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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

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Under different moisture conditions, the concurrent processes of ammonification, nitrification, denitrification, and N_2 fixation in soils treated with different levels of farmyard manure (FYM; 0, 1, 2, and 3% of soil dry weight) were analyzed. All the four processes occurred readily only under moisture conditions corresponding to 60% of maximum water-holding capacity (MWHC), and were stimulated by FYM application, whereby anaerobicity was induced by the marked increase of the respiration activity. At moisture levels higher or lower than 60% MWHC, some of the processes were suppressed. Denitrification was prevented when the MWHC was less than 60%, while the nitrification was suppressed at 100% MWHC and under flooded conditions.

The application of 1% FYM resulted in a substantial decrease of the ratios of denitrification to N_2 fixation, irrespective of the moisture levels, and the ratios increased with the increase of the levels of FYM. In each FYM application, the value of the ratio of denitrification to N_2 fixation was the highest at moisture levels of 60% or 100% MWHC.

Key Words: **denitrification, farmyard manure, moisture level, N metabolism, N_2 fixation.**

In the concurrent observation of the ammonification, nitrification, denitrification, and N_2 fixation processes in soils amended with different organic materials (OM), the presence of aerobic or anaerobic conditions was the major factor for determining the patterns of these processes (Ghani Nugroho and Kuwatsuka 1990). Aerobicity is normally regulated by the level of soil moisture which controls the rate of O_2 diffusion (Stanford et al. 1975). The amendment of OM to soil promoted the development of anaerobic microsites in the aerobic soil system (Aulakh and Rennie 1987; Rice et al. 1988).

In this study, the effects of the application rate of FYM on the concurrent processes of ammonification, nitrification, denitrification, and N_2 fixation in soils at different soil

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moisture levels were investigated.

MATERIALS AND METHODS

The soil (Dystrochrept: total C = 1.05%, total N = 0.08%) and farmyard manure (FYM: total C = 33.7%, total N = 2.4%, NH_4-N = 0.089%, NO_3-N = 0.038%) were the same as those described in our previous report (Ghani Nugroho and Kuwatsuka 1990). The soil (75 g each) was amended with four levels of FYM (0, 1, 2, and 3% on a dry weight basis) and incubated for 42 d in 125-mL Erlenmeyer flasks at 30°C at four levels of soil moisture (35, 60, and 100% MWHC, and flooded with water at a 2-cm depth). The experimental procedures for the incubation, soil analysis, and simultaneous analysis of the denitrification and N_2 fixation processes (C_2H_2 inhibition of N_2O reduction and C_2H_2 reduction to C_2H_4 methods) were practically the same as those previously described (Ghani Nugroho and Kuwatsuka 1990). Calibration flasks without injection of C_2H_2 were also assayed for the detection of N_2O and CO_2 production, and for the correction (if any) of indigenous C_2H_4 production. The mole fraction of N_2O formed during the denitrification process, which is important for assessing soil anaerobiosis, was calculated according to Knowles (1981) as follows:

Mole fraction of $N_2O = N_2O\text{-}N/(N_2O + N_2)\text{-}N$
$= N_2O$ in the absence of C_2H_2/N_2O and in the presence of C_2H_2,

the decrease of N_2O indicates a further reduction of N_2O to N_2 due to the intensification of the anaerobic conditions. The calculation of the net production of inorganic nitrogen was the same as that described in our previous report (Ghani Nugroho and Kuwatsuka 1990).

RESULTS AND DISCUSSION

Carbon mineralization and development of anaerobiosis

High rates of carbon mineralization were observed in the FYM-treated soils incubated aerobically at a moisture level of 60% MWHC. When the MWHC was higher or lower than this value, the rates of carbon mineralization decreased (Fig. 1). The CO_2 production reached its peak on the 3rd day at 35 and 60% MWHC, on the 12th day at 100% MWHC, and on the 18th day under flooded conditions, regardless of the FYM application levels.

The high respiratory activity in the FYM-treated soils at a moisture level of 60% MWHC (Fig. 1) is likely to consume the present O_2 in the localized zones within the soil matrix, creating anaerobic soil-microsites particularly in the vicinity of FYM lumps. The greater the CO_2 production the greater the opportunity for the increase of the size of the anaerobic soil-microsites. The mole fraction of N_2O in the denitrification product (Table 1) confirmed the development of anaerobiosis at a moisture level of 60% MWHC with FYM application. Smith (1980) and Klemedtsson et al. (1987) also observed this phenomenon in the aggregated soil and root environment, respectively.

