IMPACT OF *IN-SITU* PADDY STRAW BURNING ON SOIL ENZYME ACTIVITY, SOIL MICROBIAL POPULATION AND GREEN HOUSE GAS EMISSIONS IN A SANDY LOAM SOIL.

ABSTRACT

The rice - maize cropping system is one of the predominant cropping systems in Telangana state, which also generates large quantities of crop residues, whose disposal is of major concern. A large quantity of paddy straw is burnt on the farm to clear the field for succeeding crop. Burning of crop stubble causes air pollution and lead to loss of huge biomass, *i.e.* organic carbon, plant nutrients, the entire amount of C. This study was undertaken to investigate the impact of paddy straw burning on soil biological properties (enzyme activities, microbial population) and to know the amount of GHG emissions released due to burning of residue, under two tillage systems *viz.*, no tillage and conventional tillage. Results indicated that there was a significant decrease in soil microbial population, dehydrogenase activity (41.52 % in NT and 40.07 % in CT), acid and alkaline phosphatase activity and urease activity in soil due to residue burning. It also indicated that there was a rise in CO_2, CH_4 and N_2O emissions 48 hours after residue burning.

Key words: Paddy straw burning, Soil enzyme activity, Microbial Population, GHG emissions, Eta statistic

INTRODUCTION:

Rice is the most residue producing crop in Asia (826 million tons) contributing 84% of total production of the world. Traditionally, rice straw is removed from fields for use as cattle feed and other purposes in South Asia. On average, rice crop residues contain 0.7 % N, 0.23 % P and 1.75 % K. Therefore, the amount of NPK contained in rice crop residues produced is about 22.3×10^6 and 26.26×10^6 t year^{-1} in Asia and the world, respectively (Goswami *et al.*, 2019). Bacterial and fungal populations are decreased immediately and substantially in the top 2.5 cm of the soil because of residue burning. However, additional resources such as water, nutrients and bio-inoculum are required for promoting decomposition of crop residue under in situ decomposition (Thakur *et al*., 2019).

Rice straw is produced as a by-product of rice production at harvest. Rice straw is removed with the rice grains during harvest and it ends up being piled or spread out in the field depending if it was harvested manually or using machines. Ratio of straw to paddy ranges from 0.7-1.4 depending on the variety and growth. This continues to rapidly increase due to shorter turnaround time required for intensified rice cropping. The introduction of game-changing combine harvesters which solves the high labour cost associated with manual straw collection addresses only half the battle. Straw incorporation in soil for fertilization in intensive systems is also not possible with two to three crops per year because the turnaround time is too short for decomposition, resulting to poor soil fertilization properties which ultimately hinders crop establishment. With little options, open-field straw burning has increased dramatically over the last decade. (Bhuvaneshwari *et al*., 2019).

From the farmers' point of view, burning may be seen as the most suitable method of disposing of rice straw. It is not only a cost-effective method but it acts as an effective pest control procedure (Adam John, 2013). Burning of crop stubble causes air pollution and lead to loss of huge biomass, *i.e*. organic carbon, plant nutrients, the entire amount of C, approximately 80–90% N, 25% of P, 20% of K and 50% of S present in crop residues are lost in the form of various gaseous and particulate matters, resulting in atmospheric pollution and global warming (Kaur *et al*., 2019). Incorporation in the soil poses challenges in intensive systems with two to three cropping rounds per year. This is due to the insufficient time for decomposition, leaving the straw with poor fertilization properties for the soil and hindering crop establishment. As a result, open-field burning of straw has increased dramatically over the last decade, despite being banned in most rice-growing countries because of pollution and the associated health issues. Therefore, it is important to look for sustainable solutions and technologies that can reduce the environmental footprint and add value by increasing the revenues of rice production systems.

MATERIAL AND METHODS:

The experiment was conducted in a sandy clay loam soil. The initial soil sample was collected at 0-15 cm deep from random locations in the field and it was shade dried, pounded and 2 mm sieved and used for analysis of physical, physic-chemical and chemical properties of soil by adopting standard procedures. The initial soil properties of the experimental site were presented in table 1.

