

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

Knight Nthebere

knthebere@gmail.com

Professor Jayashankar Telangana State Agricultural University

Ram Prakash Tata

Professor Jayashankar Telangana State Agricultural University

Padmaja Bhimireddy

Professor Jayashankar Telangana State Agricultural University

Jayasree Gudapati

Professor Jayashankar Telangana State Agricultural University

Meena Admala

Professor Jayashankar Telangana State Agricultural University

Latha P Chandran

Indian institute of rice research

Maniyala Bhargava Narasimha Yadav

University of Agricultural Sciences

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Abstract

In a diversified cropping system, the kinds of tillage methods and weed management choices adopted exert a significant influence on soil microbiome which has a bearing on crop productivity. The synergetic impacts of such practices on 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-years CA experiment was undertaken to investigate the impact of tillage and weed management on soil microbiome and fungal diversity at 30 DAS and tasselling of maize, crop yield and identify a sustainable tillage and weed management which can provide nature-based solution. Three tillage practices; **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 weed control tactics involved; **W₁**-chemical weed control, **W₂**-chemical (herbicide) rotation, **W₃**-integrated weed management (IWM) and **W₄**-non-weeded control laid out in split-plot design. Rhizosphere soil and rhizoplane samples were collected from the respective plots at 30 DAS after herbicides application and tasselling. Analysis for microbial population, enzyme and microbial activities viz., soil basal respiration (SBR), metabolic quotient (qCO₂), microbial quotient (qMB), soil microbial biomass carbon (SMBC) and nitrogen (SMBN) was done duly following standard procedures. The rRNA gene sequencing with 18s was performed with rhizosphere soil and rhizoplane fungi isolated at tasselling. Yield was recorded at harvest. The salient findings indicated; a decline in enzyme activities, microbial population, microbial activities at initial stages (30 DAS) due to impact of herbicides which later on increased by tasseling except qCO₂ which decreased. These biological properties were higher under **T₃** and non-weeded control followed by IWM except qCO₂ which showed a decreasing trend relative to **T₁**, **T₂** and **W₁**, **W₂** at both sampling stages of maize. Kernel yield (KY) and System yield (SY) were enhanced by **T₃** and IWM, herbicides treated plots (**W₁** and **W₂**) compared to **T₁**, **T₂** and non-weeded control. *Talaromyces flavus*, beneficially rhizosphere soil inhabitant was identified in **T₃** in combination with IWM. Considering both crop productivity and soil biological assessment, **T₃** and IWM was considered as best treatment combination among all others with SY (4453 kg ha⁻¹). These findings signify the importance of adopting reduced tillage (**T₃**) and IWM for the farmer while striving for Nature-based solution.

Introduction

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

Soil microorganisms play an essential part as drivers of soil biological processes [6], which is a gain for maintenance of soil quality, agricultural sustainability and ecosystem multi-functionality. Ecosystem functions controlled by rhizosphere soil microorganisms frequently employed function-based metrics such as soil basal respiration, decomposition of soil organic matter (SOM), soil microbial activities and extracellular enzyme activity [7]. The constituent of rhizosphere soil microorganisms and function-based metrics are highly influenced by similar changing edaphic properties, thus, a suitable agricultural management practices such as irrigation, tillage, crop diversification and weed management practices can allow rhizosphere soil microorganisms to perform their different ecological functions [8–9]. Rhizosphere soil microorganisms and microbial activities change rapidly with any change in soil management practices and environmental

conditions with a short turn-over [10], and can be used as early indicators for soil health and crop yield improvement. Soil enzyme activity depends upon different abiotic factors, *viz.*, soil pH, moisture content, oxygen availability and soil texture etc [11]. These properties are subject to change depending on the intensity of tillage, weed control practices, 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 practice decline the activities of soil microbes, enzyme, change microbial diversity, shatter nutrient cycling and consequently reduce stability or resilience of soil functional status [13]. Alteration in SOM content, cropping systems could also shift the balance of rhizosphere soil enzymes, microbial activities and population into biodiversity and function [14].

A number of quantitative evaluations have been accepted universally for assessing changes in soil functional activity. For instance, 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]. The qCO_2 constitutes the metabolic level of soil microorganisms, in which greater values are indicating 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 determinant of the intensity of soil bio-geochemical processes to a certain extent. Insights of how microbial activities and population riposte to various agricultural management practices and turmoil is essential for identifying best agricultural practices which can augment, sustain soil resources and crop yield [18]. Soil enzyme activity is deemed to be indicative of specific biochemical reaction processes of the whole soil microbial activities that occur in SOM mineralization and is also important indicator of soil health, pollution and ecological restoration with a short turn-over 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 a significant increase in pre-emergence and post-emergence herbicide use in maize. However, herbicides are known to pose a significant negative or positive effect on soil microbial activities, population and diversity which in turn impact soil processes [22]. A better understanding of herbicides impact on soil enzymes dynamics, functional diversity of soil microorganisms and fungal diversity in the ecosystem 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 the changes in soil management. Thus far, several studies have already explored the influence of tillage on soil enzyme activity, microbial activities and population dynamics with CA [23], but research on direct effects of different tillage practices and weed management on such biological parameters at different crop growth stages of maize and fungal diversity at various zone level (soil rhizosphere and rhizoplane) and how crop productivity relates with soil functional metrics and biodiversity have not been extensively investigated under a diversified crop rotation system (cotton-maize-*Sesbania rostrata*) in Southern region of India. The insights on fungal diversity with CA practices under various kinds of tillage practices and weed management choices 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 and increase crop yield, restore ecosystem and inhibit pathogens). Thus, agricultural techniques such as tillage, crop residue management, crop rotation can influence diversity, and activity of microorganisms like fungi [24].

Cereal-based cropping system is a common practice in Southern regions of India, while maize yield and productivity declined monotonically under continuous intensive tillage system, and corresponding to deterioration of soil physico-chemical properties, decline of soil biological activities [11; 25]. Diversified crop rotation along-with ZT and retention of crop remains makes use of pre-crop 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 residues removal [26–27]. Thus, a reduction in tillage intensity with continuous retention of the previous crop residues and integration of chemical and cultural weed control practices in CA under a diversified cropping system may be a solution to reduce soil degradation processes, the risk of agricultural production while improving the soil functional metrics, rhizosphere soil microbial population which can have

a direct positive effect on crop productivity. Therefore, the present experiment was undertaken with these objectives; to investigate the synergetic effects of different tillage practices and weed management choices on soil microbial and enzyme activities, microbial population and diversity at various sampling stages of maize crop *i.e.*, 30 DAS and tasselling stage, to target the maize grain yield and system yield in terms of cotton equivalent yield (CEY) in a 4-years CA (8th crop cycle) experiment under cotton-maize-*Sesbania rostrata* cropping system, and to identify a suitable tillage practice and weed management option which can reduce perturbations in soil, enhance soil biological activities and harbour beneficial fungal diversity species, reduce 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 College Farm, PJTSAU, Southern Telangana Zone of India under 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*), green manure (*Sesbania rostrata*) rotations, respectively. An experiment continued from 2020 until 2023 and collection of soil samples for analysis of soil parameters and recording of yield were done after harvest of winter maize crop in 2022-23 (fourth year in the 8th crop cycle). The field trial is located at 16° 18' 17" N latitude and 78° 25' 38" E longitude. The zone is dryland with approximately 708 mm mean annual rainfall [28]. Extreme heat and humidity occur during summer months (March to fortnight of June) with mean temperature of 30 °C. Maximum temperatures often go beyond 42°C from April to May. December and January are extremely winter months with the lowest temperatures dropping as low as 10°C occasionally. Rainfall surpass 75% due to the South-West monsoon and happens between June to September [28].

Weather during the development of the crop

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

Soil characteristics

The soil of the study area falls under the soil order *Inceptisol*, sandy clay loam in texture, red chalk in color, 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, non-saline (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) in the soil surface (0–15 cm) at initiation of experiment.

Design of the experiment and treatment details

Conservation agriculture (CA) experiment was laid out in a split plot design with three tillage(s) practices in the main plots as shown in Table 1 and four weed management options in the sub-plots treatments as detailed in Table 2 and combinations of tillage and weed management were replicated thrice. For T₁: conventional tillage, the plots were prepared by ploughing two times accompanied by rotovating and seeding. T₂ during zero tillage (ZT), no-till of the soil was done *i.e.*, seeding was done directly by opening the soil followed by surface soil sealing and T₃: zero tillage (ZT) + residue retention (R), no-till of the soil, the preceding crops (cotton and *Sesbania rostrata*) residues were shredded, retained, incorporated into the soil and seeding was done directly by opening the soil accompanied by soil surface sealing with mulch from crop residues (Table 1). Weed management strategies included: W₁: chemical weed control, W₂: chemical (herbicide) rotation, W₃: integration of chemical weed control and power + 1 hand weeding (IWM) and W₄: Non-weeded control as fully elaborated in Table 2. No tillage operations and weed management were done prior to sowing of summer green manure (*Sesbania rostrata*) as it was raised up to 45 days with the intention to retain and incorporate its residues into the soil in T₃. There was no green manure (*Sesbania rostrata*) sown in T₁ plots *i.e.*, T₁ plots were fallowed during 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) = ZT + R (Sr) = zero tillage (Sr) + residue retention.

Table 2
Weed management (WM) in sub-treatments and interaction with tillage (T) in 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 pre-emergen	Diuron PE 0.75			Atrazine 1.0 kg/ha		
T ₂	-ce application PE 0.75 kg/ha fb tank mix application of pyriethionac -sodium 62.5 g/ha + quiza- lofop-ethyl 50 g/ha as PoE (Post-emergen- ce application) (2–3 weed leaf stage) fb directed spray (inter-row) of paraquat 0.5 kg/ha at 50–55 DAS. rotated with Pendimethalin 1 kg ha⁻¹ fb tank mix application of pyriethionac-sodium 62.5 g/ha + quiza- lofop ethyl 50 g/ha as PoE (2–3 weed leaf stage) fb directed spray (inter-row) 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>i.e.</i> 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 (T₂, T₃). Atrazine 1 kg ha⁻¹ PE fb tembotrione 120g/ha at 20–25 DAS as PoE (T₂, T₃). Atrazine 1 kg ha⁻¹ PE fb tembotrione 120g/ha at 20–25 DAS as PoE (T₁).	+ paraquat 600 g/ha PE fb tembotrione 120 g/ha at 20–25 DAS as PoE (T₂, T₃). Atrazine 1.0 kg/ha PE fb tembotrione 120g/ha at 20–25 DAS at PoE (T₁). 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 (T₂, T₃). Atrazine 1.0 kg/ha PE fb halo-sulfuron methyl 67.5 g/ha at 20–	Tembotri- one 120 g/ha Atrazine 50% WP 0.5 kg/ha as Early post-emergenc e) EPoE fb brush cutter at 40 DAS	One hand weeding was done after the critical period of crop-weed competi- on <i>i.e.</i> between 45–50 days after sowing.
T ₃					25 DAS as PoE (T ₁).		

