

# DIVISION S-3—SOIL MICROBIOLOGY AND BIOCHEMISTRY

## Influence of Moisture on Rice Straw Decomposition in Soils<sup>1</sup>

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### ABSTRACT

Laboratory incubation experiments were conducted for 4 months at  $22 \pm 0.5\text{C}$  with two fine-textured soils amended with doubly tagged ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) rice straw (*Oryza sativa* L.) under different moisture regimes. The moisture levels were 30, 60, and 150% of water-holding capacity, (WHC). The extent of straw decomposition was found to be a power function of time. Differential equations were derived to give rate functions for each moisture level.

Native soil organic carbon loss was depressed by added energy materials when either moisture or oxygen supply limited decomposition. At 30% WHC, priming ratios were  $< 1.0$  initially, and increased with time of incubation but did not reach unity within 4 months incubation. At 60% WHC, the ratios were  $> 1.0$  and decreased with time of incubation but remained above 1.0 throughout incubation. At 150% WHC, priming ratios were  $< 1.0$  and did not vary much during the entire decomposition period. The net release of straw-N at 60% WHC was higher than at 30% WHC. At 150% WHC, straw-N released during decomposition was lost from the system, presumably by denitrification. Soil inorganic nitrogen changes followed trends similar to net release of straw-N. The influence of moisture variables on soil organic nitrogen transformations was more pronounced than the influence of decomposing straw.

*Additional Index Words:* ammonification, nitrification, denitrification, priming effect, stable isotopes, turnover time.

MOISTURE is one of the principal environmental factors that determine the rate of organic residue decomposition by microorganisms. Although biological processes are arrested below a critical moisture level, the moisture ranges within which microorganisms multiply and metabolize substrates are rather wide (2). Most work on carbon mineralization under various moisture conditions has either been in soilless cultures of plant materials (1) or soil incubation with untagged plant materials (7). To elaborate upon the interactions of added and native soil organic matter loss during decomposition, there is a definite need for use of isotopically tagged plant materials in decomposition studies under different moisture conditions. In the present experiment,  $^{13}\text{C}$ - and  $^{15}\text{N}$ -labeled rice straw (*Oryza sativa* L.) was allowed to decompose in two rice soils at three moisture levels for 4 months. The release of C and N during decomposition under various moisture conditions was measured. Influences of straw decomposition in soil inorganic nitro-

gen changes under different moisture conditions were evaluated.

### MATERIALS AND METHODS

Doubly tagged rice straw which contained 29.8% C with 5.76%  $^{13}\text{C}$  excess and 2.80% N with 24.32 atom %  $^{15}\text{N}$  excess was utilized for the present study. This straw was obtained by growing rice plants in a closed glass chamber in which the soil was fertilized with  $^{15}\text{N}$ -enriched ammonium sulfate. Carbon-13-enriched  $\text{CO}_2$  was produced inside the chamber by decomposition of barium carbonate with lactic acid. The oxygen concentration in the chamber was maintained at or above 20%. The rice plants were harvested before maturity, which accounts for the low carbon and high nitrogen contents.

Two rice soils used in the experiments had the following properties: Sacramento clay—1.74% C, 0.16% N, and 67% moisture at saturation; Stockton adobe clay—1.69% C, 0.14% N and 54% moisture at saturation.

The moisture content at saturation, referred to hereafter as water holding capacity, (WHC), was determined by making a saturation paste.

Duplicate 25-g soil samples of each soil were weighed in 237 ml (8 oz) bottles in sufficient numbers so as to give the following treatments:

- 1) Soil control at 30% water-holding capacity, WHC;
- 2) Soil + 0.25 g straw at 30% WHC;
- 3) Soil alone at 60% WHC;
- 4) Soil + 0.25 g straw at 60% WHC;
- 5) Soil alone at 150% WHC;
- 6) Soil + 0.25 g straw at 150% WHC.

