Rapid Anaerobic Incubation Method for Measuring N Mineralization Potential in Soil.

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Received May 16, 1999

Abstract

It takes a long time to measure the N mineralization potential by the incubation method. To shorten the incubation time, the incubation temperature was raised, and to measure simultaneously the C mineralization potential, headspace air in the incubation bottle was replaced by N_2. That is, an anaerobic incubation method was proposed. Estimated amount of N mineralized in anaerobic incubation was in agreement with that in paddy incubation compared with that in upland incubation. Minimum incubation time, which was necessary to measure the rate constant accurately, was 18 days on the average, ranging from 8 to 43 days. Through anaerobic incubation at 40 ℃ , the incubation time was reduced to half of that at 35 ℃ . The N mineralization potential showed a positive correlation with the C mineralization potential in Brazilian upland soils.

Key words: N fertility, tropical soil

Introduction

The incubation method is generally applied for evaluating N fertility in soil. Stanford and Smith[8] proposed the N mineralization potential for evaluating the N fertility in upland soil. N mineralization potential was calculated from long period of 30 weeks incubation. One of shortcomings of this incubation method was that soluble organic matter and nutrients in soil were washed out. Sugihara *et al.* [9] used a simple incubation method (upland incubation), for a long period from 140 to 441 days. Inubushi *et al.* [1] used waterlogged incubation method (paddy incubation).

The rate constant of N mineralization increases when the temperature reaches the optimum value of about 70 ℃ for ammonification and 35 ℃ for nitrification. Incubation time required for measuring the N mineralization potential is inversely proportional to the rate constant. Therefore the incubation time could be shortened by raising the incubation temperature under anaerobic conditions. It is well known that the mineralization of soil N is related to the mineralization of soil organic matter. Carbon dioxide and methane are produced during the decomposition of organic carbon under anaerobic conditions.

In this study, an improved incubation method (anaerobic incubation) was proposed and both N mineralization potential and C mineralization potential were measured simultaneously.

Materials and Methods

1) *Soil samples*

Samples of 7 surface soils were collected from Japan, Malaysia and Brazil. Japanese sites consisted of an alluvial paddy field (JP) and an upland field (Ando soil) for soybean cultivation (JP) located at Japan International Research Center for Agricultural Sciences, Tsukuba, Iba-raki, Japan. Malaysian site was a paddy field (Red-yellow soil) (MP) located at Seberanperai, Penang, Malaysia. One Brazilian site consisted of an upland fallow field (Red-yellow soil) (B1), just before the onset of the NPK fertilizer experiment, located at the National Cattle Beef Research Center, EMBRAPA, Campo Grande, MS, Brazil. Another was an upland grassland field (Red-yellow soil) (B2), an upland field (Red-yellow soil) for peanut cultivation (B3) and a peaty upland grassland field (B4) located at the Agriculture Training Center, JATAK, Guatabara, SP, Brazil (Table 1).

Collected soils were immediately air-dried, passed through a 2mm sieve, and then used for the incubation experiments. Some of the JP and JU soil samples were kept approximately 5 ℃ in a refrigerator for 2 months. Similarly, some of the JP and JU soil samples were kept in a dark area at room temperature for 2 months. These samples were also used for the incubation experiments.

2) *Incubation experiments*

Four types of incubation methods were applied for the experiments.

Anaerobic incubation: a 5g aliquot of the soil samples was placed in 100mL glass bottles, and filled with 10mL of distilled water. Bottles were sealed with a butyl rubber vacuum septum. Headspace air in the bottle was replaced by N_2 by using a vacuum pump and a N_2 cylinder. Atmospheric pressure in the bottle was set approximately at 90kPa. Incubations were carried out for 3, 6, 9, 12 weeks at 40℃, and 6, 12 weeks at 30℃, respectively. After the end of incubation, the bottles were shaken thoroughly and 1mL of gas samples was removed with gas-tight syringes. The amounts of carbon dioxide and methane were analyzed. Water soluble

Table 1 Properties of soils used in the experiment

Soil A[a)]	Country	Location	Preceding Crop	Texture	pH (H2O)	Total C	Total N
						g kg^{-1}	g kg^{-1}
JU	Japan	Tsukuba	Soybean	SiL	4.1	39.6	3.30
JP	Japan	Tsukuba	Paddy Rice	SiL	6.0	17.9	1.60
MP	Malaysia	Penang	Paddy Rice	HC	4.8	24.7	2.23
B1	Brazil	Campo Grande	None	SCL	4.7	14.5	1.00
B2	Brazil	Guatapara	Peanut	LiC	6.3	14.0	1.20
B3	Brazil	Guatapara	Grass	SCL	6.1	10.3	1.00
B4	Brazil	Guatapara	Grass	SiC	4.0	212.9	14.50

a): Abbreviation

gas contents were calculated from equivalent constant at 20 ℃ , 0.9368 L L^{-1} for CO_2, 0.0331 L L^{-1} for methane. A 50 mL aliquot of 10% KCl was added and the bottles were shaken for 1 hr. The concentration of NH_4-N in the leachates was analyzed. Each sample used was duplicated.

