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Residue-retained conservation agriculture and optimized nitrogen management enhance soil biological health in a wheat-greengram-maize rotation of the Indo-Gangetic Plains

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Abstract
Soil biological indicators respond rapidly to changes in tillage, residue management, and nitrogen (N) availability, making them sensitive markers of soil health in conservation agriculture (CA). A two-year field experiment (2021-22 and 2022-23) was conducted in the Indo-Gangetic Plains of India to evaluate the effects of conservation agricultural practices (CAP) and nitrogen management practices (NMP) on soil biological properties in a wheat-greengram-maize cropping system. The experiment was laid out in a split-plot design with three CAP treatments—conventional tillage (CT), zero tillage with residue retention (ZT+R), and permanently raised beds with residue retention (PB+R)—as main plots, and seven NMP treatments (N_1-N_7) as subplots. Soil microbial biomass carbon (MBC), fluorescein diacetate hydrolytic activity (FDA), and dehydrogenase activity (DHA) were measured at the key growth stages of all three crops.

Across crops and stages, CAP exerted a dominant influence on soil biological activity, with PB+R consistently recording the highest MBC, FDA, and DHA, followed by ZT+R and CT treatments. Relative to CT, PB+R increased the pooled MBC by 4-7%, FDA activity by 6-9%, and DHA by 9-15%, indicating improved microbial habitat, substrate availability, and metabolic activity under residue-retained systems. The effects of nitrogen management were comparatively smaller; optimized N treatments (N_2 and N_5) showed marginal but significant improvements in biological indicators compared to reduced-N and control treatments. The interaction effects between CAP and NMP were mostly non-significant. The results demonstrated that residue-retained conservation agriculture, particularly permanent raised beds, is the primary driver of soil biological health in cereal-legume-cereal rotations, whereas optimized nitrogen management supports but does not substitute the residue-mediated biological benefits.

Keywords: Conservation agriculture, residue retention, permanent raised beds, microbial biomass carbon, fluorescein diacetate hydrolytic activity, dehydrogenase activity, nitrogen synchrony

Introduction

Soil biological properties are increasingly recognized as sensitive and integrative indicators of soil health because they respond rapidly to management-induced changes in organic matter inputs, soil microclimate, and disturbance intensity. Microbial biomass and enzymatic activities regulate nutrient mineralization, carbon turnover, and soil structural stabilization, thereby exerting a strong control over crop productivity and system resilience. In intensively cultivated cereal-based systems of the Indo-Gangetic Plains (IGP), continuous tillage, residue removal, and heavy dependence on mineral nitrogen fertilizers have degraded soil biological function, resulting in declining nitrogen-use efficiency, weakened nutrient synchrony, and reduced sustainability (Govaerts *et al.*, 2009; Ladha *et al.*, 2020; Bhattacharyya *et al.*, 2021) [10, 12, 2]. Conservation agriculture (CA), which is based on minimal soil disturbance, permanent soil cover through residue retention, and diversified crop rotations, has emerged as an effective strategy for restoring soil biological processes in the IGP. Residue-retained CA systems improve microbial habitat stability by moderating soil temperature, conserving moisture, and supplying labile carbon substrates, which collectively stimulate microbial biomass and enzymatic activity. Long-term experiments across Delhi, Karnal, and

Patna have consistently reported 15-32% increases in microbial biomass. carbon and substantial enhancement of dehydrogenase and other soil enzymes under CA relative to conventional tillage, indicating accelerated biological nutrient cycling and improved soil functional quality (Choudhary *et al*., 2018; Parihar *et al*., 2020; Bhattacharyya *et al*., 2021) [4, 14, 2].

Nitrogen management interacts with tillage and residue practices by influencing plant growth, root exudation, and rhizodeposition, indirectly regulating microbial activity. An optimized nitrogen supply aligned with crop demand enhances biomass production and belowground carbon inputs, thereby stimulating microbial turnover and nitrogen mineralization (Sapkota *et al*., 2017; Ladha *et al*., 2020) [16, 12]. Recent evidence suggests that reduced basal nitrogen supplemented with foliar nitrogen sources can maintain crop productivity while moderating soil nitrogen extraction, potentially supporting soil biological function under CA (Parihar *et al*., 2020) [14]. Among the foliar nitrogen options, nano urea has attracted attention because of its high use efficiency and capacity to improve nitrogen synchrony, although its effects on soil biological properties remain largely indirect and mediated through plant-soil feedbacks (FAO, 2021; Bhattacharyya *et al*., 2021) [2].

Cereal-legume-cereal rotations provide additional biological advantages by diversifying residue quality and enhancing nitrogen cycling. In the IGP, wheat-greengram-maize rotations have expanded rapidly owing to their short duration, complementary resource use, and higher annual productivity compared with continuous cereal cropping systems. The inclusion of greengram contributes to biologically fixed nitrogen, labile residues, and rhizodeposits that support microbial biomass and enzymatic activity, particularly during the transition between cereal crops (Giller, 2001; Sapkota *et al*., 2017) [8, 16]. Studies across northern India have shown that such rotations can increase mineralizable nitrogen availability and microbial biomass while improving system-level productivity and profitability (Parihar *et al*., 2020) [14].

Despite the growing evidence on CA and nitrogen management individually, system-scale information on soil biological responses under residue-retained CA combined with optimized nitrogen strategies in a wheat-greengram-maize rotation remains limited, particularly under the agroecological conditions of the IGP. Moreover, comparative evidence across multiple crops and growth stages within complete rotations is scarce. Therefore, this study aimed to quantify the effects of conservation agriculture practices and nitrogen management strategies on key soil biological indicators, including microbial biomass carbon (MBC), fluorescein diacetate hydrolytic activity (FDA), and dehydrogenase activity (DHA), across a wheat-greengram-maize cropping system. It was hypothesized that residue-retained permanent beds would exert the strongest positive influence on soil biological health, whereas optimized nitrogen management would provide secondary supportive benefits through enhanced biomass production and residue return.