The mole fraction of N_2O decreased with the increase of the levels of both the FYM application and soil moisture level (Table 1). N_2 was the main form of the denitrification product at 100% MWHC and under flooded conditions, while N_2O was the main product under 35% MWHC conditions. At a moisture level of 60% MWHC, the main form of the denitrification product was influenced by the FYM levels: N_2O was the main product in the soil without FYM application, and both N_2 and N_2O were produced by the 3% FYM application.

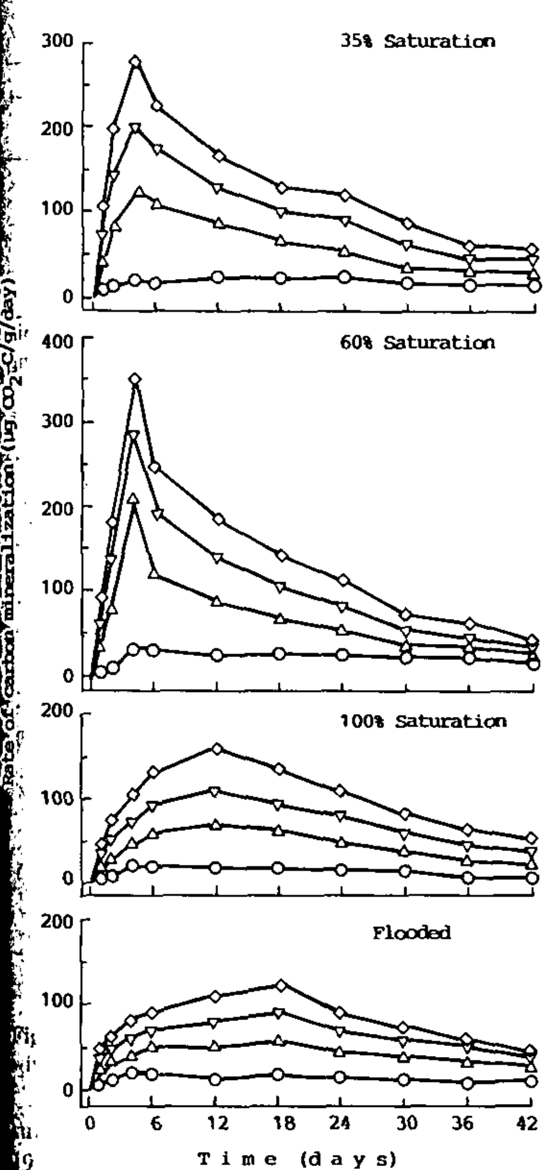

Fig. 1. Time course of the rates of carbon mineralization in soils treated with different levels of FYM and incubated under four moisture conditions. ○, control soil; △, 1% FYM; ▽, 2% FYM; ◇, 3% FYM. FYM, farmyard manure.

Concurrent processes of ammonification, nitrification, denitrification, and N_2 fixation

The effects of the soil moisture level on ammonification differed from those on the subsequent process (nitrification). At high moisture levels (100% MWHC and under flooded conditions), ammonification still occurred although at lower rates while the nitrification was completely inhibited. At lower moisture levels (35 and 60% MWHC), on the other hand, the concurrent ammonification and nitrification processes apparently occurred (Fig. 2).

Without FYM application, denitrification hardly occurred at a low soil moisture level below 60% MWHC or occurred only at low rates, and slightly increased with the increase of the soil moisture level (Fig. 3). Although denitrification was expected to be limited by the

Table 1. Average mole fraction of N_2O in the gaseous products of denitrification.[a]

FYM[b] added (%)	Average mole fraction of N_2O			
	Soil moisture levels (%)[c]			
	35	60	100	Flooded
0	0.98±0.15	0.95±0.11	0.21±0.03	0.10±0.04
1	0.94±0.12	0.76±0.08	0.11±0.04	0.04±0.02
2	0.87±0.09	0.65±0.05	0.09±0.04	0.06±0.01
3	0.85±0.10	0.58±0.07	0.08±0.03	0.05±0.02
Mean	0.91	0.73	0.12	0.06

[a] The decrease in N_2O indicates further reduction of N_2O to N_2 due to the intensification of the anaerobic conditions. [b] FYM, farmyard manure (% on dry weight basis). [c] % of saturation or maximum water-holding capacity.

