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Bao Ve Nguyen
Can Tho Univ., CanTho, Vietnam

D. C. Olk
USDA-ARS

Kenneth G. Cassman
University of Nebraska - Lincoln, kcassman1@unl.edu

Nitrogen Mineralization from Humic Acid Fractions in Rice Soils Depends on Degree of Humification

Nguyen Bao Ve, D. C. Olk,* and K. G. Cassman

ABSTRACT

Although the chemical nature of soil organic matter (SOM) is thought to affect the mineralization rate of N bound in SOM, little direct evidence exists for such effects. To test the hypothesis that the N mineralization rate is affected by the degree of SOM humification, we added equivalent amounts of humic acid (HA) N as either the labile mobile humic acid (MHA) fraction or the more humified calcium humate (CaHA) fraction to two lowland rice (*Oryza sativa* L.) soils, which were subsequently incubated under anaerobic conditions for 6 wk. The HA fractions had been chemically extracted from seven irrigated lowland rice soils from Vietnam and the Philippines. In both incubation soils, the amount of N mineralized from the added HA fractions decreased exponentially as the optical density of the HA at 465 nm (E_4, index of humification) increased (R^2 = 0.94–0.98). Mineralization of humic N was also strongly negatively associated with the amount of N or C contained in each HA fraction per kilogram of soil extracted, suggesting a more recalcitrant nature of the fractions in those soils where conditions allowed them to accumulate. Nitrogen mineralization was less for both the MHA (by 26%) and CaHA (by 41%) when incubated in the International Rice Research Institute (IRRI) soil compared with the Tanhoi soil. The most plausible explanation for this decrease is increased stabilization of the added HA by the relatively abundant Ca^{+2} in the IRRI soil. We conclude that the degree of humification of the MHA and CaHA fractions plays an important role in governing the rate of N mineralization in lowland rice soils because (i) N mineralization from these fractions was affected by their degree of humification, and (ii) the fractions are a small but important component of total soil N.

M ANAGEMENT OF INDIGENOUS SOIL N is an important issue in irrigated lowland rice systems because N mineralization from SOM provides a substantial portion of the total N taken up by a rice crop. In a companion paper (Nguyen et al., 2004), we used stepwise regression to predict parameters of net N mineralization from anaerobic incubation of 12 lowland rice soils. The prediction was improved when the independent variables of the regression included not only whole soil properties, such as total soil N, but also the properties and amounts of specific HA fractions in each soil. These results point to the relevance of chemically extracted HA fractions to achieving better understanding of process-level controls on soil N mineralization in lowland rice.

Although the chemical nature of SOM is thought to affect the mineralization rate of SOM-bound N (Stevenson and Cole, 1999), little direct evidence exists for

B.V. Nguyen, Dep. of Crop Sciences, Can Tho Univ., Can Tho, Vietnam; D.C. Olk, USDA-ARS, National Soil Tilth Laboratory, 2150 Pammel Dr., Ames, IA 50011; and K.G. Cassman, Dep. of Agronomy and Horticulture, Univ. of Nebraska, Lincoln, NE 68583-0915. Journal Series No. 14403 from the Agricultural Research Division, Univ. of Nebraska. Received 22 Nov. 2003. *Corresponding author (olk@nstl.gov).

such effects, especially for chemically extracted SOM fractions. In this study, we explore the relationship between the chemical nature of extracted HA fractions and their pattern of N mineralization when the fractions were added to two test soils and incubated. The same two fractions were examined as were used by Nguyen et al. (2004) to study soil N mineralization in lowland rice: (i) the MHA, which is a young HA fraction that is relatively N-rich and can be extracted from soil without prior removal of polyvalent cations, and the CaHA fraction, which is somewhat older than the MHA and stabilized by polyvalent cations (Olk et al., 1995, 1996).

The primary objective of these experiments was to test the hypothesis that the rate of N mineralization from these HA fractions was influenced by their degree of humification, which varies due to soil management practices and the environmental conditions in these lowland rice fields (Olk et al., 1996, 1998; Mahieu et al., 2002; Nguyen et al., 2004). A secondary objective was to determine whether N mineralization from these fractions was related to other chemical properties of these fractions or properties of the soils from which the fractions were extracted.

