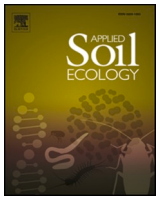
Applied Soil Ecology 193 (2024) 105128

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil



Biological soil health with conventional and qPCR based indicators under conservation agriculture based rice-wheat cropping system in Indo-Gangetic Plain

Sujit Das [a], Sunanda Biswas [a,*], B. Ramakrishnan [b], T.K. Das [c], T.J. Purakayastha [a], B.H. Gawade [d], Priya Singh [a], Partha Sarathi Ghorai [a], Saloni Tripathy [a], Kanchan Sinha [e]

[a] Division of Soil Science and Agricultural Chemistry, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India
[b] Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India
[c] Division of Agronomy, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India
[d] Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012, India
[e] Division of Agricultural Statistics, ICAR-Indian Agricultural Statistical Research Institute, New Delhi 110012, India

ARTICLE INFO

Keywords:
Biological soil health
Conservation agriculture
qPCR-based indicators

ABSTRACT

Conservation agriculture (CA) entails resource sustainability, crop productivity and climate benefits. We assessed biological soil health index (BSHI) using both conventional and state-of-the-art indicators for a long-term rice-wheat system under a regime of CA-practices in Indo-Gangetic Plains (IGP). The practices include zero till direct seeded rice (ZTDSR)–zero till wheat (ZTW), ZTDSR + wheat residue (WR)-ZTW + rice residue (RR), ZTDSR + WR + sesbania brown manuring (SBM)–ZTW + RR, ZTDSR–ZTW–zero till mungbean (ZTMB), ZTDSR + mungbean residue (MR)–ZTW + RR-ZTMB + WR, transplanted rice (TPR)-conventional till wheat (CTW)–conventional till mungbean (CTMB). Collected soil samples (0–5 cm depth) were analysed for 8 labile organic carbon pools, 8 soil enzyme activities, population of 7 microbes viz., bacteria, fungi and actinomycetes, and two microbial functions. Further, abundance of different phylogenetic groups and nutrient cycling genes was quantified by molecular based qPCR technique. In general, triple/double ZT + crop residues caused an increase in most of the pools of carbon, enzymes, and microbial population including qPCR-led genes in soils. Specifically, inclusion of mungbean residues in triple ZT and sesbania brown manuring in double ZT improved *nifH* gene abundance over other double and triple ZT treatments. Of the analysed parameters, β-glucosidase, *Bacterial amoA*, *Archaeal* 16S rRNA, *Bacteroidetes* 16S rRNA, *Bacterial* 16S rRNA, and mineralizable C were screened out as the key indicators of BSHI; its value was maximised under triple ZT with residues (ZTDSR + MR–ZTW + RR–ZTMB + WR) treatment. Attempt may be made to use the screened indicators for assessment of BSHI and upscale the identified practice for rice-wheat system in IGP.

1. Introduction

Conservation agriculture (CA) is an alternative farming system that imparts sustainability to production system ensuring resource conservation and mitigation of adverse climatic impacts (Das et al., 2014). It is based on three principles: reducing or eliminating tillage to lessen disturbance of the soil and the ecosystem, diversifying and lengthening crop rotations, and maintaining permanent ground cover (Bhattacharyya et al., 2015). Its global uptake is thus increasing. Rice-wheat (RW) cropping system, occupying ~24 Mha of land, is the foundation of food security of Asian countries (Ladha et al., 2009). Of late, productivity of the system is plateaued and proved to be a threat to long-term environmental sustainability (Bhatt et al., 2016). To overcome the problems, CA-based rice-wheat system is introduced for the farmers of the Indo-Gangetic Plains (IGP).

Widespread degradation of soil and its health under RW systems is reported from across the IGP. Depletion of organic carbon content in soils under intensive cropping with the system is one of the major reasons for such degradation. Reports are also aplenty confirming that soil organic C is one of the key indicators of soil health for different types of soils under various cropping systems including RW across the globe. Conservation agricultural practices with lot of crop residue C may

* Corresponding author.
E-mail address: sunandabiswas13@gmail.com (S. Biswas).

https://doi.org/10.1016/j.apsoil.2023.105128
Received 31 May 2023; Received in revised form 27 August 2023; Accepted 5 September 2023
Available online 10 September 2023

supplement some of the lost C and thus help in upkeeping soil health. However, such study on soil health and on its key indicators under CA practices is scarce.

'Soil health' and 'soil quality' are often used synonymously. However, while using 'soil health' we generally emphasis biological parameters of soil (Ghorai et al., 2023). Again, for keeping the soil 'living' and resilient, as is required under the present situation, we need to emphasis on biological parameters; while most of the soil health or soil quality studies made so far at different parts of the world, emphasis has been given on physical and chemical parameters because of tortuous and costly methods for measurement of biological parameters.