Table 1. Initial Soil Properties of the experimental site

S.No.	Property	Values
1.	Soil type	Black soil
2.	Sand (%)	55
3.	Silt (%)	45
4.	Clay (%)	6.6
5.	Soil Texture	Sandy loam
6.	pH	8.4
7.	EC (dSm^{-1})	0.45
8.	Organic Carbon (%)	0.57
9.	Available N ($kg\ ha^{-1}$)	177
10.	Available P ($kg\ ha^{-1}$)	15
11.	Available K ($kg\ ha^{-1}$)	280
12.	NH_4^+ - N ($mg\ kg^{-1}$)	10.44
13.	NO_3^- - N ($mg\ kg^{-1}$)	2.80
14.	Bulk Density ($Mg\ m^{-3}$)	1.30
15.	Water Holding Capacity (%)	28

Soil samples were collected before and after burning from the plots at the root zone and were analyzed immediately or were stored in refrigerator for the analysis of soil enzyme activities and soil microbial population.

MICROBIAL COUNT:

Table 2. Methods of enumeration of soil microbes

S. No	Microorganism	Method	Reference	Agar used
1	Bacteria	Serial dilution pourmethod	Thorton (1922)	Nutrient agar
2	Fungi	Serial dilution pour method	Martin (1950)	Potato Dextrose agar
3	Actinomycetes	Serial dilution pourmethod	Allen (1957)	Actinomycetes isolation agar

Number of bacteria/fungi/ actinomycetes in 1 g soil =

$$\frac{\text{No. of CFU} \times \text{dilution}}{\text{The dry weight of 1 g moist soil} \times \text{aliquot taken}}$$

SOIL ENZYME ACTIVITY:

Dehydrogenase Activity:

In screw-capped test tubes, one gram of soil samples were weighed. 50 mg $CaCO_3$ was added to the mixture, followed by addition of 2.5 ml distilled water and 1 ml of 3 % TTC. The contents of the tube were stirred and incubated at room temperature for 24 hours. After the formation of red precipitate, a few millilitres of methanol was added and shaken to dissolve it. The contents were filtered and volume was made up to 25 ml with methanol. At 485 nm, the red colour intensity was measured. (Casida *et al.*, 1964).

Phosphatase Activity:

The method described by Tabatabai and Bremner (1969) was used to measure the acid phosphatase activity, while the protocol developed by Eivazi and Tabatabai (1977) was used to estimate the alkaline phosphatase activity. A screw-capped test tube was filled with one gram of soil, 0.2 millilitres of toluene, 4 millilitres of MUB (pH 6.5 for acid phosphatase or pH 11 for alkaline phosphatase), and one millilitre of p-nitrophenyl phosphate. Samples were swirled and were incubated at 37 degrees for one hour. 1 ml of 0.5M CaCl2 and 4 ml of NaOH were added after incubation. The contents were then filtered and yellow colour intensity was measured using spectrophotometer at 420 nm. Each sample has a control where 1 ml of p-nitrophenol solution was added after the addition of 0.5M $CaCl_2$ and 4 ml of NaoH.

Urease

Five grams of soil sample was weighed into a volumetric flask (50 ml). To this, 0.2 ml of toluene followed by 9 ml of THAM buffer were added. The contents were mixed thoroughly and 1 ml of urea solution was added. The tube was stoppered and incubated at 37°C for 2 hours. To reaction was terminated using KCl - $AgSO_4$ mixture (35 ml) and the volume was made up to 50 ml. 20 ml of suspension was pipetted out after mixing the contents. The amount of NH_4 released was measured by distilling the 20 ml of suspension with 0.2 g of MgO for 4 minutes and by titrating with 0.05N H_2SO_4. 1 ml of urea solution were used as controls after the addition of KCl - $AgSO_4$ (Tabatabai and Bremner, 1972).

