



VERTICAL DISTRIBUTION OF CARBON AND NITROGEN DYNAMICS IN BLACK SOIL OF CENTRAL INDIA UNDER LONG-TERM ZERO- TILLAGE WITH DIFFERENT CROPPING SYSTEMS

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ABSTRACT

Conservation agriculture (CA) based zero-tillage system has gained prominence for its potential to improve soil health, enhance nutrient cycling, and increase carbon sequestration in soil. Knowledge about the changes in soil carbon and nitrogen dynamics under no-tillage and different cropping systems is necessary to assess the feasibility of adopting conservation agriculture to sustain soil health and productivity. This study compared conventional tillage (CT) and zero-tillage (ZT) systems, and three cropping systems (soybean-wheat, maize-wheat and maize-chickpea) on soil carbon and nitrogen dynamics. The experiment was established in 2010 on a Vertisol in Bhopal, India. After the completion of the 14th cycle of the experiment (2023-24), soil samples were collected from each plot and analysis processes were executed. The soil under ZT had found significantly higher concentrations of soil organic carbon (0.93%), total organic carbon (1.23%), very labile pool (0.55%), labile pool (0.19%), non-labile (0.36%) and total carbon (1.39%) than CT at 0-10 cm depth. Available nitrogen (229.83 kg ha⁻¹) and total nitrogen (0.133%) also had significantly higher in ZT than CT at surface soil. Tillage and cropping systems had no significant impact on soil inorganic carbon and mineral nitrogen (ammonium and nitrate nitrogen). Therefore, under Vertisols, 14 years of ZT practices are likely to improve organic carbon concentration and increase the availability of nitrogen in soil, allowing a positive trend for soil preservation and carbon sequestration in soil.

Keywords: Available nitrogen, carbon sequestration, conservation agriculture, organic carbon

INTRODUCTION

The global population is projected to reach 9.64 billion by 2050, and the demand for food will rise significantly, putting immense pressure on soil resources and ecosystems (UNDESA, 2024). This scenario calls for innovative and resilient farming approaches that ensure long-term agricultural productivity while minimizing the environmental degradation. Conservation agriculture (CA) has emerged as a cornerstone of sustainable agricultural practices, addressing the pressing dual challenges of maintaining the productivity and mitigating the environmental issues. CA-based zero-tillage (ZT)/no-tillage (NT) system has gained prominence for its potential to improve soil health, enhance nutrient cycling, and reduce greenhouse gas emissions. Nicoloso *et al.* (2021) conducted a comprehensive global meta-analysis of tilled versus no-till soils and found that NT systems showed significantly greater

soil organic carbon (SOC) and total nitrogen storage, particularly under intensified cropping regimes and in the presence of legumes.

Carbon and nitrogen dynamics are fundamental to soil fertility and ecosystem functioning. SOC is critical for maintaining soil structure, water retention, and nutrient availability, while nitrogen (N) is an essential nutrient for plant growth and productivity (Smith *et al.*, 2020). Long-term zero-tillage practices have been shown significant influence on these dynamics. For example, no-tillage systems have been shown to significantly increase soil organic matter in the topsoil - enhancing carbon storage and reducing its loss via oxidation or microbial decomposition (Meng *et al.*, 2024). Similarly, nitrogen cycling benefits from the reduced disturbance as microbial communities that drive key processes like nitrification and denitrification are less disrupted.

The dynamics of SOC is broadly categorized into labile and non-labile pools, each contributing variably to soil functions. The labile carbon pool, comprising of readily decomposable organic matter, is crucial for nutrient cycling and energy flow, while non-labile pool, which includes more stable organic compounds, plays a significant role in long-term carbon sequestration and climate change mitigation (Khan *et al.*, 2024). Improved crop management practices, like zero-tillage and straw mulch strategies, mitigating can increase the soil organic carbon and SOC fractions as compared to the conventional practices (Mishra *et al.*, 2010). The increase in the active pools (labile and less labile) of soil organic matter (SOM) in soil surface layers under no-till is much more than under conventional tillage (Aduhene-Chinbuah *et al.*, 2022).

