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RESEARCH ARTICLE

Effect of rice residue management options on soil microbial dynamics and enzyme activity under *Rabi* sunflower cultivation in an Alfisol

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Abstract

The study was conducted at the Agricultural Research Station, Tornala, in Siddipet district, Telangana, India, for two consecutive *Rabi* seasons during 2022-23 and 2023-24 to investigate the effect of *Kharif* rice residue management on soil microbial dynamics and enzyme activity under *Rabi* sunflower in a rice-sunflower cropping system. Microbial populations were higher in treatments that included incorporation of straw with adjusted ratios of straw C:N (T_5), C:P (T_6) and C:N:P (T_7) to 30:1, 30:0.3 and 30:1:0.3, respectively, or incorporation as such (T_4). The bacterial and fungal populations exhibited an increasing tendency from the time of straw integration, peaking at the sowing stage. While actinomycetes were higher in straw incorporation and peaked at 45 DAS, declining towards harvest. On the other hand, at every stage of crop growth, the residue burning + RDF (T_1) treatment continuously had the lowest microbial populations. Soil enzyme activities were significantly higher in straw incorporation treatments, with urease, dehydrogenase, β-glucosidase, acid and alkaline phosphatase peaking at 45 DAS, unlike burning and removal treatments. The enzyme activities were significantly reduced by burning the residue: urease decreased by 51.52 % and 47.93 %, dehydrogenase by 51.02 % and 44.11 %, β-glucosidase by 80.18 % and 73.74 %, acid phosphatase by 56.72 % and 48.85 % and alkaline phosphatase by 62.07 % and 61.45 % when compared to straw incorporation with C:N:P ratio adjustment (T_7) and residue retention + zero tillage + RDF (T_3). Other straw incorporation treatments (T_5, T_6) and residue removal treatment (T_2) showed noticeably greater activity of these enzymes than burning.

Keywords: burning; C:N ratio; microbial population; removal; retention; rice straw; soil enzymes

Introduction

India, is an agrarian country, produces substantial amounts of crop residues due to its diversity of crops. The Government of India estimates that approximately 500-550 million tonnes of crop residues are produced on-farm and off-farm annually in the country (1). In India, the highest generation of crop residues occurs in Uttar Pradesh, producing 60 million tonnes annually, followed by Punjab with 51 million tonnes and Maharashtra with 46 million tonnes. Nearly 70 % of the total crop residues come from cereals, including rice, wheat, maize and millets (2-3). In that, rice crop alone contributes 34 % to the crop residues (4). In India, the surplus crop residues are estimated to range between 84 and 141 million tonnes per year (5). The use of a combine harvester for rice harvesting leaves a large amount of rice straw behind. These excess residues are typically burned on the farm. In Siddipet district alone, nearly 8-12 lakh tonnes of rice residue per annum are being produced from the current level of area and production (6). This crop residue can disrupt various field operations, so it needs to be managed carefully.

Managing rice straw presents a significant challenge primarily due to the limited time available between the rice harvest and the sowing of the *Rabi* crop. Among the various residue management options, such as burning, baling, *in situ* incorporation into the soil, residue removal and complete or partial retention on the soil surface, farmers have found that burning is the simplest and most cost-effective method for clearing the field within a very short interval of time. Incorporating straw into the soil is an alternative method for managing paddy straw, but it is not commonly practiced by farmers. The rice straw is high in silica and has a high carbon-to-nitrogen (C/N) ratio, which causes the crop residues to decompose very slowly (3). Residue burning, while a quick solution has several drawbacks: it contributes to air pollution, increases evaporation rates and depletes both macronutrients (such as N, P, K, Ca and Mg) and micronutrients (including Fe, Mn, B, Zn and S) in the soil. Burning one ton of rice straw generates approximately 1460 kg of CO_2, 3 kg of particulate matter, 60 kg of CO, 199 kg of ash and 2 kg of SO_2 (2).

Removing and burning crop residues results in a significant loss of macronutrients, with up to 80 % of nitrogen (N), 25 % of phosphorus (P), 21 % of potassium (K) and 50 % of sulphur (S) being depleted from the soil. This depletion can adversely impact both crop productivity and soil health (7). Burning residue is also unsuitable because it raises the temperature of the top soil nearly from 33.8 °C to 42.2 °C, which can kill various beneficial microorganisms and further remove a large portion of the organic matter (4). Several straw management practices have been explored. Retaining crop residues on the surface or incorporating them into soil helps preserve carbon (C) and can aid in restoring the soil's ability to supply essential nutrients to plants. This approach improves microbial activities, such as nitrogen fixation and phosphorus solubilization, thereby enhancing the soil's nutrient-supplying capacity. Therefore, it is important to focus on various straw management practices and their impact on soil biological properties and carbon sequestration potential in agricultural systems.

As part of crop diversification efforts, sunflower (*Helianthus annuus* L.) has emerged as an important oilseed crop in India. During the *Rabi* season of 2022-23, it was cultivated on 0.587 lakh hectares nationwide, including 3093 hectares in Telangana. Nizamabad (1880 ha) and Siddipet (567 ha) are the key districts cultivating sunflower in the state. In 2021-22, production reached 0.24 lakh tonnes with an average productivity of 1360 kg ha^{-1}. However, with an annual sunflower seed consumption of 10.22 lakh metric tonnes in Telangana and current production meeting only 1.85 % of this demand (0.19 lakh tonnes), there is substantial scope to diversify cropping systems by expanding sunflower cultivation. Research has shown that optimal sunflower growth and yield are closely linked to a balanced and adequate supply of nitrogen and phosphorus, which play crucial roles in plant development and photosynthesis.

Straw management practices, including the adjustment of fertilizer timing and dosage, can alter the C:N or C:P ratios, potentially enabling more efficient use of straw or residue without reducing crop productivity and contributing to the improvement of soil carbon stocks. Keeping this in view, this manuscript aims to evaluate the impact of different rice residue management options on microbial dynamics and enzyme activity in a *Rabi* sunflower crop within a rice-sunflower cropping system.

Materials and Methods

Experimental site

A field experiment was carried out during *Rabi*, 2022-23 and 2023-24, at the Agricultural Research station, Tornala, in the Siddipet district, Telangana, situated at 18°06'35" N latitude and 78°44'27" E longitude and falls in a semi-arid zone with a hot and humid climate. The initial soil properties of the experimental sites are presented in Table 1.

During the *Rabi* season of 2022-23, the mean weekly maximum temperature ranged from 27.71 °C to 37.71 °C, while the mean weekly minimum temperature ranged from 17.36 °C to 24.21 °C. In the *Rabi* season of 2023-24, the mean weekly maximum temperature varied between 28.07 °C and 38.21 °C and the mean weekly minimum temperature ranged from 13.50 °C to 26.87 °C. The mean annual rainfall of Siddipet district was 784.2 mm in 2022-23 and 753.6 mm, which was mostly received during July-September with occasional rain during winter.