GHG EMISSIONS:

During the crop growing season, CO_2, N_2O, and CH_4 fluxes from the soil surface were measured using static flux chambers and a GC gas analyzer. Sampling was done before and after the burning of crop residue. At each sampling site, a 10-cm-high vented rectangular aluminium chamber with a sampling port was installed in a water channel welded to an anchor (50 40 10 cm) inserted 10 cm into the earth. Anchors were installed perpendicular to the crop row so that the crop row and inter-row were contained within each chamber. Flux measurements were usually taken between the hours of 08:00 and 12:00 to avoid daily variations in the flux pattern. Within each replication of each treatment plot, duplicate flux measurements were taken. Gas samples were taken with a syringe from inside the chambers at 0, 15, and 30 minutes following installation. Gas samples (40 mL to ensure sample overpressure in tubes) were then injected into 20-mL evacuated vials with butyl rubber septa and transferred to the laboratory in CRIDA for gas chromatography analysis. The gas chromatograph employed was a Varian 3800 fully automated equipment with an electron capture detector for quantifying N_2O and FID and TCD detectors for measuring CH_4 and CO_2, respectively. Fluxes were calculated from the linear or nonlinear increase in concentration (selected according to the emission pattern) in the chamber head space with time (Livingston and Hutchinson, 1995).

Statistical Analysis:

For comparison of mean values pre and post burning of residue, paired t test was performed using data analysis tool in microsoft excel. The eta statistic values were computed using the formula

$$t^2 / t^2 + (n+1)$$

RESULTS AND DISCUSSION:

Soil enzyme activity:

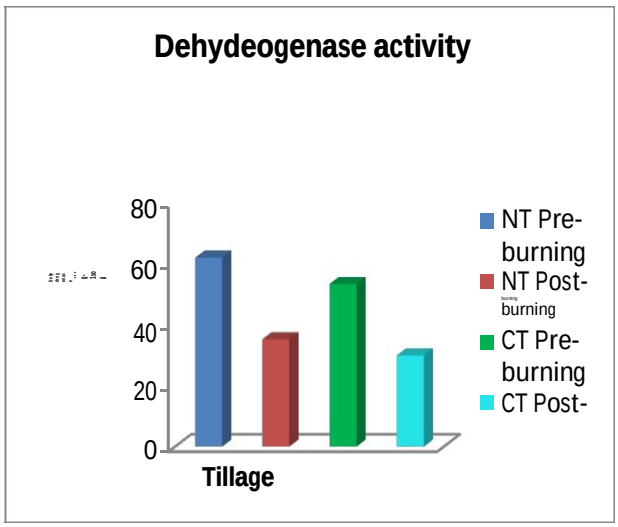
The data related to soil enzyme activity in response to residue burning was presented in figure 1. The mean values of dehydrogenase activity (fig 1a) under no tillage (61.94 µg TPF g^{-1} soil day^{-1}) was higher than conventional tillage (53.31 µg TPF g^{-1} soil day^{-1}) before burning of the residue (table 3). Dehydrogenase activity (µg TPF g^{-1} soil day^{-1}) was drastically reduced 48 hours after burning in both no tillage (35.14 µg TPF g^{-1} soil day^{-1}) and conventional tillage (29.83 µg TPF g^{-1} soil day^{-1}). Similar trend was observed in case of acid and alkaline phosphatase activity. The acid phosphatase activity (fig 1b) before burning of the residue was 91.31 µg PNP g^{-1} soil hr^{-1} and 78.74 µg PNP g^{-1} soil hr^{-1} in no tillage and conventional tillage systems respectively. Post-burning of the residue, there was a decrease in the acid phosphatase activity (as indicated by right tailed t test) in both no tillage (48.83 µg PNP g^{-1} soil hr^{-1}) and conventional tillage (28.97 µg PNP g^{-1} soil hr^{-1}). The alkaline phosphatase activity (fig 1c) before burning of the residue was 106.90 µg PNP g^{-1} soil hr^{-1} and 94.95 µg PNP g^{-1} soil hr^{-1} in no tillage and conventional tillage systems respectively. Post-burning of the residue, there was a decrease in the alkaline phosphatase activity in both no tillage (47.82 µg PNP g^{-1} soil hr^{-1}) and conventional tillage (41.18 µg PNP g^{-1} soil hr^{-1}). The mean values of urease activity (fig 1d) under no tillage (49.04 µg NH_4^+N g^{-1} soil $2hr^{-1}$) was higher than conventional tillage (40.72 µg NH_4^+N g^{-1} soil $2hr^{-1}$) before burning of the residue (table 3). Urease activity was drastically reduced 48 hours after burning (table 3) in both no tillage (20.59 µg NH_4^+ N g^{-1} soil 2 hr^{-1}) and conventional tillage (16.44 µg NH_4^+ N g^{-1} soil $2hr^{-1}$). As the t stat values are greater than t critical values for all the enzymes, it can be noted that there was significant impact of stubble burning on soil enzymatic activity in both tillage systems. Further there was decrease in the enzyme activity post burning of the residue, which was indicated by right tail t test. The eta square statistic was > 0.7 for all the enzyme activities, which indicated a large effect size of stubble burning on soil enzyme activity in both tillage systems.