Materials and Methods

The field experiment was conducted during two consecutive cropping cycles from Rabi 2021 to Kharif 2023 at the Research Farm of the ICAR-Indian Agricultural Research Institute, New Delhi, India (28°38′N, 77°09′E; 228 m above the mean sea level). The site falls within the Trans-Gangetic Plains agro-climatic zone of the Indo-Gangetic Plains and is characterized by a semi-arid, subtropical climate with hot summers, cool winters, and an average annual rainfall of approximately 800 mm, which is largely concentrated during monsoon months. The soil at the experimental site was sandy loam in texture, slightly alkaline in reaction, low in organic carbon, and medium in available phosphorus and potassium, which are characteristics typical of intensively cultivated alluvial soils of the region (Parihar *et al*., 2020; Gathala *et al*., 2021) [14, 8].

The experiment was laid out in a split-plot design with three replicates. Conservation agriculture practices were assigned to the main plots and nitrogen management practices to the sub-plots. The main plot treatments comprised conventional tillage (CT), zero tillage with residue retention (ZT+R), and permanently raised beds with residue retention (PB+R). The subplot treatments included seven nitrogen management strategies: absolute control without nitrogen, 100% recommended dose of nitrogen (RDN), 75% RDN, 75% RDN supplemented with one foliar spray of 2% prilled urea, 75% RDN supplemented with one foliar spray of nano urea, 50% RDN supplemented with two foliar sprays of 2% prilled urea, and 50% RDN supplemented with two foliar sprays of nano urea. The recommended nitrogen doses were 150 kg ha^{-1} for wheat and 180 kg ha^{-1} for maize, whereas greengram received no direct nitrogen application in any treatment to enable the assessment of residual fertility effects (Sapkota *et al*., 2017; Parihar *et al*., 2020). [16, 14]

The cropping sequence consisted of wheat during the Rabi season, greengram during the Zaid season, and maize during the Kharif season, and this sequence was repeated for two years. Wheat cultivar HD 3226, greengram cultivar PUSA 1431, and maize hybrid PJHM-1 were used in this study. Crop establishment followed treatment-specific practices: conventional tillage plots were prepared using plowing, harrowing, and planking, whereas zero tillage plots were directly sown without soil disturbance. In the permanent raised bed plots, crops were established on raised beds approximately 65 cm wide and 15 cm high, which were maintained across seasons. Crop residues of the preceding crop were retained on the soil surface at approximately 3 t ha^{-1} in the ZT+R and PB+R plots, whereas residues were completely removed in the CT plots, ensuring clear contrasts in soil cover and disturbance (Govaerts *et al*., 2009; Gathala *et al*., 2021) [10, 7].

Soil biological properties were assessed to evaluate the effects of the treatments on microbial function. Soil samples were collected from 0-15 cm depth at critical crop growth stages: 35 and 70 days after sowing (DAS) in wheat, 25 and 50 DAS in greengram, and 30 and 60 DAS in maize. Fresh soil samples were gently sieved and analyzed immediately after collection. Microbial biomass carbon was determined using the chloroform fumigation-extraction method, and biomass carbon was calculated using a conversion factor of 2.64 applied to the difference in extractable organic carbon between fumigated and non-fumigated samples (Nunan *et al*., 1998). Fluorescein diacetate hydrolytic activity, which represents overall microbial enzymatic activity, was measured by incubating soil with fluorescein diacetate substrate and quantifying fluorescein release at 490 nm (Tabatabai, 1994) [17]. Dehydrogenase activity, an indicator of microbial oxidative metabolism, was assessed by measuring the reduction of triphenyl tetrazolium chloride to

triphenyl formazan and recording the absorbance at 485 nm (Casida *et al*., 1964) [3].
All data were subjected to analysis of variance appropriate for a split-plot design, following standard statistical procedures. Year-wise and pooled analyses were conducted, and treatment means were compared using the least significant difference test at the 5% probability level. Statistical analyses were performed following the procedures described by Gomez and Gomez (1984) [9].

Results

Microbial biomass carbon (MBC)

Microbial biomass carbon responded strongly and consistently to conservation agriculture practices across all crops and sampling stages in the wheat-greengram-maize rotation (Table 1; Fig. 1). Residue-retained systems significantly enhanced MBC relative to conventional tillage, with a clear ranking of PB+R > ZT+R > CT across years and for all crops. In wheat, the pooled MBC at 35 DAS increased from 150.1 μg g^{-1} under CT to 155.7 μg g^{-1} under PB+R, while at 70 DAS, the corresponding increase was from 156.8 to 166.8 μg g^{-1}, indicating progressive microbial biomass buildup under residue retention. Similar trends were observed during the greengram phase, where PB+R recorded 150.4 and 164.1 μg g^{-1} at 25 and 50 DAS, respectively, compared with 142.1 and 155.6 μg g^{-1} in CT. In maize, MBC was consistently highest under PB+R at both 30 and 60 DAS, reaching 176.6 μg g^{-1} at 60 DAS, compared with 166.4 μg g^{-1} under CT at the same time.
Nitrogen management practices exerted statistically significant but comparatively smaller effects on MBC (Table 1; Fig.1). Treatments receiving the fully recommended nitrogen (N_2) or reduced nitrogen supplemented with nano urea (N_5) consistently recorded higher MBC than the control and reduced-N treatments, although the magnitude of increase was modest relative to CA effects. Across crops, N_2 and N_5 improved MBC by approximately 2-4% over N_1, whereas PB+R increased MBC by 6-12% over CT, highlighting the dominance of tillage-residue management in regulating microbial biomass. Most CAP × NMP interaction effects were non-significant, indicating that the nitrogen effects were largely additive rather than synergistic with CA.
The year effects were significant for all crops, with higher MBC recorded during 2022-23 than 2021-22 (Table 1), reflecting cumulative residue inputs and progressive stabilization of microbial habitats under CA. However, the relative treatment ranking remained consistent across the years, confirming the robustness of the CA-driven biological responses.

