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Stabilization of ^{13}C-Carbon and Immobilization of ^{15}N-Nitrogen from Rice Straw in Humic Fractions

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ABSTRACT

The transition from open-field burning of straw residues to alternative residue management practices may affect soil C sequestration potential and the supply of nutrients to crops. A field study of dual-labeled (^{13}C and ^{15}N) rice (*Oryza sativa* L.) residues examined the effects of winter-fallow flooding (vs. nonflooded) and straw residue incorporation (vs. untilled, open-field burned residue) on straw C and N dynamics in soil organic matter (SOM) fractions. We examined the fate of C and N in the straw, crown, and root system in the incorporated treatments and the uncombusted stubble, crown, and roots in burned treatments during 1 yr. During the winter fallow, straw residue incorporation reduced residue ^{15}N loss but increased residue ^{13}C loss compared with burning. Straw ^{13}C loss after 1 yr was unaffected by either winter flooding or straw management (77.1% of applied). Slightly more straw ^{15}N was lost of that applied in burned (65.5 ± 3.5%) compared with incorporated (52.0 ± 3.8%) during 1 yr. A greater proportion of soil-recovered ^{13}C remained as nonalkali extractable humics (humin) in burned (62.0%) compared with incorporated (40.8%). In contrast, incorporated treatments had a larger proportion of ^{15}N remaining as mobile humic acid (MHA) than burned (42.4 vs. 37.7%). Straw incorporation increased the relative retention of straw ^{15}N to ^{13}C compared with burning, indicating that straw ^{15}N additions with incorporation may increase soil organic N reserves at an even greater rate than the larger straw additions might predict. These results show that straw incorporation results in markedly different straw C and N sequestration pathways compared with untilled, open-field burned residues.

CROP RESIDUE MANAGEMENT affects the biological and chemical processes that govern the conversion of C and N to SOM and the residual availability of N to succeeding crops. Recent changes in rice straw management practices in California from open-field burning to straw incorporation with winter-fallow flooding to enhance straw decomposition have prompted a need to better understand the effects of straw management practices on the decomposition and humification of crop residue C and N. In California, rice is especially dependant on soil N for meeting crop demand (50–80% of N assimilated in fertilized fields) (Broadbent, 1979; Mikkelsen, 1987). Consequently, an enhanced knowledge of the effects of long-term soil incorporation of straw and winter flooding on crop residue C and N cycling would enable improved utilization of fertilizer and crop residue N.

Previously, we reported a sustained increase in soil microbial biomass (SMB) C, N, and labile SOM pools after four seasons of straw incorporation compared with straw burned (Bird et al., 2001; 2002). Recent work in tropical and temperate rice ecosystems has determined that the operationally-defined *mobile* humic fraction ($Na_4P_2O_7$ or 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). A better understanding of the C and N humification rates and stability of C and N in humic and fulvic acid fractions may indicate the degree to which changes in crop residue management practices might alter the C sequestration potential and the availability of soil organic N reserves in rice systems. Although numerous field studies have reported the fate of added ^{14}C-, ^{13}C-, and/or ^{15}N-labeled crop residues in situ, most investigations were limited to the measurement of N in the plant and total recovery of C and/or N in the soil (e.g., Jenkinson, 1971; Pal and Broadbent, 1975; Sørensen, 1987). Few field investigations have examined directly the pathways of crop residue C and N immobilization into the SMB, stabilization as SOM, or the fate of C and N concurrently (Stott et al., 1983; Voroney et al., 1989). Consequently, the regulation of C and N humification and turnover processes remain inadequately understood, especially in rice soils that are continuously flooded or use winter flooding.

The close relationship between crop residue C and N turnover in soil is influenced primarily by the labile C supply and the stability of the products formed by a growing microbial community (McGill et al., 1975). While it is well known that the turnover of crop residue C and N into mineral forms (CO_2 and NH_4^+/NO_3^-) or stabilization into humic substances is determined in part by the interplay between climatic factors and substrate biochemistry (Stevenson, 1994), there has been limited research on the dynamics of C relative to that of N in sequestration processes that form stable SOM fractions (Ladd et al., 1981; Voroney et al., 1989).

The traditional practice of burning eliminates 70 to 80% of the C and N held in the straw, crown and roots (Hill et al., 1999). Most of the straw C and N left after burning remains in the form of uncombusted root, crown, and stubble. This investigation focused on the dynamics of uncombusted and biologically active straw remaining after burning compared with that of chopped, incorporated straw to further elucidate the processes important to the stabilization and turnover of SOM. A field study of dual-labeled (^{13}C and ^{15}N) crop residues

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Abbreviations: GLM, general linear model; IRGA, infrared gas analyzer; IRMS, isotope ratio mass spectrometer; LF, light fraction; MFA, mobile fulvic acids; MHA, mobile humic acids; NF, nonwinter flooded; PVC, polyvinyl chloride; SMB, soil microbial biomass; SOM, soil organic matter; WF, winter flooded; WHC, water-holding capacity.

was initiated in the sixth year of a long-term rice straw management experiment. Our main objective was to assess the effects of straw incorporation and winter flooding on crop residue decomposition, microbial C and N utilization, stabilization of added crop residue C and N in SOM, and subsequent plant-N availability. These biological properties are critical to the development of straw management practices that optimize C sequestration and the long-term N supply in flooded rice soils.

MATERIALS AND METHODS

Field Site and Soil

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 (39°20′00″ N, 122°08′00″ W). The soil is classified as a fine, smectitic, superactive, thermic Sodic Endoaquerts (Willows clay) (Soil Survey Staff, 1998). The field experiment was laid out as a split-plot design with four replications. Winter-flood management (flooded vs. nonflooded) was the main-plot treatment and straw management (incorporated vs. burned) was the split-plot treatment. The four main plot treatments were: (i) burned straw and winter flooded (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. Before the initiation of the experiment in 1993, rice was cropped annually for ≈50 yr and managed with open-field burning. In October 1993, straw management practices for the field plots were implemented following grain harvest as described in Bird et al. (2001). Winter-flooded plots were flooded annually to 100- to 150-mm water depth during the fallow period (November–March). Nonwinter-flooded plots received ≈55 to 88 mm of precipitation per month during the fallow winter months (Fig. 1). Rarely would this result in >10 mm standing water for more than 2 to 3 d at a time on nonflooded fallow fields.