After thorough mixing of soil samples with straw and wetting soils with moisture at the indicated levels, the bottles were closed with rubber stoppers to which 5-ml beakers were attached to hold alkali for  $\text{CO}_2$  absorption. The samples were incubated at  $22 \pm 0.5\text{C}$  in an incubator for 4 months. The evolved  $\text{CO}_2$  was measured at 1, 2, 4, 6, 8, 10, 20, 30, 60, 90 and 121 days after incubation by precipitation of carbonate as  $\text{BaCO}_3$  and back titration of the excess alkali with standard acid. The  $\text{BaCO}_3$  precipitate was analyzed for  $^{13}\text{C}$  abundance on a mass spectrometer.

A test of this static closed system for adequacy of aeration showed that frequent opening of the bottles to replace the sodium hydroxide for absorption of  $\text{CO}_2$  prevented any inhibition of decomposition due to lack of oxygen. When bottles were opened at intervals of 12, 24, 72 and 162 hours during the initial period of most rapid decomposition the total carbon evolved was unchanged by length of interval. The coefficient of variation over all intervals was 1.8% of the total. The lowest oxygen concentration attained with the 162-hour interval was 7.4%. In the bottles used in the experiment, measured oxygen concentrations never fell below 8%.

Ten-gram soil samples were weighed into 50-ml beakers for each treatment in eight replicates. These were amended with 0.1 g straw and moisture was added in accordance with the treatment level. The 0-day weights of each beaker were recorded and incubation carried out at  $22 \pm 0.5\text{C}$  as in  $\text{CO}_2$  evolution study. Moisture loss by evaporation was made up frequently by bringing the beakers to 0-day weight by addition of water. Duplicate samples of each treatment were analyzed for inorganic nitrogen at 10, 30, 60, and 120 days of incubation. The inorganic nitrogen derived from straw was distinguished from soil organic matter derived N by analyzing the sample on a mass spectrometer using procedures described elsewhere (4).

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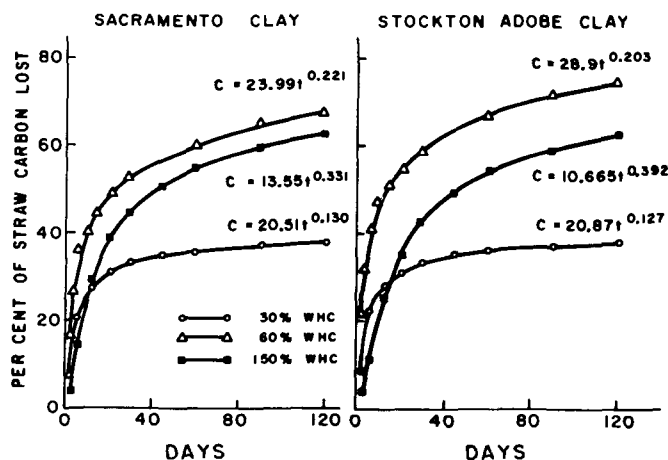


Fig. 1—Percent of straw carbon lost during course of decomposition in soils at different levels of water-holding capacity, WHC. Equations corresponding to each curve fit data for incubation period,  $6 < t < 121$  days.

### RESULTS AND DISCUSSION

Figure 1 is an illustration of the percent of straw carbon loss as a function of time. Initially for 2 weeks, straw carbon loss at 150% WHC was lowest but after that the carbon loss at 150% WHC exceeded the carbon loss at 30% WHC. Carbon loss at 60% WHC remained highest throughout the incubation period in both soils.

Regression lines obtained by log-log plots of data presented in Fig. 1 showed highly significant correlation coefficients of 0.96 or better for time period  $> 6$  days. In linear coordinates these become power functions of the form  $C = km^m$ , where  $C$  is percent of straw carbon loss in time,  $t$  days and  $k$  and  $m$  are constants. These power functions, when differentiated, yield rate functions

$$dc/dt = kmt^{(m-1)}$$

The values of constants  $km$  and  $(m-1)$  are given in Table 1. Values of  $km$  at 60% WHC indicate the highest level of microbial activity at this moisture level. Fractional and negative values of  $(m-1)$  reflect a decrease in the rate of carbon loss as a function of time, which was most pronounced at 30% WHC, followed by 60% and 150% WHC, respectively. The organic carbon balance in soil after 4 months incubation under different moisture regimes is given in Table 2. Three observations are pertinent from these data: (i) carbon loss from soils incubated alone increased with increase in moisture level, (ii) soil carbon loss in straw-amended samples was highest at 60% WHC followed by that at 150% and 30% WHC, respectively, and (iii) the net gain of carbon was highest at 30% WHC followed by 150% and 60% WHC, respectively. Straw carbon retained in soil followed a similar trend.

