60 kg
May 25, 1987	—Harvesting of horse bean	
June 2, 1987	—Plowing and seed-bed preparation	
June 9, 1987	—Application of FYM	0, 20, 50, 100 t
	—Sowing of sweet corn	
	—Application of urea	300 kg
July 2, 1987	—Application of urea	100 kg
August 21, 1987	—Harvesting of sweet corn	
September–October	—Fallow	

FYM, farmyard manure. [a] Urea was applied at the same rate in all the plots.

basis of data recorded daily.

RESULTS AND DISCUSSION

Air and soil temperature, rainfall, and soil moisture

The soil temperature increased along with the increase of the air temperature from April to August, then it gradually decreased toward October (Fig. 1). During the experimental period, the overall precipitation was considerably low (dry year). High daily precipitation occurred only sporadically throughout the experimental period, and no long-spell of rain or continuous precipitation required to maintain the soil moisture at a high level for a sufficiently long period of time was recorded (Fig. 1).

Concurrent ammonification, nitrification, denitrification, and N_2 fixation processes

The soil to which high rates of FYM had been applied contained large amounts of both NH_4-N and NO_3-N throughout the year (Fig. 2). The applied urea was hydrolyzed to NH_4-N and subsequently nitrified resulting in the pulsed inputs of NH_4-N and NO_3-N in soil which

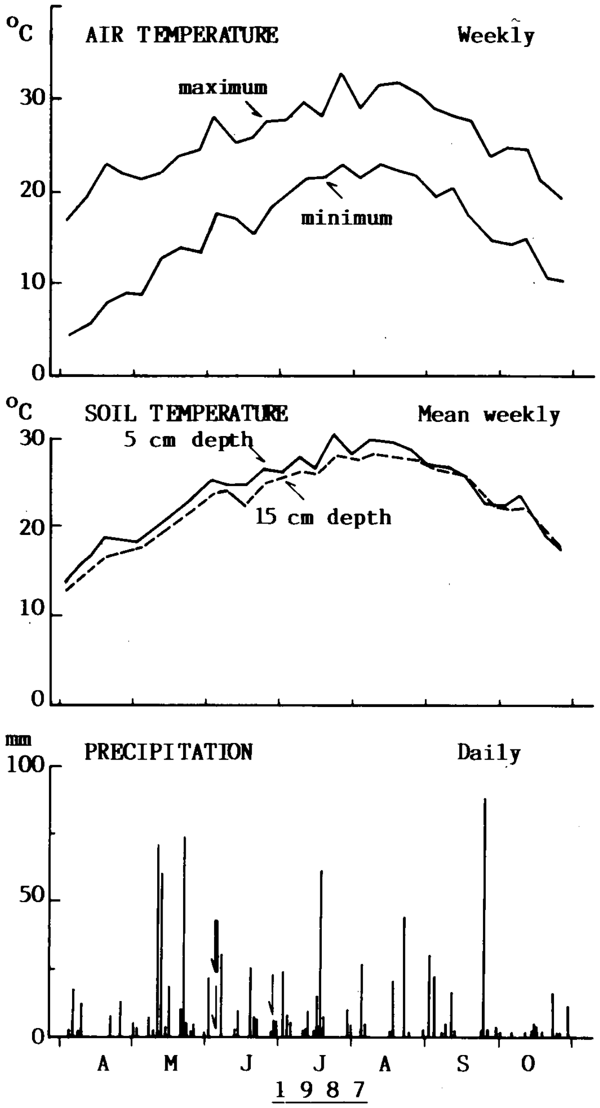

Fig. 1. Climatic data (1987). Broad arrow: incorporation of FYM (June 9). Narrow arrows: urea fertilization (June 9 and July 2). FYM, farmyard manure.