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 variety were seeded at 60 cm in between the rows and 25 cm in between the lines with 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 the hand-raking in T₁: 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 high crop population and gap filled where seeds did not emerge 13 days subsequent to seed emergence. The crop was typically developed and advanced with supplemental irrigation as the amount of rainfall received during the crop developmental period was scanty. Advocated doses 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 of potash (MOP), respectively. Application of urea and DAP were split thrice as basal, at knee height and maize tasseling period.

Sampling and standard analytical procedures

Soil physico-chemical properties

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

Soil microbial population, microbial and enzyme activities

Sampling of rhizosphere soil was done at two growth stages of maize crop (8th crop cycle) in 2022-23 during the experiment: the first, after pre-emergence, early post-emergence and post-emergence application herbicides in chemical weed control (W₁) and herbicide rotation weed management (W₂) plots at 30 DAS of maize crop and the second, at tasselling stage of maize. Composite samples were collected in respective plots in polythene bags with zip, taken to the laboratory, passed through 2 mm sieve and analysed the same day of collection from the field. The functional activity was measured in terms of soil microbial activities related to soil microbial population, soil organic matter and nitrogen cycling. Soil water content was determined according to Monteiro and Frighetto [31], and the 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 CO₂ released was trapped in NaOH and determined by HCl titration. The results were reported as milligrams of CO₂ released per kilogram of soil per hour (Eq. 1).

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

Where: V_b was the volume of HCl consumed in the blank (ml); V_s was the volume of HCl consumed in the test sample (ml); M was the HCl molarity; 6 was equivalent factor (1 ml of 0.5 N HCl is equivalent to 6 mg C-CO₂ in the NaOH solution); ds were the weight of dry soil; t was 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 done to kill the organism in the soil samples, after which the amount of readily oxidizable C in the sample was measured through standard chemical procedures. The values of SMBC are given by the carbon content of fumigated soil minus that of the non-fumigated soils, all divided by the proportion of microbial C evolved (K_{EC}). A value of 0.25 ± 0.05 was used for k_c in SMBC calculation representing the efficiency of extraction of soil microbial biomass carbon (Eq. 2).

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

Where: ECf was mg of C per kilogram of fumigated soil; ECnf was mg of C per kilogram of non-fumigated soil; K_{EC} was part of 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 CH_3Cl vapor for 24 hrs at 21°C . Both fumigated and unfumigated soil samples were extracted with 0.5 ml of 0.5 mol L^{-1} of K_2SO_4 for 30 min before gravimetric titration through ashless Whatman filter paper. Total dissolved N in the extracts was measured by persulfate digestion followed by NO_3^- determination (VCl_3 /Griess reaction). The difference in total dissolved nitrogen (TDN) in extracts of fumigated and unfumigated soils was attributed to the release (flush) of N from lysed microbial cells. Calculation for SMBN was done as per Eq. 2. A correction factor for SMBN ($K_{EN} = 0.45$) was applied for incomplete extraction of microbial N [37]. The metabolic quotient ($q\text{CO}_2$), 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 is affected. Microbial quotient (MBC: SOC) was the ratio of MBC to SOC [39].

Soil enzymatic activity

Dehydrogenase activity (DHA) was assayed according to Casida *et al.* [40] and red coloured of Triphenyl formazan (TPF) was read in spectrophotometry ($\lambda = 485\text{nm}$). Fluorescein Di-acetate activity (FDA) was estimated according to Green *et al.* [41] and the greenish-yellow coloured fluorescein was measured in spectrophotometry at wavelength of 490 nm . The urease activity was determined by quantifying the rate of release of NH_4^+ from the hydrolysis of urea as described by Tabatabai and Bremner [42]. The activity of urease was then calculated and expressed as μg of NH_4^+ released $\text{g}^{-1} \text{ soil h}^{-1}$ as described by Tabatabai and Bremner [42]. The β -galactosidase and phosphatase activity were estimated according to Eivazi and Tabatabai [43] and Tabatabai and Bremner [44]. After the incubation time appropriate to each enzyme (60 min for β -galactosidase and phosphatase), their respective substrates (*p*-nitrophenyl- β -D-galactopyranoside and *p*-nitrophenyl-phosphate) were hydrolysed into a yellow coloured *p*-nitrophenol and all determined by spectrophotometry ($\lambda = 420 \text{ nm}$ and $\lambda = 405\text{nm}$, respectively).

Rhizosphere soil and rhizoplane microbial population

Functional culturable groups of rhizosphere soil microorganisms *viz.*, *Azotobacter*, *Azospirillum*, total fungi were assessed. Rhizosphere *Azotobacter* and total fungal population were evaluated by following the protocols described in Albino and Andrade [45]. Colony counter was used for counting the colonies formed after 7 days of incubation period in BOD incubator at 30°C for *Azotobacter* and 3–5 days at 25°C in BOD incubator for total fungal population, respectively. The population was estimated as colony forming units (CFU) per gram of dry soil (Eq. 3) [46]. For enumeration of rhizosphere soil *Azospirillum* population, rhizosphere soil samples (0.1 ml aliquot) were inoculated into semi-solid nitrogen-free bromothymol blue malate medium (Nfb) according to Döbereiner and Day (1976) and incubated in BOD incubator for 3–4 days at 30°C until the formation of the pellicle in tubes containing Nfb medium and 0.1 ml of rhizosphere soil sample aliquot. *Azospirillum* was estimated by most probable number (MPN) table (s), transformed as the logarithm of most probable number per gram of soil ($\log \text{ MPN.g}^{-1}$) suggested by Alexander [47]; Woome *et al.* [48] and Cochran [49] and expressed as colony forming units (CFU g^{-1}) of soil on dry weight basis and the others (rhizosphere soil *Azotobacter* and total fungi) were transformed and expressed as logarithm of colony forming units per gram of soil ($\log \text{ CFU.g}^{-1} \text{ soil}$).

For enumeration of rhizoplane microbial population, the root samples were collected from the plant by pulling out the plant, separating the roots from the plant through cutting with the help of a knife followed by removing rhizosphere soil. The roots were collected in a polythene zip cover. Using a pair of scissors, the roots were separated and one-gram weight of the roots

was transferred into a 100 ml sterile distilled water and washed thoroughly using rotary mixer. One milliliter from 100 ml of the sample was transferred into 10 ml of the saline blanks and serial dilutions were made for each treatment following the same methodology employed for enumeration of soil microbial population, incubated in BOD incubator for 3–5 days at 25°C with dilutions of up to 10^4 . The Eq. 3 [46] was used for calculation and transformed and expressed as logarithm of colony forming units per gram of roots ($\log \text{CFU.g}^{-1} \text{ roots}$).

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

Fungal Diversity

Isolation criteria and purification

The rRNA gene sequencing with 18S was performed with fungal colonies obtained at tasseling stage of maize crop, 2022-23. Before identification, prolonged incubation of about 10–12 days of the fungal colonies grown on Rose Bengal solid agar medium at 25°C was done as to allow sporulation to occur. Based on the colour of the spores formed, classification was done and 8 plates representatives of all 12 treatment combinations were selected for purification as to obtain pure fungal strains based on abundance of the same number of the spores. These colonies which were predominant in plates, representing the treatment combinations were picked and cultured in potato dextrose (PDA) solid agar medium for 5 days in order to allow the growth of pure strains of fungal species. These 8 pure strains of fungi were sent for sequencing as to identify fungal species present in different treatment combinations of tillage and weed management.

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

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

An equal volume of phenol: chloroform: isoamly alcohol (25:24:1) was added to the tubes and 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: isoamly 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 3.0M 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 (Tris-Cl 10 mM pH 8.0, EDTA 1 mM). To remove ribonucleic acid (RNA), 5 µl of DNase, 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 used for polymerase chain reaction (PCR) amplification of 18s gene according to Baldoni *et al.* [50] along with 10pM of each primer composition of TAQ Master mix (High-Fidelity DNA Polymerase, 0.5mM dNTPs, 3.2mM MgCl₂ and PCR enzyme buffer cycling condition) (PCR clean kit). PCR cycling and amplification conditions are presented in Table 3. The primer details used are indicated in Table 4. Aligned sequence data of samples is attached in as supplementary data. The PCR product was sequenced bi-directionally according to Staden *et al.* [51] and sequencing mix composition were as follows: 10µl sequencing reaction, big dye terminator ready reaction mix: 4µl, template (100ng ul⁻¹

¹):1µl, Primer (10pmol λ⁻¹):2µl and milli Q water:3µl and PCR Conditions (25 cycles), Initial denaturation:96°C for 5 minutes, denaturation:96°C for 30 seconds, hybridization:50°C for 30 seconds and elongation : 60°C for 1.30 minutes.

Data analysis and Identification

Data was analysed by using sequencing machine: ABI 3130 genetic analyser, chemistry cycle sequencing kit: big dye terminator version 3.1" polymer and capillary array: POP_7 pol capillary array with BDTv3-KB-Denovo_v 5.2 protocol and sequence scape_ v 5.2 software reaction plate: Applied Biosystem Micro Amp Optical 96-Well Reaction plate. Identification was done by using the system software aligner to align the sequences and a comparative search of GenBank sequences in National Centre for Biotechnology Information (NCBI) was carried out using the BLASTn tool to identify the organisms and their closest neighbours. A phylogenetic tree builder used sequences aligned with 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 Weighbor with alphabet size 4 and length size 1000. The consensus sequence was deposited to the Gen Bank in NCBI to obtain accession numbers of identified organisms from type material.