Table 1—Influence of moisture variables on values of constants,  $km$  and  $(m-1)$  used in rate functions,  $[dc/dt = kmt^{(m-1)}]$

Moisture level	Sacramento clay		Stockton adobe clay	
	% WHC	$km$	$m-1$	$km$
30	2.67	-0.870	2.65	-0.873
60	5.30	-0.779	5.87	-0.797
150	4.49	-0.669	4.18	-0.608

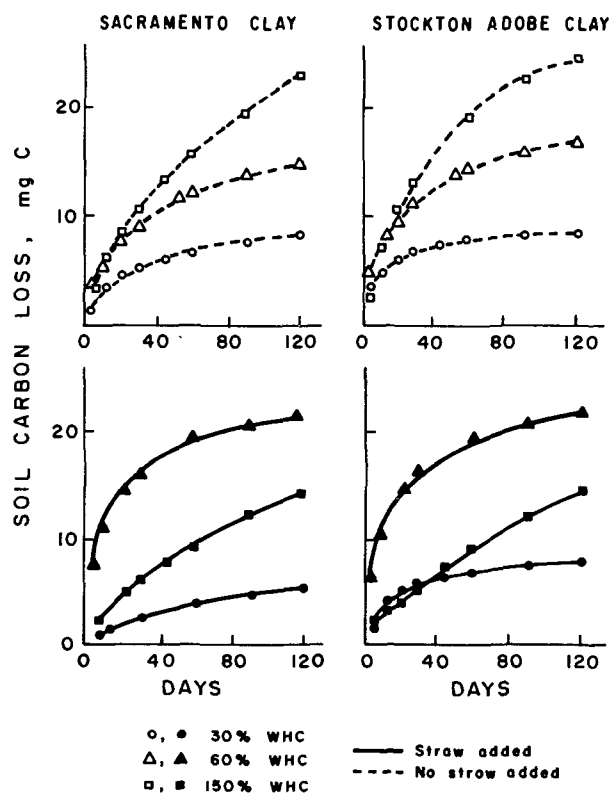


Fig. 2—Cumulative native soil carbon loss as a function of time under different moisture conditions.

The loss of native soil carbon as a function of time under different moisture conditions is depicted in Fig. 2. At 30% and 150% WHC, the soil carbon loss from straw-amended samples was always less than soil controls. At 60% WHC, straw addition accelerated soil-C loss. Thus moisture conditions modified the influence of straw on native soil organic matter loss. From the data of Fig. 2 priming ratios<sup>3</sup> were calculated for different incubation periods (Table 3). Priming ratios are defined as

$$\frac{(\text{soil carbon loss from straw-amended soil})}{(\text{carbon loss from soil alone})}$$

Priming ratios at 30% and 150% WHC were always less than 1.0, while at 60% WHC they were always  $> 1.0$ . At

Table 2—Balance sheet of organic carbon in soils incubated for 4 months under different moisture conditions

Soil	Moisture regime	Carbon added as straw	C lost in 4 months		Not gain (+) or loss (-)	Straw-C retained
			Plant	Soil		
Sacramento clay	30	0	--	8.1	- 8.1	--
		74.6	27.7	5.2	+ 41.7	46.9
	60	0	--	14.5	- 14.5	--
		74.6	50.0	20.9	+ 3.7	24.6
	150	0	--	22.9	- 22.9	--
		74.6	46.2	13.8	+ 14.6	28.4
Stockton adobe clay	30	0	--	8.4	- 8.4	--
		74.6	27.8	7.7	+ 39.1	46.8
	60	0	--	17.0	- 17.0	--
		74.6	55.5	21.2	- 2.1	19.1
	150	0	--	26.9	- 26.9	--
		74.6	46.3	14.0	+ 14.3	28.3

