Nitrogen Mineralization in Soil Amended with Compost and Urea as Affected by Plant Residues Supplements with Controlled C/N Ratios

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***Abstract*—Plant residues supply carbon-rich source but also affect nitrogen (N) dynamic in the plant–soil system. Two consecutive incubations were conducted to examine soil net N mineralization from compost and urea as affected by additions of carbon sources with controlled C/N ratios. For incubation 1–*set 1*, powder of bamboo stem (BS) was incorporated into paddy soil with increasing rates from 0 to 2457.2 mg total C/kg soil that can cover a wide range of C used in the later incubations. For incubation 1–*set 2*, either sewage sludge compost or urea was added with BS to increase their C/N ratios up to 10 and 25. Amended materials were then incorporated into paddy soil at the same rate of 100 mg total N/kg soil. For incubation 2, we compared the effectiveness of three different carbon sources, i.e., BS, powder of residual wood for building (WB) and rice straw (RS) on potential N immobilization of compost-amended sandy soil after regulating C/N values of amendments up to 25. The major results obtained were as follows: 1) The net N mineralization rates decreased with increasing amounts of added-C, indicating the immediate availability of the BS. 2) N immobilization of nitrate was greatest in treatment of BS and compost (C/N=25), whereas, N immobilization of ammonium was found in treatments of BS and urea (both C/N=10 and C/N=25). 3) N immobilization was significantly greater in the soil incorporated with BS and RS than that with WB. Our findings suggest that the mineralization–immobilization turnover (MIT) of N in soil can be controlled by adding readily available plant residues.**

***Index Terms*—ammonium, compost, immobilization, mineralization, nitrate, plant residues**

I. INTRODUCTION

Compost can serve as a soil fertilizer to supply a source of nutrients for plant uptake. Predicting nitrogen (N) mineralization from compost plays an important role from both agricultural and environmental point of view. The available N derived from sewage sludge compost has been found to depend on gross N mineralization and gross N immobilization turnover (MIT) [1]. One of the most common problems associated with utilization of composted sewage sludge in advanced agricultural technologies is the risk of nitrate (NO_3-N) leaching into the groundwater through application of fast-release N-fertilizers [2], [3].

Incorporating plant residues into soil not only improves carbon sequestration but also prevents N loss by immobilizing inorganic form into organic one [4], [5]. Materials with narrow C/N ratios can result in release of inorganic N via mineralization, whereas materials with wide C/N ratios lead to temporary N immobilization [6], [7]. Several researchers have tested the immobilization of soil N in the presence of added carbon and N sources [6], [8]-[10]. In general, to reach the highest N immobilization, the added materials need to have low N contents and high C/N ratio with easily decomposable organic carbon.

However, the diversity in quality of carbon sources together with large range in C/N ratios of amendments made it more difficult in predicting the N mineralization. From this point of view, this study was carried out to provide more information concerning the MIT in the fertilized soil using the carbon sources from common plant residues.

Our hypotheses are: 1) Incorporation of BS would change the MIT in soil in a short-term; 2) The same result would occur if BS was added to sewage sludge compost and urea; and 3) At the effective value of C/N ratio, compost with three different plant residues supplements would result in similar effects on the MIT.

II. MATERIALS AND METHODS

A. Materials Preparation and Incubation

In 2014, soil samples for incubation 1 were collected from paddy fields at a depth of 0-30 cm in Minami district, Okayama Province, Japan (*34°32'N, 133°53'E*). Incubation 2 used sandy soil which was purchased at the market. The soil was air-dried and homogenized by passing through a 2.0 mm mesh sieve. Sewage sludge compost was obtained from Green Yuki composting plant in Okayama, Japan. Three plant residues containing carbon sources, i.e., bamboo stem *(Phyllostachys bambusoides)*, residual wood for building (a mixture of Japanese cedar, *Cryptomeria japonica* and Red pine,

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Pinus densiflora, and Abies firma) and rice straw (*Oryza sativa* L.), were chopped, oven-dried at 105℃ and ground to make fine powder (called BS, WB and RS, respectively) before starting incubations. The properties of the soil, compost and materials containing carbon are given in Table I.