Plant Residue Labeling

Rice was grown to maturity and labeled uniformly with ^{13}C and ^{15}N under controlled greenhouse conditions. A soil-free medium (washed fine sand) was used to provide support and to maximize ^{15}N-uptake. Plants were labeled with $^{13}CO_2$ and $^{15}NH_4^+$ and were grown in a half-strength Hoagland solution with two times the concentration of Fe and Zn (Hoagland and Arnon, 1950). Fertilizer ^{15}N as $NH_4(SO_4)_2$ was added periodically (every 2 to 3 wk) at 9.99% atom excess. M-103, a medium-grain, short-season rice cultivar adapted to the Sacramento Valley (California Cooperative Rice Research Foundation, Biggs, CA) was grown in 300-L basins at the University of California, Davis. Labeling was accomplished in a climate-controlled, plexiglass growth chamber (Fig. 2) modified from Horwath et al. (1994). Temperature was maintained using a water-cooled heat exchanger (Horwath et al., 1994). Carbon dioxide concentrations were monitored with a LI-6200 infrared gas analyzer (IRGA) (Li-Cor, Lincoln, NE). A $^{13}CO_2$ generator was connected via a diaphragm pump (GAST, Benton Harbor, MI) to circulate $^{13}CO_2$ into the chamber. The $^{13}CO_2$ generator was a vessel containing concentrated H_2SO_4 attached to a reservoir containing 1 *M* $NaHCO_3$ solution (9.99% atom excess ^{13}C). A computer and data acquisition manager (21X Micrologger, Campbell Scientific, Inc., Logan, UT) controlled temperature and CO_2 concentration level with solenoid actuated valves (Horwath et al., 1994). Rice was exposed to one photoperiod of enriched $^{13}CO_2$ seven times during the course of its development (i.e., every 10–14 d after plant emergence from water). The labeling chamber remained over the basins for one photo period after labeling, and unlabeled $^{12}CO_2$ was maintained at ambient levels to facilitate the reassimilation of night-respired ^{13}C. Each plant received ≈13 mg ^{13}C during the labeling procedures for a final ^{13}C enrichment of 1.75% atom excess per plant. The frequent labeling and reassimilation produced plants with relatively uniform ^{13}C distribution. At maturity, the rice was dried at 25°C. Straw was uniformly enriched in ^{15}N (9.9% atom excess). Rice roots and crowns were slightly depleted in ^{13}C (443 $^0/_{00}$) compared with shoots (777 $^0/_{00}$).

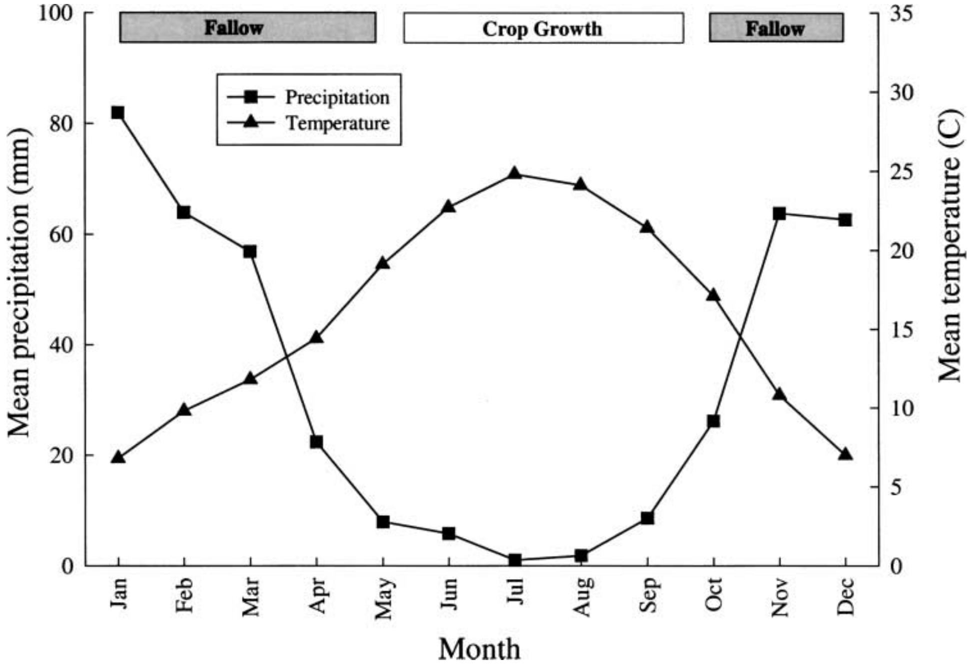

Fig. 1. Mean monthly precipitation and temperature (1961–1990) located in Colusa County, CA. Values summarized by the U.S. Western Regional Climate Center, Reno, NV.

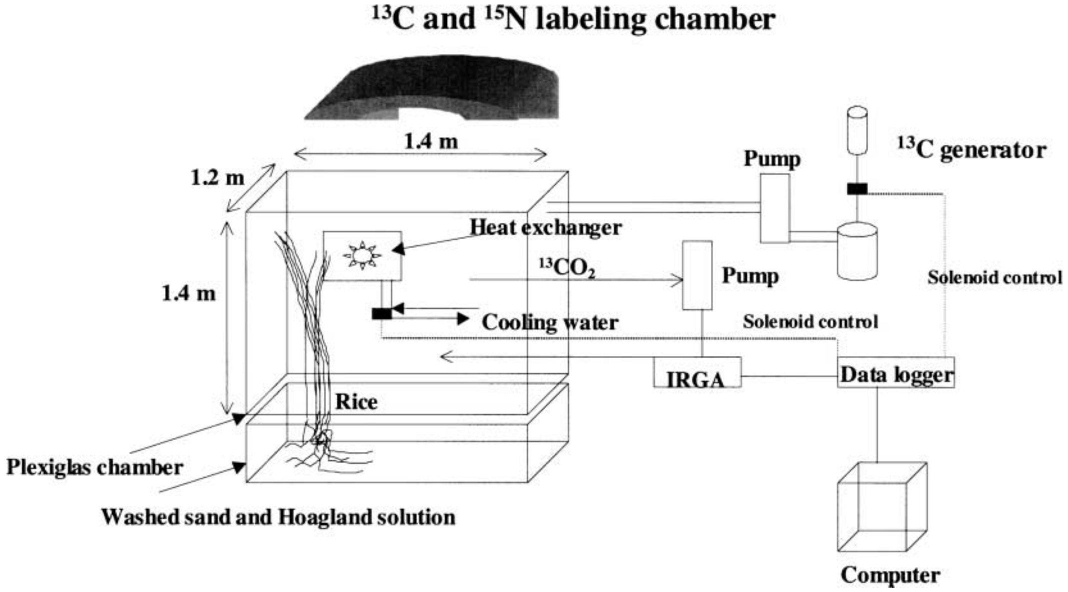

Fig. 2. Design of stable isotope ^{13}C- and ^{15}N-labeling growth chamber. Two square meters of rice (cultivar M-103) was produced in greenhouse facilities at the University of California, Davis.

^{13}Carbon- and ^{15}Nitrogen-Straw Field Study

Five successive rice crops were grown and five seasons of straw management treatments had been applied in the field study before the initiation of the labeled-straw experiment. Before the installation of the microcosms in September 1998, rice plants were removed from the soil in areas chosen for microplot installation. Microcosms [25-cm-diam. polyvinyl chloride (PVC), 20-cm height] were inserted into soil to 15 cm with a 5-cm rim above the soil surface in October 1998. In November 1998, rice straw residue, enriched in ^{13}C and ^{15}N, was added to the microcosms after field burning and straw incorporation and before winter flooding. Duplicate microcosms were installed in all treatments. One microcosm was excavated at the end of the winter-fallow period (161 d) in April 1999 and the second duplicate microcosm at crop maturity (312 d) in September 1999 immediately after soil was sampled.

