



RESEARCH ARTICLE

Effect of sunhemp green manuring on soil physicochemical properties, nutrient cycling and microbial activity in rabi maize

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Received: 11 December 2025; Accepted: 05 August 2026; Available online: Version 1.0: 30 August 2026

Cite this article: Vijay KS, Madhu BGS, Padmaja B, Kiran RG, Latha PC, Santanu K, Pooja E, Naveen L, Charan BA, Arju SA, Venkata PBK. Effect of sunhemp green manuring on soil physicochemical properties, nutrient cycling and microbial activity in rabi maize. Plant Science Today. 2026; 13(sp5): 1–9. <https://doi.org/10.14719/pst.13162>

Abstract

This field investigation was conducted during the rabi (winter) season (November 2022 to March 2023) at the College Farm, Professor Jayashankar Telangana Agricultural University (PJTU), Hyderabad, Telangana, India, to evaluate the effects of sunhemp *in situ* green manuring at 45 days after sowing (DAS) and variable nitrogen application at the tasselling stage on microbial dynamics, nutrient cycling and physicochemical properties of the rhizosphere soil of rabi maize. At 20 days after incorporation (DAI), it was found that the green-manured treatments recorded significantly higher microbial population (cellulolytic: $83.7\text{--}92.4 \times 10^3$ CFU g⁻¹; lignolytic: $206\text{--}244 \times 10^4$ CFU g⁻¹ soil) and enzymatic activities (dehydrogenase: $80.5 \mu\text{g}$ triphenyl formazan (TPF) g⁻¹ soil; fluorescein diacetate: $88.5 \mu\text{g}$ fluorescein g⁻¹ soil) compared to sole maize because of the presence of higher amount of organic matter in those treatments which is reflecting the importance of organic manures in maintaining the soil health. Similarly, in the case of available nutrients, the green manured treatments recorded significantly higher amounts of available nitrogen ($222\text{--}227$ kg ha⁻¹), phosphorus ($25.5\text{--}27.8$ kg ha⁻¹) and potassium ($316\text{--}324$ kg ha⁻¹) due to the release of nutrients from the decomposing green manure. The presence of higher organic matter in the green manured plots improved soil moisture ($21.1\text{--}22.7$ %) and soil temperature ($24.2\text{--}25.1$ °C) compared to sole maize (19.4 % moisture and 23.6 °C). Sunhemp produced a biomass of 1.33 to 1.42 t ha⁻¹, with lower levels of lignin fibre ($1.26\text{--}1.34$ %) and higher levels of cellulose and hemicellulose, indicating rapid decomposability of the organic biomass. The study revealed that *in situ* green manuring benefits the rhizosphere soil nutrient status and microbial activity, thereby improving soil health and productivity of rabi maize.

Keywords: cellulose; dehydrogenase; fluorescein diacetate; lignin; sunhemp

Introduction

Maize (*Zea mays* L.) is one of the most widely grown crops in the world and is regarded as "Queen of Cereals," due to its importance after wheat and rice in terms of cultivated area and production (1). Its high yield potential and adaptability to diverse agro-ecological regions make maize an important crop for food security. During 2023–24, maize was cultivated on approximately 203.22 million hectares globally, producing 1223.81 million tonnes with an average productivity of 3540 kg ha⁻¹ (2). In Indian agriculture, maize could significantly contribute to food security, because it is still underutilised. Maize is cultivated across approximately 11 million hectares in India, contributing 37.5 million tonnes of grain production with an average productivity of 3.4 t ha⁻¹ during 2023–24. This crop contributes ~ 8 % of the country's food supply. The importance of rabi maize has increased significantly, as its cultivated area expanded from 1.79 to 2.14 m ha over the past four

years (3). Being a high nutrient demanding crop, maize needs more plant nutrients especially nitrogen (N) to reach its maximum attainable yield potential. However, in Telangana maize is the very popular among farmers and grown as fourth most important crop. In order to utilise rabi maize's full genetic potential, recommendations suggest up to 240 kg of N ha⁻¹ (4). This demand has increased further with the introduction of improved hybrids. However, due to significant losses from processes like leaching, volatilisation, denitrification, immobilisation, erosion and adsorption onto clay colloids, managing N in maize systems continues to be a major challenge (5). Inefficiencies of N fertilisers increases the cost of production and cause environmental damage whereby production system becomes unsustainable. Hence, substitution of chemical fertilisers with nature-based products or practices makes the system more resilient, sustainable and productive (6). Leguminous green manure crops fix atmospheric N and supply organic matter to the soil thereby soil fertility could be restored. Addition of organic matter

with green manure crops like sunhemp (*Crotalaria juncea* L.) greatly increases soil organic matter and facilitates nutrient cycling while improving the soil's physical, chemical and biological properties (7). Sunhemp biomass contains approximately 2.5 % N, 0.3 % P and 1.3 % K. Its ability to produce 3–4 t ha⁻¹ of organic biomass makes it suitable as an *in situ* green manure crop. This could supply up to 30 kg of N ha⁻¹ to subsequent/ concurrent cereal crops (8). The decomposition of the included biomass gives off nutrients in the forms that plants can use, which consequently improves the effectiveness of fertilisers and keeps the soil productive (9). Since it is difficult to fulfil the high nitrogen requirement of the maize plant and at the same time to keep the soil productive, the combination of *in situ* sunhemp green manuring with an aim of N fertiliser substitution is considered as a promising strategy for increasing rabi maize yield. Therefore, this investigation aims to study the effects of incorporating intercropped sunhemp at 45 days after sowing (DAS), along with different N levels applied at the tasselling stage, on soil physiochemical properties, nutrient cycling and microbial activity. The present study hypothesised that the combined application of sunhemp and N would enhance soil quality, nutrient dynamics and microbial activity.

Materials and Methods

Experimental site and climatic conditions

College Farm (latitude of 17°32'18" N and a longitude of 78°41'31" E) of the Professor Jayashankar Telangana Agricultural University, Rajendranagar, Hyderabad, was selected as the experiment site. The experimental site falls under the Southern Telangana Agro-Climatic Zone with a semi-arid climate. The farm is situated at an elevation of 542.5 m above mean sea level.

During the experiment period, maximum and minimum temperatures ranged from 34.0–10.0 °C. The total rainfall recorded was 8.2 mm. Maximum relative humidity ranged from 75 to 92 %, while minimum relative humidity varied between 19 and 59 %. The average daily sunshine duration and wind speeds varied between 4.4–10.1 hr and from 2.0 to 4.1 km hr⁻¹, respectively. The evaporation rate during the crop growth phase was observed between 2.4–5.4 mm day⁻¹ (Fig. 1).