Waterlogged incubation: a 5g aliquot of the soil samples was placed in 100mL glass bottles, and filled with 10mL of distilled water. Bottles were sealed with a butyl rubber vacuum septum. Incubations were carried out for 3, 6, 9, 12 weeks at 40 ℃ , respectively. A 50 mL aliquot of 10% KCl was added and the bottles were shaken for 1 hr. The concentration of NH_4-N in the leachates was analyzed. Each sample used was duplicated Upland incubation: a 20g aliquot of the soil samples was placed in 200mL UM sample glass bottles, and filled to 60% of the maximum water-holding capacity with distilled water. Bottles were covered with a 0.03mm thick sheet made of polyethylene. Incubations were carried out for 4, 8, 12, 16 weeks at 35 ℃ , and 8, 16 weeks at 25 ℃ , respectively. Water content in soil was adjusted by the addition of distilled water every week. After the end of incubation, a 100 mL aliquot of 10% KCl was added and the bottles were shaken for 1 hr. NO_3-N concentration in the leachates was analyzed. Each sample used was duplicated

Paddy incubation: a 15g aliquot of the soil samples was placed in 30 mL glass bottles, and filled to the top with distilled water. Bottles were sealed with a butyl rubber septum and then closed with plastic screw caps with a hole in the center. Incubations were carried out for 3, 6, 9, 12 weeks at 40 ℃ , respectively. Excess gases in the bottles were removed by inserting a syringe needle every two weeks. After the emd of incubation, the soils and water in bottles were washed into 200 mL polyethylene bottles with 150 mL of 10% KCL. Bottles were shaken for 1 hr. The concentration of NH_4-N in the leachates was analyzed. Each sample used was duplicated.

3) *Chemical Analysis*

Soil texture and soil pH were determined by usual methods[2]. Soil total C content and soil total N content were determined with a NC analyzer (Sumika Chemicals Sumigraph NC 900). The concentration of NH4-N and NO3-N were determined with an auto analyzer (Technicon Auto Analyzer II).

Carbon dioxide and methane were analyzed with a gas chromatograph (Shimadzu GC14BPT) equipped with a thermal conductivity detector (TCD) and a 3 m by 3 mm I. D. stainless column packed with a 80/100 mesh Porapak Q.

4) *Calculation of mineralization potential, rate constant, apparent activity energy, and minimum incubation time.*

Assuming that N mineralization fitted a first order kinetic equation [8].

$$N=N_m(1-\exp^{-kt})+N_o \qquad (1)$$

where N is the amount of N mineralized (mg kg^{-1}) at time *t* (d), Nm is the N mineralization potential (mg kg^{-1}), k is a rate constant of mineralization (d^{-1}), and N_0 is the amount of N mineralized (mg kg^{-1}) at the onset. N mineralization potential and rate constant were calculated by using a non-linear least square technique [7].

Apparent activation energy, Ea (J mol^{-1}), was calculated from the relation between the temperature and rate constant[3].

$$k=A\exp(Ea/RT) \qquad (2)$$

where A is a constant (d^{-1}), R is a gas constant (8.318J K^{-1} mol^{-1}) and T is the absolute temperature (K). Combining each equation (2) at two different temperatures T_1 and T_2, where rate constants are k_1, k_2, respectively, gives equation (3).

$$Ea= R/(1/T_1-1/T_2)\cdot In(k_1/k_2) \qquad (3)$$

Minimum incubation time, T_m, which is necessary for obtaining an accurate rate constant, was calculated from the following equation developed by Sugihara *et al.*[9].

$$T_m=0.76/k \qquad (4)$$

Results and Discussion

It takes a long time to measure the N mineralization potential by the upland incubation method. To shorten the time, the temperature was increased. Since the optimum temperature for nitrification is about 35℃, it is impossible to raise the temperature above 35 ℃. Incubation under anaerobic conditions was tested where ammonification occurred. This method is well known as paddy incubation. Gas in an incubation bottle should be removed at intervals, since the rubber cap is opened by methane and CO_2 gases produced during the incubation. After the end of incubation, soil and water in the incubation bottles should be transferred to another extracting bottle. Thus the method is not convenient. In this study, for convenient handling during the incubation, the new anaerobic incubation method, in which there is a headspace in an incubation bottle, was proposed.