The rice residue additions simulated the straw remaining after fall field treatments. For the incorporated treatments, the entire labeled plant residue (roots and straw without the panicle) was chopped to 2 to 3 cm and mixed into the soil to a 10-cm soil depth. In the burned treatment, only rice residue from roots, crowns, and the lower 5-cm of stubble (including the crown) of the rice plant was added after the main plots were burned. The crowns and roots were inserted into the soil to 8- to 10-cm soil depth with the crown remaining at the soil surface. In California, burning rice fields typically combusts 70 to 80% of the C and N held in the straw, crown, and roots (Hill et al., 1999). The burn combusts mainly straw from shoots cut during combining. The root, crown, and uncut stubble remain living and do not burn significantly. Unlabeled ash from equivalent soil areas was placed in the microcosm to simulate nutrient addition from the burned straw. The total amount of C, N, ^{13}C, and ^{15}N added to the burned and incorporated treatments are presented in Table 1. The tops of the microcosms were fit with screens (0.2- and 1-cm mesh) to contain the straw in the microcosm during the winter-fallow period. In addition, a 0.5-cm screened hole was placed in the side of the PVC microcosm 2.5 cm above the soil surface to facilitate water movement while limiting straw loss. In May 1999, each of the remaining microcosms was prepared for seeding by hand-tilling to a depth of 10 cm. Unlabeled ammonium sulfate-N fertilizer was applied to the microcosms at a rate of 157 kg N ha^{-1} just before planting in May 1999. Cultivar M-202 was aerial seeded on 13 May 1999 at a rate of 190 kg ha^{-1}.

Soil Carbon and Nitrogen Analyses

Soil samples (0 to 15 cm) were collected from the microcosms using an intact core sampler (5-cm diam.) that allowed for bulk density determinations. During 1999, two soil cores per microcosm were collected on the two excavation dates (April and September) and separately fractionated into four SOM fractions. Large visible pieces of crop residue (>6 mm) were removed from the soil samples before analyses, and analyzed separately. Soil microbial biomass and inorganic N determinations were performed on field-moist soil for each of the duplicate soil cores. For SOM analyses, duplicate soil cores were combined, soil was air-dried until constant weight at 40°C, and ground using a ball-mill to pass a 250-μm sieve. Before the initiation of the field study in fall 1993, selected

Table 1. Straw treatment additions and practices applied to microcosms in November 1998. Incorporated treatments received straw, crown, and roots chopped to 2- to 3-cm lengths and incorporated to 10-cm soil depth. Unchopped crown (to 5-cm above soil surface) and roots were inserted into the soil in the burned treatment.

Straw treatment	Dry matter	Application rate					Plant component added	Tillage
		C	N	C:N	^{13}C	^{15}N		
	Mg ha^{-1}	— kg ha^{-1} —			— kg ha^{-1} —			
Burn	2.3	664	43	15.4	3.4	4.1	root, crown (to 5 cm)	none
Incorporated	11.2	3936	151	26.1	31.6	14.5	root, crown, straw	chopped, incorporated

soil physical and chemical properties were determined (Bird et al., 2001; 2002). Total C and N in the SOM fractions were determined on a Carlo-Erba CHN analyzer (Costech Analytical Technologies, Inc., Valencia, CA) (Nelson and Sommers, 1996). Soil C and N determinations were expressed on a volumetric basis (dry-soil basis). Soil microbial biomass C, N, ^{13}C, and ^{15}N contents were determined on soil samples using the chloroform-fumigation incubation method (5-d chloroform period, Horwath and Paul, 1994; Bird et al., 2001). Carbon dioxide evolved during 10 d of incubation was measured using a Horiba PIR-2000 IGRA (Horiba Ltd., Kyoto, Japan). Microbial C was calculated without use of the control and adjusted using a K_C constant of 0.41 (Voroney and Paul 1984; Horwath et al., 1996). Field moist soil subsamples and fumigated soil were extracted with 2 *M* KCl for exchangeable inorganic N (5:1 extractant:dry-soil ratio) and quantified colorimetrically on an automated N analyzer (Lachat Instruments, Milwaukee, WI) (Mulvaney, 1996). Soil microbial biomass N was calculated after subtracting the initial extractable soil NH_4^+; values were adjusted using a K_N constant of 0.57 (Jenkinson, 1988).

Soil Organic Matter Fractionation

We fractionated soil samples into four SOM fractions with varying lability: (i) light fraction (LF), (ii) MHA, (iii) mobile fulvic acid (MFA), and (iv) nonalkali extractable humic acids (humin). Soil samples from the field and soil incubation studies were fractionated by physical separation of the LF using a density fractionation procedure described in Bird et al. (2002). The heavy fraction was fractionated into three SOM fractions: MHA, MFA, and humin described by McGill and Paul (1976) and Bird et al. (2002). Briefly, soil subsamples (20 g) were initially rinsed with 200 mL 0.1 *M* HCl to remove salts, carbonates, and particulate organic matter. Soil samples were subsequently extracted with 0.4 *M* NaOH, under N_2, to yield MHA and MFA. Mobile fulvic acid solution subsamples were dialyzed to remove Na in deionized water using 1000 MW cellulose tubing (Spectrum Industries, Inc., Rancho Dominguez, CA). The remaining organic matter, humin, is bound in stable aggregates with metal polyvalent cations and clay minerals (Bird et al., 2002). The SOM fractions were analyzed for yield and isotopic enrichment (^{15}N) after lyophilizing and homogenizing. Soil and SOM solutions were maintained at 4°C during and after fractionation.

Plant Analyses

At harvest in September 1999, rice plants were collected from microcosms by cutting just above the soil surface. Grain and straw dry matter were determined after drying samples until constant weight at 60°C and separating into grain and straw biomass. Dried straw samples were ground using a Wiley mill fit to pass a 2-mm screen; followed by ball milling to pass a 250-μm sieve. Total grain and straw C and N were determined as previously described. All plant determinations were expressed on a dry-matter basis (60°C).

