

Cumulative Impact of Herbicides and Tillage on the Soil Microbiome, Fungal Diversity and Crop Productivity under Conservation Agriculture

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Abstract

In a diversified cropping system, the tillage methods and weed management practices significantly influence the soil microbiome, which affects crop productivity. The synergetic impacts of such practices on the soil microbiome in association with yield under cotton–maize–*Sesbania rostrata* rotation with CA have not been extensively explored thus far in southern India. Therefore, a 4-year CA experiment was undertaken to investigate the impact of tillage and weed management on the soil microbiome and fungal diversity at 30 DAS and on the tasselling of maize and crop yield and to identify sustainable tillage and weed management practices that can provide nature-based solutions. The three tillage practices used were **T₁**: CT(C)-CT(M)-fallow (NSr), **T₂**: CT(C)-ZT(M)-ZT(Sr) and **T₃**: ZT+R(C)-ZT+R(M)-ZT+R(Sr), and the following weed control tactics were used: **W₁**-chemical weed control, **W₂**-chemical (herbicide) rotation, **W₃**-integrated weed management (IWM) and the **W₄**-non-weeded control. Rhizosphere soil and rhizoplane samples were collected from the respective plots at 30 DAS after herbicide application and tasselling. Analysis of the microbial population and enzyme and microbial activities, viz., soil basal respiration (SBR), metabolic quotient (qCO₂), microbial quotient (qMB), and soil microbial biomass carbon (SMBC) and nitrogen (SMBN), was performed following standard procedures. rRNA gene sequencing of 18S rRNA was performed with rhizosphere soil and rhizoplane fungi isolated at tasselling. The yield was recorded at harvest. The salient findings indicated a decrease in enzyme activity, microbial population, and microbial activity at the initial stage (30 DAS) due to the impact of herbicides, which subsequently increased in response to tasselling, except for qCO₂, which decreased. These biological properties were greater in the **T₃** treatment and nonweeded control followed by IWM, except for qCO₂, which showed a decreasing trend relative to **T₁** and **T₂** and **W₁** and **W₂** at both sampling stages of maize. K yield (KY) and system yield (SY) were greater in the **T₃**, IWM, and herbicide-treated plots (**W₁** and **W₂**) than in the **T₁**, **T₂** and nonweeded control plots. *Talaromyces flavus*, a beneficial rhizosphere soil inhabitant, was identified in **T₃** in combination with the IWM. Considering both crop productivity and soil biological assessment, **T₃** and IWM were considered the best treatment combinations among all the other treatments with SY (4453 kg ha⁻¹). These findings signify the importance of adopting reduced tillage (**T₃**) and IWM for farmers while striving for nature-based solutions.

Introduction

The world's population is expected to increase to approximately 10 billion by 2050, challenging farmers to intensify production while meeting food demand—in a scenario of modest economic growth—by approximately 50% relative to 2013 [1]. These impending crises for food production are likely to cause a considerable shift to industrial farming practices. Commercial agricultural norms are associated with intensive tillage, the use of synthetic chemical fertilizers, and the use of agrochemicals, all of which have negative impacts on the quality of soil resources and biodiversity required to promote soil biological activities. Globally, approximately 10 hectares of land used for agricultural production are instantly depleted as a result of various degradation processes caused by urbanization-related agricultural systems [2]. Approximately 120 million hectares of cultivable land are regarded as degraded in India [3], which is a considerable opportunity for sustainable food production [4]. Thus, an increase in food production must always be bolstered up by a sustainable agricultural system to sustain soil resources and facilitate soil biological processes [5]. In this view, conservation agriculture (CA) is gaining momentum as a sustainable and eco-friendly production system meant to augment the soil biological functions of agroecosystems with little mechanical activity and rational utilization of chemical inputs.

Soil microorganisms play an essential role in driving soil biological processes [6], which is essential for maintaining soil quality, agricultural sustainability and multiple ecosystem functions. Ecosystem functions are controlled by rhizosphere soil microorganisms and frequently employ function-based metrics such as soil basal respiration, decomposition of soil organic matter (SOM), soil microbial activities and extracellular enzyme activity [7]. The constituents of rhizosphere soil microorganisms and function-based metrics are strongly influenced by similar edaphic properties; thus, suitable agricultural management practices, such as irrigation, tillage, crop diversification and weed management practices, can allow rhizosphere soil microorganisms to perform different ecological functions [8–9]. Rhizosphere soil microorganisms and microbial activities change rapidly with changes in soil management practices and environmental conditions with short turnover [10] and can be

used as early indicators of soil health and crop yield improvement. Soil enzyme activity depends upon different abiotic factors, viz., soil pH, moisture content, oxygen availability and soil texture [11]. These properties are subject to change depending on the intensity of tillage, weed control practices, and diverse crop species being implemented and consequently have a significant impact on soil microbial composition and enzyme activities [12]. Conventional agricultural systems with intensive tillage practices decrease the activities of soil microbes and enzymes, change microbial diversity, and delay nutrient cycling, consequently reducing the stability or resilience of the soil functional status [13]. By altering the SOM content, cropping systems could also shift the balance of rhizosphere soil enzymes, microbial activities and populations toward biodiversity and function [14].

A number of quantitative evaluations have been accepted universally for assessing changes in soil functional activity. For instance, the SMBC, SMBN, microbial quotient (qMB), soil basal respiration (SR) and metabolic quotient (qCO_2) have been broadly utilized as indicators of soil biological status [15]. qCO_2 constitutes the metabolic level of soil microorganisms, in which greater values indicate greater stress conditions; however, a rise could also indicate an input of easily degradable carbon that activates microbial activity at times [16]. The qCO_2 is based on the concept of Odum's ecosystem succession theory, which is increasingly being applied as an indicator of ecosystem development (where it declines) and of disturbance (where it theoretically increases) [17]. Thus, the activities of soil enzymes and soil basal respiration (SBR) serve as determinants of the intensity of soil biogeochemical processes. Insights into how microbial activities and population ripening occur in response to various agricultural management practices and turmoil are essential for identifying the best agricultural practices that can augment and sustain soil resources and crop yield [18]. Soil enzyme activity is deemed to be indicative of specific biochemical reaction processes of whole-soil microbial activities that occur during SOM mineralization and is also an important indicator of soil health, pollution and ecological restoration with a short turnover time [19–20]. Microbial responses in trials have often been assessed by soil enzyme activity [21]. The introduction of new-generation selective herbicides and shortage of manual labour available for manual weeding have resulted in significant increases in preemergence and postemergence herbicide use in maize. However, herbicides are known to have significant negative or positive effects on soil microbial activities, population and diversity, which in turn impact soil processes [22]. A better understanding of the impact of herbicides on soil enzyme dynamics, the functional diversity of soil microorganisms and fungal diversity in ecosystems could provide a unique opportunity for an integrated biological assessment of soils due to their crucial role in several soil biological activities, ease of measurement, and rapid response to changes in soil management. To date, several studies have explored the influence of tillage on soil enzyme activity, microbial activities and population dynamics in combination with CA [23]; however, the direct effects of different tillage practices and weed management practices on biological parameters at different crop growth stages and on fungal diversity at various zone levels (in the soil rhizosphere and rhizoplane) and on how crop productivity relates to soil functional metrics and biodiversity have not been extensively investigated under a diversified crop rotation system (cotton-maize-*Sesbania rostrata*) in the southern region of India. Insights into fungal diversity in response to CA practices under various tillage practices and weed management practices in a diversified crop rotation can aid in identifying different pathogenic and beneficial fungal species (to facilitate the breakdown of SOM, stabilize carbon and nutrients for soil health maintenance, promote plant growth, increase crop yield, restore ecosystems and inhibit pathogens). Thus, agricultural techniques such as tillage, crop residue management, and crop rotation can influence the diversity and activity of microorganisms such as fungi [24].

Cereal-based cropping systems are common practices in southern regions of India, while maize yield and productivity decline monotonically under continuous intensive tillage systems and correspond to the deterioration of soil physicochemical properties and a decrease in soil biological activities [11; 25]. Along with ZT, the diversified crop rotation and retention of crop remains use precrop effects, which lead to enhanced biological diversity and increased crop yield over continuous cropping of a single cereal-based crop(s) under intensive tillage with crop residue removal [26–27]. Thus, a reduction in tillage intensity with continuous retention of previous crop residues and the integration of chemical and cultural weed control practices in CA under a diversified cropping system may be a solution for reducing soil degradation processes and the risk of agricultural production while improving soil functional metrics and rhizosphere soil microbial populations, which can have direct positive effects on crop productivity. Therefore, the present study was performed with the following objectives: to investigate the

synergetic effects of different tillage practices and weed management practices on soil microbial and enzyme activities, microbial population and diversity at various sampling stages of maize crops, *i.e.*, 30 DAS and tasselling stage; to determine the maize grain yield and system yield in terms of cotton equivalent yield (CEY) in a 4-year CA (8th crop cycle) experiment under a cotton–maize–*Sesbania rostrata* cropping system; and to identify a suitable tillage practice and weed management option that can reduce perturbations in soil, enhance soil biological activities and harbour beneficial fungal diversity species, reduce the metabolic quotient, boost maize productivity and system CEY.

Materials and methodologies

Details and characterization of the experimental area

This current field study was undertaken at the College Farm, PJTSAU, Southern Telangana Zone of India, under the All India Coordinated Research Project (AICRP) on Weed Management implemented from 2020 in the monsoon, winter and summer seasons under cotton (*Gossypium hirsutum*), maize (*Zea mays*), and green manure (*Sesbania rostrata*) rotations, respectively. The experiment continued from 2020 until 2023, and soil samples were collected for analysis of soil parameters and recording of yield after the winter maize crop was harvested from 2022-23 (the fourth year of the 8th crop cycle). The field trial is located at 16° 18' 17" N latitude and 78° 25' 38" E longitude. The zone is a dryland zone with approximately 708 mm of mean annual rainfall [28]. Extreme heat and humidity occur during the summer months (March to fortnight of June), when the mean temperature is 30°C. Maximum temperatures often exceed 42°C from April to May. December and January are extreme winter months with the lowest temperatures dropping as low as 10°C occasionally. Rainfall surpasses 75% due to the southwest monsoon and occurs between June and September [28].

Weather during crop development

Meteorological observations taken during crop development from the station situated at the Institute of Agricultural Research (IAR), Rajendranagar, on a weekly basis are presented in Fig. 2.

Soil characteristics

The soil of the study area is classified as follows: *Inceptisol*, sandy clay loam in texture; red chalk in colour; slightly alkaline (7.82) in soil pH as a result of available lime concretion beneath the horizon; 1.23 Mg m⁻³ in bulk density; nonsaline (0.33 dS m⁻¹); medium range in soil organic carbon (6.50 g kg⁻¹); low range in available soil nitrogen (220.90 kg ha⁻¹); medium range in available soil phosphorus (22.40 g kg⁻¹); and high range in available soil potassium (408.75 kg ha⁻¹) at the soil surface (0–15 cm) at the initiation of the experiment.

Design of the experiment and treatment details

A conservation agriculture (CA) experiment was conducted in accordance with a split plot design with three tillage (s) practices in the main plots, as shown in Table 1; four weed management options were used in the subplot treatments, as detailed in Table 2; and combinations of tillage and weed management were replicated thrice. For T₁, which was subjected to conventional tillage, the plots were prepared by ploughing two times, followed by rotovating and seeding. In T₂, during zero tillage (ZT), no-tillage of the soil was performed; *i.e.*, seeding was performed directly by opening the soil followed by surface soil sealing, and in T₃, there was zero tillage (ZT) + residue retention (R) or no tillage of the soil. The preceding crop (cotton and *Sesbania rostrata*) residues were shredded, retained, and incorporated into the soil, and seeding was performed directly by opening the soil, accompanied by soil surface sealing with mulch from crop residues (Table 1). The weed management strategies used included the following: W₁: chemical weed control, W₂: chemical (herbicide) rotation, W₃: chemical weed control and power + 1 hand weeding (IWM) and W₄: non-weeded control, as fully described in Table 2. No tillage operations or weed management were performed prior to sowing summer green manure (*Sesbania rostrata*), as it was cultivated for up to 45 days with the intention of retaining and incorporating its residues into the soil in T₃. No green manure (*Sesbania rostrata*) was sown in the T₁ plots; *i.e.*, the T₁ plots were fallowed during the summer season.

Table 1
Annotation of tillage treatments with crop diversification in
the main plots

Tillage (s)	Seasons		
	Monsoon	Winter	Summer
T ₁ :	CT (C) –	CT (M) –	Fallow (NSr)
T ₂ :	CT (C) –	ZT (M) –	ZT (Sr)
T ₃ :	ZT + R (C) –	ZT + R (M) –	ZT + R (Sr)

CT(C) = conventional tillage (cotton), CT(M) = conventional tillage (maize), Fallow (NSr) = Fallow, No *Sesbania rostrata*, ZT (M) = zero tillage (maize), ZT (Sr) = *Sesbania rostrata*, ZT + R (C) = zero tillage (cotton) + residue retention, ZT + R (M) = zero tillage (Maize) + residue retention, ZT + R (Sr) = zero tillage (*Sesbania rostrata*) + residue retention.

