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Gross N Mineralization Associated with Organic Matter Application to Inceptisols and Andisols of Hokkaido, Japan, and to Ultisols of Lop Buri, Thailand

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Abstract

Applying organic material to soil is an important management method for sustainable crop production. We investigated its effect on soil nitrogen (N) mineralization in two experiments using the ^{15}N isotope dilution method. In Hokkaido, Northern Japan, we applied wheat (*Triticum aestivum*) straw to Brown Andosol (BA; US Soil Taxonomy: Andisols) and Brown Lowland soil (BLs; US Soil Taxonomy: Inceptisols). The gross rate of N mineralization increased in BA with wheat straw and adequate inorganic N, supporting the general recommendation for the application of additional N fertilizer. The relationship between the microbial biomass N (x) and the gross rate of N mineralization (y) was $y = 0.234e^{0.0751x}$ ($R^2 = 0.815$) in BA and BLs sampled during the growth season of a subsequent maize (*Zea mays*) crop. In long-term experiments with organic material application to Ultisols in Lop Buri, Central Thailand, the gross rates of N mineralization were higher in organically treated soil at a water-holding capacity (WHC) of 27%-41% and similar among soils at WHC ≈ 20%. The rates in Lop Buri soil were higher than those in Hokkaido soils, despite the former's lower total carbon levels. The results suggest high specific microbial activities in Lop Buri soil.

Discipline: Agricultural Environment
Additional key words: maize, ^{15}N, soil microbial biomass, soil moisture

Introduction

The application of organic material, such as crop residues and manure, to soil is an important method for sustainable crop production. It has many effects on the microbial, chemical, and physical properties of the soil and markedly affects its nitrogen (N) transformation (Paul & Clark 1989, Tate III 1987).

In Hokkaido, Northern Japan, the return of crop residues is important for maintaining the carbon (C) levels in the soil. Wheat straw, which has a high C/N ratio, slowly degrades in Hokkaido because of the low soil temperatures, and the net process of N immobilization from the inorganic N pool (Paul & Clark 1989) continues for a long time. Therefore, the risk of N deficiency in crops is high when wheat straw is incorporated into the soil in fields in Hokkaido (Konno et al. 1992, Sawada et al. 1968). Several researchers related the increase of microbial biomass N to the net process of N immobilization, following organic material incorporation (Aoyama & Nozawa 1993, Ocio & Brookes 1990, Ocio et al. 1991). However, the quantitative relationship between the microbial activity and the rate of N transformation in soil incorporated with cereal straw has not been clarified.

In the subhumid tropics, such as in Thailand, sustainable upland crop production requires adequate soil organic matter (OM) levels to be maintained (Greenland 1975). The application of organic material to Ultisols in Central Thailand increased maize yields and soil OM concentrations in long-term experiments (Inoue 1990, Sangtong & Katoh 2010). The accumulation of soil OM generally increases the activities of microorganisms and N transformation in soil because of the increase in the amount of substrate and the changes to soil moisture conditions (Tate III 1987). However, low-moisture

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conditions in droughts and the rapid decomposition of labile organic N, as a result of high temperatures, may result in low N mineralization rates in the crop-growing season. For the assessment of soil N mineralization activities in the subhumid tropics, evaluating the effects of the fluctuations of soil moisture and seasonal changes in microbial activity is necessary.

We investigated the effects of the application of organic materials on N mineralization in two experiments using the $^{15}N\text{-}NH_4^+$ isotope dilution method, which was developed for the analysis of N transformation related to the microbial activity in upland soils since the 1980s (Nishio 1991). The method gives the gross rate of N mineralization based on the temporal changes in ^{15}N-tagged ammonium pool size and its ^{15}N fraction caused by simultaneous processes, including mineralization and immobilization. The experiments were conducted in Hokkaido, a cool temperate region in Northern Japan, and Lop Buri, a subhumid tropic region in Central Thailand.

