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DIVISION S-3—SOIL BIOLOGY & BIOCHEMISTRY

Nitrogen Dynamics in Humic Fractions under Alternative Straw Management in Temperate Rice

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ABSTRACT

Crop residue management practices can affect N immobilization and stabilization processes important to efficient utilization of N from fertilizers, crop residues, and soil organic matter (SOM). A 2-yr, ¹⁵N-labeling field study was conducted to examine the effects of winter-fallow flooding (vs. unflooded) and straw residue incorporation (vs. burning) on the rates of sequestration and stability of specific SOM pools critical in sustaining N fertility in rice (*Oryza sativa* L.). Five SOM fractions were examined from soil samples obtained over Years 4 to 6 of a field trial: light fraction (LF), mobile humic acid (MHA), mobile fulvic acid (MFA), metal-associated humic acid (MAHA), and alkali-insoluble humics (HUM). After 4 yr of straw management treatments, soil incorporation of straw increased MHA and LF C and N compared with burned straw. Immobilization of N fertilizer peaked in all SOM fractions after one growing season (120 d) and was greatest in the MHA fraction over the 2-yr ¹⁵N study. Nitrogen fertilizer sequestration in MHA and LF was greater with straw incorporation compared with burned. Turnover of immobilized ¹⁵N-fertilizer in the stable organic components was fastest in the labile MHA and MFA fractions (7- to 9-yr half-life) compared with the half-lives of the moderately resistant MAHA fraction (53 yr) and most stable HUM fraction (153 yr). While the MAHA and HUM fractions played a significant role in N fertilizer immobilization and turnover, the MHA and LF fractions represented the primary active sink and source of sequestered N affecting both short- and long-term soil fertility.

SOIL ORGANIC N is the largest source of plant-available N for rice, representing 50 to 80% of total N assimilated by the crop (Mikkelsen, 1987; Eagle et al., 2001). In California, a recent transition in rice-straw management from open-field burning to soil incorporation of straw and winter-fallow flooding has prompted a reexamination of N immobilization-mineralization dynamics and their effects on long-term N fertility in rice. The relatively low N fertilizer-use efficiency in lowland rice systems compared with upland crops (40–60% recovery of applied N) has been attributed in part to greater soil N immobilization (Broadbent and Nakashima, 1970; Vlek and Byrnes, 1986). Long-term straw incorporation and winter flooding may alter humification processes

thereby affecting N sequestration rates into SOM fractions and its subsequent turnover.

Previous work from long-term rice management studies in tropical (Cassman et al., 1996; Bellakki et al., 1998) and temperate (Eagle et al., 2000; Bird et al., 2001) climates indicate increased plant-available soil N supply after 5 to 10 yr of straw incorporation. In our initial investigation after three seasons of straw incorporation compared with burning, greater rice N-uptake and yield in annual trials without supplemental N fertilizer was observed (Eagle et al., 2000). No change was found in total soil C and N after six seasons of straw incorporation and winter-fallow flooding (Bird et al., 2001). After many years of straw incorporation, a sustained, greater soil microbial biomass (SMB) C and N was reported (Powlson et al., 1987; Sørensen, 1987; Bird et al., 2001). An increase in SMB can affect C and N sequestration rates of fertilizer and crop residues through greater immobilization of and conversion to stable SOM as well as through greater mineralization of stabilized SOM C and N (Paul and Juma, 1981; Bird et al., 2001). Furthermore, crop residue management has affected utilization of N fertilizer in rice (Broadbent and Nakashima, 1970; Huang and Broadbent, 1989; Bird et al., 2001). These studies indicate that long-term straw management in lowland rice can affect the size and stability of the soil N supply.

Seasonal winter flooding (WF) of fallow rice fields in the temperate climate of California has been implemented to enhance habitat for migratory waterfowl in the Pacific Flyway of California and has contributed to greater straw decomposition rates (Hill et al., 1999; Bird et al., 2000). Repeated submergence and drying of rice soils has been shown to increase N losses compared with losses in continually submerged soils (Patrick and Wyatt, 1964; Kundu and Ladha, 1999). Total loss of N fertilizer was similar, however, during Years 4 through 6 of a long-term study comparing winter-fallow flooding (vs. unflooded) in temperate rice (Bird et al., 2001). Results from the tropics indicate that longer and almost continuous submergence in rice has decreased the de-

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Abbreviations: GLM, general linear model; HUM, alkali-insoluble humics; IRMS, isotope ratio mass spectrometer; LF, light fraction; MAHA, metal-associated humic acids; MHA, mobile humic acids; MFA, mobile fulvic acids; NF, nonwinter flooded; SMB, soil microbial biomass; SOM, soil organic matter; WF, winter flooded.

gree of humification of SOM fractions, thereby potentially increasing its stability (Olk et al., 1999). Consequently, repeated seasonal WF may alter the rate of N humification and the stability of SOM fractions in temperate rice soils.

The majority of residual N remaining in agricultural soils (20–40%) after cropping has limited availability to subsequent crops (<15%) primarily because of stabilization into resistant fractions of SOM (Kelley and Stevenson, 1995). Recent work in tropical and temperate rice ecosystems has determined that the mobile humic fraction ($\text{Na}_4\text{P}_2\text{O}_7/\text{NaOH}$ extractable) is influenced by agronomic practices and is more dynamic than humics associated with metal-oxides and mineral complexes (Olk et al., 1996; Devêvre and Horwath, 2001). In a laboratory incubation, California rice soils sequestered more N fertilizer in the directly extractable humic fraction than in the metal-associated humic or alkali-insoluble fractions (Devêvre and Horwath, 2001). A better understanding of the sequestration rate and stability of N into the humic and fulvic fractions may indicate the degree to which changes in crop residue management practices alter the size and availability of soil N in rice ecosystems.

A 2-yr, ^{15}N -labeling study was conducted to characterize the turnover of added fertilizer and crop residue N in the LF, MHA, MFA, MAHA, and HUM SOM fractions. Our main objective was to assess the effects of straw incorporation and WF on N sequestration and stability into specific labile (LF, MHA, MFA) and resistant (MAHA and HUM) SOM fractions. A better understanding of the N dynamics of these soil pools is critical to develop straw-management practices that lead to an optimization of long-term N supply in flooded rice soils. We hypothesize that the smaller, more active SOM fractions will account for the greater plant-available soil-N supply observed after three to four seasons of straw incorporation compared with burning. Furthermore, we hypothesize that many years of winter-fallow flooding will result in an accumulation of mobile humic N.

MATERIALS AND METHODS

Field Site

In fall 1993 winter-flood and straw-management treatments were established at a 28-ha field site located on a commercial rice farm in the northern Sacramento Valley, near Maxwell, CA (USA). The soil is classified as fine, smectitic, superactive, thermic, Sodic, Endoaquerts (Willows clay) (Soil Survey Staff, 1998). The field experiment is a split-plot design with four replications. Winter flood management (flooded vs. unflooded) is the main-plot treatment and straw management (incorporated vs. burned) is the split-plot treatment. The four treatments are: (i) burned straw and WF; (ii) burned straw and nonwinter flooded (NF); (iii) incorporated straw and WF; and (iv) incorporated straw and NF. Each of the individual plots measured 0.75 ha. Annual straw management practices in main plots were initiated following grain harvest in October 1993 and are described in Bird et al. (2001).