Experimental design and treatment details

The field experiment was carried out using a randomised block design (RBD), with 3 replications and 8 treatments (Table 1). Maize variety DHM 117 was sown in paired rows at a spacing of 30–90–30 cm × 20 cm during November 2022. Sunhemp seed at the rate of 50 kg ha⁻¹ was sown manually as green manure in two solid rows spaced at 30 cm between the maize pairs on the same day. A well-grown green manure crop with substantial biomass and rich nitrogen, phosphorus and potassium (NPK) content was incorporated *in situ* at 45 DAS to enhance soil nutrient availability. A hand-operated power weeder was used to incorporate the green manure crop in between the maize rows.

Table 1. Details of the treatments

Treatment	Treatment details
T ₁	<i>In situ</i> green manuring at 45 DAS (GM) + 0 kg N ha ⁻¹ at tasselling (T) stage
T ₂	<i>In situ</i> green manuring at 45 DAS (GM) + 10 kg N ha ⁻¹ at tasselling (T) stage
T ₃	<i>In situ</i> green manuring at 45 DAS (GM) + 20 kg N ha ⁻¹ at tasselling (T) stage
T ₄	<i>In situ</i> green manuring at 45 DAS (GM) + 30 kg N ha ⁻¹ at tasselling (T) stage
T ₅	<i>In situ</i> green manuring at 45 DAS (GM) + 40 kg N ha ⁻¹ at tasselling (T) stage
T ₆	<i>In situ</i> green manuring at 45 DAS (GM) + 50 kg N ha ⁻¹ at tasselling (T) stage
T ₇	<i>In situ</i> green manuring at 45 DAS (GM) + 60 kg N ha ⁻¹ at tasselling (T) stage
T ₈	Farmer's practice (Application of RDF without green manuring)

DAS: days after sowing; GM: green manuring; N: nitrogen; RDF: recommended dose of fertilisers.

Estimation of cell wall constituents in sunhemp

The cellulose content (%) was determined using the anthrone assay, where samples were treated with an acetic-nitric reagent, hydrolysed and reacted with an anthrone reagent (10). The absorbance was measured at 630 nm against a cellulose standard. Lignin content (%) was assessed as acid detergent lignin (ADL) from acid detergent fibre (ADF) after sequential acid hydrolysis and ashing. Hemicellulose (%) was calculated from the difference between neutral detergent fibre (NDF) and ADF. The calculations are presented as:

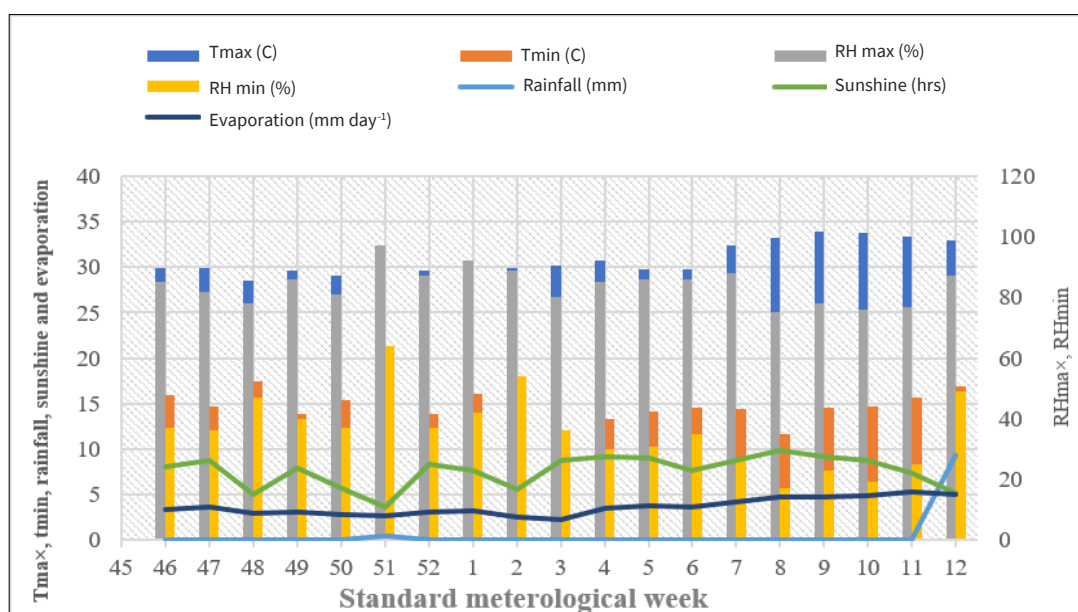


Fig. 1. Meteorological data during the crop growth season.

Cellulose = ADF - Residue after extraction with 72 % H₂SO₄
Eq. (1)

Lignin = Residue after extraction with 72 % H₂SO₄ - Ash Eq. (2)

Hemicellulose = Neutral detergent fibre (NDF) - Acid detergent fibre (ADF)
Eq. (3)

Enumeration of microbial population and analysis of enzymatic activity

Lignolytic and cellulolytic microorganisms were enumerated using enrichment culture on selective minimal salt media (11). Serial dilutions of soil suspensions were plated under aseptic conditions and colonies were counted after incubation. Microbial populations (colony-forming units (CFU) g⁻¹ soil) were calculated using:

$$\text{Microbial population (CFU g}^{-1}\text{ soil)} = \frac{\text{No. of colonies counted on plate}}{\text{Volume of sample taken} \times \text{Dilution factor}} \quad \text{Eq. (4)}$$

Dehydrogenase activity (DHA) was quantified using 2,3,5-triphenyl tetrazolium chloride (TTC) and expressed as mg triphenyl formazon (TPF) g⁻¹ soil day⁻¹. Fluorescein diacetate (FDA) hydrolysis, indicating overall microbial activity, was measured as µg fluorescein g⁻¹ soil hr⁻¹.

Statistical analysis

The data previously mentioned were collected and a statistical analysis was conducted using the analysis of variance (ANOVA) method (12). The significance of various sources of variation was assessed using the error mean square through Fisher and Snedecor's *F*-test at a probability threshold of 0.05 (13). The OPSTAT program was used to perform the statistical analysis.