Table 1. Initial properties of the experimental soil

Properties	Value
Sand (%)	66.40
Silt (%)	8.30
Clay (%)	25.30
Texture	Sandy clay loam
pH	6.5
EC (dS m^{-1})	0.16
OC (%)	0.44
N (kg ha^{-1})	209.6
P (kg ha^{-1})	39.21
K (kg ha^{-1})	237.33

Treatment imposition

Kharif season's rice (JGL-24423) was cultivated as dry direct-sown rice with all the recommended package of practices outlined by PJTSAU. During *Rabi* season, rice residue management treatments were imposed and the recommended dose of fertilizers (RDF) for sunflower is 75-90-30 kg NPK ha^{-1} with application of N in 3 splits 50 % basal and the remaining in 2 splits at 30 DAS and 55 DAS and P & K basal. The study was conducted in a randomized block design (RBD) with seven treatments *viz.* T_1: burning of rice residue 2 weeks after harvesting + RDF, T_2: rice residue removal + RDF, T_3: rice residue retention and zero till sowing of sunflower + RDF, T_4: incorporation of residue as such after harvest + RDF, T_5: adjusting C -N ratio of residue to 30:1 by applying part of the 1st dose of N through urea at the time of incorporation + the remaining recommended dose of nitrogen (RDN) in 3 splits, P and K as recommended. T_6: adjusting the C-P ratio of residue to 30:0.3 by applying part of the recommended dose of P through SSP at the time of incorporation + remaining RDP as basal & N, K as recommended and T_7: Adjusting C-N-P ratio of residue to 30:1:0.3 by applying part of the 1st dose of N through urea and part of the recommended dose of P through SSP at the time of incorporation + remaining RDN in 3 splits and P and K as recommended. All the treatments were replicated thrice during both years. The plan of layout for sunflower was made exactly same for both seasons. So, the same treatments will come on the same plots. The plot size was 8.0 × 7.2 m and the gross plot size was 57.6 m^2. Urea and SSP were used to supply N and P to sunflower as per the treatments. Muriate of potash was applied as a source of potassium.

Treatment imposition was done by quantifying the rice residue/straw (4.214, 5.186 t ha^{-1}) after harvesting *Kharif* crop. The carbon (35.75, 36.75 %), nitrogen (0.65, 0.71 %) and phosphorus (0.17, 0.22 %) contents of straw were estimated and the C:N (55:1, 51.76:1), C:P (126:1, 167.05:1) and C:N:P (55:126:1, 51.76:167.05:1) ratios were recorded during 2022-23 and 2023-24. Based on the results, before incorporation, the C:N ratio of the straw was adjusted to 30:1 by applying part of the first dose of nitrogen (284, 338 g urea $plot^{-1}$), C:P ratio of the straw was adjusted to 30:0.3 by applying part of the first dose of phosphorus (111.5, 275 g SSP $plot^{-1}$), C:N:P ratio was adjusted to 30:1:0.3 by applying part of the first dose of nitrogen and phosphorus through urea and SSP (284 g, 111.5 g $plot^{-1}$). In all the residue incorporation treatments (T_4 to T_7), rotary mulcher was run to slash down the straw and then incorporated the straw in to soil by following standard tillage operations like running cultivator twice followed by rotavation. In case of the treatments with burning/residue removal (T_1 and T_2), cultivator twice followed by rotavator was adopted. In case of T_3, control of rejuvenation of rice stubbles was done by spraying of paraquat @ 5 mL L^{-1}, rice residue was retained and zero till sowing of sunflower was taken up along with applying of the RDF as recommended.

Sunflower hybrid DRSH-1, which matures in 90-95 days was sown at the rate of 2 kg ha^{-1}. Two seeds per each hill were manually dibbled into the ground at recommended 45 cm × 20 cm spacing.

Soil sample collection

The plot-wise soil samples from 0 cm to 15 cm were collected by a core sampler at the time of straw incorporation, before sowing of sunflower crop and at 45 and 90 DAS of crop during *Rabi*, 2022-23 and 2023-24 and mixed by quartering them to make a composite sample. About 100 g of each of moist soil samples were stored at 4 °C in a refrigerator for determination of microbial population and enzyme activities.

Enumeration of soil microorganisms

Soil microbial populations were enumerated from the samples collected from 0 cm to 15 cm. The serial dilution was made to determine the microbial population in different treatments. One gram of soil was suspended in 10 mL of sterile 0.85 % saline solution and swirled for 5 min. The dilutions were made by transferring 1 mL of this suspension to subsequent 9 mL of sterile solution which shows 10^{-1} dilution. The dilutions were made up to 10^{-6}. The enumeration of microorganisms was done after culturing the organisms on specific media (Table 2). Specific media were used for growing a specific group of microorganisms of interest. The agar media were sterilized at 121 °C and 15 psi for 15 min.

One millilitre of sterile suspension was transferred to petri plates and agar media was poured into petri plates, mixed uniformly and cooled. After the media was solidified, the plates were inverted and kept in an incubator (Ferrotech sun labocare) at 50 % RH and dark condition for a specified period for different organisms (Table 2).

After the specified period, the colony forming units (cfu) were counted and enumerated by using the formula given (8).

$$\text{Number of bacteria/fungi/actinomycetes in 1 g soil} = \frac{\text{No. of cfu} \times \text{dilution}}{\text{Dry weight of 1 g moist soil} \times \text{aliquot taken}} \quad (1)$$

Enzyme activity

β-Glucosidase activity

One gram of moist soil (<2 mm) was placed in a test tube and 0.2 mL of toluene was added. After 15 min 4 mL of MUB (pH 6.2) and 1 mL of p-nitrophenyl β-glucoside (PNG) were added. The contents are mixed by swirled the test tube on a vortex mixer for a few seconds and then was incubated for 1 hr at 37 °C. After that 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M THAM buffer (pH 12) was added, the tube was swirled for about 10 sec and the soil suspension was filtered through Whatman No. 2 filter paper. After filtration, measured the yellow colour intensity with spectrophotometer (Shimadzu Corporation, Model: UV-1800) at 405 nm and the activity of β-glucosidase was expressed as µg paranitrophenol g^{-1} soil h^{-1} (9).

Urease activity

To determine the urease enzyme activity, 5 g of soil was weighed into a 50 mL volumetric flask and 0.2 mL of toluene followed by 9 mL of THAM buffer was added. Contents were mixed thoroughly and 1 mL of 0.2 M urea solution was added. The tube was Stoppard and incubated for 2 h at 37 °C. To this 35 mL of KCl-$AgSO_4$ mixture (35 mL) was added to terminate the reaction and volume was made to 50 mL with KCl-$AgSO_4$. The contents were mixed and 20 mL of suspension was pipette out. The amount of NH_4^+ released was measured by distilling the 20 mL of suspension with 0.2 g of MgO for 4 min by titrating the ammonium boric acid complex with 0.05 N H_2SO_4. Controls were done by adding 1 mL of urea solution after the addition of KCl-$AgSO_4$ (10).

Dehydrogenase activity

One gram of soil was weighed in 25 × 150 mm capacity screw-capped glass test tubes. To this 50 mg of $CaCO_3$ followed by 2.5 mL of distilled water and 1 mL of 3 % TTC were added. The contents were swirled on vortex mixer for few minutes and incubated at room temperature for 24 hr. After incubation, a 10 mL methanol was added to the contents and shaken to dissolve the red precipitate. The contents were filtered and volume was made up to 25 mL by methanol in volumetric flasks. The red colour intensity was measured at wavelength of 485 nm (blue filter) by using spectrophotometer (Model: UV-1800) (11).