Nitrogen-15 Fertilizer Study

To examine the N humification and turnover in SOM fractions, we established ^{15}N fertilizer microplots (4 by 3 m) in

each main plot prior to the fourth growing season of the field study. In May 1997, enriched urea fertilizer (9.99% ^{15}N -atom excess) was uniformly applied to the microplots at a rate of 20 kg N ha⁻¹ prior to seeding. The total amount of excess ^{15}N added was 2 kg N ha⁻¹. The cycling of labeled ^{15}N fertilizer applied in 1997 was monitored for 2 yr. At harvest in 1997 and 1998, aboveground straw contained 58.0 and 53.3 kg N ha⁻¹ and had mean ^{15}N atom excess of 0.398 and 0.036%, respectively. Straw, crop, soil, and water management practices in the microplots are described in Bird et al. (2001).

Soil Characteristics

Soil samples (0–15 cm) were collected from the microplots using a core sampler (5-cm diam.) that allowed for bulk density determinations. During 1997 through 1999 three soil cores per microplot were collected on four sampling dates: (i) 23 Apr. 1997—19 d prior to seeding, (ii) 25 Aug. 1997—crop maturity, (iii) 3 May 1998—21 d prior to seeding preplant and (iv) 18 May 1999—9 d prior to seeding preplant and separately fractionated into five SOM fractions. Additionally, soil samples were taken on 4 Nov. 1997 (14 d after incorporation and burning); only LF was determined for this sampling date. Large visible pieces of crop residue (>2 mm) were removed from the soil prior to analyses. Soil was air-dried until constant weight at 40°C and ground using a ball-mill to pass a 250- μm sieve.

Prior to the initiation of the field study in fall 1993, selected physical and chemical soil properties were determined (Bird et al., 2001). Notable soil properties include a high clay content (smectite; 510 g kg⁻¹), neutral pH (6.6), and moderate cation-exchange capacity (42 cmol kg⁻¹). Iron content was 19.9 g kg⁻¹ (DPTA-extractable Fe: 162.7 mg kg⁻¹) and Mn content was 0.57 g kg⁻¹ (DPTA-extractable Mn: 37.7 mg kg⁻¹) (Devêvre and Horwath, 2001). Total soil C and N, SMB C and N, and inorganic N were determined on these soil samples and are presented in Bird et al. (2001). Gravimetric water content was determined by drying soil subsamples at 105°C for 24 h. Soil C and N determinations are expressed on a volumetric basis. All soil determinations are expressed on a dry soil basis.

Soil Organic Matter Fractionation

We fractionated soil samples into five SOM fractions differing in their degree of stability: (i) LF, (ii) MHA, (iii) MFA, (iv) MAHA, and (v) HUM. Physical separation of the LF was accomplished using a density fractionation procedure modified after Strickland and Sollins (1987) and Boone (1994). Two aliquots of 25 mL of NaI solution (1.75 g cm⁻³) were added to 5-g soil subsamples, mixed by hand, and shaken on a reciprocal shaker for 5 min. at a rate of 180 strokes min⁻¹. Samples were centrifuged (3250 \times g, 25°C, 20 min), collected by passing the solution over an ashed (400°C, 4 h), GF/A Whatman filter (Whatman Inc., Trenton, NJ), and washed with deionized water to remove salts. Light fraction samples were dried at 105°C until constant weight (36–48 h).

The heavy fraction was fractionated into four SOM fractions: MHA, MFA (NaOH extractable), MAHA ($\text{Na}_2\text{S}_2\text{O}_8/\text{HCl}/\text{NaOH}$ extractable), and HUM (alkali non-extractable) based on methods described by McGill and Paul (1976) and Devêvre and Horwath (2001). Soil subsamples (20 g) were initially rinsed with 200 mL of 0.1 M HCl to remove salts, carbonates, and LF. Soil subsamples were subsequently extracted with 0.4 M NaOH to yield MHA and MFA. Eight 200-mL aliquots of NaOH were sequentially added under N₂, shaken for 12 h, and centrifuged (16 270 \times g, 10°C, 15 min.).

The supernatant was passed over glass wool to remove any LF not removed in the initial HCl rinse. The MHA was separated from the MFA by precipitation through the addition of concentrated HCl until pH 1.5 and centrifuged ($23\,430 \times g$, 10°C , 15 min). MFA solution was dialyzed to remove Na using 1000 MWCO cellulose-ester membrane (Spectrum Industries, Inc., Rancho Dominguez, CA) in deionized water. Residual soil samples contained large quantities of SOM after the initial NaOH extraction. The remaining SOM was bound in stable aggregates with metal polyvalent cations (Fe and Mn) and clay minerals. The MAHA fraction was extracted after adding 200-mL aliquots of 0.4 M $\text{Na}_2\text{S}_2\text{O}_3/0.1$ M HCl to reduce and remove Fe and Mn oxides. Sequential reductant rinses were performed until the Fe^{+2} concentration in the acid rinse after reduction was <150 mg $\text{Fe}^{+2} \text{L}^{-1}$ in the supernatant. Following reduction and dilute acid rinses, the residual soil was reextracted with 0.4 M NaOH, as described previously, to liberate the MAHA and MAFA fractions. The MAHA and MAFA were separated as previously described. Mobile humic acid, MAHA, and HUM fractions were desalted using 0.1 M HCl and deionized H_2O rinses. The SOM fractions were analyzed for yield and isotopic enrichment (^{15}N) after lyophilizing and ball milling. Soil and SOM extracts were stored at 4°C during and after procedures.

Total C and N in the SOM fractions were determined on a Carlo-Erba C/H/N analyzer (Costech Analytical Technologies, Inc., Valencia, CA) (Nelson and Sommers, 1982). Isotopic enrichment of the ^{15}N in SOM fractions was determined on a Europa Scientific INTEGRA isotope ratio mass spectrometer (IRMS) (PDZ Europa Ltd., Crewe, UK). Natural abundance (^{15}N) of the SOM fractions were determined on fractions separated from soil sampled in April 1997, prior to the addition of the ^{15}N -labeled fertilizer on a Geo 20/20 IRMS (PDZ Europa Ltd., Crewe, UK). The ^{15}N natural abundance of the total soil ($2.2 \pm 0.1\text{‰}$), MHA ($2.8 \pm 0.3\text{‰}$), MAHA ($3.5 \pm 0.1\text{‰}$) and HUM ($3.5 \pm 0.1\text{‰}$) fractions were similar. Nitrogen-15 atom percent excess was calculated for the MHA, MAHA, and HUM fractions by subtracting the measured ^{15}N natural abundance atom percent from the ^{15}N atom percent in each enriched SOM fraction for each individual plot. For the MFA fraction, the ^{15}N atom percent excess values were calculated using ^{15}N natural abundance values of the MHA fraction. For the LF, the measured natural abundance of rice straw (1.91‰) was used to calculate ^{15}N atom percent excess. Since bulk density was not determined on samples in April 1997, yield of SOM fractions from this initial sampling date are not presented.

