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Conservation agriculture practices impact on biological and microbial diversity in earthworm cast under maize-wheat system

Padma Angmo [a], Sandeep Sharma [a,*], H.S. Sidhu [b], K.S. Saini [c]

[a] Department of Soil Science, Punjab Agricultural University, Ludhiana, 141001, India
[b] Borlaug Institute for South Asia, Ladhowal, Ludhiana, Punjab, India, 141001
[c] Department of Agronomy, Punjab Agricultural University Ludhiana, 141001, India

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ABSTRACT

Soil degradation is a major global concern due to its negative impact on soil quality and the sustainability of agricultural resources. The conservation agriculture (CA) approach, which includes three key principles such as zero tillage, retention of crop residue and crop rotation has gained widespread adoption to help mitigate the climate change effects on agricultural soils and meet the growing demand for increased production. Earthworm communities, along with microbial activity and diversity, are highly sensitive to tillage practices. Additionally, microbial activity and diversity quickly respond to different cropping systems, making them effective indicators for detecting short-term changes in soil functioning. We therefore, assess the effects of CA innovative approached after 6-years on biological and microbial diversity within earthworm cast in maize-wheat system (MWS). The treatments consist of PBM-RN_0/ZTW-RN_0 (permanent beds No-N control-both residues removed and wheat with zero tillage); PBM+RN_0/ZTW+RN_0 (permanent beds No-N control-both residues retained)-50% of maize stover and 25% of wheat residue retained; PBM-RN_{120}/ZTW- RN_{120} (permanent beds with 120 kg N ha^{-1} both residues removed wheat with zero tillage); PBM+RN_{120}/ZTW+RN_{120} (permanent beds with 120 kg N ha^{-1} both residues retained and wheat with zero tillage) and FBM-RN_{120}/CTW-RN_{120} (fresh beds in maize/CT in wheat with 120 kg N ha^{-1} both residues removed). The result of present study showed that activities of carbon (C) cycle-related enzymes in the cast soils *viz.*, dehydrogenase (DHA), β-glucosidase (β-glu), cellulase, and xylanase were significantly higher under PBM+RN_{120}/ ZTW+RN_{120} than under PBM-RN_0/ZTW-RN_0. Specifically, the activities of these enzymes were 21.5, 26.8, and 76.5% higher under the PBM+RN_{120}/ZTW+RN_{120} treatment, respectively. Moreover, the Alk-P activity was found to be 1.3 times higher in the PBM+RN_{120}/ZTW+RN_{120} treatment than in the PBM-RN_0/ZTW-RN_0 treatment. The bacterial, fungal, and actinomycete counts in the cast soil ranged from 6.87 to 7.47 CFU (colony forming units) x 10^6 g^{-1} soil, 3.87–3.30 CFU x 10^4 g^{-1} soil, and 5.09–5.67 CFU x 10^4 g^{-1} soil, respectively. Total organic carbon (TOC) showed significant increases of 34.6% under PBM+RN_{120}/ ZTW+RN_{120} as compared to PBM-RN_0/ZTW-RN_0. The less labile C (Frac. 3), total carbohydrate carbon (TCHO), phenol oxidase (PHE) and peroxidase (PER) were observed as the sensitive indicators under different tillage, rate of nitrogen and residue management practices. This study suggests that permanent beds with crop residue retention with balance fertilization practices can be recommended and popularized to the overall improvement of soil biological pools within earthworm casts in MWS.

1. Introduction

Sustainably feeding the ever-increasing population with limited resources has become the greatest challenge faced by scientists, policymakers, and farmers. Intensive agricultural practices are upsetting the environmental functioning and deteriorating soil quality due to open field residues burning (NAAS, 2017; Srinivasa Rao et al., 2019; Sharma et al., 2021). In India, about 686 million tonnes (MT) of crop residues are produced every year (Hiloidhari et al., 2014), which can provide soil C and nutrients for crops (Bhuvaneshwari et al., 2019). In north-western India, open field burning of rice straw prefer by famers for timely wheat sowing, which depletion of natural resources, low nutrients productivity and biodiversity (Singh and Sharma, 2020; Sharma et al., 2022). To address this problem, several conservation technologies are

* Corresponding author.
E-mail address: sandyagro@pau.edu (S. Sharma).

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gaining popularity, these methods include zero-tillage, using laser to level the land, planting in beds, surface seeding, and employing automated machines to transplant rice (Singh et al., 2011; Sharma et al., 2023).

Conservation agriculture (CA) - based crop management practices improved soil physical properties, soil fertility (Gattinger et al., 2012; Sharma et al., 2022) and soil biological activity (Bera et al., 2017; Saikia et al., 2019) and therefore, sustaining the productivity (Yadvinder-Singh and Sidhu, 2014; Thind et al., 2023). The adoption of CA-based practices has led to favourable changes in soil organic carbon (SOC) and biological properties under diverse agroecological conditions (Chivenge et al., 2007; Das et al., 2013; Choudhary et al., 2018; Sharma et al., 2022). Maize can be a feasible alternative to rice in the rice-wheat system (RWS) and a potential driver for crop diversification. Maize (*Zea mays* L.) is the third most significant cereal in the world and is grown in 155 countries (Jat et al., 2019). In India, the MWS occupies around 2.9 million hectares (ha) in the Indo-Gangetic Plains (IGPs), heartland of the RWS (Jat et al., 2009). The use of 300–400 kg ha^{-1} N fertilizer to enhanced wheat and maize crops yield has resulted in a depletion of natural resources (Cui et al., 2008; Ju et al., 2009). Jat et al. (2019) revealed significantly higher system productivity under long-term CA-experiment MWS than conventional tilled (CT) systems in eastern IGPs. Crop residues management with nutrient management interventions, plays a critical role in sustaining soil health and improving SOC to the overall functioning of soil and supports plant growth (Meena et al., 2018; Wang et al., 2020a,b; Salahin et al., 2021). Practicing ZT in flat and PB in conjunction with recommended fertilization has consistently demonstrated advantages in subtropical Indian soils across various wheat-based agricultural systems (Jat et al., 2013, 2019; Parihar et al., 2019). Additionally, this approach has been shown to improved soil biota, particularly earthworm activity (Choudhary et al., 2018; Sharma and Dhaliwal, 2021).

In soil biota, earthworms hold significant importance as crucial biotic elements within the soil ecosystem (Roger-Estrade et al., 2010; Van Capelle et al., 2012). They play a vital role in the decomposition of residues, enhancing its accessibility for microorganisms and promoting nutrient turnover in CA-based cropping systems, as highlighted by Sapkota et al. (2012). Earthworm communities are recognized as essential ecosystem engineers (Blouin et al., 2013) and serve as valuable bioindicators for assessing soil quality (Peres et al., 2011). These organisms contribute positively to various ecosystem services, including pedogenesis, soil structure, water retention, nutrients cycling, climate regulation and pollution remediation (Blouin et al., 2013; Sharma and Dhaliwal, 2021). Earthworms, as heterotrophic organisms, expedite the decomposition of organic matter by augmenting the accessible surface area of such material (Seeber et al., 2008). Its influence in the soil primarily involves regulating C inputs by boosting rate of decomposition, enhancing biological activities in the drilosphere and casts, and safeguarding C within stable aggregates through different mechanisms (Bhadauria and Saxena, 2010; Roger-Estrade et al., 2010; Van Capelle et al., 2012). CT disrupts tunnels created by earthworm, eliminates the protective layer of plant debris, alters the availability of organic matter by burying leftover crop materials (Briones and Bol, 2003), and modifies soil conditions (Rosas-Medina et al., 2010). Soil biota are impacted by the CA-based cropping system through induction of habitat changes (Van Capelle et al., 2012), residues decomposition (Hendrix et al., 1992), fluctuations in moisture and temperature (Curry, 2004), and mechanical harm (Lee, 1985).