TABLE I. CHEMICAL PROPERTIES OF MATERIALS AT THE BEGINNING OF THE INCUBATION

Experimental materials	Total C (g kg^{-1})	Total N (g kg^{-1})	C/N	Inorganic N	
				NH_4-N (g kg^{-1})	NO_3-N (g kg^{-1})
Soil					
Sandy	1.0	0.2	5.0	nd	nd
Paddy	23.9	2.1	11.7	7.3×10^{-3}	9.1×10^{-3}
Nitrogen sources					
Compost	293.0	42.3	6.4	1.6	8.1×10^{-3}
Urea	200.0	467.0	0.4	–	–
Added carbon sources					
BS	290.0	–	–	–	–
WB	330.0	–	–	–	–
RS	310.0	–	–	–	–

nd, not detected; RS = bamboo stem; WB = wood for building; RS = rice straw

Soil (237 g, dry weight basis) was adjusted to 50% water holding capacity and kept in Neubauer' pots (surface area: 100 cm^2, height: 6.6 cm). For incubation 1–*set 1*, the soil was incorporated with BS at a loading rate of 0; 444.5; 957.2; 1944.5 and 2457.2 mg total C/kg soil. For incubation 1–*set 2*, the soil was amended with N at a rate of 100 mg total N/kg soil as either sewage sludge compost or urea. BS was then additionally incorporated into soil using the same loading rates as in incubation–*set 1*.

The amounts of added carbon were exactly calculated by multiplying total N by the target C/N ratios and then subtracting those values from ready carbon contents in compost or urea. The total N contents of plant residues were not accounted for this calculation. Consequently, the C/N ratios of amendments were adjusted up to 10 and 25. Treatments without BS addition were used as the controls. For incubation 2, we compared the quality BS, WB and RS using sandy amended with the same compost soil after regulating the C/N value of compost up to 25.

After mixing all materials, the pots were covered with aluminum foil perforated with five small holes to ensure gas exchange and to reduce moisture losses. The amended soil was incubated at 25℃ for twenty days. The soil samples in triplicates were destructively taken on days 10 and 20 for laboratory analysis. For this purpose, each treatment was made in six replications. Therefore, there was a total of 14 different treatments for all the incubations. Treatment details are presented in Table II.

TABLE II. TREATMENT COMBINATIONS AND WEIGHT OF INGREDIENTS

Treatments	Added C sources (mg C kg^{-1} soil)			N sources (mg N kg^{-1} soil)		C/N
	RS	WB	BS	Compost	Urea	
Incubation 1–*set 1*						
① (P-BS)	–	–	0.0	0	0	–
② (P+BS)	–	–	444.5	0	0	–
③ (P++BS)	–	–	957.2	0	0	–
④ (P+++BS)	–	–	1944.5	0	0	–
⑤ (P++++BS)	–	–	2457.2	0	0	–
Incubation 1–*set 2*						
⑥ (PC-BS)	–	–	0.0	100	0	6.4
⑦ (PC+BS)	–	–	444.5	100	0	10.0
⑧ (PC+++BS)	–	–	1944.5	100	0	25.0
⑨ (PU-BS)	–	–	0.0	0	100	0.4
⑩ (PU++BS)	–	–	957.2	0	100	10.0
⑪ (PU++++BS)	–	–	2457.2	0	100	25.0
Incubation 2						
⑫ (BS)	–	–	1944.5	100	0	25.0
⑬ (WB)	–	1944.5	–	100	0	25.0
⑭ (RS)	1944.5	–	–	100	0	25.0

BS = bamboo stem; WB = wood for building; RS = rice straw; P = paddy soil only; PC = paddy soil and compost; PU = paddy soil and urea

B. *Soil Analysis*

Inorganic N was extracted from the fresh soil samples in 2.0 *M* KCl (w: v = 1:5). The filtrate was analyzed for NH_4-N and NO_3-N according to the phenol-hypochlorite method [11] and the Vanadium (III) chloride reduction method [12], respectively, using the spectrophotometer (UV-1200, Shimadzu, Japan). All values are expressed in dry weight basis.

C. Calculation and Statistical Analysis

Net N mineralization, net ammonification and net nitrification rates were calculated for the incubation period by subtracting the initial NH_4-N and NO_3-N, respectively, from final respective N concentrations in the soil.