Results and Discussion

Physico-chemical properties of soil

At harvest, *in situ* green manuring at 45 DAS combined with variable nitrogen application at tasselling showed no statistically significant differences among treatments for pH, electrical conductivity (EC), organic carbon, bulk density, porosity, field capacity, permanent wilting point and available soil moisture. Numerically favourable trends were observed in green manured treatments (T₁–T₇) compared to sole maize (T₈). Soil pH remained neutral to slightly alkaline (7.51–7.54) near the initial 7.56, with EC values of

0.93–0.97 dS m⁻¹, all non-saline (Table 2). Organic carbon was slightly higher in green manured plots (0.39–0.42 %) due to biomass addition and microbial stimulation. Bulk density decreased (1.28–1.30 g cm⁻³) and porosity increased (49.1–49.5 %) under green manuring, while field capacity (33.7–33.8 %), permanent wilting point (16.3–16.7 %) and available moisture (17.1–17.4 %) remained comparable to initial values, reflecting improved soil structure and moisture retention. These enhancements may promote stronger crop growth, better canopy coverage and accelerate weed seed decay, reducing weed seed bank longevity (14).

Soil moisture at incorporation was higher in maize + sunhemp plots (21.1–22.7 %) than sole maize (19.4 %). This increase may be attributed to the protective canopy of sunhemp and the accumulation of biomass, which reduced direct soil exposure to solar radiation and wind, thereby minimising evaporative losses. In addition, the extensive root system and subsequent incorporation of sunhemp residues likely improved soil aggregation, porosity and infiltration, enhancing the soil's capacity to retain water. Early canopy development in the intercrop system may have further reduced soil water loss by shading the soil surface. Similar findings were reported previously (15), where a five-species cover crop mixture improved gravimetric soil water content and by another researchers, with barley and hairy vetch increasing soil moisture by 13 and 8 %, respectively, compared to fallow (16). Soil temperature trends mirrored moisture, with higher near-surface temperatures in T₁–T₇ (24.2–25.1 °C) than in T₈ (23.6 °C). The shading effect of green manure likely moderated soil temperature, consistent with previous researchers, who reported soil temperature is influenced by canopy, water and topography (17) and noting cooler day/warm night effects under cover crops (18). Cover crops can raise under-snow temperatures up to 3 °C (19) and enhance near-surface winter temperatures while lowering them in spring (20), supporting the trends observed in green manured plots.

Lignin, cellulose and hemicellulose contents

The cell wall constituents of sunhemp were studied in terms of cellulose, hemicellulose and lignin, the three basic components of crude biomass (21). The cell wall components remained the same for sunhemp raised in different treatments (Table 3). The biochemical composition basically is of the same components, but the proportions can vary between species, plants of the same species, organs of the same plant and crop conditions (22). Sunhemp was subjected to similar growth conditions and an equal amount of fertilisers in all the treatments, hence did not put up any

Table 2. Soil physico-chemical properties at the initial and harvest stages and soil moisture and soil temperature measured at 45 DAS and the grain yield of maize

Treatments	pH	EC (dS m ⁻¹)	OC (%)	BD (g cc ⁻¹)	Porosity (%)	FC (%)	PWP (%)	AM (%)	SM (%)	ST (°C)	Grain yield (kg ha ⁻¹)
T ₁	7.54	0.96	0.40	1.28	49.5	33.7	16.4	17.3	21.1	24.3	5110
T ₂	7.53	0.94	0.41	1.3	49.4	33.8	16.5	17.3	21.7	24.2	5605
T ₃	7.53	0.95	0.42	1.28	49.3	33.7	16.5	17.2	22.5	24.6	5634
T ₄	7.52	0.97	0.40	1.28	49.5	33.8	16.7	17.1	22.7	25.1	6234
T ₅	7.53	0.95	0.42	1.29	49.3	33.7	16.6	17.1	21.9	24.5	6461
T ₆	7.52	0.94	0.40	1.29	49.4	33.8	16.6	17.2	22.3	24.3	6537
T ₇	7.51	0.93	0.41	1.28	49.5	33.8	16.7	17.1	22.6	25.0	6087
T ₈	7.54	0.97	0.39	1.32	49.1	33.7	16.3	17.4	19.4	23.6	6036
S.Em ±	2.07	1.22	5.02	0.03	0.002	0.29	0.64	0.77	0.64	0.37	68.6
CD (p = 0.05)	NS	NS	NS	NS	NS	NS	NS	NS	1.94	1.13	208

FC: field capacity; PWP: permanent wilting point; AM: available moisture; SM: soil moisture; ST: soil temperature; T₁: *in situ* green manuring of sunhemp at 45 DAS (GM) + 0 kg N ha⁻¹ at tasselling (T) stage; T₂: GM + 10 kg N ha⁻¹ at T; T₃: GM + 20 kg N ha⁻¹ at T; T₄: GM + 30 kg N ha⁻¹ at T; T₅: GM + 40 kg N ha⁻¹ at T; T₆: GM + 50 kg N ha⁻¹ at T; T₇: GM + 60 kg N ha⁻¹ at T; T₈: farmer's practice.

Table 3. Nodule count (No. plant⁻¹) and weight (mg plant⁻¹) up to 45 DAS and fibre content (%) of sunhemp at 45 DAS

Treatments	Fibers			TN/ EN/ FW	Week 1			Week 2			Week 3			Week 4			Week 5			Week 6		
	CL (%)	HC (%)	LN (%)		TN	EN	FW	TN	EN	FW	TN	EN	FW	TN	EN	FW	TN	EN	FW	TN	EN	FW
T ₁	14.6	11.8	1.26	-	3.5	2.8	3.5	8.1	6.1	7.1	10.6	7.4	16.9	13.9	9.0	25.2	22.5	14.6	45.8			
T ₂	15.2	12	1.31	-	3.6	2.9	3.6	8.3	6.2	7.3	10.8	7.6	17.1	14.1	9.2	25.5	22.7	14.8	46.2			
T ₃	14.8	11.9	1.29	-	3.8	3.1	3.7	8.4	6.3	7.5	10.9	7.6	17.3	14.3	9.3	25.7	22.9	14.9	46.6			
T ₄	15.6	12.4	1.34	-	4.2	3.4	4.1	9.0	6.8	8.0	12.0	8.4	19.1	15	9.8	27.1	24.0	15.6	48.8			
T ₅	15.4	12.1	1.28	-	4.0	3.2	3.7	8.6	6.5	7.6	11.0	7.7	17.5	14.6	9.5	26.4	23.3	15.2	47.4			
T ₆	14.9	11.5	1.32	-	4.0	3.2	3.9	8.8	6.6	7.8	11.4	8.0	18.1	14.8	9.6	26.7	23.6	15.3	48.3			
T ₇	14.7	12.6	1.31	-	4.1	3.3	4.0	9.0	6.8	7.9	11.6	8.1	18.5	15.0	9.8	27.0	23.8	15.5	48.4			
S.Em ±	0.4	0.3	0.04	-	0.3	0.2	0.3	0.5	0.3	0.4	0.6	0.5	1.1	0.5	0.4	1.1	1.2	0.5	1.2			
CD (p = 0.05)	NS	NS	NS	-	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