Statistical Analysis

Main effects of WF and straw management were tested using a general linear model (GLM) designed for the split-plot design. All data are expressed as least squares means with standard errors of indicated treatments. The *F* statistics

and *P* values are indicated in text and tables for all GLM procedures. A significance level of $P < 0.05$ was set a priori as the α -level; and *P* values were specified between 0.05 and 0.20 in tables and text to facilitate data interpretation. The *P* values >0.20 are indicated simply as NS (nonsignificant) in tables. Adjusted Bonferroni *t*-tests were performed among SOM N data to compare ^{15}N enrichment mean values on specific sample dates within the sampling period and among SOM fractions for a specific sampling date. The kinetic parameters of S_1 and S_2 (initial substrate concentration for the labile [S_1] and resistant component [S_2]) and k_1 and k_2 (decay rates for the respective S_1 and S_2 components) were derived for fertilizer recovery in SOM fractions by fitting the recovery of ^{15}N fertilizer in each SOM fraction to an additive, double-exponential model (Eq. [1]).

$$\text{SOM-}^{15}\text{N} (S_t) = S_1 \times e^{(-k_1 t)} + S_2 \times e^{(-k_2 t)} \quad [1]$$

Time is expressed in years. Kinetic parameters were derived for each SOM fraction using the nonlinear procedure in SYSTAT version 7.0 (SYSTAT, 1997). Mean values for kinetic parameters of SOM fraction ($N = 16$) are presented. All statistical tests were performed using SYSTAT version 7.0 (SYSTAT, 1997).

RESULTS

Carbon and Nitrogen Distribution Among Soil Organic Matter Fractions

Soil organic matter fractionation procedures produced five labile and resistant organic fractions. Total recovery ranged from 76.6 to 79.5% of soil C and 72.0 to 77.6% of soil N (Table 1). These data represent the fourth through sixth year of the straw-management field study. The LF was the smallest SOM C and N fraction recovered. The MHA and MFA fractions together represented approximately one-third of the total soil C and N fractionated as SOM (Table 1). Nitrogen content in the MHA fraction was 2.8 (August 1997) to 3.9 (May 1999) times greater than the N content of the MFA. Similarly, the C content in the MHA fraction was 3.3 (August 1997) to 5.8 (May 1999) times greater than the C content of the MFA. After removal of polyvalent cations (i.e., Fe and Mn) and subsequent reextraction with alkali, the MAHA fraction yielded approximately one-quarter of the C and N of the MHA pool. This smaller, stabilized MAHA fraction contained 5.4 to 7.6% of soil N and 6.3 to 7.7% of soil C (Table 1). The remaining HUM fraction was the largest single SOM fraction recovered containing over one-third of the N and C in the soil 0- to 15-cm depth.

Table 1. Percentage of recovery of total soil C and N in SOM fractions in soil (0–15 cm depth). Least-squared means, standard errors ($N = 16$, averaged across treatments).

Soil fraction†	August 1997		May 1998		May 1999	
	C	N	C	N	C	N
	%					
LF	3.7 (0.2)	2.3 (0.1)	3.7 (0.2)	2.4 (0.1)	3.5 (0.2)	1.9 (0.1)
MHA	24.2 (0.7)	24.4 (0.7)	26.4 (0.6)	25.8 (0.6)	25.3 (0.4)	25.8 (0.5)
MFA	7.4 (0.4)	8.9 (0.5)	7.7 (0.3)	7.7 (0.3)	4.4 (0.1)	6.6 (0.3)
MAHA	6.3 (0.3)	6.7 (0.3)	6.3 (0.2)	5.4 (0.2)	7.7 (0.2)	7.6 (0.2)
HUM	35.0 (0.6)	29.7 (0.6)	35.4 (0.8)	31.0 (0.5)	36.9 (0.5)	35.7 (0.6)
Sum SOM fractions	76.6 (1.2)	72.0 (1.5)	79.5 (0.9)	72.3 (1.0)	77.8 (0.6)	77.6 (0.6)

† LF, light fraction; MHA, mobile humic acids; MFA, mobile fulvic acids; MAHA, metal-associated humic acids; HUM, alkali-insoluble humics.

Table 2. Effects of straw management practices on SOM fraction C contents in soil (0–15 cm depth) sampled in August 1997, May 1998 and May 1999. Least squared means, standard errors ($N = 8$, averaged across winter-flooding treatments).

Straw management	SOM fraction C content [†]				
	LF	MHA	MFA	MAHA	HUM
	kg ha ⁻¹				
	August 1997				
Burned	710 (53)	4568 (193)	1532 (113)	1240 (91)	7386 (383)
Incorporated	802 (47)	5314 (132)	1519 (173)	1350 (71)	7016 (292)
<i>P</i> values	0.099	0.007	NS	NS	0.170
	May 1998				
Burned	796 (40)	6205 (215)	1855 (142)	1580 (62)	9345 (342)
Incorporated	1032 (52)	7045 (198)	2110 (159)	1584 (80)	8466 (389)
<i>P</i> values	0.0297	0.018	NS	NS	0.124
	May 1999				
Burned	781 (59)	6195 (233)	1060 (52)	2065 (111)	9707 (312)
Incorporated	1059 (75)	7301 (409)	1222 (56)	1941 (57)	9395 (268)
<i>P</i> values	0.089	0.141	0.080	NS	NS

[†] LF, light fraction; MHA, mobile humic acids; MFA, mobile fulvic acids; MAHA, metal-associated humic acids; HUM, alkali-insoluble humics.

Straw Management Effects on Soil Organic Matter Carbon and Nitrogen

Carbon and N in SOM fractions were not affected by six seasons of winter-fallow flooding compared with NF conditions (data not shown). Consequently, SOM fraction C and N contents are presented as averages across WF treatments (Tables 2 and 3). Carbon and N yields of the MHA fraction were 12 to 16% greater with annual soil incorporation of straw compared with burning straw in August 1997 and May 1998 (Tables 2 and 3). Mobile humic acid C and N showed a similar trend because of straw-management treatment in May 1999 with higher values with straw incorporated compared with straw burned (Tables 2 and 3). At crop maturity (August 1997), LF N was similar between straw treatments and slightly larger in C with straw incorporated versus straw burned (Tables 2 and 3). In November 1997, 14 d after incorporation and burning, LF was greater in incorporated compared with burned treatments in C (1078 ± 45 vs. 811 ± 51 kg C ha⁻¹; $P = 0.023$) and N (56 ± 3 vs. 48 ± 3 kg N ha⁻¹; $P = 0.073$), respectively. Mobile fulvic acid C and N did not show clear treatment effects during the

Table 3. Effect of straw management practices on SOM fraction N contents in soil (0–15 cm depth) sampled in August 1997, May 1998 and May 1999. Least squared means, standard errors ($N = 8$, averaged across winter-flooding treatments). The *P* values >0.20 indicated as nonsignificant (NS).