Multiple sequence alignment and phylogenetic tree construction

Mega11 Version 11.0.13 and clustal W algorithm was used with the default parameters, which includes pairwise alignment with gap opening penalty – 15 gap extension and penalty – 6.06, multiple alignment with gap opening penalty – 15 and gap extension penalty – 6.06, and matrix with DNA weight Matrix – IUB Transition, weight – 0.50, use of negative matrix – off delay divergent cut off – 30.

For construction of phylogenetic tree: neighbour joining test of phylogeny method and bootstrap method were selected and number of bootstraps were 1000 in replicate and nucleotide model/method substitution type having 17 nucleotide sequences. The evolutionary distances were computed using the maximum composite likelihood method [52]. Codon positions included were 1st + 2nd + 3rd + noncoding. The maximum composite likelihood substitution including transitions + trasversions rates among sites – uniform rates pattern among Lineages – same (homogeneous), gaps/missing data treatment in pairwise deletion. There were a total of 2043 positions in the final dataset.

Table 3
Details of 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
18s Forward Primer		2 ul
18s Reverse Primer		2 ul
dNTPs (2.5mM 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
The Primer Details - The polymerase chain reaction (PCR) product size ~ 2kb

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

Crop Productivity

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

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

Statistical analysis

The data were analyzed statistically applying the analysis of variance technique dully following the ANOVA for split plot design as suggested by [53]. Critical difference for examining the treatment means for their significance was calculated at 5

per cent level of probability. Turkey's test was also used for ranking of microbial activities treatment means for their significance at 5% probability level.

Results

Soil pH and Soil organic carbon

Soil physico-chemical attributes were not significantly influenced by tillage and weed management options except Soil organic carbon (SOC) which showed a significant impact by different tillage practices. The treatment's interaction (tillage and weed management) effects on soil pH, EC and SOC were non-significant (Table 5). The ZT + R(C)-ZT + R(M)-ZT + R(Sr) treatment was observed with a significantly higher SOC (7.92 g kg⁻¹) over CT(C)-CT(M)-Fallow(NSr) and CT(C)-ZT(M)-ZT(Sr). Overall, SOC contents were higher in all the treatments compared to the initial SOC value (6.5 g kg⁻¹). Soil pH was slightly alkaline with a drop-off noticed across all the treatments over the initial soil pH value (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 - Non-weeded 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 = integration of chemical weed control + power and 1 hand weeding, C = cotton, M = maize, Sr = *Sesbania rostrata*, CD (P = 0.05) = critical difference at 5% probability level, Ns = non-significant, 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 the sampling stages (at 30 DAS after the application of herbicides and tasselling stage of maize). Soil microbial activity indices (SMAls) include soil microbial biomass carbon (SMBC), microbial biomass nitrogen (SMBN), soil basal respiration (SBR), microbial quotient (qMB) and metabolic quotient (qCO_2) (Fig. 3a, b, c, d, e and 4a, b, c, d, e). These SMAls were significantly promoted and increased by 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(NS*r*) and CT(C)-ZT(M)-ZT(*Sr*) except qCO_2 . Among weed practices, a significant increase on SMAls was observed with non-weeded control and integration of chemical weed control and power + 1 hand weeding (IWM) at both sampling stages. The herbicides applied at 30 DAS of maize in chemical weed control and chemical (herbicides) rotation, resulted in a significant reduction of SMAls, which later on increased till tasseling stage of the crop. The qCO_2 values were significantly lower under ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) in comparison with CT(C)-CT(M)-Fallow (NS*r*) and CT(C)-ZT(M)-ZT(*Sr*) at both stages of the crop. With respect to weed management choices, qCO_2 values were significantly reduced by non-weeded control and IWM over herbicides treated plots. There were no significant treatment interaction effects on SMAls observed at both periods of sampling (Fig. 3a, b, c, d, e and 4a, b, c, d, e).

At 30 DAS of maize, SMBC, SMBN, SBR, qMB were 7.52% and 26.27%, 11.01% and 28.90%, 0.64% and 17.60%, 15.15% and 15.16% significantly higher under ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) over CT(C)-ZT(M)-ZT(*Sr*) and CT(C)-CT(M)-Fallow (NS*r*), respectively. Among weed management options, after the application of pre-emergence (PE), early post-emergence (EPoE) and post-emergence (PoE) herbicides, at 30 DAS of maize, 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, 8.11–21.62% and 9.09–15.91% of qMB were higher under non-weeded control and IWM, respectively relative to chemical (herbicide) rotation and chemical weed control (Fig. 3a, b, c, d, e). At the same sampling period (30 DAS) during the crop, the ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) resulted in 7.62% and 9.64% significant reduction in qCO_2 over CT(C)-ZT(M)-ZT(*Sr*) and CT(C)-CT(M)-Fallow(NS*r*), respectively. There was also 20.24% and 32.60%, and 11.23% and 24.99% significant decrease observed in qCO_2 by non-weeded control and IWM, respectively compared to chemical (herbicide) rotation and chemical weed control (Fig. 3a, b, c, d, e).

At tasselling stage of the crop, there was an overall progressive increase of soil microbial activity indices (SMAls) due to advancement of the crop. The trends on SMAls were found to be similar to that observed at 30 DAS. Among all tillage practices, ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) had acquired 10.92% and 26.64% of SMBC, 5.53% and 19.04% of SMBN, 1.88% and 9.18% of SBR, 2.27% and 13.64% of qMB higher than 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 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%), qMB (9.09–15.91% and 14.89–21.28%) were observed under IWM and non-weeded control, respectively over chemical weed control and chemical (herbicide) rotation. During the same stage of the crop (tasselling), trends on qCO_2 were similar to that exhibited 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*). Non-weeded control and IWM also facilitated considerable decline of qCO_2 during that crop growth compared to chemical (herbicide) rotation and chemical weed control (Fig. 4a, b, c, d, e).

Soil Enzyme Activities

Adoption of ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) and non-weeded control as well as integration 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 di-acetate (FDA) and β -galactosidase (β -GaA) involved in the soil carbon (C), nitrogen (N) and phosphorus (P) cycling. This improvement in soil enzyme activities under ZT + R(C)-ZT + R(M)-ZT + R(*Sr*), non-weeded control and IWM was observed at both sampling stage of the crop, which significantly increased continuously with the crop progression. Herbicides applied at 30 DAS of the crop, after PE, EPoE, PoE resulted in a massive decrease in the activity of the soil enzymes, which later regained at tasselling stage of the crop (Table 6a, b, c).

Rhizosphere soil enzyme activity at 30 DAS of maize in CT(C)-CT(M)-Fallow (NSr) and CT(C)-ZT(M)-ZT(Sr) treatments was significantly lower over ZT + R(C)-ZT + R(M)-ZT + R(Sr). The DHA, SUA, AIP, AcP, FDA, β -GaA was 16.88% and 31.87%, 16.58% and 27.87%, 11.35% and 22.44%, 8.24% and 23.85%, 12.35% and 19.77%, 9.44% and 16.87% higher under ZT + R(C)-ZT + R(M)-ZT + R(Sr) plots over CT(C)-ZT(M)-ZT(Sr) and CT(C)-CT(M)-Fallow (NSr) plots, respectively (Table 6a, b, c). Among weed management options, DHA, SUA, AIP, AcP, FDA, β -GaA was 17.76–18.68% and 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% higher under IWM and non-weeded control, respectively over chemical weed control and chemical (herbicide) rotation at the same sampling (30 DAS) (Table 6a, b, c).

At tasselling stage, the activity of all rhizosphere soil enzymes exhibited trends similar to that observed under weed management options and tillage practices at 30 DAS. Enzyme activities increased significantly irrespective of the treatments, and tillage was the main factor which contributed on influencing the activities. At that crop growth development period (tasselling), DHA, SUA, AIP, AcP, FDA, β -GaA was 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%, 16.71% and 32.88% 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 (Table 6a, b, c). With regard to weed management choices, DHA, SUA, AIP, AcP, FDA, β -GaA was 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 over chemical (herbicide rotation) and chemical weed control at 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 on overall rhizosphere soil enzyme activities was observed at tasselling stage, and the treatment interaction effects (trends) on various enzyme activities appeared to be the same as that noticed at 30 DAS of the crop with a significantly higher enzyme activities exhibited by ZT + R(C)-ZT + R(M)-ZT + R(Sr) in combination with non-weeded control and IWM relative to all other treatment combinations (Table 6a, b, c).

Table 6a

Impact of tillage practices and weed management options on rhizosphere soil dehydrogenase ($\mu\text{g TPF. g}^{-1} \text{ dry soil. day}^{-1}$) and urease ($\mu\text{g NH}_4^+ \text{ - N. g}^{-1} \text{ dry soil. 2hr}^{-1}$) activity at two different maize 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

Treatment		Soil dehydrogenase activity			Soil urease activity			
Tillage	WM	30 DAS	Tasselling		30 DAS	Tasselling		
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 = integration of 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 5% probability level, Ns = non-significant, SE(m) = standard error of the mean.

Table 6b

Impact of tillage practices and weed management options on rhizosphere soil acid and alkaline phosphatase activity ($\mu\text{g. } p\text{-nitrophenol. g}^{-1} \text{ dry soil. hr}^{-1}$) 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 = integration of 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 5% probability level, Ns = non-significant, SE(m) = standard error of the mean.

Table 6c

Impact of tillage practices and weed management options on rhizosphere soil fluorescein di-acetate ($\mu\text{g. fluorescein. g}^{-1}$ dry soil. 3h^{-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 = integration of 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 5% probability level, Ns = non-significant, 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 choices, and the treatments (tillage and weed management) interaction effects on soil microbial and rhizoplane fungal population were significant at both sampling stages of the crop (30 DAS and tasselling) (Table 7a and b).

