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Microbial priming for *in situ* management of paddy straw and its effects on soil microbiological properties under rice-wheat cropping system

AJAY KUMAR[1], SURENDER SINGH[2], YASHBIR SINGH SHIVAY[3], SHRILA DAS[4], MADAN PAL[5] and LATA NAIN[6]

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

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ABSTRACT

The rice-wheat cropping sequence is the major cropping system of India, which generates large quantities of crop residues. The disposal of paddy straw becomes a major concern in the rice belt of India, as it is burnt to clear the field for succeeding wheat crop. Incorporation of the residues in soil is not feasible because of insufficient time between harvesting of the rice and sowing of wheat, and immobilization of the nitrogen, which causes a reduction in the subsequent crop yield. Microbial interventions to accelerate the degradation may be a viable option for the effective management of farm residues. In this study, a field experiment including three treatments: residues removal (absolute control) and straw retention (@3t/ha) with/without inoculation of fungal consortium was undertaken at the farm of Indian Agricultural Research Institute, New Delhi during 2017-18. Application of fungal consortium (*Coprinopsis cineria* LA2 and *Cyathus stercoreus* ITCC 3745) alongwith straw incorporation resulted in a significant increase in the population of microbes. Higher activities of dehydrogenase (8.13 µg TPF /g/d), Carboxymethyl cellulase (0.46 IU/g of soil/d), xylanase (0.06 IU/g of soil/ d), FDA hydrolase (4.31µg fluorescein released /g/h) and alkaline phosphatase (242.98 µg PNP/g/h) enzymes were recorded in the soil of fungal consortium treated plot. Microbial intervention in residue management increased CO_2 emission two to three fold within 15-60 days which indicates the role of inoculation in hastening the degradation process. Therefore, microbial priming can be a suitable option for *in situ* management of paddy straw.

Key words: Biodiversity, Fungal consortium, *In situ* incorporation, Microbial activity, Rice straw

Rice-wheat cropping system is India's dominant cropping system covering 10 mha area in the Indo-Gangetic plains (IGP) and contributing 50% to the total rice production (Singh and Sidhu 2014). About 75% of the rice grown is harvested mechanically in north-western parts of the IGPs, which leads to substantial amount of paddy stubbles remaining in field which interfere with sowing of wheat crop and most of the times burnt in the field. This practice appends to air pollution, increases soil erosion and decreases the efficacy of soil applied herbicides such as isoproturon (Singh and Nain 2014, Walia *et al.* 1999). Recently, incorporation of the crop residues in field became popular as it helps to maintain the nutrient status of the soil and improves the soil quality by maintaining the soil physical, chemical and biological properties. However, it also significantly increases the release of the greenhouse gases, thereby negatively affect seedling emergence and tillage (Bhatia *et al.* 2005). It also needs longer time for the degradation of paddy straw in the field. Moreover, observations of long term experiments indicate that though incorporation of rice straw in soil improves soil health significantly, it decreases the subsequent crop yields due to production of microbial phytotoxins and immobilization of the available nitrogen (Sidhu and Beri 1989, Beri *et al.* 1995, Dhiman *et al.* 2000, Mahajan and Gupta 2009).

Application of an efficient microbial consortium to soil (microbial priming) which can hasten the degradation of paddy stubbles in field may solve the problems of small farmers. In this study, a consortium of lignocellulolytic fungi was used as inoculum to accelerate the degradation of the paddy straw. Although many research groups have addressed the issue of residue incorporation/retention and its long term effect on soil physical, chemical and biological health parameters, information on the effect of microbial priming on soil microbial and biochemical processes is lacking. Moreover, the dynamics of various hydrolytic enzymes involved in the degradation of rice residues in field

[1]Present address: [2]Associate Professor (ssriari@gmail.com), Department of Microbiology, Central University of Haryana; [1]PhD Research Scholar (akjiari@gmail.com), Division of Microbiology [3]Principal Scientist (ysshivay@iari.res.in), [5]Chief Technical Officer(madanpal.sirohi@yahoo.in), Division of Agronomy; [4]Scientist (shrila34040@gmail.com), Division of Soil Science and Agricultural Chemistry; [6]Principal Scientist (latarajat@yahoo.co.in), Division of Microbiology, ICAR-IARI.

condition, is not clear. Therefore, this study addresses the problem of *in situ* management of paddy straw and effect of microbial priming on soil microbial population and enzyme activities involved in the degradation process.