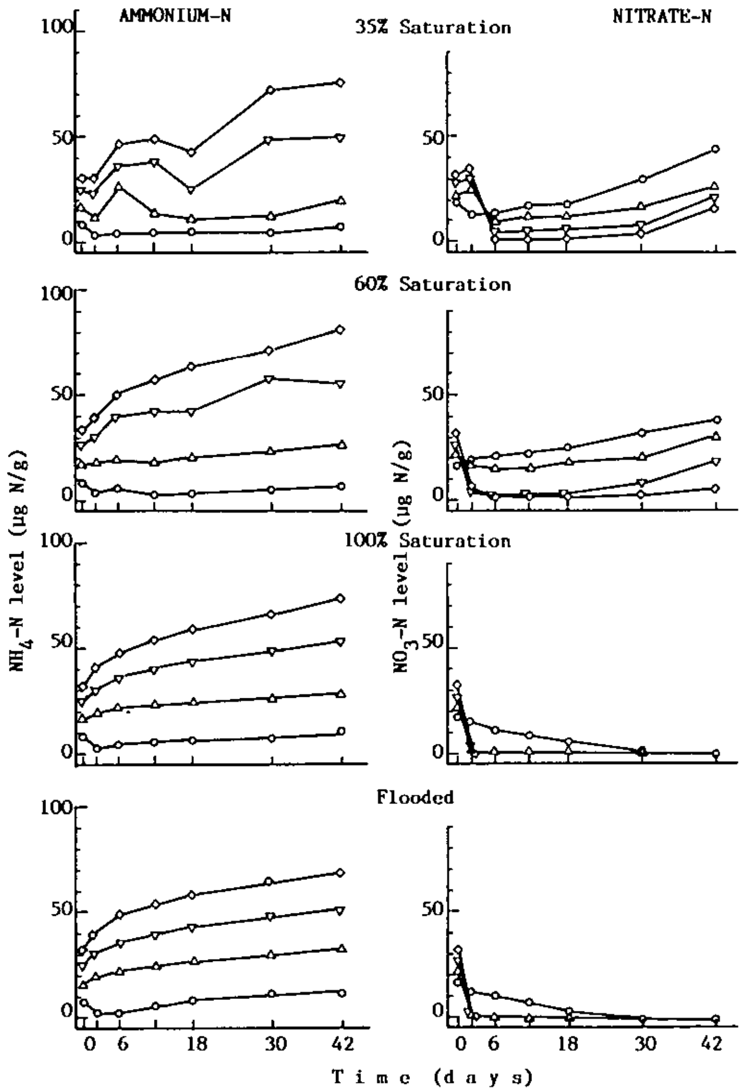

Fig. 2. Time course of the changes in the contents of NH_4-N and NO_3-N in soils treated with different levels of FYM and incubated under four moisture conditions. ○, control soil; △, 1% FYM; ▽, 2% FYM; ◇, 3% FYM. FYM, farmyard manure.