CL: cellulose; HC: hemicellulose; LN: lignin; FW: fresh weight of nodule; TN: total nodule count; EN: effective nodule count; DAS: days after sowing; T₁: *in situ* green manuring of sunhemp at 45 DAS (GM) + 0 kg N ha⁻¹ at tasselling (T) stage; T₂: GM + 10 kg N ha⁻¹ at T; T₃: GM + 20 kg N ha⁻¹ at T; T₄: GM + 30 kg N ha⁻¹ at T; T₅: GM + 40 kg N ha⁻¹ at T; T₆: GM + 50 kg N ha⁻¹ at T; T₇: GM + 60 kg N ha⁻¹ at T; T₈: farmer's practice.

variation in cell wall constituents. The cellulose, hemicellulose and lignin contents in sunhemp at the time of incorporation ranged from 14.6 to 15.6 %, 11.5 to 12.6 % and 1.26 to 1.34 %, respectively. The low lignin and high cellulose and hemicellulose contents of sunhemp indicate its easily degradable nature upon incorporation and decomposition (23). From the data, it can be inferred that up to 45 DAS, sunhemp was in the vegetative stage as it accumulated more primary cell wall constituents (cellulose and hemicellulose) than secondary cell wall components (lignin). Further, the dry biomass production of 1.33 to 1.42 t ha⁻¹ of sunhemp may be attributed to the build-up of primary cell wall constituents in higher proportion.

Nodule count

The sunhemp did not develop nodules in its roots up to 7 DAS (Table 3). Nodule development was observed after the 2nd week and a weekly assessment of total nodule count indicated that sunhemp accommodated between 3.50 to 4.20 nodules at 15 DAS (Week 2) and 22.5 to 24.0 nodules at 45 DAS (Week 6). There was a gradual increase in nodule count from week 2 to week 6. The nodule count enumerated at week 2, 3, 4, 5 and 6 ranged from 3.50–4.20, 8.11–9.00, 10.6–12.0, 13.9–15.0 and 22.5–24.0, respectively. However, 65–80 % of the total nodules were effective in sunhemp across the treatments accounting for 2.81 to 3.36 nodules at week 2 and 14.6 to 15.6 nodules at week 6. The effective nodule counts at week 2, 3, 4, 5 and 6 was 2.81–3.36, 6.08–6.75, 7.40–8.40, 9.04–9.75 and 14.6–15.6, respectively. The total and effective nodule count of the green manure crop across the treatments at the time of incorporation was 22.5 to 24.0 and 14.6 to 15.6, respectively. The above result may be ascribed to the inherent nodule-forming trait of sunhemp and its rapid growth and eventual development of enough carbon sources to meet the energy demand of the bacteria residing in the nodules to put up an effective nodule count. Similar results were obtained earlier (24–26).

Nodule weight

The nodule weight of sunhemp noted at weekly intervals (Table 3). As nodulation in sunhemp has been earmarked after week 2, the nodule weight data representing nodule count is presented from week 2 to week 6. At all the sampling dates starting from 15 DAS, sunhemp had put forth equivalent values of nodule weights among the treatments. Adequate nutrition as an external source to

sunhemp in all the treatments had not only stimulated growth but also the vigour of nodulation in sunhemp. The nodule weight recorded at weeks 2, 3, 4, 5 and 6 are 3.50–4.10, 7.10–8.00, 16.9–19.1, 25.2–27.1 and 45.8–48.8 mg, respectively. The nodule weights of the sunhemp corresponding to nodule count may be attributed to the larger size of the nodules under fertilised conditions manifesting higher nodule weights. This can be further substantiated by the increased nodule efficiency in nitrogen fixation resulting in higher nodule weights in sunhemp. The results agree with previous studies (23,27).

Cellulolytic organisms

At the time of green manure incorporation (45 DAS), the soil of the treatments accommodating maize + green manure crops (T₁–T₇) had cellulolytic organisms on par and higher. Treatment T₈ without the green manure crop showed the lowest (34.3 × 10³ CFU g⁻¹ soil) population of cellulolytic organisms and remained distinct from the aforementioned treatments (Table 4). The cellulolytic organisms in T₁ through T₇ treatments varied from 49.7 to 55.6 (× 10³ CFU g⁻¹ soil). The presence of a green manure crop between the maize pairs and the coexistence of green manure with soil bacteria, as well as the beneficial enzymes and exudates generated by the activity of plant roots and soil bacteria, may have helped the population of cellulolytic organisms increase in the T₁–T₇ treatments. A similar pattern of observations has been made at 65 DAS. The cellulolytic beings in the soil ranged from 83.7–92.4 (× 10³ CFU g⁻¹ soil) at this stage and presumably reached their maximum during the crop-growing period. The availability of more substrate due to incorporated green manure at 45 DAS and its subsequent decaying caused microbial communities to flourish and reach a maximum at this stage. This confirms the findings of previous researchers revealing that residue addition had a significant impact on the organisation of soil microbial communities (23, 28). The increase in cellulolytic microorganisms following green manure incorporation indicates active decomposition of the incorporated biomass. During this phase, decomposition releases soluble carbon compounds that stimulate microbial multiplication and enzyme production, resulting in a rapid increase in cellulolytic populations. The agreement of the present findings with earlier studies suggests that incorporation of organic residues consistently enhances microbial abundance by increasing carbon availability and creating a favourable environment for residue-degrading microorganisms. At this point,

Table 4. Effect of *in situ* green manuring and variable nitrogen split application on cellulolytic and lignolytic organisms in soil