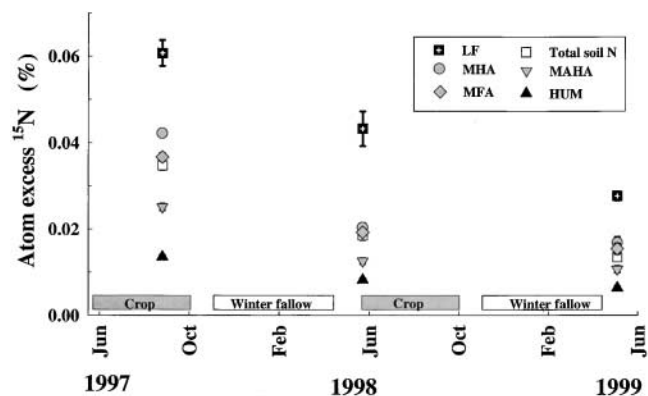
Straw management	SOM fraction N content [†]				
	LF	MHA	MFA	MAHA	HUM
	kg ha ⁻¹				
	August 1997				
Burned	38 (3)	379 (15)	152 (11)	110 (7)	503 (23)
Incorporated	42 (2)	439 (11)	148 (15)	115 (5)	540 (19)
<i>P</i> values	NS	0.006	NS	NS	0.113
	May 1998				
Burned	47 (3)	531 (25)	175 (14)	117 (5)	702 (17)
Incorporated	55 (3)	592 (22)	159 (12)	121 (6)	645 (24)
<i>P</i> values	0.095	0.109	NS	NS	0.057
	May 1999				
Burned	36 (2)	495 (20)	135 (7)	159 (20)	746 (12)
Incorporated	44 (3)	573 (23)	135 (8)	154 (6)	723 (13)
<i>P</i> values	0.099	0.043	NS	NS	NS

[†] LF, light fraction; MHA, mobile humic acids; MFA, mobile fulvic acids; MAHA, metal-associated humic acids; HUM, alkali-insoluble humics.

1997 through 1999 sampling period (Tables 2 and 3). The MAHA and HUM C and N contents showed no effect of straw incorporation (Tables 2 and 3).

Nitrogen-15 Fertilizer Sequestration and Turnover in Soil Organic Matter Fractions

The fate of ¹⁵N-labeled fertilizer (2 kg ¹⁵N ha⁻¹) added prior to planting in May 1997 was followed in SOM fractions over the course of two growing and fallow seasons. The degree of ¹⁵N-fertilizer immobilization into SOM fractions was highest after one season (August 1997) and declined thereafter in all SOM fractions (Fig. 1) as expressed by ¹⁵N atom excess (%). The enrichment of ¹⁵N in the SOM fractions followed the order of LF > MHA > MFA > MAHA > HUM (Fig. 1). The SOM fractions maintained the same relative ¹⁵N enrichments and enrichment values converged over the study period. The LF, MHA, and MFA fractions were significantly more enriched in ¹⁵N fertilizer than total soil N throughout the study period (Fig. 1). In contrast, the MAHA and HUM fractions were significantly less enriched in N fertilizer than total soil N. The MHA pool was significantly more enriched in ¹⁵N than the MFA pool on all three sampling dates ($P < 0.01$).

**Fig. 1.** Nitrogen-15 atom excess values (%) of Soil organic matter fractions, 1997 through 1999 (0–15 cm depth). Least-squares means, standard errors ($N = 16$, average of all treatments). All treatments were flooded during the cropping season while only the winter flooded treatments were flooded during the winter fallow period.

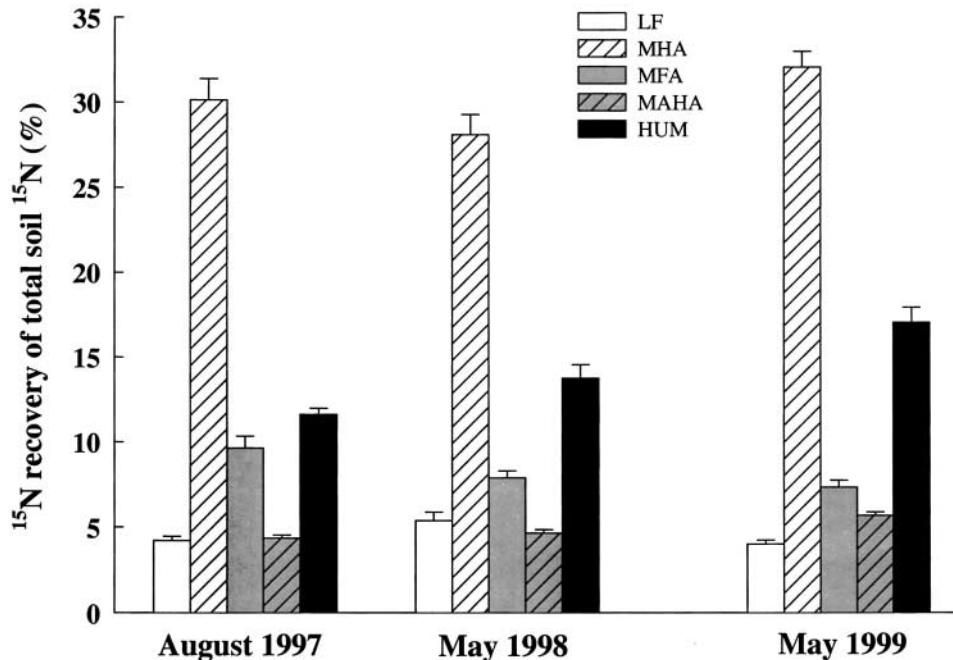


Fig. 2. Nitrogen-15 fertilizer in SOM fractions as a percentage of total ^{15}N recovery as soil N over a 2-yr period (1997–1999) in the 0- to 15-cm soil depth. Least-squares means, standard errors ($N = 16$, averaged across treatments).

The ^{15}N fertilizer content in the five SOM fractions, as a percentage of total soil ^{15}N recovery, was similar in August 1997 ($59.6 \pm 2.3\%$) and in May 1998 ($59.7 \pm 2.3\%$), and rose slightly to $66 \pm 1.6\%$ 2 yr after ^{15}N application in May 1999 (Fig. 2). The proportions of soil-recovered ^{15}N in the LF and MHA fraction were similar after one cropping season (120 d) compared with that recovered 2 yr after ^{15}N application (Fig. 2). The MHA fraction represented $\sim 25\%$ of total soil N (Table 2) but contained approximately one-third of the ^{15}N fertilizer recovered in the soil (Fig. 2). The proportion of soil-immobilized ^{15}N fertilizer recovered as MHA was the highest of all SOM fractions on all sampling dates. Fertilizer ^{15}N recovery in the MFA fraction was approximately one-third of that in the MHA pool throughout the study period (Fig. 2). The proportion of ^{15}N fertilizer recovered in the soil as MFA declined after the first sampling date (August 1997). In contrast, the proportion of soil-recovered ^{15}N fertilizer recovered in the MAHA and HUM fraction increased over the two-year period (Fig. 2).

Straw Management Effects on Nitrogen-15 Fertilizer Dynamics

Effects of straw- and WF-management treatments on the recovery of applied ^{15}N fertilizer in SOM fractions were primarily seen in the MHA and LF pools (Fig. 3 and 4; Table 4). Straw incorporation resulted in significantly greater recovery of applied ^{15}N in the LF in November 1997 and May 1998 compared with burned straw ($P < 0.05$; Fig. 3). In August 1997, LF in incorporated WF soil was more enriched in ^{15}N fertilizer compared with NF soils, resulting in a significant straw by WF interaction at crop maturity in 1997 ($P = 0.027$; Fig. 3). The recovery of ^{15}N fertilizer as MHA was significantly

greater with straw incorporation compared with straw burned in both August 1997 and May 1998 (Fig. 4 and Table 4). Two years after ^{15}N application, a similar trend was apparent with more ^{15}N recovered as MHA in incorporated plots versus burned ($P = 0.151$; Fig. 4).