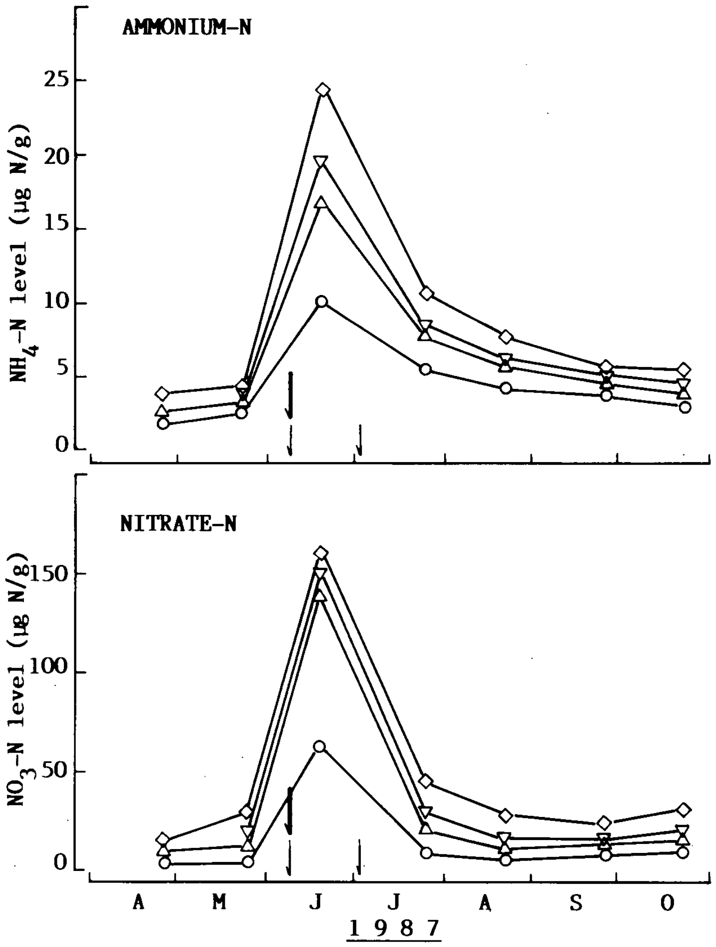

Fig. 2. Contents of NH_4-N and NO_3-N in soils after 13 yr of continuous application of FYM at different levels. Broad arrows: incorporation of FYM (June 9). Narrow arrows: urea fertilization (June 9 and July 2). ○, control; △, FYM 20 t/ha; ▽, FYM 50 t/ha; ◇, FYM 100 t/ha. FYM, farmyard manure.

Table 3. Occurrence of denitrification in upland field soils after 13 yr of continuous application of FYM at different levels of FYM in relation to the contents of NO_3-N and soil moisture.

	1987														
	April	May			June		July			Aug.		Sept.		Oct.	
	28	15	22	25	5	19	3	17	24	7	21	11	25	9	23
	Without FYM														
Moist.[a]	30	40	35	36	30	33	70	55	35	33	32	30	40	30	28
NO_3-N[b]	2.2		2.5		64.6			6.8		6.5		8.4		10.0	
Denit.[c]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	With FYM 20 t/ha														
Moist.	37	56	45	55	40	50	77	60	47	53	40	41	55	40	38
NO_3-N	8.5		9.2		140.8			23.7		7.2		11.1		15.6	
Denit.	nd	nd	nd	nd	nd	1.78	1.30	1.0	nd	nd	nd	nd	nd	nd	nd
	With FYM 50 t/ha														
Moist.	42	60	50	58	45	52	80	69	50	55	45	46	60	42	40
NO_3-N			17.2		149.9			31.7		11.4		13.1		24.7	
Denit.	nd	nd	nd	nd	nd	1.89	1.67	1.2	1.1	nd	nd	nd	nd	nd	nd
	With FYM 100 t/ha														
Moist.	45	62	55	60	50	55	85	75	55	60	50	50	60	45	43
NO_3-N	10.7		28.3		160.4			44.6		24.7		30.3		33.6	
Denit.	nd	nd	nd	nd	nd	3.56	2.89	2.0	2.0	1.2	nd	nd	nd	nd	nd

nd, undetectable; FYM, farmyard manure; MWHC, maximum water holding capaciity. [a] Soil moisture (% of MWHC); [b] NO_3-N (g N/g); [c] denitrification (kg N/ha/d).

may affect the denitrification as well as N_2 fixation processes. However, it should be noted that the plants (sweet corn) also utilized NO_3-N and competed with the denitrifiers for the NO_3-N produced during the nitrification process.

