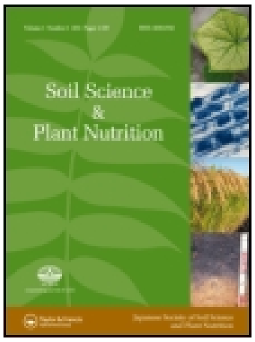

Soil Science and Plant Nutrition

ISSN: 0038-0768 (Print) 1747-0765 (Online) Journal homepage: http://www.tandfonline.com/loi/tssp20

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To cite this article: Sutopo Ghani Nugroho & Shozo Kuwatsuka (1990) Concurrent observation of several processes of nitrogen metabolism in soil amended with organic materials, Soil Science and Plant Nutrition, 36:2, 215-224, DOI: 10.1080/00380768.1990.10414986

To link to this article: http://dx.doi.org/10.1080/00380768.1990.10414986

Published online: 04 Jan 2012.

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

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Received September 16, 1988

Under aerobic and anaerobic conditions, the concurrent ammonification, nitrification, denitrification, and N_2 fixation activities in soils treated with several organic materials were determined. All activities were apparently observed only under aerobic conditions, particularly in the soils treated with organic materials with a low C/N ratio. In the case of a high C/N ratio, several processes were delayed due to the high activity of N immobilization, while under anaerobic conditions, the nitrification activity for the supply of NO_3-N was so limited that the occurrence of denitrification was inhibited.

The apparent ratio of nitrification to ammonification was higher under aerobic than anaerobic conditions. The ratio of nitrification to denitrification was also higher under aerobic than anaerobic conditions due to the complete inhibition of the nitrification process under anaerobic conditions. The ratio of denitrification to N_2 fixation was higher under aerobic than anaerobic conditions. It was shown that it is difficult to predict the ratios of these processes only based on the soil C content or the C/N ratio of amended materials. These ratios are controlled by the C/N ratio of amended materials, as well as by the constituents of amended materials and the levels of NH_4-N and NO_3-N.

Key Words: **aerobic and anaerobic conditions, denitrification, N metabolism, organic material amendment.**

Numerous reports on the N metabolism in soil have mostly focused on a single process, rather than on interrelations among the various processes. Any one of the N cycling processes inevitably leads to other processes (Tiedje et al. 1981), since the end product of one process may become the substrate of another (Campbell and Lees 1967).

The concurrent processes of ammonification, nitrification, denitrification, and N_2 fixation in soil are of special interest. This microbial N transformation is highly dependent

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upon the supply of available C and energy for soil microorganisms (Staaf and Berg 1981). Therefore, the effect of organic materials on the N transformation is exerted directly through the stimulation of microbial proliferation and indirectly through ecological modifications such as changes in pH, aeration, nutrient availability, etc. (Haynes 1986). These processes of N metabolism occur simultaneously in a certain soil ecosystem. However, there are few reports on the correct evaluation of the ratios of these biochemical reactions under identical soil conditions at the same time, in particular the ratio of denitrification to N_2 fixation which occur in opposite direction in the N flow in soil.

The laboratory study described here, which was conducted under aerobic and anaerobic conditions with a constant soil moisture content, was designed firstly to analyze the effects of various organic materials and soil aeration conditions on the ammonification, nitrification, denitrification, and N_2 fixation processes and secondly, to evaluate the ratios of these biochemical reactions, particularly the ratio of denitrification to N_2 fixation in soil amended with organic materials.

MATERIALS AND METHODS

Soils and organic materials. The soil was collected from an upland field in Nagoya University Farm that had never been subjected to organic material amendment. Composite soil samples were collected from the upper 15 cm layer, and passed through a 2-mm mesh sieve under moist field conditions. The soil is a Red-Yellow soil with clay loam (Dystrochrept, Table 1). The organic materials used were rice straw (RS), rice straw compost (RSC), farmyard manure (FYM), and sawdust-cow-feces compost (SDCFC). Organic materials (OM) were air-dried, finely ground, and the chemical components were analyzed (Table 2).