Decline in dehydrogenase enzyme due to residue burning indicates lower microbial activity in the soil (Kumar *et al*., 2019). The decrease in the alkaline phosphatase activity due to residue burning was supported by the study conducted by Ajwa *et al*. (2000), who found the decrease in alkaline phosphatase activity after burning of the residue. Any direct effects due to physical destruction of the microbial

population by burning would lead to decreases in microbial populations after burning in the top soil layer of the soil profile. The effects of residue burning are probably caused by volatile losses of readily available C compounds that are important energy sources for microbial activity which, in turn, could affect the accumulation of soil enzymes.

Table 3. Comparison of Soil enzyme activities from pre and post burning of crop residue from both tillage systems

Soil enzyme activity	NT		CT	
	Pre-burning	Post-burning (48 hrs after burning)	Pre-burning	Post-burning (48 hrs after burning)
Dehydrogenase				
Dehydrogenase (µg TPF g^{-1} soil day^{-1}) - Mean	61.94	35.14	53.31	29.83
Stdev	4.02	3.58	5.13	3.08
SEm	1.64	1.46	2.10	1.26
t stat (5)	10.020		14.434	
t critical (one tailed (lower/right tail)	2.015		2.015	
Eta square	0.93		0.97	
Acid Phosphatase				
Acid Phosphatase (µg PNP g^{-1} soil hr^{-1}) Mean	91.31	48.83	78.74	28.97
Stdev	6.92	5.30	10.38	6.67
SEm	2.83	2.16	4.24	2.72
t stat (5)	17.022		7.558	
t critical (one tailed (lower/right tail))	2.015		2.015	
Eta square	0.98		0.89	
Alkaline phosphatase				
Alkaline phosphatase (µg PNP g^{-1} soil hr^{-1}) Mean	106.90	47.82	94.95	41.18
Stdev	4.59	7.10	6.52	5.17
SEm	1.87	2.90	2.66	2.11
t stat (5)	19.146			16.813
t critical (one tailed (lower/right tail)	2.015			2.015
Eta square	0.98			0.98
Urease				
Urease (µg NH_4^+N g^{-1} soil $2hr^{-1}$) Mean	49.04	20.59	40.72	16.44
Stdev	8.20	5.37	6.01	4.92
SEm	3.35	2.19	2.45	2.01
t stat (5)	6.644		9.343	
t critical (one tailed (lower/right tail)	2.015		2.015	
Eta square	0.86		0.93	



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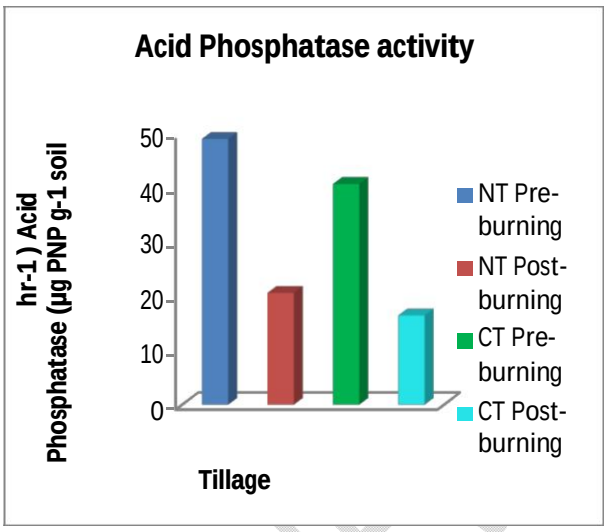