Earthworm casts are hotspots for microbial activity, housing diverse microbial communities that are vital for organic matter decomposition and nutrient cycling (Aira et al., 2009). This makes earthworms essential for studying microbial interactions and processes in CA systems. Additionally, casts contain higher concentrations of nitrogen, phosphorus, and other essential nutrients that are readily available to plants (Edwards and Bohlen, 1996), making them crucial for assessing nutrient dynamics in CA systems (Sharma and Dhaliwal, 2021). Soil microorganisms are central to the regulation of the transformation of nutrients, organic residues decomposition, and improvement in soil structure and fertility (Hartmann et al., 2009; Wang et al., 2021a,b). Soil enzymes are considered an important role in agroecosystems, regulating the soil biogeochemical cycles and released of important soil nutrients (e.g., C, N, P) (Mencel et al., 2022). Soil enzymes participate in the decomposition of organic matter, releasing or binding the trace nutrients and maintaining soil fertility for optimum plant growth (Evon et al., 2021; Sharma et al., 2024a). The decomposition of organic matter, nutrients cycling, soil enzymes and soil biota are all enhanced by CA-based practices (Castellano-Hinojosa and Strauss, 2020; Niewiadomska et al., 2020; Sharma et al., 2024b). The specific objective of the study to identified specify the key soil biological indicators of soil quality from earthworm cast samples under different CA and CT practices in MWS. We hypothesized that soil biological quality indicators in earthworm cast would probably be influenced by various CA and CT based method, rate of nitrogen and residue management practices. The soil quality indicators obtained can be valuable for evaluating the biological quality of soils in various agro-ecological regions and cropping systems.

2. Material and methods

2.1. Brief description of experimental site

The experiment was conducted at the research farm, Borlaug Institute for South Asia Ladhowal, Ludhiana located in Indian Punjab (30°59′ latitude and 75°40′ longitude) at an elevation of 229 m above mean sea level. The climate is sub-tropical and semi-arid, with hot and dry summers with 680 mm average annual rainfall.

2.2. Experimental design and treatments

The field experiment on tillage, nitrogen application, and residue management in MWS was laid out in a randomized block design with three replications. The experimental design comprised the following: T1: PBM-RN_0/ZTW-RN_0-permanent beds No-N control-both residues removed and wheat with zero tillage; T2: PBM+RN_0/ZTW+RN_0 permanent beds No-N control-both residues retained)−50% of maize stover and 25% of wheat residue retained; T3: PBM-RN_{120}/ZTW-RN_{120}-permanent beds with 120 kg N ha^{-1} both residues removed wheat with zero tillage; T4: PBM+RN_{120}/ZTW+RN_{120}-permanent beds with 120 kg N ha^{-1} both residues retained and wheat with zero tillage and T5: FBM-RN_{120}/CTW-RN_{120} -Fresh beds in maize/CT in wheat with 120 kg N ha^{-1} both residues removed.

2.3. Tillage, rate of nitrogen application and management practices in maize and wheat

2.3.1. Maize

2.3.1.1. Fertilizer management. The whole of phosphorus (26 kg P ha^{-1}) and potassium (50 kg K ha^{-1}) required were applied at planting using di-ammonium phosphate, single super phosphate in no N control, and potash muriate, respectively. The total N applied was 150 kg ha^{-1}, with 24 kg N ha^{-1} being di-ammonium phosphate, and the remaining N (126 kg N ha^{-1}) being applied as urea. In FBM-RN_{120}/CTW-RN_{120} urea N was applied in two splits i.e., 21–25 and 40–45 days after sowing (DAS). Applying N as urea in five equal split doses at 10-day intervals beginning at 20 DAS using subsurface drip irrigation (SDI). To control broad-leaf weeds, the herbicide Atrazine at rates of 1.25 kg ha^{-1}as Atrataf 50WP was applied to every treatment within two days of sowing. Likewise, Deltamethrin at the rate 200 ml ha^{-1} as Decis was applied to every treatment to control the pest management (Anonymous 2022).

2.3.1.2. Irrigation water management. Fresh bed plots of FBM-RN_{120}

were irrigated with approximately 75 mm of water before tillage. Seedbed preparation for both maize and wheat required a specific sequence of operations, including discing, cultivating, and planking. These steps were carefully timed to ensure optimal moisture levels in the field. Maize hybrid P3396 was sown in the third week of July with a seed rate of 20 kg ha^{-1}. Wheat varieties HD 2957 was sown in second week of November with seed rate of 45 kg $acre^{-1}$.

2.3.2. Wheat

2.3.2.1. Fertilizer management. Similar to maize, whole of phosphorus and potassium were applied at the time of sowing. However, the total N applied was 120 kg ha^{-1}, with 24 kg N ha^{-1} applied as di-ammonium phosphate (DAP) and the remaining N (96 kg N ha^{-1}) applied as urea. Fertilizer N was applied in two equal splits at crown root initiation (21–25 DAS) and the maximum tillering stage (40–45 DAS) prior to irrigation in FBM-RN_{120}/CTW-RN_{120}. N was applied under SDI in five equal splits, each at a 15-day interval, beginning 21 days after sowing. The post-emergence herbicides Topik (clodinafop 15% WP) at a rate of 400 g ha^{-1} and Algrip (metsulfuron) at a rate of (@) 25 g ha^{-1} were applied between 25 and 30 DAS. was performed using Precautionary sprays of propiconazole and dimethoate 30% EC at a rate of 500 ml ha^{-1} were used for pest management in all treatments (Anonymous 2023).

2.3.2.2. Irrigation water management. After the harvest of maize, a pre-irrigation of 75 mm was provided to the conventional till plot CTW-RN_{120} before the preparation of the seedbed for wheat. Subsequently, the fresh maize beds were removed, and a conventional flat seedbed for wheat was established through two rounds of discing, tilling, and planking.

2.4. Earthworm cast sampling and method for enzymes study

Samples of earthworm cast were collected from the surface following the maize harvest in each plot. The chosen field revealed the existence of two earthworm species within the Megascolecidae family, namely *Lampito mauritii* and *Metaphire posthuma*. Samples from the earthworm cast in each treatment plot were collected and subsequently sifted through a 2 mm sieve after slight moistening. These samples were then preserved at −4 °C in a deep freezer for various biological analyzes (Table 1). Dehydrogenase (DHA) activity was determined by measuring the release of triphenyl formazan (TPF) through the reduction of 2,3,5-triphenyl tetrazolium chloride (TTC) (Casida 1964). Fluorescein diacetate activity (FDA) was assessed using the FDA hydrolysis assay (Adam and Duncan, 2001). β-Glucosidase (β-glu) activity was estimated using the p-nitrophenyl method as detailed by Tabatabai and Bremmer (1969). Alkaline phosphatase activity was evaluated following the method outlined by Tabatabai and Bremmer (1969). Phenol and peroxidase activities were measured according to the procedures described by Shi et al. (2006). Total polysaccharides carbon (TPC) and total carbohydrate carbon (TCHO) were determined using standardized procedures (Lowe, 1993; Chebhire and Mundie 1966). Total and easily extractable glomalin were determined using 50 mM and 20 mM sodium citrate as extractants, following the method described by Wright and Upadhyaya (1998).

Table 1
The method used for the analysis of different cast biochemical properties.

Biochemical properties	Brief description of method used	Reference (s)
Dehydrogenase activity	Triphenyl formazan (TPF) is produced by the reduction of 2,3,5 tetrazolium chloride (TTC)	Casida (1964)
Fluorescein diacetate activity	FDA hydrolysis assay, which hydrolyzes colorless FDA to release a colored end product fluorescein	Adam & Duncan (2001)
Alkaline phosphate activity	p-nitrophenyl method	Tabatabai & Bremner (1969)
β-glucosidase activity (β-GLU)	p-nitrophenyl method	Eivazi & Tabatabai (1988)
Total glomalin and easily extractable glomalin (TG) and (EEG)	Total and easily extractable glomalin is extracted from soil using 50 mM and 20 mM sodium citrate as extractant. Protein content is determined by Lowry et al. (1951) method	Wright & Upadhyaya (1998)
Phenol oxidase activity (PHE)	The oxidized reaction product is determined after soil is incubated with (L-dihydroxy phenylalanine (DOPA)	Shi et al. (2006)
Peroxidase activity (PER)	The oxidized reaction product is determined after soil is incubated with (L-dihydroxyphenylalanine (DOPA) and hydrogen peroxide (H_2O_2)	Shi et al. (2006)
Total polysaccharides carbon (TPC)	Phenol sulphuric acid method	Lowe (1993)
Total carbohydrate carbon (TCHO)	Phenol-method without acid hydrolysis	Safarik & Santruckova (1992)
Soil organic carbon (SOC) pools	Frac. 1 (Very labile SOC) = Oxidizable organic C under $12NH_2SO_4$, Frac. 2 (Labile SOC) =The difference in oxidizable C between 18 N and 12 N $12NH_2SO_4$, Frac. 3 (Less labile SOC) =The difference in oxidizable C between 24 N and $18NH_2SO_4$, Frac. 4 (Nonlabile SOC) =the difference between total SOC and 24 N H_2SO_4	Chan et al., 2001
Total organic carbon (TOC)	Wet digestion method	Snyder & Trofymow (1984)