MATERIALS AND METHODS

Microbial cultures: Two hyper lignocellulolytic indigenous fungi namely *Coprinopsis cinerla* LA2 and *Cyathus stercoreus* ITCC 3745 were selected for *in situ* degradation of crop residues on the basis of their colonization potential on wheat/paddy straw (Gaind *et al.* 2007, Nain *et al.* 2018). Inoculums of both the fungi were raised separately at 30°C for 7 days on sorghum grains, mixed in 1:1 ratio and applied @3kg/ha.

Experimental location and climate: The experiment was conducted in the rice-wheat cropping system at Agronomy research field, Indian Agricultural Research Institute, New Delhi during 2017-18, located in 28.63° N latitude, 77.15° E longitude and situated at an altitude of 216 m (709 ft) m above mean sea level. Details of the soil characteristics have been described in the Table 1.

Experimental setup: Rice variety Pusa Basmati 1509 was the preceding crop grown in the field. The crop was harvested mechanically and the stubbles (approx. 40-50 cm) along with all the residues were left in the field. Discing followed by rotavator was used for field preparation, after the application of fungal inoculum. The following three treatments [Residue removal (T1) (Absolute control), Residue retained (T2) @3 t/ha and Residue + fungal consortium (T3)] were maintained in a plot size of 13 m × 5 m. A basal dose of N @ 30 kg/ha was applied to the straw along with fungal inoculum to avoid nutrient immobilization. Soil samples were collected with a hand auger from a depth of 0-10 cm from different locations before and after treatment at different time intervals (0, 7, 15, 30 and 60 days). Ten random samples from each replication were pooled together to get a composite sample and analyzed in triplicate. After 25 days of inoculum application, wheat variety HD 2967 was sown in the field with recommended dose of N, P and K (120:60:60 kg/ha) fertilizers.

Enumeration of culturable microbial population: Culturable population of bacteria, fungi, actinobacteria and other functional groups in the treatments was enumerated by serial dilution method, followed by plating on selective media. Nutrient Agar for bacteria, Rose Bengal Agar for fungi, Kenknight and Munaier's Agar for actinobacteria, Pikovskaya's medium for Phosphate solubilizers (Atlas 2010) and CMC agar (Teather and Wood 1982) for cellulose degraders were used for enumeration. The plates were incubated at 30°C for 1-4 days and the colonies counted and presented as cfu/g of soil.

Table 1 Properties of the soil

Parameters	Value
Soil texture	sand 15%, silt 38% and clay 47%
pH	7.86
Electrical conductivity	0.113 ds/m
Total Nitrogen	307.5 Kg N/ha
Total Phosphorus	58.99 Kg P/ha
Total Potassium	368.11 Kg K /ha

Quantification of soil biological parameters: Dehydrogenase activity was determined following the method of Casida Jr *et al.* (1964) using TTC (3%) as a substrate and expressed as µg TPF/ g/d. The β-glucosidase activity was estimated using p- nitrophenyl β- glucopyranoside (p-NPG) as the substrate and expressed as µmoles of p-nitrophenol released/ g soil/h (IU/g soil) (Eivazi and Tabatabai 1988). The cellulase and xylanase enzyme activities were determined using carboxy methyl cellulose and xylan as substrate and reducing sugars were estimated by the methodology of *Nelson-Somogyi* (Deng and Tabatabai 1994, Nelson 1944) and DNSA method (Miller 1959, Kanazawa & Miyashita 1986) respectivily. Both enzyme activities were expressed as IU/g soil/d. Alkaline phosphatase activity (µg p-nitrophenol/g soil/h) was determined using p-nitrophenyl phosphate as the substrate and amount of p-nitrophenol released was recorded by estimating the absorbance at 440 nm (Tabatabai and Bremner 1969). Basal soil respiration was estimated through carbon dioxide evolution method (Stotzky 1965, Anderson 1982). FDA hydrolysis was estimated by using fluorescein diacetate as substrate and the amount of fluorescein released was quantified recording absorbance at 490 nm. FDA hydrolase activity was represented as µg of fluorescein released/g soil/h (Green *et al.* 2006).