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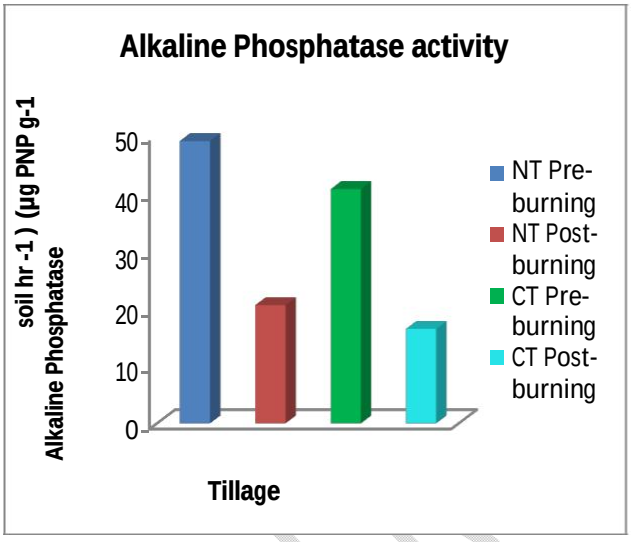

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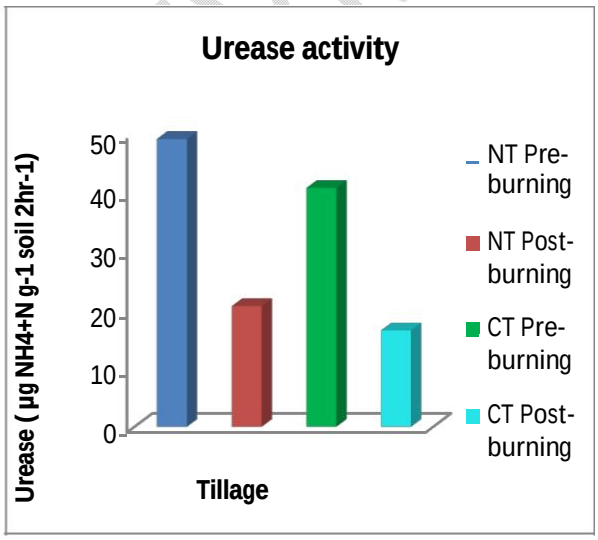


1d

Fig. 1. Comparison of Soil enzyme activity (1a. Dehydrogenase; 1b Acid phosphatase; 1c. Alkaline phosphatase; 1d. Urease). pre and post-burning of residue

Microbial population:

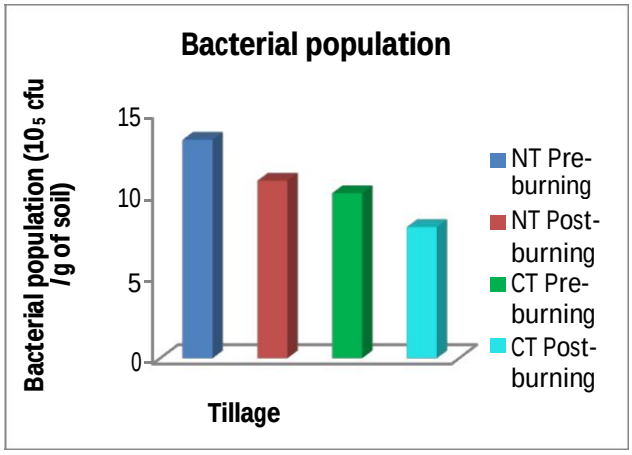
The data pertaining to microbial population is given in table 4. The bacterial population (fig. 2a) before burning was higher in no tillage (18.83 X 10^5 cfu /g of soil) than conventional tillage (13.25 X 10^5 cfu /g of soil). Post-burning there was a decline in bacterial population in both no tillage (9.08 X 10^5 cfu /g of soil) and conventional tillage system (7.17 X 10^5 cfu /g of soil). The fungal population (fig. 2c) before burning was higher in no tillage (13.33 X 10^3 cfu /g of soil) than conventional tillage (10.08 X 10^3 cfu /g of soil). Post-burning there was a decline in fungal population in both no tillage (10.83 X 10^3 cfu /g of soil) and conventional tillage system (8 X 10^3 cfu /g of soil). Actinomycetes population (fig. 2b) before burning was higher in no tillage (20.08 X 10^4 cfu /g of soil) than conventional tillage (13.25 X 10^4 cfu /g of soil). Post-burning there was a decline in actinomycetes population in both no tillage (13.50 X 10^4 cfu /g of soil) and conventional tillage system (9.08 X 10^4 cfu /g of soil). The population of bacteria, fungi

and actinomycetes declined after burning. As the t stat values are greater than t critical values for microbial population, it can be noted that there was significant impact of stubble burning on soil microbial population. Further there was decrease in the microbial population post burning of the residue, which was indicated by right tail t test. The eta square statistic was > 0.7 for bacterial population in both the tillage systems and actinomycetes population in conventional tillage, which indicated a large effect size of stubble burning on population of these organisms. The eta square statistic was > 0.6 for fungal population in both tillage systems and actinomycetes population in no tillage, which indicated a moderate effect size of stubble burning on population of these organisms.