2.5. Microbial count

Using serial dilution spread plate technique bacteria, fungi, and *Actinomycetes* were enumerated on nutrient agar medium, rose bengal agar medium, and Kenknight's medium, respectively. The dilutions series used were 10^{-6} to 10^{-7} for Bacteria, 10^{-3} to 10^{-4} for Fungi, and 10^{-3} to 10^{-5} for actinomycetes. The media were sterilized for 20 min at 15 psi and 121 °C in an autoclave (Dhingra and Sinclair, 1993; Ganguly et al., 2019). Following serial dilution, pure cultures were maintained at 4 °C as stock, streaked as sub culture on slants in every 6 weeks, and preserved for further isolation and characterization (Chattaraj et al., 2023; Ganguly et al., 2024). All the biochemical characterizations of bacteria isolated from the earthworm guts can confirm microorganisms identified at the morphological level (Table 2).

2.6. Statistical analysis

The data were analyzed using analysis of variance (ANOVA) on different biological pools and microbial counts in the earthworm cast in randomized block design. The least significant difference (LSD) and pearson correlation were used for multiple comparisons of treatment means using R software. The ggplot2 package v4.2.1 (Wickham H, 2016) within R Studio was used to generate boxplots. Principal component analysis (PCA) and the determination of relative variable importance, based on mean increase error, were carried out on the dataset using the 'XLSTAT' (add-on for MS-Excel) software.

3. Results

3.1. Earthworm cast enzyme activities

The tillage, rate of nitrogen and residue management practices were significantly enhanced enzyme activities in the earthworm cast of maize-

Table 2
Biochemical characterization of bacteria isolated from the earthworm guts.

Isolate No.	Color and shape	Gram staining	Methyl red (MP)	Catalase	Nitrate reduction	Citrate	Oxidase
1	Brown+ small wrinkle	Negative	Negative	Negative	Positive (+)	Negative	Negative
2	Creambrown+Wrinkle	Negative	Negative	Negative	Negative	(+)	Negative
3	Creamy wrinkle	Negative	Negative	Negative	(++)	Negative	Negative
4	Round brown	Positive	(++++)	Negative	Negative	Negative	Negative
5	Shiny brown	Negative	(+++)	Positive	(++)	(+)	Negative

wheat system (Figs.1 and 2). The enzymatic activities in the soils under PBM+RN_{120}/ZTW+RN_{120} were found to be significantly ($p < 0.05$) higher than that in, PBM-RN_0/ZTW-RN_0, except phenol oxidase and peroxidase activities. The C cycle related enzymatic activity such as DHA, FDA, β-glu were increased by 21.5, 13.9, 2.1%; 163.1, 115.6, 61.7% and 76.5, 44.95, 30.6% with PBM+RN_{120}/ZTW+RN_{120}, PBM-RN_{120}/ZTW-RN_{120} and FBM-RN_{120}/CTW-RN_{120} over PBM-RN_0/ZTW-RN_0, respectively. PBM+RN_{120}/ZTW+RN_{120} exhibited a significantly greater Alk-P activity than PBM-RN_0/ZTW-RN_0. There was a 1.3-fold increase in Alk-P activity under PBM+RN_{120}/ZTW+RN_{120} than PBM-RN_0/ZTW-RN_0. Phenol oxidase and peroxidase activity was highest under PBM-RN_{120}/CTW-RN_{120} and lowest was under PBM+RN_{120}/ZTW+RN_{120}.; Phenol oxidase was 58, 50%, 25% higher with FBM-RN_{120}/CTW-RN_{120}, PBM-RN_0/ZTW-RN_0, PBM+RN_0/ZTW+RN_0 over PBM+RN_{120}/ZTW + RN_{120}. The corresponding increase for peroxidase was 130.7, 76.9, 23.1%, respectively (Figs.1 and 2). Total polysaccharides carbon (TPC) and TCHO were significantly ($p < 0.05$) higher in PBM+RN_{120}/ZTW+RN_{120} compared to PB-R-N0 N/ZT. In the earthworm cast soils, TPC varied from 11.1 to 17.6 g kg^{-1} and TCHO from 2.2 to 2.9 g kg^{-1}. The TG was higher by 40.6, 33.7, 4.3% in PBM+RN_{120}/ZTW+RN_{120}, PBM-RN_{120}/ZTW- RN_{120} and FBM-RN_{120}/CTW-RN_{120} than PB-R-N0 N/ZT. The corresponding values for EEG were 73.6, 47.2, and 29.9%, respectively (Figs.1 and 2).

3.2. Microbial diversity within earthworm cast

The maximum bacteria, fungus and actinomycetes counts in cast were significantly ($p < 0.05$) higher under PBM+RN_{120}/ZTW+RN_{120} than PBM-RN_0/ZTW-RN_0 (Table 3). The bacteria count in cast was ranged from 6.87 to 7.47 CFU x 10^6 g^{-1} soil, fungus 3.87–3.30 CFU x 10^4 g^{-1} soil and Actinomycetes 5.09–5.67 CFU x 10^4 g^{-1} soil. The bacterial and fungal counts in PBM+RN_{120}/ZTW+RN_{120}, PBM-RN_{120}/ZTW-RN_{120} and FBM-RN_{120}/CTW-RN_{120} treatments were higher by 8.7%, 5.4%, 5.1%, and 17.3%, 13.3% and 7% than PBM-RN_0/ZTW-RN_0, respectively. However, there was no significant difference among the actinomycetes counts in the earthworm cast.

3.3. Soil carbon pools

The impact of tillage, rate of nitrogen, and residue management practices on the increase of C-pools and total organic carbon (TOC) in cast soils was significant as shown in Table 4. The findings of this study indicate that Frac. 1 represented the smallest C fraction, while Frac. 4 exhibited the largest C fraction associated with the cast samples. Implementing conservation-based practices such as PBM+RN_{120}/ZTW+RN_{120} led to significantly ($p < 0.05$) higher C fractions and TOC content compared to PBM-RN_0/ZTW-RN_0. Frac. 1, Frac. 2, Frac. 3, Frac. 4, and TOC showed significant increases of 41.3%, 34.6%, 23.4%, 37.6%, and 34.6% respectively, under PBM+RN_{120}/ZTW+RN_{120} than PBM-RN_0/ZTW-RN_0.

3.4. Principal component analysis

Analysis of principal components (PCs) of the assessed earthworm cast biochemical variables showed that first and second component explained 88.33% and 7.06% of the total variance (Fig. 3). The combined variability explained by the two PCs was 95.40% (PC1 and PC2) and 90.97% (PC1 and PC3), with PC1 contributing 88.33%, PC2 contributing 7.06%, and PC3 contributing 2.63%. While phenol oxidase and peroxidase activity were the only two variables that did not correlate positively with PC1, and Frac. 3 showed the highest loading value (0.99) on PC1. Among the variables DHA, FDA, Alk-P, l-ASP, total carbohydrate, total polysaccharide carbon, total organic carbon, MBC, basal soil respiration, fraction 1 to 4, and easily extractable glomalin had a significant contribution to PC1 while polyphenol oxidase and peroxidase activity contributed to PC2. In PC3, MBC showed highest 0.54 loading value (Table 5). PCA also clearly separated the PBM-RN_0/ZTW-RN_0, PBM+RN_0/ZTW+RN_0 treatments from PBM-RN_{120}/ZTW- RN_{120}, PBM+RN_{120}/ZTW+RN_{120} and FBM-RN_{120}/CTW-RN_{120} treatments. The Frac. 3 was significantly ($p < 0.01$) correlated to TCHO ($r = 0.97^{**}$), Frac. 4 ($r = 0.98^{*}$), Frac. 1 ($r = 0.99^{**}$) while Frac. 3 was non-significantly correlated with PHE and PER (Fig. 4). The PCA showed the contribution of Frac. 3 towards soil quality index (SQI) was maximum under PBM+RN_{120}/ZTW+RN_{120} and lowest was observed under PBM-RN_0/ZTW-RN_0 (Fig. 5). Maximum contribution by Frac. 3 to SQI was observed under PBM+RN_{120}/ZTW+RN_{120} (0.838) and lowest (0.753) under PBM-RN_0/ZTW-RN_0. Similarly, the contribution by PHE to SQI was highest (0.066) under PBM+RN_{120}/ZTW+RN_{120} and lowest (0.059) was observed under PBM-RN_0/ZTW-RN_0. While, the highest contribution of PER towards SQI was observed under PBM+RN_{120}/ZTW+RN_{120} and lowest under PBM-RN_0/ZTW-RN_0. The contributions of Frac. 3, TCHO, PHE and PER to SQI in casts were 47%, 46%, 4% and 3% (Fig. 6). Radar graph depicting the contribution (%)as influenced by tillage, rate of nitrogen and residue management on soil quality has been shown in Fig. 7.