One-way and two-way ANOVA were used to determine statistical significant differences between treatments. The mean values of treatments were compared according to Fisher's protected least significant difference (LSD) test at $P < 0.05$ using EXCEL ® macro add-ins DSAASTAT version 1.512 [13].

III. Results and Discussion

A. Incubation 1–Set 1: The Effect of BS on Inorganic N in the Paddy Soil

The NO_3-N in the paddy soil (P) increased rapidly during the first half and then maintained stability during the second half of the incubation time. Meanwhile, the change in NH_4-N was relatively small throughout the entire incubation period (Fig. 1). The results show that NO_3-N was more strongly affected by BS addition than NH_4-N. The NO_3-N concentration significantly decreased with increasing loading rates of BS, suggesting a preferential immobilization of nitrate rather than ammonium N (NH_4-N).

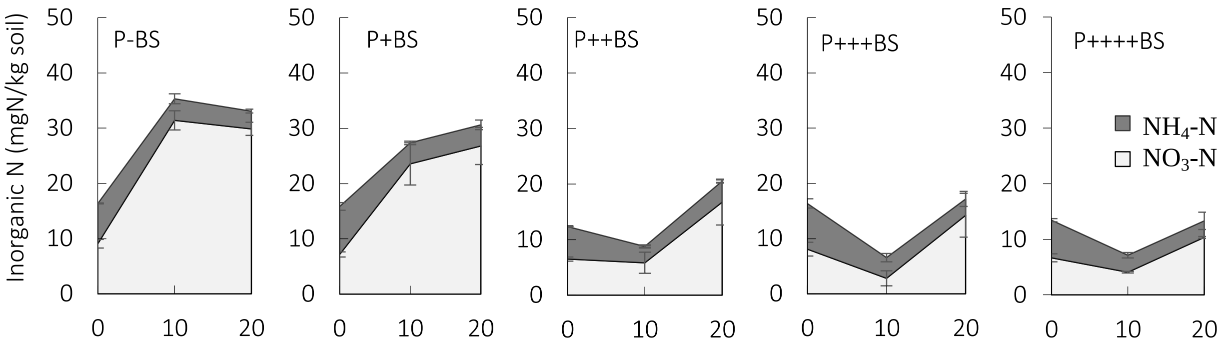

Figure 1. Changes in inorganic N in paddy soil as affected by added BS. Error bars represent standard deviation of the mean (n = 3).

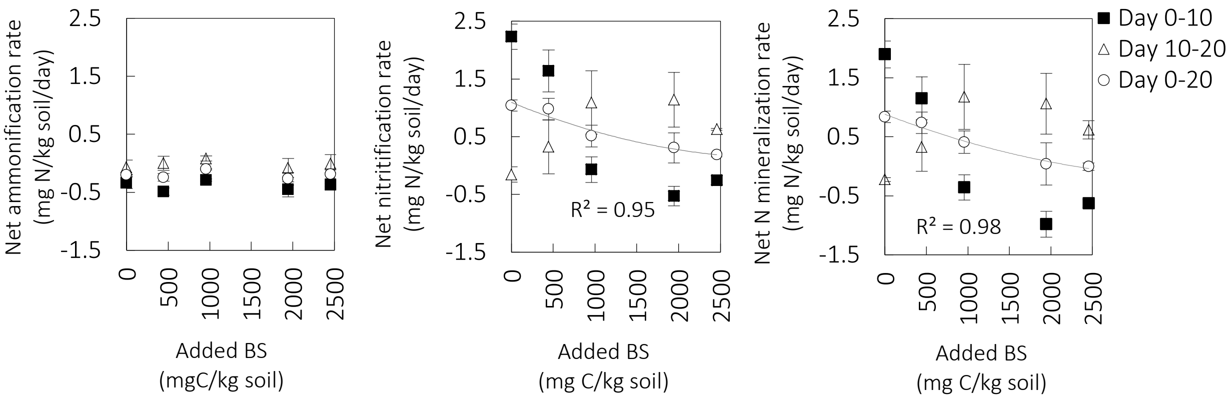

Figure 2. Relationship between net ammonification, net nitrification, net N mineralization rates and amount of added BS (mg C/kg soil). Error bars represent standard deviation of the mean (n = 3). r-squared values indicate the correlation during the whole incubation period of 0-20 day.