MATERIALS AND METHODS

Selection of Soils

The MHA and CaHA were extracted from seven of the 14 lowland rice soils used by Nguyen et al. (2004). To ensure a representative subset of the 14 soils, we hypothesized that potential N mineralization of the humic fractions was related to the MHA-N fraction, which is the ratio of the amount of N contained in the MHA per kilogram of extracted soil (MHA-N) to the sum of MHA-N and CaHA-N. This hypothesis was based on the interpretation by Nguyen et al. (2004) and Olk et al. (1998) that in the better-aerated, low-N soils (such as the Guimba and Philippine Rice Institute [PhilRice] soils), young humic materials such as the MHA are more readily decomposed than in soils that remain under anoxic conditions for most of the year, resulting in accumulation of a more recalcitrant form of young humic materials in the anoxic soils. Thus, the 14 soils were grouped by their MHA-N fraction and two to three soils were selected from each of three groups: (i) MHA-N fraction <0.50, (ii) MHA-N fraction ≈0.50, and (iii) MHA-N fraction >0.50. Of the seven selected soils, Guimba is from a farmer's field in Central Luzon, Philippines, PhilRice is from the Long-Term Fertility Experiment (LTFE) at the Philippine Rice Research Institute in Central Luzon, IRRI is from the LTFE at the International Rice Research Institute in Luzon, Cuulong is from a long-term experiment in the Mekong Delta of Vietnam, and Cantho, Huongso, and Tanhoi are from farmers' fields in the Mekong Delta and

Table 1. Tropical lowland rice soils used for extraction of the humic acid fractions.†

Soil	Soil organic C	Total soil N	pH	Clay	Silt	CEC‡	1 M ammonium acetate-extractable cations			
							Ca	Mg	K	Na
	——— g kg⁻¹ soil ———			— g kg⁻¹ soil —			——— cmol$_c$ kg⁻¹ soil ———			
Guimba	12.1	1.00	7.7	360	430	27.1	18.8	12.3	0.10	0.56
PhilRice	15.5	1.25	6.6	400	505	25.4	18.0	8.4	0.11	0.56
Tanhoi§	21.2	2.09	6.0	680	300	22.6	10.8	10.7	0.36	1.13
Cuulong	32.6	2.96	5.5	560	436	22.0	11.1	8.1	0.23	0.48
IRRI§	22.0	1.84	6.5	550	355	35.7	18.2	11.8	1.51	1.44
Cantho	26.4	2.34	5.3	445	545	15.9	9.3	2.8	0.24	0.28
Huangso	14.1	1.36	6.3	200	530	4.2	2.3	1.5	0.09	0.50

† Data are from Nguyen et al. (2004), who also reported the analyses used to obtain the data.
‡ Cation-exchange capacity.
§ Also used as an incubation soil.

Hue region of central Vietnam. These soils have a wide range of organic C, total N, and other soil chemical and physical properties (Table 1).

The MHA and CaHA fractions that were extracted from these seven "extraction soils" were added separately to the IRRI and Tanhoi soils to determine their N mineralization patterns during anaerobic incubations. These two "incubation soils" were selected because they had near neutral pH and low K-fixing capacity, which are properties conducive for amendment with HA and measurement of subsequent N mineralization. Both incubation soils had soil organic C of about 20 g kg⁻¹, total soil N of about 2 g kg⁻¹, and high clay contents (Table 1). The IRRI soil had a higher cation-exchange capacity than the Tanhoi soil due to a higher exchangeable Ca content (Table 1). For each incubation soil a composite soil was made from field replicates and used for all mineralization incubations.

Extraction and Characterization of MHA and CaHA fractions

The MHA and CaHA were extracted from three field replicates of each soil as described by Nguyen et al. (2004), following the procedure of Olk et al. (1996). Briefly, 18 g of air-dried soil was incubated in 180 mL of 0.25 M NaOH for 20 h and then centrifuged. The supernatant was decanted and acidified to pH 1.95 to precipitate the MHA. The soil residue was washed twice with 0.0025 M CaCl$_2$ to remove fine clays in suspension that had combined with soluble MHA, then decalcified by repeated 0.1 M HCl washes. The CaHA was subsequently extracted from the soil with 0.25 M NaOH using the same protocol as for the MHA. To reduce inorganic contamination, both the MHA and CaHA were shaken in an HF/HCl solution for 3 d with daily solution replacement. The MHA and CaHA were H⁺–saturated by dialyzing for 3 d against HCl solutions and water. Then the MHA and CaHA were frozen, lyophilized, and stored as a dry powder.

The C and N concentrations of the MHA and CaHA were determined by dry combustion on a CHN automated elemental analyzer. Per kilogram of extracted soil, the amounts of N or C contained in the extracted MHA (MHA-N or MHA-C) and CaHA (CaHA-N or CaHA-C) were estimated as the products of the N and C concentrations of each HA fraction and their mass extracted per kilogram of extracted soil. Optical density at 465 nm was determined on solutions of 3 mg HA-C solubilized in 30 mL of 0.05 M NaHCO$_3$ (Chen et al., 1977). Ash content was determined gravimetrically after heating to 700°C for 3 h. All values reported for C and N concentration are corrected for ash content.