Statistical analysis: The data were analyzed statistically by using randomized block design (RBD) as outlined in Panse and Sukhatme (1954) and the test of significance was done at the 5% level.

RESULTS AND DISCUSSION

An experiment was conducted in a field with a history of rice-wheat cropping system to study the effect of straw management practices (straw removed, straw retention and microbial inoculation on paddy stubbles) on soil microbial dynamics and hydrolytic enzyme activities. Several microbial activities parameters were also estimated in the soil collected from field at different intervals of time (0 to 60 days) to analyse the progress of straw degradation in field.

Microbiological analysis of soil samples

Total heterotrophic microbial population: The populations of soil microorganisms such as bacteria, fungi, actinobacteria, cellulose degraders and phosphorous solubilizing bacteria were quantified. The result revealed an increasing trend in the bacterial population in each treatment at 7th and 15th day of sampling (Fig 1). The bacterial population significantly increased and was found to be highest in the fungal consortium treated plot at the 15th (8.82 log cfu/g soil) and 30th day (8.95 log cfu/g soil). Fungal population recorded significantly higher values in

the fungal inoculated plot compared to the residues removed and retained plot. At the 30th day after treatment, fungal population was the highest (4.93 log cfu/g soil) in fungal inoculated + residue retained plot followed by residue retained plot (4.79 log cfu/g soil), while lowest population was recorded in residue removed plot (4.69 log cfu/g soil). An increasing trend in the actinobacterial population was observed between 7 to 15 days in all the treatments. However, among the three treatments fungal treated plots showed significantly higher population compared to others treatment at the 7th day (5.25 log cfu/g soil) and 15th day (5.19 log cfu/g soil). The population of phosphate solubilizing microorganisms (PSB) was also enumerated (Fig 2). A declining trend with time was recorded, however, the population of PSB was found to be highest at the 30th day. A significant difference in the population of cellulose degraders was recorded and highest population was recorded in fungal treated plot (5.12 log cfu/g soil) indicating the proliferation of inoculated fungi on paddy stubbles (Fig 2).

Soil analyses revealed that the population of the microorganisms increased due to the microbial priming; this resulted in an enhancement in the rate of the decomposition of the rice residues between 7-15 days after inoculation. Sannathimmappa *et al.* (2015) treated rice straw with combination of cow dung slurry @5% + *Trichoderma harzianum* @5 kg/ha + *Pleurotus sajor caju*@5 kg/ ha and recorded significant influence on straw degradation as well as concomitant increase in N- fixing and P- solubilizing microorganisms in the soil. The overall bacterial population ranged between 1.8×10^8 to 8×10^8 cfu/g of soil in their study.