Incubation. Six sets (each of 20 flasks) of duplicate treatments and one set (20 flasks) of duplicate calibration flasks were adopted initially. Soil samples (75 g each on a dry weight basis) were placed in 125-ml Erlenmeyer flasks, to which 2% (on dry weight basis) of OM (RS, RSC, FYM, and SDCFC) was added in powder form, and thoroughly mixed. Then, distilled water was added to reach a soil moisture content corresponding to 60% of maximum water holding capacity (MWHC). The flasks without OM were treated in the same way. Each flask was sealed with a butyl rubber stopper. The flasks were then evacuated and reflushed several times with a gas mixture of N_2 and O_2 (4 : 1) or with N_2 to give 1 atm for aerobic or anaerobic conditions, respectively. Afterwards the six sets of flasks were incubated at 30°C for designated periods (Table 3).

Analysis. The C_2H_2 inhibition of N_2O reduction and C_2H_2 reduction was used for

Table 1. Properties of the soil samples used.

Properties	Values
Texture	Clay loam
pH	6.4
Total carbon (%)	1.05
Total nitrogen (%)	0.08
NH_4-N (μg/g)	8.7
NO_3-N (μg/g)	16.2
Available P_2O_5 (ppm)	168.0
MWHC[a] (ml/100 g)	43.0

[a] Maximum water holding capacity.

Table 2. Chemical components of the organic materials used.[a]

(% of oven dry weight)

Chemical component	RS	RSC	FYM	SDCFC
Hot-water soluble	13.5	17.4	28.9	12.2
Lignin	14.5	15.0	11.9	28.5
Hemicellulose	31.2	36.9	25.0	11.7
Cellulose	32.1	11.5	30.0	34.4
Ash[b]	8.7	19.2	4.2	13.2
Total C	36.2	17.0	33.7	41.7
Total N	0.7	1.7	2.4	2.5
C/N ratio	52	10	14	17
NH_4-N	0.013	0.615	0.089	0.025
NO_3-N	0.012	0.128	0.038	0.115

[a] Analyzed by Harper and Lynch's method (Harper and Lynch 1981). [b] Ash remaining after sequential extractions. RS, rice straw; RSC, rice straw compost; FYM, farmyard manure; SDCFC, sawdust-cow-feces compost.

Table 3. Description of total incubation period and time of sampling for different sets.

Set number	Total incubation period (day)	Time of sampling for	
		Inorganic N analysis, on day(s)	N_2O and C_2H_4, on day(s)
With C_2H_2			
1	0	0	—
2	6	6	1, 2, 3, 4, 5, 6
3	12	12	8, 10, 12
4	18	18	14, 16, 18
5	30	30	21, 24, 27, 30
6	42	42	33, 36, 39, 42
Without C_2H_2			
7 (calibration flasks)	42	—	1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 21, 24, 27, 30, 33, 36, 39, 42

the simultaneous measurement of denitrification and N_2 fixation (Yoshinari et al. 1977). The flasks were subjected to 1-day assay in the presence of 5% (v/v) of acetylene. Calibration flasks without injection of acetylene were also assayed for correction, if any, of indigenous C_2H_4 production. Time of assay for different sets is shown in Table 3. Prior to the assay, the flasks were evacuated and replaced several times with a gas mixture of Ar and O_2 (4 : 1) or Ar to 1 atm for aerobic or anaerobic conditions, respectively. Acetylene (5%, v/v) was injected after an equal amount of the head space gas was removed from the flask. After 24 h, a 0.2 ml portion of the gas sample in the flask was analyzed for N_2O and C_2H_4 using a GC equipped with a TCD and a FID, and a column of Poropak N and Molecular Sieve 5A, respectively. Column temperature was kept at 50°C, and injection and detector temperature was 100°C. After the assay, the head space gas of the flasks was replenished with a gas mixture of N_2 and O_2 (4 : 1) or with N_2 to reach 1 atm for aerobic and anaerobic conditions, respectively, and the flasks were then returned to the incubator.

After the designated period of incubation, the soil in each flask was well mixed and the determination of the content of inorganic N and total Kjeldahl N was immediately carried out or otherwise the soil was stored at −15°C until analysis. A 10-g (on dry weight basis) portion of the samples was extracted with 2 M KCl for the determination of the content of inorganic N, and another 10-g portion for the determination of total Kjeldahl N. A portion of the samples was air-dried for the determination of the total C content by a dry combustion method using a Yanagimoto MT-500 CN corder. The remaining soil was used for water content determination.