Humic Acid Addition and In Vitro Soil Nitrogen Mineralization Procedure

Because the HA fractions were H⁺–saturated during their extraction from soil, their addition to the IRRI and Tanhoi soils could cause soil acidification. Hence before their addition to the IRRI and Tanhoi soils, the extracted MHA and CaHA fractions were saturated with K⁺ by solubilizing HA containing 100 mg N in 10 mL of 0.05 M KOH in a beaker with constant mixing by a magnetic stirrer. After solubilization, the HA solution was adjusted to pH 7 with dilute HCl, then frozen, lyophilized, ground, and stored as a dry powder. Nitrogen concentrations of the K⁺–saturated HA samples were measured again to estimate the addition rate of each HA sample to the incubation soils based on an equivalent N addition level of 200 mg N kg⁻¹ incubation soil. This rate was selected to represent the increase in total soil N that can occur under field conditions following the introduction of double- or triple-cropping of irrigated lowland rice (Cassman et al., 1995; Witt et al., 2000). The mass of HA (K⁺–free basis) that was added to the incubation soils ranged from 3.48 to 4.26 g MHA kg⁻¹ incubation soil and from 3.89 to 5.60 g CaHA kg⁻¹ soil.

Quantities of each MHA and CaHA fraction corresponding to 0.4 mg N were mixed into 2 g of dry soil in 20-mL glass test tubes with three replications. These tubes were stored in an incubator overnight at 10°C. The next day each tube received 1 mL of cold (10°C) water and was flushed with N$_2$ + 5% (v/v) CO$_2$ gas for 20 s at a flow rate of 2.5 mL s⁻¹. Tubes were stoppered immediately and placed in an incubator for 2 d at 10°C to allow chemical equilibration between HA and soil. After 2 d, 1 g of dry K⁺–saturated resin was added to each tube followed by 14 mL of deionized water. Resin was kept on the surface of the soil to avoid interactions between the amended HA and resin. The tubes were flushed with N$_2$ + 5% CO$_2$ gas and stoppered as above, then placed in an incubator at 30°C.

The triplicate tubes of each soil and MHA or CaHA combination were extracted for mineralized NH$_4$ at 0, 21, and 42 d after resin addition, following the procedure described by Nguyen et al. (2004). Briefly, at sampling time the contents of each tube were transferred to a 50-mL centrifuge tube via three 5-mL washes of 4 M KCl. This solution was shaken and centrifuged. The supernatant was filtered and acidified before storage. Soil was extracted again with KCl to recover residual NH$_4$. The NH$_4$–N concentration in each extract was determined spectrophotometrically (Kempers and Zweers, 1986) and summed for both soil washes. As a control, triplicate tubes containing soil without HA addition were also extracted at each sampling time for both incubation soils. Extractable NH$_4$ in the control samples was subtracted from extractable NH$_4$ in the HA-amended samples to determine the amount of N mineralized from the added HA.

Statistical Analysis

The relationship between N mineralization of HA fractions added to the IRRI incubation soil and N mineralization of HA fractions added to the Tanhoi incubation soil was evaluated by

Table 2. Selected properties of the mobile humic acid (MHA) and calcium humate (CaHA) fractions extracted from seven tropical lowland rice soils for addition to two incubation soils.†

Soil	MHA fraction	N content		C content		N concentration		C concentration		C/N ratio		E_4‡	
		MHA	CaHA	MHA	CaHA	MHA	CaHA	MHA	CaHA	MHA	CaHA	MHA	CaHA
		g kg^{-1} extraction soil				g kg^{-1} humic acid							
Guimba	0.15	0.022	0.130	0.21	2.14	57.5	35.7	538	575	9.4	16.1	2.05	13.3
PhilRice	0.37	0.074	0.124	0.76	1.87	53.3	37.3	541	554	10.2	14.9	2.24	10.0
Tanhoi	0.52	0.178	0.167	1.79	2.14	53.7	43.7	540	554	10.0	12.7	3.62	10.7
Cuulong	0.52	0.464	0.432	4.90	5.02	52.1	51.4	542	573	10.4	11.2	7.77	16.6
IRRI	0.58	0.213	0.155	2.41	2.18	47.0	40.0	539	552	11.5	13.8	3.58	9.8
Cantho	0.71	0.451	0.184	5.00	2.45	49.8	42.8	546	564	11.0	13.2	5.21	11.0
Huongso	0.71	0.212	0.085	2.15	0.99	54.2	48.6	526	529	9.7	10.9	3.36	8.1

† Data are from Nguyen et al. (2004), who also reported the analyses used to obtain the data.
‡ Optical density at 465 nm. Units are optical density units (g HA-C L^{-1})$^{-1}$.

correlation analysis. Relationships between the amount of N mineralized from the added HA fractions and specific chemical properties of the HA fractions or of the extraction soils were evaluated by regression analysis. Curvilinear relationships were identified using the TableCurve 2D software package, Version 4.0 (SPSS Inc., Chicago, IL). This software evaluates a family of curvilinear functions and identifies the model with the smallest residual sum of squares. Stepwise regression (SAS Institute, 1990) was used to determine which properties of whole soil significantly influenced the cumulative N mineralized from the HA fractions after 3 and 6 wk. Independent variables entered the model at a significance level of 0.15.