Acid and alkaline phosphatases

The phosphatase activity was determined by the procedure given in the previous study (12). It involved the colorimetric estimation of p-nitrophenyl (PNP) released by phosphatase activity. For this 1 g of soil was weighed into a screw-capped test tube and 0.2 mL toluene were added followed by 4 mL of MUB (pH 6.5 for acid phosphatase or pH 11 for alkaline phosphatase) and 1 mL of p-nitrophenyl phosphate. Samples were swirled and incubated for 1 hr at 37 °C. After incubation, 1 mL of 0.5M $CaCl_2$ and 4 mL of 0.5M NaOH were added. The contents were filtered and the intensity of yellow colour was measured under spectrophotometer (Model: UV -1800) at 420 nm. Controls were done by except adding 1 mL of p-nitrophenol solution after the addition of 0.5M $CaCl_2$ and 4 mL of 0.5M NaOH.

Statistical analysis

The data recorded on microbial population and enzymes activity during study were statistically analyzed duly following the analysis of variance (ANOVA) technique for randomized block design (field experiments) (13) in OPSTAT. The statistical significance was tested with 'F' test at 0.05 level of probability and wherever the 'F' value was found significant, critical difference (CD, $p \leq 0.05$) was worked out to test the significance of difference between means.

Table 2. Details of techniques used for enumeration of microbial population

S.No	Organism	Media used	Dilution used	Temperature	Incubation time
1	Bacteria	Nutrient agar	10^5	37 °C	24 hr
2	Actinomycetes	Rose Bengal agar	10^4	30 °C	48 hr
3	Fungi	Ken-Knight and Munair's media	10^3	25 °C	72 hr

Results and Discussion

Microbial population

Soil microbial populations were significantly influenced by residue management practices across crop growth stages in both years. No notable differences among treatments were observed after the *Kharif* rice harvest and before straw incorporation, removal, or burning during the 2022-23 and 2023-24 *Rabi* seasons. Sowing coincided with 15 days after straw incorporation (T_4-T_7), the next day after straw burning (T_1) or removal (T_2) and 7-8 days post-harvest in zero-till sunflower (T_3). In straw-incorporated treatments, with or without C:N (T_5), C:P (T_6), or C:N:P (T_7) adjustments, peak bacterial and fungal populations occurred at sowing, increasing from incorporation to sowing and declining thereafter. Actinomycetes peaked at 45 DAS before gradually decreasing. Straw burning reduced microbial populations immediately, unlike straw removal. Pooled analysis (Table 3-5) showed no significant year-by-treatment interaction; thus, results were based on pooled data.

Bacteria

Mean data from *Rabi* 2022-23 and 2023-24 (Table 3) showed that T_7 recorded the highest bacterial population at all stages sowing (32.17 * 10^5 cfu g^{-1}), 45 DAS (29.17 * 10^5) and 90 DAS (25.50 * 10^5) followed closely by T_5 with 30.17 * 10^5, 28.67 * 10^5 and 23.33 * 10^5 cfu g^{-1}, respectively. Both were statistically at par and superior to other treatments. T_3 had slightly lower values but was at par with T_5. T_6 recorded 26.17 * 10^5, 25.00 * 10^5 and 22 * 10^5 cfu g^{-1} at sowing, 45 and 90 DAS, respectively and was inferior to T_7 and T_5 but on par with T_4 (25.33 * 10^5, 24.17 * 10^5 and 20.17 * 10^5). All straw-incorporated and zero-till treatments outperformed residue removal (T_2) and burning (T_1). T_2 showed moderate bacterial populations, while T_1 consistently recorded the lowest at all stages.

Generation of heat due to burning crop residues might result in higher temperature and may have deleterious effects on microbial survival. The reduction of bacterial populations in the topsoil layer of burnt plots by more than 50 % compared to areas where straw was retained and recovery of population of most of groups after 30-60 days of burning indicating the resilience of microbial populations and high buffering capacity of soil (14). The burning of straw stubble heats the soil surface (0 to 3 cm) up to 50-70 °C immediately after the burning and numbers of heterotrophic microorganisms declined by up to 77 % in the topsoil and 9 % decrease in the 5-10 cm layer (15). Organic fertilizers generally promote microbial populations; incorporation of organic materials significantly enhance bacterial growth. Immediately following the addition of fresh organic carbon substrates, microbes transition from a state of dormancy, becoming active and multiplying rapidly (16). Previous studies reported that the addition of fresh straw increased organic carbon (C), nitrogen (N) and the soil C/N ratio, which likely stimulated microbial growth (17). Micro-organisms showed an increase in viable cell numbers, peaking at 45 days after sowing (DAS) and declining by 90 DAS. The decrease in microbial population as the crop reached maturity was due to the reduced availability of substrates (18).

Fungi

Pooled data from *Rabi* 2022-23 and 2023-24 (Table 4) showed that T_7 had the highest fungal population at all stages with 25 * 10^3 cfu g^{-1} at sowing, 22.67 * 10^3 at 45 DAS and 20.83 * 10^3 at 90 DAS, closely followed by T_5 (24 * 10^3, 21.67 * 10^3 and 19.83 * 10^3). Both were statistically at par and superior to other treatments. T_3 recorded slightly lower values but was at par with T_5. T_6 (21.00 * 10^3, 19.17 * 10^3, 17.50 * 10^3) was inferior to T_7 and T_5 but on par with T_4 (20.33 * 10^3, 18.17 * 10^3, 16.33 * 10^3). All straw incorporation and zero-tillage treatments outperformed residue removal (T_2) and burning (T_1). T_2 showed moderate fungal populations, while T_1 consistently recorded the lowest values at all stages.

Results of the current study corroborate with previous findings, who observed a significant influence of incorporating straw with inorganic nitrogen on microbial density in the soil at various growth stages of rice (19). Research indicated that the highest fungal population at 45 DAS was observed with the incorporation of straw combined with inorganic fertilizers (18). While a significant decline in the fungal population following crop residue burning, with a more pronounced decrease compared to

Table 3. Bacterial population in soil at different growth stages of sunflower with rice straw management options

Treatment	Bacterial population (× 10^5 cfu g^{-1} soil)											
	At incorporation			At sowing			At 45 DAS			At 90 DAS		
	2022- 23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled
T_1: Residue Burning + RDF	13.00	12.67	12.83	6.67	7.00	6.83	8.00	8.67	8.33	7.67	8.33	8.00
T_2: Residue Removal + RDF	12.33	12.33	12.33	12.00	13.00	12.50	13.67	12.33	13.00	10.33	10.00	10.17
T_3: Residue Retention + ZT SF + RDF	13.33	13.33	13.33	29.00	29.67	29.33	27.33	28.00	27.67	22.33	23.67	23.00
T_4: Residue incorporation as such + RDF	13.06	13.67	13.37	25.00	25.67	25.33	23.67	24.67	24.17	19.67	20.67	20.17
T_5: Adjustment of C:N of residue to 30:1 before	13.00	13.67	13.33	30.33	30.00	30.17	28.67	28.67	28.67	22.67	24.00	23.33
T_6: Adjustment of C:P of residue to 30:0.3 before	13.38	13.33	13.36	26.33	26.00	26.17	24.67	25.33	25.00	21.67	22.33	22.00
T_7: Adjustment of C:N:P of residue to 30:1:0.3 before	13.67	13.67	13.67	32.00	32.33	32.17	29.00	29.33	29.17	25.33	25.67	25.50
SE(m) ± for years			0.36			0.42			0.40			0.40
SE(m) ± for treatments	0.95	0.96	0.68	1.22	1.00	0.79	1.09	1.02	0.75	1.07	1.04	0.75
SE(m) ± for years * treatments			0.96			1.11			1.06			1.05
CD (p = 0.05) for years			NS			NS			NS			NS
CD (p = 0.05) for treatments	NS	NS	NS	3.75	3.08	2.30	3.37	3.15	2.19	3.31	3.19	2.18
CD (p = 0.05) for years * treatments			NS			NS			NS			NS
CV (%)	12.60	12.55	12.57	9.15	7.40	8.31	8.55	7.90	8.23	10.03	9.33	9.68

cfu: colony forming unit.