Treatments	Cellulolytic organisms ($\times 10^3$ CFU g^{-1} soil)					Lignolytic organisms ($\times 10^4$ CFU g^{-1} soil)				
	At incorporation	20 DAI	40 DAI	60 DAI	Harvest	At incorporation	20 DAI	40 DAI	60 DAI	Harvest
T ₁	49.7	83.7	58.7	46.2	38.8	135.7	206.0	148.4	96.8	55.5
T ₂	50.3	85.1	61.6	49.2	40.7	138.2	211.0	153.1	99.8	58.2
T ₃	51.1	86.8	64.0	50.9	42.9	140.9	218.0	158.0	100.5	61.6
T ₄	52.9	88.8	66.4	52.7	43.4	144.0	226.0	165.8	105.2	67.3
T ₅	53.5	89.3	71.4	58.5	51.0	146.9	230.0	172.1	117.8	78.3
T ₆	54.7	90.2	73.8	60.7	53.2	150.0	236.0	176.0	123.2	81.0
T ₇	55.6	92.4	75.2	62.3	55.3	152.5	244.0	181.0	127.0	86.7
T ₈	34.3	59.1	41.2	35.0	23.7	85.9	107.0	78.9	54.0	31.3
S.Em $\pm$	2.3	3.0	1.8	1.7	2.2	6.3	13.2	3.6	4.0	3.6
CD ($p = 0.05$)	7.1	9.0	5.4	5.2	6.7	19.0	40.2	10.9	12.2	10.8

DAI: days after incorporation; T₁: *in situ* green manuring of sunhemp at 45 DAS (GM) + 0 kg N ha⁻¹ at tasselling (T) stage; T₂: GM + 10 kg N ha⁻¹ at T; T₃: GM + 20 kg N ha⁻¹ at T; T₄: GM + 30 kg N ha⁻¹ at T; T₅: GM + 40 kg N ha⁻¹ at T; T₆: GM + 50 kg N ha⁻¹ at T; T₇: GM + 60 kg N ha⁻¹ at T; T₈: farmer's practice.

the data also showed that, with the exception of T₈, all other treatments (T₁–T₇) were on par or higher, with microbial communities ranging from 83.7 to 92.4 ($\times 10^3$ CFU g^{-1} soil). As stated in the previous paragraph, the crops in T₁–T₇ are maize + green manure. While T₈ with sole maize had the fewest cellulolytic organisms (59.1×10^3 CFU g^{-1} soil) present in the soil.

Results at 65 DAS confirm that green manuring has a positive impact on regulating the soil environment and enhancing soil biology. The cellulolytic population decreased from 41.2 to 75.2 ($\times 10^3$ CFU g^{-1} soil) to 23.7 to 55.3 ($\times 10^3$ CFU g^{-1} soil), according to the observations made at 85 DAS (40 DAI), 105 DAS (60 DAI) and harvest (75 DAI). After 20 DAI (65 DAS), there was a decline in the cellulolytic population, indicating their mortality and potential soil nitrogen mineralisation. The soil cellulolytic population varied considerably between the treatments at all phases past 65 DAS (85, 105 DAS and at harvest). The cellulolytic population had shown a gradual increase from T₁ to T₇ treatments, where nitrogen split (60 kg ha⁻¹) to be applied at the tasselling stage of maize is scheduled from 0 to 60 kg N ha⁻¹, respectively. The treatments T₅, T₆ and T₇, however, seemed comparable and revealed a larger population of cellulolytic organisms. The cellulolytic population in these treatments ranged from 71.4–75.2, 58.5–62.3 and 51.0–55.3 $\times 10^3$ CFU g^{-1} soil at 85, 105 DAS and at harvest, respectively. In T₁ to T₇ treatments, the addition of green manure and irrigation may have generated ideal conditions with enough substrate and temperature for the cellulolytic organisms to thrive. Variable N application from 0 to 60 kg N ha⁻¹ during the tasselling stage in increments of 10 kg N ha⁻¹ may be the reason for gradual rise in cellulolytic population from T₁ to T₇. Further, the application of nitrogen split in higher doses at 40, 50 and 60 kg N ha⁻¹ at the tasselling stage in T₅, T₆ and T₇ treatments, respectively may have improved the substrate (carbon) in the soil, maintaining higher cellulolytic populations despite their mortality after 65 DAS. The least number of populations were maintained in T₈ at 85, 105 DAS and harvest accommodated with maize just applied with advised nitrogen. These populations were 41.2, 35.0 and 23.7×10^3 CFU g^{-1} soil, respectively at the above-mentioned stages of sampling.

Lignolytic population

Sunhemp being a herbaceous annual crop with appreciable stem biomass by 45 DAS that had sustained a lignolytic population in the soil. The treatments showed significant differences for the lignolytic population in the soil when observed at different stages (45, 65, 85, 105 DAS and harvest). Observations made before incorporation of

the green manure at 45 DAS indicated that the treatments T₁–T₇ with a population of 135.7–152.5 $\times 10^4$ CFU g^{-1} of soil, were on par and higher concerning lignolytic organisms (Table 4). The left-alone treatment T₈ was inferior with the least population of 85.9×10^4 CFU g^{-1} soil and remained different from the other treatments. This pattern is similar to that observed in the cellulolytic population.

By 65 DAS (20 DAI), the incorporated green manure started decaying confirmed by the increase in the lignolytic population reaching its maximum as was seen with cellulolytic beings. However, the trend observed at the time of incorporation of green manure (45 DAS) continued at 65 DAS with T₁ to T₇ treatments being with equivalent and higher population ranging from 206–244 $\times 10^4$ CFU g^{-1} soil. While T₈ is the least with a population of 107×10^4 CFU g^{-1} soil. Later to 65 DAS, the lignolytic population started declining towards harvest. At all the later stages (85 (40 DAI), 105 (60 DAI) DAS and harvest), the lignolytic population was highest in T₅, T₆ and T₇ which were treated with 40, 50 and 60 kg N ha⁻¹, respectively at the tasselling stage in addition to green manure incorporation. The lignolytic population at 85, 105 DAS and harvest in the above treatments ranged from 172.1–181.0, 117.8–127.0 and 78.3–86.7 $\times 10^4$ CFU g^{-1} soil, respectively. This was in line with that obtained for the cellulolytic population. Green manure addition enriched with N application at the tasselling stage in higher amounts than T₁, T₂, T₃ and T₄ might have increased the availability of substrate for the microbial communities to multiply at an increased rate. This can also be confirmed by the gradual increase in microbial communities from T₁ to T₇ treatments. The maintenance of higher lignolytic populations in T₅, T₆ and T₇ may be attributed to improved nitrogen availability, which supports microbial growth and enzyme synthesis during residue decomposition. Nitrogen is often a limiting nutrient during the degradation of lignin-rich materials; therefore, additional nitrogen application may have enhanced microbial utilisation of the incorporated sunhemp residues and sustained larger lignolytic populations. The gradual increase in lignolytic organisms from T₁ to T₇ further suggests a positive interaction between residue incorporation and nitrogen availability in regulating microbial activity. On the other side, T₈ with the application of recommended nitrogen and no green manuring resulted in the least population (78.9, 54.0 and 31.3×10^4 CFU g^{-1} soil) of lignolytic organisms at 85, 105 DAS and harvest.