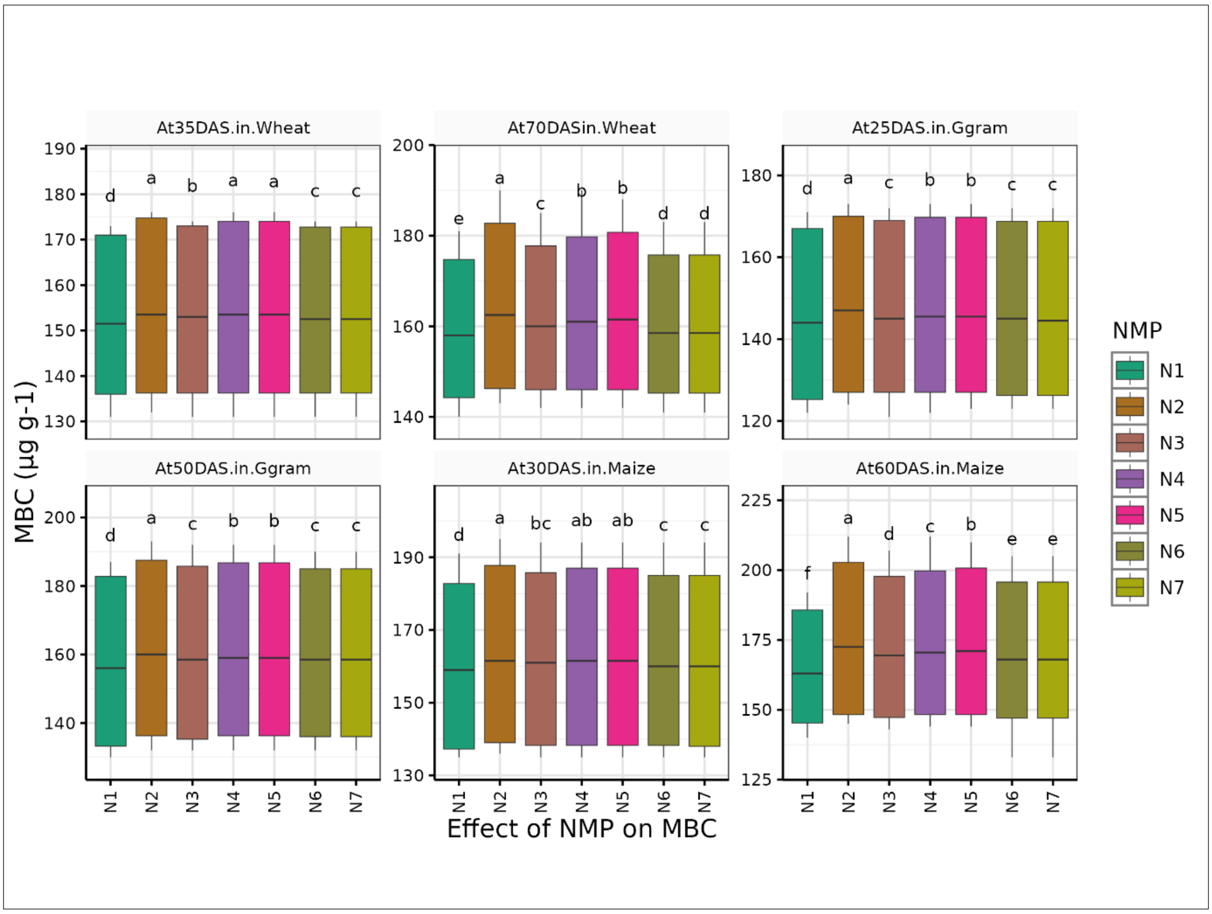

Fig 1: Effect of nitrogen management practices on microbial biomass carbon (MBC) across the wheat-greengram-maize cropping system (pooled over 2021-22 and 2022-23).

Table 1: Effect of conservation agricultural practices and nitrogen management on microbial biomass carbon (MBC, µg g^{-1} soil) at different crop growth stages in a wheat-greengram-maize cropping system (pooled over 2021-22 and 2022-23).

