Winter Flooding of Harvested Rice Fields
Alters Soil and Rice Straw Enzymatic Activity and Soil Microbial Community Structure

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ABSTRACT

Winter flooding of rice fields can manage rice straw and weeds, and provide a habitat for migratory waterfowl. This study was conducted on a Tunica clay to assess the interactions of flooding and tillage on soil/ straw enzymatic activity and soil microbial community structure as measured by total fatty acid methyl ester (FAME). A split plot design was used consisting of 14 weeks of flooding (November to February) or non-flooded as mainplots, and rice straw remaining on the soil surface or incorporated by tillage as subplots. Straw sampled from flooded plots had higher fluorescein diacetate (FDA) hydrolysis and β-glucosidase activity during flooding compared to straw from non-flooded plots. FDA hydrolysis and dehydrogenase, were greater in flooded plots compared to non-flooded plots with no effect of tillage. Effects of flooding on β-glucosidase activity in soil were inconsistent. PCA analysis of total FAMEs indicated that the soil microbial community was altered in response to flooding but not tillage. In flooded plots 26% of the original rice straw remained, while in non-flooded plots 51% of the rice straw remained. The stimulation of microbial activity and altered soil microbial communities can contribute to enhanced rice straw degradation under flooded conditions.

MATERIALS & METHODS

The experiment was conducted on a Sharkey clay, Mississippi State DREC, Stoneville, MS, previously cropped to rice. A split plot design of flood and non-flood (main plots) and tilled and not tilled (subplots) replicated in four blocks, was used. Tillage incorporated rice residues to a depth of 15cm and water levels in flooded plots were maintained at 15 cm from November 7th to February 14th. Initial pre-flooding soil samples (0-2.5 cm) were taken on November 6, and soils sampled monthly through April. Soil and straw enzyme activity was determined within 48 hours or sampling, and FAME analysis was conducted on samples stored at -80ºC. Rice straw residues was determined from a meter square in each plot in November and again in April prior to soybean planting. Soil and straw samples were dried (60ºC) and weighed to determine moisture content and mass of straw residues.

ENZYME ACTIVITY
* Fluorescein Diacetate (FDA) Hydrolysis (esterases, lipases etc.) determined on both soil and rice straw (Schnurer & Rosswall, 1978).
* Triphenyl Tetrazolium Chloride (TTC) Dehydrogenase - Assayed on soil with yeast extract (Weaver et al. 2004).
* β Glucosidase assayed using p-nitrophenyl-β-D-glucoside (Eivazi & Tabatabai, 1988) on soil and rice straw

MICROBIAL COMMUNITY STRUCTURE
A protocol similar to Shutter and Dick (2000) was used. Methyl-ester linked fatty acids were extracted from soil with 0.2 N methanolic KOH at 37 ºC. After incubation, contents were neutralized and FAMEs extracted in hexane, and concentrated. FAMEs were identified and quantified using an Agilent 6890 gas chromatograph and the MIDI EUKARYOTE protocol. Microbial community structure was assessed using PCA on FAMEs present in > 50% of the samples and > 0.5% molar percent. Following PCA, the contributions of flooding and tillage and interactions between flooding and tillage on PCs were analyzed using SAS PROC MIXED. Pearson's correlations determined FAMEs contributing to the principal components.

Winter flooding of experimental rice plots

Table 1. Effects of winter flooding and tillage on soil fluorescein diacetate hydrolytic activity*

Treatment	11-05	12-05	01-06	02-06	03-06
Flooded	5.7	51.3	50.8	56.1	27.6
No-flood	8.4	18.4	21.7	29.3	26.3
Tilled	7.2	29.1	37.4	44.9	30.0
Not-tilled	6.9	40.5	35.0	40.5	26.9
Pr > F					
Flood	0.127	<0.001	<0.001	0.054	0.804
Till	0.826	0.134	0.736	0.647	0.994
Flood x Till	0.917	0.516	0.083	0.417	0.994

* nmole fluorescein formed g^{-1} soil h^{-1}

Table 2. Effects of winter flooding and tillage on soil TTC-dehydrogenase* activity

Treatment	11-05	12-05	01-06	02-06	03-06
Flooded	60	69	485	274	211
No-flood	65	60	75	193	115
Tilled	63	64	284	241	148
No-Tilled	61	64	275	225	178
Pr > F					
Flood	0.684	0.493	<0.001	<0.001	0.001
Till	0.870	0.994	0.832	0.152	0.254
Flood*Till	0.492	0.753	0.579	0.906	0.801