The reduction in microbial population may be due to the generation of heat due to burning crop residues resulting in higher temperature; this may have deleterious effects on microbial survival. Similar results were recorded by Kumar *et al*. (2019). Helgason *et al*. (2009) reported that conservation tillage practices increase bacterial and fungal population in the soil.

Table 4. Comparison of soil microbial population from pre and post burning of crop residue from both tillage systems

Soil Microbial population	NT		CT	
	Pre-burning	Post-burning (48 hrs after burning)	Pre-burning	Post-burning (48 hrs after burning)
Bacteria				
Bacteria (10^5 cfu /g of soil) Mean	18.83	9.08	13.25	7.17
Stdev	1.21	1.86	1.08	0.93
SEm	0.49	0.76	0.44	0.38
t stat (5)	27.129		12.864	
t critical (one tailed (lower/right tail)	2.015		2.015	
Eta square	0.99		0.96	
Actinomycetes				
Actinomycetes (10^4 cfu /g of soil) Mean	20.08	13.50	13.25	9.08
Stdev	2.65	1.87	1.54	1.07
SEm	1.08	0.76	0.63	0.44
t stat (5)	3.929		4.829	
t critical (one tailed (lower/right tail)	2.015		2.015	
Eta square	0.69		0.77	
Fungi				
Fungi (10^3 cfu /g of soil) Mean	13.33	10.83	10.08	8.00
Stdev	1.72	1.03	1.74	1.30
SEm	0.70	0.42	0.71	0.53
t stat (5)	2.565		4.110	
t critical (one tailed (lower/right tail)	2.015		2.015	
Eta square	0.48		0.71	



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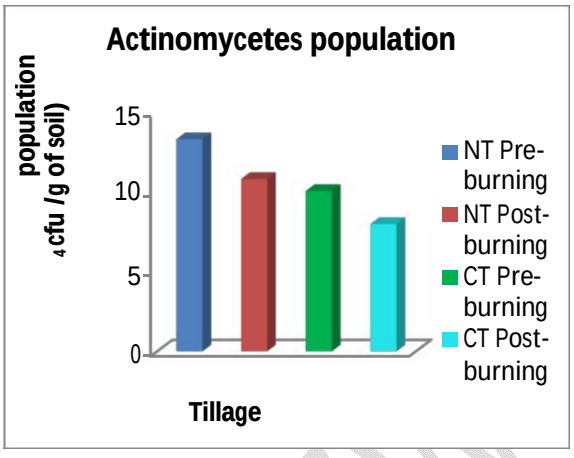

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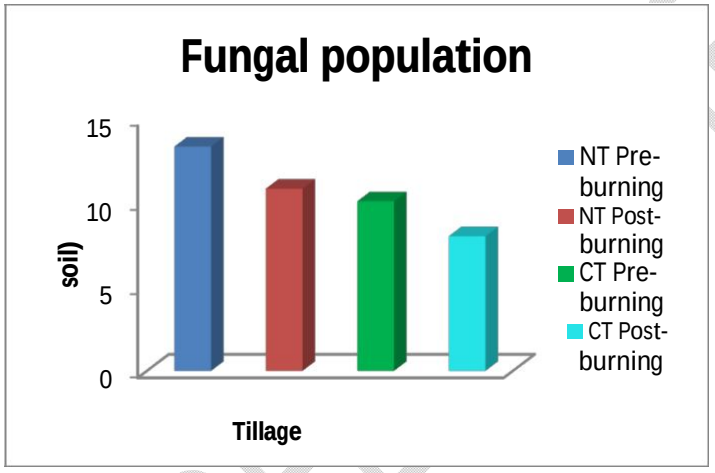