Table 2
Weed management (WM) in subtreatments and interaction with tillage (T) in the main treatments

Monsoon (Cotton)				Winter (Maize)			
W ₁ :	W ₂ : Herbicide Rotation (Every year)	W ₃ :	W ₄ :	W ₁ :	W ₂ : Herbicide Rotation (Every year)	W ₃ : IWM	W ₄ :
Chemical Weed Control		IWM	Non-weeded Control	Chemical Weed Control			Non-weeded Control
T ₁	Diuron preemergent	Diuron PE 0.75			Atrazine 1.0 kg/ha		
T ₂	-ce application PE 0.75 kg/ha fb tank mix application of pyriithiobac -sodium 62.5 g/ha + quiza-lofop-ethyl 50 g/ha as PoE (Postemergence application) (2–3 weed leaf stage) fb directed spray (interrow) of paraquat 0.5 kg/ha at 50–55 DAS.	kg/ha fb tank mix application of pyriithiobac-sodium 62.5 g/ha + quiza-lofop-ethyl 50 g/ha as PoE (2–3 weed leaf stage) fb directed spray (interrow) of paraquat 0.5 kg/ha at 50–55 DAS. rotated with Pendimethalin 1 kg ha ⁻¹ fb tank mix application of pyriithiobac-sodium 62.5 g/ha + quiza-lofop ethyl 50 g/ha as PoE (2–3 weed leaf stage) fb directed spray (interrow) of paraquat 24% SL 0.5 kg/ha at 50–55 DAS.	Diuron PE 0.75 kg/ha fb mechanical brush cutter twice at 25 and 60 DAS. One hand weeding was done after the critical period of crop-weed competition, i.e., between 45–50 days after sowing)	Atrazine 1.0 kg/ha + paraquat 600 g/ha PE fb tembotrione 120 g/ha at 20–25 DAS as PoE (T ₂ , T ₃). Atrazine 1 kg ha ⁻¹ PE fb tembotrione 120 g/ha at 20–25 DAS as PoE (T ₁). rotated with Atrazine 1.0 kg/ha PE fb halo-sulfuron methyl 67.5 g/ha at 20–25 DAS as PoE (T ₁).	+ paraquat 600 g/ha PE fb tembotrione 120 g/ha at 20–25 DAS as PoE (T ₂ , T ₃). Atrazine 1.0 kg/ha PE fb tembotrione 120 g/ha at 20–25 DAS at PoE (T ₁). rotated with Atrazine 1.0 kg/ha + paraquat 600 g/ha PE fb halosulfuron-methyl 67.5 g/ha at 20–25 DAS as PoE (T ₂ , T ₃). Atrazine 1.0 kg/ha PE fb halo-sulfuron methyl 67.5 g/ha at 20–25 DAS as PoE (T ₁).	Tembotri-one 120 g/ha Atrazine 50% WP 0.5 kg/ha as Early post-emergence EPoE fb brush cutter at 40 DAS	One hand weeding was done after the critical period of crop-weed competition, i.e., between 45–50 days after sowing.
T ₃							

T₁ = conventional tillage (CT) – conventional tillage (CT) – Fallow, T₂ = conventional tillage (CT) – zero tillage (ZT) – zero tillage (ZT), T₃ = zero tillage (ZT) + R (residue retention) – zero tillage (ZT) + R (residue retention) – zero tillage (ZT) + R (residue retention), IWM = integration of chemical weed control and power + 1 hand weeding.

Sowing and fertilizer application

The DHM 117 maize seeds were seeded at 60 cm between the rows and 25 cm between the rows, with a net field plot size of 41.3 m² in 10 rows for each plot. Prior to seeding, the experimental plots were ploughed two times, accompanied by rotovating and levelling with manual raking in T₁; these plots were conventionally tilled plots, while the maize seeds were dibbled with no-till in ZT plots. The quantity of the maize seeds utilized for sowing was 20 kg ha⁻¹. The crop was thinned in the portions of the plots with a high crop population, and the gaps were filled where the seeds did not emerge 13 days after seed emergence. The crop was typically developed and advanced with supplemental irrigation because the amount of rainfall received during the crop developmental period was limited. Advocated dose fertilizers (ADFs) for N:P:K (200:60:50 kg ha⁻¹)

were supplied to raise the crop through urea, di-ammonium phosphate (DAP) and muriate potash (MOP). Urea and DAP were applied thrice—as basal, at knee height and during the maize tasselling period.

Sampling and standard analytical procedures

Soil physicochemical properties

Composite soil samples were randomly collected in triplicate from each treatment plot at a depth of 0–15 after the maize crop was harvested during the 8th crop cycle in April 2023. These collected soil samples were air-dried well under shade, processed through a wooden hammer, passed through a 0.5 mm sieve, and analysed for soil organic carbon by following standard methods described by Walkley and Black [29]. For the analysis of soil pH, a 2 mm sieve was used to sieve the soil samples, and analysis was performed according to Jackson [30].

Soil microbial population and microbial and enzyme activities

Sampling of rhizosphere soil was performed at two growth stages of maize crops (8th crop cycle) in 2022-23 during the experiment: the first, after preemergence, early postemergence and postemergence application of herbicides in chemical weed control (W_1) and herbicide rotation weed management (W_2) plots at 30 DAS in the maize crop; the second, at the tasselling stage. Composite samples were collected from the respective plots in polythene bags with zippers, taken to the laboratory, passed through a 2 mm sieve and analysed on the same day as collection from the field. The functional activity was measured in terms of soil microbial activities related to the soil microbial population, soil organic matter and nitrogen cycling. The soil water content was determined according to Monteiro and Frighetto [31], and this information was utilized in calculating the evaluated parameters.

Soil microbial activity

Soil basal respiration (SBR) was measured in a closed jar incubated for 24 hours at 26°C [32]. The CO_2 released was trapped in NaOH and determined by HCl titration. The results are reported as milligrams of CO_2 released per kilogram of soil per hour (Eq. 1).

$$\text{SBR} = \frac{(V_b - V_s) \cdot M \cdot 6.1000}{ds \cdot t} \quad (1)$$

where V_b is the volume of HCl consumed in the blank (ml); V_s is the volume of HCl consumed in the test sample (ml); M is the HCl molarity; 6 is the equivalent factor (1 ml of 0.5 N HCl is equivalent to 6 mg C- CO_2 in the NaOH solution); ds is the weight of dry soil; and t is the time of incubation.

Total soil microbial biomass carbon (SMBC) was determined by following the procedure of fumigation extraction [33–34], in which the soil was fumigated with chloroform-free ethanol in a desiccator. Overnight fumigation (24 hours) of chloroform was performed to kill the organisms in the soil samples, after which the amount of readily oxidizable C in the sample was measured through standard chemical procedures. The SMBC values are given as the carbon content of fumigated soil minus that of nonfumigated soil; all the values are divided by the proportion of microbial C evolved (K_{EC}). A value of 0.25 ± 0.05 was used for k_c in the SMBC calculation, representing the efficiency of the extraction of soil microbial biomass carbon (Eq. 2).

$$\text{SMBC (mg kg}^{-1} \text{ soil)} = \frac{(EC_f - EC_{nf})}{K_{EC}} \quad (2)$$

where EC_f is mg of C per kilogram of fumigated soil, EC_{nf} is mg of C per kilogram of nonfumigated soil, and K_{EC} is part of the microbial C evolved (0.25 ± 0.05).

Soil microbial biomass nitrogen (SMBN) was determined by the CH_3Cl fumigation-extraction technique as described by Brookes et al. [35] and Amato and Ladd [36]. Aliquots of fresh soil (5 g) were fumigated in a glass desiccator with ethanol-free

CH₃Cl vapour for 24 hrs at 21°C. Both fumigated and unfumigated soil samples were extracted with 0.5 ml of 0.5 mol/L K₂SO₄ for 30 min before gravimetric titration through ashless Whatman filter paper. The total dissolved N in the extracts was measured by persulfate digestion followed by NO₃⁻ determination (VCl₃/Griess reaction). The difference in total dissolved nitrogen (TDN) between the fumigated and unfumigated soil extracts was attributed to the release (flush) of N from the lysed microbial cells. The calculation of SMBN was performed according to Eq. 2. A correction factor for SMBN (K_{EN} = 0.45) was applied for incomplete extraction of microbial N [37]. The metabolic quotient (qCO₂), the ratio between SBR and SMBC [38], was employed to obtain the efficiency of substrate consumption by microorganisms as a stress indicator when the microbial biomass was affected. The microbial quotient (MBC:SOC) was the ratio of the MBC to the SOC [39].

Soil enzymatic activity

Dehydrogenase activity (DHA) was assayed according to Casida et al. [40], and the red colour of triphenyl formazan (TPF) was determined via spectrophotometry (λ = 485 nm). Fluorescein diacetate (FDA) activity was estimated according to Green et al. [41], and the amount of greenish-yellow fluorescein was measured via spectrophotometry at a wavelength of 490 nm. The urease activity was determined by quantifying the rate of release of NH₄⁺ from the hydrolysis of urea as described by Tabatabai and Bremner [42]. The activity of urease was subsequently calculated and expressed as μg of NH₄⁺ released g⁻¹ soil h⁻¹ as described by Tabatabai and Bremner [42]. The β -galactosidase and phosphatase activities were estimated according to Eivazi and Tabatabai [43] and Tabatabai and Bremner [44]. After the appropriate incubation time for each enzyme (60 min for β -galactosidase and phosphatase), their respective substrates (*p*-nitrophenyl- β -D-galactopyranoside and *p*-nitrophenyl-phosphate) were hydrolysed into the yellow colour *p*-nitrophenol, which was determined by spectrophotometry (λ = 420 nm and λ = 405 nm, respectively).

Rhizosphere soil and rhizoplane microbial population

Functional culturable groups of rhizosphere soil microorganisms, *viz.*, *Azotobacter*, *Azospirillum*, and total fungi, were assessed. Rhizosphere *Azotobacter* and the total fungal population were evaluated following the protocols described in Albino and Andrade [45]. A colony counter was used to count the colonies that formed after 7 days of incubation in a BOD incubator at 30°C for *Azotobacter* and 3–5 days at 25°C in a BOD incubator for the total fungal population. The population density was estimated as colony forming units (CFU) per gram of dry soil (Eq. 3) [46]. For enumeration of the rhizosphere soil *Azospirillum* population, rhizosphere soil samples (0.1 ml aliquots) were inoculated into semisolid nitrogen-free bromothymol blue malate medium (Nfb) according to Döbereiner and Day (1976) and incubated in a BOD incubator for 3–4 days at 30°C until the pellicles formed in tubes containing Nfb medium and 0.1 ml of rhizosphere soil sample aliquot. *Azospirillum* abundance was estimated by the most likely number (MPN) table (s), which was transformed to the logarithm of the most likely number per gram of soil (log MPN.g⁻¹) suggested by Alexander [47], Woormer et al. [48] and Cochran [49] and expressed as colony-forming units (CFU g⁻¹) of soil on a dry weight basis, and the others (rhizosphere soil *Azotobacter* and total fungi) were transformed and expressed as the logarithm of colony-forming units per gram of soil (log CFU.g⁻¹ soil).

For enumeration of the rhizoplane microbial population, root samples were collected from the plants by removing the plants, separating the roots from the plants through cutting with the help of a knife, and removing the rhizosphere soil. The roots were collected in a polythene zip cover. Using a pair of scissors, the roots were separated, and one gram of the roots was transferred to 100 ml of sterile distilled water and washed thoroughly using a rotary mixer. One milliliter from 100 ml of the sample was transferred into 10 ml of saline blanks, and serial dilutions were made for each treatment following the same methodology employed for enumeration of the soil microbial population. The samples were incubated in a BOD incubator for 3–5 days at 25°C with dilutions of up to 10⁴. Eq. 3 [46] was used for calculation, after which the results were transformed and are expressed as the logarithm of colony-forming units per gram of roots (log CFU.g⁻¹ roots).

$$\text{Number of total fungi/Azotobacter in 1 g soil} = \frac{\text{No. of CFU} \times \text{dilution}}{\text{The dry weight of 1 gm moist soil} \times \text{aliquot taken}} \quad (3)$$

Fungal Diversity

Isolation criteria and purification

rRNA gene sequencing of 18S was performed on fungal colonies obtained at the tasselling stage from the maize crop 2022-23. Before identification, the fungal colonies were incubated for approximately 10–12 days on rose bengal solid agar media at 25°C to allow sporulation to occur. Based on the colour of the spores formed, classification was performed, and 8 representative plates of all 12 treatment combinations were selected for purification to obtain pure fungal strains based on the abundance of the same number of spores. These colonies, which were predominant on plates and represented the treatment combinations, were picked and cultured in potato dextrose (PDA) solid agar medium for 5 days to allow the growth of pure strains of fungal species. The 8 pure fungal strains were subjected to sequencing to identify the fungal species present in the different treatment combinations for tillage and weed management.

Deoxyribonucleic acid (DNA) extraction and polymerase chain reaction (PCR) amplification of 18S gene partial sequencing

Deoxyribonucleic acid (DNA) extraction was performed by picking sample and isolating genomic DNA from those samples (pure fungal strains). The DNA was placed in a mortar and homogenized with 1 ml of extraction buffer, after which the homogenate was transferred to a 2 ml-microfuge tube.

An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added to the tubes, which were mixed well by gently shaking the tubes. The tubes were centrifuged at room temperature for 15 min at 14,000 rpm. The upper aqueous phase was collected in a new tube, and an equal volume of chloroform:isoamyl alcohol (24:1) was added and mixed. The upper aqueous phase obtained after centrifuging at room temperature for 10 min at 14,000 rpm was transferred to a new tube. The DNA was precipitated from the solution by adding 0.1 volume of 3.0 M sodium acetate (pH 7.0) and 0.7 volume of isopropanol. After 15 minutes of incubation at room temperature, the tubes were centrifuged at 4°C for 15 minutes at 14,000 rpm. The DNA pellet was washed twice with 70% ethanol and then very briefly with 100% ethanol and air-dried. The DNA was dissolved in TE (10 mM Tris-Cl [pH 8.0], 1 mM EDTA). To remove ribonucleic acid (RNA), 5 µl of DNase or free RNase A (10 mg ml⁻¹) was added to the DNA.

After extraction of the total DNA, different quantities of DNA extracted from the treatment samples (154, 155, 169, 170, 175, 179 and 198 ng µl⁻¹) were subjected to polymerase chain reaction (PCR) amplification of the 18 s gene according to Baldoni et al. [50] along with 10 pM of each primer mixture (High-Fidelity DNA Polymerase, 0.5 mM dNTPs, 3.2 mM MgCl₂ and PCR enzyme buffer cycling condition) (PCR Clean Kit). The PCR cycling and amplification conditions are presented in Table 3. The sequences of primers used are listed in Table 4. The aligned sequence data of the samples are included in the supplementary data. The PCR products were sequenced bidirectionally according to Staden et al. [51], and the sequence mix used was as follows: 10 µl of sequencing mixture, 4 µl of Big Dye Terminator-ready Reaction Mix, 1 µl of template (100 ng µl⁻¹), 2 µl of primer (10 pmol λ⁻¹), 3 µl of milliquoise water and 25 cycles of PCR conditions. The mixture was subjected to initial denaturation at 96°C for 5 minutes, denaturation at 96°C for 30 seconds, hybridization at 50°C for 30 seconds and elongation at 60°C for 1.30 minutes.

Data analysis and identification

The data were analysed by using a sequencing machine (ABI 3130 genetic analyser), a chemistry cycle sequencing kit (big dye terminator version 3.1", a polymer and a capillary array (POP_7 pol capillary array) with a BDTv3-KB-Denovo_v 5.2 protocol and a sequence scape_v 5.2 software reaction plate (Applied Biosystem Micro Amp Optical 96-Well Reaction plate). Identification was performed by using the system software aligner to align the sequences, and a comparative search of GenBank sequences in the National Centre for Biotechnology Information (NCBI) was carried out using the BLASTn tool to identify the organisms and their closest neighbours. The phylogenetic tree builder used sequences aligned with the system software aligner, and a distance matrix was generated using the Jukes–Cantor corrected distance model. When generating

the distance matrix, only alignment model positions were used; alignment inserts were ignored, and the minimum comparable position was 200. The tree was created using a neighbor with an alphabet size of 4 and a length of 1000. The consensus sequence was deposited in the GenBank in the NCBI database to obtain accession numbers of identified organisms from the type material.