RESULTS

Nitrogen Mineralization of the MHA and CaHA

The proportion of extracted HA N that was contained in the MHA, the MHA-N fraction, was <40% for both the Guimba and PhilRice soils (Table 2). In contrast, the other five extraction soils had an MHA-N fraction ranging from 52 to 71%. The MHA of the Guimba and PhilRice soils also had the smallest E_4 values, and the Guimba soil had the highest N concentration of the MHA. After a 6-wk incubation in both incubation soils, the amount of N mineralized per unit N added as MHA was on average 18 to 116% greater for MHA extracted from the Guimba and PhilRice soils than for MHA extracted from the other soils (Table 3). Nitrogen mineralization from extracted CaHA did not differ in a consistent pattern between extraction soils with small or large MHA-N fractions.

After a 6-wk incubation, cumulative N mineralized was two to five times greater per unit N added as MHA

than per unit N added as CaHA, and most of this difference occurred during the first 3 wk of incubation (Table 3). For example, after a 3-wk incubation in the IRRI soil, N mineralized ranged from 111 to 230 g NH$_4$–N kg^{-1} MHA-bound N and from 17 to 43 g NH$_4$–N kg^{-1} CaHA-bound N. From 3 to 6 wk, cumulative N mineralized from added MHA and CaHA slowed dramatically, and the decrease was greater for the MHA. The coefficient of variation for N mineralization from the HA fractions across extraction soils was greater for MHA than for CaHA in both time periods. After 6 wk, cumulative N mineralization varied among extracted soils by 1.7- to 3.2-fold for the four combinations of HA fraction and incubation soil.

The relative pattern of cumulative N mineralized from HA was generally similar whether the HA was incubated in the Tanhoi soil or the IRRI soil. Thus, there was a close linear correlation between the quantity of N mineralization from N added as MHA to the IRRI soil and N mineralization from N added as MHA to the Tanhoi soil at both 3 (Fig. 1a) and 6 wk (Fig. 1b). In contrast, the relationship between N mineralization from N added as CaHA in the two incubation soils was more clustered. The quantities of N mineralized from both HA fractions were substantially less when incubated in the IRRI soil than in the Tanhoi soil. For example, after the 6-wk incubation, N mineralized from MHA added to the IRRI soil was on average 26% less than from MHA added to the Tanhoi soil; for CaHA, the decrease was 41% in the IRRI soil compared with the Tanhoi soil (Table 3).

Table 3. Nitrogen (NH$_4$–N) mineralized from the mobile humic acid (MHA) and calcium humate (CaHA) fractions extracted from seven tropical lowland rice soils and added to the IRRI and Tanhoi soils for incubation under anaerobic conditions.