The spraying of the herbicides either in rotation or repeatedly in every alternate year as pre-emergence (PE), early post-emergence (EPoE) and post-emergence (PoE), at 30 DAS of maize, suppressed the growth and population of the microorganisms. Among all weed management options, at 30 DAS of the crop, rhizosphere soil *Azotobacter* (*Azot*), *Azospirillum* (*Azosp*), total fungal (TF), rhizoplane total fungal population (RF) was 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% superior under IWM and non-weeded control, respectively than under chemical weed control and chemical (herbicide) rotation (Table 7a, and b). In terms of all different tillage systems, rhizosphere soil *Azot*, *Azosp*, TF, rhizoplane TF population was 1.51% and 2.81%, 1.60% and 3.43%, 1.61% and 2.75%, 3.69% and 6.39% higher under ZT + R(C)-ZT + R(M)-ZT + R(Sr) over CT(C)-ZT(M)-ZT(Sr) and CT(C)-CT(M)-Fallow (NSr), respectively observed at 30 DAS (Table 7a, and b). Population of rhizosphere soil microorganisms and rhizoplane total fungi (TF) increased significantly at tasselling period of the crop, noticed in all the treatments, and tillage was the principal factor influencing a progressive rise of microbial population. At that growth stage of maize crop (tasselling), population of rhizosphere soil *Azot*, *Azosp*, TF, rhizoplane TF was 1.20% and 1.80%, 1.21% and 2.23%, 2.38% and 4.75%, 3.12 and 4.45% greater under ZT + R(C)-ZT + R(M)-ZT + R(Sr) than CT(C)-ZT(M)-ZT(Sr) and CT(C)-CT(M)-Fallow (NSr), respectively (Table 7a, and b). A significant difference with a continuous increase on overall microbial population was observed in all weed management options possibly due to microorganism's recovery from herbicidal injury at tasselling. The pattern for the growth of both rhizosphere soil and rhizoplane microbial counts at that crop growth stage (tasselling) resembled the trends observed at 30 DAS of the crop.

During the initial stage of crop development (30 DAS), the treatments (tillage and weed management) interactions effects on rhizosphere soil *Azotobacter* (*Azot*) counts were 1.91–3.39%, 3.62–4.26%, 3.88–4.31%, *Azospirillum* (*Azosp*) counts were 2.90–4.24%, 2.71–4.30%, 4.32–6.36%, total fungal (TF) counts were 2.68–4.25%, 2.51–3.20%, 3.89–4.35%, rhizoplane total fungal (TF) counts were 2.64–3.84%, 3.41–7.56%, 8.33–9.31% superior under ZT + R(C)-ZT + R(M)-ZT + R(Sr) in combination with non-weeded control, CT(C)-ZT(M)-ZT(Sr) combined with non-weeded control, CT(C)-CT(M)-Fallow (NSr) coupled with non-weeded control over ZT + R(C)-ZT + R(M)-ZT + R(Sr) on interaction with IWM and chemical weed control or chemical (herbicide) rotation, CT(C)-ZT(M)-ZT(Sr) in combination with IWM and chemical weed control or chemical (herbicide) rotation, CT(C)-CT(M)-Fallow (NSr) on interaction with IWM and chemical or chemical (herbicide) rotations, respectively (Table 7a and b). At tasselling stage of the crop, all microbial counts were observed with a further significant surge irrespective of the treatment combinations. Among all the treatment interactions, at tasselling of maize, ZT + R(C)-ZT + R(M)-ZT + R(Sr) in combination with unweeded control was observed with a significantly higher 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 other treatment combinations (Table 7a and b).

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

Table 7a
Impact of tillage practices and weed management options on rhizosphere soil *Azotobacter* and *Azospirillum* population (log CFU g⁻¹ soil) at two different maize growth stages.

Log of <i>Azotobacter</i> (log CFU/g 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 = integration of 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 5% probability level, Ns = non-significant, 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 7b

Impact of tillage practices and weed management options on rhizosphere soil and rhizoplane total fungal population 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 = integration of 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 5% probability level, Ns = non-significant, 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

The sub-culturing of the fungi from the culture plates was done prior to sequencing to purify the fungal strains, depicted in Fig. 5, and agarose gel electrophoresis images of total Deoxyribonucleic acid (DNA) and polymerase chain reaction (PCR) amplified product of 18s rRNA gene are illustrated in Fig. 6a, b and c. The fungi were identified based on nucleotide sequence homology of 18s rRNA gene presented in Table 8. The results of 18s rRNA gene sequencing indicated that *Talaromyces flavus* var. *flavus* (5-PJTSAU-KNIGHT-23) was identified under 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 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 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 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 (MSA) of data are attached as supplementary data.

Table 8
Impact of tillage and weed management practices on fungal diversity at tasselling stage of winter maize.

S.NO	Isolate ID	Treatment combination	Fungal name	Identity (%)	Accession numbers
Rhizosphere soil fungal microbe (s)					
1	1-PJTSAU-KNIGHT-23	T ₁ W ₁	<i>Aspergillus niger</i>	100.00%	PP177339
	1-PJTSAU-KNIGHT-23	T ₁ W ₂	<i>Aspergillus niger</i>		
	1-PJTSAU-KNIGHT-23	T ₁ W ₄	<i>Aspergillus niger</i>		
2	2-PJTSAU-KNIGHT-23	T ₁ W ₃	<i>Aspergillus niger</i>	100.00%	PP177340
3	3-PJTSAU-KNIGHT-23	T ₂ W ₁	<i>Aspergillus terreus</i>	99.33%	PP177341
4	4-PJTSAU-KNIGHT-23	T ₂ W ₂	<i>Apiospora serenensis</i>	98.47%	PP177342
	4-PJTSAU-KNIGHT-23	T ₃ W ₂			
5	5-PJTSAU-KNIGHT-23	T ₂ W ₃	<i>Talaromyces flavus</i> var. <i>flavus</i>	99.63%	PP177343
	5-PJTSAU-KNIGHT-23	T ₃ W ₃			
6	6-PJTSAU-KNIGHT-23	T ₂ W ₄	<i>Zasmidium cellare</i>	100.00%	PP177344
7	7-PJTSAU-KNIGHT-23	T ₃ W ₁	<i>Penicillium limosum</i>	99.88%	PP177345
	7-PJTSAU-KNIGHT-23	T ₃ W ₄			
Rhizoplane fungal microbe (s)					
8	8-PJTSAU-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 = Integration of chemical weed control and power + 1 hand weeding (IWM); 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 a significant influence 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 non-significant for kernel yield (KY) (Table 9).

A significantly higher KY (6801 kg ha^{-1}) was recorded under ZT + R(C)-ZT + R(M)-ZT + R(Sr), while lower KY (6014 kg ha^{-1}) was observed with CT(C)-CT(M)-Fallow(NSr). Adoption of chemical weed control and chemical (herbicide) rotation resulted in significantly higher KY (7245 kg ha^{-1} and 7324 kg ha^{-1}), followed by integration of chemical weed control and power + 1 hand weeding (IWM) with KY of 6722 kg ha^{-1} . The significantly lower KY (4099 kg ha^{-1}) was exhibited by 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 then added to the monsoon cotton yield of 4th year to arrive at the cotton equivalent yield of the cotton maize system (system CEY) for 4th year. The data on system CEY is presented in Table 9.

The ZT + R(C)-ZT + R(M)-ZT + R(Sr) was observed with significantly higher system CEY (3775 kg ha^{-1}) compared to CT(C)-ZT(M)-ZT(Sr) and CT(C)-CT(M)-Fallow(NSr) with system CEY of 3517 kg ha^{-1} 3328 kg ha^{-1} , respectively (Table 9). Among weed management strategies, significantly higher system CEY (4157 kg ha^{-1}) was noticed with IWM over chemical (herbicide) rotation, chemical weed control and non-weeded control with system CEY of 4065 kg ha^{-1} , 4018 kg ha^{-1} and 1921 kg ha^{-1} , respectively (Fig. 6a). Based on tillage and weed management interactions, ZT + R(C)-ZT + R(M)-ZT + R(Sr) in combination with IWM recorded a significantly higher system CEY (4453 kg ha^{-1}), and the least system CEY of 1767 kg ha^{-1} and 1848 kg ha^{-1} was noticed with CT(C)-ZT(M)-ZT(Sr) on interaction with non-weeded control and CT(C)-CT(M)-Fallow(NSr) in combination with non-weeded control, respectively in comparison with all other treatment combinations (Table 9). The CT(C)-CT(M)-Fallow(NSr) and all weed management combinations were also observed with a lower system CEY (Table 9).

Table 9

Grain Yield of maize and system yield in terms of system cotton equivalent yield (CEY) as influenced by tillage practices and weed management (WM) options after 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
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CT = conventional tillage, ZT = zero tillage; R = crop residue retention; IWM = integration of chemical weed control + power and 1 hand weeding, C = cotton, M = maize, Sr = *Sesbania rostrata*, CD (P = 0.05) = critical difference at 5% probability level, Ns = non-significant, SE(m) = standard error of the mean.

Discussions

Soil pH and soil organic carbon

Among all other soil factors, tillage and weed management strategies contributed in the alteration of soil organic carbon (SOC). The SOC concentration in the soil surface soil under no-till with at least 30% maintenance of the crop debris is less prone to depletion due to lower soil disturbance and cumulative crop residues, thus, yielding a higher SOC [54]. This greater SOC exhibited by ZT + R(C)-ZT + R(M)-ZT + R(Sr) could be associated with 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] under CA-based maize–legume cropping sequence. In zones where soil and weather conditions are conducive for the production of biomass, and where adverse crop yield effects are unnoticed, then CA practices demonstrate greater quantity of SOC comparative to CT managed systems, more especially in the top soil. CT transpose the soil, shatter the soil clods, and exposes SOM to wetting-drying phenomena resulting in the reduction of SOC contents [56]. The reduction in SOC levels observed under CT(C)-CT(M)-Fallow(NSr) could be the result of continuous removal of crop leftovers, and primary and secondary tillage implements employed for ploughing which disturbed the soil aggregation and promoted susceptibility of the soil to erosion. Thus, CA-based practices like 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. Conservation tillage practices which retain crop debris tend to stabilize the soil pH conditions, and elevate SOC conducive for soil microbial composition contrary to the CT with continuous disposal of crop residues away.