Ghani Nugroho and Kuwatsuka (1990) reported that anaerobic incubation induced the denitrification of NO_3-N supplied by FYM amendment in slopy soil, suggesting the occurrence of a denitrification flush after rainfall in the field, as reported by Sexstone et al. (1985). Although field measurements were not always conducted immediately after precipitation, particularly after heavy rainfall, it is likely that the denitrification of NO_3-N supplied by the FYM amendment occurred. Thus, a major part of the denitrification detected in the present study was considered to have originated from the nitrification-denitrification coupled reaction. This process generally requires a moderately high soil moisture content (at around 60% MWHC), supply of available substrates, and NH_4-N (for nitrification). However, since high soil moisture contents following a long-spell of rain were seldom recorded (Fig. 1, Table 3), it is considered that denitrification could not be detected at the time of sampling (Fig. 3).

A high rate of denitrification was detected in the FYM-treated plots during the period from June to July when the NO_3-N content in soil increased after the simultaneous application of FYM and urea (Figs. 2 and 3). The denitrification rates increased with the increase of the rate of FYM application (from 20 to 100 t/ha), ranging from 1.00 to 3.56 kg N_2O-N/

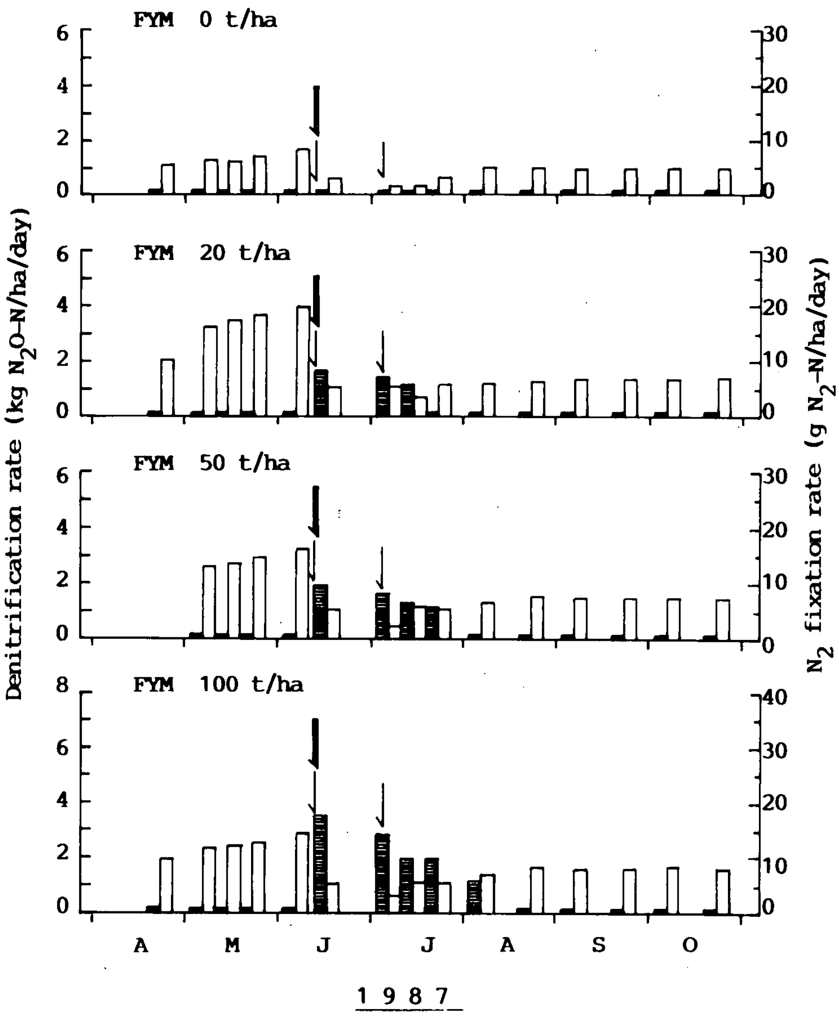

Fig. 3. Rates of denitrification and N_2 fixation measured simultaneously *in situ* in soils after 13 yr of continuous application of FYM at different levels. Broad arrows: incorporation of FYM (June 9). Narrow arrows: urea fertilization (June 9 and July 2). ▭, N_2 fixation; ▤, denitrification. FYM, farmyard manure.