Dehydrogenase activity

Among all enzymes in the soil environment, dehydrogenases are one of the most important enzymes and are used as an indicator of

overall soil microbial activity (29–31), because they occur intracellularly in all living microbial cells (32–34). In line with the data on microbial communities, the DHA increased up to 65 DAS and later decreased towards harvest (Table 5). At all the stages of observation, the DHA was significant among the treatments. At the time of green manure incorporation (45 DAS), the DHA was comparable and higher for T₁ to T₇ treatments ranging from 38.9 to 47.5 µg TPF g⁻¹ soil 24 hr⁻¹. These treatments accommodating maize + green manure in the system have favoured the growth of microbial communities. The rooting activity of the accompanying green manure crop and the enzymatic activity of the co-existing bacteria have provided a favourable situation for the microbial communities to multiply. However, the T₈ treatment having sole maize remained different from the other treatments with the lowest DHA (32.4 µg TPF g⁻¹ soil 24 hr⁻¹).

The DHA rose to the maximum at 65 DAS and ranged from 67.3 to 80.5 µg TPF g⁻¹ soil 24 hr⁻¹ from T₁ through T₇ treatments. The DHA was on par and higher in these treatments. T₈ continued to be the last one with the lowest (41.3 µg TPF g⁻¹ soil 24 hr⁻¹) DHA in the soil. The incorporation of green manure at 45 DAS and its subsequent decomposition has increased the substrate availability for microbial growth resulting in their increased population and hence DHA in T₁ to T₇ treatments. However, the raising of sole maize without any green manure crop in T₈ caused a less favourable environment for the microbial communities to flourish, hence less population and DHA. Later to 65 DAS up to harvest, the DHA diminished indicating the decrease in soil microbial population towards harvest. In the subsequent phases (85, 105 DAS and harvest), the differences among the treatments became more conspicuous with enhanced DHA in T₅, T₆ and T₇ treatments than in the remaining. The DHA ranged from 68.4 to 71.0, 55.4 to 58.0 and 42.7 to 45.2 µg TPF g⁻¹ soil 24 hr⁻¹ at 85, 105 DAS and harvest, respectively. Green manuring and nitrogen supplementation in graded increments at 10 kg N ha⁻¹ from T₁ to T₇ have increased the DHA gradually. Further, higher doses of nitrogen beyond 40 kg ha⁻¹ might have enhanced the soil substrate (carbon) for the microbial communities to multiply, thus increasing the DHA in T₅, T₆ and T₇ treatments. Conversely, T₈ continued to be the least treated with the lowest (36.0, 29.0 and 26.8 µg TPF g⁻¹ soil 24 hr⁻¹, respectively) DHA at 85, 105 DAS and harvest.

Fluorescein diacetate hydrolysis enzyme activity

Assessment of hydrolysis of fluorescein diacetate (3', 6'- diacetyl fluorescein (FDA)) has been suggested as a prospective method for the determination of total microbial activity because it includes

several enzyme classes, including lipases, esterases and proteases (35). The inclusion of green manure crops in the maize system had shown a positive effect on the soil microbial activity, as noted from the data of cellulolytic and lignolytic organisms. Similar to DHA, FDA activity was higher and on par in T₁ through T₇ treatments with maize + green manure crops (Table 5). The FDA in these treatments ranged from 65.6 to 77.0 µg fluorescein g⁻¹ soil 0.5 hr⁻¹ at 30 DAS. Maize with no green manure crop (T₈) reported the lowest (49.2 µg fluorescein g⁻¹ soil 0.5 hr⁻¹) FDA.

A similar trend continued till 65 DAS, with T₁ to T₇ being comparable and higher with 76.3–88.5 µg fluorescein g⁻¹ soil 0.5 hr⁻¹ FDA. The FDA reached its maximum at 65 DAS in line with DHA. T₈ continued to exhibit the least (59.4 µg fluorescein g⁻¹ soil 0.5 hr⁻¹) FDA at 65 DAS. The peak FDA activity at 65 DAS coincided with the period of active decomposition of incorporated sunhemp biomass. During this phase, microbial communities are generally characterised by high enzymatic activity owing to rapid nutrient transformation and organic matter turnover. The subsequent decline towards harvest may be attributed to depletion of readily decomposable organic compounds and reduced microbial demand as decomposition progressed. Obvious differences were noted at later stages, i.e., at 85, 105 DAS and harvest. After 65 DAS, the FDA diminished towards harvest. Among the treatments, T₅, T₆ and T₇ performed equivalently and reported higher FDA ranging from 73.2 to 78.9, 57.3 to 64.0 and 44.1 to 49.3 µg fluorescein g⁻¹ soil 0.5 hr⁻¹ at 85, 105 DAS and harvest, respectively. The conspicuous and higher performance of the above treatments than the remaining may be attributed to the application of higher amounts of nitrogen above 40 kg N ha⁻¹ at the tasselling stage in addition to green manuring. On the other hand, maize in the T₈ treatment, though it received the recommended amount of N at all the critical stages, it missed out on the additional benefit from green manuring, hence reporting the lowest (85 DAS- 48.1; 105 DAS- 38.1; and harvest - 26.3 µg fluorescein g⁻¹ soil 0.5 hr⁻¹) soil FDA throughout the crop growth period.