Net production of inorganic N was calculated on the basis of gross changes in the content of NH_4-N and NO_3-N between the initial period and the end of incubation and was added to the value of total N loss by denitrification. The total denitrification represents the 42-day-integrated values of 1-day gaseous N losses recovered as N_2O inhibited with C_2H_2. Accordingly, the total N_2 fixation represents the 42-day-integrated values of 1-day production of C_2H_4 from the reduction of C_2H_2 divided by the fixed value of 3.

RESULTS AND DISCUSSION

Organic matter decomposition

At 30°C and 60% of MWHC, the rates of OM decomposition under aerobic conditions were consistently higher than under anaerobic conditions (Fig. 1) with differences ranging from 4.8 to 8.9% of C loss during the 42-day period (Table 4). The differences mostly occurred in the initial period, during which organic C rapidly disappeared, due to the role of O_2 in the microbial metabolism. Under anaerobic conditions, the decomposition process of OM was largely dependent on the activity of obligate and facultative anaerobes which require a much lower amount of energy and are less efficient than aerobes (Yoshida 1975), so that OM was incompletely metabolized to induce the accumulation of intermediate products.

The rate of organic C decomposition was correlated positively with the content of hot-water soluble materials, hemicellulose and cellulose of added OM, and negatively with their lignin contents (Tables 2 and 4). There was no correlation between the rate of decomposition and the C/N ratio of the added OM (Table 4). In this case the concept of regulation effect of the C/N ratio of the added OM on the rates of substrate decomposition may not be valid, since it does not adequately characterize the availability of C to soil

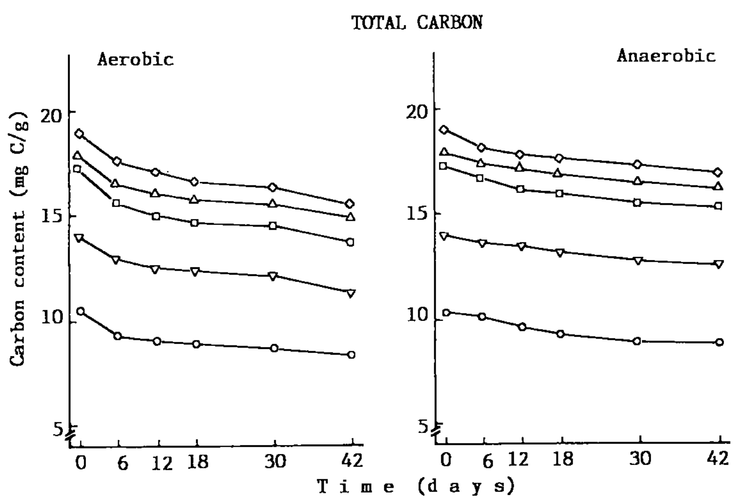

Fig. 1. Time course of the changes in the total carbon content in soil amended with different organic materials at 2% rate and incubated under aerobic and anaerobic conditions. ○, control; △, rice straw (RS); ▽, rice straw compost (RSC); □, farmyard manure (FYM); ◇, sawdust-cow-feces compost (SDCFC).

Table 4. Total carbon loss during 42 days of incubation.

Treatment	Total C loss (%)
Aerobic	
Control	19.2
RS	16.9
RSC	17.2
FYM	20.3
SDCFC	16.5
Anaerobic	
Control	14.4
RS	8.5
RSC	10.7
FYM	12.2
SDCFC	10.0

RS, rice straw; RSC, rice straw compost; FYM, farmyard manure; SDCFC, sawdust-cow-feces compost.