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, soil basal respiration (SBR), metabolic quotient (qCO₂) and microbial quotient (qMB) were employed as to produce an extensive assessment of microbial activity. The results of this present investigation demonstrated that zero tillage (ZT) with retention of the crop residues had acquired a significantly greater microbial activity (Table 2a, b, c) probably due to ample additive-free materials drawn from the crops which can become a vital component for rapid metabolic reaction to external sources of carbon and slowed down the figure(s) of metabolic quotient (qCO₂), thereby facilitating microbiomes to utilize large quantities of additive-free substrates for proliferation in lieu of respiration utilization purpose. The increased qMB under ZT + R(C)-ZT + R(M)-ZT + R(Sr) apparently showed the efficiency of soil microbes on utilizing sources of carbon materials for their survival and growth as opposed to tillage practices with 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 requirement, that 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 increased as the crop advanced, particularly with the incorporation of non-chemical cultural weed control practices and the adoption of no-till practices along with 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 get enriched with specific microorganisms and nutrients necessary to perform the activities related to SOM cycling. Thus, this increase in SMBN, SMBC, SBR and qMB might also be the result of root proliferation, exudation and crop litter fall which serves as substrates

for the activity of microorganisms, and also favorable bio-physical climate 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, favored by the prevalence of soil microorganisms, likely contributed to this observed enhancement. Koné *et al.* [57] had also discovered that soil microorganisms which in turn may improve biological activity in the rhizosphere are higher plants with extensive rooting system and well-spread root hairs [58], and those characteristics are found to yield in the exudation of vast amounts of organic compounds and consequently promote a rise in SMBC and SMBN [59]. These results of this present investigation 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 of the crop (s) as the most suitable strategy for sustenance of 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] had also indicated that SMBN contents were superior under no-till and/or reduced tillage systems in comparison with conventionally tilled soil, which also agrees with the results of this study.

Conventional tillage disrupts the soil aggregates, expose the soil and make it more prone to erosion, and herbicides may stimulate or activate the soil microbial biomass. In this present experiment, intensive tillage and herbicides spraying have caused a drastic decrease on SMBC observed after pre-emergence, post emergence application of herbicides at 30 DAS of the crop and that could be due to more soil disturbance and herbicides inhibiting factor. Modak *et al.* [63] had also reported a reduction in SMBC after herbicides application owing to adverse effect of herbicides on microbial population, while in treatments without involvement of herbicides more SMBC and SMBN were recorded relative to the treatments with herbicides component. In like manner, Pertile *et al.* [64] observed a significant reduction in SMBC and SMBN following the application of flumioxazin, imazethapyr herbicides as compared to the control. The positive response of conservation tillage practices as compared to conventional tillage systems were probably due to higher levels of C substrates available for microorganism growth, as well as better soil physical conditions and higher water retention due to the altered land configurations and applied residues [65].

It is evident that soil disturbance as a result of intensive tillage had a significant impact on increasing the qCO_2 mean values. Likewise, herbicides applied as pre-emergence and post-emergence greatly increased the qCO_2 mean values in this study. The lower metabolic quotient (qCO_2) values observed under ZT which leaves the crop residues in the soil could be an indicator of less energy requirement of microorganisms. Similarly, Engell *et al.* [66] reported lower qCO_2 values. indicating a low demand of the microbial community for energy maintenance. This discovery is in accordance with the meta-analysis of Zuber and Villamil [67] under similar soil field conditions as in this present experiment, and had indicated that sandy clay loam soils have lower qCO_2 values under NT over CT, although the impact of tillage were found to be low in soils having very finer particles. Less values of qCO_2 is an indication of conducive conditions for predominance of microbial activity [66].

The results of this current investigation on qCO_2 are also supported by Jiang *et al.* [68], who noticed a significant rise in qCO_2 in the 0 – 20 cm soil depth under conventional tillage system. Similarly, in the study of Aziz *et al.* [69], the qCO_2 was up to 50% higher in a conventional tillage than in a no-tillage system. 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 could be interpreted as a positive priming on decomposition of the labile soil organic carbon pool, following addition of readily degradable carbon substrates to soil [16]. In this present investigation, higher qCO_2 is associated with low values of SMBC under conventionally tilled plots and herbicides treated plots, and likely to reflect stress and poor conditions related to physical soil disturbance.

Weed management involving herbicides were found to have increased qCO_2 indicating stress or disorder probably due to the detrimental effects of applied herbicides on soil microbial population. In congruence with the findings of this present study, Pertile *et al.* [64] observed increased metabolic quotient during the first 15 days of herbicides application, although

their study was conducted under soil incubation conditions attributed to indicate an initial negative effect of the herbicides on soil microorganisms. Since the application of chemical compounds in the soil needs an adaptation of soil microbial biomass that uses their reserves to degrade these compounds, C from microbial biomass becomes lost ultimately, thus, increasing the qCO_2 .

Rhizosphere Soil Enzyme Activity

Maintenance of the crop left-overs in zero tillage (ZT) plots, cultural weed control tactics and SOC preservation in conservation agriculture positively influenced the biomass production and activated soil microbes by modifying the provision of the substrate. Rhizosphere soil enzymes such as dehydrogenase (DHA), fluorescein di-acetate (FDA), β -galactosidase (β -GaA), alkaline phosphatase (AIA) and acid phosphatase (AcA) play an essential part in the breakdown of carbon in the soil [71] and urease (SUA) 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 (non-weeded control and IWM) are liable to encourage metabolic reactions necessary for the development of biotic microorganisms, which ultimately advanced the activity of urea hydrolysis and carbon (C) cycling, *i.e.*, greater succession in C and nitrogen (N) which can aid in 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 for soil microorganism's constituents [74]. Since rhizosphere soil enzymes are secreted by specific groups of microbes, the diversity of crops plays a key role on 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), non-weeded control and IWM treatments had acquired higher activity of rhizosphere enzymes. Diversification of crops (cotton-maize-*Sesbania rostrata*) in rotation resulted in a significant modification in the activity of enzymes probably due to greater variety of crops having greater distinct litter falls and rhizosphere exudation, thus, ZT + R(C)-ZT + R(M)-ZT + R(*Sr*), non-weeded control and IWM treatments under these diverse cropping systems have shown a huge influence on rhizosphere soil functional diversity. The addition of crop residues through crop residue retention and ZT resulted in a higher soil enzyme activity relative to intensive tillage with continuous crop residues removal. The activity of overall enzymes increased significantly with crop growth advancement possibly due to secretion of beneficial nutrients, decomposition of organic substrates and herbicides degradation.

It can be inferred that, a significant reduction on rhizosphere soil DHA, FDA, SUA, AIA, AcA, β -GaA was more pronounced where herbicides were applied for weed management. The reduction of these enzymes was in the order; chemical (herbicide) rotation followed by chemical weed control and IWM ($W_1 > W_2 > W_3$) observed at 30 DAS of maize. Higher rhizosphere soil enzyme activities observed with ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) relative to CT(C)-CT(M)-Fallow (*NSr*) or CT(C)-ZT(M)-ZT(*Sr*) at 30 DAS of maize could be associated with partial inhibition of pre-emergence 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 that of Priya *et al.* [75] who observed that herbicides significantly inhibited the soil DHA after application at 15 DAS although their study was conducted under soil incubation conditions. Modak *et al.* [63] had also recorded the maximum values of DHA under two treatments of weed management without involvement of herbicides *viz.*, weedy check and hoeing and weeding twice. Varsha *et al.* [76] findings on SUA are in support with the observations obtained in this present study in which SUA was noticed with a decreasing trend following herbicide application, and the activity regained normalcy with the increase in 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] had also revealed a reduction in acid (AcA) and alkaline (AIA) phosphatase activity in all herbicide treatment plots on 15 days after herbicide application, but on 45 days after herbicide application, an increase AcA and AIA was observed. This might be due to the change in species composition

of soil micro-organisms and variation in the availability of organic substrate. Madsen *et al.* [78] results also concur with these of present investigation in which the highest FDA was recorded in system where the soil was covered with winter crop residues due to added organic matter (OM) and lowest FDA occurred in conventional systems (the system without no crop cover and treated with herbicides) due to very low input of OM and usage of herbicides and pesticides. Shahid *et al.* [79] also reported adverse effects of herbicides application on inhibiting the secretion of β -GaA. Thus, it may be deduced that from this study, herbicide treatments resulted in a significant decline on rhizosphere soil enzyme activities in comparison with herbicides untreated plots (non-weeded control plots) of soil samples.

Water plays an essential and complex role in the activity of enzymes. Assessed enzymes activity involved in SOM cycling and Urea hydrolysis in this study increases with increasing soil water content up to near field capacity, followed by a decreasing trend thereafter [80]. In the case of our experiment, soil water content was maintained at field capacity level through supplemental irrigation, thus, enzyme activity followed increasing trend irrespective of the treatments. Savant *et al.* [80] also observed a higher rate of urea hydrolysis in soil at field capacity than in wetted soils after 24h of incubation. It has been indicated in several studies that soil DHA is significantly influenced by water content, and declined with the decrease in soil humidity [81]. In connection to that, DHA reached higher values at lower soil water potential [81], so in the case of winter season during maize there was low rainfall amount, thus, lower oxygen diffusion rate and redox potential conditions, however, field moisture level was maintained with supplemental irrigation and relative humidity was also increasing which could be the reasons for higher DHA noticed in this study irrespective of the treatments. Wang *et al.* [82] observed a reduction in SUA due to flooding as a result of high amount of rainfall and this was probably due to an increase in metal ions under reduced conditions, which decreased SUA. So, during the sampling periods in winter, there was an increase in humidity and no flooding conditions due to scanty rainfall, therefore irrigation was provided for the development of the crop and also for retention of the field moisture content, and that might be the reason for obtaining higher SUA irrespective of the treatments.