Nitrogen is a key limiting factor for attaining stable crop yields in dryland cropping systems (Sainju *et al.*, 2009). Any management practice that changes soil organic C influences soil organic N since the C/N ratio of stable SOM has a relatively narrow range (10:1-12:1). Tillage management affects the storage and availability of soil nitrogen and may, therefore, influence the crop yield and quality. Several studies have shown that conservation tillage, such as no-tillage, usually increases soil N content as compared to conventional tillage under dryland agriculture (Dikgwatlhe *et al.*, 2014). The soil mineral N ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) content is the available N pool for crop uptake. ZT can improve the availability of N to crops in long term by increasing soil N retention and the labile N pool in upper soil layers (Sun *et al.*, 2015). This study provides detailed assessment of carbon and nitrogen fractions across soil depths under long-term zero-tillage in Vertisols of central India. By simultaneously examining the labile and non-labile carbon pools alongside nitrogen dynamics, it offers new insights into how ZT enhances soil fertility, carbon sequestration, and nitrogen-use efficiency, thereby demonstrating its potential as a climate-smart strategy for sustainable dryland agriculture.

MATERIALS AND METHODS

Description of experiment

The experiment was conducted during *Kharif* (rainy) and *Rabi* (winter) seasons of 2023-24 on black soil in Central India as part of the ongoing long-term conservation agriculture study initiated in June 2010 at the research farm of ICAR-Indian Institute of Soil Science, Bhopal, India (Fig. 1). The experimental site is located at an altitude of 23° 18' N and longitude of 77° 24' E at 485 m masl elevation. The climate of Bhopal region is hot sub-humid, with average precipitation of 1100-1400 mm, mostly received during monsoon months from mid-June to September. Monthly meteorological data, including rainfall, pan evaporation, and maximum and minimum temperatures, collected during the study period is given in Fig. 2. The soil at the experimental site was identified as clayey Vertisol (isohyperthermic, typic Haplustert), with the top 0-15 cm layer comprising of 58% clay, 22% silt, and 20% sand. The field features a uniform, gently sloping topography with good drainage, and the pH range of soil at 0-30 cm depth was between 7.9 and 8.2.

The experiment was conducted in a factorial randomized block design, with two tillage operations [conventional tillage (CT) and zero tillage (ZT)] and three cropping systems (soybean-wheat, maize-