In contrast to the MHA and LF fractions, straw incorporation had no significant effect on the recovery of applied ^{15}N fertilizer in MFA or MAHA fractions (Fig. 4 and Table 4). While ^{15}N recovery as MFA and MAHA was not significantly different between straw incorporated and burned ($P < 0.05$) in May 1998, the MAHA fraction had significantly greater ^{15}N atom excess with incorporated straw ($0.019 \pm 0.001\%$) compared with burned straw ($0.015 \pm 0.001\%$; $P = 0.012$). Similarly, ^{15}N atom excess in the MFA fraction was slightly greater with incorporated straw ($0.020 \pm 0.001\%$) compared with burned straw ($0.018 \pm 0.002\%$; $P = 0.130$) in May 1998. The HUM-N fraction was not affected by straw incorporation or winter-fallow flooding (Table 4).

To estimate the initial ^{15}N concentrations of the labile and stable components (S_1 and S_2) and their turnover rates (k_1 and k_2) for each SOM fraction, the amounts of fertilizer- ^{15}N over the 2-yr period (1997–1999) were fit to an additive, double-exponential decay function (Eq. 1; Table 5). A double exponential model was used instead of a single-exponential since it provided a better fit to the data illustrated in Fig. 4. Because of the relatively high variability of the data by sample date compared with the rate of change, kinetic parameters were estimated using all treatments ($N = 16$) for each SOM fraction. All SOM fractions had similar half-lives in the labile ^{15}N pool ($S_1 = 0.36$ – 0.90 yr). The half-lives (k_2) of the stable component (S_2) of immobilized ^{15}N fertilizer in the MHA and MFA fractions were similar (8.9 and 6.9 yr, respectively). The MFA had a much greater

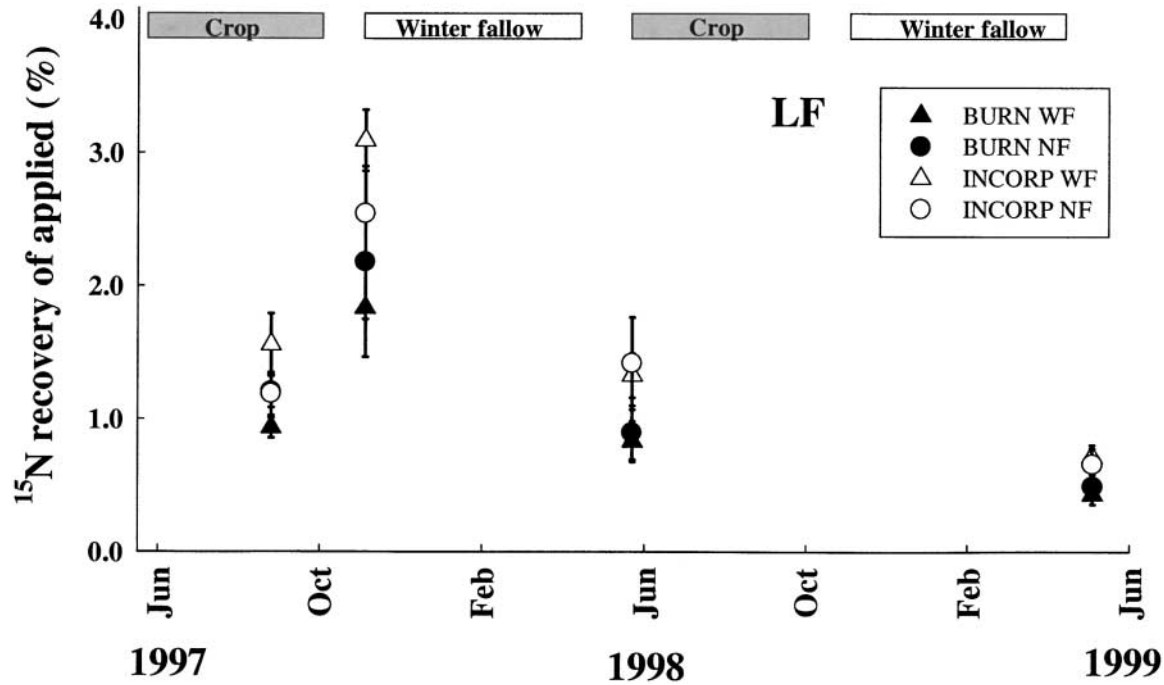


Fig. 3. Percentage of recovery of applied ¹⁵N fertilizer in LF over a 2-yr period (1997–1999) in the 0- to 15-cm soil depth when straw was incorporated or burned and the fields were winter-flooded (WF) or nonwinter flooded (NF). Least-squares means, standard errors (*N* = 4).