Multiple sequence alignment and phylogenetic tree construction

The default parameters were used for the Mega11 Version 11.0.13 and clustalW algorithm, which included pairwise alignment with a gap opening penalty of 15, a penalty of 6.06, multiple alignments with a gap opening penalty of 15 and a gap extension penalty of 6.06. Additionally, the default parameters included a matrix with a DNA weight matrix (IUB transition), a weight of 0.50, and a negative matrix with an off-delay divergent cut-off of 30.

For construction of the phylogenetic tree, the neighbour joining test of the phylogenetic method and bootstrap method were used, and 1000 bootstrap replicates were used; moreover, the nucleotide model/method substitution type included 17 nucleotide sequences. The evolutionary distances were computed using the maximum composite likelihood method [52]. The codon positions included were 1st + 2nd + 3rd + noncoding. The maximum composite likelihood substitution included transitions + transversion rates among sites – uniform rate pattern among lineages – same (homogeneous) gap/missing data treatment in pairwise deletion. There was a total of 2,043 positions in the final dataset.

Table 3
Details of the PCR cycling and amplification conditions

Cycling Conditions		
Initial Denaturation	3 minutes at 94°C	30 Cycles
Denaturation	1 minute at 94°C	
Annealing	1 minute at 50°C	
Extension	2 minutes at 72°C	
Final Extension	7 minutes at 72°C	
PCR Amplification conditions		Volume
DNA		1 ul
18 s Forward Primer		2 ul
18 s Reverse Primer		2 ul
dNTPs (2.5 mM each)		4 ul
10X Taq DNA polymerase Assay Buffer		10 ul
Taq DNA Polymerase Enzyme (3U ml ⁻¹)		1 ul
Water		30 ul
Total reaction volume		50 ul

Table 4
Primer details - The polymerase chain reaction (PCR) product size was ~ 2 kb

S. No	Oligo Name	Sequence (5'→3')	Tm (°C)	GC- Content
1	18 sForward	TCCTGAGGGAAACTTCG	47	52.94%
2	18 s Reverse	ACCCGCTGAACTTAAGC	47	52.94%

Crop Productivity

The grain yield for maize in each net plot was recorded by weighing the sun-dried produce before threshing, and the yield was expressed in kg ha^{-1} . Similarly, the maize stover in the net plot area was cut, and the sun-dried weight was expressed in kg ha^{-1} . Cotton was the first crop, followed by maize and *Sesbania rostrata* in the cropping system; therefore, the system yield was computed in terms of the cotton equivalent yield (CEY) (the monsoon seed cotton yield after the 4th year used in the calculation is attached as supplementary data) using Eq. 4, as follows:

$$\text{System CEY (kg ha}^{-1}\text{)} = \frac{\text{Economical yield of a maize crop (kg ha}^{-1}\text{)} \times \text{Price (Rs kg}^{-1}\text{) of the same crop, i.e., maize}}{\text{Price (Rs kg}^{-1}\text{) of cotton}} \quad (4)$$

Statistical analysis

The data were analysed statistically by applying the analysis of variance technique following the ANOVA for split plot design as suggested by [53]. The significance of critical differences in the treatment means was calculated at the 5 percent level of probability. Turkey's test was also used for ranking microbial activity treatment means because of the significance of the differences at the 5% probability level.

Results

Soil pH and soil organic carbon

Soil physico-chemical attributes were not significantly influenced by tillage or weed management options except for soil organic carbon (SOC), which was significantly impacted by the different tillage practices. The interaction effects (tillage and weed management) on the soil pH, EC and SOC were not significant (Table 5). The ZT + R(C)-ZT + R(M)-ZT + R(Sr) treatment had a significantly greater SOC (7.92 g kg^{-1}) than did the CT(C)-CT(M)-Fallow(NSr) and CT(C)-ZT(M)-ZT(Sr) treatments. Overall, the SOC contents were greater in all the treatments than in the initial treatment (6.5 g kg^{-1}). The soil pH was slightly alkaline, with a decrease observed across all the treatments over the initial soil pH range (Table 5).

Table 5
Impact of tillage and weed management options on soil pH and soil organic carbon (SOC) after harvest of winter maize (8th crop cycle).

Treatments	pH	SOC (g kg ⁻¹)
	0–15 cm	0–15 cm
Tillage practices		
Initial (s)	7.82	6.50
T ₁ : CT(C)-CT(M)-Fallow (NSr)	7.15	6.71
T ₂ : CT(C)-ZT(M)-ZT(Sr)	7.14	7.26
T ₃ : ZT + R(C)-ZT + R(C)-ZT + R(Sr)	7.04	7.92
SE(m)±	0.05	0.15
CD(P = 0.05)	NS	0.60
Weed management options		
W ₁ - Chemical weed control	7.11	7.29
W ₂ - chemical (herbicide) rotation	7.09	7.30
W ₃ - IWM	7.13	7.34
W ₄ - Nonweeded control	7.11	7.25
SE(m)±	0.08	0.19
CD(P = 0.05)	Ns	Ns
Interactions (TxW) CD(P = 0.05)	Ns	Ns

CT = conventional tillage, ZT = zero tillage; R = crop residue retention; IWM = chemical weed control + power and 1 hand weeding; C = cotton; M = maize; Sr = *Sesbania rostrata*; CD (P = 0.05) = critical difference at the 5% probability level; Ns = nonsignificant; SE(m) = standard error of the mean.

Soil Microbial Activity

Tillage and weed management practices exerted a significant influence on overall soil microbiological activity at all sampling stages (at 30 DAS after the application of herbicides and during the tasselling stage of maize). The soil microbial activity indices (SMAs) included soil microbial biomass carbon (SMBC), microbial biomass nitrogen (SMBN), soil basal respiration (SBR), the microbial quotient (qMB) and the metabolic quotient (qCO₂) (Fig. 3a, b, c, d, e and 4a, b, c, d, e). These SMAs were significantly promoted and increased by the adoption of ZT + R(C)-ZT + R(M)-ZT + R(Sr) at both sampling stages of the crop relative to CT(C)-CT(M)-Fallow(NSr) and CT(C)-ZT(M)-ZT(Sr), but not qCO₂. Among weed practices, a significant increase in SMAs was observed with non-weeded control and the combination of chemical weed control and power + 1-hand weeding (IWM) at both sampling stages. The herbicides applied at 30 DAS to maize via chemical weed control and chemical (herbicide) rotation resulted in a significant reduction in the SMAs, which later increased until the tasselling stage of the crop. The qCO₂ values were significantly lower in the ZT + R(C)-ZT + R(M)-ZT + R(Sr) treatment than in the CT(C)-CT(M)-Fallow (NSr) and CT(C)-ZT(M)-ZT(Sr) treatments at both stages of the crop. With respect to weed management practices, the qCO₂ values were significantly lower in the non-weeded control and IWM plots than in the herbicide-treated plots. There were no significant treatment interaction effects on the SMAs observed during either period of sampling (Fig. 3a, b, c, d, e and 4a, b, c, d, e).

At 30 DAS, for maize, the SMBC, SMBN, SBR, and qMB were significantly greater (7.52% and 26.27%, 11.01% and 28.90%, 0.64% and 17.60%, 15.15% and 15.16%, respectively) under ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) than under CT(C)-ZT(M)-ZT(*Sr*) and CT(C)-CT(M)-Fallow (NS*r*). Among weed management options, after the application of preemergence (PE), early postemergence (EPoE) and postemergence (PoE) herbicides at 30 DAS, 21.41–21.72% and 2.93–3.23% of SMBC, 20.00–21.40% and 14.21%–15.71% of SMBN, 13.73–23.16% and 9.21–19.70% of SBR, and 8.11–21.62% and 9.09–15.91% of qMB were greater under nonweeded control and IWM, respectively, than under chemical (herbicide) rotation and chemical weed control (Fig. 3a, b, c, d, e). During the same sampling period (30 DAS), the ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) treatment resulted in significant reductions in the qCO₂ concentration compared with that in the CT(C)-ZT(M)-ZT(*Sr*) and CT(C)-CT(M)-Fallow(NS*r*) groups. There were also significant decreases of 20.24%, 32.60%, 11.23% and 24.99% in the qCO₂ concentration in the non-weeded control and IWM treatment groups, respectively, compared to those in the chemical (herbicide) rotation and chemical weed control groups (Fig. 3a, b, c, d, e).

At the tasselling stage, there was an overall progressive increase in the soil microbial activity indices (SMAls) due to the advancement of the crop. The trends in the SMAls were similar to those observed at 30 DAS. Among all the tillage practices, ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) had 10.92% and 26.64% SMBC, 5.53% and 19.04% SMBN, 1.88% and 9.18% SBR, and 2.27% and 13.64% qMB higher than those of CT(C)-ZT(M)-ZT(*Sr*) and CT(C)-CT(M)-Fallow(NS*r*), respectively (Fig. 4a, b, c, d, e). With respect to weed management choices, higher percentages of SMBC (10.83–16.11% and 14.46–19.54%), SMBN (11.80–12.94% and 8.29–9.47%), SBR (5.36–9.67% and 1.58–6.06%), and qMB (9.09–15.91% and 14.89–21.28%) were observed under IWM and non-weeded control, respectively, than under chemical weed control and chemical (herbicide) rotation. During the same stage of the crop (tasselling), the trends in qCO₂ were similar to those at 30 DAS, with a further significant decrease observed under ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) relative to CT(C)-ZT(M)-ZT(*Sr*) and CT(C)-CT(M)-Fallow(NS*r*). The non-weeded control and IWM also facilitated a considerable decrease in qCO₂ during crop growth compared to chemical (herbicide) rotation and chemical weed control (Fig. 4a, b, c, d, e).

Soil Enzyme Activities

The addition of ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) and the nonweeded control, as well as the combination of chemical weed control and power + 1 hand weeding (IWM), improved the activities of rhizosphere soil dehydrogenase (DHA), urease (SUA), alkaline and acid phosphatase (AIP and AcP), fluorescein diacetate (FDA) and β -galactosidase (β -GaA), which are involved in soil carbon (C), nitrogen (C) and phosphorus (P) cycling. This improvement in soil enzyme activity in the ZT + R(C)-ZT + R(M)-ZT + R(*Sr*), nonweeded control and IWM treatment groups was observed at both sampling stages, during which the activity significantly increased continuously with crop progression. Herbicides were applied at 30 DAS; after PE, EPoE, and PoE resulted in a massive decrease in the activity of the soil enzymes, which subsequently returned to their initial levels at the tasselling stage (Table 6a, b, c).

Rhizosphere soil enzyme activity at 30 DAS in maize in the CT(C)-CT(M)-Fallow (NS*r*) and CT(C)-ZT(M)-ZT(*Sr*) treatments was significantly lower than that in the ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) treatment. The DHA, SUA, AIP, AcP, FDA, and β -GaA concentrations were 16.88% and 31.87%, 16.58% and 27.87%, 11.35% and 22.44%, 8.24% and 23.85%, and 12.35% and 19.77%, 9.44% and 16.87% greater, respectively, in the ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) plots than in the CT(C)-ZT(M)-ZT(*Sr*) and CT(C)-CT(M)-Fallow (NS*r*) plots (Table 6a, b, c). Among the weed management options, DHA, SUA, AIP, AcP, FDA, and β -GaA had 17.76–18.68%, 30.28–31.06%, 10.24–11.79% and 25.51–26.79%, 7.37–9.29% and 18.80–20.48%, 2.41–3.81% and 21.12–22.26%, 4.31–4.88% and 24.26–24.71%, 3.89–5.18% and 24.82–25.83% greater under IWM and non-weeded control, respectively, than under chemical weed control and chemical (herbicide) rotation at the same sampling (30 DAS) (Table 6a, b, c).

At the tasselling stage, the activities of all the rhizosphere soil enzymes exhibited trends similar to those observed under the weed management options and tillage practices at 30 DAS. Enzyme activities increased significantly irrespective of the treatment, and tillage was the main factor influencing these activities. During the crop growth development period (tasselling), for DHA, SUA, AIP, AcP, FDA, and β -GaA, the percentages were 10.85% and 20.61%, 10.19% and 15.67%, 15.77% and 28.56%,

5.23% and 23.24%, 21.17% and 35.97%, and 16.71% and 32.88% greater, respectively, under ZT + R(C)-ZT + M)-ZT + R(*Sr*) than under CT(C)-ZT(M)-ZT(*Sr*) and CT(C)-CT(M)-Fallow (*NSr*) (Table 6a, b, c). With regard to weed management choices, for DHA, SUA, AIP, AcP, FDA, and β -GaA, 16.30-16.96% and 20.55–21.18%, 3.95–9.52% and 7.39–12.76%, 12.03–16.10% and 21.52–25.15%, 4.63–5.79% and 8.00-9.09%, 12.64–17.04% and 15.02–19.30%, 5.55–7.89% and 10.53–12.74% higher under IWM and non-weeded control, respectively, than chemical (herbicide rotation) and chemical weed control at the tasselling stage (Table 6a, b, c).

Tillage practices (main treatments) and weed management options interaction effects on DHA was 19.09–25.99%, 9.20–31.97%, 16.87–39.04%, SUA was 7.18–19.25%, 14.32–32.72%, 29.59–32.17% AIP was 7.34–16.98%, 13.22–22.34%, 26.37–34.90%, AcP was 16.04–22.84%, 15.02–18.57%, 27.52–28.01%, FDA was 8.71–19.76%, 21.65–28.80%, 27.41–33.96%, β -GaA was 20.87–26.22%, 26.24–28.48%, 18.21–24.62% higher under ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) in combination with non-weeded control, CT(C)-ZT(M)-ZT(*Sr*) on interaction with non-weeded control, CT(C)-CT(M)-Fallow (*NSr*) in combination with non-weeded control over ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) in combination with IWM and chemical weed control or chemical (herbicide) rotation, CT-ZT-ZT on interaction with IWM and chemical weed control or chemical (herbicide) rotation, CT(C)-CT(M)-Fallow(*NSr*) coupled with IWM and chemical weed control or herbicide rotation, respectively observed at 30 DAS of the crop (Table 6a, b, c). A progressive increase in overall rhizosphere soil enzyme activity was observed at the tasselling stage, and the treatment interaction effects (trends) on the activities of various enzymes appeared to be the same as those observed at 30 DAS, with significantly greater enzyme activity observed in the ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) treatment in combination with the non-weeded control and IWM relative to all the other treatment combinations (Table 6a, b, c).

Table 6a Impact of tillage practices and weed management options on rhizosphere soil dehydrogenase

($\mu\text{g TPF. g}^{-1}$ dry soil. day^{-1}) and urease ($\mu\text{g NH}_4^+ \text{- N. g}^{-1}$ dry soil. 2 hr^{-1}) activity on two different maize plants growth stages.