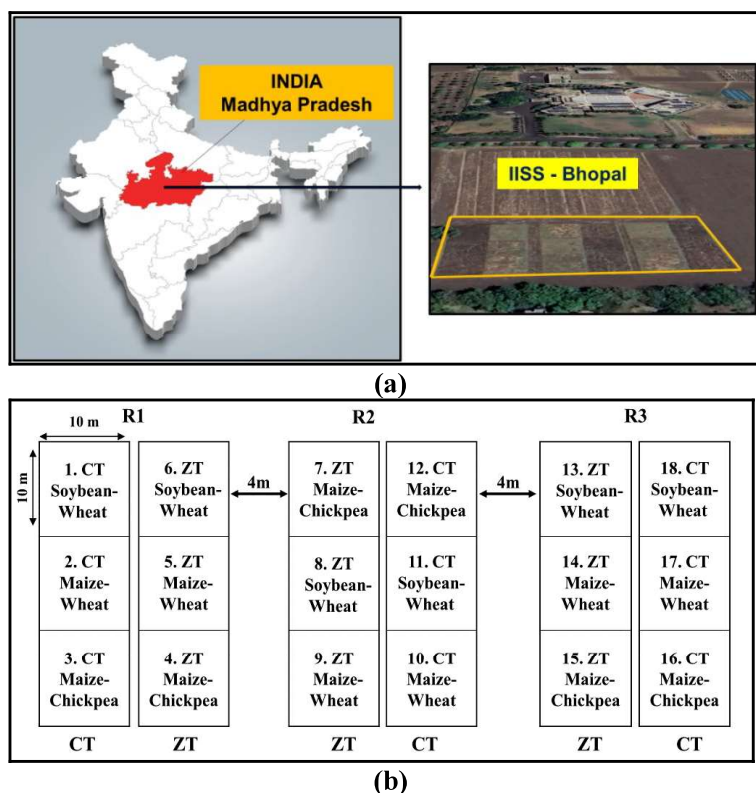


Fig. 1: a. Location of the experiment; b. Layout of the experiment

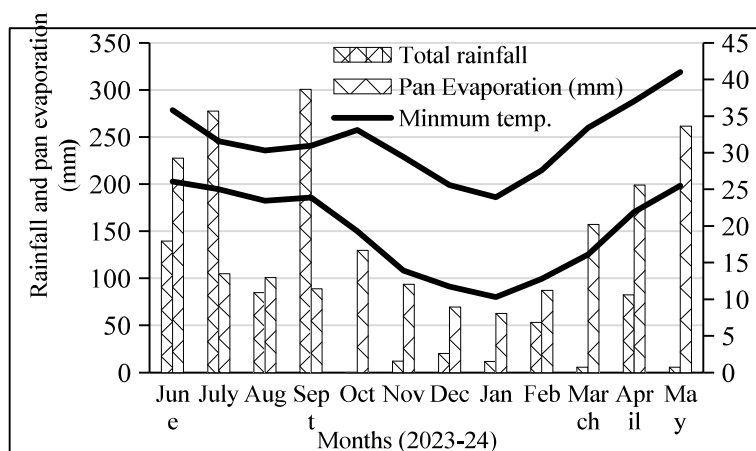


Fig. 2: Monthly metrological data during experiments

dried in a well-ventilated area to reduce moisture content while maintaining soil integrity for analysis. After drying, the soil was sieved through 0.5 and 0.25 mm mesh sieves to obtain fine particles suitable for precise laboratory analysis. This sieving process ensured the removal of larger debris and facilitated the accurate estimation of various forms of carbon and nitrogen present in the soil.

Analytical methods

For soil organic carbon (SOC) analysis, wet digestion method was used (Walkley and Black, 1934). Available nitrogen in soil was determined by alkaline potassium permanganate method (Subbiah and Asija, 1956). Different organic pools of soil carbon were determined under an increasing gradient of oxidizing conditions using three sulphuric acid (H_2SO_4)-aqueous solution ratios of 0.5:1, 1:1 and 2:1

wheat and maize-chickpea). These treatments were replicated three times (Fig. 1b), with each plot measuring 10 x 10 m². CT involved summer ploughing after residue removal and 3-4 tillage operations by a cultivator, followed by sowing crops. ZT involved sowing directly in undisturbed soil using a Happy seeder. In ZT system, treatment plots for maize and wheat crops retained residue with a height of 30 cm after harvest. Fertilizer was applied at the recommended rates of N: P₂O₅: K₂O, with 120:60:40 kg ha⁻¹ for wheat and maize, 30:60:30 kg ha⁻¹ for soybean, and 40:60:30 kg ha⁻¹ for chickpea in both tillage systems.

Soil sampling

Soil samples were collected once during 2023-24 cropping cycle, immediately after *rabi* crop harvest in April 2024. This post-*rabi* sampling was chosen because it represented the residual effect of complete annual cropping sequence (*kharif* + *rabi*) under each tillage and cropping system. Soil was sampled at 0-10, 10-20, 20-30 cm depth to capture vertical stratification of SOC and N pools that typically differ between ZT (surface-enriched) and CT (subsurface-distributed) systems. The composite samples were air-

corresponding to 12, 18 and 24 N H₂SO₄, respectively (Chan *et al.*, 2001). The amount of carbon thus estimated lead to the partition of total organic carbon (TOC) into the following four different organic carbon pools of decreasing oxidizability: i. active very labile pool - Organic C oxidizable by 12 N H₂SO₄; ii. active labile pool - the difference in C oxidizable by 18 N and that by 12 N H₂SO₄; iii. passive less labile pool - the difference in C oxidizable by 24 N and that by 18 N H₂SO₄; iv. passive non-labile pool - the difference between TOC and C oxidizable by 24 N H₂SO₄.