Table 4. Fungal population in soil at different growth stages of sunflower with rice straw management options

Treatment	Fungal population (× 10^3 cfu g^{-1} soil)											
	At incorporation			At sowing			At 45 DAS			At 90 DAS		
	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled
T_1: Residue Burning + RDF	8.33	8.50	8.42	4.67	6.33	5.50	7.33	7.67	7.50	9.67	8.33	9.00
T_2: Residue Removal + RDF	8.50	8.83	8.67	12.00	13.00	12.50	12.67	13.00	12.83	12.00	12.33	12.17
T_3: Residue Retention + ZT SF + RDF	7.83	8.00	7.92	23.00	23.00	23.00	19.67	21.33	20.50	18.00	20.00	19.00
T_4: Residue incorporation as such + RDF	8.33	8.83	8.58	20.33	20.33	20.33	18.00	18.33	18.17	15.33	17.33	16.33
T_5: Adjustment of C:N of residue to 30:1 before incorporation	7.50	7.67	7.58	23.67	24.33	24.00	21.67	21.67	21.67	19.00	20.67	19.83
T_6: Adjustment of C:P of residue to 30:0.3 before incorporation	9.00	8.67	8.83	21.00	21.00	21.00	19.33	19.00	19.17	17.00	18.00	17.50
T_7: Adjustment of C:N:P of residue to 30:1:0.3 before incorporation	9.67	9.50	9.58	24.67	25.33	25.00	22.33	23.00	22.67	20.67	21.00	20.83
SE(m) ± for years			0.22			0.30			0.35			0.35
SE(m) ± for treatments	0.46	0.68	0.41	0.78	0.79	0.55	0.94	0.90	0.65	0.96	0.91	0.66
SE(m) ± for years * treatments			0.58			0.78			0.92			0.94
CD (p = 0.05) for years			NS			NS			NS			NS
CD (p = 0.05) for treatments	NS	NS	NS	2.39	2.44	1.62	2.91	2.76	1.90	2.96	2.81	1.94
CD (p = 0.05) for years * treatments			NS			NS			NS			NS
CV (%)	9.35	13.74	11.79	7.28	7.20	7.24	9.45	8.77	9.11	10.45	9.41	9.92

cfu: colony forming unit.

Table 5. Actinomycetes population in soil at different growth stages of sunflower with rice straw management options

Treatment	Actinomycetes population (× 10^4 cfu g^{-1} soil)											
	At incorporation			At sowing			At 45 DAS			At 90 DAS		
	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled
T_1: Residue Burning + RDF	11.67	11.33	11.50	7.67	8.00	7.83	8.00	8.33	8.17	9.33	10.00	9.67
T_2: Residue Removal + RDF	11.33	11.00	11.17	16.33	17.67	17.00	14.33	16.00	15.17	11.67	13.33	12.50
T_3: Residue Retention + ZT SF +RDF	10.97	11.30	11.13	24.33	23.33	23.83	25.33	26.00	25.67	23.67	23.33	23.50
T_4: Residue incorporation as such + RDF	11.96	12.29	12.13	21.33	19.67	20.50	20.33	22.67	21.50	19.33	20.33	19.83
T_5: Adjustment of C:N of residue to 30:1 before	11.00	13.67	12.33	25.67	24.33	25.00	27.00	27.67	27.33	24.00	24.33	24.17
T_6: Adjustment of C:P of residue to 30:0.3 before	11.33	11.67	11.50	22.67	20.33	21.50	22.33	23.67	23.00	20.33	21.67	21.00
T_7: Adjustment of C:N:P of residue to 30:1:0.3 before	12.00	13.00	12.50	26.33	25.00	25.67	28.33	28.67	28.50	24.33	25.67	25.00
SE(m) ± for years			0.23			0.35			0.40			0.37
SE(m) ± for treatments	0.58	0.62	0.43	0.93	0.94	0.66	1.08	1.04	0.75	1.17	0.77	0.70
SE(m) ± for years * treatments			0.60			0.94			1.06			0.99
CD (p = 0.05) for years			NS			NS			NS			NS
CD (p = 0.05) for treatments	NS	NS	NS	2.88	2.90	1.93	3.32	3.21	2.19	3.61	2.37	2.04
CD (p = 0.05) for years * treatments			NS			NS			NS			NS
CV (%)	8.79	8.96	8.88	7.85	8.24	8.04	8.97	8.25	8.60	10.70	6.73	8.85

cfu: colony forming unit.

bacteria, likely due to fungi lower heat resistance (2). However, 90 days after burning, the highest fungal population was observed in plots where residue was incorporated and retained, followed by those where residue was removed and burned.

Actinomycetes

Residue management significantly influenced soil actinomycetes population at all crop stages. Pooled data from *Rabi* 2022-23 and 2023-24 (Table 5) showed T_7 had the highest population at sowing (25.67 * 10^4 cfu g^{-1}), 45 DAS (28.50 * 10^4) and 90 DAS (25 * 10^4), closely followed by T_5 (25 * 10^4, 27.33 * 10^4 and 24.17 * 10^4), with both being statistically at par and superior to others. T_3 showed slightly lower values than T_7 but was comparable to T_5. T_6 (21.50 * 10^4, 23 × 10^4, 21 * 10^4) was inferior to T_7 and T_5 but on par with T_4 (20.50 * 10^4, 21.50 * 10^4, 19.83 * 10^4). All straw incorporation and zero-tillage treatments outperformed residue removal (T_2) and burning (T_1). T_2 recorded moderate populations, while T_1 showed the lowest at all stages.

Burning crop residues significantly raises soil temperature, reaching up to 55 ℃, which leads to a marked decline in actinomycetes population also. In contrast, retaining crop residues, rather than removing them, leads to higher population counts of total bacteria, fungi and actinomycetes under both zero tillage and conventional tillage practices (20). The highest soil microbial populations were observed in the zero tillage with straw mulch (T_3) treatment, compared to treatments involving straw burning or removal. This aligns with previous studies indicating that zero tillage creates favourable conditions by maintaining optimal temperatures and retaining moisture, which promotes the growth and proliferation of soil microbes (21). The mulching effect of straw left on the soil surface likely contributed to these improved microbial conditions.

Soil enzymes activity

Soil microbial enzymatic activity was significantly affected by rice residue management. Before straw burning, removal, or incorporation during *Rabi* 2022-23 and 2023-24, no significant differences were observed among treatments. However, straw incorporation treatments (T_5, T_6, T_7) and residue retention + ZT + RDF (T_3) showed the highest enzyme activity at 45 DAS, which increased after incorporation and declined towards harvest. In contrast, enzyme activity dropped immediately after straw burning and remained low in the removal treatment. Pooled data showed no year-by-treatment interaction; thus, results were based on pooled analysis.