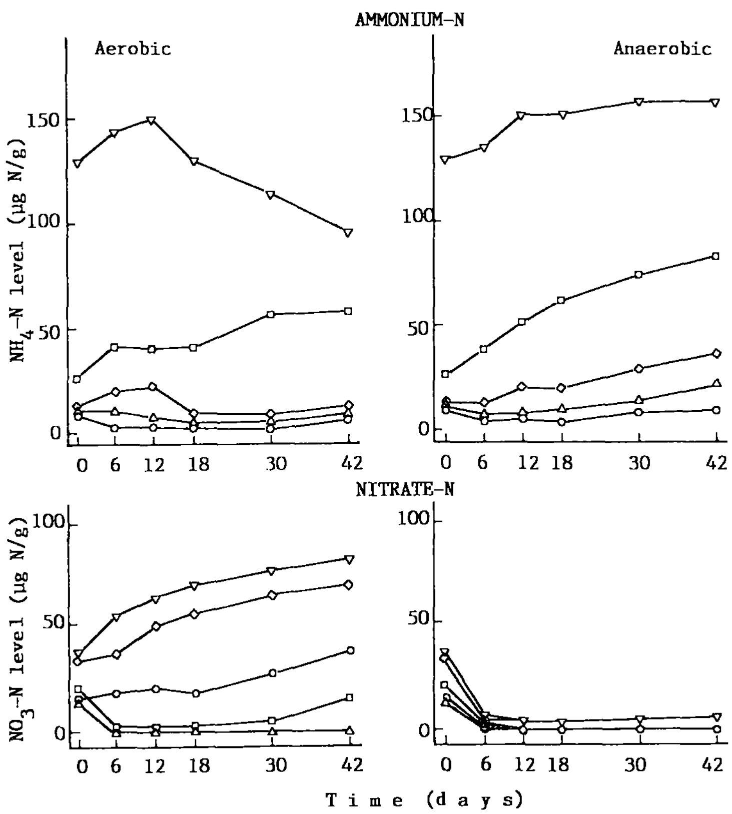

Fig. 2. Time course of the changes in the levels of NH_4-N and NO_3-N in soil amended with different organic materials at 2% rate and incubated under aerobic and anaerobic conditions. ○, control ; △, rice straw (RS); ▽, rice straw compost (RSC); □, farmyard manure (FYM); , sawdust-cow-feces compost (SDCFC).

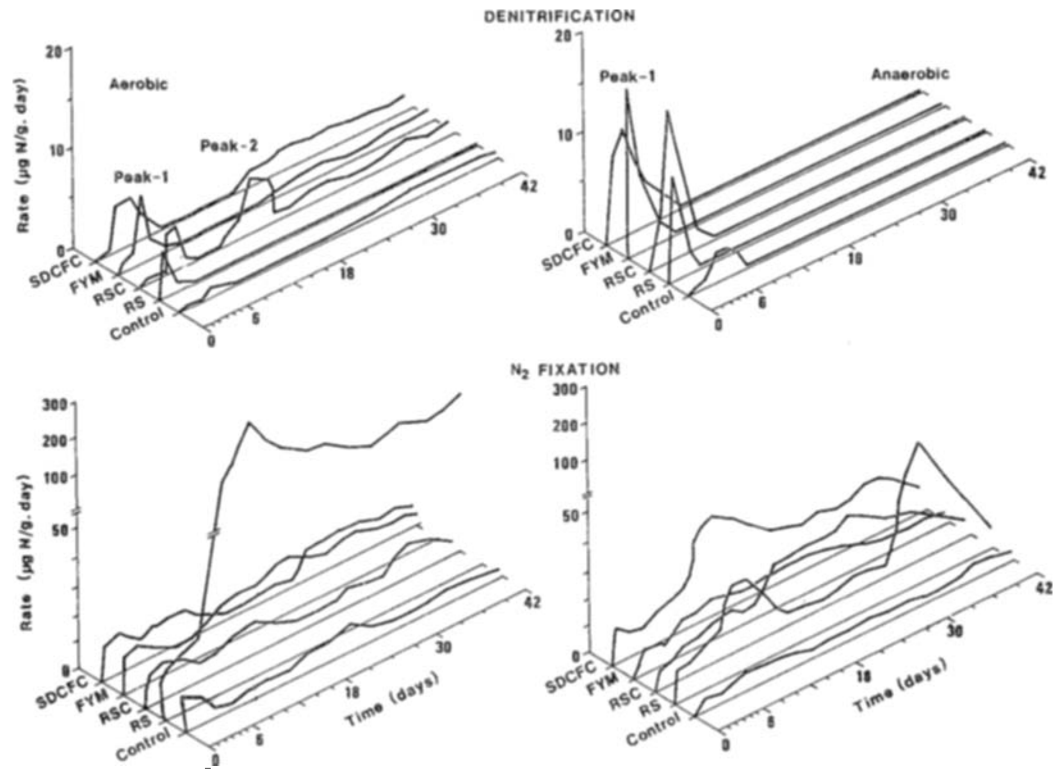

Fig. 3. Time course of the rates of denitrification and N_2 fixation measured simultaneously in soil amended with different organic materials at 2% rate and incubated under aerobic and anaerobic conditions. RS, rice straw; RSC, rice straw compost; FYM, farmyard manure; SDCFC, sawdust-cow-feces compost.

microorganisms (Parr and Papendick 1978).