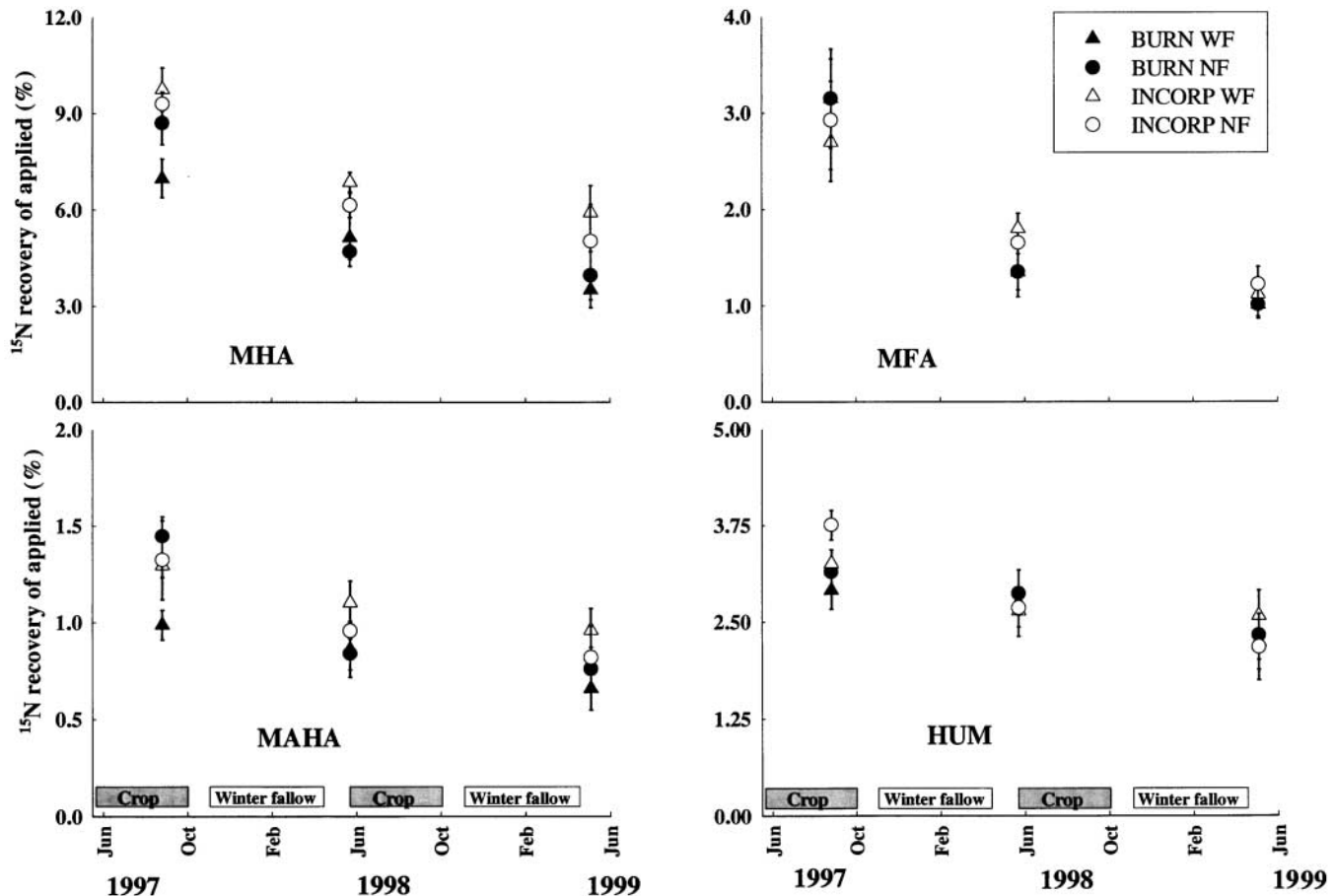


Fig. 4. Percentage of recovery of applied ¹⁵N fertilizer in MHA, MFA, MAHA, and HUM fractions over a 2-yr period (1997–1999) in the 0- to 15-cm soil depth when straw was incorporated or burned and the fields were winter-flooded (WF) or nonwinter flooded (NF). Least-squares means, standard errors (*N* = 4).

Table 4. Significance of winter flooding and straw incorporation on recovery of applied ¹⁵N fertilizer in SOM fractions, 1997 through 1999 (0–15 cm depth). Two kilograms of ¹⁵N per hectare added in May 1997. The *P* values >0.20 indicated as nonsignificant (NS).

Effect	<i>F</i> statistic/ <i>P</i> value	Sample date					
		Aug-97	May-98	May-99	Aug-97	May-98	May-99
		MHA†			MFA‡		
Winter flood (WF)	<i>F</i>	4.0	3.1	0.7	1.4	1.6	6.4
	<i>P</i>	0.140	0.177	NS	NS	NS	0.086
Straw (S)	<i>F</i>	6.0	6.9	2.7	1.0	2.2	1.9
	<i>P</i>	0.049	0.040	0.151	NS	0.191	NS
WF × S	<i>F</i>	2.6	<0.1	<0.1	0.2	<0.1	0.1
	<i>P</i>	0.157	NS	NS	NS	NS	NS
		MAHA§			HUM¶		
WF	<i>F</i>	4.0	0.5	0.1	1.5	0.2	3.0
	<i>P</i>	0.140	NS	NS	NS	NS	0.185
S	<i>F</i>	0.2	4.1	1.1	1.3	0.2	0.1
	<i>P</i>	NS	0.088	NS	NS	NS	NS
WF × S	<i>F</i>	2.2	0.5	0.5	<0.1	<0.1	0.3
	<i>P</i>	0.188	NS	NS	NS	NS	NS

† Mobile humic acid.
 ‡ Mobile fulvic acid.
 § Metal-associated humic acid.
 ¶ Alkali-insoluble humics.

proportion of immobilized N in the labile component (*S*₁) compared with the stable component (*S*₂) than that observed in the MHA, which had slightly more in the labile component (Table 5). In contrast, the proportions of ¹⁵N in the labile and stable component of the MAHA fraction were similar. The HUM fractions showed a greater proportion of ¹⁵N in the stable fraction compared with the labile component. Turnover of the stable ¹⁵N component (*k*₂) was fastest in the MHA and MFA fractions compared with intermediately resistant MAHA and highly resistant HUM fraction (Table 5).

DISCUSSION

Active Soil Organic Matter Fractions

Four years of soil incorporation of crop residues increased LF and MHA C and N compared with straw burned. Furthermore, more N fertilizer was immobilized in the MHA and LF pools in straw incorporated treatments compared with burned. As total soil C and N contents were similar among treatments after 6 yr of treatments (Bird et al., 2001), the more labile LF and MHA represented the active N pools most important in both sequestering and resupplying recently added N from crop residues and fertilizer. The significant role of the LF and MHA N pools in cycling recently added N are consistent with our previously reported results of a sustained increase in the SMB C and N pools (Bird et

al., 2001) and greater plant-available soil N after 3 to 4 yr of straw incorporation compared with straw burning (Eagle et al., 2000).

We showed a rapid enrichment of the LF in all treatments prior to crop residue incorporation (August 1997) as a result of root turnover, abiotic reactions and biological immobilization by the SMB. The microbial component of the LF is well illustrated by recent work showing the N sink characteristics of the LF in a long-term tillage study (Whalen et al., 2000). The LF is thought to be composed of partially degraded plant residues coated with varying degrees of C and N compounds including polysaccharides, proteins, humic and fulvic acids, and a substantial microbial component (Paul and Clark, 1996). Approximately 2 wk after straw incorporation and burning (November 1997), LF C, N, and ¹⁵N enrichment rose substantially. This rapid enrichment in the LF in November 1997 was greater than expected as much of the residual N and C was present as large particles (>2 mm) and was removed prior to SOM fractionation. Root turnover, additions of straw chaff from harvest operations, and LF-associated SMB immobilization may have been responsible for the ¹⁵N enrichment in the LF in November 1997. While the LF represented a small amount of the total soil C, N, and ¹⁵N recovered in the surface soil (0–15 cm), its high activity was apparent in the effect of straw management on LF yield, ¹⁵N enrichment, and the rapid turnover of immobilized ¹⁵N fertilizer.

Table 5. Initial SOM ¹⁵N content (*S*₁ and *S*₂), decay rates (*k*₁ and *k*₂), and half-lives of SOM-¹⁵N over the 2-yr period calculated using the additive, double exponential equation: SOM - ¹⁵N = *S*₁ × e^(-*k*₁*t*) + *S*₂ × e^(-*k*₂*t*). Functions fit using all treatments (*N* = 16).

SOM N fraction†	Regression parameters					
	<i>S</i> ₁	<i>k</i> ₁	<i>t</i> _{1/2} (<i>S</i> ₁)	<i>S</i> ₂	<i>k</i> ₂	<i>t</i> _{1/2} (<i>S</i> ₂)
	kg ¹⁵ N ha ⁻¹	yr ⁻¹	yr	kg ¹⁵ N ha ⁻¹	yr ⁻¹	yr
MHA	0.130	1.90	0.36	0.105	0.078	8.9
MFA	0.053	1.37	0.51	0.021	0.100	6.9
MAHA	0.015	1.35	0.51	0.015	0.013	53.3
HUM	0.033	0.77	0.90	0.040	0.005	138.6

† LF, light fraction; MHA, mobile humic acids; MFA, mobile fulvic acids; MAHA, metal-associated humic acids; HUM, alkali-insoluble humics.