4. Discussion

4.1. Earthworm cast enzyme activities

Soil management and crop production practices e.g. tillage, fertilization, cropping systems have differential effect on the production of C substrates, which resulted in enhanced C pools and biological properties with variable impact on crop productivity (Sharma et al., 2022; Thind et al., 2023). According to Mathieu et al. (2015), the capacity of soils to sequester C from the atmosphere to support more efficient soil biological activities is determined by the equilibrium between the rate and extent of the deposition of photosynthates and the respiration rate of decomposer micro-organisms. In addition, biomass of roots has more resistance to decomposition and mineralization as they are crucial for maintaining soil health and ensuring food security by supporting a sustainable production system (Rasse et al., 2005; Anantha et al., 2018). The ligno-cellulosic root exudates also affect the physiological activities and very labile and labile C pools (Bhattacharyya et al., 2007), which proliferate microbial activities through rapid C decomposition (Yan et al., 2013). Soil biota activity and abundance are key markers of soil quality (Li et al., 2021), and microbial processes regulate residue decomposition, nutrient release in soil for maintaining crop productivity. In the present study, enzyme activities in earthworm cast were significant increase with tillage, rate of N application and residue management practices as compared to CT with residue removal. These differences were ascribed to differences in luxuriant root proliferation, nutrient rich

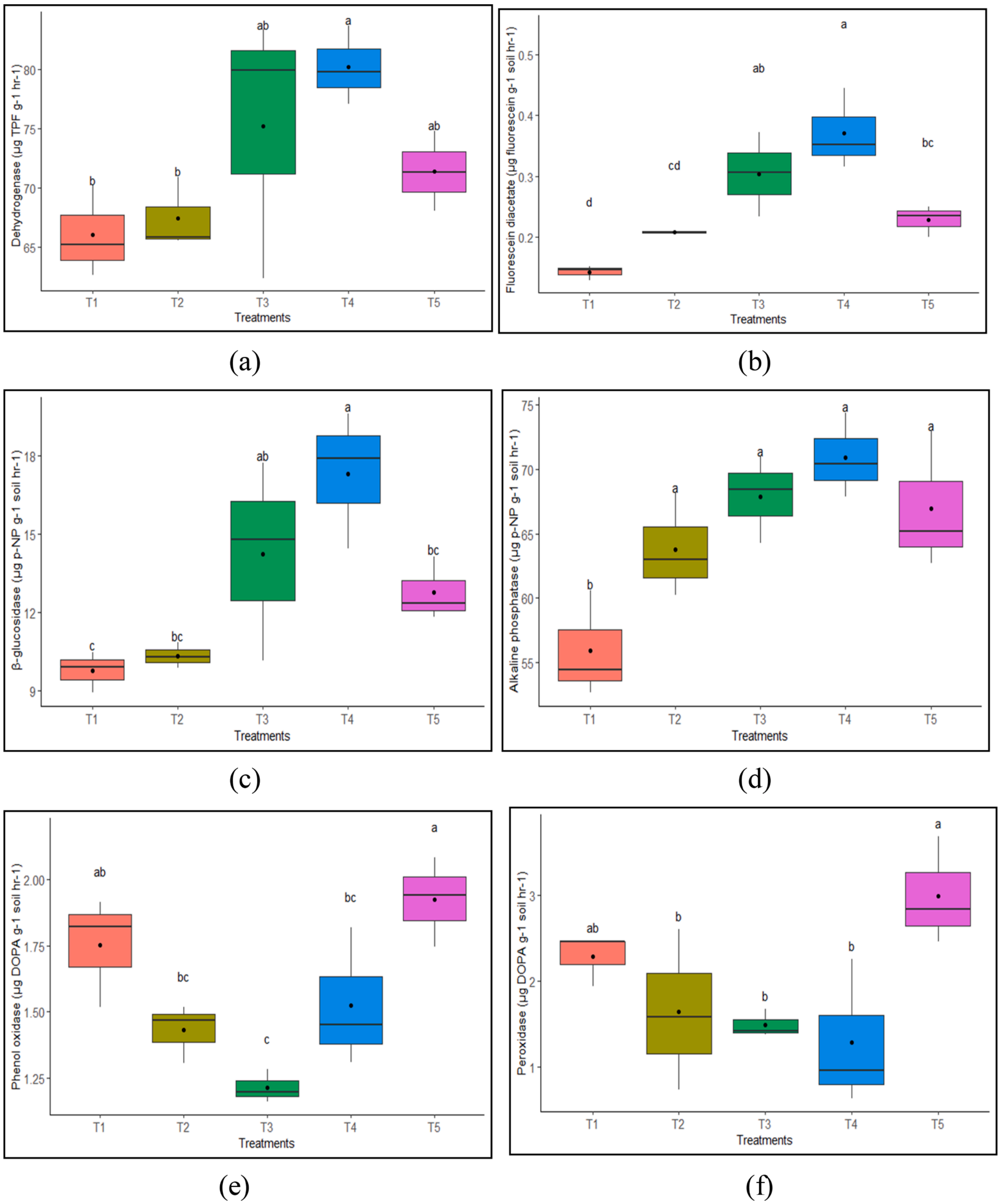

Fig. 1. The effect of tillage, rate of nitrogen and residue management practices on cast a) Dehydrogenase b) FDA c) β-glucosidase d) Alkaline phosphatase e) Phenol oxidase f) Peroxidase. Means boxplots with different letters are significantly different from each other at 0.05 probability level. Each individual dot represents an observation recorded from each plot.

environment under residue managed field conditions (Wallenius et al., 2011), which significantly impacts the nutrients cycle and soil quality (Allison et al., 2007; Choudhary et al., 2018). Extracellular enzymes in soil are indeed critical for biogeochemical nutrient cycling and productivity of soil ecosystems. (Lopes et al., 2021), which is the key link between soil micro-organisms and soil nutrients (Li et al., 2009; Xu et al., 2018). The greater soil enzyme activities observed in casts can be attributed to a variety of factors, including the presence of enzymes from the earthworms themselves and the supply of a nutrient-rich substrate that promotes microbial and microfaunal growth (Zhang et al., 2000).