Isotope Analyses

Natural abundance values ($^{13}C/^{15}N$) of plant, soil, SOM fractions, and inorganic N were determined from samples from a previous experiment (Bird et al., 2002). Unlabeled soil and plant samples were analyzed on a Europa Scientific Geo 20/20 isotope mass spectrometer (IRMS) (PDZ Europa Ltd., Crewe, UK). Labeled inorganic N extracts (NH_4^+ and NO_3^-) and KCl extracts from CFI were diffused onto ashed, Whatman GF/A filter paper using the teflon tape method described by Stark and Hart (1996). Isotopic enrichment of the ^{15}N in diffused samples, and ^{13}C and ^{15}N in whole soil, plant, straw, and SOM fractions were determined on a Europa Scientific INTEGRA IRMS (PDZ Europa Ltd., Crewe UK). Respired C ($^{13}CO_2$) samples from CFI were analyzed on a Trace Gas Inlet System interfaced to the GEO 20/20 IRMS. The recovery of ^{13}C and ^{15}N in soil and plant pools analyzed by mass spectrometry was calculated with the following equations for C and N, initially in $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰):

$$\delta^{13}C = [(R_{\text{sample}}/R_{\text{standard}}) - 1]\ 1000, \text{ and} \quad [1]$$

$$\delta^{15}N = [(R_{\text{sample}}/R_{\text{standard}}) - 1]\ 1000, \quad [2]$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$ for C and N, respectively. Values were calculated relative to the international standard Vienna-Pee Dee Belemnite for ^{13}C ($R = 0.0112372$) and for atmospheric N_2 for ^{15}N ($R = 0.0036765$). Atom % values presented for ^{15}N were calculated for samples highly enriched in ^{15}N from the fractional abundance of ^{15}N.

$$\text{Atom \%} = [R/(R + 1)]\ 100 \quad [3]$$

The ^{13}C and ^{15}N enrichments were used to determine the fraction (f) of turnover of new C and N entering these pools (Balesdent and Mariotti, 1996):

$$f = (\delta - \delta_0)/(\delta_1 - \delta_0), \quad [4]$$

where δ_0 and δ are the $\delta^{13}C$ values of a soil fraction at the beginning (i.e., natural abundance values) and end of the experiment (or sampling time point) and δ_1 is the ^{13}C signal of the substrate added. Similar calculations were done for ^{15}N.

Statistical Analyses

Main effects of winter flooding and straw management were tested using a general linear model (GLM) designed for the split-plot design. All data were expressed as least squares means with standard errors of indicated treatments. Fisher (F) statistics and P values were 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 greater than 0.05 in Tables are listed as nonsignificant. Studentized t tests were performed among soil and plant C and N fraction data to compare ^{13}C and ^{15}N enrichment mean values on specific sample dates within incorporated and burned treatments and between sampling dates for the percentage recovery of straw ^{13}C and ^{15}N. Since proportional data are often not normally distributed, analyses on percentage data were performed after log-transformation. All statistical tests were performed using SYSTAT version 7.0 (SPSS Inc., Chicago, IL).

RESULTS

Soil Carbon and Nitrogen Pool Sizes

Total soil C and N were unaffected by straw or flooding management practices (Tables 2 and 3) at the end of 6 yr. The alkali-insoluble humin fraction represented the largest proportion of SOM recovered in all treatments, 52.4 ± 0.9% and 50.4 ± 0.8% of total C and N reserves, respectively (averaged across sample dates). The MHA fraction, the second largest soil C and N fraction examined (25.8 ± 0.6% and 27.3 ± 0.6% of total soil C and N), was significantly greater with annual straw incorporation than with burned (Tables 2 and 3). The labile LF-C, LF-N, and SMB-N pools, like the MHA fraction, were significantly greater with straw incorporation than open-field burning.

Table 2. Quantities of various C fractions from soil sampled at a depth of 0 to 15 cm in April and September 1999 when straw was incorporated or burned and the fields were winter flooded (WF) or nonwinter flooded (NF). All treatments were flooded during the cropping season. Least-squares means, values in parentheses are standard errors ($N = 4$). *P* values greater than 0.05 are indicated as nonsignificant (NS).

Straw management	Fallow flood management	Soil C fraction					
		Microbial	LF†	MHA‡	MFA§	Humin	Soil
		kg ha^{-1}					
		April—Preplant					
Burned	WF	499 (74)	645 (63)	5 123 (200)	1 221 (288)	12 178 (705)	22 488 (1 167)
Burned	NF	481 (52)	541 (76)	5 901 (351)	1 030 (111)	11 986 (536)	23 140 (1 246)
Incorporated	WF	555 (74)	869 (30)	6 031 (141)	1 300 (239)	10 888 (277)	23 634 (626)
Incorporated	NF	634 (52)	788 (64)	6 666 (530)	1 447 (402)	12 092 (753)	23 514 (239)
Main effects							
P values	WF	NS	NS	NS	NS	NS	NS
	Straw	NS	0.011	0.013	NS	NS	NS
	WF × S	NS	NS	NS	NS	NS	NS
		September—Crop maturity					
Burned	WF	579 (80)	742 (57)	5 755 (206)	975 (184)	12 599 (437)	23 585 (1 062)
Burned	NF	492 (43)	665 (54)	5 433 (383)	1 422 (278)	12 962 (710)	23 377 (2 233)
Incorporated	WF	627 (22)	850 (38)	6 540 (320)	1 567 (148)	11 588 (473)	23 388 (1 355)
Incorporated	NF	599 (23)	703 (79)	6 008 (286)	1 076 (189)	11 849 (601)	22 187 (1 118)
Main effects							
P values	WF	NS	0.005	NS	NS	NS	NS
	Straw	NS	NS	0.009	NS	0.028	NS
	WF × S	NS	NS	NS	NS	NS	NS

† LF = light fraction.
‡ MHA = mobile humic acids.
§ MFA = mobile fulvic acids.

While winter flooding did not affect C and N contents of the larger, more stable SOM fractions, LF-C (September) and inorganic N (April and September) were greater with annual winter flooding (Tables 2 and 3). A significant winter flood by straw interaction was present for inorganic N on both sampling dates in 1999. This interaction resulted in greater inorganic N with winter flooding and straw incorporation.

Straw ^{13}Carbon and ^{15}Nitrogen Dynamics during Winter Fallow

At the end of the winter-fallow period (November–April 1999; 161 d in situ), more straw ^{13}C and ^{15}N were recovered in the straw incorporated treatment compared with the burned ($P = 0.001$). The total percentage recovery of applied ^{13}C (soil and >6 mm particulate straw), however, was almost two times greater for the

Table 3. Quantities of various N fractions from soil sampled at a depth of 0 to 15 cm in April and September 1999 when straw was incorporated or burned and the fields were winter-flooded (WF) or nonwinter flooded (NF). All treatments were flooded during the cropping season. Least-squares means, values in parentheses are standard errors ($N = 4$). *P* values greater than 0.05 are indicated as nonsignificant (NS).

Straw management	Fallow flood management	Soil N fraction						
		Inorganic	Microbial	LF†	MHA‡	MFA§	Humin	Soil
		kg ha^{-1}						
		April—Preplant						
Burned	WF	11.8 (1.0)	75 (2)	31.1 (2.5)	463 (21)	105 (22)	970 (36)	1935 (113)
Burned	NF	1.7 (0.5)	71 (7)	27.4 (3.3)	535 (31)	90 (20)	975 (28)	1978 (96)
Incorporated	WF	29.4 (5.6)	102 (9)	35.1 (1.8)	543 (3)	106 (24)	918 (30)	2068 (31)
Incorporated	NF	2.4 (0.4)	91 (9)	38.8 (1.2)	591 (44)	137 (30)	996 (42)	2018 (17)
Main effects								
P values	WF	0.006	NS	NS	NS	NS	NS	NS
	Straw	0.032	0.032	0.035	0.011	NS	NS	NS
	WF × S	0.041	NS	NS	NS	NS	NS	NS
		September—Crop maturity						
Burned	WF	3.7 (0.2)	76 (7)	34.5 (2.8)	500 (21)	92 (19)	979 (29)	1901 (70)
Burned	NF	4.2 (0.8)	65 (5)	33.3 (3.7)	467 (35)	143 (32)	1021 (39)	1886 (180)
Incorporated	WF	7.5 (0.5)	75 (4)	38.9 (1.9)	573 (27)	150 (19)	938 (44)	1939 (103)
Incorporated	NF	4.3 (0.1)	77 (5)	35.8 (5.2)	530 (23)	107 (22)	980 (26)	1804 (72)
Main effects								
P values	WF	0.010	NS	NS	NS	NS	NS	NS
	Straw	0.007	NS	NS	0.002	NS	NS	NS
	WF × S	0.011	NS	NS	NS	NS	NS	NS

† LF = light fraction.
‡ MHA = mobile humic acids.
§ MFA = mobile fulvic acids.