Treatment		Soil dehydrogenase activity			Soil urease activity			
Tillage	WM	30 DAS	Tasselling		30 DAS		Tasselling	
T ₁ : CT(C)-CT(M)-Fallow (NSr)	W ₁	21.50	43.22		31.79		65.37	
	W ₂	21.90	48.10		33.00		68.76	
	W ₃	29.32	56.24		32.06		70.84	
	W ₄	35.27	62.81		46.87		77.00	
T ₂ : CT(C)-ZT(M)-ZT(Sr)	W ₁	27.35	51.67		34.87		67.47	
	W ₂	27.71	49.84		35.07		74.43	
	W ₃	36.50	66.98		44.41		78.05	
	W ₄	40.20	67.71		51.83		80.33	
T ₃ : ZT + R(C)-ZT + R(C)-ZT + R(Sr)	W ₁	37.00	63.89		48.03		79.69	
	W ₂	35.28	62.11		44.63		82.41	
	W ₃	38.57	68.01		51.30		85.99	
	W ₄	47.67	70.94		55.27		86.28	
Tillage (Main plots)								
T ₁ : CT(C)-CT(M)-Fallow (NSr)	27.00		52.59		35.93		70.49	
T ₂ : CT(C)-ZT(M)-ZT(Sr)	32.94		59.05		41.55		75.07	
T ₃ : ZT + R(C)-ZT + R(C)-ZT + R(Sr)	39.63		66.24		49.81		83.59	
Weed Management (Subplots)								
W ₁ - Chemical weed control	28.62		52.93		38.23		70.84	
W ₂ - chemical (herbicide) rotation	28.30		53.35		37.57		75.20	
W ₃ - IWM	34.80		63.74		42.59		78.29	
W ₄ - Non-weeded control	41.05		67.15		51.32		81.20	
	SE(m) ±	CD (P = 0.05)	SE(m) ±	CD (P = 0.05)	SE(m) ±	CD (P = 0.05)	SE(m) ±	CD (P = 0.05)
Tillage	1.83	6.29	1.24	4.99	0.79	3.10	1.23	4.85
Weed Management	0.08	0.45	0.35	1.87	0.84	2.50	0.61	1.81
Interactions								
W at same level of T	2.02	6.48	1.02	11.07	1.46	4.33	1.06	3.14
T at same level of W	0.31	1.36	1.00	11.09	1.49	4.42	1.54	4.56

CT = conventional tillage, ZT = zero tillage; R = crop residue retention; IWM = chemical weed control + power and 1 hand weeding; WM = weed management; C = cotton; M = maize; Sr = *Sesbania rostrata*; CD (P = 0.05) = critical difference at the 5% probability level; Ns = nonsignificant; SE(m) = standard error of the mean.

Table 6

b: Impact of tillage practices and weed management options on rhizosphere soil acid and alkaline phosphatase activity (μg . p -Nitrophenol. g^{-1} dry soil. hr^{-1}) at two different maize growth stages.

Treatment		Acid phosphatase activity				Alkaline phosphatase activity			
Tillage	WM	30 DAS	Tasselling		30 DAS	Tasselling			
T ₁ : CT(C)-CT(M)-Fallow (NSr)	W ₁	45.54	122.75		117.18	221.05			
	W ₂	46.14	123.12		120.63	226.27			
	W ₃	45.85	129.64		132.52	241.76			
	W ₄	63.26	130.51		179.99	251.70			
T ₂ : CT(C)-ZT(M)-ZT(Sr)	W ₁	56.42	148.85		143.27	260.44			
	W ₂	57.35	153.19		146.79	277.31			
	W ₃	58.88	157.89		160.09	275.21			
	W ₄	69.29	164.89		184.48	296.25			
T ₃ : ZT + R(C)-ZT + R(C)-ZT + R(Sr)	W ₁	63.17	162.56		176.55	306.33			
	W ₂	59.25	152.59		160.50	300.42			
	W ₃	64.47	167.72		179.13	337.04			
	W ₄	76.79	176.40		193.32	373.14			
Tillage (Main plots)									
T ₁ : CT(C)-CT(M)-Fallow (NSr)	50.20		126.51		137.58	235.20			
T ₂ : CT(C)-ZT(M)-ZT(Sr)	60.49		156.20		157.25	277.30			
T ₃ : ZT + R(C)-ZT + R(C)-ZT + R(Sr)	65.92		164.82		177.38	329.23			
Weed Management (Subplots)									
W ₁ - Chemical weed control	55.04		144.72		145.66	262.61			
W ₂ - chemical (herbicide) rotation	54.25		142.97		142.64	268.00			
W ₃ - IWM	56.40		151.75		157.25	284.67			
W ₄ - Non-weeded control	69.78		157.27		179.38	307.03			
	SE(m) ±	CD (P = 0.05)	SE(m) ±	CD (P = 0.05)	SE(m) ±	CD (P = 0.05)	SE(m) ±	CD (P = 0.05)	
Tillage	0.80	3.15	2.34	9.18	3.06	12.02	6.38	25.04	
Weed Management	0.66	1.96	1.43	4.24	3.07	9.13	4.56	13.56	
Interactions									

Treatment	Acid phosphatase activity				Alkaline phosphatase activity			
Tillage	WM	30 DAS	Tasselling		30 DAS	Tasselling		
W at same level of T	1.14	3.39	2.47	7.35	5.32	15.81	7.9	23.48
T at same level of W	1.27	3.78	3.17	9.42	5.53	16.44	9.36	27.79

CT = conventional tillage, ZT = zero tillage; R = crop residue retention; IWM = chemical weed control + power and 1 hand weeding; WM = weed management; C = cotton; M = maize; Sr = *Sesbania rostrata*; CD (P = 0.05) = critical difference at the 5% probability level; SE(m) = standard error of the mean.

Table 6

c: Impact of tillage practices and weed management options on rhizosphere soil fluorescein diacetate ($\mu\text{g. fluorescein. g}^{-1}$ dry soil. $^3\text{ h}^{-1}$) and β -galactosidase ($\text{nmol } p\text{-nitrophenol. g}^{-1}$ dry soil. hr^{-1}) activity at two different maize growth stages.

Treatment		Fluorescein di-acetate activity			β-galactosidase activity			
Tillage	WM	30 DAS	Tasselling		30 DAS	Tasselling		
T ₁ : CT(C)-CT(M)-Fallow (NSr)	W ₁	118.91	149.97		118.25	152.92		
	W ₂	130.70	172.35		120.58	159.79		
	W ₃	120.94	186.95		128.31	168.79		
	W ₄	180.06	190.21		156.88	187.71		
T ₂ : CT(C)-ZT(M)-ZT(Sr)	W ₁	132.83	199.73		128.74	192.67		
	W ₂	135.92	204.54		129.38	206.47		
	W ₃	146.18	220.52		132.76	210.06		
	W ₄	186.57	236.42		180.00	221.15		
T ₃ : ZT + R(C)-ZT + R(C)-ZT + R(Sr)	W ₁	169.96	240.19		148.76	243.49		
	W ₂	152.56	244.31		140.50	237.73		
	W ₃	173.57	303.62		150.70	249.57		
	W ₄	190.14	304.35		190.44	266.22		
Tillage (Main plots)								
T ₁ : CT(C)-CT(M)-Fallow (NSr)	137.65		174.87		131.01	167.30		
T ₂ : CT(C)-ZT(M)-ZT(Sr)	150.38		215.30		142.72	207.59		
T ₃ : ZT + R(C)-ZT + R(C)-ZT + R(Sr)	171.56		273.12		157.60	249.25		
Weed Management (Subplots)								
W ₁ - Chemical weed control	140.57		196.63		131.92	196.36		
W ₂ - chemical (herbicide) rotation	139.73		207.07		130.15	201.33		
W ₃ - IWM	146.90		237.03		137.26	213.17		
W ₄ - Non-weeded control	185.59		243.66		175.48	225.03		
	SE(m) ±	CD (P = 0.05)	SE(m) ±	CD (P = 0.05)	SE(m) ±	CD (P = 0.05)	SE(m) ±	CD (P = 0.05)
Tillage	2.88	11.30	4.17	16.38	0.96	3.76	3.01	11.82
Weed Management	3.02	8.97	4.37	12.97	1.62	4.81	3.08	9.14
Interactions								

Treatment	Fluorescein di-acetate activity				β-galactosidase activity			
Tillage	WM	30 DAS	Tasselling		30 DAS	Tasselling		
W at same level of T	5.23	15.53	7.56	22.46	2.80	8.33	5.33	15.84
T at same level of W	5.36	15.94	7.76	23.07	2.61	7.75	5.51	16.38

CT = conventional tillage, ZT = zero tillage; R = crop residue retention; IWM = chemical weed control + power and 1 hand weeding; WM = weed management; C = cotton; M = maize; Sr = *Sesbania rostrata*; CD (P = 0.05) = critical difference at the 5% probability level; SE(m) = standard error of the mean.

Microbial population

Rhizosphere soil microbial and rhizoplane fungal counts were significantly influenced by different tillage practices and weed management practices, and the interaction effects of the tillage and weed management practices on the soil microbial and rhizoplane fungal populations were significant at both sampling stages (30 DAS and tasselling) (Table 7a and b).

The spraying of the herbicides either in rotation or repeatedly every other year, such as preemergence (PE), early postemergence (EPoE) and postemergence (PoE), at 30 DAS suppressed the growth and population of the microorganisms. Among all weed management options, at 30 DAS, the rhizosphere soils of *Azotobacter* (*Azot*), *Azospirillum* (*Azosp*), and total fungal (TF) and total fungal (RF) rhizoplane populations were 0.44–0.66% and 3.62–3.84%, 1.40–1.63% and 4.51–4.74%, 0.47–0.70% and 3.63–3.85%, 1.79–2.04% and 6.55–6.80% greater under IWM and nonweeded control, respectively, than under chemical weed control and chemical (herbicide) rotation (Table 7a, and b). In terms of all the different tillage systems, the rhizosphere soil *Azot*, *Azosp*, TF, and rhizoplane TF populations were 1.51% and 2.81%, 1.60% and 3.43%, 1.61% and 2.75%, and 3.69% and 6.39% greater under ZT + R(C)-ZT + R(M)-ZT + R(Sr) than under CT(C)-ZT(M)-ZT(Sr) and CT(C)-CT(M)-Fallow (NSr), respectively, at 30 DAS (Table 7a, and b). The population of rhizosphere soil microorganisms and rhizoplane total fungi (TF) increased significantly during the tasselling period of the crop in all the treatments, and tillage was the principal factor influencing the progressive increase in the microbial population. At that growth stage, for maize crops (tasselling), the populations of rhizosphere soil *Azot*, *Azosp*, TF, and rhizoplane TF were 1.20% and 1.80%, 1.21% and 2.23%, 2.38% and 4.75%, and 3.12 and 4.45% greater, respectively, in the ZT + R(C)-ZT + R(M)-ZT + R(Sr) treatment than in the CT(C)-ZT(M)-ZT(Sr) and CT(C)-CT(M)-Fallow (NSr) treatments (Table 7a, and b). A significant difference with a continuous increase in the overall microbial population was observed for all weed management options, possibly due to microorganism recovery from herbicidal injury at tasselling. The patterns of the growth of both the rhizosphere soil and rhizoplane microbial counts at that crop growth stage (tasselling) resembled the trends observed at 30 DAS.

During the initial stage of crop development (30 DAS), the interaction effects of the various treatments (tillage and weed management) on the rhizosphere soil *Azotobacter* (*Azot*) were 1.91–3.39%, 3.62–4.26%, and 3.88–4.31%; *Azospirillum* (*Azosp*) counts were 2.90–4.24%, 2.71–4.30%, and 4.32–6.36%; total fungal (TF) counts were 2.68–4.25%, 2.51–3.20%, and 3.89–4.35%; rhizoplane total fungal (TF) counts were 2.64–3.84%, 3.41–7.56%, and 8.33–9.31%; and the results were superior to those of the ZT + R(C)-ZT + R(Sr) treatment in combination with the non-weeded control, CT(C)-ZT(M)-ZT(Sr) combined with the non-weeded control, and CT(C)-CT(M)-Fallow (NSr) coupled with the non-weeded control over the ZT + R(C)-ZT + R(M)-ZT + R(Sr). At the tasselling stage of the crop, all microbial counts increased further, regardless of the treatment combination. Among all the treatment interactions, at tasselling of maize, ZT + R(C)-ZT + R(M)-ZT + R(Sr) in combination with the unweeded control had a significantly greater rhizosphere soil microbial and rhizoplane fungal population, which was closely and statistically followed by the interaction of ZT + R(C)-ZT + R(M)-ZT + R(Sr) and IWM in comparison with all the other treatment combinations (Table 7a and b).

The fungal population observations indicated that the rhizosphere soil fungal counts were greater than the rhizoplane fungal counts during both sampling periods (30 DAS and tasselling) in the maize crop (Table 7a and b).

Table 7

a: Impact of tillage practices and weed management options on rhizosphere soil *Azotobacter* and *Azospirillum* populations (log CFU g⁻¹ soil) at two different maize growth stages.

Treatment		<i>Azotobacter</i> population				<i>Azospirillum</i> population			
Tillage	WM	30 DAS	Tasselling			30 DAS	Tasselling		
T ₁ : CT(C)-CT(M)-Fallow (NSr)	W ₁	4.44	4.89			4.12	4.79		
	W ₂	4.46	4.91			4.13	4.81		
	W ₃	4.45	4.92			4.21	4.85		
	W ₄	4.64	4.93			4.40	4.86		
T ₂ : CT(C)-ZT(M)-ZT(Sr)	W ₁	4.50	4.94			4.23	4.87		
	W ₂	4.51	4.95			4.24	4.88		
	W ₃	4.53	4.96			4.30	4.89		
	W ₄	4.70	4.98			4.42	4.89		
T ₃ : ZT + R(C)-ZT + R(C)-ZT + R(Sr)	W ₁	4.62	5.01			4.34	4.90		
	W ₂	4.56	5.03			4.29	4.93		
	W ₃	4.63	5.05			4.35	4.95		
	W ₄	4.72	5.08			4.48	4.99		
Tillage (Main plots)									
T ₁ : CT(C)-CT(M)-Fallow (NSr)	4.50		4.91			4.22	4.83		
T ₂ : CT(C)-ZT(M)-ZT(Sr)	4.56		4.94			4.30	4.88		
T ₃ : ZT + R(C)-ZT + R(C)-ZT + R(Sr)	4.63		5.00			4.37	4.94		
Weed Management (Subplots)									
W ₁ - Chemical weed control	4.52		4.91			4.23	4.85		
W ₂ - chemical (herbicide) rotation	4.51		4.94			4.22	4.87		
W ₃ - IWM	4.54		4.95			4.29	4.89		
W ₄ - Non-weeded control	4.69		5.00			4.43	4.91		
	SE(m) ±	CD (P = 0.05)	SE(m) ±	CD (P = 0.05)		SE(m) ±	CD (P = 0.05)	SE(m) ±	CD (P = 0.05)
Tillage	0.004	0.015	0.004	0.014		0.004	0.016	0.003	0.012
Weed Management	0.003	0.008	0.001	0.002		0.003	0.010	0.001	0.003
Interactions									

Treatment	<i>Azotobacter</i> population				<i>Azospirillum</i> population			
Tillage	WM	30 DAS	Tasselling		30 DAS	Tasselling		
W at same level of T	0.005	0.014	0.001	0.004	0.006	0.017	0.002	0.005
T at same level of W	0.006	0.016	0.004	0.011	0.006	0.019	0.003	0.010

CT = conventional tillage, ZT = zero tillage; R = crop residue retention; IWM = chemical weed control + power and 1 hand weeding; WM = weed management; C = cotton; M = maize; Sr = *Sesbania rostrata*; CD (P = 0.05) = critical difference at the 5% probability level; SE(m) = standard error of the mean.