β-Glucosidase activity

Pooled data (Fig. 1) showed that T_7 recorded the highest β-glucosidase activity at sowing (23.25 µg), 45 DAS (39.81 µg) and 90 DAS (31.81 µg p-nitrophenol g^{-1} hr^{1}), followed closely by T_5 (20.48, 37.52 and 29.12 µg), with both treatments being statistically at par and superior to others. T_6 showed slightly lower values (19.20, 35.96 and 26.00 µg) but was comparable to T_5. T_3 (18.62, 30.04 and 25.16 µg) was inferior to T_7 and T_5, but on par with T_4 (16.86, 26.92 and 23.37 µg). All straw-incorporated and zero-till treatments outperformed residue removal (T_2) and burning (T_1). T_2 showed moderate activity (12.47, 15.75 and 8.38 µg), while T_1 had the lowest (6.82, 7.89 and 7.20 µg) across all stages.

β-Glucosidase enzyme is an important indicator of soil quality, reflecting past biological activity and the soil ability to stabilize organic matter. Additionally, it can help assess the impact of different management practices on soil health. β-Glucosidase serves as a key indicator of a soil ecosystem's capacity to decompose crop residues and release simple sugars for heterotrophic microbes (2). Its activity is vital in carbon cycling, facilitating the hydrolysis of cellobiose and supplying a crucial energy source for soil biological processes. The products generated by the enzyme β-glucosidase are considered crucial energy sources for soil microorganisms. As a result, β-glucosidase activity serves as a valuable indicator of soil organic matter dynamics. The above data showed that β-glucosidase activity increased gradually as the crop's growth stage from at incorporation to 90 DAS in treatments where straw was incorporated. This finding aligns with earlier studies, who also observed higher β-glucosidase activity with straw incorporation combined with fertilizer (22). This effect was attributed to a rise in microbial biomass, might be due to the supply of substrate in the form residue and mineral fertilizers which led to increased enzyme production and enhanced decomposition of the residue through carbon mineralization. No tillage and residue retention are critical for boosting β-glucosidase activity. No tillage combined with residue retention resulted in the highest β-glucosidase activity (23). Higher β-glucosidase activity due to crop residue retention in a corn-soybean cropping system under a no-tillage system. β-Glucosidase activity and microbial populations increased with the incorporation of straw and as time progressed (24). To fulfil their carbon needs, microorganisms released carbon-degrading enzymes to break down straw carbohydrates. The addition of nitrogen fertilizer supplied essential mineral nitrogen, promoting microbial growth and the production of these enzymes. Over time, enzyme production continued to rise due to the greater availability of cellulose and lignin degradation products (17). In line with our findings, β-glucosidase activity was significantly increased in straw when supplemented with N and NP fertilizers compared to straw alone was also found in earlier studies (25). Previous studies found that straw incorporation markedly increased β-glucosidase activity compared to biochar applications or control treatments (26). The enzyme activity increased over time, with lower levels at the initial stages of decomposition and higher levels later (27). Initially, easy to decompose compounds were broken down, followed by the decomposition of cellulose and other resistant materials by carbon-decomposing enzymes. Although the addition of nitrogen fertilizer did not accelerate the decomposition process, it provided essential energy for microbes, as enzyme secretion is an energy-intensive activity. β-Glucosidase activity was significantly lower in T_1 after burning. This may be due to loss of organic matter and killing of microorganism in the soil due to burning (2).

Urease

Pooled data (Fig. 2) showed that urease activity was unaffected by year or treatment-year interaction. The highest urease activity was recorded in T_7 treatment at all stages with 14.01 µg NH_4^+-N g^{-1} soil 2 hr^{-1} at sowing, 28.52 µg NH_4^+-N g^{-1} soil 2 hr^{-1} at 45 DAS and 26.48 µg NH_4^+-N g^{-1} soil 2 hr^{-1} at 90 DAS. It was closely followed by T_5 treatment with 13.30, 27.14 and 25.29 µg NH_4^+-N g^{-1} soil 2 hr^{-1}, T_6

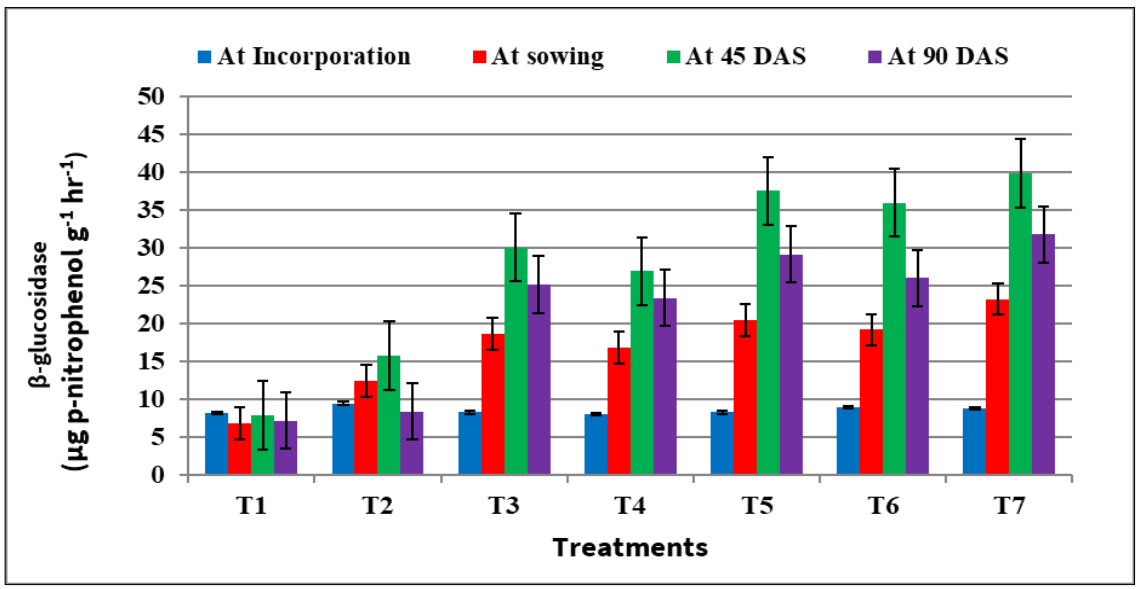

Fig. 1. Effect of rice residue management on β-glucosidase activity.

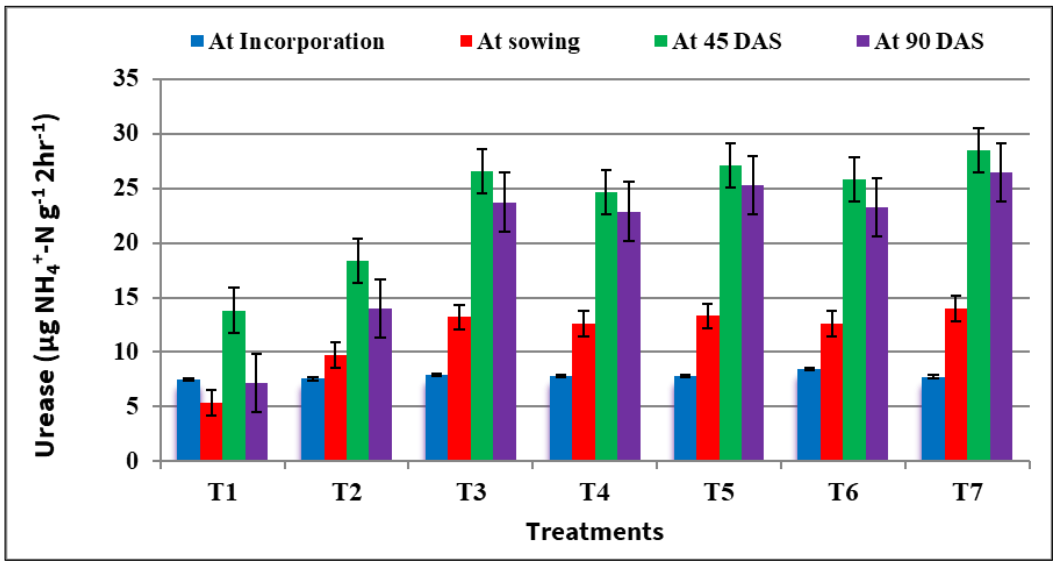

Fig. 2. Effect of rice residue management on urease activity.