Table 4. Rates of N_2 fixation measured *in situ* in upland fied soils after 13 yr of continuous application of FYM at different levels in relation to the contents of NH_4-N and soil moisutre.

	1987														
	April	May			June		July			Aug.		Sept.		Oct.	
	28	15	22	25	5	19	3	17	24	7	21	11	25	9	23
	Without FYM														
Moist.[a]	30	40	35	36	30	33	70	55	35	33	32	30	40	30	28
NH_4-N[b]	1.9		2.5		10.3			5.8		4.2		3.9		3.3	
N_2 fix.[c]	5	6	6	7	9	3	2	1	3	5	5	5	5	5	5
	With FYM 20 t/ha														
Moist.	37	56	45	55	40	50	77	60	47	53	40	41	55	40	38
NH_4-N	2.5		3.0		17.1			7.9		6.8		3.6		4.0	
N_2 fix.	11	17	19	20	21	5	5	3	6	6	6	6	6	6	6
	With FYM 50 t/ha														
Moist.		60	50	58	45	52	80	69	50	55	45	46	60	42	40
NH_4-N			3.3		20.0			8.2		6.8		4.4		4.6	
N_2 fix.		15	15	17	18	5	2	5	6	7	7	7	7	7	7
	With FYM 100 t/ha														
Moist.	45	62	55	60	50	55	85	75	55	60	50	50	60	45	43
NH_4-N	2.9		3.6		24.7			10.7		7.9		5.4		5.6	
N_2 fix.	11	13	13	14	16	6	3	6	6	8	8	8	8	8	8

FYM, farmyard manure; MWHC, maximum water holding capacity. [a] Soil moisture (% of MWHC); [b] NH_4-N (g N/g); [c] N_2 fixation rate (g N/ha/d).

ha/d (Fig. 3). In soils under long-term arable cropping and grassland, the denitrification rates were reported to range from 0.70 to 1.50 kg N_2O-N/ha/d (Bijay-Singh et al. 1989). Ryden and Lund (1980) recorded a sharp peak of denitrification after the application of plant residues up to 3.40 kg N_2O-N/ha/d.

The positive effect of long-term application of FYM on the N_2 fixation activity of soil was observed especially in April and May. The effect on the increase, however, was observed only up to the application rate of 20 t FYM/ha, and further increase in the application rates did not affect it further. The N_2 fixing rate was also controlled by climatic factors, in particular during the period from April to May (Fig. 3). The rate increased with the increase of the soil temperature. Figure 3 shows that the maximum N_2 fixing rate was in the range of 13.0 to 21.0 g N_2-N/ha/d (in the control plot the rate was less than 9.0 g N_2-N/ha/d), values which were considered to be rather low (Knowles 1977). This phenomenon was likely to be due to the relatively dry soil in which the moisture content (Table 4) was not high enough to restrict aeration (Hill et al. 1990). In addition, the low C/N ratio of FYM which induced N mineralization, and the application of urea inhibited the N_2 fixing activity, as previously reported (Fig. 3) (Nohrstedt 1988; Ghani Nugroho and Kuwatsuka 1990, 1992b, c). This inhibitory effect lasted during a long period of time and was still significant in the last measurement (October) when the level of NH_4-N in soil had decreased to the original low level.

Total bacteria, denitrifiers, and N_2 fixers

The number of total bacteria in the FYM-treated plots was larger than that in the control plot throughout the experimental period. The number consistently increased with the increase of the rate of FYM application (Fig. 4), particularly in June as a response to