Considering the importance of 'soil health' vis-à-vis 'soil quality', in the present study, in a first, we tried to evaluate biological soil health index for a long-term CA-based RW system measuring a large number of biological parameters. For example, we measured a large number of labile soil organic carbon pools used as energy materials by microbes, a number of soil enzyme activities, population of bacteria, fungi, actinomycetes, nematodes etc. and a few soil functions. Besides, using state-of-the-art technique (qPCR method), we also made quantitative evaluation of the abundances of particular phylogenetic groups of microorganisms in soil. The qPCR method allows for a quantitative evaluation of the abundances of particular phylogenetic groups of microorganisms in soil in a very short amount of time (Fierer et al., 2005). These are done for their sensitivities towards a regime of CA-practices over the conventional ones. We, therefore, hypothesised that long-term conservation agricultural practices with different levels of zero tillage, crop residues incorporation and green manuring have distinct effects on labile pools of SOC, soil enzymatic activities, and community structure and functions of soil microorganisms which, in turn, influence, biological health of soils. With this background, we addressed the following objectives in the present study: i) to assess the effects of a regime of conservation agricultural practices on various labile pools of soil organic carbon, enzymes activities, and community structure and functions of soil microbes, ii) to identify key biological soil health indicators and develop biological soil health index, and iii) to derive relationships between soil organic carbon and selected biological soil health indicators. The novelty of the present investigation lies in development of biological soil health index by taking both conventional and state-of-the-art biological parameters into consideration and based on this identification of the best CA-practices for upscaling among the rice-wheat growing farmers of South and South East Asian countries.

2. Materials and methods

2.1. Study area and experimental details

The experiment was laid out in a split plot design with three replications in 2010 at the ICAR-Indian Agricultural Research Institute (IARI), New Delhi (28°35′N, 77°12′E, altitude 228 m above mean sea level). The site experiences a sub-tropical and semi-arid climate with hot and dry summers and cold winters. May and June are the hottest months, while January is the coldest with maximum and minimum temperatures of 40.0 to 46.0 and 6.0 to 8.0 °C, respectively. Eighty per cent of the site's total rainfall (710 mm) is received during July to September. Its soil (Typic Haplustept) had a sandy loam texture with pH 7.8, organic carbon 0.51 %, electrical conductivity 0.64 dS m^{-1}, available N 272.6 kg ha^{-1}, available P 10.2 kg ha^{-1}, and available K 208 kg ha^{-1}. There were six treatments with double and triple zero tillage (ZT), and with and without crop residues (Table 1). The double/triple ZT systems with crop residues were considered as the conservation agricultural (CA) practices with all its three principles. The triple cropping system is not usually followed by farmers of the region; hence, it was taken as a futuristic treatment to compare with the traditional transplanted puddled rice (TPR) – conventionally tilled wheat (CTW) system. Rice (Arize 6129 Gold, duration 115–125 days) was sown by using a previously-calibrated multi-crop planter with 20 kg seed ha^{-1} at 20 cm × 5 cm spacing and at 2–3 cm depths of soil. Under transplanted rice (TPR) system, seed were sown in nursery beds with 40 kg seed per 1000 m^2 nursery area for transplanting of 1 ha (with TPR-CTW). Transplanting was done manually with 25 days old seedlings in TPR treatment. In all ZT treatments, wheat (HDCSW 18, duration 140–145 days) was sown after a pre-sowing irrigation by using a turbo seeder (inclined plate seed metering system) in 20 cm × 5 cm spacing with 100 kg seed ha^{-1}. Summer mungbean (SML 832, duration 65–75 days) was sown after wheat harvest by using a turbo seeder under ZT conditions. Irrigation was applied through border strip method. The standing water of 2–2.5 cm was maintained in TPR through irrigation almost at every 3 days. Irrigation at 50 % soil moisture depletion was applied in DSR. Recommended doses of 120 kg N, 60 kg P_2O_5 and 40 kg K_2O ha^{-1} as urea, single super phosphate, and muriate of potash, respectively were applied to each of rice and wheat crops. Thirty per cent of the recommended N and the full doses of P and K were applied as basal. Remaining amount of N was applied in two equal halves at active tillering and panicle initiation stages. Diammonium phosphate was applied at the rate of 100 kg ha^{-1} to mungbean as basal.

Table 1
Details of the treatments and their operation.