The table mean values are in log CFU g⁻¹ soil from log transformation of exponential (10³) values from CFU g⁻¹ soil (oven dry basis) taken from plate counts.

Table 7

b: Impact of tillage practices and weed management options on the total fungal population of the rhizosphere soil and rhizoplane at two different maize growth stages.

Treatment		Rhizosphere soil total fungal population (log CFU g ⁻¹ soil)			Rhizoplane total fungal Population (log CFU g ⁻¹ roots)			
Tillage	WM	30 DAS	Tasselling		30 DAS	Tasselling		
T ₁ : CT(C)-CT(M)-Fallow (NSr)	W ₁	4.18	4.38		3.70	4.21		
	W ₂	4.20	4.39		3.71	4.24		
	W ₃	4.19	4.42		3.74	4.30		
	W ₄	4.37	4.45		4.08	4.39		
T ₂ : CT(C)-ZT(M)-ZT(Sr)	W ₁	4.24	4.46		3.79	4.31		
	W ₂	4.25	4.49		3.81	4.32		
	W ₃	4.27	4.51		3.96	4.35		
	W ₄	4.38	4.61		4.10	4.41		
T ₃ : ZT + R(C)-ZT + R(C)-ZT + R(Sr)	W ₁	4.34	4.60		4.05	4.43		
	W ₂	4.28	4.61		4.01	4.45		
	W ₃	4.35	4.64		4.06	4.49		
	W ₄	4.47	4.68		4.17	4.58		
Tillage (Main plots)								
T ₁ : CT(C)-CT(M)-Fallow (NSr)	4.24		4.41		3.81	4.29		
T ₂ : CT(C)-ZT(M)-ZT(Sr)	4.29		4.52		3.92	4.35		
T ₃ : ZT + R(C)-ZT + R(C)-ZT + R(Sr)	4.36		4.63		4.07	4.49		
Weed Management (Subplots)								
W ₁ - Chemical weed control	4.25		4.48		3.85	4.32		
W ₂ - chemical (herbicide) rotation	4.24		4.50		3.84	4.34		
W ₃ - IWM	4.27		4.52		3.92	4.38		
W ₄ - Non-weeded control	4.41		4.59		4.12	4.46		
	SE(m) ±	CD (P = 0.05)	SE(m) ±	CD (P = 0.05)	SE(m) ±	CD (P = 0.05)	SE(m) ±	CD (P = 0.05)
Tillage	0.003	0.014	0.006	0.024	0.007	0.029	0.005	0.022

Treatment		Rhizosphere soil total fungal population (log CFU g ⁻¹ soil)				Rhizoplane total fungal Population (log CFU g ⁻¹ roots)			
Tillage	WM	30 DAS	Tasselling			30 DAS	Tasselling		
Weed Management	0.003	0.007	0.001	0.004		0.004	0.013	0.002	0.006
Interactions									
W at same level of T	0.004	0.013	0.003	0.008		0.008	0.023	0.003	0.010
T at same level of W	0.005	0.015	0.007	0.019		0.010	0.030	0.006	0.018

CT = conventional tillage, ZT = zero tillage; R = crop residue retention; IWM = chemical weed control + power and 1 hand weeding; WM = weed management; C = cotton; M = maize; Sr = *Sesbania rostrata*; CD (P = 0.05) = critical difference at the 5% probability level; SE(m) = standard error of the mean.

The table mean values are in log CFU g⁻¹ soil/roots from log transformation of exponential (10³) values from CFU g⁻¹ soil (oven dry basis)/roots taken from plate counts.

Fungal Diversity

Sub-culturing of the fungi from the culture plates was performed prior to sequencing to purify the fungal strains, as depicted in Fig. 5, and agarose gel electrophoresis images of total deoxyribonucleic acid (DNA) and polymerase chain reaction (PCR) products of the 18s rRNA gene are shown in Fig. 6a, b and c. The fungi were identified based on nucleotide sequence homology of the 18 s rRNA gene **are** presented in Table 8. The results of 18S rRNA gene sequencing indicated that *Talaromyces flavus* var. *flavus* (5-PJTSAU-KNIGHT-23) was identified under the T₃: ZT + R(C)-ZT + R(M)-ZT + R(Sr) and W₃: IWM combinations (T₃W₃) and T₂: CT(C)-ZT(M)-ZT(Sr) on interaction with IWM (T₂W₃). The other species of rhizosphere soil fungal and rhizoplane fungal isolates, viz., *Aspergillus niger*, *Penicillium limosum*, *Aspergillus terreus*, *Apiospora serenensis*, *Zasmidium cellare*, and *Ochraceocephala foeniculi*, were identified under the T₁: CT(C)-CT(M)-Fallow (NSr), T₂: CT(C)-ZT(M)-ZT(Sr) and T₃: ZT + R(C)-ZT + R(M)-ZT + R(Sr) tillage (main treatments) in combination with the W₁: chemical weed control, W₂: chemical (herbicide) rotation and W₄: non-weeded control (sub-treatments) (Table 8). The isolate ID (8-PJTSAU-KNIGHT), which was isolated abundantly from the rhizoplane zone across all the tillage and weed management treatments, was identified as *Penicillium limosum*. Phylogenetic tree(s) of all the 8 identified fungal species and multiple sequence alignments (MSAs) of the data are included in the supplementary data.

Table 8

Impact of tillage and weed management practices on fungal diversity at the tasselling stage of winter maize.

Rhizosphere soil fungal microbe (s)					
S.NO	Isolate ID	Treatment combination	Fungal name	Identity (%)	Accession numbers
1	1-PJ TSAU-KNIGHT-23	T ₁ W ₁	<i>Aspergillus niger</i>	100.00%	PP177339
	1-PJ TSAU-KNIGHT-23	T ₁ W ₂	<i>Aspergillus niger</i>		
	1-PJ TSAU-KNIGHT-23	T ₁ W ₄	<i>Aspergillus niger</i>		
2	2-PJ TSAU-KNIGHT-23	T ₁ W ₃	<i>Aspergillus niger</i>	100.00%	PP177340
3	3-PJ TSAU-KNIGHT-23	T ₂ W ₁	<i>Aspergillus terreus</i>	99.33%	PP177341
4	4-PJ TSAU-KNIGHT-23	T ₂ W ₂	<i>Apiospora serenensis</i>	98.47%	PP177342
	4-PJ TSAU-KNIGHT-23	T ₃ W ₂			
5	5-PJ TSAU-KNIGHT-23	T ₂ W ₃	<i>Taloromyces flavus</i> var. <i>flavus</i>	99.63%	PP177343
	5-PJ TSAU-KNIGHT-23	T ₃ W ₃			
6	6-PJ TSAU-KNIGHT-23	T ₂ W ₄	<i>Zasmidium cellare</i>	100.00%	PP177344
7	7-PJ TSAU-KNIGHT-23	T ₃ W ₁	<i>Penicillium limosum</i>	99.88%	PP177345
	7-PJ TSAU-KNIGHT-23	T ₃ W ₄			
Rhizoplane fungal microbe (s)					
8	8-PJ TSAU-KNIGHT-23	Abundant in all T & W combinations	<i>Ochraceocephala foeniculi</i>	96.55%	PP177346

Main treatments: T₁ = CT(C)-CT(M)-Fallow (NSr); T₂ = CT(C)-ZT(M)-ZT(Sr); T₃ = ZT + R(C)-ZT + R(M)-ZT + R(Sr); **Sub-treatments:** W₁ = chemical weed control; W₂ = chemical (herbicide) rotation; W₃ = chemical weed control and power + 1 hand weeding; W₄ = non-weeded control. T = Tillage; W = Weed Management, CT = Conventional Tillage, ZT = Zero Tillage; C = Cotton; M = Maize; Sr = *Sesbania rostrata*.

The details of the entire sequence along with accession numbers can be accessed through the following link:

<https://submit.ncbi.nlm.nih.gov/subs/?search=SUB14162715>

Crop productivity

Maize Grain Yield and System Productivity (Cotton Equivalent Yield)

Tillage and weed management practices exerted significant influences on maize grain yield (kernel yield) and system productivity in terms of cotton equivalent yield (CEY) (Table 9). The treatment interaction effects on CEY were significant and not significant for kernel yield (KY) (Table 9).

A significantly greater KY (6801 kg ha^{-1}) was recorded in the ZT + R(C)-ZT + R(M)-ZT + R(Sr) treatment, while a lower KY (6014 kg ha^{-1}) was observed in the CT(C)-CT(M)-Fallow(NSr) treatment. The adoption of chemical weed control and chemical (herbicide) rotation resulted in significantly greater KY (7245 kg ha^{-1} and 7324 kg ha^{-1}), followed by chemical weed control and power + 1 hand weeding (IWM) with a KY of 6722 kg ha^{-1} . A significantly lower KY (4099 kg ha^{-1}) was exhibited by the non-weeded control (Table 9).

The maize grain yield, which was recorded from different tillage-weed management treatment combinations, was converted into cotton equivalent yield (CEY) considering the monetary equivalence. The winter CEY was subsequently added to the monsoon cotton yield of the 4th year to arrive at the cotton equivalent yield of the cotton–maize system (system CEY) for the 4th year. The data on the system CEY are presented in Table 9.

ZT + R(C)-ZT + R(M)-ZT + R(Sr) exhibited a significantly greater CEY (3775 kg ha^{-1}) than did CT(C)-ZT(M)-ZT(Sr) and CT(C)-CT(M)-Fallow(NSr), with a CEY of 3517 kg ha^{-1} and 3328 kg ha^{-1} (Table 9). Among the weed management strategies, IWM had a significantly greater system CEY (4157 kg ha^{-1}) than chemical (herbicide) rotation, chemical weed control or non-weeded control, with system CEYs of 4065 kg ha^{-1} , 4018 kg ha^{-1} and 1921 kg ha^{-1} , respectively (Fig. 6a). Based on the tillage and weed management interactions, in combination with the IWM, ZT + R(C)-ZT + R(M)-ZT + R(Sr) had a significantly greater CEY (4453 kg ha^{-1}), and the lowest CEY values (1767 kg ha^{-1} and 1848 kg ha^{-1} , respectively) were observed with CT(C)-ZT(M)-ZT(Sr) in combination with the non-weeded control and CT(C)-CT(M)-Fallow(NSr) in combination with the non-weeded control in comparison with all the other treatment combinations (Table 9). The CT(C)-CT(M)-Fallow(NSr) and all weed management combinations were also associated with a lower system CEY (Table 9).

Table 9

The grain yield of maize and the system yield in terms of system cotton equivalent yield (CEY) were influenced by tillage practices and weed management (WM) options after the 4th year in the 8th crop cycle under conservation agriculture.

Treatment Interaction		kernel yield	System (CEY)	
Tillage	WM	(kg ha ⁻¹)	(kg ha ⁻¹)	
T ₁ : CT(C)-CT(M)-Fallow (NSr)	W ₁	6822	3756	
	W ₂	6854	3801	
	W ₃	6354	3908	
	W ₄	4025	1848	
	W ₁	7133	4005	
T ₂ : CT(C)-ZT(M)-ZT(Sr)	W ₂	7662	4187	
	W ₃	6558	4109	
	W ₄	3559	1767	
T ₃ : ZT + R(C)- ZT + R(M)- ZT + R(Sr)	W ₁	7780	4292	
	W ₂	7456	4206	
	W ₃	7253	4453	
	W ₄	4713	2157	
Tillage practices				
T ₁ : CT(C)-CT(M)-Fallow (NSr)	6014		3328	
T ₂ : CT(C)-ZT(M)-ZT(Sr)	6228		3517	
T ₃ : ZT + R(C)-ZT + R(C)-ZT + R(Sr)	6801		3775	
Weed Management options				
W ₁ - Chemical weed control	7245		4018	
W ₂ -chemical (herbicide rotation)	7324		4065	
W ₃ - IWM	6722		4157	
W ₄ - Non -weeded control	4099		1921	
	SE(m)±	CD(P = 0.05)	SE(m)±	CD(P = 0.05)
Tillage	144.83	568.66	18.69	73.38
Weed Management	126.98	377.28	40.29	119.71
Interactions				
W at same level of T	219.94	Ns	69.79	207.35
T at same level of W	239.28	Ns	63.26	187.96

CT = conventional tillage, ZT = zero tillage; R = crop residue retention; IWM = chemical weed control + power and 1 hand weeding; C = cotton; M = maize; *Sr* = *Sesbania rostrata*; CD ($P = 0.05$) = critical difference at the 5% probability level; Ns = nonsignificant; SE(m) = standard error of the mean.

Discussion

Soil pH and soil organic carbon

Among all the other soil factors, tillage and weed management strategies contribute to the alteration of soil organic carbon (SOC). The SOC concentration in the soil surface soil under no-till conditions with at least 30% maintenance of crop debris is less prone to depletion due to lower soil disturbance and cumulative crop residue, thus yielding a greater SOC content [54]. This greater SOC content exhibited by ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) could be associated with the continuous adoption of ZT, accrual retention and incorporation of the preceding cotton and *Sesbania* crops into the soil for consecutive years, which resulted in soil aggregation enhancement and shielded the soil against SOC loss. Similar results were presented by Bitew et al. [55] for a CA-based maize–legume cropping sequence. In zones where soil and weather conditions are conducive to the production of biomass and where adverse crop yield effects are unnoticed, CA practices result in greater quantities of SOC than CT-managed systems, especially in the topsoil. CT transposes the soil, displaces the soil clods, and exposes SOM to wetting-drying phenomena, resulting in a reduction in SOC content [56]. The reduction in SOC levels observed under CT(C)-CT(M)-Fallow(*NSr*) could be the result of the continuous removal of crop leaves, and primary and secondary tillage are employed for ploughing, which disturbs soil aggregation and promotes susceptibility to erosion. Thus, CA-based practices such as zero tillage (ZT) are directly associated with the maintenance of crop residues and nutrient management, which in turn impacts SOC accumulation and dynamics under diversified cropping systems. In contrast to CT, conservation tillage practices, which retain crop debris, tend to stabilize soil pH conditions and elevate SOC, which is conducive to increasing the soil microbial composition through the continuous disposal of crop residues.