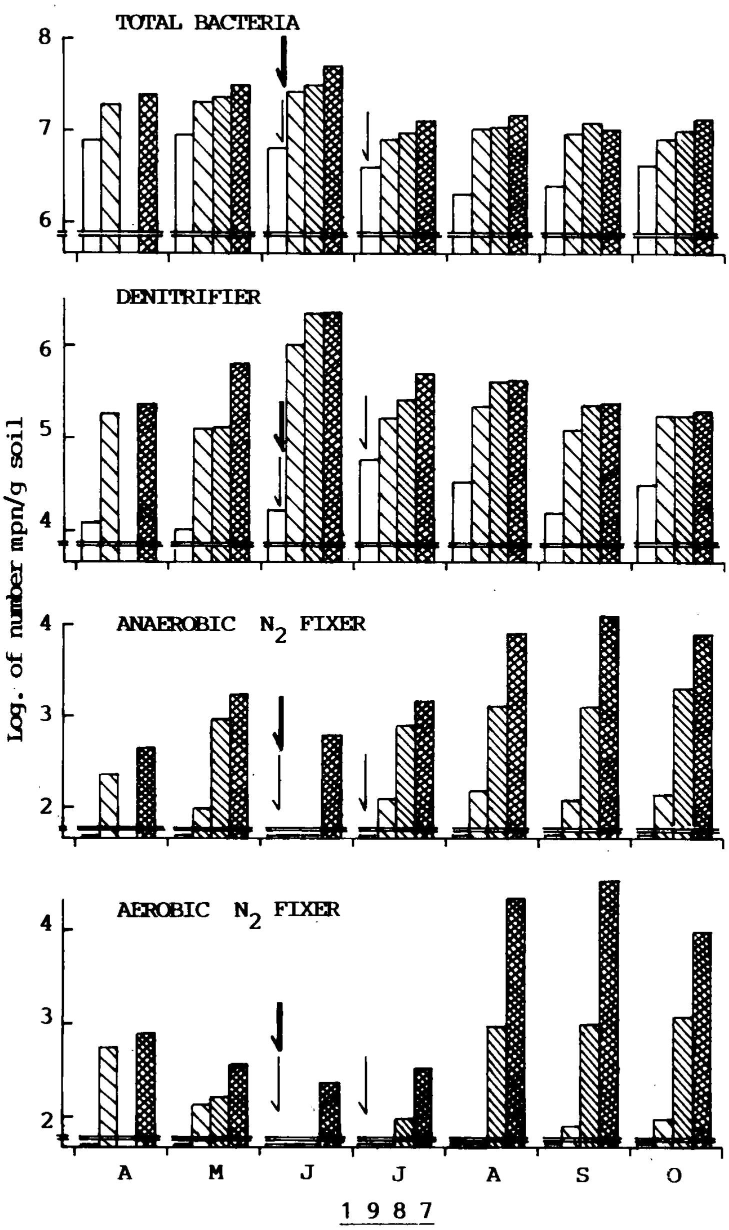

Fig. 4. Changes in the numbers of total bacteria (A), denitrifiers (B), and N_2 fixers (C) in soils after 13 yr of continuous application of FYM at different levels. Broad arrow: incorporation of FYM (June 9). Narrow arrows: urea fertilization (June 9 and July 2). ▭, control; ▧, FYM 20 t/ha; ▧, FYM 50 t/ha; ▩, FYM 100 t/ha. FYM, farmyard manure.

the latest FYM application (on June 9).

The effect of urea application on the number was not clear in both the control and the FYM-treated plots as in the laboratory experiments (Ghani Nugroho and Kuwatsuka 1992b).

Long-term FYM application increased the number of denitrifiers preferentially (Fig. 4). The number of denitrifiers also increased substantially by the latest FYM application, which corresponded to the increase of the number of total bacteria. These results suggested that the denitrifiers competed effectively with other heterotrophs for the substrates supplied by the FYM application.

Long-term FYM application increased the number of N_2 fixers by 100 times (Fig. 4). A large number was observed in particular in the 100 t/ha FYM-treated plots. The number of N_2 fixers and its proportion to the number of total bacteria significantly decreased by the application of urea (Fig. 4). This phenomenon may be ascribed to the competitive suppression of N_2 fixers by other microorganisms, since the N_2 fixers lost their competitive advan-