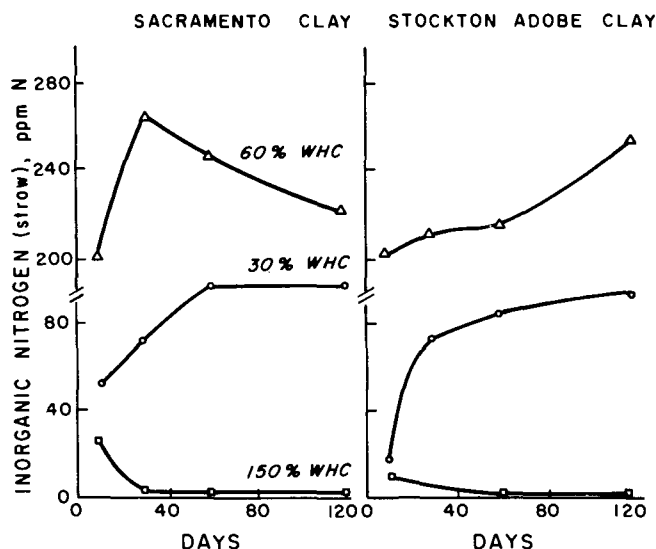


Fig. 3—Influence of moisture levels on the net release and loss of inorganic nitrogen from straw during 4 months' decomposition.

30% WHC, priming ratios increased from 0.32 to 0.64 during 4 months decomposition in Sacramento clay and from 0.57 to 0.92 in Stockton adobe clay. At 60% WHC priming ratios in both soils decreased with time but remained greater than 1.0 throughout 4 months incubation.

It is clear from these data that priming action is positive only when moisture and aeration conditions are near optimum for proliferation of heterotrophic microorganisms active in decomposition of added substrate. Under less than optimum conditions, the decomposition rate of soil organic matter was retarded much more than substrate carbon. Hence at 30% and 150% WHC, a negative priming effect was apparent. It is also evident from these data that the priming effect is large in the beginning and diminishes with the time of incubation as the supply of added substrate decreases.

Native organic matter loss followed kinetics similar to added plant materials under all moisture regimes. Highly significant correlation coefficients ( $0.97 < r < 0.997$ ) were found for straight lines obtained by plotting log (soil carbon loss) versus log (time). The constants  $A'$  and  $m'$  for the general expression for soil carbon loss,  $A't^{m'}$ , are given in Table 4 which hold good for the indicated time periods. Higher values of the constant  $A'$  in straw-treated samples at 60% WHC indicate a higher level of microbial activity. Values of  $A'$  at 30% and 150% WHC were less in straw-amended treatments than in corresponding control soils, as expected.

Table 3—Influence of moisture variables on priming ratios

Soil	Moisture level % WHC	Days of incubation				
		10	30	61	91	121
		Priming ratios				
Sacramento clay	30	0.32	0.52	0.6	0.62	0.64
	60	2.06	1.8	1.59	1.49	1.45
	150	0.63	0.58	0.58	0.61	0.60
Stockton adobe clay	30	0.5	0.84	0.86	0.89	0.92
	60	1.64	1.53	1.40	1.33	1.30
	150	0.50	0.38	0.45	0.50	0.52

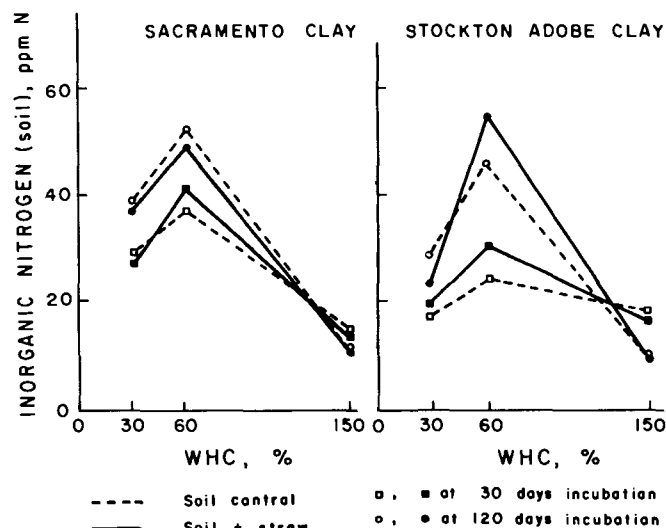


Fig. 4—Soil inorganic nitrogen changes as a function of moisture content with and without incorporation of straw.