Soil available nutrients

During the crop growth period, soil available N, P and K was increased up to tasselling stage thereafter a declining trend was observed till harvest (Table 6). Nitrogen fertilisation at sowing, 45 DAS, knee high, pre-tasselling and tasselling stages and the *in situ* mineralisation of the biomass derived from green manure, might have contributed to higher soil NPK levels compared to the initial status up to tasselling. In our study, maize + sunhemp (green manure) intercropping (T₁–T₇) consistently maintained higher NPK concentrations than sole maize (T₈) during the early and mid-

Table 5. Effect of *in situ* green manuring and variable nitrogen split application on DHA and FDA in soil

Treatments	DHA (µg TPF g ⁻¹ soil 24 hr ⁻¹)					FDA (µg fluorescein g ⁻¹ soil 0.5 hr ⁻¹)				
	At incorporation	20 DAI	40 DAI	60 DAI	Harvest	At incorporation	20 DAI	40 DAI	60 DAI	Harvest
T ₁	38.9	67.3	55.5	43.0	34.4	65.6	76.3	61.6	44.0	34.7
T ₂	39.2	69.1	56.3	45.1	35.9	67.1	78.2	63.0	46.0	36.1
T ₃	42.0	72.7	59.3	48.0	37.4	69.4	80.8	66.0	50.0	38.1
T ₄	44.0	74.2	62.5	50.3	38.4	71.5	81.3	68.0	51.0	40.0
T ₅	45.6	77.8	68.4	55.4	42.7	73.1	83.9	73.2	57.3	44.1
T ₆	45.8	79.6	70.3	56.3	43.5	74.1	85.6	75.7	60.0	46.1
T ₇	47.5	80.5	71.0	58.0	45.2	77.0	88.5	78.9	64.0	49.4
T ₈	32.4	41.3	36.0	29.0	26.8	49.2	59.4	48.1	38.1	26.3
S.Em ±	3.0	4.6	1.5	1.0	1.1	4.0	4.8	2.2	2.4	2.4
CD (p = 0.05)	9.1	14.0	4.6	3.0	3.3	12.1	14.7	6.7	7.2	7.2

DHA: dehydrogenase activity; FDA: fluorescein diacetate hydrolytic activity; TPF: triphenyl formazan; DAI: days after incorporation; T₁: *In situ* green manuring of sunhemp at 45 DAS (GM) + 0 kg N ha⁻¹ at tasselling (T) stage; T₂: GM + 10 kg N ha⁻¹ at T; T₃: GM + 20 kg N ha⁻¹ at T; T₄: GM + 30 kg N ha⁻¹ at T; T₅: GM + 40 kg N ha⁻¹ at T; T₆: GM + 50 kg N ha⁻¹ at T; T₇: GM + 60 kg N ha⁻¹ at T; T₈: farmer's practice.

Table 6. Soil available nitrogen, phosphorus and potassium at different stages of maize in response to *in situ* green manuring at 45 DAS and variable nitrogen split application at the tasselling stage

Treatments	Soil available nitrogen (N), phosphorus (P ₂ O ₅) and potassium (K ₂ O) in kg ha ⁻¹ at different stages of maize																	
	Knee high			At incorporation (45 DAS)			Pre-tasselling			Tasselling			Grain filling			Harvest		
	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O
T ₁	187.0	18.8	318.0	207.0	22.5	306.0	213.0	24.0	312.0	225.0	25.5	316.0	189.0	20.6	312.0	164.0	17.5	303.0
T ₂	189.0	19.3	319.0	209.0	22.9	307.0	215.0	24.4	314.0	227.0	25.9	317.0	194.0	20.9	314.0	169.0	17.9	304.0
T ₃	185.0	19.6	322.0	205.0	23.2	309.0	213.0	24.7	315.0	223.0	26.2	318.0	197.0	21.5	314.0	172.0	18.0	305.0
T ₄	189.0	21.5	327.0	209.0	24.8	317.0	215.0	26.3	321.0	227.0	27.8	324.0	212.0	23.8	325.0	187.0	19.8	318.0
T ₅	185.0	20.3	323.0	208.0	23.8	313.0	214.0	25.3	316.0	226.0	26.8	320.0	203.0	21.8	314.0	178.0	18.3	307.0
T ₆	186.0	20.7	326.0	206.0	24.1	314.0	212.0	25.6	317.0	224.0	27.1	322.0	205.0	22.2	315.0	180.0	18.6	309.0
T ₇	184.0	21.2	326.0	204.0	24.6	316.0	210.0	26.1	320.0	222.0	27.6	324.0	208.0	23.3	320.0	183.0	19.3	313.0
T ₈	182.0	17.1	314.0	201.0	20.8	301.0	207.0	21.6	308.0	219.0	22.2	313.0	201.0	18.2	309.0	176.0	16.5	302.0
S.Em ±	1.9	1.2	4.0	2.1	1.2	5.0	2.1	1.4	3.9	2.3	1.2	3.4	2.0	0.4	3.0	1.8	0.3	2.6
CD (p = 0.05)	5.8	3.6	12.0	6.3	3.7	15.3	6.4	4.2	11.9	7.1	3.5	10.4	6.1	1.2	9.0	5.4	0.8	7.9

DAS: days after sowing; N: nitrogen; P₂O₅: phosphorus (expressed as phosphorus pentoxide); K₂O: potassium (expressed as potassium oxide); T₁: *In situ* green manuring of sunhemp at 45 DAS (GM) + 0 kg N ha⁻¹ at tasselling (T) stage; T₂: GM + 10 kg N ha⁻¹ at T; T₃: GM + 20 kg N ha⁻¹ at T; T₄: GM + 30 kg N ha⁻¹ at T; T₅: GM + 40 kg N ha⁻¹ at T; T₆: GM + 50 kg N ha⁻¹ at T; T₇: GM + 60 kg N ha⁻¹ at T; T₈: farmer's practice.

growth stages. Specifically, soil available NPK ranged from 222–227, 25.5–27.8 and 316–324 kg ha⁻¹, respectively, at the tasselling stage. However, sole maize consistently recorded the lowest NPK levels across these stages. The higher nutrient availability in T₁–T₇ compared with T₈ indicates the contribution of green manure beyond fertiliser inputs alone. In addition to supplying nutrients through decomposition, sunhemp residues likely reduced nutrient losses and improved nutrient retention through increased soil organic matter and microbial activity. Similar observations have been reported in green-manuring systems where decomposition of leguminous biomass enhanced nutrient availability and nutrient-use efficiency.