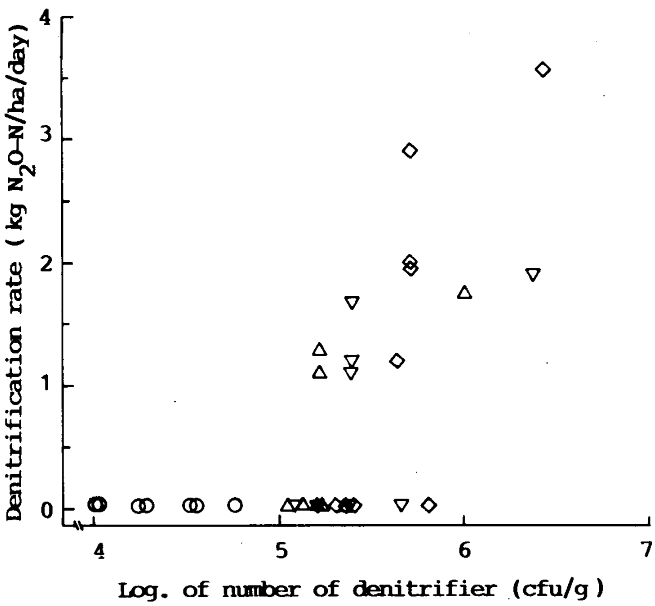

Fig. 5. Relationship between the number of denitrifiers and the rates of denitrification in soils after 13 yr of continuous application of FYM at different levels. ○, control; △, FYM 20 t/ha; ▽, FYM 50 t/ha; ◇, FYM 100 t/ha. FYM, farmyard manure.

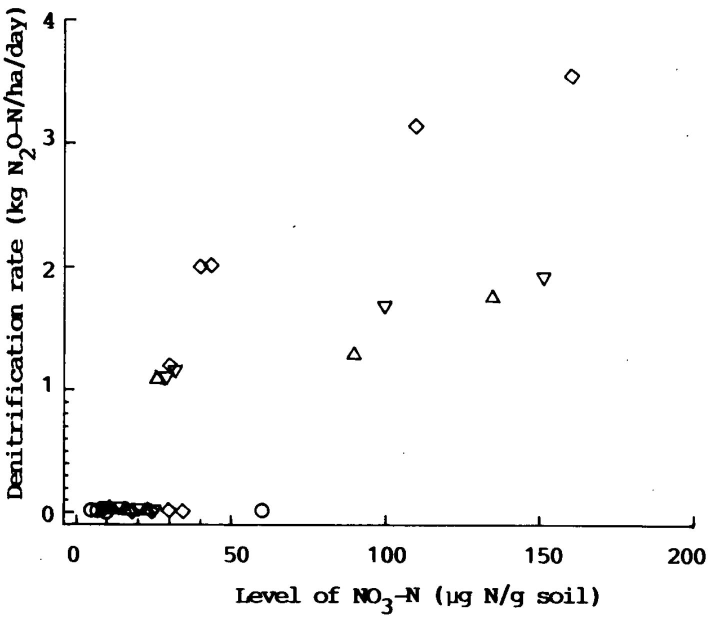

Fig. 6. Relationship between the content of NO_3-N and the rate of denitrification in soils after 13 yr of continuous application of FYM at different levels. ○, control; △, FYM 20 t/ha; ▽, FYM 50 t/ha; ◇, FYM 100 t/ha. FYM, farmyard manure.

tage to fix the atmospheric N_2 as reported by Kolb and Martin (1988).

Relationship between the denitrification and nitrogen fixing processes and the populations of denitrifiers, and N_2 fixers

The latest FYM application considerably increased the number of total bacteria as well as denitrifiers (Fig. 4). Their high respiratory O_2 consumption was expected to contribute to the formation of the anaerobic microsites even under relatively drier soil conditions ($<60\%$ MWHC). At the same time, the fertilizer N (urea) was hydrolyzed to NH_4-N and subsequently nitrified which eventually led to the increase of the NO_3-N level in soil (Fig. 2). These conditions were conducive to denitrification through the nitrification-denitrification coupled reaction in the FYM-treated plots from June to July (Fig. 3). The presence of a large number of denitrifiers was not necessarily associated with the high rate of denitrification (Fig. 5). However the denitrification rate increased proportionally with the increase of the