| Humic acid extracted from soil	0–3 wk				3–6 wk				0–6 wk			
	MHA		CaHA		MHA		CaHA		MHA		CaHA	
	IRRI	Tanhoi	IRRI	Tanhoi	IRRI	Tanhoi	IRRI	Tanhoi	IRRI	Tanhoi	IRRI	Tanhoi
	g NH$_4$–N kg^{-1} humic acid-N											
Guimba	230	388	32.5	56.8	79	65	24	45	309	453	56	102
PhilRice	216	363	35.4	58.9	74	55	32	43	290	418	68	102
Tanhoi	185	312	28.2	68.4	69	52	32	38	254	364	60	106
Cuulong	111	132	16.9	35.2	28	9	18	33	139	141	35	68
IRRI	134	243	30.3	62.5	61	41	28	42	195	284	58	105
Cantho	117	187	41.3	55.1	35	27	22	40	152	214	64	96
Huongso	159	256	43.0	69.9	73	47	28	44	232	303	71	114
Mean	164	269	32.5	58.1	60	42	26	41	224	311	59	99
Std dev.	47	92	8.8	11.6	20	19	5	4	66	111	12	15
CV, %	29	34	27	18	34	45	20	10	29	36	20	15
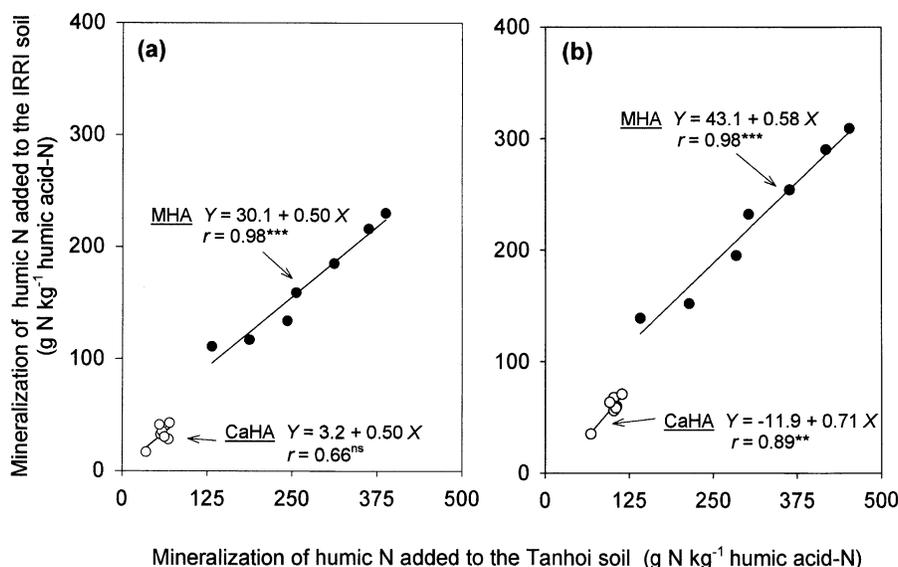
Fig. 1. The correlation between the amount of N mineralized from the mobile humic acid (MHA) fraction or the calcium humate (CaHA) fraction that were extracted from seven tropical lowland rice soils and incubated in the IRRI soil versus Tanhoi soil under anaerobic conditions at 30°C for (a) 3 and (b) 6 wk. ** and *** indicate significance at 1 and 0.1% levels, respectively; ns is not significant.

Nitrogen Mineralization as Influenced by Chemical Properties of the Humic Acid Fractions

Nitrogen mineralized from each HA fraction was negatively associated with the HA N, the amount of N contained in each humic fraction per kilogram of extraction soil, for Tanhoi (Fig. 2a) and IRRI (Fig. 2b) incubations. For the MHA, these relationships were best described by exponential equations of the form:

$$Y = a + b\exp(-X/c) \qquad [1]$$

where Y is the amount of N mineralized from the added MHA, X is the MHA-N content per kilogram of extraction soil, and a, b, and c are constants. Corresponding data for the CaHA fraction were best fit by a power function of the form:

$$Y = a + bX^c \qquad [2]$$

where Y is the amount of N mineralized from the added CaHA, X is the CaHA-N content per kilogram of extraction soil, and a, b, and c are constants. Fits were closer for the MHA than for the CaHA. The coefficients of determination between HA-C content per kilogram of extraction soil as the independent variables and mineralized N from added HA fractions as the dependent variables, were as high as when the independent variables were HA-N content per kilogram of extraction soil (data not shown). The degree of fit for these regressions was comparable in both incubation soils despite the smaller amount of N mineralization from humic N added to the IRRI soil.

The cumulative amounts of N mineralization at 6 wk were pooled for both fractions and regressed against the E_4 optical density values using the exponential model of Eq. [1]. The amounts of N mineralized from the MHA and CaHA were strongly negatively associated with the E_4 values (Fig. 3a,b). Nitrogen mineralization from the MHA fraction was also negatively associated

with the C/N ratio of the MHA, but the data were not significantly fit by linear, power, or exponential equations (data not shown). No trend was apparent between N mineralization from the CaHA fraction and the C/N ratio of the CaHA.

Although N mineralization from the added MHA and CaHA was also significantly associated with soil organic C and total soil N of the extraction soils (Table 1), the coefficients of determination were much weaker than for the relationships shown in Fig. 2 and 3. For example, coefficients of determination for exponential relationships (Eq. [1]) with N mineralized from the MHA at either 3 or 6 wk as the dependent variable ranged from 0.71 to 0.80 for soil organic C and from 0.73 to 0.78 for total soil N. Simple linear regressions with soil organic C or total soil N were slightly weaker than the exponential and power functions. Other properties of the extraction soils, including particle-size fractions and exchangeable cations, did not have significant linear relationships with N mineralized from the MHA and CaHA, except for positive associations of soil pH with MHA mineralization in both incubation soils and at both sampling times ($R^2 = 0.82$–0.84).