* nmole Triphenyl formazan formed g^{-1} soil h^{-1}

Table 3. Effects of winter flooding and tillage on soil β-glucosidase activity

Treatment	Jan 06	Feb 06	Mar 06
Flooded	86	114**	78
No-flood	81	70	112***
Tilled	67	93	100
No-Tilled	99*	90	91
Pr > F			
Flood	0.429	0.003	0.001
Till	0.024	0.766	0.584
Flood*Till	0.057	0.775	0.518

* nmole p-nitrophenol released g^{-1} soil h^{-1}

Fig 1. Effects of winter flooding on straw FDA and β-glucosidase activity

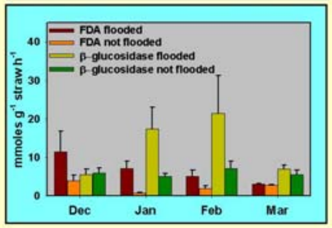
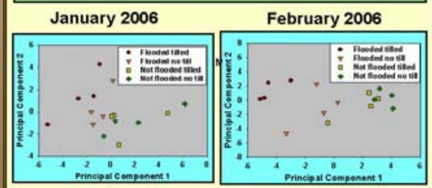


Table 4. Contribution of flooding and tillage to principal component analysis of soil microbial community structure

	12-05	01-06	02-06	03-06
PC1		Pr > F		
Flood	0.996	0.023	0.003	0.276
Till	0.406	0.373	0.029	0.135
Flood x till	0.447	0.366	0.336	0.006
PC2				
Flood	0.254	0.063	0.894	0.097
Till	0.573	0.501	0.349	0.606
Flood x till	0.098	0.416	0.075	0.299

Fig 2. Effect of flooding / tillage on FAME based soil microbial community structure



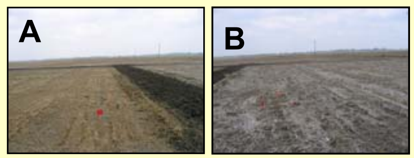


Fig 3. Fields in April 2006
A: Non Flooded Plot B: Flooded Plot

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RESULTS

Soil Enzyme Activity

- Flooding significantly increased soil FDA activity (Table 1), however FDA hydrolysis returned to non-flooded levels on flood removal. There was no significant effect of straw incorporation on FDA activity in soil.
- Likewise flooding increased TTC-dehydrogenase activity (Table 2), however this did not occur until January to March, again there was no effect of straw incorporation on soil dehydrogenase activity
- Mixed effects of flooding on soil β-glucosidase activity were found higher activity was observed in flooded soil in only February, while activity was greater in non flooded plots in March after removal of the flood.
- Rice straw FDA hydrolysis was significantly greater (Pr >0.05 to Pr >0.01 level) from 12-05 to 02-06 (Figure 1), but not different when the flood was removed in March.
- β-glucosidase activity was significantly greater in flooded rice straw in January and February compared to non-flooded straw (Fig 1) and levels returned to similar levels when the flood was removed.

Soil Microbial Community Structure

- Significant effects of flooding on soil microbial community structure based on Total FAMEs were observed two months after flooding and were not found on removal of the flood (Table 4, Fig 2a,b). Tillage had only minor effects on PC1 in 02-06 (Fig 2b)
- Although in January and February different FAMEs contributed to PC1 and PC2, mostly monounsaturated and terminally branched FAMEs positively correlated with PC1 and eukaryotic FAMEs (18:2w6c 20:5w3c, and 16:1w5c) negatively correlated with PC1 and PC2.

Rice Straw Degradation

- A 14 week flood reduced rice straw residues mass. In flooded plots 26% of the original rice straw remained, compared to 51% in non-flooded plots (Fig 3).

CONCLUSIONS

Flooding significantly effected soil and rice straw hydrolytic enzymes and soil dehydrogenase activities indicating a more active microbial population under flooded conditions. Likewise flooding altered soil microbial community structure. Similar effects of flooding on altering the soil and rice straw microbial community have been observed in California and Japanese field studies. Such shifts in microbial activity and populations may be key to enhanced microbial straw degradation potential under flooded conditions.

Winter flooding may alleviate the need for burning of rice straw, provide alternative income (Duck hunting) and improve environmental quality for Mississippi Delta rice farmers.

Sustainable rice production