Microbial population

Soil organic matter (SOM) is a crucial driver for microbial population and diversity, and can affect the microbial counts [83–84]. Rhizosphere soil *Azospirillum* (*Azosp*), *Azotobacter* (*Azot*), and total fungal (TF) population was higher where crop residues were retained probably due to more addition of SOM 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). Less disturbance of soil favors formation and stabilization of macroaggregates to improve and protect habitat for the microbial population. Zero-till (ZT) increases soil aggregation by reducing soil disturbance and increasing SOM, and possibly the growth of microbes that bind soil particle and micro-aggregates together [85]. Nitrogen-free bacterial fixers require aerobic environments to obtain their resources for N_2 -fixation [86]. Several workers have also reported that the change from conventional to zero tillage only alters the distribution of SOM along the soil profile [87–88]. The observations recorded in this experiment on rhizosphere soil microbial and rhizoplane fungal population also indicated that the counts increased significantly with the crop advancement ascribed to the progressive mineralization of the litter fall from the crops, root exudates and added crop residues and enhanced availability of organic substrates which served as food and helped in increasing the population of nitrogen-fixing microorganisms [89]. The study by Singh *et al.* [90] also reported the stimulation of *Azotobacter spp* in upper soil layers under minimum tillage attributed to increased availability of nutrients and root proliferation. Our results concur with that of Verma *et al.* [83] who observed an increased trend in the preponderance of *Azospirillum* with an increase in organic carbon level from 0.2- 1.0%. The findings of this study also collaborate with Bashan *et al.* [91] who found that SOM had a definite role in the survival of *Azospirillum* strains.

Azospirillum shows a trend to move toward locations where the presence of O_2 is ideal for its metabolism (low O_2 concentration) [92]. So, an increase in the *Azospirillum* population with an increase in moisture content (maintained through supplemental irrigation prior collection of rhizosphere soil samples) was noticed in the current experiment. The same

results were reported by Belaid *et al.* [93] who observed that when moisture content was increased, there was an increase in the *Azospirillum* population along with time.

Rhizoplane total fungi inhabit between the root system of the crops and connected directly with the plant metabolism. Higher counts of Rhizoplane total fungi were also obtained in ZT which incorporate the residues probably be due to the release of organic exudates and more plant nutrient absorption and exchange with the root system of the crops. Tillage operations incorporate crop residues, prepare the seed- bed, alleviate compaction, improve nutrient mineralization, 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 microbe [96]. Odunfa [97] noted that some fungi need specific nutrient substances for growth and hence are host specific. Oyeyiola [98] had also pointed out several fungal microflora populations in the rhizoplane, but in Okro (*Abelmoschus esculentus*) crop.

It was observed that the application of herbicides at pre-emergence reduced the population of rhizosphere soil microbes drastically compared to post-emergence application at 30 DAS which could probably be due to the herbicide's direct application into the soil rhizosphere, while the population decrease after post-emergence application of herbicides was not that much due to leaf foliage and emerged weeds interception, the herbicides applied might have not reached fully reached the soil. The population of rhizosphere soil microbes was higher in 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 of less harmful effect of post emergence herbicides on microorganisms may be more foliage of crops at 22 DAS (at the time of herbicides application) which covers the soil surface resulting in less dropping and absorption of applied herbicides in the soil. On the contrary, the deposition of pre-emergence herbicides in the soil are relatively higher attributed to the exposed bare soil surface at the time of pre-emergence herbicide spraying. Further, they had noted that the effects of herbicides on the soil microflora are normally most severe immediately after their application.

The application of post-emergence herbicides (fenoxaprop- p-ethyl and ethoxy sulfuron) which were sprayed at 20 days after crop emergence did not suppress the growth of micro-organisms such as nitrogen-free bacteria, which was visualized by their respective population at 20, 30 and at 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 their findings are in support of this present investigation. Barman *et al.* [100] also reported that the *Azotobacter* count decreased under herbicide treatments compared to control, and could not regain its population indicating higher susceptibility to the herbicide. Similar inhibitory effect of other pre-emergence herbicides *viz.*, alachlor and atrazine on *Azotobacter* count was reported by Konstantinovic *et al.* [101]. The results of this experiment also indicated that various herbicides evinced high potency on inhibiting growth of fungi. The inhibition of mycelial growth of fungi by herbicide application was consistent with previous studies [102–103]. Similarly, Eze [104] had found that Glyphosate, Paraquat, Atrazine and Linuron were more effective on inhibiting the mycelial growth of all the rhizoplane fungi screened than 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 the plant systems play an essential role 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, crop rotation, etc., influence the physical and chemical properties of the soil where microorganisms like fungi inhabit, thus, affecting their abundance, diversity, and activity [24]. The adoption of zero tillage (ZT) and conservation tillage (ZT + crop residue retention) with integration of chemical weed control and power + 1 hand weeding (IWM) as soil

management practices tend to harbor beneficial fungal species while improving and maintaining soil health and quality in a long run. *Talaromyces flavus* var. *flavus* having been identified under CT(C)-ZT(M)-ZT(*Sr*) and ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) in combination with integration of chemical weed control and power + 1 hand weeding (IWM) (T_2W_3 and T_3W_3) in the rhizosphere soil has been newly reported as beneficial fungal species inhabitant in the soil (soil stabilizer) and plant growth promoting fungi (PGPF) with high potential to inhibit other pathogenic fungal species (bio-control agent) while benefiting the plant and the soil [106–110]. However, CT(C)-CT(M)-Fallow(NSr), 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 were found to have contained general pathogenic fungal species (*Aspergillus niger*, *Penicillium limosum*, *Aspergillus terreus*, *Apiospora serenensis*, *Zasmidium cellare*, *Ochraceocephala foeniculi*), which were earlier reported to have adverse effects on soil health and productivity.

It is evident that conventionally tilled plots with crop residues removal in combination with non-weeded control and all herbicides treat plots, and the application of the herbicides, no-weed control regardless of any tillage combination, changed and produced pathogenic fungal microbes which agrees with the results of Bhardwaj *et al.* [111] on studying the impact of herbicides in irrigated tropical rice field, in which predominance of pathogenic fungi (*Humicola*, *Nigrospora*, *Paramyrothecium*, *Mariannaea*, *Ceratobasidium*, *Funneliformis*, *Aspergillus*, *Pseudorhizophila*, and *Lecytophthora*) were identified with unweeded control and herbicides treated plots, indicating adverse effects of herbicides and high weed density population on microbial dynamics.

These results obtained on fungal diversity signifies the importance of conservation tillage and minimum tillage coupled with IWM under conservation agricultural practice as compared to conventional tillage in combination with herbicides and hand weed removal only at critical period of weed competition.

Crop productivity

Maize Grain Yield and System Productivity (Cotton Equivalent Yield)

The better growth/development of crops and increased yield rely to a large extent on the tillage practices, as these play a crucial role in determining the development of the crop's rooting system, the soil volume explored by the roots for moisture and nutrients, the availability of air, and the regulation of soil temperature, among other factors. The importance of crop-weed interaction in determining the competition faced by the crop plants for the light, moisture and space is well-established. Confined root growth lead 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 CT, is inconsistent and impacted substantially by cropping systems followed by aridity index, crop residue maintenance, ZT duration, and weed management strategies [113]. In this present investigation, maize grain, and harvest index demonstrated higher values when subjected to the ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) treatment in comparison to other tillage methods. This superior performance can be interconnected to the development of robust, deep-rooted systems in the crops facilitated by the practice of zero tillage.

The implementation of ZT is thought to augment the nutrient absorption capacity of the 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 proves especially crucial during the post-tasselling stage of the maize crop, which coincided with a hot 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 the crop development. The research outcomes by You *et al.* [114] also indicated that short-term reduced tillage (rotary-till and no-till) and residue incorporation enhanced soil properties and 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 as to how yield can be improved.

Long-term conventional tillage always hinders root growth and root to shoot ratio [117]. No-till enhance root biomass, shoot biomass, regulate shoot to root ratio and increase yield in comparison with plow-till and rotary-till [118–119]. Residue incorporation can also enhance crop biomass and yield due to enhanced soil buffer capacity [120–121]. The post-emergence tank-mix combination of atrazine and tembotrione herbicide was applied at recommended rates in both chemical weed control and chemical (herbicide) rotation which resulted in effective weed control and no phyto-toxicity. The absence of phytotoxic effects suggests the efficacy and safety of the tembotrione and atrazine combination in weed management, contributing to better crop performance. Poor crop performance was also observed under non-weeded control which ultimately reflected in yield. This could be due to high weed density at critical crop growth stage which out competed with the crop for available moisture, nutrient, light and rooting space. Ganapathi *et al.* [122] also recorded higher kernel, harvest index and least weed dry weight with IWM compared to 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 observed that when pre-emergence herbicide was applied 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 this present investigation, who noticed that Nicosulfuron application and one hand weeding with a hoe at 15 DAS led to greater kernel yield, whereas the least kernel yield was obtained from non-weeded control. In the current study, there was an increase in corn yield and system CEY when employing a 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 both chemical and cultural mechanical control tactics, along with the moisture and nutrient preservation facilitated by no-till practices that retained crop residues. These results are supported by Ahmad *et al.* [124] who had deduced that maize can flourish when cultivated in zero tillage either with application of atrazine, glyphosate or with hand weeding (HW) at 40 DAS alternative to manual weeding in spring seasons to attain higher grain yield.

Identification of treatment performance on the basis of system cotton equivalent yield (CEY) and maize grain yield along-with microbial population and fungal diversity, microbial and enzyme activities.

The winter maize grain yield recorded from different tillage weed management treatment combinations was converted into cotton equivalent yield (CEY) considering the monitory equivalence. The winter CEY was then added to the monsoon cotton yield in the 4-year 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 remunerative tillage, weed management and tillage-weed management combination with relatively higher system (CEY), maize grain yield, microbial and enzyme activities, microbial population and fungal diversity. This data is presented in figure (s) 3a, b, c, 4a, b, c, 7a-h and Table 7a, b, 8 and 9.

Tillage and weed management exerted a significant effect on 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 non-weeded control (T_3W_4) recorded higher microbial and enzyme activities, diverse group of pathogenic fungal species, microbial population, except metabolic quotient which was lower, but crop productivity of this treatment combination was significantly lower compared to all other treatment combinations. Conventional tillage (CT) in combination with all weed management choices recorded lower microbial and enzyme activities, diverse group of pathogenic fungal species, microbial population except metabolic quotient which was higher under this treatment combinations. However, the crop productivity in CT along-with all weed management options was higher compared to ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) in combination with non-weeded control, which indicate higher productivity but poor soil health indicated by soil biological attributes. The SY in terms of CEY was recorded higher under ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) in combination with IWM which indicated that adoption ZT + R(C)-ZT + R(M)-ZT + R(*Sr*) with IWM practices can maintain the status of soil microbiological attributes at higher level, harbour beneficial fungal species and increase the farmers productivity. So, implementing zero tillage with retention of crop residues in CA together with IWM aids on improving the soil health and can optimise productivity to the farmer in cotton-maize-*Sesbania rostrata* cropping system.