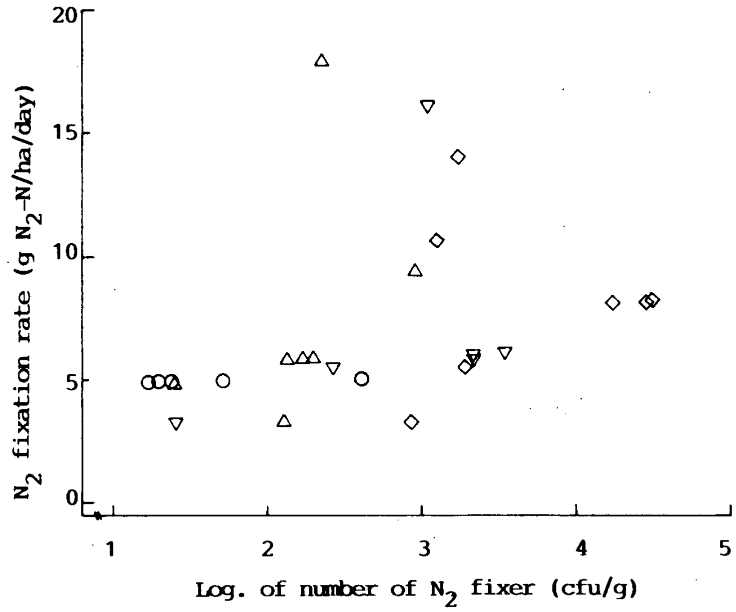

Fig. 7. Relationship between the number of N_2 fixers and the rate of N_2 fixation in soils after 13 yr of continuous application of FYM at different levels. ○, control; △, FYM 20 t/ha; ▽, FYM 50 t/ha; ◇, FYM 100 t/ha. FYM, farmyard manure.

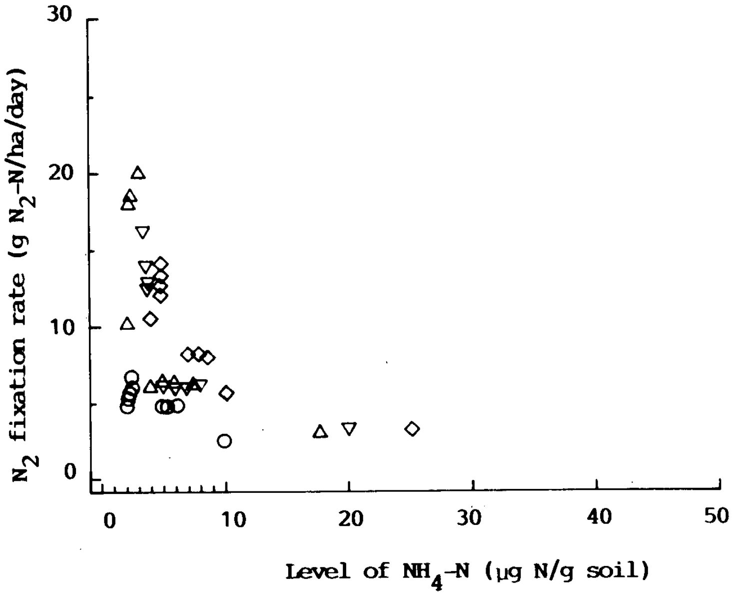

Fig. 8. Relationship between the content of NH_4-N and the rate of N_2 fixation in soils after 13 yr of continuous application of FYM at different levels. ○, control; △, FYM 20 t/ha; ▽, FYM 50 t/ha; ◇, FYM 100 t/ha. FYM, farmyard manure.

number of denitrifiers when a sufficient amount of NO_3-N and anaerobic conditions were provided in the period from June to July ($Y=-5.73+1.32$ log [No. of denitrifiers], $r=0.76^{**}$). The increase of the NO_3-N content in soil appeared to result in the increase of the rate of denitrification (Fig. 6).

Although comparatively larger numbers of N_2 fixers were observed with the increase of the rate of FYM application (Fig. 4), the N_2 fixing rate reached maximum values when the application of FYM was 20 t/ha (Fig. 3). The rate of N_2 fixation also was not positively correlated with the number of N_2 fixers (Fig. 7), due to the inhibitory effect of the high level of available inorganic-N on the nitrogenase activity, while the number of N_2 fixers was less influenced. When the level of NH_4-N in soil was higher than 5.0 μg N/g soil the N_2 fixing activity (Fig. 8) was inhibited.