Materials and methods

1. Experimental plots and sample preparation

(1) Hokkaido, Japan

Experimental microplots were set up in a field at the Hokkaido Agricultural Research Center, National Agriculture and Food Research Organization, in Memuro, Hokkaido (143°03′E, 42°53′N). In May 1995, open-ended polyvinyl chloride pipes (diameter: 0.3 m; length: 0.4 m) were buried vertically with 0.03 m protruding above the ground. Red subsoils of Brown Andosol (BA; US Soil Taxonomy: Andisols), which contained very little humus, were placed in the bottom of each pipe; it was then filled up to ground level with 0.3 m of topsoil from the plow layer of BA or Brown Lowland soil (BLs; US Soil Taxonomy: Inceptisols). After maize was grown in 1995, the top 0.15 m of the soil in all the pipes was removed and replaced with the same type of soil in May 1996. In 1996, three treatments in triplicate were set out in a completely randomized design. In the treatment "SN," 500-g air-dried spring wheat straw (length: 0.03 m-0.05 m) m^{-2} and 14-g N (ammonium sulfate: 65 g) m^{-2} were incorporated. In the treatment "S1/4N," 500-g air-dried straw m^{-2} and 3.6-g N (ammonium sulfate: 17 g) m^{-2} were incorporated. In the treatment "1/4N," no straw but 3.6-g N (ammonium sulfate: 17 g) m^{-2} were incorporated. In all the pipes, 36-g P_2O_5 (lime superphosphate: 202 g) m^{-2}, 20-g K_2O (potassium sulfate: 40 g) m^{-2}, and 11-g MgO (magnesium sulfate: 45 g) m^{-2} were mixed into the top 0.15 m of the soil. The straw and inorganic fertilizer were incorporated, and maize seeds were sown on May 27. The incorporated straw initially contained 433-g C and 3.5-g N kg^{-1} oven-dried matter. The selected properties of the soils that filled up each pipe without the incorporation of straw are provided in Table 1.

After thinning, the maize plants and soils were sampled three times during the growing season. The plants were uprooted, and the topsoil samples (0 m-0.15 m) were collected in a tube auger (diameter: 0.05 m). After sieving (< 2 mm), the measurement of the microbial biomass N in the soil samples commenced during the evening of the sampling day. The next morning, the measurements of the gross N mineralization rates in the soil samples started.

(2) Lop Buri, Thailand

Long-term experiments on OM application were set up in 1976 at the Phraphuttabat Technical Service Center in Lop Buri Province, Central Thailand (100°47′E, 14°43′N) (Inoue 1990). In the experimental field, maize was sown from mid- to late-May each year with and without inorganic fertilizer application, and mung beans (*Vigna radiata*) were sown just before the maize harvest in September and grown until November (Sangtong & Katoh 2010). We selected four plots. In the compost plot, the city's municipal compost was incorporated into the soil in April of each year at a rate of 2 kg $m^{-2}y^{-1}$ (1980-1988) and 0.625 kg $m^{-2}y^{-1}$ (1989-1995). In the rice straw plot, rice straw was mulched onto the soil at 400 g $m^{-2}y^{-1}$ just after maize sowing. In the mimosa plot, the mimosa (*Mimosa invisa*) seed was sown as an intercrop between maize rows in 1981, and the mimosa reappeared every year. The mung beans were not sown. The control plot received no organic amendment.

In 1994, the topsoil (0 cm-10 cm) was collected in January (dry season) in parts of each plot that had not

Table 1. Selected properties of the soils in the micro-plots in Hokkaido

Soil	WHC* (g kg^{-1})	Clay (g kg^{-1})	Total C (g kg^{-1})	Total N (g kg^{-1})	C/N ratio	pH** (H_2O)	Inorg. N** (mg kg^{-1})
Brown Andosol	850	230	30.3	2.6	11.6	5.3	13.7
Brown Lowland soil	520	270	13.5	1.4	9.5	5.9	5.6

*Water-holding capacity

**Values of soil samples obtained before the incorporation of straw with fertilizer on 22 May 1996 received inorganic fertilizer and in August 1994 (rainy season) between the rows of maize in the same plots. The soil samples were sieved (< 2 mm). Each rainy season, soil samples were divided into three portions and dried under an electric fan to moisture contents of 40 g-60 g, 60 g-80 g, and 90 g-100 g water per 1 kg oven-dry soil. The selected properties of these soils are provided in Table 2.