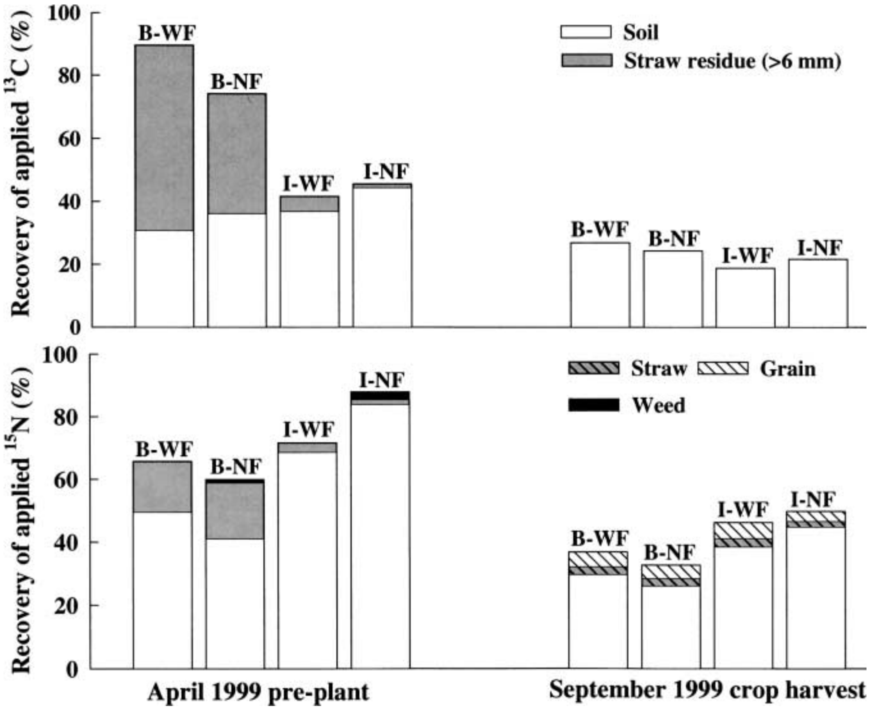

Fig. 3. Recovery of ^{13}C (top) and ^{15}N (bottom)-crop residue as a percentage of that applied in November 1998 from soil sampled in April 1999 and September 1999. Soil recoveries were from soil sampled to the 0- to 15-cm depth when straw was incorporated (I) or burned (B) and the fields were winter flooded (WF) or nonwinter flooded (NF) (N = 4).

burned straw treatment (82.5 ± 4.0%) than for straw incorporated (44.0 ± 2.3%) ($P = 0.001$; Fig. 3). A large proportion of intact crown and root biomass (>6 mm) remained in the burned treatments while most of the visible straw in the incorporated treatments had decomposed. Treatments did not result in significant differences in the percentage of applied ^{13}C recovered in the soil (<6 mm) (Fig. 3). A significant winter flood by straw interaction was present ($F = 9.1$; $P = 0.024$) for the total percentage recovery of applied ^{13}C (soil and >6 mm). Winter flooding resulted in greater total recovery of applied ^{13}C in burned treatments but had little effect in incorporated treatments (Fig. 3).

In contrast to ^{13}C, total percentage recovery of applied ^{15}N (soil and >6 mm) was greater in incorporated (78.5 ± 4.3%) compared with burned (62.3 ± 2.4%) treatments in April ($P = 0.001$; Fig. 3). The percentage of applied ^{15}N recovered in the soil (<6 mm) was also greater in incorporated compared with burned treatments (Fig. 3). A small percentage of ^{15}N was recovered as volunteer annual bluegrass (*Poa annua* L.) in the NF treatments.

At the end of the five-month winter-fallow period, the majority of ^{13}C was recovered in the soil as humin and MHA (Table 4). The humin and MHA fractions were not significantly affected by straw management and averaged 51.8 ± 3.2% and 21.2 ± 0.9%, respectively, of soil-recovered ^{13}C in April. The percentage of applied ^{13}C recovered in soil as LF-C was affected by straw management and represented a greater proportion of soil-recovered ^{13}C in the incorporated (16.2 ± 1.8%) compared with burned (9.0 ± 1.7%) treatments (Table 4).

Percentage recovery of applied ^{15}N in soil as SOM was greatest in the MHA fraction for all treatments (Table 5). With soil incorporation of straw, recovery of applied ^{15}N in soil as MHA and LF was greater than for burned treatments. Nonwinter-flooded conditions resulted in greater recovery of applied ^{15}N in soil as LF than winter flooding (Table 5). In contrast to the LF, winter flooding increased the recovery of straw-^{15}N as inorganic N in April 1999 compared with nonflooded fallow.

Straw ^{13}Carbon and ^{15}Nitrogen Dynamics during Crop Season

One year after application, more straw ^{13}C and ^{15}N were recovered in straw incorporated treatments compared with burned (September 1999; $P = 0.001$). Similar to April 1999, treatments did not result in significant differences in the percentage of applied ^{13}C recovered in the soil at the end of 1 yr (Fig. 3). The percentage recovery of applied ^{15}N in the soil for straw incorporated plots (41.7 ± 3.9%) exceeded straw burned (27.9 ± 2.8%) at the end of 1 yr, but this difference was not significant at a $P < 0.05$ ($P = 0.061$; Fig. 3).

The percentage recovery of applied ^{13}C in soil as SOM was affected by straw incorporation and winter flooding (Table 4). In the humin fraction, the percentage of soil-recovered ^{13}C was greater in the burned treatment compared with straw incorporated. In contrast, the percentage of ^{13}C recovered as MHA- and SMB-C showed an opposite effect of straw management (Table 4). The partitioning of straw ^{13}C recovered in soil as SOM fractions changed during the summer period (April–Septem-

Table 4. Distribution of ^{13}C recovered from soil (0- to 15-cm depth) among various fractions sampled in April and September 1999. Least-squares means, values in parentheses are standard errors ($N = 4$). *P* values greater than 0.05 are indicated as nonsignificant (NS).