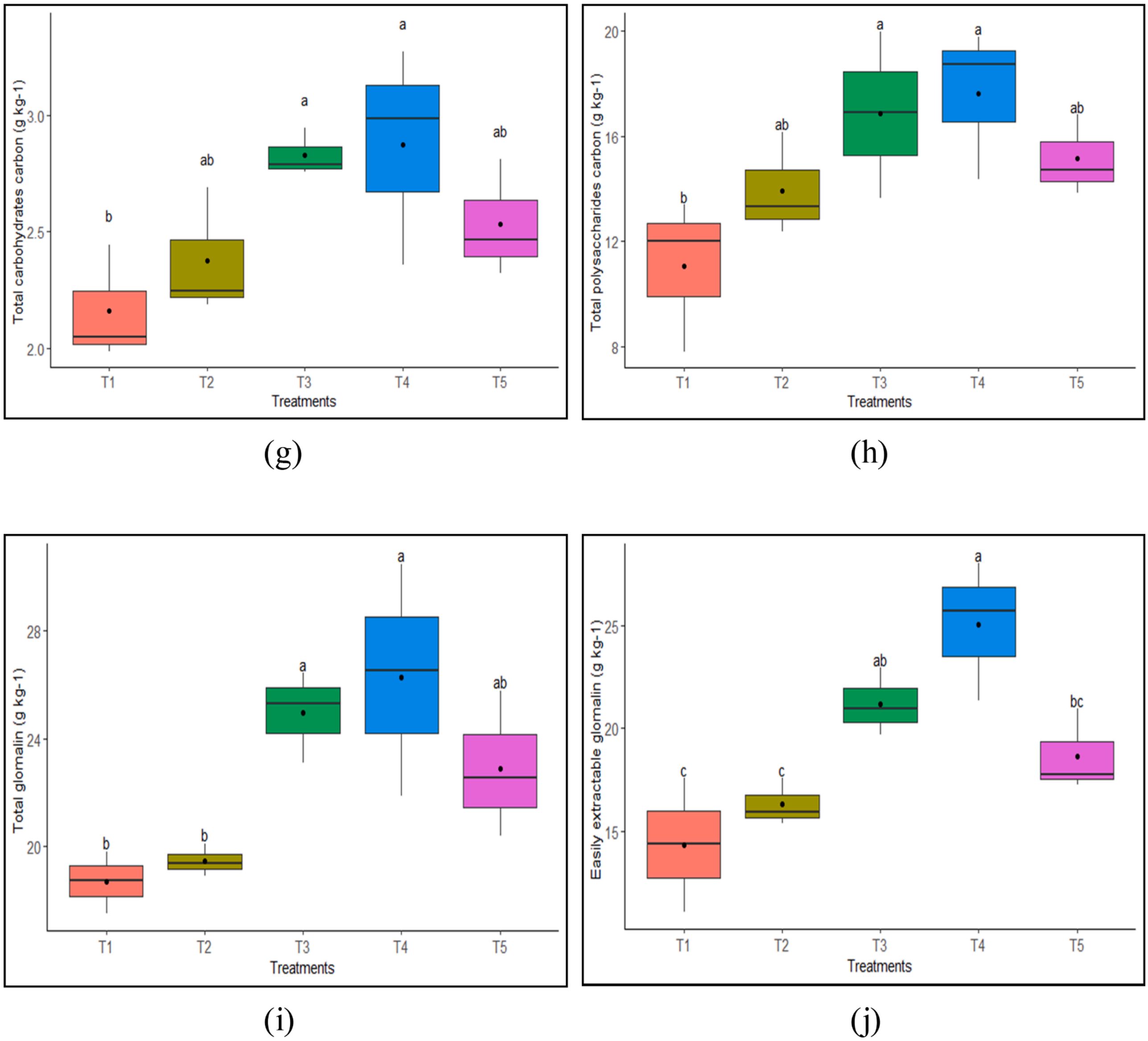

Fig. 2. The effect of tillage, rate of nitrogen and residue management practices on cast g) Total carbohydrate carbon h) Total polysaccharides carbon i) Total glomalin j) Easily extractable glomalin. Means boxplots with different letters are significantly different from each other at 0.05 probability level. Each individual dot represents an observation recorded from each plot. T1;PBM-RN_0/ZTW-RN_0; T2;PBM+RN_0/ZTW+RN_0; T3 PBM-RN_{120}/ZTW- RN_{120}; T4 PBM+RN_{120}/ZTW+RN_{120} and T5;FBM-RN_{120}/CTW-RN_{120}.

Table 3
Effect of tillage, rate of nitrogen and residue management practices in maize-wheat systems on microbial diversity within earthworm cast.

S. No.	Treatments	Bacteria (CFU x 10^6 g^{-1} soil)	Fungus (CFU x 10^3 g^{-1} soil)	Actinomycetes (CFU x 10^4 g^{-1} soil)
1	PBM-RN_0/ZTW-RN_0	6.87^{b}	3.30^{b}	5.09^{a}
2	PBM+RN_0/ ZTW+RN_0	7.05^{ab}	3.39^{ab}	5.22^{a}
3	PBM-RN_{120}/ ZTW-RN_{120}	7.24^{ab}	3.74^{ab}	5.49^{a}
4	PBM+RN_{120}/ ZTW+RN_{120}	7.47^{a}	3.87^{a}	5.67^{a}
5	FBM-RN_{120}/ CTW-RN_{120}	7.22^{ab}	3.53^{ab}	5.38^{a}

Table 4
Effect of tillage, rate of nitrogen and residue management practices in maize-wheat systems on carbon pools of cast.

	Treatments	Frac. 1	Frac. 2	Frac. 3	Frac. 4	TOC
1	PBM-RN_0/ZTW-RN_0	0.46^{c}	0.78^{a}	0.94^{a}	3.19^{c}	5.38^{c}
2	PBM+RN_0/ZTW+RN_0	0.50^{bc}	0.80^{a}	0.97^{ab}	3.43^{c}	5.70^{c}
3	PBM-RN_{120}/ZTW-RN_{120}	0.63^{a}	0.96^{a}	1.10^{ab}	4.31^{ab}	7.01^{a}
4	PBM+RN_{120}/ZTW+RN_{120}	0.65^{a}	1.05^{a}	1.16^{b}	4.39^{a}	7.24^{a}
5	FBM-RN_{120}/CTW-RN_{120}	0.58^{bc}	0.89^{a}	1.01^{b}	3.96^{b}	6.45^{b}

Tao et al. (2009) found significantly greater DHA activities in casts compared to the surrounding soil during both RWS, primarily due to higher soil bacterial and fungal biomass (Tiwari et al., 1989). Other research has indicated that earthworm casts enhance carbohydrate C (Ross and Cairns 1982), phosphatase (Tiwari et al., 1989), and DHA activities (Kizilkaya 2008). The increase in FDA activity with residue can be attributed to the growth of microbial activity due production of