Treatment	**MBC (µg g^{-1})**					
	Crop growth stage					
	Wheat		Greengram		Maize	
	35DAS	**70DAS**	**25DAS**	**50DAS**	**30DAS**	**60DAS**
Conservation Agricultural Practices						
CT	150.12 ± 18.41^{c}	156.79 ± 15.07^{c}	142.07 ± 19.18^{c}	155.60 ± 23.14^{c}	157.67	166.40 ± 24.69^{c}
ZT + R	154.98 ± 18.61^{b}	161.95 ± 16.81^{b}	147.71 ± 21.71^{b}	160.55 ± 25.51^{b}	162.07 ± 24.30^{b}	172.12 ± 25.62^{b}
PB + R	155.74 ± 18.80^{a}	166.76 ± 18.08^{a}	150.43 ± 21.23^{a}	164.12 ± 26.39^{a}	167.50 ± 25.98^{a}	176.55 ± 29.01^{a}
SEm±	0.18	0.09	0.14	0.18	0.12	0.19
CD (P = 0.05)	0.59	0.3	0.46	0.57	0.39	0.61
Nitrogen Management Practices						
N_1	152.44 ± 18.34^{d}	159.33 ± 16.30^{e}	145.56 ± 21.16^{d}	157.50 ± 25.20^{d}	160.56 ± 24.14^{d}	165.00 ± 21.99^{f}
N_2	154.44 ± 19.84^{a}	164.61 ± 19.09^{a}	147.83 ± 21.43^{a}	161.33 ± 26.13^{a}	163.44 ± 25.44^{a}	175.50 ± 29.03^{a}
N_3	153.67 ± 19.03^{b}	161.78 ± 16.93^{c}	146.56 ± 21.52^{c}	160.17 ± 25.77^{c}	162.56 ± 24.98^{bc}	172.33 ± 27.18^{d}
N_4	154.17 ± 19.58^{a}	162.94 ± 18.18^{b}	147.11 ± 21.61^{b}	160.83 ± 25.88^{b}	163.00 ± 25.32^{ab}	173.89 ± 28.02^{c}
N_5	154.11 ± 19.51^{a}	163.28 ± 18.43^{b}	147.22 ± 21.35^{b}	160.83 ± 25.88^{b}	163.00 ± 25.32^{ab}	174.22 ± 28.30^{b}
N_6	153.22 ± 18.60^{c}	160.44 ± 16.31^{d}	146.44 ± 21.27^{c}	159.94 ± 25.52^{c}	162.17 ± 24.94^{c}	170.44 ± 27.22^{e}
N_7	153.22 ± 18.60^{c}	160.44 ± 16.31^{d}	146.44 ± 21.27^{c}	160.00 ± 25.47^{c}	162.17 ± 24.94^{c}	170.44 ± 27.22^{e}
SEm±	0.13	0.13	0.17	0.17	0.24	0.24
CD (P = 0.05)	0.36	0.37	0.49	0.49	0.66	0.66
Year						
2021-22	135.25 ± 2.48^{b}	145.52 ± 3.02^{b}	126.30 ± 2.76^{b}	135.41 ± 2.50^{b}	138.49 ± 2.72^{a}	145.89 ± 3.38^{b}
2022-23	171.97 ± 2.94^{a}	178.14 ± 6.11^{a}	167.17 ± 4.62^{a}	184.76 ± 5.12^{a}	186.33 ± 5.68^{b}	197.49 ± 8.03^{a}
SEm±	0.07	0.07	0.09	0.09	0.13	0.13
CD (P = 0.05)	0.19	0.2	0.26	0.26	0.36	0.36
CAP × NMP	NS	NS	NS	NS	NS	1.15
Year × CAP	NS	0.43	0.66	0.81	0.56	0.87
Year × NMP	0.51	0.52	NS	0.7	NS	0.94
Year × CAP × NMP	NS	NS	NS	NS	NS	NS