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Comparison of (2a) Bacterial population (10^5 cfu /g of soil) (2b) Actinomycetes population (10^4 cfu /g of soil) (2c) Fungal population (10^3 cfu /g of soil) from pre and post burning of crop residue from both tillage systems

GHG EMISSIONS:

The data related to GHG emissions is presented in table 5. Before residue burning, the CO_2 emissions in CT (0.44 µg C/g of soil/ hr) were higher than NT (0.26 µg C/g of soil/ hr). Post-burning resulted in release of greater amounts of CO_2 in both the tillage systems *i.e.*, NT (33.55 µg C/g of soil/ hr) and CT (49.35 µg C/g of soil/ hr). Methane emissions (µg C/g of soil/ hr) in NT and CT prior to burning of the residue was 16.87 µg C/g of soil/ hr and 25.81 µg C/g of soil/ hr respectively. After burning of the residue, there was increase in methane emissions in NT (41.58 µg C/g of soil/ hr) and CT (40.71 µg C/g of soil/ hr). N_2O emissions (µg N/g of soil/ hr) in NT and CT before burning of residue was 14.79 and 11.56 respectively. There was a rise in N_2O emissions post residue burning in both NT and CT which was 38.71 µg N/g of soil/ hr and 38.17 respectively µg N/g of soil/ hr. As the t stat values are greater than t critical values for GHG emissions, it can be noted that there was significant impact of stubble burning on GHG emissions. The t stat value was negative for all the three gases which indicates that mean values of gases pre-burning of the stubble were less than post-burning. Further there was increase in the GHG emissions post burning of the residue, which was indicated by left tail t test. The eta square statistic was > 0.7 for all the three gas emissions in both the tillage systems, which indicated a large effect size of stubble burning on GHG emissions.

There was an increase in CO_2, CH_4 and N_2O emissions after burning and these results were in accordance with the results obtained by Jumpen *et al.*, 2018 who found that open field rice residue

burning leads to 5.34 ± 2.33 megaton (Mt) of CO_2 release 44 ± 14 kiloton (kt) of CH_4 and 2 ± 2 kt of NO_X release into the atmosphere. Methane is produced during biomass burning in field due to incomplete combustion. The results are in agreement with Gadde *et al.*, 2009 who reported that open field burning of rice straw and other crop residues emits species such as CO_2, nitrous oxide (N_2O), CH_4, CO, non-methane hydrocarbons (NMHC), NOx, SO_2, particulate matter (PM) and few others species. As huge piles of dried crop residues start burning in the field at a time there is scarcity of oxygen supply during the process because the rate of burning is much faster than that of supply of oxygen to the residue heap from the surrounding. As a result, there is a deficiency of oxygen, which triggers an incomplete combustion of residues resulting in the methane production and subsequent emission (Bhattacharya and Barman, 2018). Carbon dioxide may be produced due to complete combustion of the crop residues. It is assumed that the N contained in crop residues is to stimulate N_2O emissions (IPCC, 1997).

Table 5. Comparison of GHG emissions from pre and post burning of crop residue from both tillage systems

GHG emissions	NT		CT	
	Pre-burning	Post-burning (48 hrs after burning)	Pre-burning	Post-burning (48 hrs after burning)
CO_2				
CO_2 Emissions (µg C/g of soil/ hr) Mean	0.26	33.55	0.44	49.35
Stdev	0.15	1.96	0.09	2.18
SEm	0.06	0.80	0.04	0.89
t stat (5)	-44.866		-54.537	
t critical (one tailed (Upper/left tail)	2.015		2.015	
Eta square	1.00		1.00	
N_2O				
N_2O Emissions (µg N/g of soil/ hr) Mean	14.79	38.71	11.56	38.17
Stdev	0.96	1.45	1.09	1.01
SEm	0.39	0.59	0.45	0.41
t stat (5)	-55.293		-44.963	
t critical (one tailed (Upper/left tail)	2.015		2.015	
Eta square	1.00		1.00	
CH_4				
CH_4 Emissions (µg C/g of soil/ hr) Mean	16.87	41.58	25.81	40.71
Stdev	1.03	1.01	0.83	1.51
SEm	0.42	0.41	0.34	0.62
t stat (5)	-58.341		-32.961	
t critical (one tailed (Upper/left tail)	2.015		2.015	
Eta square	1.00		0.99	

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