A key process by which N is immobilized in soils involves microbial uptake and processing of added N and the subsequent conversion into more stable humic and fulvic substances. We hypothesize that greater amounts of MHA C, N, and ^{15}N because of annual straw incorporation were due primarily to the microbiological pathway, as opposed to the abiotic reactions of NH_4^+ and SOM and clay minerals. Overall, N immobilization and stabilization of N added as NH_4^+ involve a number of simultaneous biotic and abiotic processes (Stevenson, 1994). In rice soils, soil texture, mineralogy, cation composition and concentration, and SOM content have been suggested as factors that influence the degree of abiotic immobilization in soils (Burge and Broadbent, 1961; Norman and Gilmour, 1987; Tsutsuki and Ponnamperna, 1987). Stabilization of C and N via the microbiological pathway may be the primary source of SOM N under conditions where the SMB is active and N limited (Voroney et al., 1989; Ladd et al., 1995; Knicker et al., 1997). The SMB immobilized significant amounts ^{15}N fertilizer both at the end of the first cropping season (6.7% of applied) in August 1997 and 2 yr after ^{15}N application (2.4% of applied) and was greater with straw incorporation compared with burned (Bird et al., 2001). Additionally, SMB C and N were consistently greater in straw incorporated compared with burned treatments (Bird et al., 2001). Furthermore, N fertilizer sequestration in the MHA fraction was greater when straw was incorporated compared with burned prior to the incorporation of the ^{15}N -labeled straw (120 d, August 1997). These results suggest that N fertilizer and root exudates, immobilized by the consistently larger SMB with soil incorporation of straw (Bird et al., 2001), was a major contributing factor in the conversion of N fertilizer into MHA-N over the first cropping season of the ^{15}N study.

The importance of the MHA fraction in immobilization of N fertilizer in our field study is consistent with results reported from a 160-d incubation with ^{15}N -labeled fertilizer using the same soil (Devèvre and Horwath, 2001). Devèvre and Horwath (2001) reported that directly extractable humics (equivalent to the combined MHA and MFA fractions) had the highest percentage (up to 50%) of the N fertilizer recovered after 160 d. Their soil incubation results may have overestimated N fertilizer recovery as organic N because of very low N losses and the absence of plant uptake and root turnover. They attributed their results to increased microbial turnover in the absence of available C. Our results indicate a close relationship between C and N in the increased LF and MHA yields because of straw incorporation. Similar relative sequestration rates of C and N into humic substances have been reported by Olk et al. (1996), who reported parallel trends of greater C and N after 20+ yr of double and triple rice crops compared with single crop rotations in the tropics. Together, these laboratory and field studies clearly indicate that the MHA and MFA fractions are the most active site of C and N stabilization in temperate rice soils.

The MHA, in contrast to the MFA fraction, was the most affected SOM fraction because of straw incorporation as illustrated by greater C, N, and ^{15}N fertilizer contents compared with straw burned. The turnover

rates of ^{15}N in the MHA and MFA fractions were generally similar over the 2 yr as described by double-exponential equation fit. The main difference in ^{15}N turnover of the two mobile SOM fractions was apparent in the much higher proportion of ^{15}N in the labile component (S_1) of the MFA fraction than in the resistant (S_2) component compared with the distribution of ^{15}N between components in the MHA fraction. Consequently, the similar decay constants (k_1 and k_2) and relative half-lives of the MFA and MHA fractions suggest that the turnover of MFA- ^{15}N was more rapid than the MHA- ^{15}N . The ^{15}N enrichment MFA pool may have been higher than that of the MHA fraction prior to 120 d. This possibility may explain, in part, the higher recoveries of applied ^{15}N in the $\text{Na}_4\text{P}_2\text{O}_7$ -extractable MFA compared with MHA reported by McGill and Paul (1976). Alternatively, this result may be because of the SOM extraction method employed. Greater recovery of N fertilizer was reported in labile fulvics than humics using $\text{Na}_4\text{P}_2\text{O}_7$, while similar recovery of added ^{15}N was found in the KOH extractable fulvic and humic fractions after a 7-d laboratory incubation (He et al., 1988). Related problems associated with SOM extraction procedures have been reported (Jenkinson, 1971; Stevenson, 1994; Honda, 1996). Nevertheless, SOM-fractionation methodologies that yield labile humic and fulvic substances have provided insight into humification dynamics in intensified rice systems in the tropics (Olk et al., 1996; Olk et al., 1999) and form the foundation of our understanding of the stabilization of N fertilizer and the dynamics of SOM in agricultural soils (Kelly and Stevenson, 1995).

The amount of ^{15}N fertilizer recovered the MHA fraction is similar in size to that of the SMB, however, the turnover rate (half-life) of immobilized ^{15}N in the SMB (0.65-yr half-life—single exponential decay model; Bird et al., 2001) was significantly faster than that of the overall decay rate of the MHA or MFA fractions over the same time period. This result indicates that while some SMB ^{15}N may have been extracted as MHA, their significant differences in ^{15}N turnover suggest minimal overlap.

Winter flooding had little effect on LF, MHA, or MFA fraction yields or on N fertilizer sequestration in both burned and incorporated treatments. A current concern with intensified rice cultivation in the tropics (2–3 crops per year with almost continuous flooding) has been the accumulation of resistant organic N in humic fractions (Cassman et al., 1995; Olk et al., 1996). Greater organic N stabilization is hypothesized to result from condensation reactions involving low molecular weight N compounds and free phenols that accumulate under continuous submergence (Olk et al., 1999). In temperate rice areas, such as California, single crop sequences with winter-fallow flooding have two aerated periods in the fall and spring (each 60 to 75 d). These two aerated periods may have allowed for the degradation of any apparent accumulation of abiotically immobilized N during the winter-flooded fallow period. Additionally, we have reported greater plant-available N, both prior to and during the growing season after 4 to 6 seasons of WF compared with NF conditions (Bird et al., 2001).

Chemically and Physically Protected Soil Organic Matter Fractions

The importance of the most stable, or so-called inert SOM fraction, with estimated turnover rates in thousands of years, has been reconsidered in modeling efforts (Falloon et al., 2000). The MAHA fraction, extracted with 0.4 M NaOH after removal of metal oxides, has been described as older and more humified than the directly extractable MHA and MFA fractions (McGill and Paul, 1976; Olk et al., 1996; Devêvre and Horwath, 2001). Our results showed substantially lower ^{15}N fertilizer enrichment and no significant effect of straw management practices on the C and N content of the MAHA fraction. While straw incorporation showed a slightly greater amount of MAHA- ^{15}N than with burning in May 1998, this trend was not present prior to or after this sampling date. The resistance of the immobilized fertilizer-N in the MAHA fraction was evident in the slower turnover rate (k_2) of the stable component (S_2) compared with turnover rate (k_2) of the MHA and MFA fractions. Similarly, low ^{15}N enrichments were reported for the metal-associated humic and fulvic fraction (i.e., the combined MAHA and MFA fractions) from a 160-d soil incubation study (Devêvre and Horwath, 2001). The relative proportion of ^{15}N recovered in the soil as MAHA- ^{15}N rose over time, indicating either the transformation of MHA- or MFA- ^{15}N to MAHA or the relatively high stability of the MAHA compared with MHA and MFA. Utilization of a second base extraction after removal of sequioxides liberated MAHA fractions both greater and smaller than the respective MHA fractions depending on the soil characteristics (McGill and Paul, 1976). Regardless of soil type, McGill and Paul (1976) found approximately one-half the enrichment of added N fertilizer in the MAHA (nonmobile) fraction compared with the MHA and MFA fractions. Olk et al. (1996) defined a calcium-humate fraction as humics extracted after initial base extraction and CaCl_2 addition of tropical rice soils. The calcium humate was found to be older and more humified than MHA, but represented a similar size of the total C and N pools (Olk et al., 1996). Most studies of SOM dynamics usually stop extractions of humic substances after the initial base extraction. Researchers often consider the remaining nonextractable portion as a single pool, even when this fraction represents more than half of the soil C and N. Our approach, by further separating the metal-bound humic substances (MAHA) from that of the mineral-bound (HUM) provides a greater degree of resolution in describing N immobilization and turnover of organic N stabilized by metal oxides.