The soil total carbon and total nitrogen in soil were measured through dry combustion with a CHNS Analyzer (EURO EA 3000/1F). In the elemental analyser's combustion tube, soil samples were oxidized at a high temperature of 1150°C in presence of O₂ gas, using tungsten oxide as a catalyst. A thermal conductivity detector sequentially measures the desired components with the assistance of specific adsorption columns. The soil inorganic carbon (SIC) was calculated by subtracting total organic carbon from total carbon. Ammonium (NH₄) and nitrate (NO₃) nitrogen were analysed using flow injection analysis. The NH₄ and NO₃ content in dry soil extracts were measured by using a continuous flow autoanalyzer (FIAstar- 5000 FOSS Analyzer), integrating the Griess-Ilosvay method for NO₃ determination (Keeney and Nelson, 1983) and the Solorzano method for NH₄ analysis (Solorzano, 1969).

Statistical analysis

The experimental data collected during the study were analysed using standard statistical methods, specifically the analysis of variance (ANOVA), following the procedures outlined by Gomez and Gomez (1984). For parameters that exhibited significant differences at 5% level of significance, the critical difference (CD) values were computed. The CD value is a quantitative measure to compare treatment means and identify the minimum difference required for the variations to be considered statistically meaningful.

RESULTS AND DISCUSSION

Carbon dynamics in soil

Organic carbon is an indicator of soil health and a storehouse of all essential plant nutrients. Soil organic carbon (SOC) and total organic carbon (TOC) analysis revealed significantly higher amount of SOC and TOC under ZT (0.93 and 1.23%) as compared to CT (0.81 and 1.07%) at surface soil, respectively (Table 1). Conservation agriculture (CA) is associated with higher SOC and TOC levels in surface layer of soil because of a rise in the amount of crop residue (Dikgwatlhe *et al.*, 2014; Marahatta *et al.*, 2014) and less soil disturbance, which lowers organic matter oxidation and enhances SOC storage (Sarker *et al.*, 2024). In contrast, in conventional tillage practice, regular and frequent ploughing causes physical breakdown, overturning of soil and disruption of soil aggregates that lead to the oxidation of soil organic matter and erosion, which lowers SOC content in surface soil (Zheng *et al.*, 2018). Higher organic carbon contents in upper layers of ZT systems than CT might lead to the prevention of loss of soil organic carbon by gaseous emissions (Bono *et al.*, 2007). Protection of soil surface from raindrop impact and erosion is invaluable in attaining higher TOC content in the surface layers of ZT plots (Chivenge *et al.*, 2007). The study showed that SOC and TOC had higher concentrations in maize-wheat cropping system (1.01 and 1.34%) as compared to other cropping systems in ZT. This might be due to the higher residue retention on soil surface in maize-wheat cropping system than soybean-wheat and maize-chickpea cropping systems. As soil depth increased, the SOC and TOC concentration dropped in the order of 0-10 cm > 10-20 cm > 20-30 cm because the organic matter in the form of crop residue inputs is predominantly concentrated at soil surface and the decomposition rate of organic matter is slower in deeper soil (Parihar *et al.*, 2016).

For better understanding of the processes involved in carbon storage and loss, the soil organic carbon was divided into four pools with different levels of oxidizability *viz.*, very labile (C_{VL}) and

Table 1: Effect of long-term no-tillage practice on organic, inorganic and total carbon in soil