Comparative significance of denitrification for N_2 fixation

Since the soil moisture content was usually below 60% MWHC in the field (Table 3), the denitrification rates were relatively low. The N_2 fixing rate increased with the amount of FYM applied. As a result, the ratio of denitrification to N_2 fixation was considerably low ranging from 0.5×10^{-4} to 1.0×10^{-4}. However, denitrification became substantial during the short-period following the simultaneous application of FYM and urea in June and July. Since this practice markedly decreased the N_2 fixation activity, the ratio of N_2 fixation to denitrification decreased, ranging from 685 to 133 during this period of time.

Acknowledgments. Gratitude is extended to Dr. M. Kimura for his invaluable suggestions during the study and constructive comments in reviewing the manuscript.

REFERENCES

Bijay-Singh, Ryden, J.C., and Whitehead, D.C. 1989: Denitrification potential and actual rates of denitrification in soils under long-term grassland and arable cropping. *Soil Biol. Biochem.*, **21**, 897-901

Burford, J.R. and Stefanson, R.C. 1973: Measurement of gaseous losses of nitrogen from soils. *Soil Biol. Biochem.*, **5**, 133-141

Ghani Nugroho, S. and Kuwatsuka, S. 1990: Concurrent observation of several processes of nitrogen metabolism in soil amended with organic materials. I. Effect of different organic materials on ammonification, nitrification, denitrification, and N_2 fixation under aerobic and anaerobic conditions. *Soil Sci. Plant Nutr.*, **36**, 215-224

Ghani Nugroho, S. and Kuwatsuka, S. 1992a: Concurrent observation of several processes of nitrogen metabolism in soil amended with organic materials. II. Effect of farmyard manure on ammonification, nitrification, denitrification, and N_2 fixation at different levels of soil moisture. *Soil Sci. Plant Nutr.*, **38**, 593-600

Ghani Nugroho, S. and Kuwatsuka, S. 1992b: Concurrent observation of several processes of nitrogen metabolism in soil amended with organic materials. III. Changes in microbial populations following application of ammonium-nitrogen. *Soil Sci. Plant Nutr.*, **38**, 601-610

Ghani Nugroho, S. and Kuwatsuka, S. 1992c: Concurrent observation of several processes of nitrogen metabolism in soil amended with organic materials. IV. Regulatory effects of ammonium- and nitrate-nitrogen on denitrification and N_2 fixation. *Soil Sci. Plant Nutr.*, **38**, 611-617

Hill, N.M., Patriquin, D.G., and Sircom, K. 1990: Increased oxygen consumption at warmer temperatures favours aerobic nitrogen in plant litters. *Soil Biol. Biochem.*, **22**, 321-325

Knowles, R. 1977: The significance of asymbiotic dinitrogen fixation by bacteria. *In* A Treatise on Dinitrogen Fixation, Sect. IV A-Z, Ed. R.W.F. Hardy and A.H. Gibson, p. 33-83, John Wiley, New York

Kolb, W. and Martin, P. 1988: Influence of nitrogen in the number of N_2 fixing and total bacteria in the rhizosphere. *Soil Biol. Biochem.*, **20**, 221-225

Nohrstedt, H.O. 1988: Effect of liming and N-fertilization on denitrification and N_2-fixation in an acid coniferous forest floor. *Forest Ecol. Manage.*, **24**, 1-13

Roper, M.M. 1983: Field measurements of nitrogenase activity in soils amended with wheat straw. *Aust. J. Agric. Res.*, **34**, 725-739

Ryden, J.C. and Lund, L.J. 1980: Nature and extent of directly measured denitrification losses from some irrigated vegetable crop production units. *Soil Sci. Soc. Am. J.*, **44**, 505-511

Scott Smith, M. and Tiedje, J.M. 1979: Phases of denitrification following oxygen depletion in soil. *Soil Biol. Biochem.*, **11**, 261-267

Sexstone, A.J., Parkin, T.B., and Tiedje, J.M. 1985: Temporal response of soil denitrification rates to rainfall and irrigation. *Soil Sci. Soc. Am. J.*, **49**, 99-103

Soderstrom, B., Baath, E., and Lundgren, B. 1983: Decrease in microbial activity and biomasses owing to nitrogen amendments. *Can. J. Microbiol.*, **29**, 1500-1506

Yoshida, S. 1984: Non-destructive determination of nitrogen fixing activity in upland plants and fields by acetylene reduction technique. *Jpn. J. Crop. Sci.*, **53**, 450-454 (in Japanese)