Conclusion

On the basis of four years' investigation on cumulative effect 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 non-weeded control (only one hand weeding at critical period of weed competition) and integration of chemical weed control and power + 1 hand weeding (IWM) significantly enhanced soil enzymatic and microbial activities, microbial population and decreased metabolic quotient (qCO_2), whereas conventionally tilled (CT) and chemical treated plots resulted in a drastic reduction of soil enzymatic and microbial activities, microbial population and increased qCO_2 at both sampling period (30 DAS and tasselling) during maize growth. The ZT with and without crop residues incorporation in combination with IWM harboured beneficial soil inhabitant fungal species; *Talaromyces flavus* (soil stabilizer, plant growth promoter, soil pathogenic fungal inhibitor), while CT on interaction with overall weed management led to production of pathogenic fungal species identified at tasselling stage of maize. Maize grain yield and system yield in terms of cotton equivalent yield (CEY) were higher under ZT with retention of crop left-overs and, IWM, chemical weed control and chemical (herbicides) rotation plots relative to CT with crop residues removal and non-weeded control treatments. Based on treatment combination effects maize grain yield, there was no significant effect ($P = 0.05$). ZT with crop residues maintenance (ZT + R(C)-ZT + R(M)-ZT + R(Sr)) on interaction with IWM recorded significantly higher system CEY (4453 kg ha^{-1}) followed by ZT + R in combination with chemical weed control and chemical (herbicide) rotation with system CEY of 4292 kg ha^{-1} and 4206 kg ha^{-1} , respectively. Among tillage practices, ZT + R(C)-ZT + R(M)-ZT + R(Sr) recorded higher system CEY over 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 higher under ZT + R(C)-ZT + R(M)-ZT + R(Sr) in combination with IWM which indicated that adoption of conservation tillage with IWM practices augment important soil microbiological attributes, harbour beneficial fungal species and give good productivity to the farmer in a long-run. Even though ZT + R(C)-ZT + R(M)-ZT + R(Sr) on interaction with non-weeded control had shown a positive response on increasing soil microbiological parameters and activities, crop productivity was very low, so, adoption of ZT + R(C)-ZT + R(M)-ZT + R(Sr) in CA along with IWM helps in improving the soil health and can optimise productivity to the farmer in a long-run in 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 line)

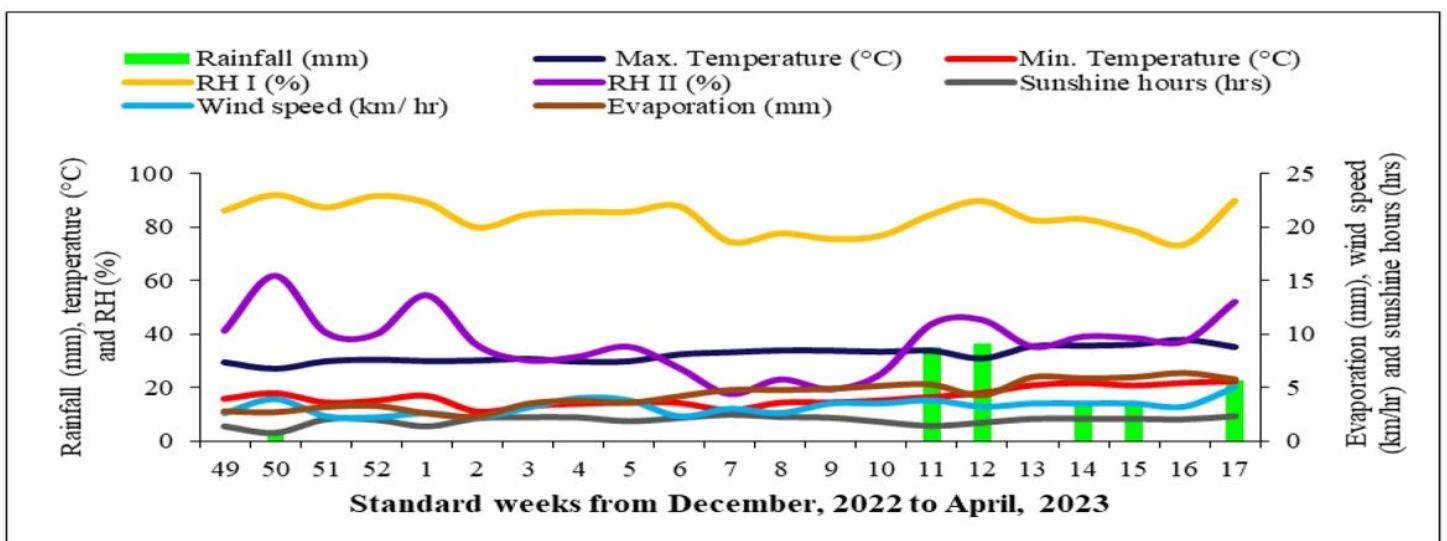


Figure 2

Weekly-base mean meteorological observations during maize development

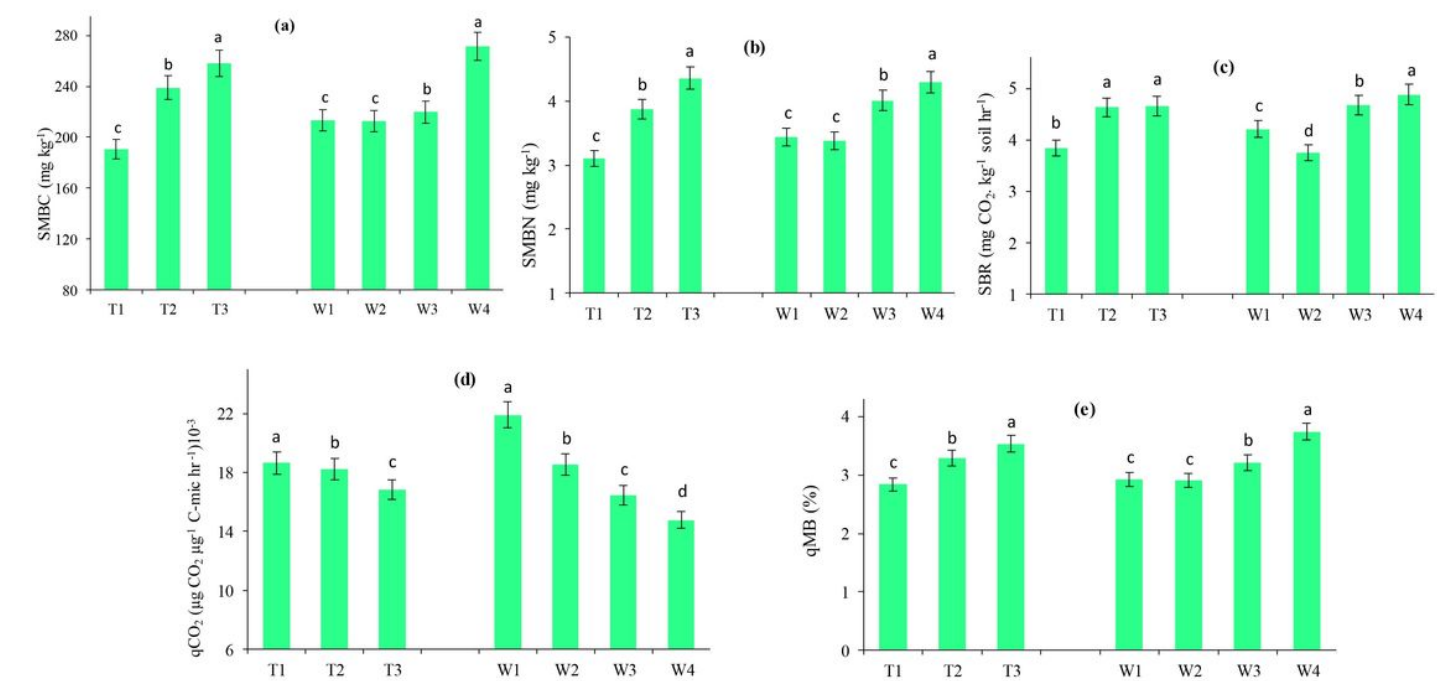


Figure 3

Impact of tillage practices and weed management options of 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), microbial quotient-qMB (e) at 30 days after sowing (DAS) of maize crop (8th crop cycle). Means having distinct symbols demonstrate significant variances between the treatments at 5% probability level (Tukey's test) and means having the same symbols indicate no significant variances among the treatment means at 5% probability level. Refer to table 1 and 2 for treatment details.