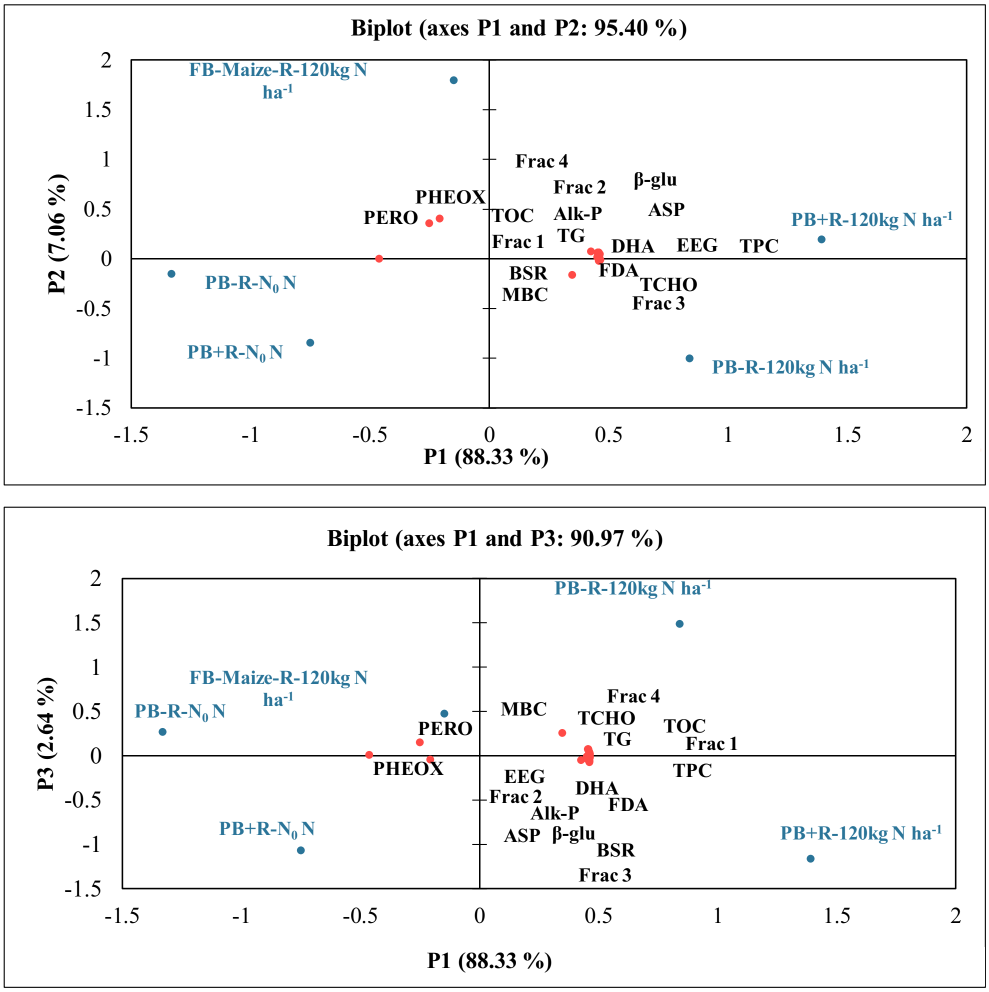

Fig. 3. Bi-plots of principle component analysis (PCA) on the soil properties in earthworm cast.
PBM-RN$_0$/ZTW-RN$_0$ (Permanent beds No-N control-both residues removed and wheat with zero tillage); PBM+RN$_0$/ZTW+RN$_0$ (Permanent beds No-N control-both residues retained)−50% of maize stover and 25% of wheat residue retained; PBM-RN$_{120}$/ZTW- RN$_{120}$ (Permanent beds with 120 kg N ha^{-1} both residues removed wheat with zero tillage); PBM+RN$_{120}$/ZTW+RN$_{120}$ (Permanent beds with 120 kg N ha^{-1} both residues retained and wheat with zero tillage) and FBM-RN$_{120}$/CTW-RN$_{120}$ (Fresh beds in maize/CT in wheat with 120 kg N ha^{-1} both residues removed), DHA: Dehydrogenase activity, FDA: Fluorescein diacetate activity, Alk-P: Alkaline Phosphatase activity, β-glu: β-glucosidase activity, l-ASP: l-asparaginase activity, PHEOX: Phenol oxidase activity, PERO: Peroxidase activity, MBC: Microbial biomass carbon, BSR: Basal soil respiration, TCHO: Total carbohydrate carbon, TPC: Total polysaccharide carbon, TOC: Total organic carbon Frac 1: Fraction 1, Frac 2: Fraction 2, Frac 3: Fraction 3, Frac 4: Fraction 4

volatile organic compounds by maize residues which also effects the microbial diversity and activity, biochemical processes and enzyme activities (Caravaca and Roldan 2003). Incorporation of maize residues into soil can significantly impact on nutrients cycling through secretion of large quantities of enzymes by soil microbes (Elfstrand et al., 2007).

The Alk-P is involved in the conversion of organic to inorganic phosphorus and is also linked with the production of micro-flora and fauna residues (Juma and Tabatabai, 1988). The presence of earthworms was associated with increased Alk-P activity due to readily available microbial biomass input supplies for higher available P in the soil (Aira et al., 2007). Furthermore, minimizing soil disturbance plays a crucial role in preserving earthworm communities, leading to positive impacts on their life cycles and reproductive processes and ultimately accelerates the cast enzymes activities (Tsiafouli et al., 2015). Another reason behind the increase in cast enzyme activities is due to the addition of crop residue, earthworms get more C substrate and enhance their activities (Mouni et al., 2019). Conversely, CT resulted in lower macro-organism activities due to the mixing of soil during ploughing, which disturbs their habitats, life cycle and disrupts their food sources (Blanco-Canqui and Lal 2010). The presence of abundant nutrient supply and large surface area facilitated the growth and reproduction of the microbes, thereby exhibited a higher microbial proliferation within the casts (Parthasarathi et al., 2007). In earthworm cast (EWC), (Jat et al., 2022a,b) reported that CA-based practices than CT enhanced DHA and Alk-P activity in earthworm cast due to symbiotic interaction with soil microbiota by transforming crop residues into EWC-containing enzymes and microorganisms (Choudhary et al., 2018). Kumar et al. (2020a,b) observed significantly higher FDA and Alk-P in earthworm cast than the bulk soil under ZT. In their study, earthworm cast has 2.1 times FDA, 1.33 times Alk-P, and 1.09 times urease activity than surrounding soils due to soil disturbance facilitates the maintenance of a stable soil microenvironment (Sun et al., 2016). Whereas, CT involves a physical perturbation leads to a disruption of aggregates, loss in essential nutrient pools and soil microorganisms which locking up earthworm in the soil clods (Briones and Schmidt 2017).

4.2. Microbial diversity within earthworm cast

The gut of an earthworm functions as a natural bioreactor, resulting in the excretion of material with a microbial density that is 1000 times higher than the surrounding soil (Savin et al., 2004). Furthermore, the increase in microbial count in earthworm guts and the cast is attributed

Table 5
Loading values and percent contribution of assayed biochemical variables in tillage, rate of nitrogen and residue management practices by the principal component analysis.

	PC1		PC2		PC3	
Soil Variables	Loading values	Contribution of variables	Loading values	Contribution of variables	Loading values	Contribution of variables
DHA	0.987	4.79	0.080	0.391	−0.070	0.812
FDA	0.989	4.81	−0.045	0.126	−0.139	3.17
Alk-P	0.913	4.10	0.163	1.630	−0.108	1.92
β-glu	0.974	4.67	0.141	1.230	−0.098	1.58
ASP	0.985	4.77	0.031	0.059	−0.119	2.32
PHE	−0.445	0.975	0.867	**46.2**	−0.090	1.34
PER	−0.540	1.43	0.772	**36.6**	0.324	17.2
TG	0.983	4.75	0.143	1.26	0.110	2.00
EEG	0.988	4.80	0.073	0.332	−0.122	2.44
TCHO	0.989	**4.81**	−0.031	0.060	0.084	1.17
TPC	0.968	4.60	0.034	0.072	−0.015	0.038
MBC	0.743	2.71	−0.340	7.12	0.547	49.2
BSR	0.984	4.76	−0.037	0.085	−0.057	0.542
TOC	0.980	4.72	0.135	1.12	0.138	3.12
Frac. 1	0.979	4.71	0.111	0.759	0.159	4.16
Frac. 2	0.985	4.77	0.119	0.876	−0.037	0.224
Frac. 3	0.993	**4.85**	−0.028	0.048	−0.002	0.001
Frac. 4	0.975	4.68	0.127	0.996	0.158	4.12
Eigenvalue		20.3		1.62		0.607
Variability (%)		88.3		7.064		2.639
Cumulative%		88.3		**95.3**		98.0

DHA: Dehydrogenase activity, FDA: Fluorescein diacetate activity, Alk-P: Alkaline Phosphatase activity, β-glu: β-glucosidase activity, l-ASP: l-asparaginase activity, PHEOX: Phenol oxidase activity, PERO: Peroxidase activity, MBC: Microbial biomass carbon, BSR: Basal soil respiration, TCHO: Total carbohydrate carbon, TPC: Total polysaccharide carbon, TOC: Total organic carbon. Frac 1: Fraction 1, Frac 2: Fraction 2, Frac 3: Fraction 3, Frac 4: Fraction 4.