The single largest C and N pool fractionated was the HUM fraction. While most SOM fractionation methodologies retain over half of the soil C and N as humin (Stevenson, 1994; Kelley and Stevenson, 1995), our study found approximately one-third of the soil C and N as HUM. The relatively low yield in this pool is likely because of our two-step extraction and our use of 0.4 M NaOH, which liberates more C and N as humics

compared with other, milder extractants. While the HUM pool was slightly larger than the MHA pool, the enrichment of ^{15}N fertilizer in this mineral-bound pool was much lower than all other SOM fractions over the 2-yr study. Further, the turnover of sequestered ^{15}N in the HUM pool was substantially slower than the MHA and MAHA fractions. The double exponential decay equation fit to the ^{15}N recovery in the HUM fraction resulted in the only SOM fraction with less labeled N in the labile component (S_1) than in the resistant component (S_2). Furthermore, the resistant component (S_2) of the HUM had a half-life three times larger than the MAHA pool and >10 times larger than the MFA and MHA fractions. These results are consistent with other work showing that the HUM or humin fraction is the most refractory and stable portion of soil C and N (Stevenson, 1994). Our findings of ^{15}N immobilization in the HUM fraction were lower than those reported using similar extraction methodologies in laboratory incubations that have been as high as 23% (He et al., 1988 [7 d]; Devêvre and Horwath, 2001 [160 d]). We found a peak of <4% of ^{15}N fertilizer as HUM after 120 d. This proportion of sequestered N fertilizer was 17% of that recovered in the soil after 2 yr (average of treatments), which falls within the range of 13 to 18% after two cropping sequences reported by McGill and Paul (1976). A substantial portion of sequestered N fertilizer recovered in the HUM fraction in this study may have entered as NH_4^+ fixation to clay minerals, as this fraction was not separated from clay minerals in our study.

The 2:1 type smectite clays of this soil have been shown to fix large amounts of added ammonium fertilizers. This fixed N was more available than fixed-N with vermiculite- and illite-dominated clay soils (Alison et al., 1953; Norman and Gilmour, 1987). Devêvre and Horwath (2001), using a H_2O_2 oxidation procedure, estimated from 1 to 6% of the fertilizer-derived HUM- ^{15}N (referred to as nonalkali extractable humics) was the result of clay fixation. Additionally, our extraction procedures may have resulted in contamination of the HUM fraction with SMB and particulate SOM. The degree of this potential contamination was likely low given the distinctly lower immobilization rate and slower turnover rate of ^{15}N fertilizer in the HUM as compared with the MHA, MFA, and MAHA fractions.

Our SOM-fractionation methodology was intended to isolate operationally defined fractions that could be meaningfully related to organic soil pools in situ that differed in their N fertilizer dynamics. As evident by the recovery of ~75% of total C and N in the five SOM fractions, our fractionation methodology did not isolate all organic C and N compounds. Not included in the five SOM fractions are 0.1 M HCl soluble and low molecular weight C and N compounds (e.g., polysaccharides, amino sugars, and proteins) that were either discarded with the initial 0.1 M HCl rinse or passed through the 1000 D dialysis tubing used for the isolation of MFA. A potentially more substantial loss of organic C and N occurred during the reduction and removal of Fe and Mn to liberate the MAHA fraction. The total amount of C and N left in the residual soil after the removal of

the LF, MHA, and MFA fractions contained on average 9% more soil C and 12% more soil N than sum of the MAHA and HUM fractions (data not shown; Bird, 2001). This 9 to 12% loss includes the MAFA C and N fraction that was not quantified in this study. Loss of soil C and N in the dithionate-HCl rinses during the removal of Fe and Mn oxides has been reported to be in the range of 3 to 5% of soil N and 7 to 9% of added ^{15}N fertilizer 2 yr after ^{15}N addition (McGill and Paul, 1976). Therefore, if our relative losses of ^{15}N to total soil N lost during the metal oxide removal were similar to that reported by McGill and Paul (1976), the N discarded in the dithionate-HCl rinses might have resulted in a greater loss of ^{15}N compared with soil N. Consequently, we may have underestimated the ^{15}N enrichment of the MAHA and HUM fractions. Without examining the characteristics of the ^{15}N lost in these fractionation steps it is difficult to know whether the ^{15}N lost was chemically similar to MAHA. While not complete, the SOM fractionation methodology employed in this study resulted in the quantitative description of the majority of soil organic N into components whose differing sequestration and turnover dynamics could be related to both the short-term effects of crop management practices and the protective abilities of metal-oxides and minerals.

The proportions of soil C and N recovered among the SOM fractions was generally similar among sample dates showing reproducibility of the extraction procedure. Two exceptions were observed with the May 1999 sampling date. The percent of soil C and N recovered as MFA was lower in May 1999 than in the two previous sampling dates (Table 1). The higher C and N contents in the MFA fraction in May 1998 compared with May 1999 may be because of a shorter period of aeration in April through May 1998 because of an unusually wet winter fallow period (El Niño 1997–1998). The longer period of aeration after the winter fallow in May 1999 may have allowed for greater microbial degradation of MFA C and N than in 1999. Additionally, the proportion of soil C and N recovered in the MAHA fraction was higher in May 1999 than in 1997 or 1998. This slightly higher recovery of soil C and N as MAHA was likely because of methodological variability encountered during the repeated reduction steps. While generally representing similar proportions of the total soil C and N pools, the content of SOM fractions (kg ha^{-1} to 15-cm soil depth) differed by date primarily due the wide fluctuations in soil bulk density at different soil moisture contents. Soil bulk density in this clay-rich soil was lowest during the growing season (0.73 g cm^{-3}) in August 1997 and highest prior to planting in May 1998 (0.95 g cm^{-3}) and May 1999 (1.01 g cm^{-3}) (Bird, 2001). As the soil bulk density was also affected by straw management practices (Bird, 2001) we presented C and N SOM fraction contents on a volumetric basis.

CONCLUSIONS

Our results show that annual WF did not affect the rates of C, N, or N-fertilizer immobilization or its turn-

over rate in either straw burned or incorporated treatments after six seasons. Straw incorporation resulted in a greater C and N as MHA and LF after four seasons compared with burning. Immobilization of N fertilizer was greatest in the MHA fraction. While the effects of straw-management treatments were restricted to the more active MHA and LF, a significant amount of ^{15}N fertilizer was incorporated into the MFA, MAHA, and HUM fractions. The SOM fractions displayed a slow decline of ^{15}N stabilized in the resistant component of MHA and MFA (6–9 yr half-life), MAHA (53-yr half-life), and HUM (135-yr half-life). The enhanced stability of the N fertilizer sequestered in the metal-bound MAHA and the mineral-bound HUM fraction was evident after 2 yr. These results are consistent with the greater plant-available soil N supply and larger SMB C, N, and immobilized ^{15}N fertilizer found after 3 to 4 yr of soil incorporated of straw compared with straw burned. Repeated straw incorporation has built an active soil N reservoir suggesting the potential for reduced N fertilizer needed for optimum rice yields after 4 yr of straw incorporation compared with burned. Our results show the value in examining specific humic fractions in interpreting the availability of fertilizer and crop residue N in relation to changes in residue management and short- and long-term soil fertility.

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