Straw management	Fallow flood management	Soil C fraction				
		Microbial	LF†	MHA‡	MFA§	Humin
		% of soil recovered ^{13}C				
		April—Preplant				
Burned	WF	ND¶	7.0 (1.7)	21.0 (1.9)	7.5 (3.5)	58.5 (5.0)
Burned	NF	ND	11.1 (1.7)	20.5 (1.4)	3.5 (1.7)	59.5 (12.5)
Incorporated	WF	ND	19.7 (1.9)	21.5 (0.5)	4.6 (0.6)	46.2 (1.8)
Incorporated	NF	ND	12.7 (1.6)	21.7 (2.7)	4.7 (1.2)	44.9 (3.7)
Main effects						
P values	WF		NS	NS	NS	NS
	Straw		0.021	NS	NS	NS
	WF × S		NS	NS	NS	NS
		September—Crop maturity				
Burned	WF	3.1 (0.5)	15.0 (3.5)	18.9 (1.0)	7.8 (1.8)	59.9 (5.2)
Burned	NF	7.2 (2.9)	20.7 (6.8)	10.2 (3.2)	11.1 (0.7)	64.1 (7.8)
Incorporated	WF	11.9 (1.4)	11.8 (1.6)	30.8 (0.5)	8.2 (0.5)	39.5 (3.8)
Incorporated	NF	10.2 (2.7)	13.6 (2.3)	27.6 (0.8)	5.7 (1.1)	42.1 (1.8)
Main effects						
P values	WF	NS	NS	NS	NS	0.008
	Straw	0.025	NS	0.008	NS	0.003
	WF × S	NS	NS	NS	NS	NS

† LF = light fraction.
‡ MHA = mobile humic acids.
§ MFA = mobile fulvic acids.
¶ Not determined.

ber 1999) and was affected by straw management treatments (Table 4). The proportion of soil-recovered ^{13}C as MHA decreased from April to September in burned and increased in incorporated treatments ($P < 0.05$). The ^{13}C enrichment of total soil and SOM fractions decreased from April to September in straw incorporated treatments ($P < 0.05$; Table 6). In contrast, ^{13}C enrichments were similar on both sampling dates for total soil and SOM fractions in the straw burned treatments.

The recovery of applied ^{15}N in soil as MHA and humin was affected by straw management treatments 1 yr after application. More ^{15}N was partitioned into the humin fraction in burned treatments, whereas more ^{15}N was partitioned into the MHA fraction in incorporated treatments (Table 5). The ^{15}N enrichment of all soil N pools examined were lower in September than April, with the exception of the LF in the burned treatments ($P <$ 0.05; Table 6).

At crop maturity, the recovery of ^{15}N in rice, considered as a percentage of applied and as a percentage of total ^{15}N recovered in April 1999, was not affected by treatments (Fig. 3 and Table 7). A significant winter flood × straw interaction was present for total rice-

Table 5. Distribution of ^{15}N recovered from soil (0- to 15-cm depth) among various fractions sampled in April and September 1999. Least-squares means, values in parentheses are standard errors ($N = 4$). *P* values greater than 0.05 are indicated as nonsignificant (NS).

Straw management	Fallow flood management	Soil N fraction					
		Inorganic	Microbial	LF†	MHA‡	MFA§	Humin
		% of soil recovered ^{15}N					
		April—Preplant					
Burned	WF	4.1 (0.5)	13.2 (3.1)	2.6 (0.2)	29.0 (2.1)	6.3 (1.2)	21.7 (2.5)
Burned	NF	0.4 (0.1)	33.8 (9.5)	6.4 (1.3)	32.9 (3.5)	6.4 (1.4)	22.7 (3.9)
Incorporated	WF	6.3 (1.1)	16.7 (5.5)	5.6 (0.5)	34.5 (1.1)	5.7 (1.1)	22.8 (0.6)
Incorporated	NF	0.4 (0.1)	11.8 (4.6)	7.0 (0.3)	39.0 (2.7)	7.7 (1.7)	26.0 (1.4)
Main effects							
P values	WF	0.003	NS	0.019	NS	NS	NS
	Straw	NS	NS	0.026	0.017	NS	NS
	WF × S	NS	NS	NS	NS	NS	NS
		September—Crop maturity					
Burned	WF	0.5 (0.1)	12.4 (2.6)	4.6 (0.7)	38.6 (0.9)	6.6 (1.6)	31.6 (2.1)
Burned	NF	0.5 (0.1)	8.1 (1.7)	7.5 (1.9)	36.7 (3.0)	9.7 (1.5)	34.5 (1.8)
Incorporated	WF	1.0 (0.1)	10.7 (1.3)	5.2 (0.7)	42.4 (0.6)	9.7 (1.0)	25.9 (1.1)
Incorporated	NF	0.4 (0.1)	7.3 (1.1)	7.9 (1.5)	42.3 (0.8)	7.7 (1.8)	31.1 (1.2)
Main effects							
P values	WF	NS	NS	NS	NS	NS	0.035
	Straw	NS	NS	NS	0.047	NS	0.005
	WF × S	0.020	NS	NS	NS	NS	NS

† LF = light fraction.
‡ MHA = mobile humic acids.
§ MFA = mobile fulvic acids.

Table 6. Enrichment of δ^{13}C (‰) and ^{15}N atom excess(%) in plant and soil (0- to 15-cm depth) pools sampled in April (preplant) and September (crop maturity) when straw was incorporated or burned (averages across winter-flooding treatments) the previous fall. Least-squares means, values in parentheses are standard errors ($N = 8$). Different letters following values correspond to differences among ^{15}N or ^{13}C enrichment values within straw management treatments and sampling date are significantly different ($P < 0.05$).

Plant and soil pool	Burned straw		Incorporated straw	
	April	September	April	September
		^{13}C, ‰		
Rice residue (>6 mm)	502.60 (21.52) a	ND	543.07 (27.90) a	ND†
Total soil	−22.07 (0.39) c	−23.27 (0.51) d	23.53 (3.18) c	−0.71 (3.91) b
SMB‡	ND	−20.40 (1.01) bc	ND	67.82 (7.10) a
LF§	−9.71 (3.82) b	−7.28 (4.61) a	193.91 (11.07) b	69.29 (16.51) a
MHA¶	−22.69 (0.41) c	−24.41 (0.49) e	13.14 (1.69) d	0.44 (3.64) b
MFA#	−22.83 (1.07) c	−20.80 (0.57) b	13.07 (2.17) d	3.60 (3.82) c
Humin	−21.53 (0.54) c	−22.74 (0.70) cd	19.59 (2.05) c	−5.99 (3.34) d
		^{15}N atom excess, %		
Grain	ND	0.26 (0.03) a	ND	0.72 (0.06) b
Rice residue (>6 mm)	7.62 (0.12) a	ND	7.57 (0.13) a	ND
Total soil	0.09 (0.01) e	0.06 (0.01) e	0.54 (0.03) e	0.33 (0.04) f
Inorganic N	0.55 (0.01) b	0.14 (0.02) c	2.07 (0.08) b	0.67 (0.07) c
SMB	0.71 (0.14) b	0.19 (0.03) b	1.92 (0.35) b	0.92 (0.09) a
LF	0.27 (0.05) c	0.20 (0.04) abc	1.88 (0.15) b	1.07 (0.19) ab
MHA	0.11 (0.01) d	0.09 (0.01) d	0.72 (0.04) c	0.47 (0.05) d
MFA	0.12 (0.01) d	0.08 (0.01) d	0.60 (0.03) d	0.42 (0.05) e
Humin	0.04 (0.01) f	0.04 (0.01) f	0.28 (0.02) f	0.18 (0.02) g

† ND = not determined.
‡ SMB = soil microbial biomass.
§ LF = light fraction.
¶ MHA = mobile humic acids.
# MFA = mobile fulvic acids.