It is inferred from Fig. 3 that net mineralization of straw-N was higher at 60% WHC than at 30% WHC. The straw-N mineralized at 150% WHC did not accumulate and was lost from the system, presumably by denitrification. Application of straw did not influence the soil inorganic nitrogen content appreciably at any of the moisture levels. Soil inorganic nitrogen at 30% and 60% WHC increased with length of incubation period, being highest at 60% WHC in 120 days. Soil inorganic nitrogen decreased with time at 150% WHC, due possibly to loss of soil nitrates by denitrification (Fig. 4).

The extent of straw decomposition during 4 months incubation at different moisture levels decreased in the following order: 60%, 150%, 30% WHC. Computation of turnover times as listed in Table 5 involved setting the equations of Fig. 1 equal to 100% and solving for  $t$ . At 30% WHC, biological activity was severely limited by moisture supply and hence the turnover time for straw carbon was very long. At 60% WHC or higher turnover time became even less

Table 4—Values of constants,  $A'$  and  $m'$ , for equations describing soil carbon loss with general expression,  $A't^{m'}$

Moisture level % WHC	Straw loading rate %	Sacramento clay		Stockton adobe clay		Equations fitted to data for time period
		$A'$	$m'$	$A'$	$m'$	
30	0	1.69	0.329	3.63	0.177	30 < t < 121
	1	0.53	0.482	2.41	0.242	
60	0	2.04	0.420	2.64	0.399	6 < t < 121
	1	5.72	0.284	4.70	0.332	
150	0	1.41	0.585	1.62	0.595	13 < t < 121
	1	0.78	0.600	0.36	0.769	

Table 5—Predicted values of straw carbon turnover time under different moisture regimes

Moisture levels % WHC	Nature of priming effect	Time required for priming ratio = 1	
		Sacramento clay	Stockton adobe clay
years			
30	-	5.6	1.5
60	+	5.4	15.3
150	-	$2.8 \times 10^4$	16.1

than 2 years and was less in Stockton adobe clay than in Sacramento clay. It is speculated from these data that under field conditions where moisture availability is ample, straw applied at rates corresponding to 11.2 to 22.4 metric tons ha<sup>-1</sup> year<sup>-1</sup> would not accumulate, whereas under moisture limiting conditions carbonaceous straw materials would likely build up. When moisture limits microbial proliferation even the most readily utilizable components of straw are slowly metabolized. Bartholomew and Norman (2) reported that threshold moisture for straw decomposition was between 10 and 20% for different species. At 30% WHC the moisture contents of Sacramento clay and Stockton adobe clay were 20% and 16% of the soil weight, respectively. These values were close to threshold moisture levels for microbial activity. Recently, Chen and Alexander (3) presented evidence that drought susceptible bacteria can rarely grow in media with low activity of water. It is therefore expected that at 30% WHC only drought resistant flora multiplied and participated in decomposition processes. At 60% WHC, moisture and aeration conditions were close to optimum for microbial activity. High moisture levels such as at 150% WHC reduce microbial activities by hindering movement of air and reducing oxygen supply to microorganisms (1). For about 2 weeks the extent of straw decomposition at 150% WHC on both soils was even lower than at 30% WHC because of limited oxygen supply. Thus the available energy materials in the straw were not lost rapidly at 150% WHC, and therefore a decline in decomposition rate was not as steep as at 60% WHC. There was no evidence of moisture loss from the closed containers at any moisture level.