Further evidence of immobilization during the first 10 days of incubation in the presence of BS with loading rates over 957.2 mg C/kg soil is shown in Fig. 2. The net N mineralization rates and net nitrification rates during 20 days of incubation were positively correlated with amount of BS additions (r^2=0.98 and r^2=0.95, respectively). However, the net ammonification rate was not clearly affected by amount of added BS (Fig. 2).

B. Incubation 1 – Set 2: The Effect of BS and N Fertilizers with Controlled C/N Ratios on Inorganic N in Paddy Soil

The changes in NH_4-N and NO_3-N in soil amended with N fertilizers and BS are shown in Fig. 3. In treatments amended with urea (PU), the NO_3-N increased continously during the 20 days of incubation. However, in treatments amended with compost (PC), the NO_3-N rapidly increased during the first half and decreased during the second half of incubation. The NH_4-N gradually decreased with inbucation time in PC treatments, but significantly increased during the first half and then decreased during the second half time of incuation in PU treatments. Thus, the changes in NH_4-N and NO_3-N in compost amended soil was similar to those observed in aforementioned paddy soil. The increase in NO_3-N was remarkably inhibited at the treatment PC+++BS (C/N=25) only. Meanwhile, NH_4-N was affected by BS addition in both treatments PU++B (C/N=10) and PU++++B (C/N=25).

In regards to mineralization rates, the net N mineralization rates were significantly higher in urea than compost-amended treatments. N immobilization was found in the treatment PC+++BS (C/N=25) due to lowering net nitrification rate (0.20 mg N/kg soil/day) as compared to the control treatment PC-B (1.64 mg N/kg soil/day). Meanwhile, net N mineralization rates significantly decreased from 3.53 mg N/kg soil/day to 1.67 mg N/kg soil/day in the treatment PU++BS (C/N=10) and 1.62 mg N/kg soil/day in the treatment PU++++BS (C/N=25) (Table III).

C. Incubation 2: Potential N Immobilization Using Different Carbon Sources

The sandy soil amended with compost had initial NH_4-N of 3.81 mg N/kg soil and negligible NO_3-N (data not shown). The NH_4-N slightly decreased to 2.00, 3.27 and 2.66 mg N/kg soil after 20 days of incubation in the absence of BS, WB and RS, respectively. However, NO_3-N in treatment WB increased from 0 to 6.66 mg N/kg soil on day 10 and 10.62 mg N/kg soil on day 20. Consequently, the total inorganic N in the treatment WB was significantly higher than those in the treatments of BS and RS (Table IV). Similarly, compared to BS and RS, the treatment WB had significantly greater net nitrification and net N mineralization rates. The results also show that the rates of net ammonification, net nitrification and net N mineralization did not differ significantly between two treatments BS and RS (Table V).