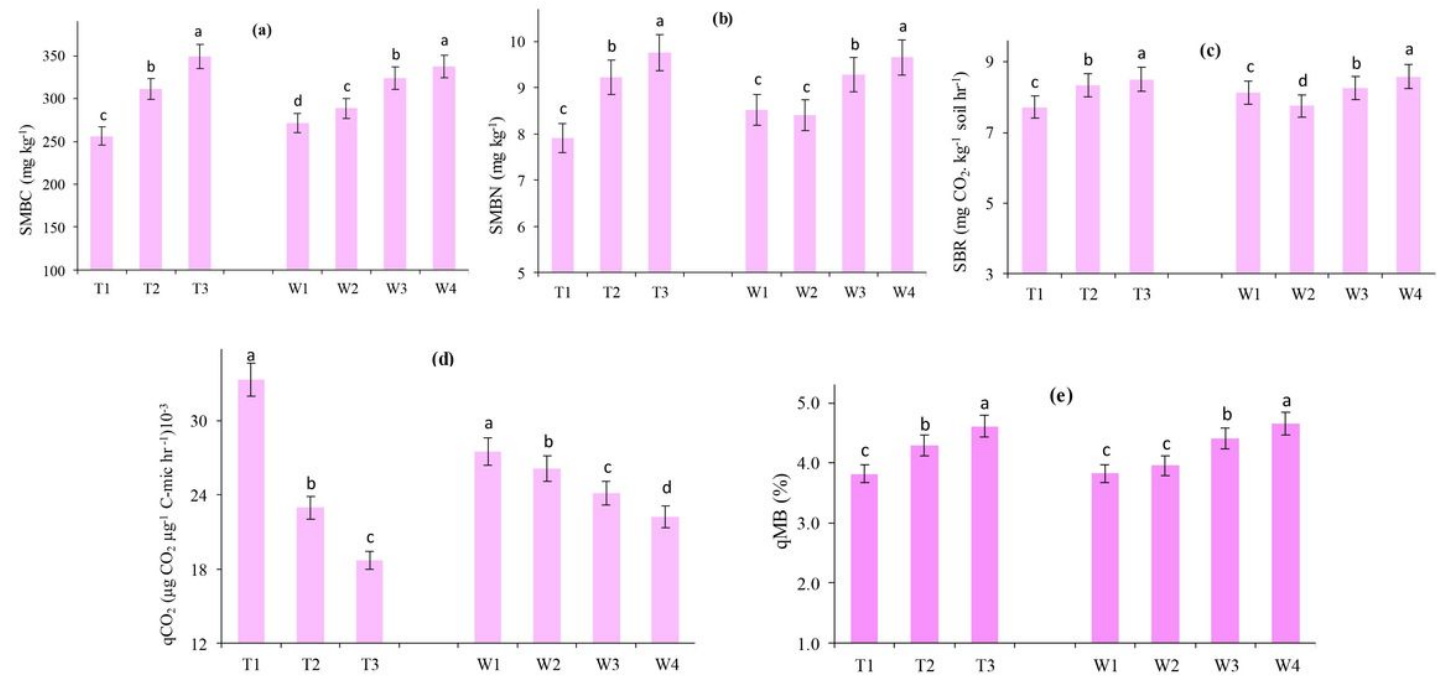


Figure 4

Impact of tillage practices and weed management options of 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), microbial quotient-qMB (e) at 30 tasselling stage of maize crop (8th crop cycle). Means having distinct symbols demonstrate significant variances between the treatments at 5% probability level (Tukey's test) and means having the same symbols indicate no significant variances among the treatment means at 5% probability level. Refer to table 1 and 2 for treatment details.

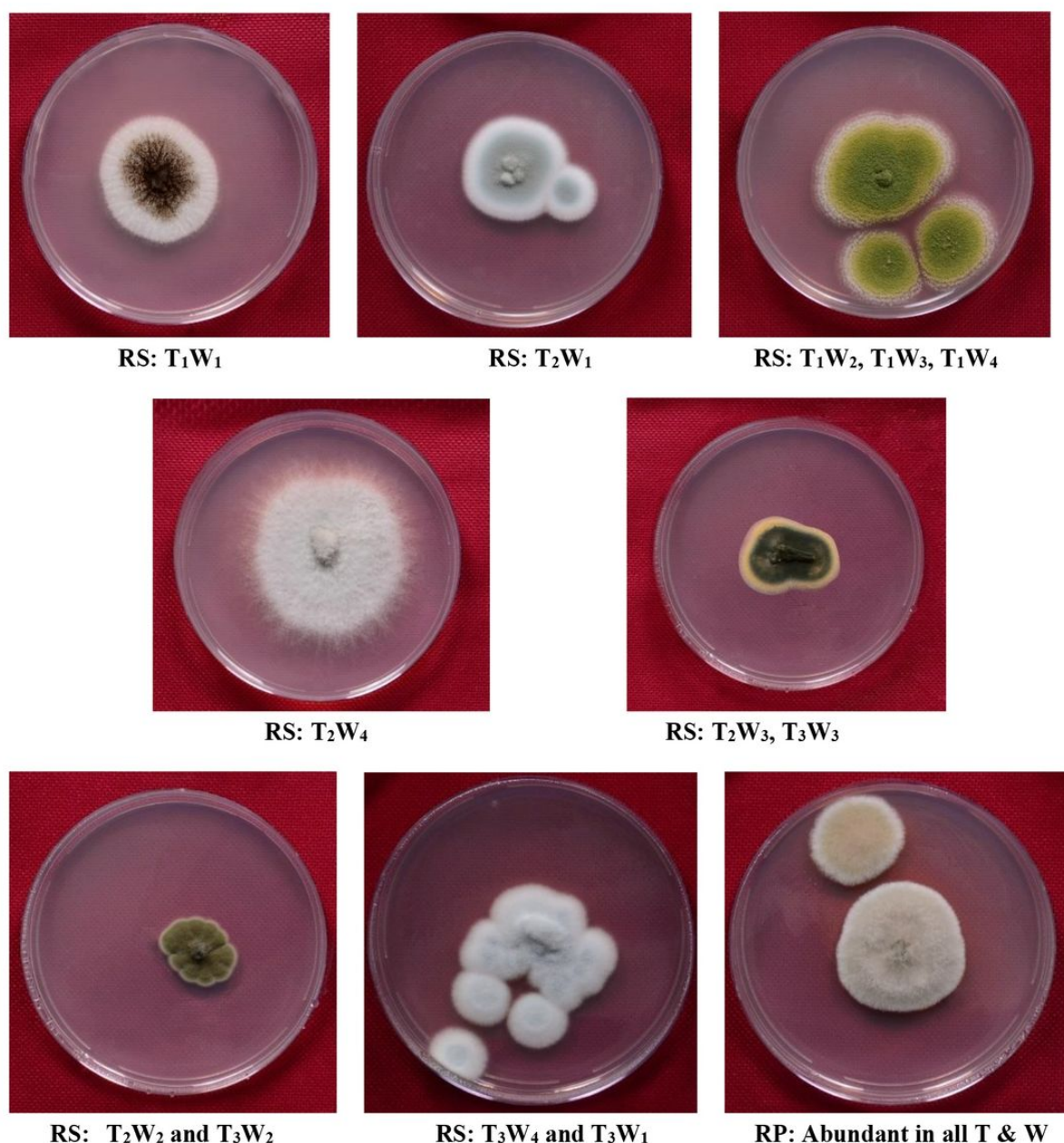


Figure 5

Treatment's representation of various cultured rhizosphere soil and rhizoplane fungal species grown in PDA from the colonies enumerated at 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 rostrate*; W₂ = Chemical (herbicide) rotation; W₃ = Integration of chemical weed control and power + 1 hand weeding (IWM); W₄ = Non-weeded Control. T= Tillage; TW=tillage and weed management treatment interaction.

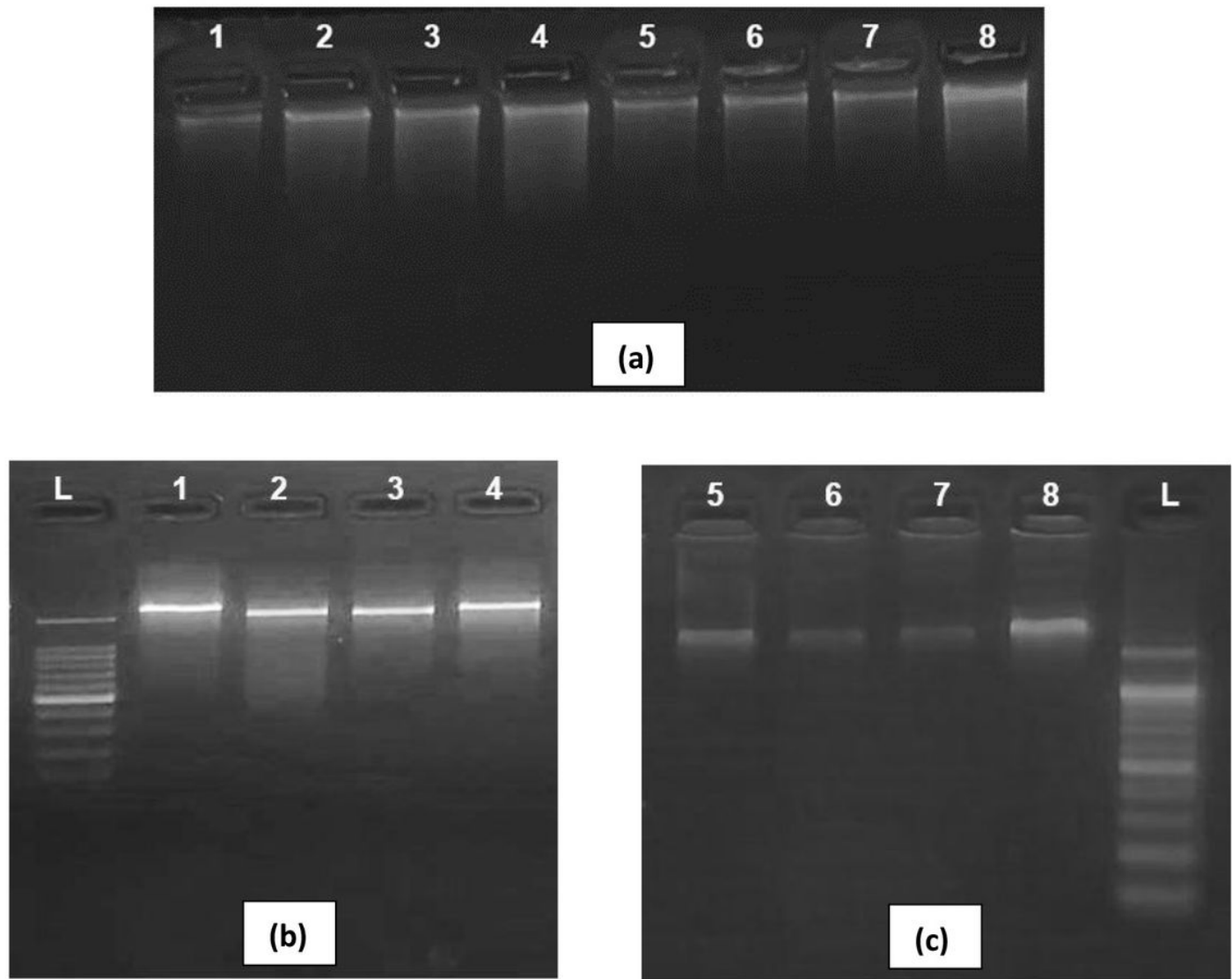


Figure 6

Agarose gel electrophoresis of genomic DNA extracted from isolated fungi

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

Supplementary Files

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