At first, gas in the headspace was compared. Fig. 1 shows the time course of N mineralization in the anaerobic incubation in which headspace gas was replaced by N_2 and waterlogged incubation in which headspace gas consisted of air. The amount of NH_4-N increased with increasing incubation time under anaerobic conditions. As a result, it became possible to calculate the N mineralization potential and rate constant. Since NH_4-N concentration decreased after 6 to 9 weeks in waterlogged incubation, calculation of N mineralization potential was impossible. Table 2 shows the N mineralization potential and rate constant in upland incubation, paddy incubation and anaerobic incubation. Compared to paddy incubation, anaerobic incubation gave a lower N mineralization potential and higher rate constant. Table 3 shows the estimated amount of mineralized N at 35 days and 70 days calculated from the N mineralization potential and rate constant at 40 ℃. The values at 70 days in anaerobic incubation were close to those in paddy incubation.

Apparent activation energy in N mineralization and minimum incubation time where an error in rate constant was less than 5% are shown in Table 4. Apparent activation energy was higher in anaerobic incubation. Mean value of apparent activation energy in upland incubation was almost same as that in other measurements [3,5,6]. Similarly, the value in anaerobic incubation was close to that of other measurements

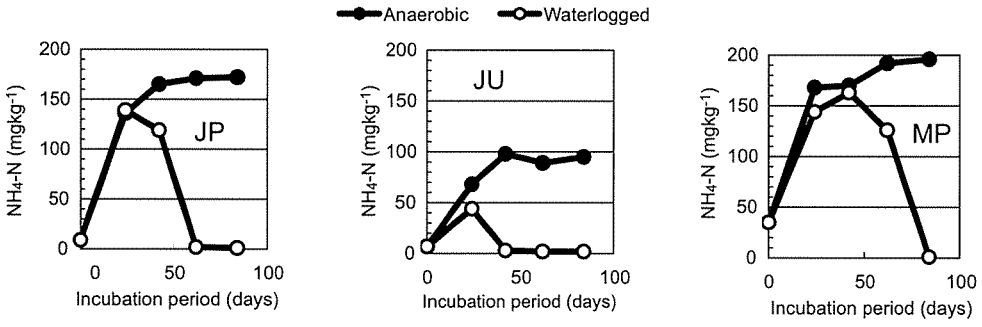

Fig.1. Time course of ammonium nitrogen during incubation at 40 ℃.

Table 2. Calculation of N mineralization potential (N_m), rate constant (k_{35}) at 35 ℃, and rate constant (k_{40}) at 40 ℃

	Upland incubation			Paddy incubation		Anaerobic incubation		
Soil	N_m	k_{35}	k_{40}	N_m	k_{40}	N_m	k_{35}	k_{40}
	$mgkg^{-1}$	d^{-1}	d^{-1}	$mg\,kg^{-1}$	d^{-1}	$mg\,kg^{-1}$	d^{-1}	d^{-1}
JP	210	0.017	0.024	180	0.046	164	0.038	0.072
JU	125	0.016	0.028	147	0.015	91	0.021	0.059
MP	127	0.025	0.034	187	0.028	155	0.045	0.090
B1	35	0.026				45	0.011	0.018
B2	52	0.016				72	0.019	0.037
B3	87	0.012				71	0.030	0.062
B4	110	0.018				434	0.017	0.039

Table 3. Estimation of amount of N mineralized in soil at 40 ℃ , which was calculated from N mineralization potential and rate constant

Soil	35 days			70 days		
	Upland	Paddy	Anaerobic	Upland	Paddy	Anaerobic
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
JP	109	143	153	172	181	171
JU	78	60	82	114	103	97
MP	98	141	180	132	195	190

Table 4. Calculation of apparent activation energy (Ea) and minimum incubation time (.T_m.)

Soil	Upland incubation		Paddy incubation	Anaerobic incubation		
	Ea.	T_m. 35 ℃	T_m 40℃	Ea	T_m. 35 ℃	40 ℃
	J mol^{-1}	day	day	J mol-1	day	day
JP	57000	46	17	100000	20	11
JU	91000	48	51	170000	27	13
MP	46000	30	27	111000	17	8
B1	78000	29		79000	69	43
B2	74000	48		104000	39	21
B3	42000	64		115000	25	12
B4	10000	42		132000	44	19
Mean	57000	44	32	116000	36	18

Table 5. Effect of storage method on N mineralization potential, rate constant, and estimated amount of N mineralized from soil

Soil	Storage method	N mineralization potential	Rate constant	Estimated amount of N mineralized[a)]	
				35days	70days
		mg kg^{-1}	d^{-1}	mg kg^{-1}	mg kg^{-1}
JP					
	None	164	0.072	153	171
	Refrigerator	183	0.065	171	188
	Room temperature.	189	0.066	180	197
JU					
	None	91	0.059	82	97
	Refrigerator	120	0.020	64	94
	Room temperature	116	0.024	74	102

a): Estimated amount of N mineralized from soil was calculated from the N mineralization potential and rate constant at 40 ℃

in paddy incubation [1,4]. Minimum incubation time in anaerobic incubation at 35 ℃ was 36 days on the average, and that at 40℃ was 18 days on the average, ranging from 8 to 43 days. Minimum incubation time was shortened half by increasing the incubation temperature by 5 ℃ in anaerobic incubation.