Stepwise regression analysis using properties of the extracted soils as independent variables provided similar predictions of cumulative N mineralization as did the amount of N contained in the HA fraction per kilogram of extraction soil (Fig. 2) for (i) MHA incubated in the Tanhoi soil for 3 wk, (ii) MHA incubated in both soils for 6 wk, and (iii) CaHA incubated in the IRRI soil for 6 wk. In all three MHA cases, soil organic C and clay content were negatively and positively, respectively, associated with humic N mineralization, and soil organic C was the more important predictor. For all other incubation times of MHA and CaHA in either incubation soil, stepwise regression identified either soil organic C or clay content as the sole soil property that was significantly associated with cumulative N mineral-
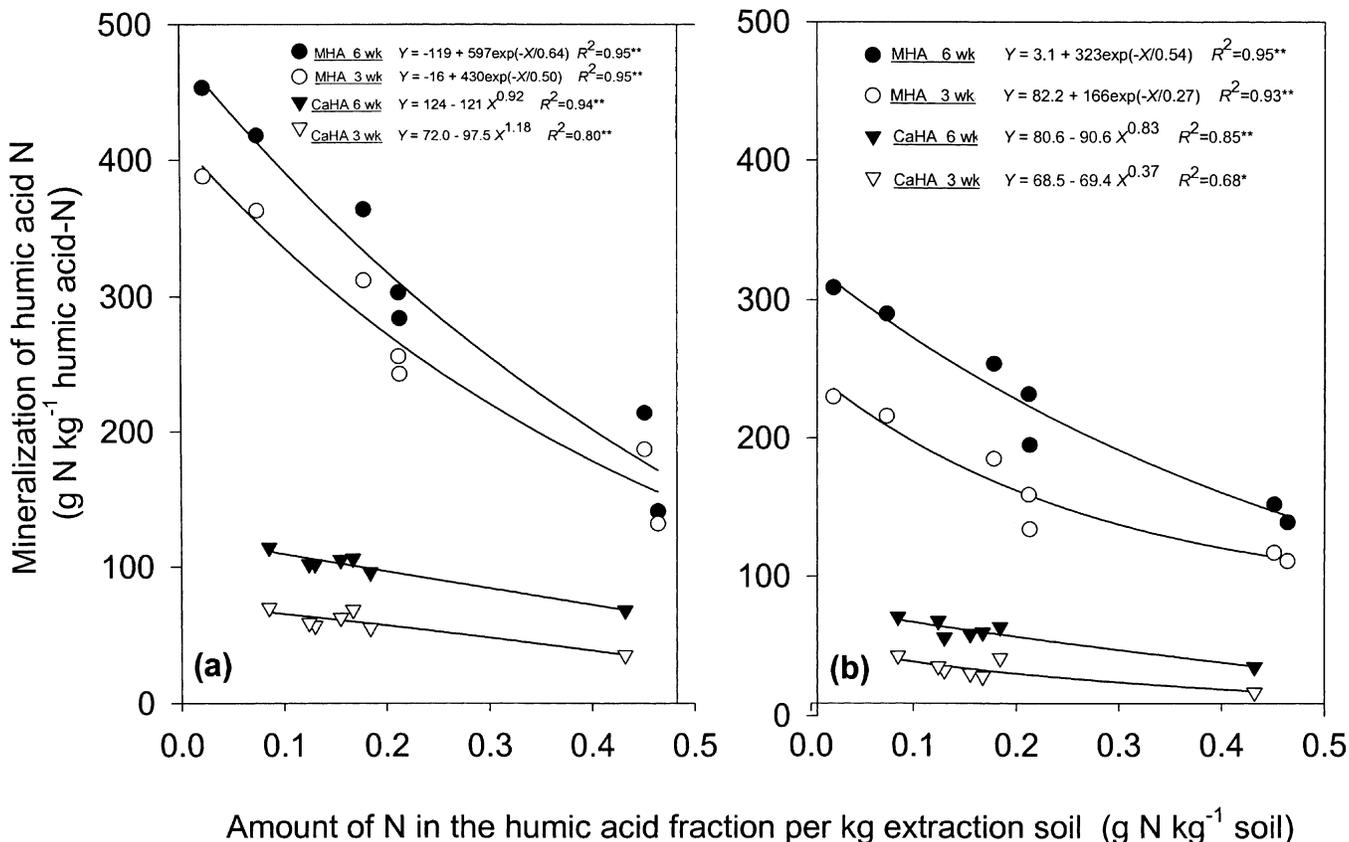
Fig. 2. The relationship between the amount of humic acid N mineralized and the amount of N contained in the humic acid fraction per kg extraction soil for the mobile humic acid (MHA) fraction and the calcium humate (CaHA) fraction that were extracted from seven tropical lowland rice soils and incubated in the (a) Tanhoi and (b) IRRI soils under anaerobic conditions at 30°C for 3 and 6 wk. * and ** indicate significance at 5 and 1% levels, respectively.
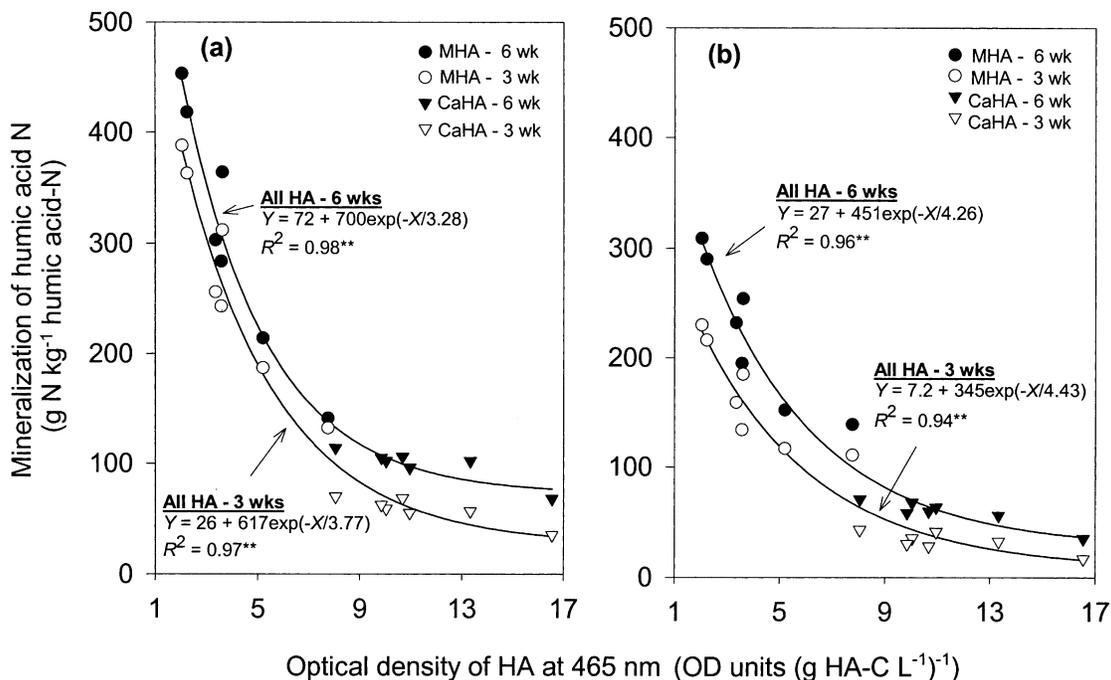