The microbial population involved in decomposition at 150% WHC consists mainly of bacteria, facultative and obligate anaerobes, in the reduced zone. The growth of aerobic microorganisms is limited under such conditions to the surface zone where oxygen may penetrate. Despite the restriction in oxygen supply to microorganisms in the reduced zone at 150% WHC, the metabolism of substrate remained more rapid than at 30% WHC. The predicted values of turnover time were lower at 150% WHC than at 60% WHC in both soils. This extrapolation is in concordance with higher values of exponent *m* (Fig. 1) at 150% WHC than at 60% WHC.

Under moisture conditions when biological decomposition of added straw was limited by either low moisture or oxygen supply the soil-C loss from straw-amended soils was invariably lower than respective soil controls. An attempt to find the time required for priming ratio to reach unity was made by extrapolation of parabolic functions fitted to data presented in Fig. 2 for each moisture level. The predicted length of time periods when the priming effect is completely overcome are abstracted in Table 6. The calculated value in Sacramento clay at 150% WHC for the time required to overcome the negative priming effect was impossibly long. At 30% and 150% WHC the negative effect was overcome in lesser time in Stockton adobe clay than in Sacramento clay. It is concluded from these findings that moisture conditions influence the nature, magnitude and duration of priming effect. It is noteworthy that straw-C at 150% WHC was calculated to be lost com-

Table 6—Time required for priming ratios to reach unity at different moisture levels

Moisture level	Straw-C added	Turnover time	
		Sacramento clay	Stockton adobe clay
% WHC	mg	years	
30	74.6	537	623
60	74.6	1.75	1.24
150	74.6	1.15	0.83

pletely within about a year in both soils (Table 5) but the depressive effect of straw on soil organic matter loss persisted much longer. This points up the need for caution in extrapolating too far beyond the measured data. The heterotrophic microflora developed by application of straw competed for available moisture and oxygen with those involved in decomposition of soil humus. Hence, the native soil-C loss was lessened by straw application at 30% and 150% WHC. When the supply of both moisture and oxygen was ample such as at 60% WHC, the heterotrophic activity induced by added straw complemented the activity of microflora that were effective in decomposing native soil organic matter.

At 60% WHC, C-inputs as straw roughly equalled C-output as CO<sub>2</sub>. A net gain of C in soils was evident after 4 months incubation at 30% and 150% WHC. Considerable amounts of straw-C remained in soil under all moisture conditions, being highest at the lowest moisture level. More straw-C was retained in Sacramento clay than in Stockton adobe clay at each moisture level, possibly because of higher clay content in the former than in the latter. Also aerobic populations of microorganisms assimilate more substrate carbon than anaerobic ones (6).

At 150% WHC, about 62% of straw-C was oxidized to CO<sub>2</sub> level during 4 months incubation. However, there was negligible accumulation of mineralized nitrogen in either soil at this high moisture level. It is well known that ammonification proceeds in wet submerged soils with degradation of complex organic molecules even when oxygen supply is low. Under partially aerobic conditions such as at 150% WHC, NH<sub>4</sub>-N may be oxidized to NO<sub>3</sub>-N in the microenvironment near the surface. The nitrate thus produced may be utilized by facultative anaerobes as a terminal electron acceptor when their demands for oxygen are not fully met. Heterotrophic denitrifiers derive energy and carbon by oxidation of available energy materials. Thus application of straw at 150% WHC made conditions ideal for energy-requiring denitrification processes. It is, therefore, concluded that mineralized straw-N at 150% WHC was subject to nitrification which was simultaneously accompanied by denitrification. This is in keeping with findings of Bremner and Shaw (5) where they reported that the critical moisture level for denitrification was 60% WHC. At 100% WHC, they measured a loss of 50% of the added nitrate by denitrification within 3 weeks.

Undoubtedly moisture-level is a critical environmental factor in determining soil and straw carbon loss and nitrogen release. The fate of mineralized nitrogen is also very much dependent on moisture level. Where rice residues are managed by soil incorporation, moisture availability must be taken into account for decomposition processes.

On Sacramento clay and Stockton adobe clay in California where most precipitation is received during winter season rice straw may be incorporated right after the crop harvest before the onset of rainfall for effective decomposition. Otherwise, it may be incorporated before the planting of rice where adequate moisture is available and gas formation is not a problem. Each time of incorporation has its own limitations and would depend on several environmental and management factors.

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