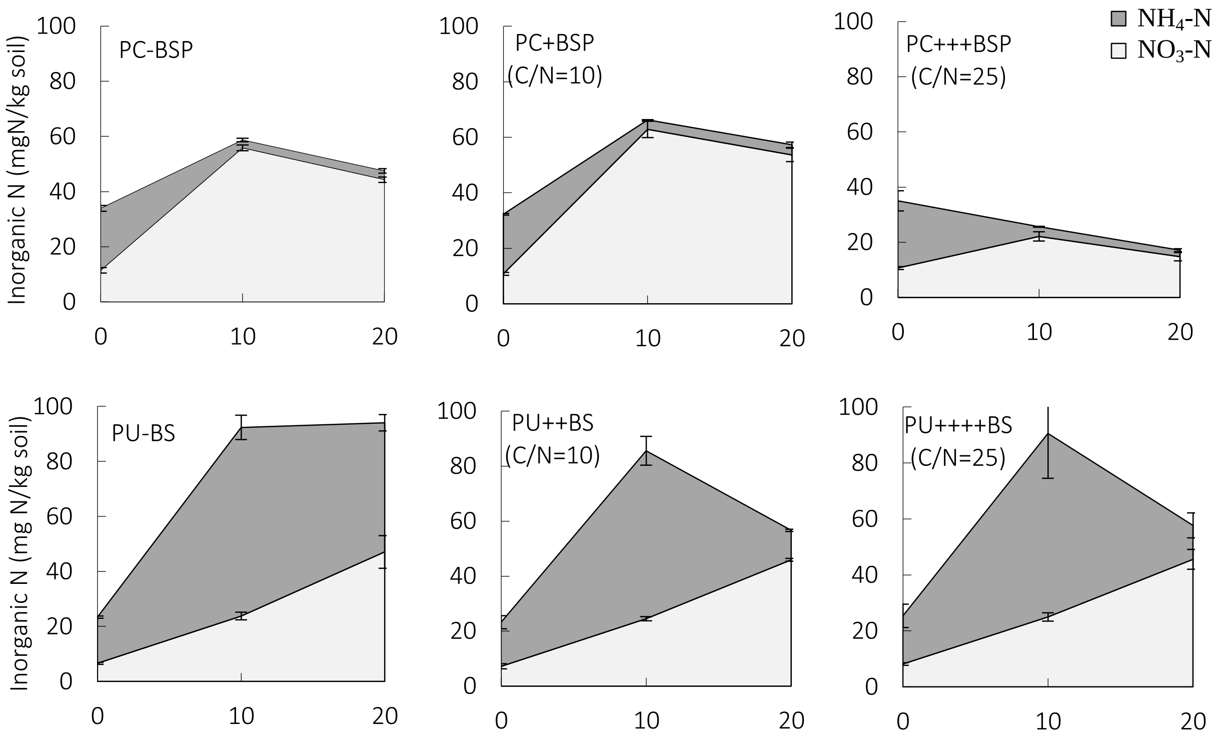

Figure 3. Changes in inorganic N in fertilized soil as affected by added BS with C/N= 10 and C/N=25. Bars represent standard deviation of the mean (n = 3).

TABLE III. NET AMMONIFICATION, NET NITRIFICATION AND NET N MINERALIZATION RATES OF N-FERTILIZED SOIL AS AFFECTED BY ADDED BS WITH C/N=10 AND C/N=25

Treatments	Net ammonification rate (mg N/kg soil/day)		Net nitrification rate (mg N/kg soil/day)		Net N mineralization rate (mg N/kg soil/day)	
	Compost	Urea	Compost	Urea	Compost	Urea
Control (no BS)	-0.97 c	1.51 a	1.64 b	2.03 a	0.68 d	3.53 a
C/N=10 (less BS)	-0.89 c	-0.27 b	2.14 a	1.94 ab	1.25 c	1.67 b
C/N=25 (more BS)	-1.10 c	-0.25 b	0.20 c	1.87 ab	-0.89 e	1.62 bc
Probability for:						
N sources	<0.001**		<0.001**		<0.001**	
BS levels	<0.001**		<0.001**		<0.001**	
N sources x BS levels	<0.001**		<0.001**		<0.001**	

Values within two columns for each parameter followed by the same letter are not significantly different according to LSD test

TABLE IV. SOIL INORGANIC N AS AFFECTED BY COMPOST AND CARBON SOURCES ADDITION AT CONTROLLED C/N = 25

Added C sources	10 days of incubation			20 days of incubation		
	NH_4-N	NO_3-N	Total	NH_4-N	NO_3-N	Total
BS	1.84	0.99	2.83 b	2.00	1.24 b	3.24 b
WB	3.29	6.66	9.95 a	3.27	10.62 a	13.89 a
RS	2.13	1.02	3.15 b	2.66	1.40 b	4.06 b
P > F	ns	ns	*	ns	**	**
LSD	1.75	5.93	5.11	1.27	4.68	4.55

Values within the same column followed by the same letter are not significantly different according to LSD test. All values are expressed in unit of mg N/kg soil

TABLE V. SOIL NET AMMONIFICATION RATE, NET NITRIFICATION RATE AND NET N MINERALIZATION RATE AS AFFECTED BY COMPOST AND CARBON SOURCES ADDITION AT CONTROLLED C/N = 25