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

The ammonification of organic N occurred both under aerobic and anaerobic conditions. The nitrification, however, was affected by the presence of O_2 as NH_4-N was converted to NO_3-N only under aerobic conditions (Fig. 2). This observation supports the assumption that both aerobes and anaerobes are related with the ammonification process, while only aerobes are involved in the nitrification process. Consequently, under aerobic conditions, it is likely that there is a delicate balance between NH_4-N and NO_3-N levels at any time (Fig. 2, Aerobic) and possibly also a competition between heterotrophs and autotrophs for inorganic N and O_2. On the other hand, under anaerobic conditions, the low N requirement for microbial growth (Acharya 1935) led to a more rapid release of NH_4-N (Fig. 2, Anaerobic) than would ordinarily be expected on the basis of the low rate of OM decomposition (Fig. 1, Anaerobic). In the RSC-treated soil, the (NH_3+NO_3) content decreased from the 12th day to 42nd day, presumably because the application of RSC increased the NH_3 content in the soil, and the nitrification and the subsequent denitrification were more active than the ammonification in the soil. The control soil showed a low but steady rate of N mineralization resulting in actual N release. Likewise, the application of RSC, FYM, and SDCFC (all of which having a C/N ratio <17) to soil contributed to the actual release of

Table 5. Relation between production of inorganic N and N_2 fixation.

Treatment	Production of inorganic N for 42 days		N_2 fixation for 42 days (ng N/g)
	Net (μg N/g)	Contribution of OM (μg N/g)	
Aerobic			
Control	36.6	—	87
RS	−7.0	−43.6	6,973
RSC	72.4	35.8	380
FYM	50.7	14.1	650
SDCFC	91.5	54.9	110
Anaerobic			
Control	7.7	—	210
RS	11.4	3.7	889
RSC	34.8	27.1	794
FYM	61.8	54.1	675
SDCFC	41.2	33.5	740

OM, organic matter; RS, rice straw; RSC, rice straw compost; FYM, farmyard manure; SDCFC, sawdust-cow-feces compost.

Table 6. Concurrent NH_4-N and NO_3-N formation, denitrification, and N_2 fixation during the 7 to 42 day period.

Treatment	Formation of		Denitrification, Peak-2 (μg N/g)	N_2 fixation (ng N/g)
	NH_4-N (μg N/g)	NO_3-N (μg N/g)		
Aerobic				
Control	+3.4	+19.1	15.0	56
RS	+2.0	−0.9	0.0	6,825
RSC	−57.9	+29.1	60.0	316
FYM	+19.2	+16.9	9.0	582
SDCFC	−2.7	+37.1	36.7	62
Anaerobic				
Control	+10.9	−0.4	2.6	172
RS	+15.7	0.0	0.0	787
RSC	+25.7	0.0	0.0	733
FYM	+48.0	0.0	0.0	680
SDCFC	+28.9	0.0	0.0	698

RS, rice straw; RSC, rice straw compost; FYM, farmyard manure; SDCFC, sawdust-cow-feces compost.

N under both aerobic and anaerobic conditions (Table 5). In contrast, since the application of RS (C/N ratio 52) resulted in a high N immobilization activity the activities of nitrification and subsequent denitrification were delayed.