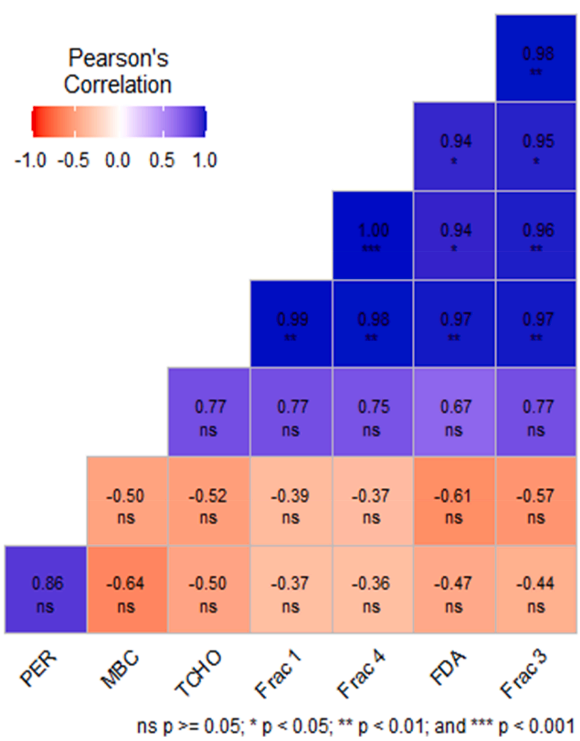

Fig. 4. Pearson's correlation of highly weighted variables within casts.
PER: Peroxidase activity, MBC: Microbial biomass carbon, FDA: Fluoresceine diacetate, Frac 3: Fraction 3, Frac 1: Fraction 1, Frac 4: Fraction 4, TCHO: Total carbohydrate carbon.

due to the large quantity of high-quality residue applied in this maize-bean system as well as root inputs likely serve as key food sources and shelter for the earthworms which significantly influence the earthworms' microbes (Fonte et al., 2010). While the bacteria, fungi and actinomycetes counts were lower under residue removal CT because earthworms are more sensitive to tillage operation due to physical disruption, degradation of natural resources and agricultural residue inputs and changes in soil moisture and temperature regimes. Pulleman et al. (2005) observed higher earthworm activity in the straw addition long-term farming systems which suggested that straw addition stimulates earthworm activity and increases the organic C contents of earthworms. Raised-bed planting creates an optimal growing environment that enhances soil health, supports beneficial microbial and enzymatic activities, and leads to better plant growth compared to flat planting (Patino-Zuniga et al., 2009). In the present study, bacterial counts increased significantly along gut sections of the earthworms identified. Bacteria exhibit a faster growth rate than fungi and play a more significant role in the initial phases of decomposition, whereas fungi are more prominent in the later stages of decomposition (Wang et al., 2021a,b). This aligns with previous findings that earthworm casts tend to exhibit higher bacterial counts than soil (Daniel and Anderson 1992; Pederson and Hendriksen 1993). Furthermore, Parthasarathi and Ranganathan (1999) reported that the enhancement of microbial population in the casts can be attributed to several factors. Firstly, the casts contain a high concentration of nutrients, providing an abundant food source for the microbes. Secondly, as the casts pass through the digestive system of worms, the microbes present in them multiply, contributing to the overall increase in microbial population. Thirdly, the optimal moisture levels in the casts create a favourable environment for microbial growth and activity. Lastly, the large surface area of the casts offers an ideal habitat for the microbes, facilitating their feeding and multiplication. Tillage process promotes the decay of crop residue by creating uniform soil litter conditions. Conversely, under ZT and PB with residue retention, causing a slow release of nutrients over extended period, which enhances soil invertebrates activity (Errouissi et al., 2011). Luo et al. (2020) reported that long-term CA practices improved bacterial community composition significantly due to reducing soil disturbance, slower decomposition of soil organic matter, and increasing the soil C content. Additionally, ZT and residue management practices act as barrier that prevents soil moisture loss and nutrients (Zhang et al., 2019).

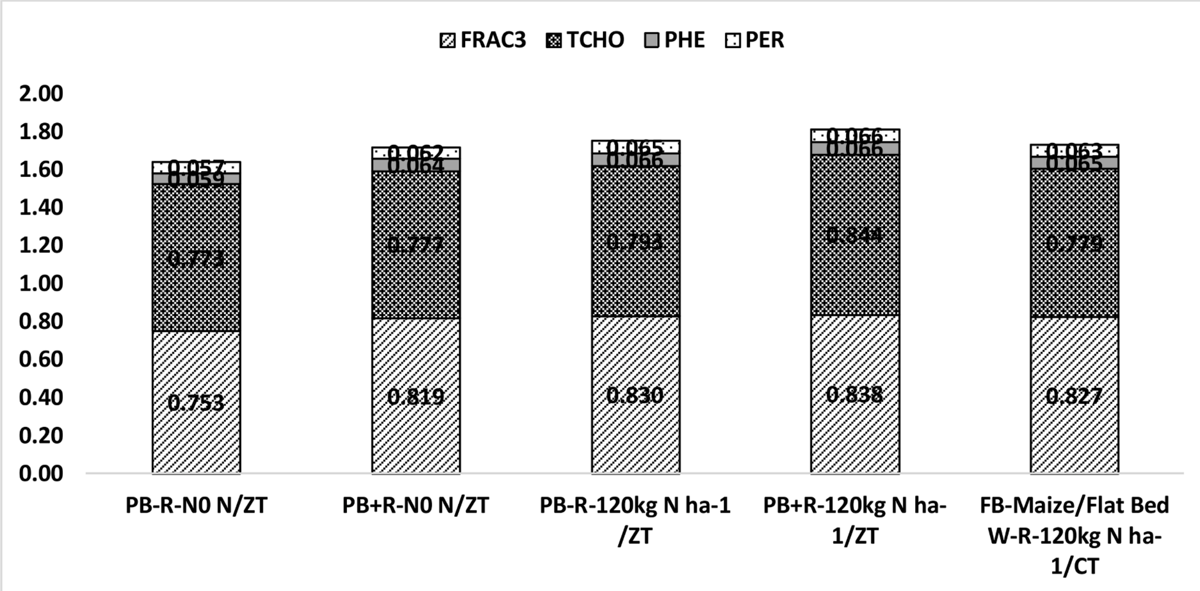

Fig. 5. Effect of tillage, rate of nitrogen and residue management practices on soil quality index.
PBM-RN$_0$/ZTW-RN$_0$ (Permanent beds No-N control-both residues removed and wheat with zero tillage); PBM+RN$_0$/ZTW+RN$_0$ (Permanent beds No-N control-both residues retained)−50% of maize stover and 25% of wheat residue retained; PBM-RN$_{120}$/ZTW- RN$_{120}$ (Permanent beds with 120 kg N ha^{-1} both residues removed wheat with zero tillage); PBM+RN$_{120}$/ZTW+RN$_{120}$ (Permanent beds with 120 kg N ha^{-1} both residues retained and wheat with zero tillage) and FBM-RN$_{120}$/CTW-RN$_{120}$ (Fresh beds in maize/CT in wheat with 120 kg N ha^{-1} both residues removed) FRAC3: Fraction 3, TCHO: Total carbohydrate carbon, PHE: Phenol oxidase activity, PER: Peroxidase activity

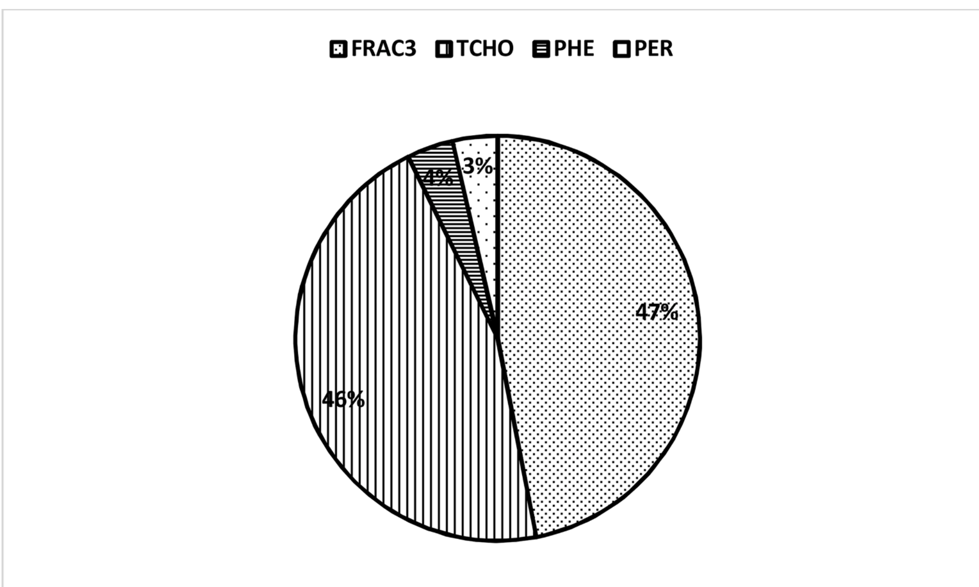

Fig. 6. Contribution of the selected soil quality indicators to SQI.
FRAC 3: Fraction 3, TCHO: Total carbohydrate carbon, PHE: Phenol oxidase activity, PER: Peroxidase activity