Soil microbial activity

The potential influence of several biogeocenotic services in the soil environment can be expressed to a certain degree by the activities of microbes; thus, the soil basal respiration (SBR), metabolic quotient (qCO_2) and microbial quotient (qMB) were employed to perform an extensive assessment of microbial activity. The results of the present investigation demonstrated that zero tillage (ZT) with retention of crop residues resulted in significantly greater microbial activity (Table 2a, b, c), probably due to the ample additive-free materials drawn from the crops, which can become vital components for rapid metabolic reactions to external sources of carbon and slow down the figure(s) of the metabolic quotient (qCO_2), thereby facilitating the utilization of large quantities of additive-free substrates by microbiomes for proliferation rather than for respiration utilization purposes. The increased qMB under ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) apparently indicated the efficiency of soil microbes in utilizing sources of carbon materials for survival and growth as opposed to tillage practices, in which continuous crop residue remains (CT(C)-ZT(M)-ZT(*Sr*) and CT(C)-CT(M)-Fallow(*NSr*), which can exert a small impact on soil microbes to a certain extent on biological oxygen requirements, might equilibrate the proportion of carbon dioxide released by microbes to microbial biomass.

The observed results also suggest that soil microbial biomass carbon (SMBC) and nitrogen (SMBN), SBR and qMB increase as crops advance, particularly with the incorporation of nonchemical cultural weed control practices and the adoption of no-till practices along with the continuous retention of previous crop residues. This increase can be attributed to the most active and reproductive stage of the crop in which the rhizosphere begins to become enriched with specific microorganisms and nutrients necessary to perform activities related to SOM cycling. Thus, the increases in SMBN, SMBC, SBR and qMB might also be the result of root proliferation, exudation and crop litterfall, which serve as substrates for the activity of microorganisms and favourable biophysical climates created for microbes under ZT, promoting better soil functional diversity. Additionally, the availability of energy and nutrient resources and the limited oxidation of soil organic carbon, favoured by the prevalence of soil microorganisms, likely contributed to this observed enhancement. Koné et al. [57] reported that soil

microorganisms that in turn may improve biological activity in the rhizosphere include more plants with extensive rooting systems and more well-spread root hairs [58], and these characteristics are found to contribute to the exudation of vast amounts of organic compounds and consequently promote an increase in SMBC and SMBN [59]. These results are supported by Chaudhari et al. (2020), who observed the combination of zero tillage + residue incorporation with inter-cultivation (IC) + hand weeding (HW) at 15, 30 and 45 DAS as the most suitable strategy for sustaining the greatest soil microbial biomass. Less soil disturbance under conservation tillage and crop residue retention/incorporation tend to improve aggregation in soil and SMBC, possibly due to a rise in SOC [60–61]. Hazarika et al. [62] also indicated that, compared with those in conventionally tilled soil, the SMBN content in the till and/or reduced tillage systems was greater, which is also consistent with the results of this study.

Conventional tillage disrupts soil aggregates, exposes the soil and increases the susceptibility to erosion, and herbicides may stimulate or activate the soil microbial biomass. In the present study, intensive tillage and herbicide spraying caused a drastic decrease in SMBC after preemergence and after the emergence of herbicides at 30 DAS, possibly due to increased soil disturbance and herbicide inhibition. Modak et al. [63] also reported a reduction in SMBC after herbicide application owing to the adverse effects of herbicides on the microbial population, while in treatments without the involvement of herbicides, more SMBC and SMBN were recorded relative to those in treatments with herbicide components. Similarly, Pertile et al. [64] observed a significant reduction in SMBC and SMBN following the application of flumioxazin and imazethapyr herbicides compared to those in the control. The positive response of conservation tillage practices compared to conventional tillage systems was probably due to higher levels of C substrates available for microorganism growth, as well as better soil physical conditions and greater water retention due to altered land configurations and applied residues [65].

Soil disturbance as a result of intensive tillage had a significant impact on increasing the mean qCO_2 . Likewise, the application of herbicides before and after emergence greatly increased the mean qCO_2 value in this study. The lower metabolic quotient (qCO_2) values observed under ZT, which leaves crop residue in the soil, could be an indicator of the lower energy requirements of microorganisms. Similarly, Engell et al. [66] reported lower qCO_2 values. These findings indicate a low demand for energy maintenance by the microbial community. This discovery is in accordance with the meta-analysis of Zuber and Villamil [67] under similar soil field conditions as in the present study and indicated that sandy clay loam soils have lower qCO_2 values under NT than under CT, although the impact of tillage was found to be low in soils with very fine particles. Low values of qCO_2 are an indication of conducive conditions for the predominance of microbial activity [66].

The results of the present study on qCO_2 are also supported by Jiang *et al.* [68], who reported a significant increase in qCO_2 at 0–20 cm soil depth under a conventional tillage system. Similarly, in the study of Aziz *et al.* [69], the qCO_2 was up to 50% greater under conventional tillage than under no-tillage. A lower qCO_2 reflected improved physiological conditions resulting from amended organic matter, while a higher qCO_2 indicated soil degradation under intensive land use [70]. On the other hand, a rise in qCO_2 might not only be ascribed to microbial stress but also be interpreted as a positive priming on the decomposition of the labile soil organic carbon pool following the addition of readily degradable carbon substrates to the soil [16]. In the present investigation, higher qCO_2 was associated with low values of SMBC in conventionally tilled plots and herbicide-treated plots and is likely to reflect stress and poor conditions related to physical soil disturbance.

Weed management involving herbicides was found to increase qCO_2 , indicating stress or disorder, probably due to the detrimental effects of applied herbicides on the soil microbial population. In accordance with the findings of the present study, Pertile *et al.* [64] observed an increase in the metabolic quotient during the first 15 days of herbicide application, although their study was conducted under soil incubation conditions, indicating an initial negative effect of the herbicides on soil microorganisms. Since the application of chemical compounds in the soil requires an adaptation of soil microbial biomass that uses their reserves to degrade these compounds, C from microbial biomass ultimately becomes lost, thus increasing qCO_2 .

Rhizosphere soil enzyme activity

Maintenance of crop leftovers in zero tillage (ZT) plots, cultural weed control tactics and SOC preservation in conservation agriculture positively influence biomass production and activated soil microbes by modifying the provision of the substrate. Rhizosphere soil enzymes such as dehydrogenase (DHA), fluorescein diacetate (FDA), β -galactosidase (β -GaA), alkaline phosphatase (AIA) and acid phosphatase (AcA) play essential roles in the breakdown of carbon in the soil [71], and urease (SUA) plays a role in the hydrolysis of urea. The present investigation clearly indicated that ZT + R(C)-ZT + R(M)-ZT + R(Sr) and cultural weed control methods (nongweeded control and IWM) are likely to encourage the metabolic reactions necessary for the development of biotic microorganisms, which ultimately advance the activity of urea hydrolysis and carbon (C) cycling, *i.e.*, greater succession of C and nitrogen (N), which can aid in the accrual of microbial biomass [72].

The functional groups of culturable microbes are often interlinked with C and N cycling activities and are related to rhizosphere soil enzymes [73], demonstrating that alterations in SOC fractions can become the chief driver of soil microorganism constituents [74]. Since rhizosphere soil enzymes are secreted by specific groups of microbes, the diversity of crops plays a key role in enhancing the activity of enzymes, which agrees with the results of this study in which ZT + R(C)-ZT + R(M)-ZT + R(Sr) (zero tillage with diverse crop residues retained in the soil surface), nonweeded control and IWM treatments resulted in increased activity of rhizosphere enzymes. The diversification of crops (cotton-maize-*Sesbania rostrata*) in rotation resulted in a significant modification in the activity of enzymes, probably due to the greater variety of crops having greater distinct litterfall and rhizosphere exudation; thus, the ZT + R(C)-ZT + R(M)-ZT + R(Sr), nonweeded control and IWM treatments under these diverse cropping systems have shown a large influence on rhizosphere soil functional diversity. The addition of crop residues through crop residue retention and ZT resulted in greater soil enzyme activity than intensive tillage with continuous crop residue removal. The activity of overall enzymes increased significantly with crop growth advancement, possibly due to the secretion of beneficial nutrients, the decomposition of organic substrates and herbicide degradation.

It can be inferred that the significant reductions in the rhizosphere soil DHA, FDA, SUA, AIA, AcA, and β -GaA concentrations were more pronounced when herbicides were applied for weed management. The order of reduction in the activity of these enzymes was chemical (herbicide) rotation followed by chemical weed control and IWM rotation ($W_1 > W_2 > W_3$) at 30 DAS. The higher rhizosphere soil enzyme activities observed in the ZT + R(C)-ZT + R(M)-ZT + R(Sr) treatment group relative to those in the CT(C)-CT(M)-Fallow (NSr) or CT(C)-ZT(M)-ZT(Sr) treatment groups at 30 DAS could be associated with the partial inhibition of preemergence herbicides reaching the soil. This inhibition is likely a result of the presence of crop residues (cotton crop residues utilized for maize) on the soil surface in ZT + R(C)-ZT + R(M)-ZT + R(Sr). These results concur with those of Priya *et al.* [75], who observed that herbicides significantly inhibited soil DHA after application at 15 DAS, although their study was conducted under soil incubation conditions. Modak *et al.* [63] also recorded the maximum values of DHA under two weed management treatments without the use of herbicides, *viz.*, weedy checks, hoeing and weeding twice. The findings of Varsha *et al.* [76] on SUA support the observations obtained in the present study, in which SUA decreased following herbicide application, and the activity returned to normal over time. This might be due to the herbicide effect on microbial population stabilized after time or the herbicide themselves adsorbed irreversibly on soil colloids with an increase in time, resulting in decreased inhibition. Similarly, Raj *et al.* [77] revealed a reduction in acid (AcA) and alkaline (AIA) phosphatase activity in all herbicide treatment plots 15 days after herbicide application, but 45 days after herbicide application, increases in AcA and AIA were observed. This difference might be due to the change in the species composition of the soil microorganisms and variation in the availability of the organic substrate. The results of Madsen *et al.* [78] also concur with those of the present investigation, in which the highest FDA was recorded in systems where the soil was covered with winter crop residues due to added organic matter (OM) and the lowest FDA occurred in conventional systems (systems without crop cover and treated with herbicides) due to the very low input of OM and usage of herbicides and pesticides. Shahid *et al.* [79] also reported the adverse effects of herbicide application on the secretion of β -GaA. Thus, it may be deduced that, from the results of this study, herbicide treatments resulted in a significant decrease in rhizospheric soil enzyme activity in comparison with that in the untreated plots (non-weeded control plots) of soil samples.

Water plays an essential and complex role in the activity of enzymes. The activity of the enzymes involved in SOM cycling and urea hydrolysis assessed in this study increased with increasing soil water content up to near field capacity, followed by a decreasing trend thereafter [80]. In the case of our experiment, the soil water content was maintained at the field capacity level

through supplemental irrigation; thus, the enzyme activity increased throughout the treatment. Savant *et al.* [80] also observed a greater rate of urea hydrolysis in soil at field capacity than in wetted soils after 24 h of incubation. Several studies have indicated that soil DHA is significantly influenced by water content and decreases with decreasing soil humidity [81]. In addition, DHA reached higher values at lower soil water potentials [81]. In the winter season, there was a low rainfall amount; thus, a lower oxygen diffusion rate and redox potential were observed; however, the field moisture level were maintained with supplemental irrigation, and the relative humidity also increased, which could be the reason for the higher DHA observed in this study irrespective of the treatments. Wang *et al.* [82] observed a reduction in SUA due to flooding as a result of a high amount of rainfall, which was probably due to an increase in metal ions under reduced conditions, which decreased SUA. Therefore, during the sampling periods in winter, there was an increase in humidity and no flooding conditions due to scant rainfall; therefore, irrigation was provided for the development of the crop and for retention of the field moisture content, which might be the reason for obtaining higher SUA levels irrespective of the treatment.

Microbial population

Soil organic matter (SOM) is a crucial driver of microbial population size and diversity and can affect microbial counts [83–84]. Similarly, the rhizosphere soils of *Azospirillum* (*Azosp*), *Azotobacter* (*Azot*), and total fungal (TF) populations were greater where crop residues were retained, probably due to the greater addition of organic matter through crop residues and the reduced tillage and access to a steady source of organic carbon to support the microbial population compared to conventional tillage (CT). A decreased disturbance of soil favours the formation and stabilization of macroaggregates to improve and protect habitats for microbial populations. Zero-till (ZT) increases soil aggregation by reducing soil disturbance and increasing SOM and possibly increasing the growth of microbes that bind soil particles and microaggregates together [85]. Nitrogen-free bacterial fixers require aerobic environments to obtain resources for N₂ fixation [86]. Several workers have also reported that the change from conventional to zero tillage alters the distribution of SOM only along the soil profile [87–88]. The observations recorded in this study on rhizosphere soil microbial and rhizoplane fungal populations also indicated that the counts increased significantly with crop advancement due to the progressive mineralization of litterfall from crops, root exudates and added crop residues and the increased availability of organic substrates, which serve as food and help increase the population of nitrogen-fixing microorganisms [89]. Singh *et al.* [90] also reported the stimulation of *Azotobacter* spp. in upper soil layers under minimum tillage, which was attributed to increased availability of nutrients and root proliferation. Our results concur with those of Verma *et al.* [83], who observed an increasing trend in the prevalence of *Azospirillum* with an increase in the organic carbon concentration from 0.2–1.0%. The findings of this study are consistent with those of Bashan *et al.* [91], who reported that SOM had a definite role in the survival of *Azospirillum* strains.

Azospirillum species tend to move toward locations where O₂ is ideal for metabolism (at low O₂ concentrations) [92]. Therefore, an increase in the *Azospirillum* population with an increase in moisture content (maintained through supplemental irrigation prior to collection of rhizosphere soil samples) was observed in the present study. The same results were reported by Belaid *et al.* [93], who observed that when the moisture content increased, the *Azospirillum* population increased over time.