Added C sources	Net ammonification rate (mg N/kg soil/day)	Net nitrification rate (mg N/kg soil/day)	Net N mineralization rate (mg N/kg soil/day)
BS	-0.09	0.06 b	-0.03 b
WB	-0.03	0.53 a	0.50 a
RS	-0.06	0.07 b	0.01 b
P > F	ns	**	**
LSD	6.32	0.23	0.23

Values within the same column followed by the same letter are not significantly different according to LSD test

D. Discussion

In the incubation 1–*set 1*, the NO_3-N was rapidly immobilized in the presence of BS. Thus, when the bio-available plant residues were supplied together with available N source, the heterotrophic organisms in native soil could use carbon as a source of energy and N for increasing their population. These microbial assimilations induced a temporary reduction of inorganic N in soil. Winsor and Pollard [6] found a similar pattern of rapid N immobilization within two days in soils treated with starch and sucrose. Their results indicated that the N immobilization was greater in soil treated with higher levels of added carbon compounds.

The increase in immobilization with increasing C/N ratios as observed in the incubation 1-*set 2* has been reported in other articles [6], [8], [14]. At a given C/N ratio, the preferable assimilation for either NH_4-N or NO_3-N might depend upon the N source and the quantity of added carbon. For example, the preference for nitrate was virtually complete at wider value of C/N ratio of 25 (more carbon added) in the case of soil amended with compost, whereas ammonia was easily assimilated at narrower value of C/N ratio of 10 (less carbon added) in the case of soil amended with urea. It is noted that NO_3-N content in current paddy soil was much higher than to NH_4-N content after 10 and 20 days of incubation. However, when both inorganic N forms of NH_4-N and NO_3-N were present in soil, such as treatments with urea, ammonia appeared to be more suitable for microbial immobilization than NO_3-N. A preference for ammonia over nitrate was also reported by Recous *et al.* [15], who indicated that in soil added with glucose at a rate of 500 mg C/kg soil, the amount of ^{15}N immobilized was much higher in [^{15}N] Urea, $^{15}(NH_4)_2SO_4$ and $^{15}NH_4NO_3$ than in $NH_4{}^{15}NO_3$ treatments. One possible reason was that the amount of carbon needed to immobilize one unit of N was larger with nitrate than that with ammonia as suggested by Ahmad *et al.* [14].

At a wider C/N of 25, the potential N immobilization was greater in soil incorporated with BS or RS rather than that with WB, suggesting the difference in immediate bio-availability of added carbon sources. These results implied that, either BS, RS may contain more labile carbon sources or WB may contain carbon compounds that are recalcitrant to decomposition, like lignin content [16]. Upadhyaya *et al.* conducted a 90-day laboratory incubation on soil amended with freshly fallen bamboo foliage from two different species in India [17]. They found that amendments of foliage bamboo did not contribute to the increasing N mineralization in the soil due to a readily available supply of C to soil microbes resulting in heterotrophic immobilization. In a recent study, Abbasi et al. compared impact of different plant residues on nitrogen mineralization–immbobilization turnover and carbon content of a soil incubated under laboratory conditions [18]. They found that incorporation of leguminous and non-leguminous plant residues strongly modifies the mineralization–immobilization of soil. For example, incorporation of plant residues with high C/N ratios and lignin contents like *Zea mays* root resulted in immobilization of N, whereas those with low C/N ratios like *Glycine max* shoot resulted in net N mineralization.

IV. CONCLUSION

Our study confirmed that incorporating bio-available carbon sources like bamboo stem powder and rice straw powder into N-fertilized soil caused a temporary decrease in N mineralization or reveal an N immobilization. The immobilization of NO_3-N was greatest in treatment of BS and compost (C/N=25), whereas, the immobilization of NH_4-N was found in treatments of BS and urea (both C/N=10 and C/N=25). Although the C/N ratios are useful to predict the potential N immobilization, the carbon quality is likely the more important factor to control the soil N mineralization–immobilization turnover in soil.

Because the mineralization versus microbial immobilization was found to depend upon the different N sources, the quantity and the quality of added carbon sources, further studies is needed to clarify the changes in N dynamics in soil–plant ecosystem after their application. We will discuss this subject in the next paper.

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