Soil microbial activity parameters: Soil microbial activities are considered as indices of microbial proliferation in the soil. Microorganisms present in the soil produce extracellular enzymes, which mineralize organic matter and release carbon dioxide and other nutrients in forms which can be assimilated by microbes. Dehydrogenase is an oxido-reductase enzyme present in all viable microbial cells. This enzyme is considered as a sensitive indicator of soil quality (Nannipieri 1994) and a valid biomarker to indicate changes in total microbial activity (TMA) due to changes in soil management practices (Ceccanti *et al.* 1993, Roldan *et al.* 2004). An increasing trend with time was recorded in dehydrogenase activity in all the three treatments, but fungal inoculated soil showed the highest dehydrogenase activity (8.13 µg TPF/ g /d) after 15 days among all the three treatments. Although, dehydrogenase showed an increasing trend up to 60 days, this may be due to rhizospheric effect of wheat crop (Fig 3a). FDA hydrolase activity generally indicates the overall status of extracellular enzymes and highest FDA hydrolase (4.31µg fluorescein released/g/h) was recorded in treatment T3 (straw inoculated with fungal consortium) at 15 days, indicating fast degradation of straw (Fig 3b). This may be due to higher microbial activities in residue retained plot because of active degradation of paddy straw by the inoculated fungal consortium.

Carboxymethylcellulase (CMCase) and xylanase enzymes which act on cellulose and hemicellulose components of straw significantly increased in the sample from the fungal inoculated plot. The cellulase activity increased gradually from 0 to 60 days in all the three treatments. The highest CMCase activity was recorded in the sample from the fungal inoculated residues at the 15, 30 and 60 days (0.11, 0.25, 0.46 IU/ g soil/ d). This may be due to greater production of cellulolytic enzymes by the fungal consortium (Fig 4). The xylanase activity also showed the increasing trend from 0 to 60 days. The highest xylanase activity was recorded in fungal treated

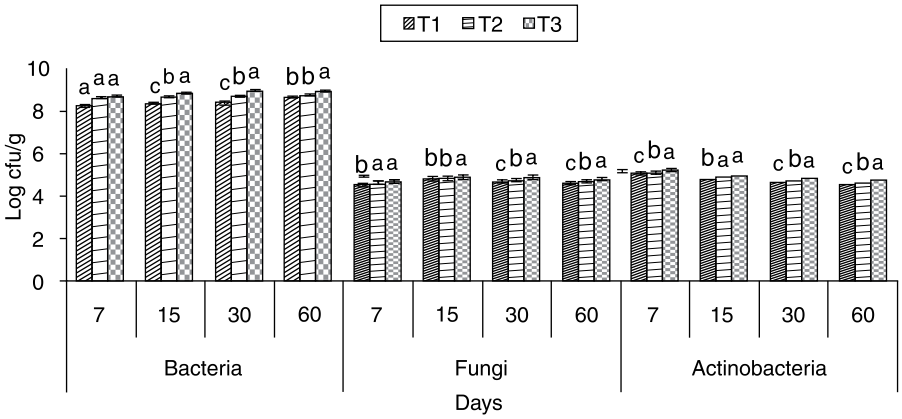

Fig 1 Population of soil bacteria, fungi and actinobacteriaas affected by straw management practices. T1: Straw removed, T2: Straw retained, T3: Straw retained + microbial inoculation.

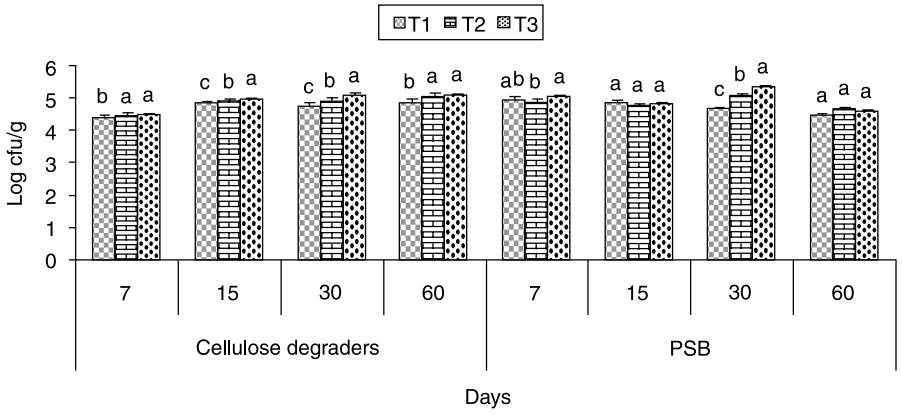

Fig 2 Population of cellulose degraders and phosphate solubilizing bacteria (PSB) as affected by straw management practices. T1: Straw removed, T2: Straw retained, T3: Straw retained + microbial inoculation.