with 12.59, 25.81 and 23.27 µg NH_4^+-N g^{-1} soil 2 hr^{-1}, T_3 with 13.20, 26.56 and 23.75 µg NH_4^+-N g^{-1} soil 2 hr^{-1} and T_4 with 12.59, 24.70 and 22.89 µg NH_4^+-N g^{-1} soil 2 hr^{-1} at sowing, 45 DAS and 90 DAS, respectively and superior over residue burning and removal. The T_2 treatment recorded significantly higher urease activity with 9.69 µg NH_4^+-N g^{-1} soil 2 hr^{-1} at sowing, 18.34 µg NH_4^+-N g^{-1} soil 2 hr^{-1} at 45 DAS and 13.99 µg NH_4^+-N g^{-1} soil 2 hr^{-1} at 90 DAS compared to the residue burning (T_1) treatment recorded lowest activity of urease with 5.36, 13.83 and 7.18 µg NH_4^+-N g^{-1} soil 2 hr^{-1} at sowing, 45 DAS and 90 DAS, respectively.

Urease is a crucial enzyme among soil enzymes, playing a key role in the transformation, biological turnover and bioavailability of nitrogen (28). The results of the present study align with findings of previous studies, who found that incorporating straw significantly enhances soil enzyme activity levels up to 45 days after sowing, in comparison to treatments without straw (18). The enhanced urease activity observed with zero tillage and residue retention may be attributed to improved soil aggregation and aeration, which create favourable conditions for microbial proliferation (3). The application of straw as mulch can enhance the population of soil microbes, increase soil carbon and nitrogen levels and create an energy-rich, favourable environment for the growth of soil enzymes (29). In comparison to no straw incorporation, incorporating of straw significantly boosted the activity of soil urease in soil. These increases are likely due to both the growth of microorganisms and the enhanced activity of these microbes, driven by improved resource availability and changes in microbial community composition (30). The incorporating straw into the surface soil significantly increased the activity of urease enzyme (31). The researchers reported that urease activity increased under no tillage and residue retention due to enhanced soil organic carbon, organic nitrogen and overall biological activity (23).

Dehydrogenase

Data from Fig. 3 showed that the highest DHA was recorded in T_7 treatment at all stages *viz*., 22.43, 32.01 and 25.59 µg TPF g^{-1} soil day^{-1} at sowing, 45 DAS and 90 DAS, respectively and it was closely followed by T_5 treatment (T_5) with DHA of 21.01 µg TPF g^{-1} soil day^{-1} at sowing, 29.85 µg TPF g^{-1} soil day^{-1} at 45 DAS and 24.60 µg TPF g^{-1} soil day^{-1} at 90 DAS. Both the treatments were at par and superior over rest of the treatments. The T_3 treatment recorded DHA lesser than T_7 and at par with T_5 treatments, with 19.92, 28.06, 23.69 µg TPF g^{-1} soil day^{-1} at sowing, 45 DAS and 90 DAS. The T_6 treatment recorded DHA of 18.01 µg TPF g^{-1} soil day^{-1} at sowing, 24.68 µg TPF g^{-1} soil day^{-1} at 45 DAS and 21.75 µg TPF g^{-1} soil day^{-1} at 90 DAS at harvest, respectively and this was statistically inferior to T_7 and T_5 and on par with T_4 treatment with 16.71 µg TPF g^{-1} soil day^{-1}, 23.15 µg TPF g^{-1} soil day^{-1} and 20.73 µg TPF g^{-1} soil day^{-1} at sowing, 45 DAS and 90 DAS, respectively. All the straw incorporated treatments and zero till treatments outperformed residue removal and residue burning treatments. The T_2 treatment recorded 14.07 µg TPF g^{-1} soil day^{-1} at sowing, 20.38 µg TPF g^{-1} soil day^{-1} at 45 DAS and 16.61 µg TPF g^{-1} soil day^{-1} at 90 DAS, respectively and was superior to T_1 treatment which had the lowest DHA, recording (9.05 µg TPF g^{-1} soil day^{-1}, 15.68 µg TPF g^{-1} soil day^{-1} and 11.94 µg TPF g^{-1} soil day^{-1}) at sowing, 45 DAS and 90 DAS, respectively. Overall, all straw incorporation and zero-tillage treatments outperformed residue removal and residue burning treatments.

The dehydrogenase is an oxido-reductase enzyme found in all viable microbial cells. It is a key indicator of total microbiological activity and is commonly used as a reliable biomarker for soil quality across various agricultural management practices (2). It is a key indicator of living and respiring soil microbial populations, reflecting the viable cell counts of various microbial groups. Biological oxidation of organic compounds is generally a dehydrogenation process and thus, dehydrogenases are important enzymes during the biological oxidation of organic compounds. The dehydrogenase enzyme is involved in oxidizing soil organic matter by transferring protons (H^+) and electrons (e^-) from substrates to acceptors. These processes are integral to the respiration pathways of soil microorganisms and are closely linked to soil type. The activity of dehydrogenase in soil reflects an active microbial population and offers insights into the soil capacity to support biochemical processes that are vital for maintaining soil fertility. The dehydrogenase assay demonstrated that the enzyme activity was affected by external nutrient inputs from inorganic fertilizers and the incorporation of residues. The dehydrogenase, urease and phosphatase activities were higher in soil samples treated with rice straw and nitrogen levels (18). However, a decrease in these soil enzymatic activities was observed as the crop approached maturity. The increased dehydrogenase activity in soils managed with zero tillage with residue retention due to greater moisture availability, which results from improved organic matter and more stable soil aggregates (3).