From the grain-filling stage to harvest, soil NPK gradually declined. This decline may be attributed to increased crop uptake and reduced nutrient mineralisation. The decline in available NPK from grain filling to harvest can be primarily attributed to increased nutrient uptake by maize during reproductive development, when demand for nutrients is greatest for grain formation and translocation. Moreover, as decomposition progressed, the quantity of readily mineralisable organic matter decreased, resulting in a lower rate of nutrient release than crop removal. However, treatments combining N-fertilisation at 30 and 60 kg ha⁻¹ with green manuring (T₄ and T₇) maintained comparatively higher available NPK (Grain-filling: N 212–208, P 23.8–23.3, K 325–320 kg ha⁻¹; Harvest: N 187–183, P 19.8–19.3, K 318–313 kg ha⁻¹). Similarly, treatments with medium N dose (T₅ and T₆) showed comparable values. Treatments with 0–10 kg N ha⁻¹ (T₁ and T₂) recorded the least available NPK at the grain filling to harvest (Grain-filling: N 189–194, P 21.8–22.2, K 309–312 kg ha⁻¹; Harvest: N 164–169, P 16.5–18.6, K 302–309 kg ha⁻¹). These results suggested that green manuring not only enhanced soil nutrient availability but also reduce the soil NPK depletion during the reproductive to hard dough phase. It was attributed to higher nutrient mineralisation and microbial activity, resulted from sunhemp green manuring, which also aided in nutrient recycling from deeper soil layer. Furthermore, the deep-rooted nature of sunhemp may have facilitated nutrient retrieval from deeper soil layers and their subsequent return to the surface soil through biomass incorporation. This nutrient recycling effect,

coupled with enhanced microbial activity and organic matter turnover, likely contributed to the reduced depletion of available N, P and K observed in green-manured treatments.

Correlation analysis

The correlation matrix (Fig. 2) between grain yield of maize and the parameters pertaining to soil bio-chemical health revealed a strong, significant and positive association among cellulolytic organisms, lignolytic organisms and FDA hydrolytic activity across incorporation, intermediate and harvest stages. It implied that residue decomposition is facilitated the microbial enzyme activity or in other words availability of labile substrate with low carbon (C) : N biomass through green manure enhanced the soil microbial activity, led to the higher mineralisation. Thereby, microbial parameters were also positively correlated with soil available N and maize grain yield. The positive association among cellulolytic organisms, lignolytic organisms and FDA activity indicates that decomposition of incorporated sunhemp residues stimulated both microbial proliferation and enzymatic activity. Enhanced microbial activity accelerated the breakdown of organic residues and nutrient mineralisation, resulting in greater soil N availability. The strong correlation between available N and grain yield further suggests that microbial-mediated nutrient release played a key role in supporting maize productivity. Thus, enhanced microbial activity facilitated efficient nutrient mineralisation and improved crop performance. Particularly, the strong linkage between available N and grain yield. Hence, microbial-driven N cycling is crucial in maize productivity which may reduce the full dependency on chemical fertilisers. Overall, the results demonstrated that active decomposer communities through residue incorporation not only improved soil health but also facilitated effective nutrient release which may synchronise the crop demand. The observed relationships further highlight the importance of soil biological processes in linking residue decomposition with nutrient availability and crop performance. The synchronised release of nutrients from decomposing sunhemp biomass appears to have improved nutrient supply during critical crop growth stages, thereby contributing to higher grain yield and improved soil biochemical health.

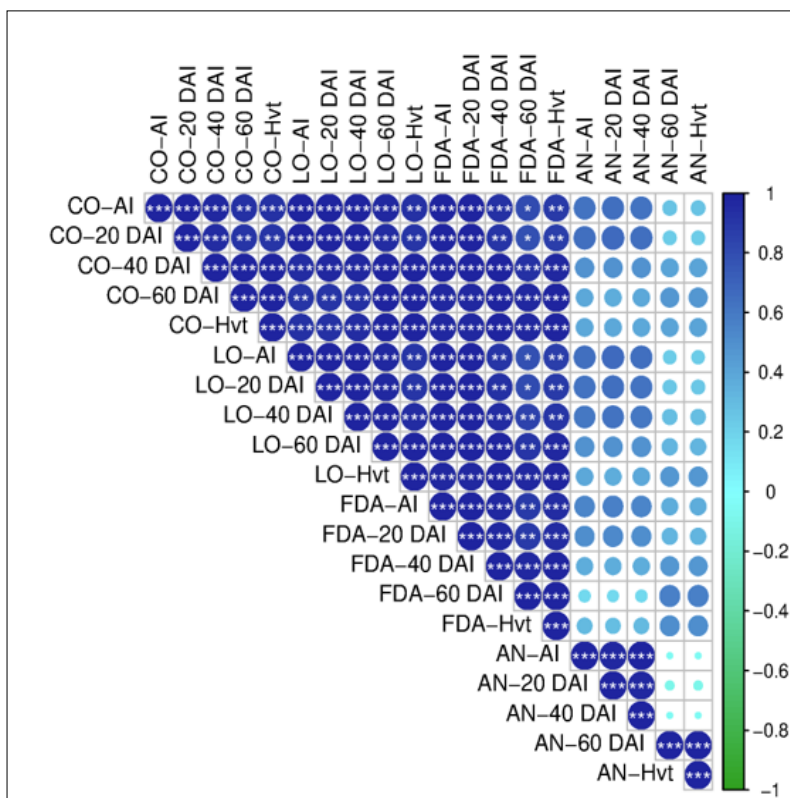


Fig. 2. Correlation matrix between cellulolytic organisms, lignolytic organisms, FDA, soil available nitrogen and maize grain yield.

CO: cellulolytic organisms; LO: lignolytic organisms; FDA: fluorescein diacetate hydrolytic activity; AN: available nitrogen; DAI: days after incorporation; Hvt: harvest; AI: at incorporation. Significant at $p \leq 0.05$; significant at $p \leq 0.01$; *significant at $p \leq 0.001$.

Conclusion

Our overall observation indicates that *in situ* sunhemp green manuring coupled with nitrogen (N) application at tasselling stage, resulted in higher soil biological activity, nutrient cycling and overall soil health in maize growing system. Especially, significant increases in cellulolytic and lignolytic bacterial populations along with dehydrogenase activity (DHA) and diacetyl fluorescein (FDA) activity were observed. Consequently, higher levels of available N, phosphorus (P) and potassium (K) were maintained during critical growth stages. Organic carbon, bulk density and soil moisture status were also improved. The approach reduces dependence on mineral fertilisers and strengthens microbial resilience and maize productivity. Thus, integration of sunhemp green manuring in maize not only reduces the fertiliser dependency after tasselling stage but also improves soil health and environmental stability.

Acknowledgements

The authors are thankful to Professor Jayashankar Telangana Agricultural University (PJTU) and the Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana, for providing all support and facilities to conduct this research.

Authors' contributions

SVK conducted the original research, performed the data analysis and wrote the original manuscript. GSMB, BP and GKR conceptualised, supervised and reviewed the research. PCL, SK, EP, NL and CBA participated in the original research. ASA contributed to writing and editing the manuscript. KVPB participated in the laboratory work. All authors have read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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