Table 2: Effect of conservation agricultural practices and nitrogen management on fluorescein diacetate (FDA) hydrolytic activity (µg fluorescein g soil $^{-1}$ h^{-1}) at different crop growth stages in a wheat-greengram-maize cropping system (pooled over 2021-22 and 2022-23).

Treatment	**FDA (µg fluorescein g^{-1} soil hr^{-1})**					
	Crop growth stage					
	Wheat		Greengram		Maize	
	35DAS	**70DAS**	**25DAS**	**50DAS**	**30DAS**	**60DAS**
Conservation Agricultural Practices						
CT	12.37 ± 0.79^{c}	13.86 ± 0.76^{c}	10.10 ± 0.28^{c}	12.18 ± 1.04^{c}	12.18 ± 0.27^{c}	13.59 ± 0.72^{c}
ZT + R	12.59 ± 0.82^{b}	14.25 ± 0.63^{b}	10.93 ± 0.86^{b}	12.69 ± 1.39^{b}	12.46 ± 0.28^{b}	14.24 ± 1.00^{b}
PB + R	12.88 ± 0.84^{a}	14.59 ± 0.73^{a}	11.32 ± 1.01^{a}	13.04 ± 1.27^{a}	12.86 ± 0.40^{a}	14.53 ± 0.99^{a}
SEm±	0.18	0.1	0.01	0.23	0.01	0.01
CD (P = 0.05)	0.58	0.34	0.02	0.74	0.04	0.04
Nitrogen Management Practices						
N_1	11.88 ± 1.12^{g}	13.97 ± 0.69^{d}	10.63 ± 0.96^{d}	12.49 ± 1.27^{e}	12.32 ± 0.41^{e}	13.77 ± 1.07^{f}
N_2	13.06 ± 0.74^{a}	14.42 ± 0.82^{a}	10.94 ± 0.93^{a}	12.79 ± 1.35^{a}	12.65 ± 0.41^{a}	14.40 ± 0.87^{a}
N_3	12.64 ± 0.67^{d}	14.20 ± 0.75^{c}	10.77 ± 0.96^{c}	12.65 ± 1.30^{c}	12.49 ± 0.42^{c}	14.11 ± 1.00^{d}
N_4	12.80 ± 0.75^{c}	14.32 ± 0.78^{b}	10.84 ± 0.96^{b}	12.72 ± 1.32^{b}	12.57 ± 0.42^{b}	14.23 ± 0.93^{c}
N_5	12.91 ± 0.72^{b}	14.37 ± 0.81^{b}	10.86 ± 0.96^{b}	12.74 ± 1.34^{ab}	12.59 ± 0.44^{b}	14.33 ± 0.89^{b}
N_6	12.53 ± 0.65^{e}	14.16 ± 0.76^{c}	10.73 ± 0.93^{c}	12.53 ± 1.30^{d}	12.44 ± 0.42^{d}	14.02 ± 1.04^{e}
N_7	12.49 ± 0.72^{f}	14.19 ± 0.78^{c}	10.73 ± 0.94^{c}	12.54 ± 1.31^{d}	12.46 ± 0.44^{d}	13.98 ± 1.11^{e}
SEm±	0.24	0.24	0.02	0.24	0.01	0.02
CD (P = 0.05)	0.66	0.66	0.04	0.66	0.04	0.04
Year						
2021-22	11.91 ± 0.53^{a}	13.55 ± 0.34^{a}	10.09 ± 0.23^{b}	11.43 ± 0.28^{a}	12.21 ± 0.26^{b}	13.26 ± 0.42^{b}
2022-23	13.32 ± 0.36^{b}	14.91 ± 0.36^{b}	11.48 ± 0.84^{a}	13.85 ± 0.53^{b}	12.79 ± 0.36^{a}	14.98 ± 0.54^{a}
SEm±	0.13	0.13	0.01	0.13	0.01	0.01
CD (P = 0.05)	0.36	0.36	0.02	0.36	0.02	0.02
CAP × NMP	NS	NS	NS	NS	NS	NS
Year × CAP	0.82	0.48	0.03	1.04	0.05	0.05
Year × NMP	0.94	0.94	NS	NS	NS	0.06
Year × CAP × NMP	NS	1.63	NS	NS	NS	NS