Rhizoplane fungi inhabit the root systems of crops and are connected directly to plant metabolism. Higher counts of Rhizoplane total fungi were also detected in ZT plants than in the other strains, which incorporated the residues, probably due to the release of organic exudates and increased plant nutrient absorption and exchange with the root system of the crops. Tillage operations incorporate crop residues, prepare seed beds, alleviate compaction, improve nutrient mineralization, and reduce weeds, pests and pathogens [94–95]. Conventional tillage (ploughing) continually exposes deeper soil to wet–dry and freeze–thaw cycles at the surface, thus increasing macroaggregate turnover, disrupting the existing pore network and ultimately favouring soil erosion [10]. These alterations in soil structure have direct effects on the physical habitat of microbes, with fungi often being detrimentally affected through the destruction of their hyphal network. Increased structural diversity of root systems and changes in SOM and nutrient inputs through plant litter and rhizodeposition can alter porosity, aeration and aggregate stability, providing more diverse niches for microbes [96]. Odunfa [97] noted that some fungi need

specific nutrient substances for growth and hence are host specific. Oyeyiola [98] also noted several fungal microflora populations in the rhizoplane but not in the Okro (*Abelmoschus esculentus*) crop.

The application of herbicides before emergence drastically reduced the population of rhizosphere soil microbes compared to that after emergence at 30 DAS, which could be due to the direct application of herbicides to the soil rhizosphere. However, the decrease in population after postemergence herbicide application was not large due to leaf foliage and weed emergence; moreover, the herbicides applied might not have fully reached the soil. The population of rhizosphere soil microbes was greater in the non-weeded control (no herbicide application), probably due to soil coverage by the weeds. Tapas *et al.* [99] also reported that one of the reasons for the less harmful effects of postemergence herbicides on microorganisms may be the greater foliage of crops at 22 DAS (at the time of herbicide application), which covers the soil surface, resulting in a decrease in and absorption of applied herbicides in the soil. In contrast, the deposition of preemergence herbicides in the soil is relatively greater than that in the exposed bare soil surface at the time of preemergence herbicide spraying. Furthermore, they noted that the effects of herbicides on the soil microflora are normally most severe immediately after their application.

The application of postemergence herbicides (fenoxaprop-p-ethyl and ethoxy sulfuron), which were sprayed at 20 days after crop emergence, did not suppress the growth of microorganisms such as nitrogen-free bacteria, which was visualized by their respective populations at 20, 30 and 50 DAS in rhizosphere soil and weed control practices. Hand weeding was found to be most appropriate for microbial population increase in soil, followed by herbicide application [99], and these findings support the present investigation. Similarly, Barman *et al.* [100] reported that the *Azotobacter* count decreased in response to herbicide treatments compared to that in the control group and that *Azotobacter* could not reach this level, indicating increased susceptibility to the herbicide. Konstantinovic *et al.* reported similar inhibitory effects of other preemergence herbicides, *viz.*, alachlor and atrazine, on the *Azotobacter* count [101]. The results of this study also indicated that various herbicides strongly inhibited the growth of fungi. The inhibition of fungal mycelial growth by herbicide application was consistent with the findings of previous studies [102–103]. Similarly, Eze [104] reported that glyphosate, paraquat, atrazine and linuron were more effective at inhibiting the mycelial growth of all the rhizoplane fungi screened than was primextra.

Fungal Diversity

A Vast fungal diversity has been interlinked with plant systems, *viz.*, epiphytic, endophytic and rhizosphere fungi. All these fungi, in association with plant systems, play essential roles in plant growth, crop yield and soil health improvement[105]. Agricultural techniques such as tillage, crop residue management through retention and incorporation into the soil, and crop rotation influence the physical and chemical properties of the soil inhabited by microorganisms such as fungi, thus affecting their abundance, diversity, and activity [24]. The adoption of zero tillage (ZT) and conservation tillage (ZT + crop residue retention) with the integration of chemical weed control and power + 1-hand weeding (IWM) as soil management practices tends to harbour beneficial fungal species while improving and maintaining soil health and quality in the long run.

Talaromyces flavus var. flavus was identified under CT(C)-ZT(M)-ZT(*Sr*) and ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) in combination with chemical weed control and power + 1 hand weeding (IWM) (T_2W_3 and T_3W_3) in the rhizosphere soil; these plants have been newly reported as beneficial fungal species inhabiting the soil (soil stabilizer) and plant growth-promoting fungi (PGPF) with high potential to inhibit other pathogenic fungal species (biocontrol agents) while benefiting the plant and the soil [106–110]. However, the CT(C)-CT(M)-Fallow(NS*r*), CT(C)-ZT(M)-ZT(*Sr*) and ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) treatments in combination with chemical (herbicide) rotation and chemical weed control generally produced pathogenic fungal species (*Aspergillus niger*, *Penicillium limosum*, *Aspergillus terreus*, *Apiospora serenensis*, *Zasmidium cellare*, and *Ochraceocephala foeniculi*), which were previously reported to have adverse effects on soil health and productivity.

It is evident that in combination with crop residue removal, crop residue removal occurs in combination with nonweed control and herbicide treatment in plots, and the application of herbicides or no-weed control, regardless of the tillage combination, caused changes and the production of pathogenic fungal microbes, which agrees with the results of Bhardwaj *et al.* [111] on the impact of herbicides in irrigated tropical rice fields, in which predominant pathogenic fungi (*Humicola*, *Nigrospora*, *Paramyrothecium*, *Mariannaea*, *Ceratobasidium*, *Funneliformis*, *Aspergillus*, *Pseudorhizophora*, and *Lecythophora*) were

identified with unweeded control and herbicide-treated plots, indicating the adverse effects of herbicides and high weed density populations on microbial dynamics.

These results on fungal diversity signify the importance of conservation tillage and minimum tillage coupled with IWM under conservation agricultural practices compared to conventional tillage in combination with herbicides and hand weed removal only during critical periods of weed competition.

Crop productivity

Maize Grain Yield and System Productivity (Cotton Equivalent Yield)

The improved growth/development of crops and increased yield largely rely on tillage practices, as these practices play a crucial role in determining the development of a crop's rooting system, the soil volume explored by roots for moisture and nutrients, the availability of air, and the regulation of soil temperature, among other factors. The importance of crop-weed interactions in determining the competition faced by crop plants for light, moisture and space is well established. Confined root growth leads to decreased nutrient uptake and poor crop growth [112]. The meta-data analysis of ZT with residue retention indicated that the effect on crop yields in comparison with that in CT was inconsistent and was impacted substantially by cropping system, aridity index, crop residue maintenance, ZT duration, and weed management strategy [113]. In the present investigation, the maize grain and harvest indices were greater in the ZT + R(C)-ZT + R(M)-ZT + R(Sr) treatment than in the other tillage methods. This superior performance can be interconnected with the development of robust, deep-rooted systems in crops facilitated by the practice of zero tillage.

The implementation of ZT is thought to augment the nutrient absorption capacity of crops, thereby fostering their physiological growth and overall development. Furthermore, the preservation of crop residues on the soil surface under the ZT + R(C)-ZT + R(M)-ZT + R(Sr) treatment likely contributed to the enhanced retention and availability of soil moisture. This aspect is especially crucial during the posttasselling stage of the maize crop, which coincided with a warm period from mid-March to May. Given the limited moisture conditions during this period, supplemental irrigation was applied to ensure optimal soil moisture levels throughout crop development. Research by You *et al.* [114] also indicated that short-term reduced tillage (rotary-till and no-till) and residue incorporation enhanced soil properties; spring maize grain yield; growth and attributes; and increased root biomass and shoot ratio. Furthermore, the interaction of tillage and residue treatments can increase crop biomass and yield [115–116]. A number of previous studies conducted on short-term conservation tillage have not paid full attention to how yield can be improved.

Long-term conventional tillage always hinders root growth and the root-to-shoot ratio [117]. No-till enhances root biomass and shoot biomass, regulates the shoot-to-root ratio and increases yield in comparison with plough-till and rotary-till [118–119]. Residue incorporation can also enhance crop biomass and yield due to enhanced soil buffering capacity [120–121]. The postemergence tank-mix combination of atrazine and tembotrione herbicides was applied at recommended rates in chemical weed control and chemical (herbicide) rotation, which resulted in effective weed control and no phytotoxicity. The absence of phytotoxic effects suggests the efficacy and safety of the combination of tembotrione and atrazine for weed management, which contributes to improved crop performance. Poor crop performance was also observed under non-weeded control, which was ultimately reflected in yield. This could be due to the high weed density at the critical crop growth stage, which outcompeted the crop for available moisture, nutrients, light and rooting space. Ganapathi *et al.* [122] also recorded higher kernel and harvest indices and lower weed dry weight with IWM than with the use of only advocated herbicides and non-weeded treatments due to less weed infestation. Similar results were obtained by Kumar *et al.* [123], who reported that the application of preemergence herbicide followed by one rotary hoeing at 35 DAS led to increased grain yield. The results of Ahmad *et al.* [124] concur with the findings of the present investigation, who noted that nicosulfuron application and one-way weeding with a hoe at 15 DAS led to greater kernel yield, whereas the lowest kernel yield was obtained from the non-weeded control. In the present study, there was an increase in corn yield and system CEY when employing zero tillage with crop residue retention (ZT + R(C)-ZT + R(M)-ZT + R(Sr)) and IWM, chemical weed control and chemical (herbicide) rotation. This

improvement could be attributed to the synergistic effects of efficient weed management achieved through the use of chemical and cultural mechanical control tactics, as well as moisture and nutrient preservation facilitated by no-till practices that retain crop residues. These results are supported by Ahmad *et al.* [124], who deduced that maize can flourish when cultivated under zero tillage either with the application of atrazine or glyphosate or with hand weeding (HW) at 40 DAS as an alternative to manual weeding in spring seasons to attain higher grain yield.

Treatment performance was assessed on the basis of system cotton equivalent yield (CEY) and maize grain yield, microbial population and fungal diversity, and microbial and enzyme activities.

The winter maize grain yield recorded from the different tillage weed management treatment combinations was converted into cotton equivalent yield (CEY) considering the minority equivalence. The winter CEY was then added to the monsoon cotton yield in the 4 years to arrive at the cotton equivalent yield of the cotton–maize system (system CEY) for the fourth year. The system CEY and enzyme activities were used to evaluate the different tillage practices, weed management options and various tillage-weed management treatment combinations to identify a combination of remunerative tillage, weed management and tillage-weed management practices with a relatively higher system (CEY), maize grain yield, microbial and enzyme activities, microbial population and fungal diversity. These data are presented in figure (s) 3a, b, c, 4a, b, c, 7a-h and Tables 7a, b, 8 and 9.

Tillage and weed management had significant effects on the soil microbiological properties and system yield (SY) in terms of cotton equivalent (CEY). Even though ZT + R(C)-ZT + R(M)-ZT + R(Sr) in combination with the non-weeded control (T_3W_4) had higher microbial and enzyme activities, diverse groups of pathogenic fungal species and microbial populations, except for the metabolic quotient, were lower than those in the other treatment combinations, but the crop productivity in the ZT + R(M)-ZT + R(M)-ZT + R(M)-ZT + R(Sr) treatment combination was significantly lower than that in the other treatment combinations. In combination with all weed management methods, CT had lower microbial and enzyme activities, diverse groups of pathogenic fungal species, and microbial populations, but not metabolic quotients, which were greater under these treatment combinations. However, the crop productivity in the CT treatment combined with all weed management options was greater than that in the ZT + R(C)-ZT + R(M)-ZT + R(Sr) treatment combined with the non-weeded control, which indicates increased productivity but poor soil health, as indicated by the soil biological attributes. The SY in terms of CEY was greater in the ZT + R(C)-ZT + R(M)-ZT + R(Sr) treatment in combination with the IWM, which indicated that the adoption of ZT + R(C)-ZT + R(M)-ZT + R(Sr) in combination with IWM practices can maintain the status of soil microbiological attributes at higher levels, harbour beneficial fungal species and increase farmer productivity. Therefore, implementing zero tillage with the retention of crop residues in CA together with IWM aids in improving soil health and can optimize productivity for farmers in cotton–maize–*Sesbania rostrata* cropping systems.

Conclusion

On the basis of four years of investigation into the cumulative effects of tillage and weed management options on microbial activity and population, fungal diversity, enzyme activities, and crop productivity under conservation agriculture (CA), the following conclusions can be drawn: conservation tillage in combination with nonweeded control (only one-way weeding at the critical period of weed competition) and the combination of chemical weed control and power + 1-hand weeding (IWM) significantly enhanced soil enzymatic and microbial activities and a microbial population and decreased metabolic quotient (qCO_2), whereas conventionally tilled (CT) and chemically treated plots resulted in a drastic reduction in soil enzymatic and microbial activities and in the microbial population and increased qCO_2 during both the sampling period (30 DAS and tasselling) during maize growth. ZT with and without crop residue incorporation in combination with IWM harboured beneficial soil inhabitant fungal species, *Talaromyces flavus* (a soil stabilizer, plant growth promoter, and soil pathogenic fungal inhibitor), while CT, which interacts with overall weed management, led to the production of pathogenic fungal species identified at the tasselling stage of maize. The maize grain yield and system yield in terms of cotton equivalent yield (CEY) were greater under ZT, which included the retention of crop leftovers; IWM, chemical weed control; and chemical (herbicidal) rotation plots than under CT, crop residue removal; and non-weeded control treatments. There was no significant effect of the

combination of treatment on maize grain yield ($P=0.05$). ZT with crop residue maintenance (ZT+R(C)-ZT+R(M)-ZT+R(Sr)) in combination with IWM had a significantly greater system CEY (4453 kg ha^{-1}), followed by ZT+R in combination with chemical weed control and chemical (herbicide) rotation, with system CEYs of 4292 kg ha^{-1} and 4206 kg ha^{-1} , respectively. Among the tillage practices, ZT+R(C)-ZT+R(M)-ZT+R(Sr) had a greater system CEY than did CT(C)-CT(M)-Fallow(NSr) and CT(C)-ZT(M)-ZT(Sr). With regard to weed management options, IWM had higher system CEY. The SY in terms of CEY was greater in the ZT+R(C)-ZT+R(M)-ZT+R(Sr) treatment in combination with IWM, which indicated that the adoption of conservation tillage with IWM practices augments important soil microbiological attributes, harbours beneficial fungal species and provides good productivity to farmers in the long-term. Even though the interaction of ZT+R(C)-ZT+R(M)-ZT+R(Sr) with non-weeded control had a positive effect on increasing soil microbiological parameters and activities, crop productivity was very low; therefore, the adoption of ZT+R(C)-ZT+R(M)-ZT+R(Sr) in CA along with IWM helps improve soil health and can optimize productivity in a long-term cotton-maize-*Sesbania rostrata* cropping system.