Table 2. Selected properties of Lop Buri soils sampled in the rainy season in 1994

Treatment	pH (H_2O)	Total C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	WHC* ($g\ kg^{-1}$)
Compost	8.2	13.0	1.1	550
Rice straw	7.8	7.5	1.2	500
Mimosa	6.9	11.0	0.6	520
Control	7.8	6.4	0.6	410

*Water-holding capacity

2. Measurement of gross N mineralization rate

We added 5 mL of ammonium sulfate solution containing 2-mg N (20.6 atom% ^{15}N as $(^{15}NH_4)_2SO_4$) to the Hokkaido soil samples, equivalent to 100 g oven-dried weight, and 4 mL of ammonium sulfate solution containing 2-mg N (70.4 or 35.2 atom% ^{15}N) to the Lop Buri soil samples, equivalent to 100 g oven-dry weight. Distilled water was added to adjust the moisture content to 58%-64% of the water-holding capacity (WHC) in BA, 46%-50% in BLs, and 50% in the dry season sample of the Lop Buri soil and to 18%-24%, 27%-36%, and 36%-41% in the rainy season samples of the Lop Buri soil.

The ^{15}N-amended soils were placed in 500-mL flasks with rubber stoppers and incubated for 2 d (BA and Lop Buri soil) or 4 d (BLs), with average values of soil temperature at 0.1 m depth for 10 d before the same date as the sampling date in 1993, 1994, and 1995 in the Hokkaido experiment and at 30°C in the Lop Buri experiment. The concentration of ammonium N and nitrate N and the ^{15}N isotope ratio of ammonium in the soils were analyzed three to five times during incubation. The gross rates of N mineralization were calculated using the equations proposed by Nishio et al. (1985).

3. Measurement of soil microbial biomass N

The microbial biomass N in the soil samples was measured using the fumigation–extraction method proposed by Brookes et al. (1985), with minor modifications (Nira 2003, Ocio & Brookes 1990). Biomass N was calculated by multiplying (increased amount of organic N plus ammonium N in K_2SO_4 extracts by fumigation) by 2.22.

4. Chemical and statistical analyses

The N content of the plant material was determined using the Kjeldahl method with a modification to the salicylic acid–thiosulfate digestion method (Bremner & Mulvaney 1982). The content of organic N plus ammonium N in the K_2SO_4 extracts of the soil for the measurement of the microbial biomass N, after digestion with selenyl chloride as a catalyst, was measured using the flow injection analysis by the indophenol method (Scheiner 1976). The concentrations of ammonium and nitrate in the KCl extracts of the soil samples were determined using the flow injection analysis by the indophenol method (Scheiner 1976) and the cadmium–copper reduction method (Wood et al. 1967). The ^{15}N isotope ratio of ammonium was determined by emission spectrometry using a ^{15}N-analyzer (JASCO N-150) (Kano et al. 1974).

Analysis of variance followed by Tukey's test was performed using the GLM procedure of the SAS software (SAS Institute Inc., Cary, NC, USA).

Results

1. Experiment in Hokkaido, Japan

(1) Maize growth and N uptake

In BA, maize plant biomass and plant N uptake were significantly lower on August 21 and September 30 in the S1/4N plot than those in the 1/4N plot (Table 3). In BLs, both were marginally lower in the S1/4N plot than those in the 1/4N plot. In both soils, SN significantly increased plant biomass and N uptake, except in the early growth stage in BA, relative to S1/4N. Plant N uptake was always lower in BA than in BLs.