N uptake (soil plus residual straw N). This interaction resulted in greater N uptake in the WF soils compared with the NF soils in straw incorporated treatments and less in the WF soils in straw burned treatments (Table 7).

^{13}Carbon Loss:^{15}Nitrogen Loss Ratios

The ratios of straw ^{13}C loss:^{15}N loss during each time period were calculated for each plot ($N = 16$; Table 8). During the winter-fallow period, ^{13}C loss was lower than ^{15}N loss for the burned treatments, while ^{13}C loss was substantially greater than ^{15}N loss in the incorporated treatments. The loss of ^{13}C relative to that of ^{15}N for the summer cropping period was reversed from that of the winter-fallow period. Overall, the net result of these opposing trends resulted in a straw ^{13}C:^{15}N loss ratio of 1.5 with straw incorporation and 1.1 with straw burning (Table 8). Winter flooding decreased the ^{13}C:^{15}N loss ratio compared with NF during the winter period and increased the ^{13}C:^{15}N loss ratio during the summer period. Consequently, no significant net effect of winter flooding was observed in the ^{13}C:^{15}N loss ratio during the course of 1 yr (Table 8).

DISCUSSION

Straw Carbon and Nitrogen Decomposition

All straw management practices examined in this study facilitated the decomposition of enough residual straw residues for optimal seed bed preparation and produced similar grain yields under average fertilizer N rates (Eagle et al., 2000). As expected, more straw ^{13}C and ^{15}N were retained in the soil as SOM with straw incorporation (straw, stubble, crown, and roots) compared with straw (stubble, crown, and roots) in the burned treatment as more straw dry matter was applied in the straw incorporated treatment. The loss of applied ^{13}C remaining after burning (74%) and incorporation (80%) are consistent with values reported for crop residue decomposition after 1 yr in temperate climates (Jenkinson and Rayner, 1977; Aita et al., 1997).

Loss of applied straw ^{13}C during the winter fallow (November–April) was substantially reduced in burned (17%) compared with incorporated (56%) treatments. In burned treatments, slower labeled straw decomposition resulted in more straw recovered as intact crowns

Table 7. Total rice plant N uptake and ^{15}N-crop residue uptake as a percentage of total ^{15}N recovered (April 1999) as affected by incorporated or burned straw, winter flooding (WF), and nonwinter flooding (NF) fields at crop maturity (September 1999). Least-squares means, values in parentheses are standard errors ($N = 4$). P values greater than 0.05 are indicated as nonsignificant (NS).

Plant component	Straw management treatment				Main effects		
	Burn WF	Burn NF	Incorporated WF	Incorporated NF	WF	Straw	WF × Straw
		N uptake, kg ha^{-1}				*P* values	
grain	65.0 (4.8)	69.2 (2.4)	94.7 (3.5)	69.0 (9.6)	NS	0.046	0.043
straw	42.2 (1.9)	50.8 (3.5)	61.7 (0.3)	47.1 (5.0)	NS	NS	0.017
total	107.2 (6.4)	120.0 (5.1)	156.4 (3.6)	116.1 (13.3)	NS	0.039	0.021
		% of ^{15}N recovered from April					
grain	7.1 (2.2)	6.6 (0.7)	7.1 (1.0)	3.6 (0.6)	NS	NS	NS
straw	4.0 (1.2)	4.0 (2.7)	4.0 (0.5)	2.2 (0.2)	NS	NS	NS
total	11.1 (3.3)	10.6 (1.0)	11.1 (1.5)	5.8 (0.8)	NS	NS	NS

Table 8. Relative loss of ^{13}C- and ^{15}N-crop residue as a percentage of that applied in November 1998 during the initial winter-fallow period (November–April), summer crop period (May–September) and the entire 11 mo in situ study. Loss excludes plant assimilated ^{15}N, and soil ^{13}C and ^{15}N below 15-cm depth. Least-squares means, values in parentheses are standard errors ($N = 4$). P values greater than 0.05 are indicated as nonsignificant (NS).

	Straw management treatment				Main effects		
Time period	Burn WF†	Burn NF‡	Incorporated WF	Incorporated NF	WF	Straw	WF × S
	C:N loss ratio				*P* values		
Winter	0.28 (0.1)	0.62 (0.1)	2.41 (0.6)	8.30 (4.9)	0.020	0.002	NS
Summer	3.02 (1.0)	1.74 (0.1)	1.09 (0.2)	0.70 (0.1)	0.007	0.014	NS
1 yr	1.17 (0.1)	1.12 (0.1)	1.51 (0.1)	1.63 (0.2)	NS	0.005	NS

† WF = winter flooded.
‡ NF = nonwinter flooded.

and root biomass (>6 mm) than recovered as total soil ^{13}C. Similarly, field tillage (wet-rolling) increased straw decomposition during the winter fallow compared with untilled rice straw under WF conditions in California (Bird et al., 2000). Uncombusted straw crowns and stubble in burned treatments had limited soil contact compared with incorporation, thereby reducing microbial degradation. In contrast, percentage loss of applied ^{13}C was greater during the summer in burned treatments compared with incorporated treatments. At this stage, remaining surface straw was incorporated into the soil following seedbed preparation in all treatments allowing for increased soil contact and exposure to decomposition processes, especially for surface residues in the burned treatment. As straw ^{13}C was already converted into SMB- and SOM-C in incorporated treatments by early spring, decomposition of straw was consequently slower than in burned treatments during the growing season.

While the slower initial degradation of the residues remaining after burning may have been due to the effects of tillage, the differences in C quality may also have contributed to slower initial loss rates. As suggested by Balesdent and Balabane (1996), the relatively slow loss of root-derived C may be due to greater amounts of complex C compounds such as lignin and structural carbohydrates.

While winter flooding did not affect the loss of applied straw ^{13}C at the end of 1 yr, decomposition of straw was slower in WF than NF soils in the burned treatments during the winter fallow. As anaerobic decomposition is generally considered less energetically efficient than aerobic (Broadbent, 1979), this result was expected. Winter flooding, however, did not affect the percentage loss of applied ^{13}C in incorporated plots. Flooded soil conditions have either shown no effect on straw C loss rates compared with aerobic conditions (Neue and Scharpenseel, 1987; Devêvre and Horwath, 2000) or have shown increased straw C mineralization rates (Murayama, 1984). Flooded conditions during a laboratory incubation of straw added to soil from this study site depressed total CO_2–C mineralization rates during the first 60 d compared with 50% water-holding capacity (WHC), but was similar thereafter (Devêvre and Horwath, 2000). Loss of C as methane from flooded soils, however, greatly exceeded those produced at 50% WHC and contributed ≈9% of the total C emissions at 25°C under flooded conditions (Devêvre and Horwath, 2000). Thus, greater methane loss in WF treatments may partly explain the lack of observed differences in total C loss due to winter flooding with straw incorporation.