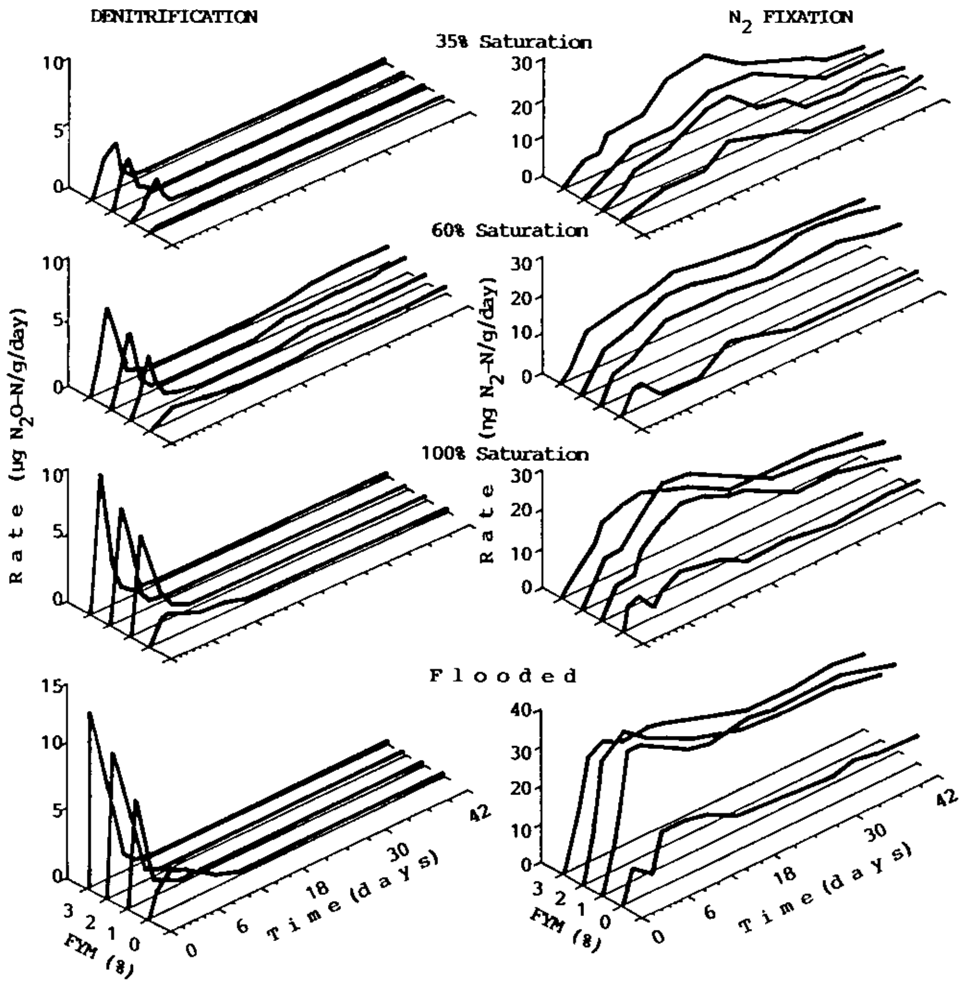

Fig. 3. Time course of the rates of denitrification and N_2 fixation measured simultaneously in soils treated with different levels of FYM and incubated under four moisture conditions. FYM, farmyard manure.

supply of available carbon and NO_3-N concentration (Myrold and Tiedje 1985; Malhi et al. 1990), the supply of available C rather than NO_3-N limited the denitrification in the soil without FYM application. Similar findings were reported by Bijay-Singh et al. (1988). There were two peaks of denitrification in the soil at a moisture level of 60% MWHC, during the 0-6 d (Peak-1) and 18-42 d (Peak-2) periods of incubation. The increase of the rate of FYM application not only stimulated the maximum level of denitrification in Peak-1, but also advanced its occurrence (Table 2). The increase of the application rates of FYM resulted in higher levels of denitrification in Peak-1 when the moisture level increased.

At a 30% MWHC level, the FYM application induced denitrification in Peak-1, which it did not increase the denitrification level in Peak-2. At 100% MWHC and water flooded conditions, on the other hand, the virtual absence of NO_3-N after approximately 3 d of incubation limited the denitrification in Peak-2 in the FYM-treated soils. Only at 60% MWHC was the denitrification in Peak-2 associated with NO_3-N formation (nitrification), suggesting that both aerobic and anaerobic zones coexisted in soil and that the conditions

Table 2. Occurrence of the maximum rate of denitrification in Peak-1.

Moisture levels (%)[b]	Denitrification rate (μg N/g/d)			
	FYM added (%)[a]			
	0	1	2	3
35	0.0	1.1 (4)	1.5 (3)	1.9 (3)
60	0.5 (4)[c]	4.3 (3)	5.4 (3)	6.2 (2)
100	2.0 (4)	6.5 (2)	8.0 (2)	9.2 (1)
Flooded	2.5 (3)	7.8 (1)	9.6 (1)	11.2 (1)

[a] FYM, farmyard manure (% on dry weight basis). [b] % of saturation or maximum water-holding capacity.
[c] ( ), day(s) after incubation.

Table 3. Total N_2 fixation for 42 d period.