Fig. 3. The relationship between the amount of humic acid (HA) N mineralized and the optical density at 465 nm for the mobile humic acid (MHA) fraction and the calcium humate (CaHA) fraction that were extracted from seven tropical lowland rice soils and incubated in the (a) Tanhoi and (b) IRRI soils under anaerobic conditions at 30°C for 3 and 6 wk. ** indicates significance at 1% level.

ization, and the coefficients of determination were considerably smaller than those of the humic acid N content per kilogram of extraction soil.

Nitrogen and C concentrations per unit of HA were significantly associated with N mineralized from the MHA or CaHA in only two cases: (i) the N concentration per unit of MHA (and hence also mass of MHA added) was positively associated with N mineralized from the MHA after a 3-wk incubation in the IRRI soil ($R^2 = 0.55$, $P < 0.05$), and (ii) the C concentration per unit of CaHA was negatively associated with N mineralized from the CaHA after a 3-wk incubation in the Tanhoi soil ($R^2 = 0.55$, $P < 0.05$).

DISCUSSION

Soil biochemical processes are assumed to influence the rate of N mineralization (Stevenson and Cole, 1999), yet direct evidence is sparse for process-level controls that apply across a range of environments. Likewise, N mineralization of specific SOM fractions has not been consistently related to their quantity or chemical composition other than their C/N ratio, and most or all of this work has been based on physical separation of SOM fractions (Christensen, 1992; Feller and Beare, 1997; Whalen et al., 2000). In general, fundamental relationships between N cycling and biochemical properties of whole soil have not been established except for the inhibition of N mineralization by an abundance of phenolic compounds in coniferous forest soils, organic soils, and grassland soils (Burge and Broadbent, 1961; Schimel and Firestone, 1989; Bending and Read, 1996; Knicker et al., 2000).

In this study, N mineralization from the added MHA was greater than that from the added CaHA for each of the seven extraction soils and for incubations in both the IRRI and Tanhoi soils. This reproducibility suggests that the MHA is a distinctly more labile fraction than the CaHA in all soils. This finding is consistent with previous, more detailed studies of the chemical composition of these two HA fractions and speculation about their contributions to nutrient cycling (Mahieu et al., 2002; Olk and Cassman, 2002; Nguyen et al., 2004).