(2) Gross rate of N mineralization and microbial biomass N in soils

In BA, the gross rate of N mineralization in soil was always significantly larger in SN than that in the other treatments (Table 4). The rates were marginally larger in S1/4N than in 1/4N, except on September 30. In BLs, they were significantly larger in SN than in 1/4N on September 30.

The treatment had no effect on the microbial biomass N, except for a decrease in SN of BLs on August 21, which had no obvious reason (Table 4).

The relationship between the microbial biomass N and the gross rate of N mineralization in all samples was calculated as $y = 0.234e^{0.0751x}$ ($R^2 = 0.815$; Fig. 1). The microbial biomass N and the gross rates of N mineralization were always larger in BA than in BLs (Table 4).

Table 3. Effect of straw incorporation on maize plant biomass production and N uptake in the Hokkaido experiment in 1996

Treatment	Plant mass (g per pot)			Plant N uptake (mg per pot)		
	July 9	Aug 21	Sep 30	July 9	Aug 21	Sep 30
Brown Andosol						
SN	0.4a	42a(A)	152a	nd*	686a(A)	1,498a
S1/4N	0.3a	24b(B)	84c	nd	327b(C)	1,070b
1/4N	0.2b	36ab(A)	108b	nd	430b(B)	1,222ab
Brown Lowland soil						
SN	1.6a	72a	181a	nd	1,045a	2,099a(A)
S1/4N	0.6b	31b	99b	nd	442b	1,262a(B)
1/4N	1.0ab	37b	107b	nd	570b	1,272a(B)

Values indicated by the same letter within a column are not significantly different at the $P < 0.05$ level (abc) or the $P < 0.10$ level (ABC)(Tukey's test).
*Not determined

Table 4. Effect of straw incorporation on gross rates of N mineralization and amount of microbial biomass N in Hokkaido soils in 1996

Treatment	Gross N mineralization rate (mg kg^{-1}d^{-1})			Microbial biomass N (mg kg^{-1})		
	July 9 (19°C)*	Aug 21 (21°C)*	Sep 30 (16°C)*	July 9	Aug 21	Sep 30
Brown Andosol						
SN	8.06a	5.81a	4.15a	49.0a	36.3a	32.3a
S1/4N	3.51b	3.17b	1.83c	32.7a	32.3a	35.7a
1/4N	2.66b	2.45b	2.76b	35.0a	30.7a	36.0a
Brown Lowland soil						
SN	1.23a	1.30a	0.82a	20.9a	11.9b	18.2a
S1/4N	1.28a	1.01a	0.52ab	18.0a	18.8a	15.7a
1/4N	0.83a	1.12a	0.20b	15.5a	18.6a	14.1a

Values indicated by the same letter within a column are not significantly different at the $P < 0.05$ level (abc) or the $P < 0.10$ level (ABC)(Tukey's test).
*Incubation temperature

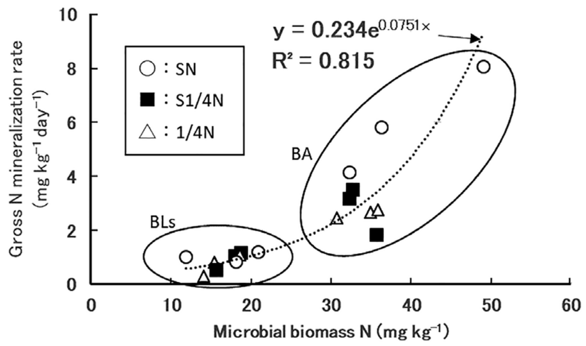

Fig. 1. Relationship between the size of microbial biomass N and the gross rate of N mineralization in the soils from the experiment in Hokkaido
The points indicate data from samples with three treatments (△: 1/4N, ■: S1/4N, ○: SN) at three sampling times in Brown Andosol (BA) and Brown Lowland soil (BLs).