Denitrification occurred rapidly upon incubation in all the treatments, particularly under anaerobic conditions (Fig. 3). The first phase of the high rate of denitrification (named Peak-1) lasted for only 3 to 6 days due to the disappearance of the NO_3-N initially present in the first 6 days (Fig. 3). Murakami (1987) reported that most of the NO_3-N initially present was lost in the step of Peak-1. Thereafter, the second phase of the low-rate of denitrification (named Peak-2) appeared or did not appear. Under anaerobic conditions, the virtual absence of NO_3-N and the inhibition of the nitrification process were the major limiting factors for the occurrence of Peak-2 in all the treatments. In contrast, under aerobic conditions, Peak-2 emerged in association with the NO_3-N formation, except for the

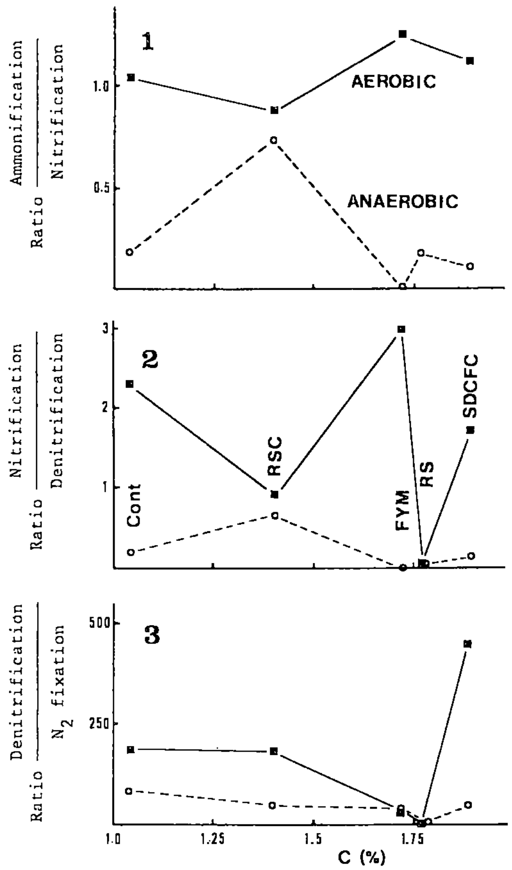

Fig. 4. Relation between the total carbon content of soil and the ratios of ammonification to nitrification (1), nitrification to denitrification (2), and denitrification to N_2 fixation (3). RS, rice straw; RSC, rice straw compost; FYM, farmyard manure; SDCFC, sawdust-cow-feces compost.

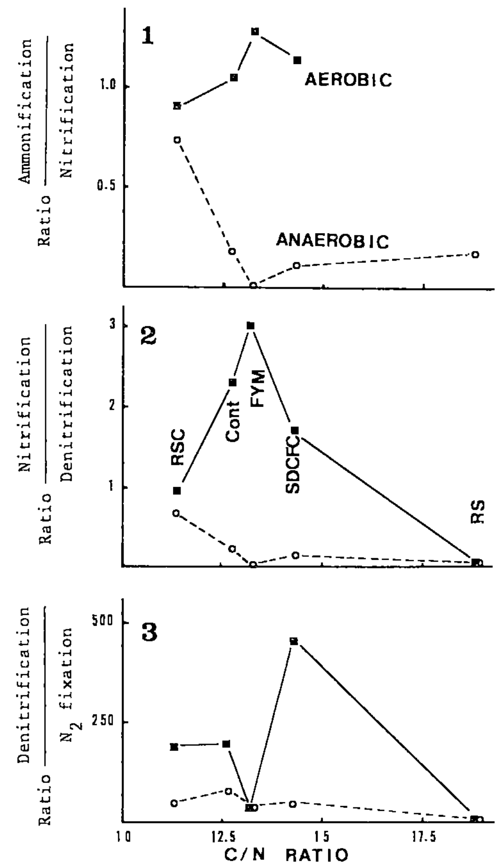

Fig. 5. Relation between the soil C/N ratios and the ratios of ammonification to nitrification (1), nitrification to denitrification (2), and denitrification to N_2 fixation (3). RS, rice straw; RSC, rice straw compost; FYM, farmyard manure; SDCFC, sawdust-cow-feces compost.

RS-treated soil. The activity of the reductases catalyzing the denitrification process is generally inhibited by O_2 (Kakami et al. 1985) and the derepression of the activity of the denitrifying enzymes is considered to require strict anaerobiosis. However, the activity may persist under some aerobic conditions (Hernandes and Rowe 1987). It has been reported that the biogenic production of N-gases may occur in well-aerated soils (Broadbent and Clark 1972; Starr et al. 1974). The presence of denitrification under aerobic conditions has been ascribed to the existence of anaerobic microsites within the aerobic soil matrix (Burford and Stefanson 1973) which create the conditions favorable for the occurrence of nitrification-denitrification coupled reactions (Patrick 1982). Anaerobic microsites can develop even in drier soils when the biological oxygen requirement is high due to the application of OM (Broadbent and Clark 1972; Scott Smith and Tiedje 1979).