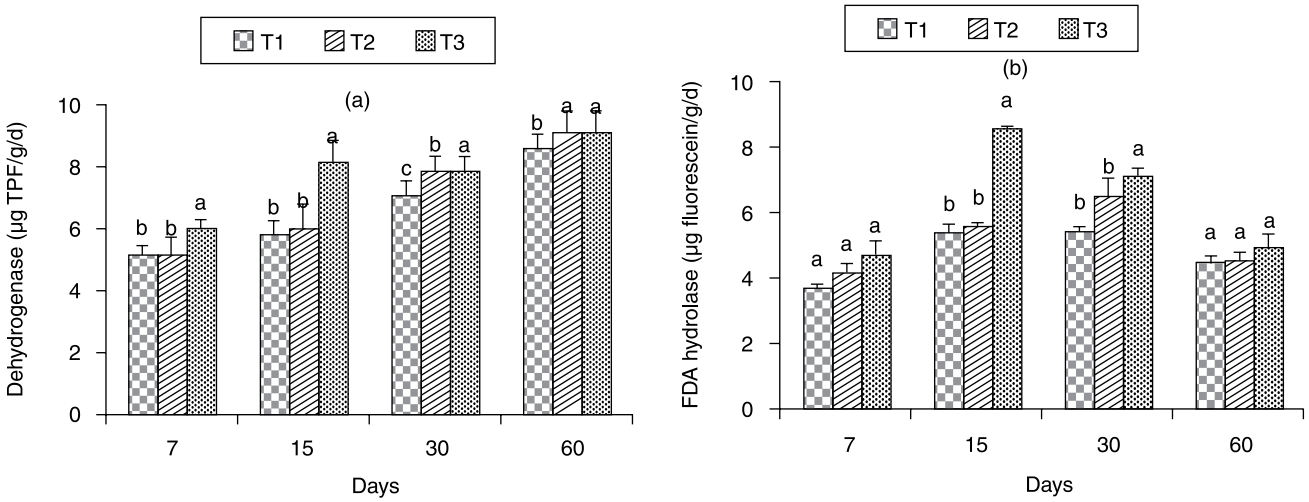

Fig 3 Effects of straw management practices on soil microbial activities (a) Dehydrogenase activity and (b) FDA hydrolase activity. T1: Straw removed, T2: Straw retained, T3: Straw retained + microbial inoculation.