Moisture levels (%)[b]	Total N_2 fixation (ng N/g)			
	FYM[a] added (%)			
	0	1	2	3
35	94	258	311	368
60	93	528	635	542
100	150	513	428	354
Flooded	361	1,135	927	769

[a] FYM, farmyard manure (% on dry weight basis). [b] % of saturation of maximum water-holding capacity.

Table 4. Concurrent NH_4-N and NO_3-N formation, denitrification, and N_2 fixation during the period of 7 to 42 d.

FYM[a] levels (%)	Formation of NH_4-N (μg N/g)	Formation of NO_3-N (μg N/g)	Denitrication Peak-2 (μg N/g)	N_2 fixation (ng N/g)
		35% saturation[b]		
0	+2.2	+28.6	0.0	76
1	−7.8	+17.4	0.0	232
2	+15.3	+16.3	0.0	285
3	+31.3	+15.1	0.0	348
		60% saturation		
0	+0.8	+15.1	14.4	62
1	+9.5	+16.2	9.0	470
2	+14.6	+18.6	8.6	577
3	+32.8	+4.6	6.8	494
		100% saturation		
0	+5.8	−10.0	8.1	113
1	+6.9	0.0	0.0	465
2	+18.6	0.0	0.0	375
3	+24.4	0.0	0.0	307
		Flooded		
0	+9.3	−10.1	5.1	307
1	+8.1	0.0	0.0	976
2	+17.4	0.0	0.0	767
3	+21.0	0.0	0.0	650

[a] FYM, farmyard manure (% on dry weight basis). [b] Or maximum water-holding capacity.

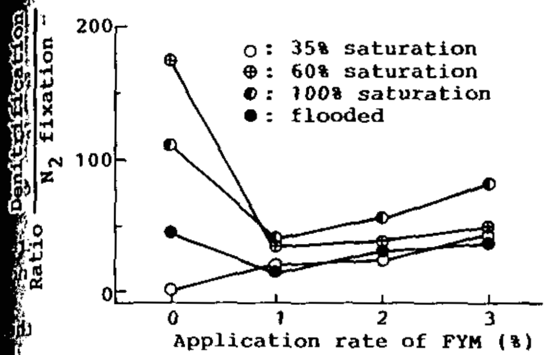

Fig. 4. Relationship between the application rates of FYM and the ratios of denitrification to N_2 fixation in soil incubated under four moisture conditions.

favorable for the concurrent occurrence of both nitrification and denitrification may be provided. This observation likely confirmed the findings of Kokufuta et al. (1988) and Rice et al. (1988).

Without FYM application, N_2 fixation occurred only at a very low rate (Fig. 3), probably due to the lack of available substrates in soil. Application of FYM to soil increased the N_2 fixation for 42 d (Table 3). In contrast with general observations, a high level of FYM (low C/N ratio) application adversely decreased the N_2-fixing activity presumably due to the relatively large production of inorganic N. The available inorganic N can normally be used preferentially as N source for the metabolism by N_2 fixers as Kolb and Martin (1988) suggested, thus hampering the N_2-fixing activity. The N_2-fixing activity increased with the increase of the soil moisture level, suggesting that N_2 fixation (whether by aerobic, facultative anaerobic, or strict anaerobic N_2 fixers) proceeded more actively under poor rather than good aeration conditions as reported by Barrow and Jenkinson (1962).

The concurrent ammonification, nitrification, denitrification, and N_2 fixation processes were observed only at a moisture level of 60% MWHC, particularly during the Peak-2 period of 7 to 42 d (Table 4).

Dominant processes for denitrification and N_2 fixation were evaluated on the basis of their ratios at different soil moisture and FYM levels. The application of 1% FYM resulted in a substantial decrease of the ratios of denitrification to N_2 fixation, regardless of the moisture levels, but the ratios increased gradually with the increase of the FYM application rates (Fig. 4). At each FYM application level, the ratio showed the highest value at moisture levels of 60 or 100% MWHC. As described in our previous report (Ghani Nugroho and Kuwatsuka 1990), the FYM-treated soils contained only a small amount of NO_3-N but high levels of substrates such as water soluble materials, lignin and cellulose, which may have induced the decrease of the denitrifying and increase of the N_2-fixing activities, respectively.

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