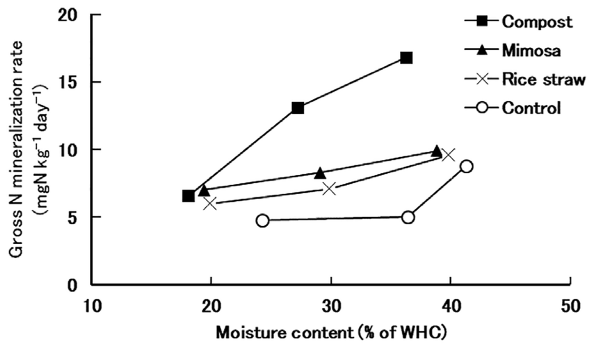

Fig. 2. Effect of moisture content on the gross rate of N mineralization in the Lop Buri soils sampled in the rainy season

2. Analysis of soil sampled at Lop Buri, Thailand

The gross rates of N mineralization rose as the water content increased to WHC of 36%-41% corresponding to 200 g water (soil with organic treatments) and 170 g water (control soil) per 1 kg dried soil (Fig. 2). Particularly, the rate in soil with compost markedly increased. The rates at WHC of 18%-24% (100 g water per 1 kg dried soil) were similar among all soils. They were higher in soils with higher total C (TC) level, i.e., soils that had received organic treatment, except in low-moisture conditions (Fig. 3).

In the dry season, the gross rates of mineralization were lower in soils with WHC of 50% from the compost and mimosa plots than in soils with WHC of 36%-39% in the rainy season (Table 5; Fig. 2). The rate in soil from the rice straw plot was higher in the dry season than in the rainy season. The gross mineralization rates in soil from the control plot were similar in both seasons.

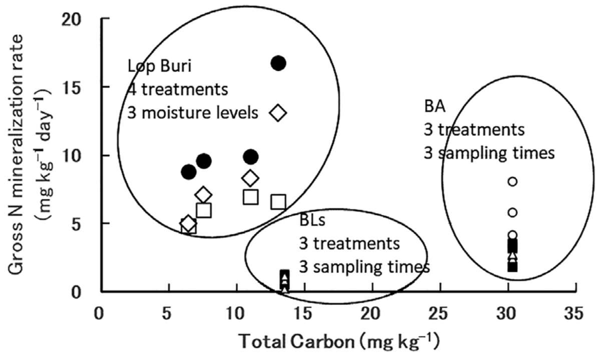

Fig. 3. Relationship between the total carbon content and the gross rate of N mineralization in the soils from the experiments in Hokkaido and Lop Buri
The points indicate data from the samples with three treatments (△: 1/4N, ■: S1/4N, ○: SN) at three sampling times in Brown Andosol (BA) and Brown Lowland soil (BLs) and the samples fixed at three moisture conditions (●: WHC of 36%-41%, ◇: 27%-36%, □: 18%-24%) from four organic matter application plots (control, rice straw, mimosa, compost) in Lop Buri in the rainy season.

Table 5. Gross N mineralization rates in Lop Buri soils sampled in the dry season in 1994

Treatment	Gross N mineralization rate (mg kg^{-1} day^{-1})
Compost	8.8
Rice straw	12.6
Mimosa	3.1
Control	6.0

Values were gained at WHC of 50%.

Discussion

1. Hokkaido, Japan

The incorporation of wheat straw significantly increased the gross rate of N mineralization in BA plots that also received adequate amounts of inorganic N (Table 4). The C/N ratios of materials incorporated into the soil was 13 in SN (calculated from 472 g wheat straw (oven-dry weight basis) m^{-2} and 14 g N as ammonium sulfate m^{-2}), but it was 38 in S1/4N. As the addition of crop residues with C/N ratio > 20-30 can cause net N immobilization (Alexander 1961), an additional application of N fertilizer has been recommended to release available N without immobilizing external N (Matsushiro & Satoh 1981, Paul & Clark 1989). The suppression of N release was inferred from the decreased N uptake of maize in the S1/4N treatment in BA (Table 3). In this plot, the gross rate of N immobilization might have increased as a result of the incorporation of wheat straw without the suppression of gross N mineralization. An additional application of N fertilizer caused increased maize N uptake in addition to increased N immobilization (Table 3). Furthermore, these results confirm that an increase in the gross rate of N mineralization (Table 4) contributes to the release of available N when additional N fertilizer is applied to soil along with the incorporation of wheat straw. In BLs, wheat straw and fertilizer N caused little suppression of maize N uptake or changes in the gross rate of N mineralization (Table 3). The low microbial biomass present in BLs (Table 4) might not have markedly increased. The smaller pore space in BLs, reflecting the lower WHC (Table 1), is associated with a smaller microbial biomass than that in BA (Nira 2003).