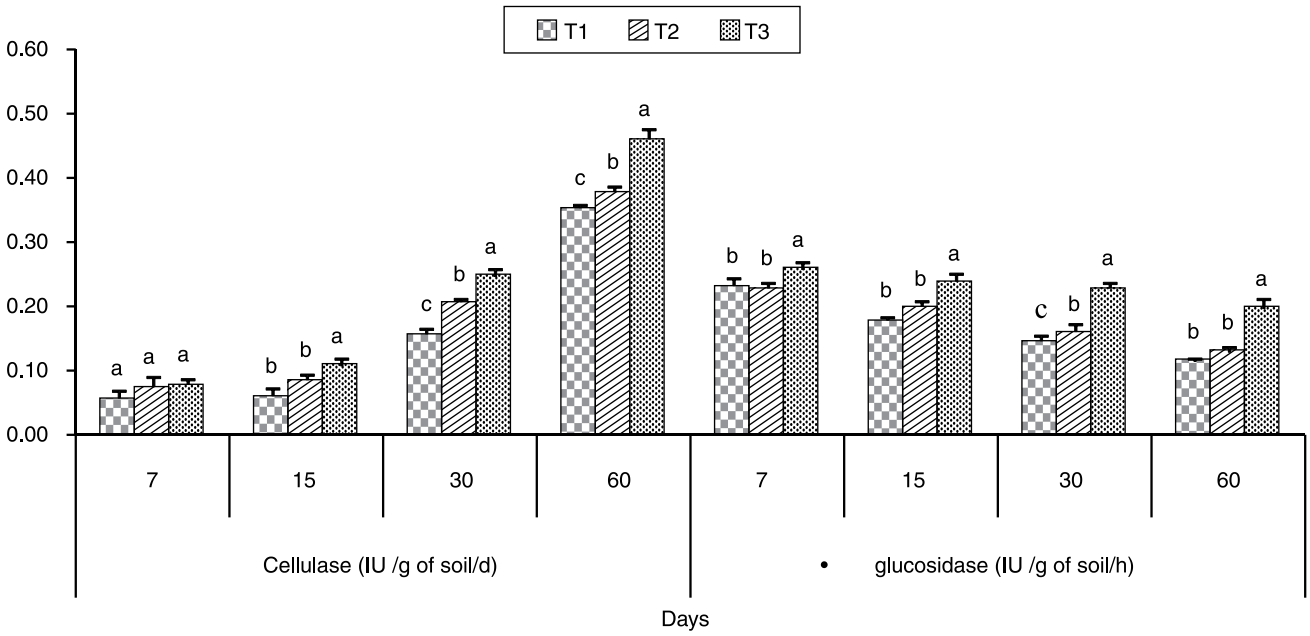

Fig 4 Effects of straw management practices on soil hydrolytic enzyme (cellulase and β glucosidase). T1: Straw removed, T2: Straw retained, T3: Straw retained + microbial inoculation.

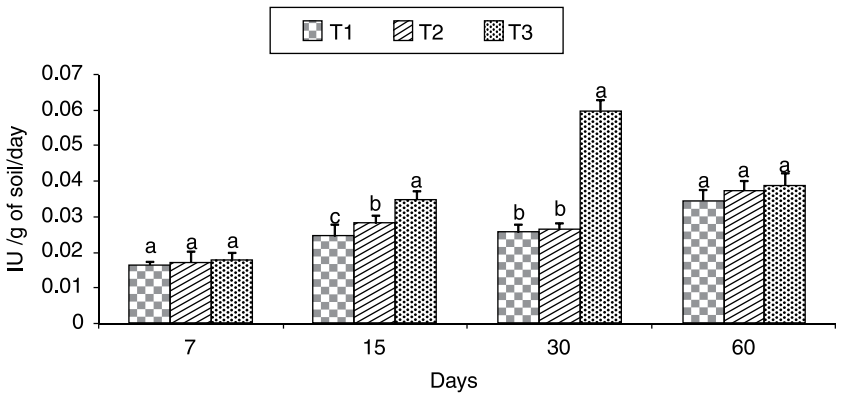

Fig 5 Effects of straw management practices on soil xylanase activity. T1: Straw removed,T2: Straw retained, T3: Straw retained + microbial inoculation.

plot (0.06 IU /g of soil/day) on the 30th days (Fig 5). β-glucosidase enzyme is an important indicator of the ability of a given soil ecosystem to degrade crop residues as it provides simple sugars for the heterotrophic microbial population (Stott *et al.* 2010). β-glucosidase activity was significantly reduced from 0 to 60 days after treatment. The reduction in β-glucosidase activity may be due to decreasing concentration of cellobiose, as a result of exhaustion of undegraded residues with time. Highest β glucosidase activity was recorded in microbes treated plot followed by residue retained

Table 2 Effects of straw management practices on alkaline phosphatase activity (µgPNP/g/h) in soil

Treatments	Days			
	7	15	30	60
T1: (Straw removed)	164.12	169.94	145.76	69.44
T2: (Straw retained)	171.13	221.36	156.33	74.01
T3: (Straw retained+ microbes*)	179.04	242.98	225.37	73.84
SEm±	1.08	1.81	3.30	1.27
CD (P=0.05)	12.5	21.96	50.93	NS