Our results indicate that, in the short term, winter flooding decreased decomposition of intact straw at or above the soil surface and had no net effect on soil-incorporated residues. The winter-flooding effect on ^{13}C sequestration, however, did not persist at the end of 1 yr. In addition, winter flooding had no effect on total soil C, or on smaller more responsive SOM fractions such as MHA at the end of 6 yr of winter-flooding treatments. Consequently, winter flooding of fallow rice fields does not appear to be an effective method to accumulate C in rice soils, but may be worthwhile for waterbird habitat enhancement (Elphick and Oring, 1998).

Total percentage loss of applied straw ^{15}N in all treatments was slightly higher, but consistent, with values reported at the end of 1 yr for medic (*Medicago littoralis* Rohde ex Loisel.) (35–45%, Ladd et al., 1981) and wheat (*Triticum aestivum* L.) residues (48–52%, Voroney et al., 1989). In contrast to straw ^{13}C dynamics, loss of applied straw ^{15}N was slightly higher in burned (65.5 ± 3.5%) compared with incorporated (52.0 ± 3.8%) treatments at the end of 1 yr. While this difference was not significant ($P = 0.085$), this trend was also apparent in recovery of applied ^{15}N as soil N at the end of 1 yr. A high rep × straw error may have resulted in a Type II error for this comparison. The frequently saturated soils in temperate, flooded rice systems presumably contributed to higher N losses from the plant-soil system than unflooded soils. Loss pathways were most likely a combination of denitrification, ammonia volatilization, and leaching of ^{15}N below the 15-cm soil depth. We suspect, however, that loss of straw-derived N below the 15-cm soil depth was small (<10% of applied) based on previous work that showed little movement of applied ^{15}N fertilizer into the 15- to 90-cm depth 2 yr after application (Bird et al., 2001).

The higher loss of ^{15}N relative to ^{13}C for straw in burned treatments compared with incorporated at the end of 1 yr was the net result of markedly different ^{13}C loss to ^{15}N loss patterns during the two time periods. During the winter fallow, the burned treatments had low ^{13}C:^{15}N loss ratios (0.3 to 0.6). In contrast, ^{15}N was more highly conserved in the soil than ^{13}C with straw incorporation during the winter-fallow period. The low

^{13}C loss relative to ^{15}N in the burned treatments during the winter fallow emphasized the high proportion of intact straw residues and low microbial processing of applied straw C.

The straw × winter-flooding interaction observed in total rice N uptake was consistent with rice N uptake and yield observed at this site (Eagle et al., 2000). While the total amount of soil N recovered in the rice crop was greater in incorporated treatments compared with burned, the percentage of applied ^{15}N recovered in the plant was similar among treatments. With an average uptake of 6.6% of applied straw N (averaged across treatments), straw N clearly plays a small but important role in supplying rice N under N-fertilized conditions. Ultimately, a slightly greater percentage of applied ^{15}N remained in the soil in straw incorporated treatments compared with burned at the end of 1 yr. These results suggest that more straw ^{15}N immobilized after straw incorporation was conserved in the soil as SOM than in burned treatments. Therefore, with time, more soil-immobilized straw N may be supplied to subsequent rice crops.

Soil-Microbial-Biomass-Carbon and Nitrogen

The increased SMB in incorporated compared with burned treatments was consistent with those reported previously during Years 4 through 6 of this field study (Bird et al., 2001). The sustained, larger SMB fraction in incorporated treatments was due, in part, to the larger labile MHA and LF fractions observed after 4 yr of straw incorporation compared with burned (Bird et al., 2002). While the SMB-C and N pool represented a small proportion (2 to 4%) of total C and N, the flux of ^{13}C and ^{15}N through the SMB was very high. This is illustrated well by the higher ^{13}C and ^{15}N enrichments in the SMB than all humic fractions on both sampling dates. The similar ^{15}N enrichment of SMB to the inorganic N and LF-N indicate their close source-sink relationship. Furthermore, similarity of the relative ^{15}N enrichments of the SMB to the LF at the end of 1 yr indicates that straw LF-^{15}N was a major source of SMB-N in all treatments. In contrast, the ^{13}C enrichment of the SMB in the burned treatment was significantly less than the LF at the end of 1 yr. The SMB in the burned treatment appeared to be more dependent on native soil C than LF-C.

Carbon and Nitrogen Stabilization and Turnover in Soil Organic Matter

Soil organic matter fractionation in combination with C and N stable isotope methodologies has provided a wealth of information on the important processes of humification in soils (McGill et al., 1975; Stott et al., 1983; Devêvre and Horwath, 2001). Our data show that at the end of 161 d in situ, 80 to 95% of soil-recovered straw ^{13}C and ^{15}N ($<$6 mm) was present in microbial and humic substances. The relative humification pathway of straw ^{13}C was markedly different than straw ^{15}N into SOM fractions. Furthermore, the pathways of ^{13}C and ^{15}N into and among SOM fractions varied significantly among straw and WF treatments.

More straw ^{13}C entered the resistant humin fraction relative to straw ^{15}N, while more straw ^{15}N was stabilized as MHA than straw ^{13}C. These trends in ^{13}C and ^{15}N partitioning, which were present in all treatments, suggest divergent humification pathways for C and N from straw byproducts. Further research in this area, however, is needed to consider whether these trends are maintained with time, if these observations are apparent in the humification pathways of other ecosystems, and if other SOM fractionation procedures reflect similar results.

Our results indicate that crop residue management practices not only can affect soil C and N stabilization rates, but can also affect the relative contributions of C and N to different SOM fractions. At the end of 1 yr, the proportions of ^{13}C and ^{15}N recovered in the soil as MHA were greater in straw incorporated compared with burned treatments. Similarly, the proportion of ^{13}C recovered in the soil as humin was greater in burned treatments compared with incorporated. The labeled straw addition results were consistent with the longer-term effects (i.e., 6 yr) of straw management practices observed in greater total MHA-C, MHA-N, and slightly lower total humin-C with straw incorporation compared with straw burning. The implications of these findings suggest that the MHA-C, MHA-N, and humin-C fractions contain sizable portions of recent straw additions and reflect, most significantly, recent agronomic practices.

Mobile humic acid N is an important source of crop N needs in temperate rice (Bird et al., 2002). Rice straw residue incorporation during six seasons has contributed substantially to this source of plant-available N and may warrant a reduction in fertilizer N needs compared with annual straw burning. While total soil C and N have not increased at the end of 6 yr of straw incorporation of residues, the trends apparent in greater MHA-, SMB-, and LF-C pools suggest that, in the long term, soil incorporation of residues may have a significant impact on soil C accumulation.

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