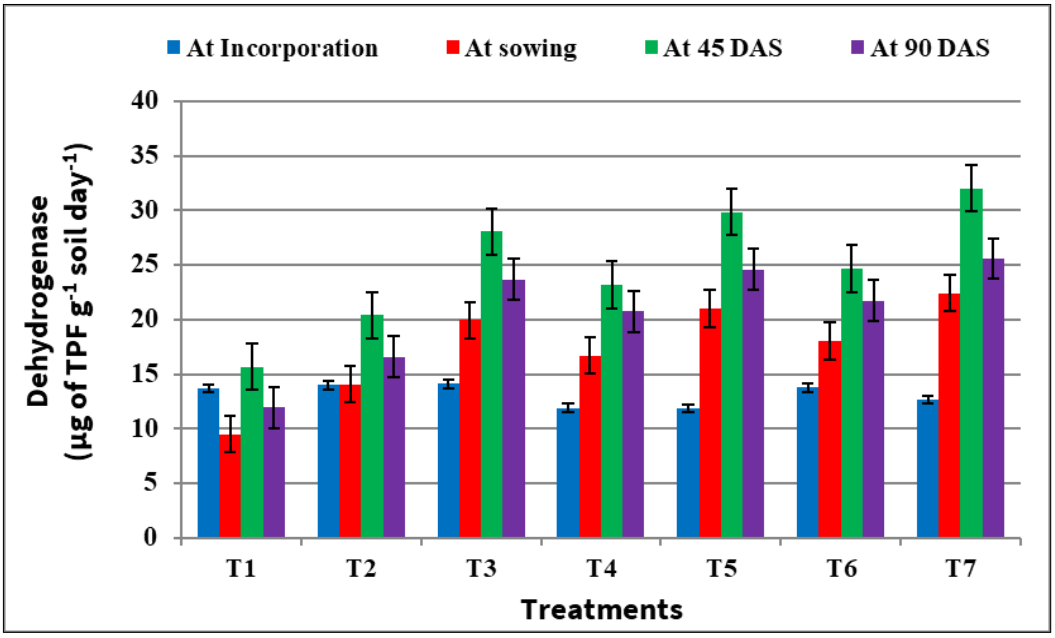

Fig. 3. Effect of rice residue management on dehydrogenase activity.

Acid phosphatase

Fig. 4 shows that an initial increased in residue incorporation treatments, peaking at 45 DAS and declined subsequently. Conversely, in treatments where residues were burned or removed, acid phosphatase activity steadily decreased. Acid phosphatase activity in residue incorporation treatments rose by approximately 45-56 % between sowing and flowering. Prior to residue incorporation or before burning of residue or removal, there was non-significant difference in acid phosphatise activity during both years.

The pooled data (Fig. 4) showed that significantly highest acid phosphatase was recorded in T_5 treatment at all growth stages *viz*., 60.62, 90.51 and 60.01 µg PNP g^{-1} soil hr^{-1} at sowing, 45 DAS and 90 DAS, respectively and it was statistically on par with T_7 treatment with acid phosphatase activity of 56.77 µg PNP g^{-1} soil hr^{-1} at sowing, 88.07 µg PNP g^{-1} soil hr^{-1} at 45 DAS and 57.84 µg PNP g^{-1} soil hr^{-1} at 90 DAS. Both treatments (T_7 and T_5) were superior to all other treatments. The T_6 treatment recorded slightly lower acid phosphatase activity than T_5 but on par with T_7 treatment with values of 54.62 µg PNP g^{-1} soil hr^{-1}, 84.21 µg PNP g^{-1} soil hr^{-1} and 55.10 µg PNP g^{-1} soil hr^{-1} at sowing, 45 DAS and 90 DAS, respectively. The T_3 treatment recorded acid phosphatase activity of 54.11 µg PNP g^{-1} soil hr^{-1} at sowing, 74.51 µg PNP g^{-1} soil hr^{-1} at 45 DAS and 52.51 µg PNP g^{-1} soil hr^{-1} at 90 DAS. This was statistically inferior to the T_5 and T_7 treatments but on par with T_4 treatment, which recorded acid phosphatase activity of 52.30µg PNP g^{-1} soil hr^{-1}, 70.85 µg PNP g^{-1} soil hr^{-1} and 50.95 µg PNP g^{-1} soil hr^{-1} at sowing, 45 DAS and 90 DAS, respectively. The T_2 treatment was performed better than the residue burning (T_1) treatment with acid phosphatase activity values of 46.93 µg PNP g^{-1} soil hr^{-1} at sowing, 57.43 µg PNP g^{-1} soil hr^{-1} at 45 DAS and 43.15 µg PNP g^{-1} soil hr^{-1} at 90 DAS. T_1 recorded the lowest acid phosphatase activity with values of 16.11 µg PNP g^{-1} soil hr^{-1}, 38.11 µg PNP g^{-1} soil hr^{-1} and 31.84 µg PNP g^{-1} soil hr^{-1} at sowing, 45 DAS and 90 DAS, respectively.

The findings of the present study align with earlier studies, indicating that stubble burning reduces enzyme activity in the soil (32). The intense heat from burning residues raises soil temperature to approximately 33.8-42.2 °C, leading to the death of beneficial microorganisms and a subsequent decline in DHA, acid phosphatase and alkaline phosphatase activity. While increased organic matter in topsoil stimulates enzyme activity. The incorporating straw into the soil significantly increased the activity of enzymes like urease and acid phosphatase enzymes,

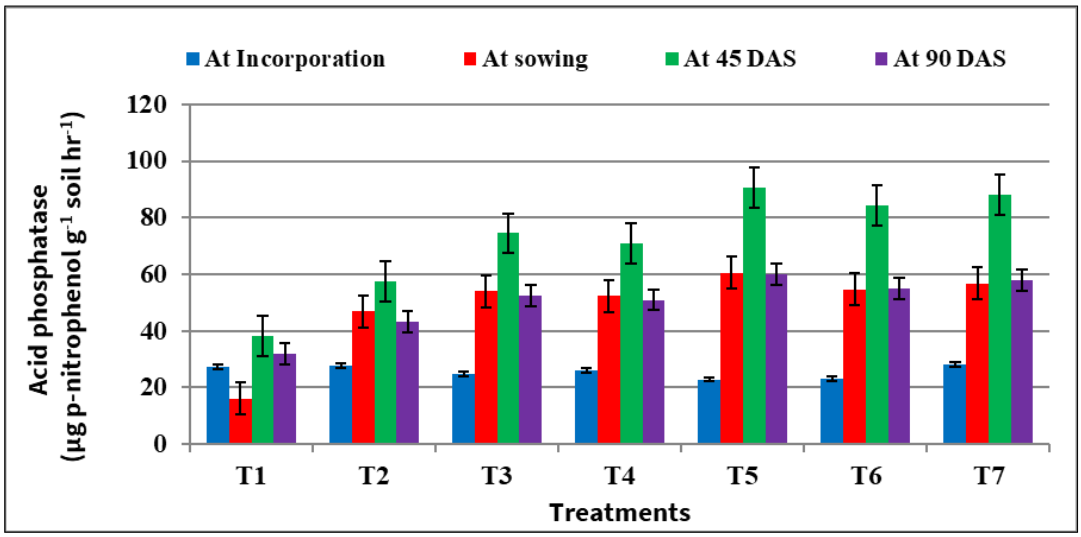

Fig. 4. Effect of rice residue management on acid phosphatase activity.

crucial for breaking down straw and recycling nutrients back into the soil (31). The *in situ* crop residue burning leads to the killing of the organisms and hence reduce the acid phosphatise activity (33). Burning intensity significantly impacted soil temperature. High intensity burning reached 450 °C, while moderate and low intensity burns attained 350 °C and 250 °C, respectively. These elevated temperatures led to the deactivation of hydrolytic enzymes, consequently decreasing overall soil enzyme activity.