We could not determine the gross rate of N immobilization; its evaluation was hard to conduct using the ^{15}N isotope dilution technique because it proportionally increases with the addition of further ^{15}N-ammonium (Nishio 1991). Recous et al. (1999) reported that the gross rates of N mineralization and N immobilization were increased by the incorporation of wheat straw based on the injection of ^{15}N into the soil during field incubation. In recent years, adding ^{15}N-ammonium to a maximum of 20% of the initial NH_4^+ pool has been recommended (Braun et al. 2018). An accurate method of analyzing gross N immobilization is required.

The exponential correlation between the microbial biomass and the gross rate of N mineralization among all samples indicates that both the size of the microbial biomass and the activity of N mineralization per unit biomass control the gross rate of N mineralization (Fig. 1). Puri and Ashman (1998) reported that the microbial biomass N remained stable throughout the year despite variations in the gross N mineralization rate in acidic woodland soil. These facts also suggest that measuring microbial biomass alone is insufficient in estimating N mineralization activity in soil. Measuring the specific microbial activity and biomass is instead necessary.

2. Lop Buri, Thailand

The Lop Buri soils that had received organic treatments might hold larger microbial biomass because soils with higher TC contents and higher WHC are able to hold larger microbial biomass (Nira 2003). Therefore, the higher gross rates of N mineralization in the soils with organic treatments (Fig. 2) might have been a consequence of the larger population of microorganisms.

Maize yields often decrease in drought years in Central Thailand, but mulching with plant residues results in a high stable yield by maintaining suitable soil moisture conditions (Inoue 1990). The rate of N release from microorganisms would markedly change with the fluctuations in soil moisture. In a previous incubation experiment with Lop Buri soil in which rice straw, green manure, and compost were applied, only slight CO_2 release and small net rates of N mineralization were evident at WHC of 20% at 30°C (Suzuki 1985). In our evaluation, the gross rates of N mineralization in all plots were similar at WHC ≈ 20% (Fig. 2) but higher than those in the Hokkaido BLs (Fig. 3). The field soil moisture was 4.1%-17.5% from mid-June to September 1979 in a drought year and 9%-12.8% from mid-July to August 1976 in a normal year (Igarashi et al. 1985); these values are equivalent to a WHC of 7%-31%. In the field, the lower rates of mineralization than in our incubation experiment at WHC of 17% and the slight effect of organic material application on N release could persist for a much longer period because of the low moisture levels.

The low gross rates of N mineralization in soils from the compost and mimosa plots in the dry season (Table 5) might have resulted from the rapid consumption of easily mineralizable OM during the crop-growing season at high soil temperature. However, the high gross rate in the soil of the rice straw plot (Table 5) suggests that rice straw, with a higher C/N ratio, needs a longer time for decomposition and thus provides easily mineralizable organic N in the dry season.

The gross rates of N mineralization in the Lop Buri soil were higher than those in the Hokkaido soils at temperatures simulating field conditions, despite the lower TC levels (Fig. 3). The high rate of mineralization could not be explained only by the size of the microbial biomass, as the TC content in the soils was small. Araragi (1985) described a large microbial population with high cellulase and protease activities; this supports and is consistent with the results of our study, in which the mineralization of OM was rapid despite the low nutrition levels in Thai soils. Further studies are needed to clarify whether the activity of N mineralization per unit biomass is high in Thai soils.

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