Tillage system (TS)	Cropping system (CS)	Soil organic carbon (SOC%)			Total organic carbon (TOC%)			Soil inorganic carbon (SIC%)			Total carbon (TC%)		
		0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30
		cm	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm
CT	Soybean-wheat	0.85	0.68	0.57	1.13	0.90	0.76	0.16	0.19	0.14	1.29	1.09	0.90
	Maize-wheat	0.81	0.67	0.59	1.08	0.89	0.78	0.16	0.17	0.11	1.24	1.07	0.90
	Maize-chickpea	0.76	0.68	0.56	1.00	0.90	0.74	0.19	0.11	0.19	1.19	1.02	0.93
	Mean	0.81	0.68	0.57	1.07	0.90	0.76	0.17	0.16	0.15	1.24	1.06	0.91
ZT	Soybean-wheat	0.86	0.70	0.60	1.14	0.93	0.79	0.24	0.13	0.11	1.38	1.06	0.91
	Maize-wheat	1.01	0.69	0.60	1.34	0.92	0.80	0.15	0.21	0.15	1.49	1.13	0.95
	Maize-chickpea	0.93	0.70	0.58	1.22	0.93	0.78	0.09	0.16	0.15	1.30	1.08	0.92
	Mean	0.93	0.69	0.59	1.23	0.92	0.79	0.16	0.17	0.14	1.39	1.09	0.93
CD _{0.05}	TS	0.04*	ns	ns	0.07*	ns	ns	ns	ns	ns	0.06*	ns	ns
	CS	0.04*	ns	ns	0.09*	ns	ns	ns	ns	ns	0.08*	ns	ns
	TS x CS	0.06*	ns	ns	0.13*	ns	ns	ns	ns	ns	ns	ns	ns

NS- No significance difference, * - significance difference at the 5% level of significance

labile (C_L) active pool which is easily oxidizable and serves as nutrient sources, and less labile (C_{LL}) and non-labile carbon (C_{NL}) passive pool, which is the slow pool with decadal change intervals of time and is important for C sequestration in soil. The present study revealed that across all soil depths, the C pools in soil varied in the order of C_{VL} (0.55 and 0.50%) > C_{NL} (0.36 and 0.32%) > C_L (0.19 and 0.13%) > C_{LL} (0.13 and 0.12%) in both (ZT and CT) tillage systems (Table 2). All the four organic carbon pools showed decrease with increase in soil depth (Rangel *et al.*, 2008), but a relatively lower decrease was observed in non-labile (C_{NL}) pools with soil depth as compared to other pools. It was also observed that C_{VL} pool in ZT practice (0.55%) was significantly higher at soil surface than that in CT (0.50%), which might be due to the prevailing soil moisture status in ZT helping in the decomposition of soil organic matter, resulting from net changes in C_{VL} carbon pool (Khosa *et al.*, 2025). Significantly higher labile carbon content was observed at 0-10 and 10-20 cm soil depth in ZT (0.19 and 0.18%) as compared to CT (0.13 and 0.15%). This may be attributed to the additional crop residues and minimum soil disturbance, which increases soil carbon; and the labile carbon pool mainly contributed to the addition of plant material, including above and below ground biomass and living organisms (Bayer *et al.*, 2006). Long-term zero-tillage practices significantly affected the non-labile

Table 2: Effect of long-term no-tillage practice on different pools of organic carbon in soil

Tillage system (TS)	Cropping system (CS)	Different pools of organic carbon in soil											
		Very labile carbon (C_{VL} %)			Labile carbon (C_L %)			Less labile carbon (C_{LL} %)			Non labile carbon (C_{NL} %)		
		0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30
		cm	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm
CT	Soybean-wheat	0.51	0.33	0.23	0.12	0.15	0.14	0.14	0.14	0.14	0.32	0.23	0.23
	Maize-wheat	0.52	0.34	0.21	0.14	0.16	0.14	0.10	0.13	0.12	0.33	0.27	0.21
	Maize-chickpea	0.47	0.30	0.22	0.13	0.15	0.16	0.11	0.13	0.13	0.29	0.25	0.17
	Mean	0.50	0.32	0.22	0.13	0.15	0.14	0.12	0.13	0.13	0.32	0.25	0.21
ZT	Soybean-wheat	0.50	0.28	0.24	0.21	0.19	0.15	0.10	0.14	0.14	0.37	0.29	0.21
	Maize-wheat	0.58	0.31	0.23	0.18	0.18	0.16	0.15	0.15	0.13	0.41	0.25	0.23
	Maize-chickpea	0.59	0.32	0.22	0.19	0.15	0.15	0.14	0.12	0.13	0.29	0.27	0.20
	Mean	0.55	0.31	0.23	0.19	0.18	0.15	0.13	0.14	0.14	0.36	0.27	0.21
CD _{0.05}	TS	0.05*	ns	ns	0.05*	0.02*	ns	ns	ns	ns	0.04*	ns	ns
	CS	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.05*	ns	ns
	TS x CS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