Declarations

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Figures



Figure 1

Satellite view of the experimental field (36 plots inside demarcated with yellow lines)

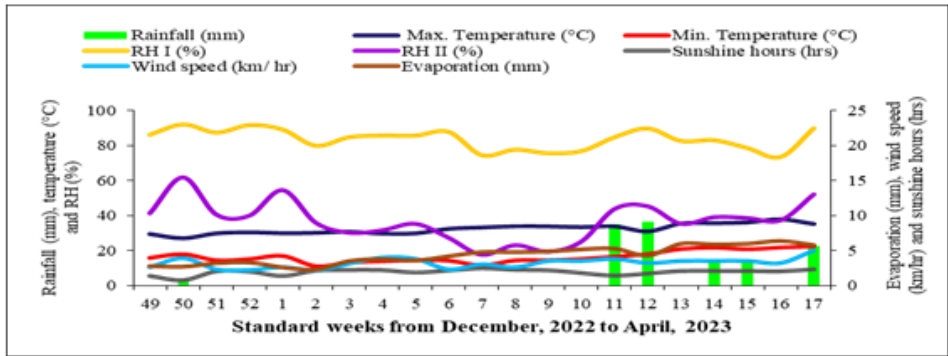


Figure 2

Weekly base mean meteorological observations during maize development

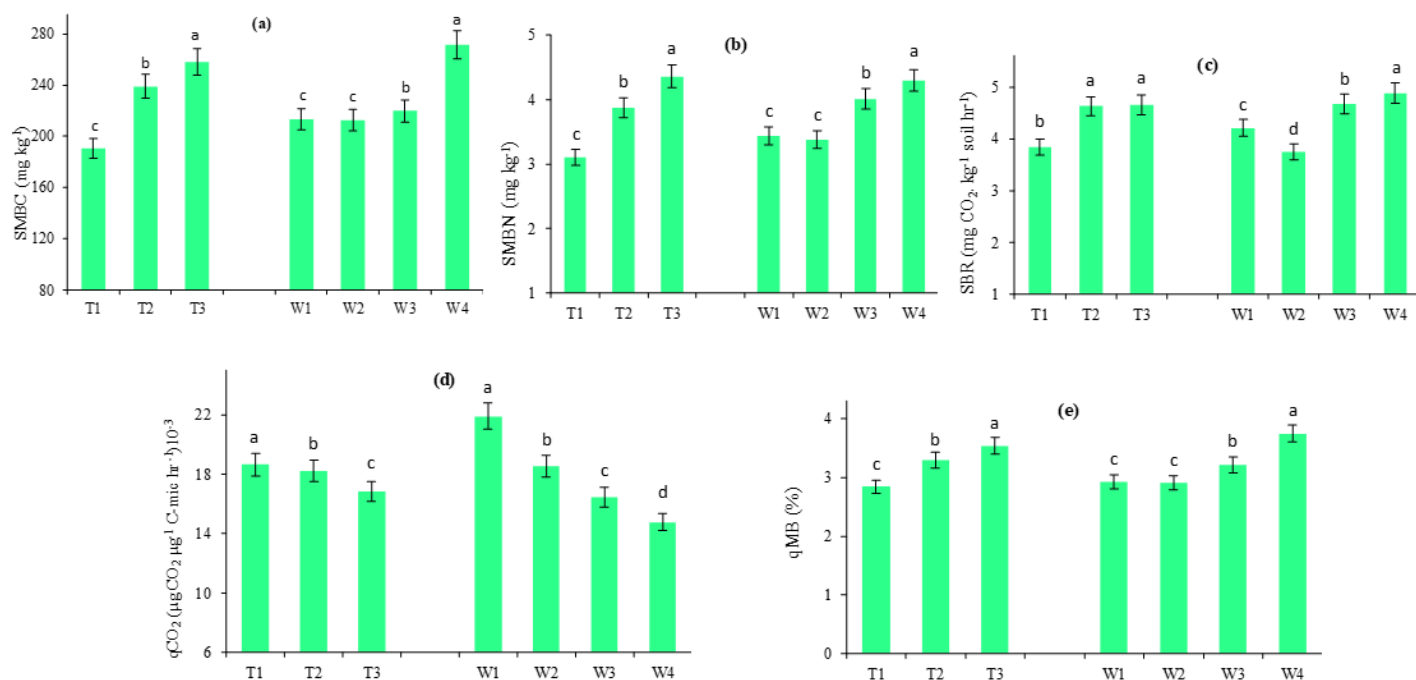


Figure 3

Impact of tillage practices and weed management options on soil microbial activity, viz., soil microbial biomass carbon-SMBC (a), soil microbial biomass nitrogen-SMBN (b), soil basal respiration-SBR (c), metabolic quotient-qCO₂ (d), and microbial quotient-qMB (e), at 30 days after sowing (DAS) on the maize crop (8th crop cycle). Means with distinct symbols demonstrate significant differences between the treatments at the 5% probability level (Tukey's test), and means with the same symbols indicate no significant differences among the treatment means at the 5% probability level. Refer to Tables 1 and 2 for treatment details.

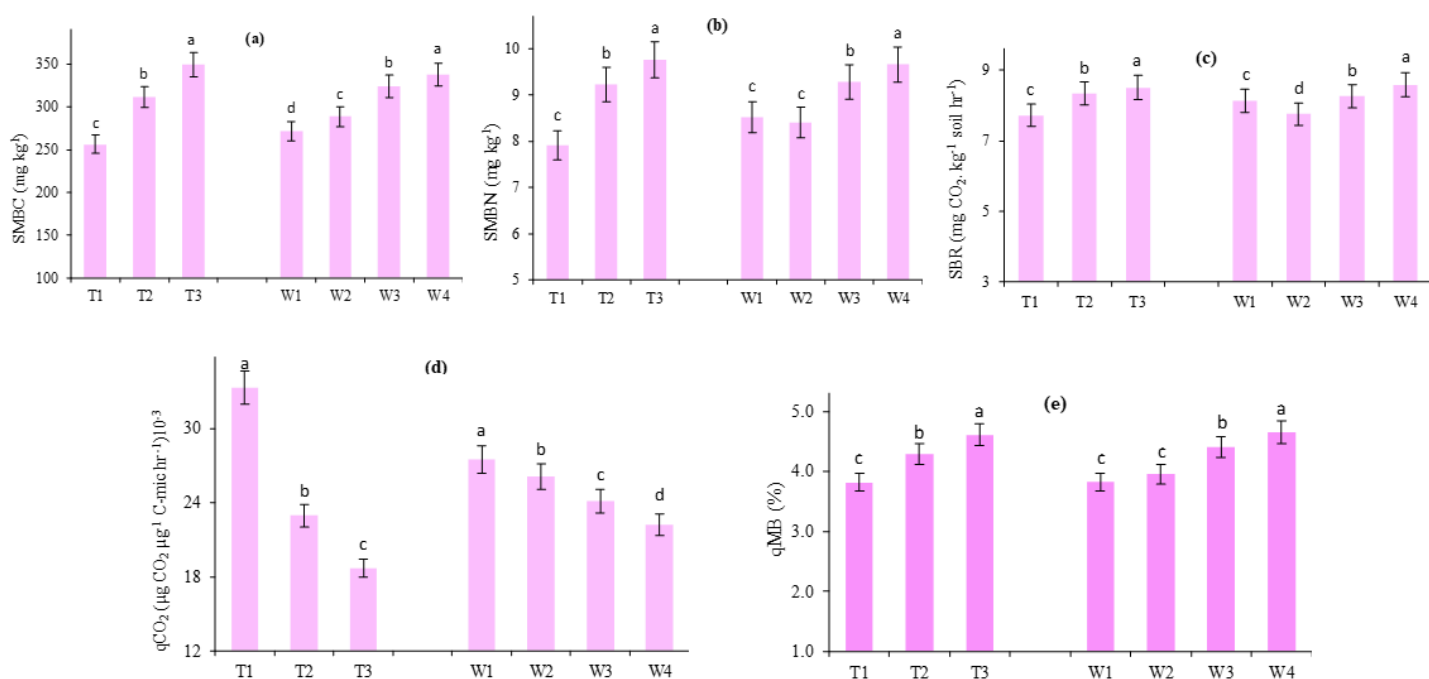


Figure 4

Impact of tillage practices and weed management options on soil microbial activity, viz., soil microbial biomass carbon-SMBC **(a)**, soil microbial biomass nitrogen-SMBN **(b)**, soil basal respiration-SBR **(c)**, metabolic quotient-qCO₂ **(d)**, and microbial quotient-qMB **(e)**, at the 30-tasselling stage of the maize crop (8th crop cycle). Means with distinct symbols demonstrate significant differences between the treatments at the 5% probability level (Tukey's test), and means with the same symbols indicate no significant differences among the treatment means at the 5% probability level. Refer to Tables 1 and 2 for treatment details.

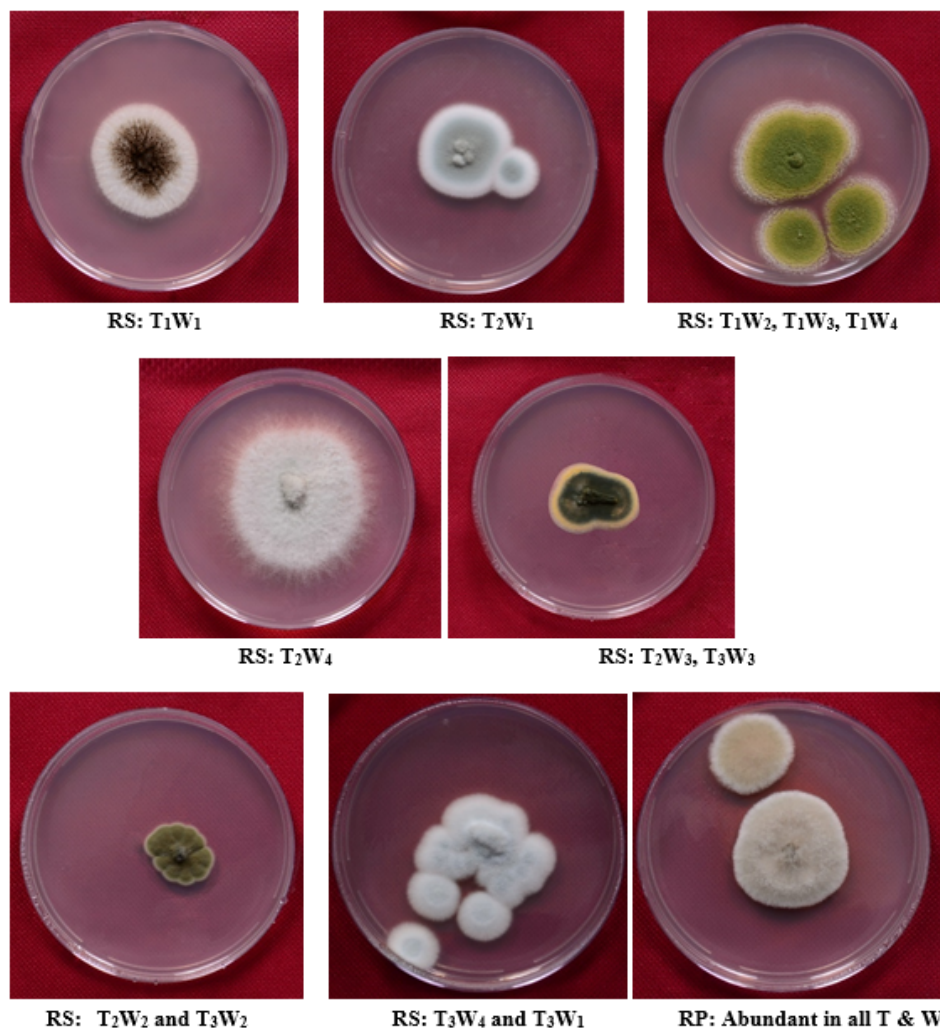


Figure 5

Treatments of various cultured rhizosphere soil and rhizoplane fungal species grown on PDA from the colonies enumerated at the tasselling stage of maize. RS: rhizosphere; RP: rhizoplane; T: tillage; W: weed management; T₁ = CT(C)-CT(M)-Fallow (NSr); T₂ = CT(C)-ZT(M)-ZT(Sr); T₃ = ZT+R(C)-ZT+R(M)-ZT+R(Sr); W₁ = chemical weed control; C= cotton; M= maize; Sr= *Sesbania rostrata*; W₂ = chemical (herbicide) rotation; W₃ = integration of chemical weed control and power + 1 hand weeding (IWM); W₄ = nonweeded control. T= tillage; TW=tillage and weed management treatment interaction.

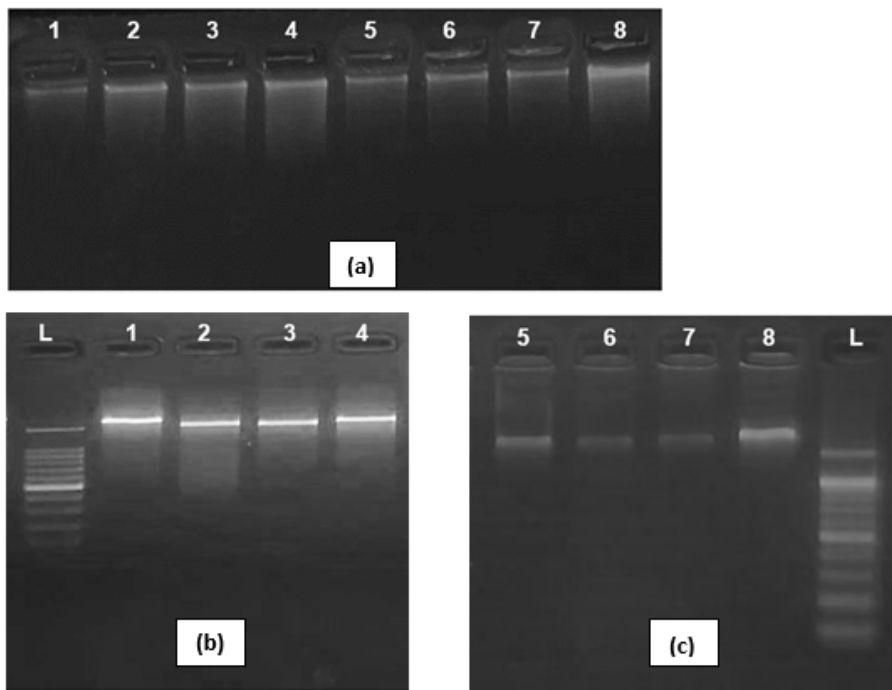


Figure 6

Agarose gel electrophoresis of genomic DNA extracted from isolated fungi

Agarose gel electrophoresis of the PCR-amplified product (18S rRNA gene).

Supplementary Files

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