Nitrogen fixation occurred under both aerobic and anaerobic conditions (Fig. 3). Since N_2 fixation is a reductive process involving highly O_2-sensitive enzymes, the N_2 fixers develop a protective mechanism against O_2 under aerobic conditions (Granhall 1981).

Application of OM increased the total N_2 fixation for the 42 day period (Table 5). While in the absence of OM, the N_2 fixation occurred only at very low rates. The lack of available C and energy seemed to be the major limiting factor for the asymbiotic N_2 fixation in the control soil as reported by Postgate and Hill (1979). The N_2 fixation was governed by the apparent N mineralization-immobilization process (Table 5). When the amendment with organic materials resulted in the apparent N immobilization (*e.g.* in the RS-treatment), the N_2 fixation was stimulated and vice versa.

Concurrent ammonification, nitrification, denitrification, and N_2 fixation activities were apparently observed only under aerobic conditions, particularly during the period from 7 to 42 days in all the OM-treated and non-treated soils except for the RS-treated soil (Table 6). In the RS-treated soil under aerobic conditions, nitrification and subsequent denitrification were retarded due to the high activity of N immobilization, while in all the treatments under anaerobic conditions, as the absence of O_2 inhibited the nitrification, denitrification could not occur due to the limited supply of NO_3-N as the source of denitrification.

Dominant N metabolism processes under amendment with various organic materials and ratio

Simultaneous measurements of the concurrent ammonification, nitrification, denitrification, and N_2 fixation activities enable to evaluate the ratios of these processes under identical soil conditions at the same time.

The ratio of nitrification to ammonification was higher under aerobic than anaerobic conditions (Figs. 4 and 5). It remained rather constant under aerobic conditions, irrespective of the levels of the C and value of C/N ratio. Since the values of both nitrification and ammonification in the RS-treated soil were negative under aerobic conditions, the calculation of the ratio was omitted. The high ratio of the RSC-treated soil under anaerobic conditions may be ascribed to the fact that the immobilization process was active compared with ammonification due to the high NH_4-N content in this soil (Fig. 2).

The ratio of nitrification to denitrification was also higher under aerobic than anaerobic conditions, presumably due to the complete inhibition of the nitrification process under anaerobic conditions. No clear tendency was observed for the C content or C/N ratio under aerobic conditions (Figs. 4 and 5). The low ratio of the RS-treated soil under aerobic conditions was due to the low amount of NO_3-N and the high C/N ratio of amended rice straw. The ratio of the RSC-treated soil was low under aerobic conditions and high under anaerobic conditions, respectively, since nitrification was active due to the high content of NH_4-N under aerobic conditions. The presence of a high ratio under anaerobic conditions was difficult to explain as the soil contained a large amount of NO_3-N and denitrification was expected to be high.

The ratio of denitrification to N_2 fixation was higher under aerobic than anaerobic conditions (Figs. 4 and 5). As in the case of the ratio of nitrification to denitrification, there was no clear relation between the ratios, and the levels of C or C/N. The zero ratios observed in the RS-treated soil under both aerobic and anaerobic conditions were ascribed to the fact that the rice straw with a very high C/N ratio brought about low denitrifying and high N_2 fixing activities, respectively. The ratio under aerobic conditions was low in the FYM-treated soil. The soil contained a small amount of NO_3-N and high level of substrates such as hot-water soluble materials, lignin and cellulose, which may have induced the low denitrifying and high N_2 fixing activities. In contrast to the FYM-treated soil, the ratio of the SDCFC-treated soil under aerobic conditions was high, presumably because the soil contained a large amount of NO_3 and small amount of substrate.

Thus this experiment showed that the ratios of nitrification to ammonification, nitrification to denitrification, and denitrification to N_2 fixation could not be easily predicted only on the basis of the soil C content or the C/N ratio of amended materials. They were controlled by the C/N ratio of the amended materials, as well as by the constituents of the amended materials and the levels of NH_4-N and NO_3-N.