NS - No significance difference, * - significance difference at the 5% level of significance

pool of SOC at upper surface of soil. The results indicated that C_{NL} pool was significantly higher in ZT (0.36%) than CT (0.32%) systems at 0-10 cm soil depth. The accumulation of C_{NL} organic carbon pool in ZT plays a critical role in enhancing soil resilience and long-term carbon sequestration. The stabilization of SOC within soil aggregates under ZT reduces the availability of carbon for microbial decomposition, leading to greater carbon retention in non-labile pools (Lehmann and Kleber, 2015).

Soil inorganic carbon (SIC) refers to the carbon stored in soils in the form of carbonate minerals, such as calcium carbonate and magnesium carbonate. Unlike SOC, SIC is not derived from organic material but rather from geological and chemical processes. The study revealed that tillage and cropping systems had no remarkable effects on SIC. Across all soil depths, SIC values ranged from 0.09 to 0.24% and 0.11 to 0.19% under ZT and CT practices, respectively (Table 1). Inorganic carbon stocks in soil remained largely unchanged under long-term no-tillage systems in semi-arid regions (Luo *et al.*, 2010). This finding suggests that zero-tillage did not influence the dynamics of SIC, which is often more stable and less responsive to management practices as compared to the SOC.

The impact of tillage systems and cropping systems on soil total carbon (TC) in ZT system demonstrated significantly higher TC (1.39%) at surface soil only than in CT system (1.24%) (Table 1). Among the cropping systems, maize-wheat recorded highest TC within ZT system, with values of 1.49% at 0-10 cm soil depth. This may be attributed to the higher biomass inputs from maize and wheat residues than other cropping systems with lower residue input contributing less to soil carbon accumulation (Powelson *et al.*, 2011).

Nitrogen dynamics in soil

The beneficial effect of ZT in terms of higher nitrogen (N) availability may be due to the crop residue retention on soil surface, arresting their leaching losses by reducing the decomposition of surface residues, and larger soil N mineralization potential as compared to CT (Yadev *et al.*, 2021). The analysis of soil available N demonstrated a significant increase in NT (229.83 kg ha⁻¹) which was 5.28% higher than CT (217.68 kg ha⁻¹) at 0-10 cm soil depth (Table 3). Residue retention on soil surface in ZT acts as a mulch and provides favourable conditions for microbial process, resulting in higher available N in soil (Choudhary *et al.*, 2018). Residue retention maintains favourable temperature and moisture conditions that regulate the processes of organic matter decomposition and nutrient cycling, resulting in higher N availability (Jat *et al.*, 2018). The present study revealed that tillage had no significant effect on ammonium nitrogen (NH₄-N) and nitrate nitrogen (NO₃-N) in soil (Table 3). NH₄-N decreased with increase in soil depth, but NO₃-N content was comparatively lower in 0-10 cm than 10-20 cm, and 20-30 cm soil depth which could be due to the NO₃-N leaching from upper surface to the lower surface. The study revealed that the NH₄-N values were slightly higher in

Table 3: Effect of long-term no-tillage practice on available NH₄-N, NO₃-N and total nitrogen in soil