Nitrogen mineralization from the added HA fractions was closely related to the amounts of N and C contained in the HA fractions per kilogram of extraction soil and the E_4 optical density value of the HA. These relationships were based on regressions that included two incubation soils and seven extraction soils with a wide range of chemical and physical properties, which indicates a reproducible relationship between humic acid chemistry and the mineralization potential of humic N. Most noteworthy were the strong negative associations of N mineralization with E_4 (Fig. 3). Further, stepwise regressions by Nguyen et al. (2004) found that (i) the CaHA-E_4 was negatively associated with two rate constants for net N mineralization in these lowland rice soils, and (ii) the E_4 values for both HA fractions were negatively associated with the size of a readily mineralizable N pool. An inverse relationship between N mineralization potential and the E_4 value is consistent with the positive correla-

tion of the E_4 value with the degree of humification as determined by several spectroscopic analytical methods (Mahieu et al., 2002) and the greater recalcitrance of more humified humic materials to microbial decomposition (Zech et al., 1997; Stevenson and Cole, 1999). Yet E_4 has not previously been linked to N mineralization, despite considerable work by Kumada (1987), Schnitzer and Lévesque (1979), and others that described optical density at visible light wavelengths as an index of humic lability.

The amount of N mineralized from either HA fraction was also negatively associated with the amount of N contained in that fraction per kilogram of extracted soil. Of the seven extraction soils, the MHA of three relatively anoxic soils, Cuulong, Cantho, and IRRI, had the largest E_4 values and largest N contents per kilogram of extraction soil, yet the smallest amounts of N mineralization per unit N. Conversely, the MHA of two more aerated soils, Guimba and PhilRice, had the smallest E_4 values and smallest N contents per kilogram of extraction soil yet the largest amounts of N mineralization per unit N, indicating the sensitivity of the quantity and chemical composition of this SOM fraction to recent soil conditions.

Mineralization of HA-bound N was considerably less when the same HA fraction was incubated in the IRRI soil than in the Tanhoi soil. We attribute this difference to stronger stabilization of the added HA by Ca^{+2} in the IRRI soil, because this soil had 70% more extractable Ca than did the Tanhoi soil. Previous studies have demonstrated Ca-induced stabilization of organic materials and SOM against microbial degradation (Baldock and Skjemstad, 2000; Kononova, 1961), and stepwise regressions by Nguyen et al. (2004) associated exchangeable Ca in these rice soils with formation or stabilization of SOM into the more recalcitrant CaHA fraction at the expense of the labile MHA fraction. In addition, the impedance of N mineralization from HA added to the IRRI soil compared with the Tanhoi soil was proportionately greater for the CaHA fraction (41% decrease) than for the MHA fraction (26% decrease), suggesting that the polyvalent cation-bound CaHA was more amenable to stabilization by the IRRI soil than was the MHA. Based on this body of evidence, we propose that Ca was a primary agent for stabilization of the added HA, especially for the CaHA.

Calcium-induced stabilization of amended HA was further suggested by the results of an ancillary study in which N mineralization was measured during aerobic incubation of the same MHA and CaHA fractions after addition to the IRRI and Guimba soils. These two incubation soils had equally high levels of exchangeable Ca, and, accordingly, the amount of N mineralization from added HA differed less between them than in this study between the IRRI and Tanhoi incubation soils. Specifically, after 6 wk aerobic incubation, MHA-N mineralization was on average only 8% greater in the Guimba soil than in the IRRI soil, and mineralization of CaHA-N was 36% less in the Guimba soil than in the IRRI soil (data not shown).

There was no evidence for any inhibition of microbial

activity in the IRRI soil that would have slowed N mineralization from the amended HA. The amount of N mineralization in the unamended control soils was 30% higher in the IRRI soil than in the Tanhoi soil during the first 3 wk, and it was similar in both soils from 3 to 6 wk, suggesting uninhibited microbial activity in the IRRI soil. Both soils had favorable pH values and SOM levels. Other soil properties that may promote stabilization of HA, such as clay and SOM, were also similar in both soils.

The experimental design of this study was not intended to quantify the contributions of the MHA and CaHA to whole soil N mineralization. In fact, rapid N mineralization during the 6-wk incubation of this study suggests that the incubation method did not simulate field conditions. After the 6-wk incubation, as much as 45% of the humic N had been mineralized, suggesting incomplete restabilization of the HA that was added to the incubation soils. By comparison, extractions of the MHA from an irrigated lowland rice field indicated net mineralization of only about 10% of the N contained in the MHA during the first 53 d of a rice cropping period (Olk and Cassman, 2002). Nevertheless, the results of this study, in conjunction with those of Nguyen et al. (2004), indicate that these HA fractions are significant contributors to N mineralization of whole soil as measured using the anaerobic incubation procedure, and that the rate of N mineralization from these HA fractions depends on their chemical composition, which in turn is influenced by recent field management and soil moisture regime.

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