Fluorescein diacetate (FDA) hydrolytic activity

Fluorescein diacetate hydrolytic activity, an integrative indicator of total microbial enzymatic activity, showed pronounced enhancement under residue-retained CA systems across all crops and growth stages (Table 2; Fig. 2). In wheat, pooled FDA activity at 35 DAS increased from 12.37 µg g^{-1} h^{-1} under CT to 12.88 µg g^{-1} h^{-1} under PB+R, while at 70 DAS the increase was from 13.86 to 14.59 µg g^{-1} h^{-1}. The effect was equally evident in greengram, where the combination of PB and R recorded the highest FDA activity at both 25 DAS (11.32 µg g^{-1} h^{-1}) and 50 DAS (13.04 µg g^{-1} h^{-1}), significantly exceeding the CT. In maize, PB+R maintained superior FDA activity throughout the season, reaching 14.53 µg g^{-1} h^{-1} at 60 DAS compared with 13.59 µg g^{-1} h^{-1} under CT.

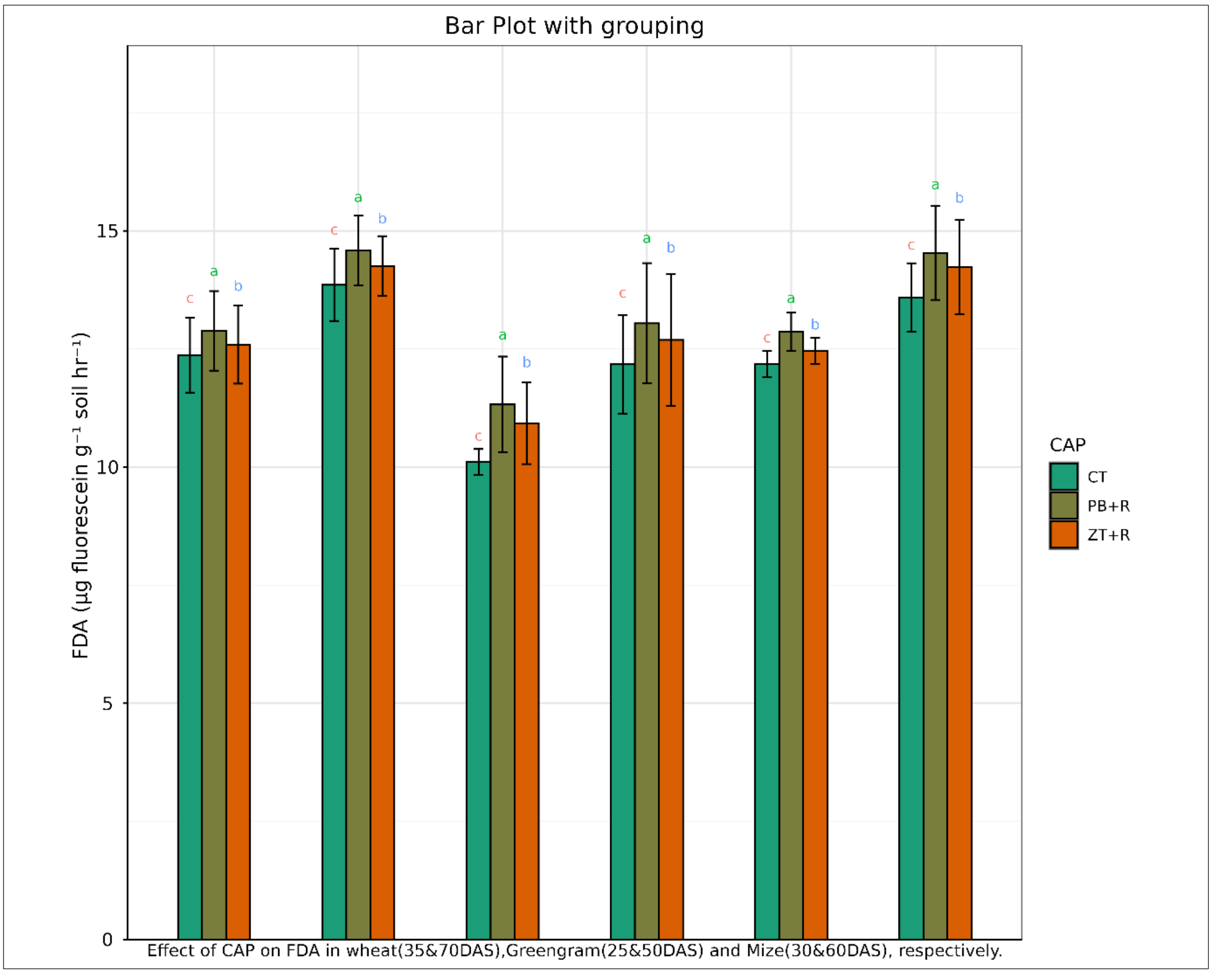

Fig. 2: Effect of conservation agricultural practices on fluorescein diacetate (FDA) hydrolytic activity across the wheat-greengram-maize cropping system (pooled over 2021-22 and 2022-23).

Nitrogen management on FDA activity were significant but secondary to the effects of CA (Table 2). N_2 consistently recorded the highest FDA values across crops, followed closely by N_5, indicating that the optimized nitrogen supply supported microbial enzymatic activity. However, the differences among the nitrogen treatments were relatively narrow, and CAP × NMP interactions were largely non-significant, reinforcing that residue retention and reduced disturbance were the primary drivers of enzymatic activity. FDA activity increased significantly in the second year across all crops, particularly during the greengram and maize phases (Table 2), reflecting the cumulative biological benefits of residue retention and improved soil microclimate under CA systems.

Dehydrogenase activity (DHA)

Among the measured biological indicators, dehydrogenase activity exhibited the strongest and most consistent response to conservation agriculture (Table 3; Fig. 3). PB+R recorded significantly higher DHA than ZT+R and CT. across all crops and growth stages. In wheat, pooled DHA increased from 93.7 µg TPF g^{-1} day^{-1} under CT to 102.1 µg TPF g^{-1} day^{-1} under PB+R at 35 DAS, and from 108.9 to 120.1 µg TPF g^{-1} day^{-1} at 70 DAS in response to PB+R. During the greengram phase, DHA under PB+R reached 102.7 µg TPF g^{-1} day^{-1} at 50 DAS, compared to 94.2 µg TPF g^{-1} day^{-1} under CT. In maize, PB+R maintained the highest DHA throughout the season, reaching 117.3 µg TPF g^{-1} day^{-1} at 60 DAS, which was 9.3% higher than that of CT.

Nitrogen management influenced DHA more strongly than MBC and FDA, although the effect size was smaller than that of CA (Table 3). N_2 consistently recorded the highest DHA across crops, followed by N_5, indicating improved microbial oxidative metabolism under an optimized nitrogen supply. Reduced-N treatments without supplementation (N_6 and N_7) resulted in lower DHA, particularly during the later growth stages. Nevertheless, CAP × NMP interactions were non-significant, confirming that the nitrogen effects were supportive rather than determinative.

The year effects were significant for DHA in all crops, with higher activity recorded during 2022-23, particularly in maize (Table 3). This reflects the cumulative biological activation under sustained residue retention and reduced disturbance.