4.3. Soil carbon pools

Carbon pools in soil represent equilibrium between C inputs (crop residue, root biomass, exudates, and manure) and C losses (crop removal, mineralization, respiration) (Benbi et al., 2015; Liang et al., 2023). In the present study, the increase in earthworm cast C pools and TOC under PBM+RN$_{120}$/ZTW+RN$_{120}$ could be attributed to the integration of crop residue retention, earthworm feeding and casting, and microbial diversity. Additionally, the adoption of ZT, PB, and continuous crop residue retention for the long term has improved earthworm proliferation, soil microbe structure and C mineralization resulted in higher nutrients availability (Jat et al., 2022a,b). In their study, Bottinelli et al. (2010) eloquently described the crucial role of earthworms in soil ecology, highlighting their ability to modify the environment to meet their ecological needs. Furthermore, it has been observed that earthworm casts contribute significantly to the formation of soil aggregates (Kamau et al., 2020), which in turn, is crucial for the stabilization of organic C (Wiesmeier et al., 2019). According to Van Groenigen et al. (2019), their meta-analysis concluded that earthworm casts are highly fertile and contain 40–48% more nutrients (N, P, K and C) compared to bulk soil. Within the earthworm's gut, microorganisms ingested by the worm produce exoenzymes such as lipases, chitinases

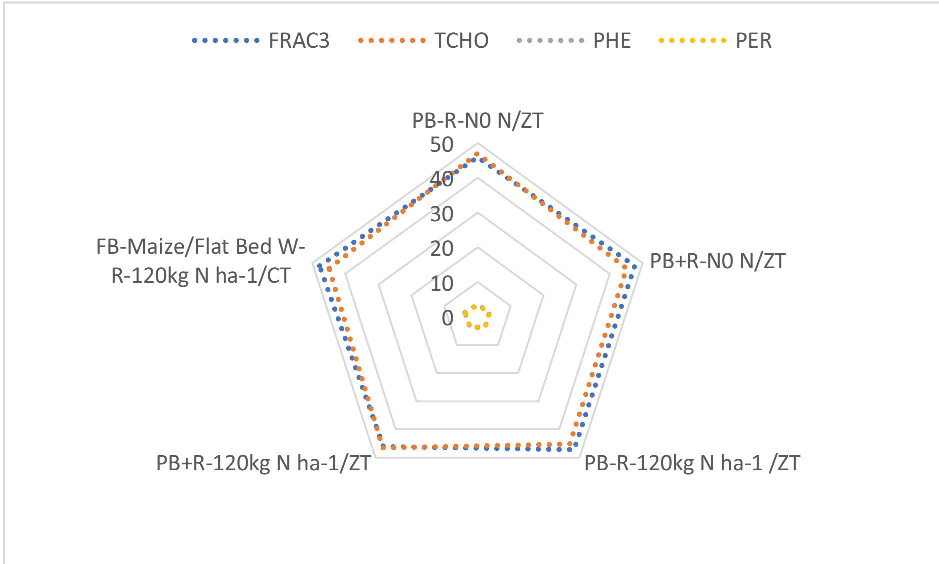

Fig. 7. Radar graph depicting the contribution (%) of selected key indicators to soil quality as effect of tillage and residue management practices. PBM-RN_0/ZTW-RN_0 (Permanent beds No-N control-both residues removed and wheat with zero tillage); PBM+RN_0/ZTW+RN_0 (Permanent beds No-N control-both residues retained)−50% of maize stover and 25% of wheat residue retained; PBM-RN_{120}/ZTW- RN_{120} (Permanent beds with 120 kg N ha^{-1} both residues removed wheat with zero tillage); PBM+RN_{120}/ZTW+RN_{120} (Permanent beds with 120 kg N ha^{-1} both residues retained and wheat with zero tillage) and FBM-RN_{120}/CTW-RN_{120} (Fresh beds in maize/CT in wheat with 120 kg N ha^{-1} both residues removed), FRAC3: Fraction 3, TCHO: Total carbohydrates carbon, PHE: Phenol oxidase activity, PER: Peroxidase activity

and cellulases. These enzymes facilitate the breakdown of complex organic matter, particularly in fresh straw, as it passes through the gut (Wu et al., 2018). As a result, earthworms are capable of converting recalcitrant C compounds into more easily assimilated C compounds in their castings (Aira et al., 2006; Chen et al., 2015). In the present study, C pools were significantly higher under PBM+RN_{120}/ZTW+RN_{120} as compared to PBM-RN_0/ZTW-RN_0. This increase may be attributed to the conversion of easily labile C into a more stable C form. Similar to other studies, TOC, its labile C pools and soil aggregation were improved under CA-based management practices, which supports to enhance microbial communities for higher soil enzyme activities (Sharma et al., 2019; Saikia et al., 2019; Babu et al., 2023). Another reason might be that casts were formed through positive interactions between mineral soil fractions and SOM higher production of bacterial polysaccharides and fungal hyphae (Alvear et al., 2005; Bhadauria and Saxena 2010; Fontana et al., 2015). We observed a lower amount of TOC under the CT, which can be attributed to the loss through C mineralization during tillage operations. In CT plots, C mineralization is faster due to the exposure resulting from soil inversion and aggregate breakdown (Alvear et al., 2005). Enhancing the storage or sequestration of C in the soil not only holds significant potential for mitigating increases in atmospheric carbon dioxide (Wiesmeier et al., 2019) but also can improve soil quality and improved crop productivity (Lal., 2004).

4.5. Principal component analysis

The effect of residue management and CA-based practices on microbial activity can be statistically positive correlated between soil respiration and DHA activity (Saikia et al. 2019; Sharma et al. 2022). The retention of residues provided C based compound like cellulose and lignin into the soils and break down these residues through production of ligninolytic and lignin-cellulolytic enzymes by soil microbes (Singh et al., 2018). Choudhary et al. (2018) reported that the SQI was higher by 90% in MWS compared to RWS, 22% in ZT with residue as compared to CT with residue removal. Similarly, Chellapa et al. (2021) demonstrated that SOC was significantly correlated with soil enzyme activities in the soil as labile C is the primary source of energy for microorganisms. A substantial association between enzyme and SOC may be attributable to enhanced C associated microbial activity (Sandhu et al., 2019) as the cause of greater enzymatic activity in ZT as compared to CT. In addition, TPC, PHE and TCHO were provides C-containing molecules and other nutrients that can stimulate the growth of microbial populations. Furthermore, enzyme activity significantly correlates with organic C because higher levels of C indeed support increased microbial biomass and activity in ecosystems. In addition, increased organic matter stabilizes and protects extracellular enzymes (Balota et al., 2004). The significant link between various enzyme activities and C fractions may be attributed to soil enzymes binding to clay and humic colloids and forming humus-enzyme or clay-enzyme complexes, which safeguard soil enzyme function (Klose and Tabatabai 1999).

5. Conclusions

These results showed that CA-based practices like permanent beds with crop residue retention with balance fertilization have a significant impact on earthworm cast enzymes, microbial diversity, and C pools in MWS. The observed high microbial activity in PBM+RN_{120}/ZTW+RN_{120} is driven by improved substrate availability, suggests a reduced risk of nutrient losses. Additionally, minimizing soil disturbance plays a crucial role in preserving earthworm communities, leading to positive impacts on their life cycles and reproductive processes, and ultimately accelerating cast enzyme activities. Our study identified that Fraction 3, total carbohydrate carbon, phenol oxidase, and peroxidase are reliable indicators for evaluating and distinguishing the most sustainable crop residue management practices in MWS. These findings highlight the importance of these metrics in guiding sustainable agricultural practices. However, further research is necessary to explore additional soil health-related quality indices that are sensitive to various management practices. Expanding this research within diverse conservation agriculture-based crop rotations will provide a more comprehensive understanding of soil health and sustainability in different agricultural contexts. By building on these insights, future studies can enhance our ability to develop and implement agricultural practices that not only improve crop productivity but also foster long-term ecological balance and soil health.

CRediT authorship contribution statement

Padma Angmo: Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Data curation. **Sandeep Sharma:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. **H.S. Sidhu:** Conceptualization, Methodology. **K.S. Saini:** Writing – review & editing.

Declaration of competing interest

None.

Data availability

No data was used for the research described in the article.

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