Tillage system (TS)	Cropping System (CS)	Available nitrogen (kg ha ⁻¹)			Ammonium (NH ₄) nitrogen (kg ha ⁻¹)			Nitrate (NO ₃) nitrogen (kg ha ⁻¹)			Total nitrogen (TN%)		
		0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30
		cm	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm
CT	Soybean-wheat	220.37	179.80	162.74	19.08	17.24	16.26	37.13	87.28	88.87	0.123	0.109	0.087
	Maize-wheat	217.20	182.96	164.55	19.36	16.99	16.53	36.26	83.89	87.02	0.120	0.104	0.088
	Maize-chickpea	215.34	181.74	162.44	17.08	18.79	15.06	37.97	91.56	87.14	0.114	0.100	0.087
	Mean	217.64	181.50	163.24	18.51	17.67	15.95	37.12	87.58	87.68	0.119	0.105	0.087
ZT	Soybean-wheat	228.19	183.95	165.12	17.79	17.37	15.13	35.74	91.55	88.94	0.135	0.104	0.084
	Maize-wheat	237.01	184.62	165.92	21.52	18.61	17.19	34.65	87.12	83.60	0.139	0.104	0.082
	Maize-chickpea	224.31	180.27	163.91	18.46	18.67	16.00	37.05	87.99	85.78	0.126	0.103	0.087
	Mean	229.83	182.94	164.99	19.26	18.22	16.11	35.82	88.89	86.11	0.133	0.104	0.084
CD _{0.05}	TS	4.91*	ns	ns	ns	ns	ns	ns	ns	ns	0.008*	ns	ns
	CS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	TS x CS	ns	Ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

NS- No significance difference, * - significance difference at the 5% level of significance

ZT (19.26 kg ha⁻¹) than CT (18.51 kg ha⁻¹) at 0-10 cm soil depth (López-Bellido *et al.*, 2014; Sharifi *et al.*, 2008), but NO₃-N were lower in ZT (35.82 kg ha⁻¹) than CT (37.12 kg ha⁻¹) at 0-10 cm soil depth (Sainju *et al.*, 2009). Other studies have revealed that tillage increased the moisture diffusion path, causing the leaching of soil NO₃-N (Jiang *et al.*, 2005). In contrast to our findings, conservative tillage practices improved soil NO₃-N contents over conventional tillage (Sadiq *et al.*, 2021; Thimmaiah *et al.*, 2023). Crop residue can reduce N leaching and volatilization losses by minimizing the soil temperature and finally improving soil NO₃-N in soil under conservation tillage management practices (Rani *et al.*, 2017). The accumulation of crop residues near soil surface stratifies microbial activity and conserves more soil N (Schomberg and Jones, 1999). The study demonstrated that total nitrogen (TN) was higher in ZT (0.133%) than CT (0.119%) in surface soil (Table 3). Further, the concentration of TN in soil decreased with increase in soil depth which may be attributed to the low amount of decomposed organic matter in deeper soil (Avanzi and Marina, 2011; Bohoussou *et al.*, 2022).

Conclusion: The study highlights that long term zero-tillage significantly increases total organic carbon stocks in soil by reducing soil disturbance and promoting residue retention on soil surface as compared to the conventional tillage. Increased very-labile and labile carbon under ZT plays a critical role in soil fertility and nutrient cycling because these pools are energy source for soil microbes and contributes to the immediate availability of nutrients. Higher non-labile carbon pools in ZT exhibited higher carbon sequestration potential of CA, contributing to long-term carbon storage in soil. This study demonstrates that continuous zero-till is an effective management practice in the central India to conserve soil nitrogen. ZT had higher total nitrogen and enhances nitrogen availability in soil by reduces losses through leaching and volatilization, compared to conventional tillage. The conservation of soil N with continuous zero-till management indicates improved N use efficiency of the cropping system. These benefits make zero-tillage an attractive and practical option for farmers in central India which improve soil health, reduce input costs, and enhancing resilience to climatic variability.

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Author contributions: SR designed the study, collected and analysed the data, and prepared original draft. RSC supervised the project, managed resources and reviewed and edited the manuscript; NKS carried out field investigation, data curation, and manuscript revision. JS contributed to data interpretation and critically reviewed the manuscript; SKT reviewed and edited the manuscript.

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