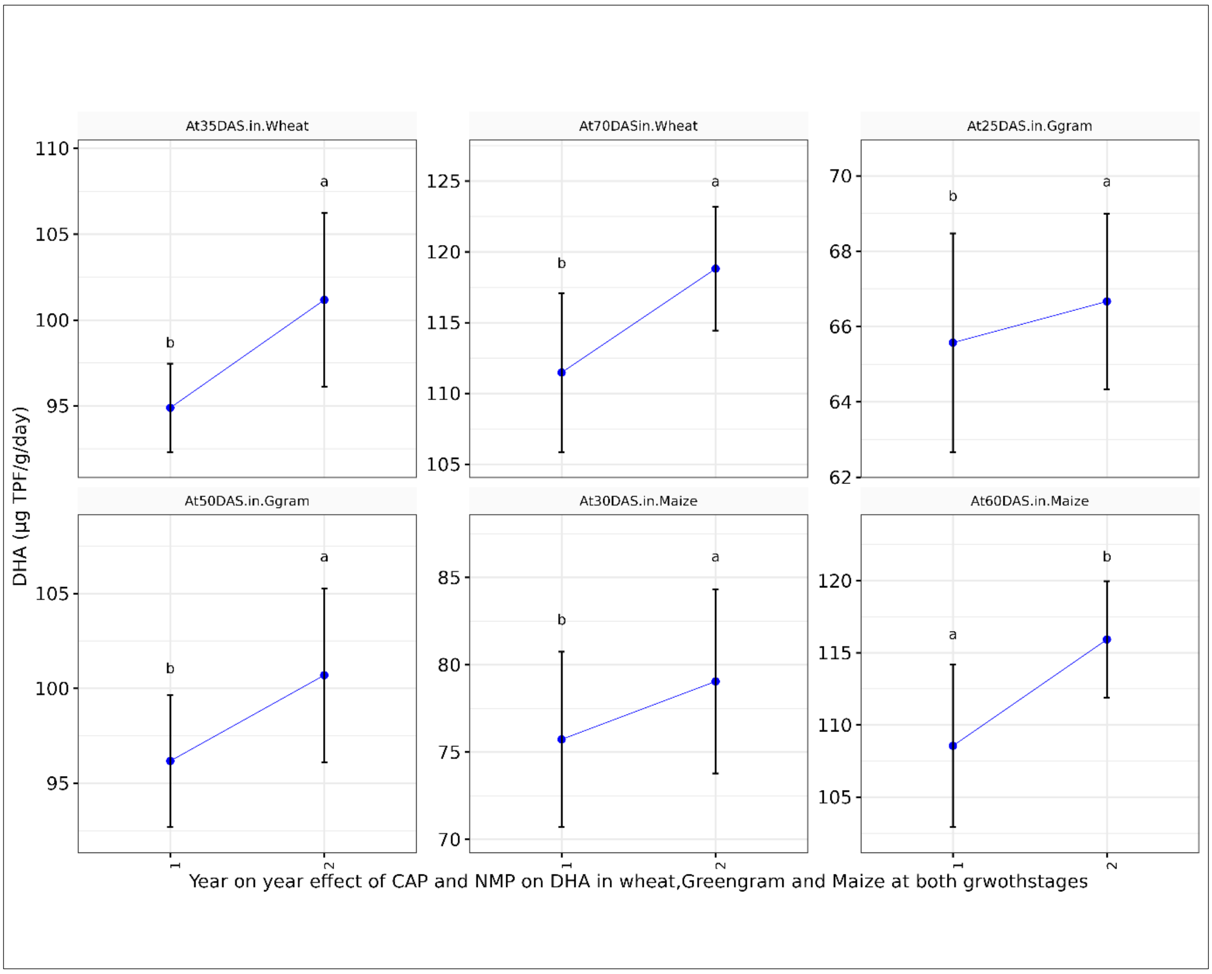

Fig. 3: Year-wise effect of conservation agricultural practices (CAP) and nitrogen management practices (NMP) on dehydrogenase activity (DHA) across the wheat-greengram-maize cropping system (pooled over 2021-22 and 2022-23).

Integrated response across the cropping system

Across all biological indicators and crops, conservation agriculture consistently followed the order PB+R > ZT+R > CT, demonstrating that residue retention combined with minimal disturbance is the dominant driver of soil biological health in the wheat-greengram-maize rotation. Importantly, enhanced microbial activity under CA was maintained even during the greengram phase, which received no direct nitrogen application, highlighting the role of residual fertility and residue-mediated biological buffering. Nitrogen management provided secondary benefits, with N_2 and N_5 marginally enhancing biological activity, but it could not compensate for the absence of residue retention and reduced tillage.

Table 3: Effect of conservation agricultural practices and nitrogen management on dehydrogenase activity (DHA, µg TPF g^{-1} soil 24 h^{-1}) at different crop growth stages in a wheat-greengram-maize cropping system (pooled over 2021-22 and 2022-23).

Treatment	**DHA (µg TPF/g/day) in Wheat**					
	Crop growth stage					
	Wheat		Greengram		Maize	
	35DAS	**70DAS**	**25DAS**	**50DAS**	**30DAS**	**60DAS**
Conservation Agricultural Practices						
CT	93.71 ± 2.03^{c}	108.95 ± 5.13^{c}	64.12 ± 1.97^{c}	94.17 ± 2.27^{c}	71.98 ± 2.68^{c}	107.26 ± 5.59^{c}
ZT + R	98.24 ± 3.84^{b}	116.38 ± 3.26^{b}	65.81 ± 2.24^{b}	98.45 ± 3.41^{b}	77.05 ± 2.99^{b}	112.19 ± 4.50^{b}
PB + R	102.14 ± 4.86^{a}	120.10 ± 3.84^{a}	68.43 ± 1.82^{a}	102.69 ± 3.43^{a}	83.14 ± 2.87^{a}	117.26 ± 3.39^{a}
SEm±	0.17	0.1	0.1	0.12	0.14	0.22
CD (P = 0.05)	0.54	0.34	0.33	0.4	0.44	0.72
Nitrogen Management Practices						
N_1	95.67 ± 4.98^{f}	112.28 ± 5.82^{f}	63.00 ± 2.11^{e}	95.22 ± 4.37^{f}	73.44 ± 5.40^{f}	107.83 ± 5.86^{e}
N_2	100.00 ± 4.81^{a}	117.11 ± 6.28^{a}	68.78 ± 1.93^{a}	101.28 ± 4.24^{a}	80.17 ± 4.82^{a}	115.28 ± 5.76^{a}
N_3	97.89 ± 5.18^{d}	115.11 ± 6.27^{d}	66.06 ± 2.04^{c}	98.28 ± 4.28^{d}	77.17 ± 5.01^{d}	112.44 ± 5.71^{c}
N_4	98.67 ± 5.29^{c}	115.94 ± 6.40^{c}	67.11 ± 2.37^{b}	99.17 ± 4.50^{c}	78.44 ± 5.08^{c}	113.44 ± 5.95^{b}
N_5	99.17 ± 4.97^{b}	116.44 ± 6.27^{b}	67.56 ± 1.89^{b}	100.06 ± 4.39^{b}	79.67 ± 5.26^{b}	113.72 ± 6.27^{b}
N_6	97.22 ± 4.98^{e}	114.44 ± 6.11^{e}	65.00 ± 2.03^{d}	97.39 ± 4.49^{e}	76.28 ± 4.91^{e}	111.22 ± 5.78^{d}
N_7	97.61 ± 5.04^{de}	114.67 ± 6.13^{e}	65.33 ± 2.03^{d}	97.67 ± 4.35^{e}	76.56 ± 4.97^{e}	111.72 ± 5.62^{d}
SEm±	0.15	0.15	0.17	0.14	0.17	0.24
CD (P = 0.05)	0.43	0.44	0.47	0.39	0.49	0.66