Alkaline phosphatase

The pooled mean (Fig. 5) alkaline phosphatase activity indicate that significantly highest alkaline phosphatase activity was recorded in (T_5) treatment at all crop growth stages with values of 80.24 µg PNP g^{-1} soil hr^{-1} at sowing, 125.23 µg PNP g^{-1} soil hr^{-1} at 45 DAS and 67.10 µg PNP g^{-1} soil hr^{-1} at 90 DAS. It was on par with T_7 treatment with values of 77.30 µg PNP g^{-1} soil hr^{-1} at sowing, 115.23 µg PNP g^{-1} soil hr^{-1} at 45 DAS and 64.08 µg PNP g^{-1} soil hr^{-1} at 90 DAS. Both treatments (T_7 and T_5) were statistically on par with each other and superior to all other treatments. The T_3 treatment recorded slightly lower alkaline phosphatase activity than T_5but on par with T_7 treatment with values of 72.17 µg PNP g^{-1} soil hr^{-1}at sowing, 113.39 µg PNP g^{-1} soil hr^{-1} at 45 DAS and 62.99 µg PNP g^{-1} soil hr^{-1}at 90 DAS. The T_6 treatment recorded alkaline phosphatase activity of 74.57µg PNP g^{-1} soil hr^{-1} at sowing, 110.05 µg PNP g^{-1} soil hr^{-1} at 45 DAS and 59.81µg PNP g^{-1} soil hr^{-1} at 90 DAS. This was statistically inferior to T_7 and T_5 treatments but on par with T_4 treatment, which recorded alkaline phosphatase activity of 67.71 µg PNP g^{-1} soil hr^{-1} at sowing, 101.86 µg PNP g^{-1} soil hr^{-1} at 45 DAS and 56.51 µg PNP g^{-1} soil hr^{-1} at 90 DAS. Overall, all straw incorporation and zero-tillage treatments outperformed residue removal and residue burning treatments. The T_2 treatment recorded alkaline phosphatase activity of 55.11 µg PNP g^{-1} soil hr^{-1} at sowing, 85.53 µg PNP g^{-1} soil hr^{-1} at 45 DAS and 50.08 µg PNP g^{-1} soil hr^{-1} at 90 DAS, which were superior to the residue burning (T_1) treatment. T_1 recorded the lowest fungal population, with values of 33.78 µg PNP g^{-1} soil hr^{-1} at sowing, 43.71 µg PNP g^{-1} soil hr^{-1} at 45 DAS and 31.74 µg PNP g^{-1} soil hr^{-1} at 90 DAS.

The lowest alkaline phosphatase activity was observed at sowing, 20 days after the removal of residues or burning treatments. Both acid and alkaline phosphatase activities peaked at 45 DAS, likely due to the higher microbial population present at this stage. By harvest time, enzyme activity decreased, which may be attributed to lower soil moisture content was an unfavourable condition for enzyme release and reduced root system growth, leading to diminished enzyme activity.

In the current study, alkaline phosphatase activity was notably higher than that of acid phosphatase across all treatments. This increased activity of alkaline phosphatase is likely due to the neutral to alkaline pH of the experimental soil. Soil pH plays a significant role in regulating the activity of these enzymes (32). The alkaline reaction of the soil may have further enhanced the activity of alkaline phosphatase compared to acid phosphatase. Alkaline phosphatase activities are primarily derived from microbial sources, making this enzyme a useful indicator for short-term changes in microbial activity. Following burning, there was a significant reduction in alkaline phosphatase activity, which then declined progressively over time. Residue burning can lead to a reduction in alkaline phosphatase activity and this might be attributed to heat from burning which can physically destroy microorganisms in the soil, particularly in the topsoil layer, reducing their ability to produce alkaline phosphatase. Additionally, residue burning may lead to the volatilization of readily available carbon compounds that are important for microbial energy. The loss of these compounds can decrease microbial activity and, consequently, the production of soil enzymes like alkaline phosphatase. A significant decrease in alkaline phosphatase activity following burning, compared to the levels measured prior to the burning (2). The addition of straw as mulch enhance the concentration of soil carbon and nitrogen and provide energy as well as a favourable environment for the growth of the population of soil microbes, may increase soil enzymes (29). Adding residue inputs with increasing carbon sources can potentially boost soil microbial activity (34). In turn, phosphatase activities show positive correlations with both microbial biomass and soil activity. The increased alkaline phosphatase activities under ZT with residue retention could be attributed to higher organic matter, which continuously provides an energy source for microbial activity (3). The increase in phosphatase activity was primarily due to enhanced microbial growth and soil organic matter enrichment under no tillage with residue retention, compared to conventional tillage and residue removal (23). This increase in phosphatase activity leads to higher phosphorus availability and improved soil fertility. The straw incorporation increased phosphatase activity, while mineral fertilizer had an inhibitory effect (35). Organic amendments, on the other hand, enhanced phosphatase activity due to the increased availability of substrates.

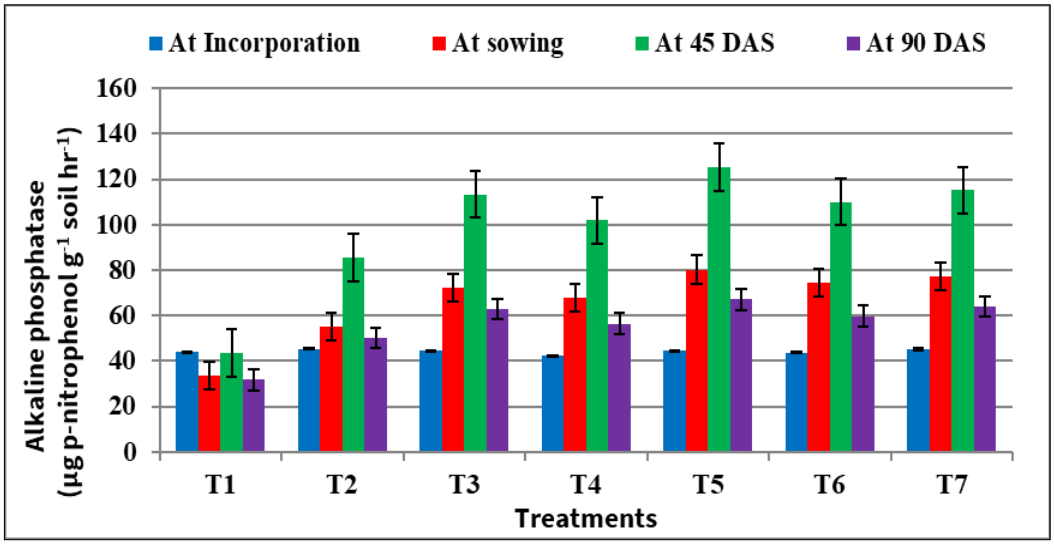

Fig. 5. Effect of rice residue management on alkaline phosphatase activity.

Conclusion

The results of the present study indicated that adopting different rice residue management practices in combination with inorganic fertilizers led to significant improvements in microbial dynamics and enzymatic activity. These practices also enhanced soil organic carbon and its fractions, ultimately boosting sunflower productivity in rice-based systems compared to residue burning or removal in the light-textured soils of Siddipet district, Telangana. Notably, treatments involving C:N:P ratio adjustment of the residue to 30:1:0.3 (T_7) and C:N ratio adjustment to 30:1 (T_5) before incorporation through partial application of recommended nitrogen and phosphorus resulted in significantly higher microbial populations and enzyme activities in sunflower cultivated on Alfisols. These treatments proved more effective than straw burning or removal in promoting microbial enzymatic activity.

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Authors' contributions

VP experimented, collected data, performed statistical analysis and drafted the whole manuscript. SS, GJ, ST and MVR development of the concept, supervision and corrected the draft. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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