Table 5 shows the relationship between storage methods and N mineralization potential, rate constant and estimation of the amount of N mineralized. After 2 months of storage, the N mineralization potential increased and the rate constant decreased. Estimated amount of N mineralized during the 70-day incubation period of soil kept in a refrigerator was closer to that of soil before storage than that of soil placed at room temperature. Therefore it was necessary to start the incubation immediately, and soil should be kept in a refrigerator.

Fig. 2 shows the relationship between the C mineralization potential and N mineralization potential in Brazilian soils. Although there were only 4 samples, the N mineralization potential showed a correlation with the C mineralization potential. Mineralization potential of both N and C could thus be measured simultaneously by the new anaerobic

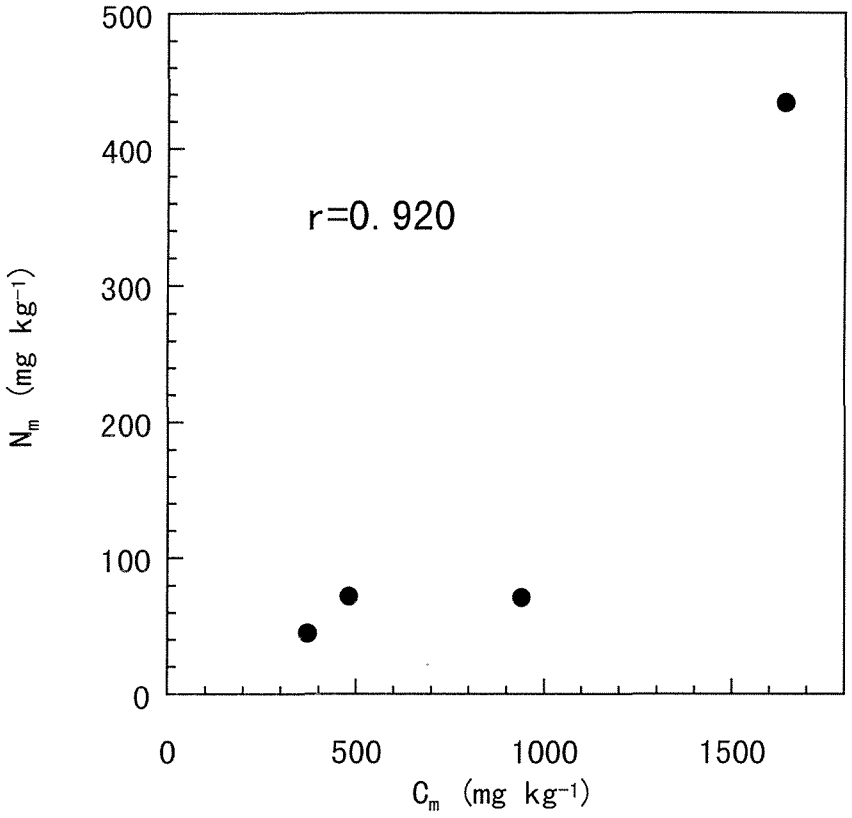

Fig.2. Relationship between C mineralization potencial (C_m) and N mineralization (N_m) in Brazilian soils.

incubation method.

Acknowledgements

I thank Dr. Katsuyuki Minami for his helpful suggestions, Dr.Kazuyuki Yagi, Mr. Takeshi Watanabe and Mr. Takayuki Ishikawa for their assistance in the chemical analysis, Dr. Souichi Nakayama and Dr. Tsutomu Kanno for their assistance in sample collection.

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嫌気培養法による土壌の窒素無機化ポテンシャルの測定

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要約

培養により土壌の窒素無機化ポテンシャルを測定するには長時間を要する。培養時間を短縮するために培養温度を上げ、ヘッドスペースを窒素に置換することにより炭素の無機化ポテンシャルも同時に測定可能となる嫌気培養法を考案した。嫌気培養による窒素無機化量は畑培養より水田培養の値に近かった。正確な速度定数を求めるのに必要な培養日数は平均で18日、8～43日の範囲になった。40℃で培養することにより35℃で培養した場合に較べて培養期間が半分に短縮可能となった。ブラジルの畑土壌では炭素無機化ポテンシャルと窒素無機化ポテンシャルは正の相関を示した。

キーワード：窒素肥沃度、 窒素無機化、 熱帯土壌