Acknowledgments. Gratitude is extended to Dr. M. Kimura (Associate Professor) of the authors' laboratory for his invaluable suggestions and constructive comments in reviewing this manuscript.

REFERENCES

Acharya, C.N. 1935: Studies on the anaerobic decomposition of plant materials. III. Comparison of the course of decomposition of rice straw under anaerobic, aerobic and partially aerobic conditions. *Biochem. J.*, **29**, 1116-1120

Broadbent, F.E. and Clark, F.E. 1972: Denitrification in sealed soil plant systems. II. Effect of soil water content and form of applied nitrogen. *Plant Soil*, **37**, 129-140

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

Campbell, N.E.R. and Lees, H. 1967: The nitrogen cycle. *In* Soil Biochemistry, Vol. 1, Ed. A.D. McLaren and G.H. Peterson, p. 194-215, Marcel Dekker, New York

Granhall, U. 1981: Biological nitrogen fixation in relation to environmental factors and functioning of natural ecosystems. *In* Terrestrial Nitrogen Cycles, Ecol. Bull., Vol. 33, Ed. F.E. Clark and T. Rosswall, p. 131-134, Swedish Natural Science Research Council, Stockholm

Harper, S.H.T. and Lynch, J.M. 1981: The chemical components and decomposition of wheat straw leaves, internodes and nodes. *J. Sci. Food Agric.*, **32**, 1057-1062

Haynes, R.J. 1986: The decomposition process. Mineralization, immobilization, humus formation, and degradation. *In* Mineral Nitrogen in the Plant-Soil System, Ed. R.J. Haynes, p. 52-126, Academic Press, London

Hernandes, D. and Rowe, J.J. 1987: Oxygen regulation of nitrate uptake in denitrifying *Pseudomonas aeruginosa. Appl. Environ. Microbiol.*, **53**, 745-750

Kakami, Y., Pacaud, B., and Nishimura, N. 1985: Inhibition of denitrification by oxygen in *Paracoccus holodenitrificans. J. Ferment. Technol.*, **63**, 437-442

Murakami, T. 1987: Soil nitrogen source associated with the early stage of N_2O evolution. *Soil Sci. Plant Nutr.*, **33**, 143-146

Parr, J.F. and Pappendick, R.I. 1978: Factors affecting the decomposition of crop residues by microorganisms. *In* Crop Residue Management Systems, Ed. W.R. Oschwald, p. 101-129, ASA/CSSA/SSSA, Madison, Wisconsin

Patrick, W.H. 1982: Nitrogen transformation in submerged soils. *In* Nitrogen in Agricultural Soils, Ed. F. J. Stefanson, p. 449-466, ASA, Madison, Wisconsin

Postgate, J.R. and Hill, S. 1979: Nitrogen fixation. *In* Microbial Ecology. A Conceptual Approach, Ed. J. M. Lynch and N.J. Poole, p. 191-213, Blackwell, Oxford

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

Staaf, H. and Berg, B. 1981: Plant litter input to soil. *In* Terrestrial Nitrogen Cycles, Ecol. Bull., Vol. 33, Ed. F.E. Clark and T. Rosswall, p. 147-167, Swedish Natural Science Research Council, Stockholm

Starr, J.L., Broadbent, F.E., and Nielsen, D.R. 1974: Nitrogen transformations during continuous leaching. *Soil Sci. Soc. Am. Proc.*, **33**, 283-289

Tiedje, J.M., Sorensen, J., and Chang, Y.-Y.L. 1981: Assimilatory and dissimilatory nitrate reduction. Perspective and methodology for simultaneous measurement of several nitrogen cycle processes. *In* Terrestrial Nitrogen Cycles, Ecol. Bull., Vol. 33, Ed. F.E. Clark and T. Rosswall, p. 331-342, Swedish Natural Science Research Council, Stockholm

Yoshida, T. 1975: Microbial metabolism of flooded soils. *In* Soil Biochemistry, Vol. 3, Ed. E.A. Paul and A.D. McLaren, p. 83-112, Marcel Dekker, New York

Yoshinari, T., Hynes, R., and Knowles, R. 1977: Acetylene inhibition of nitrous oxide reduction and measurement of denitrification and nitrogen fixation in soil. *Soil Biol. Biochem.*, **9**, 177-183