*CoprinopsiscineriaLA2 and Cyathussterocoreus ITCC 3745

Table 3 Effects of straw management practices on carbon dioxide evolution (mg CO_2 evolved/100g soil/day) from soil

Treatments	Days			
	7	15	30	60
T1: (Straw removed)	4.03	6.23	6.60	7.70
T2: (Straw retained)	4.40	6.31	6.97	9.17
T3: (Straw retained + microbes*)	5.02	11.73	20.90	25.50
SEm±	0.42	0.34	0.45	1.10
CD (P=0.05)	NS	1.19	1.62	1.86

*CoprinopsiscineriaLA2 and Cyathussterocoreus ITCC 3745

treatment whereas lowest β -glucosidase activity was recorded in residue removed treatment at all the sampling intervals (Fig 4).

The activities of alkaline phosphatases are mostly of microbial origin (Tabatabai and Bremner 1969, Frankenberger and Dick 1983). Therefore, this enzyme can be used as an indicator for short term changes in microbial activities. Alkaline phosphatase activity was significantly higher in microbial inoculated field as compared to residues retained and removed at 15 and 30 days (242.98 and 225.37 µgPNP/g/h respectively). However, the activity of alkaline phosphatase was gradually increasing up to 15 days. This may be due to increase in the available phosphorus content of the soil (Table 2). Two to three fold increase in CO_2 evolution was recorded in fungal inoculated residue plot from 15-60 days of treatments (Table 3). This may be due to higher microbial population and better colonization of the fungi inoculated in T3 leading to increase in degradation as well as more release of CO_2 due to metabolic surge.

Correlation analyses: The results of this study revealed significant role of inoculated microbes in accelerating the degradation of the paddy straw. Correlation analysis also confirmed these observations. After 15 and 30 days of inoculation, bacterial population positively correlated with the activities of dehydrogenase (r=0.98), FDA hydrolase (r=0.98) cellulase (r=0.95) xylanase (r=0.99), β-glucosidase (r=0.99) and CO_2 evolution (r=0.97). Similarly the fungal population showed a positive correlation with the dehydrogenase (r=0.96), FDA hydrolase (r=0.95), cellulase (r=0.97), xylanase (r=1), β-glucosidase (r=0.98) activities and CO_2 evolution (r=0.94). Actinobacteria population also highly correlated with the alkaline phosphatase (r=0.98), cellulase (r=0.88), xylanase(r=0.77) activities. The population of cellulose degrading microorganisms positively correlated with the dehydrogenase (r=0.98), FDA hydrolase (r=0.98), cellulase (r=0.94), xylanases (r=0.99), glucosidase (r=0.99) activities and CO_2 evolution (r=0.97). The analyses also confirmed the important role of inoculated microbes in the accelerated degradation of the rice residues. All the five enzyme activities significantly correlated with the microbial population, reflective of active degradation and CO_2 evolution from paddy straw mediated by the fungal consortium.

The management of paddy straw residues and stubbles after the harvesting of the rice crop is becoming a major problem among the farmers. In the north-west part of India, farmers are left with no option, except burning of the residues in field as there is less time gap between the harvesting of the paddy and sowing of wheat in November. Burning is responsible for the air pollution and health problems. Our study illustrates that microbial priming can assist *in situ* residues management, and can be an effective alternative to hasten the degradation rate of the residues and improve soil physico-chemical and biological properties. All the enzyme activities were found to be more pronounced in the fungal inoculated treatment as compared to the other plots. Microbial intervention in residues management also increased the metabolism and CO_2 evolution, which validates the role of inoculated microbes. In future, microbial mediation coupled with mechanical intervention, comprising provision for uniform application of microbial formulations may help in the complete degradation of retained straw in field within 15-20 days of straw incorporation. Such an intervention in an environmentally friendly manner obviates the need for burning of valuable crop residues.

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