Year						
2021-22	94.89 ± 2.57^{b}	111.48 ± 5.61^{b}	65.57 ± 2.90^{b}	96.17 ± 3.48^{b}	75.73 ± 5.02^{b}	108.56 ± 5.62^{a}
2022-23	101.17 ± 5.05^{a}	118.81 ± 4.36^{a}	66.67 ± 2.33^{a}	100.70 ± 4.58^{a}	79.05 ± 5.26^{a}	115.92 ± 4.04^{b}
SEm±	0.08	0.08	0.09	0.07	0.09	0.13
CD (P = 0.05)	0.23	0.23	0.25	0.21	0.26	0.36
CAP × NMP	NS	NS	NS	NS	NS	NS
Year × CAP	NS	0.48	0.47	0.57	0.62	1.02
Year × NMP	NS	NS	NS	NS	NS	NS
Year × CAP × NMP	NS	NS	NS	NS	NS	NS

Discussion

The present study demonstrates that soil biological properties in the wheat-greengram-maize cropping system were regulated mainly by conservation agricultural practices (CAP), whereas nitrogen management practices (NMP) exerted secondary effects. Across crops and stages, residue-retained conservation agriculture, particularly permanent raised beds with residue retention (PB+R), consistently increased microbial biomass carbon (MBC), fluorescein diacetate (FDA) hydrolytic activity, and dehydrogenase activity (DHA) relative to zero tillage with residue retention (ZT+R) and conventional tillage (CT) (Tables 1-3; Figs. 1-3). This confirms that biological indicators respond rapidly to shifts in residue-derived substrate supply, microclimate buffering, and reduced physical disturbance under the CA.

Higher MBC under PB+R and ZT+R reflects improved carbon inputs and microbial habitat stability promoted by residue retention and minimal soil disturbance. Residues provide labile and particulate organic carbon, whereas reduced tillage preserves aggregates and microbial niches, limiting organic matter oxidation. Similar increases in microbial biomass under CA-based systems in the Indo-Gangetic Plains have been reported, where cereal-legume rotations enhanced surface carbon dynamics and biological nutrient immobilization (Choudhary *et al*., 2018; Parihar *et al*., 2020; Ladha *et al*., 2020) [4, 14, 12]. The persistence of the PB+R > ZT+R > CT ranking across wheat, greengram, and maize indicates a system-driven benefit rather than a crop-specific effect on the system.

The higher FDA activity under PB+R throughout the rotation (Table 2; Fig. 2) indicates a stronger overall enzymatic capacity and faster turnover of organic substrates under residue-retained management. FDA generally increased from early to later sampling stages across crops, consistent with the progressive stimulation of microbial function as root activity and residue decomposition advanced. These responses are consistent with evidence that residue cover stabilizes temperature-moisture regimes and supports enzyme-mediated nutrient cycling in CA systems (Govaerts *et al*., 2009; Corbeels *et al*., 2018) [10, 5].

Among the indicators, DHA showed the most consistent and pronounced improvement under CA (Table 3; Fig. 3), reflecting the enhanced oxidative metabolism and respiration of active microbial communities. Comparable stimulation of microbial redox activity under CA has been observed in cereal systems, where residue retention and reduced tillage improve biological functioning and nitrogen turnover potential (Bhattacharyya *et al*., 2021; Gathala *et al*., 2021) [2, 7]. The stronger DHA response relative to the MBC suggests that CA increases microbial metabolic intensity along with microbial biomass.

Nitrogen management influenced the biological properties modestly compared to CAP. Treatments receiving full nitrogen (N_2) and reduced basal nitrogen supplemented with nano urea (N_5) tended to record slightly higher MBC, FDA, and DHA than low-N or un-supplemented treatments, but effect sizes remained smaller than CAP-driven differences (Tables 1-3; Figs. 1-3). This indicates that NMP affects soil biology mainly via indirect pathways, such as crop growth, rhizodeposition, and residue return, rather than through rapid structural changes in the soil environment. Notably, CAP × NMP interactions were generally non-significant for FDA and DHA, and for MBC at most stages; the main exception was MBC in maize at 60 DAS (Table 1), suggesting that under warm-season conditions, the nitrogen strategy may more strongly modulate residue-driven microbial responses.

The greengram phase sustained higher biological activity under CA despite receiving no direct nitrogen input, emphasizing the buffering role of residues and stable moisture conditions in supporting microbial pools during the summer pulse. Overall, the data provide strong evidence that residue-retained CA—particularly PB+R—is the dominant driver of biological soil health in this rotation, whereas optimized nitrogen strategies (N_2 or N_5) complement CA by supporting nitrogen synchrony and residue-mediated microbial functioning across the system.

Conclusion

PB+R consistently produced the highest soil biological health across the wheat-greengram-maize rotation, increasing microbial biomass carbon (MBC), fluorescein diacetate (FDA) hydrolytic activity, and dehydrogenase activity (DHA) compared to ZT+R and CT at all measured crop stages. ZT+R remained intermediate, confirming that residue retention with reduced disturbance is essential for sustaining the microbial activity. The effects of nitrogen management were smaller than those of CAP, but N_2 and N_5 generally maintained slightly higher biological activity than low-N and control treatments, indicating a supportive role through improved crop growth and residue/rhizodeposition inputs. CAP × NMP interactions were mostly non-significant, with the notable exception of maize MBC at 60 DAS, suggesting that warm-season microbial responses may be more sensitive to nitrogen strategies under CA. Overall, residue-retained CA, especially PB+R, is the primary pathway for biological soil restoration in the Indo-Gangetic Plains, and it is best complemented by optimized nitrogen management, such as N_2 (full RDN) or N_5 (75% RDN + nano urea), to sustain biological functioning across the system.

Declarations

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- **Conflict of interest:** The authors declare no conflicts of interest.
- **Data availability:** The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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