Treatment	Operations performed	Treatment short forms
Zero till direct seeded rice (ZTDSR)–zero till wheat (ZTW)	Zero till sowing of DSR (20 kg seed ha^{-1}) and ZTW (100 kg seed ha^{-1}) was done using a turbo seeder with row spacing of 20 cm both for rice and wheat.	ZTDSR-ZTW
ZTDSR + wheat residue (WR)-ZTW + rice residue (RR)	In addition to ZTDSR–ZTW, 40 % wheat residue was maintained in rice crop and 40 % rice residue was retained in wheat crop.	ZTDSR + WR–ZTW + RR
ZTDSR + WR + sesbania brown manuring (SBM)–ZTW + RR	In addition to ZTDSR+WR–ZTW + RR, *Sesbania bispinosa* (20 kg ha^{-1}) was grown as a co-culture for about 25 days after sowing (DAS) producing above-ground dry biomass weight of 1.5–2.0 t ha^{-1} and provided ~30–35 kg N ha^{-1}.	ZTDSR + WR + SBM-ZTW + RR
ZTDSR–ZTW–zero till mungbean (ZTMB)	In addition to ZTDSR-ZTW, mungbean (*Vigna radiata*) cv. SML 832, short duration (60–65 days) variety was grown after wheat. Mungbean was sown by using a turbo seeder in mid-April produced shoot dry biomass ~1.6–2.0 t ha^{-1} and ~50–55 kg N ha^{-1} to soil.	ZTDSR-ZTW-ZTMB
ZTDSR + mungbean residue (MR)–ZTW + RR-ZTMB + WR	In addition to the ZTDSR–ZTW-ZTMB, after picking the matured pods of mung bean, whole mungbean biomass was ploughed.	ZTDSR + MR–ZTW + RR–ZTMB + WR
Transplanted rice (TPR)-conventional till wheat (CTW)–conventionally tilled mungbean (CTMB)	In TPR, two diskings and two harrowings were done and then soil was puddled. For CTW two disking and two harrowing were done and wheat (100 kg seed ha^{-1}) was sown at 20 cm row spacing with seed drill followed by conventional tillage mungbean.	TPR-CTW-CTMB

2.2. Sampling and methods of analysis

After harvesting of rice in October 2020, triplicate soil samples (at

0–5.0 cm layer) were collected from each of the replications/plots (42.0 m × 4.0 m) of the selected six treatments (Table 1). During soil sampling and sample preparation process, visible pieces of crop residues and gravels were removed. Each of the collected soil samples was divided into three parts: one was kept in refrigerator at 4 °C for analysis of biological parameter; the second was air-dried and processed for analysis of the labile pools of carbon; and the third part was processed for analysis of the physical parameters.

To assess the changes in physico-chemical properties of the experimental soils, bulk density (BD) (Blake and Hartge, 1986), mean weight diameter (MWD) (Yoder, 1936), pH (Page et al., 1982), $KMnO_4$ oxidizable nitrogen (nitrogen) (Subbiah and Asija, 1956), Olsen phosphorus (phosphorus) (Olsen, 1954), NH_4OAc extractable potassium (potassium) (Hanway and Heidel, 1952), and $CaCl_2$ extractable sulphur (sulphur) (Chesnin and Yien, 1951) were estimated. Different fractions of organic carbon in the soils viz., dichromate oxidizable organic C (SOC) (Walkley and Black, 1934), organic C of different lability (Chan et al., 2001), water soluble organic carbon (WSOC) (McGill et al., 1986), hot water extractable organic carbon (HWEOC) (Ghani et al., 2003), permanganate oxidizable organic carbon (KMOC) (Tirol-Padre and Ladha, 2004), and total organic carbon (TOC) (Schollenberger, 1927) were also measured. Further, different components of biological soil health viz., microbial biomass C (MBC) (Vance et al., 1987), mineralizable C (MINC) (Franzluebbers and Arshad, 1996), respiratory quotient (RQ) and a few enzyme activities such as dehydrogenase (DHA) (Dick et al., 1996), acid (ACP) and alkaline phosphatase (ALKP) (Tabatabai and Bremner, 1969), arylsulphatase (ARS) (Tabatabai and Bremner, 1970), urease (URS), amidase (AMDS), β-glucosidase (BGA) and fluorescein diacetate hydrolase (FDA) activity (Dick et al., 1996) of the soils under different treatments were assessed. Microbial population viz., total bacteria (Zuberer, 1994), actinomycetes (ACTNM) (Himedia, 2009), fungi (Martin, 1950), N fixing bacteria (NFB) (Jensen, 1930), phosphate solubilizing bacteria (PSB) (Pikovskaia, 1948), cellulolytic bacteria (CLB) (Wirth and Ulrich, 2002) were also enumerated by serial dilution and pour plate technique. Population of nematodes (NMT) were further isolated from 100 g of soil of each of the samples by 'decanting and sieving' technique (Cobb, 1918).

The extraction of DNA of the total soil community of the samples was done using 'Nucleopore GDNA soil kit' (Genetix, New Delhi, India). To quantify its concentration (extracted DNA), Nanodrop 3300 spectrofluorometry (Waltham, Massachusetts, USA) was used. The extracted DNA sample was then stored at −20 °C for further analysis The abundances of the gene copies of 16S rRNA that are specific for *Alpha proteobacteria*, *Betaproteobacteria*, *Bacteroidetes*, *Archaeal* 16S and *Bacterial* 16S and two functional genes of N cycling such as *nifH* and *amoA* of bacteria (Fierer et al., 2005; Regan et al., 2017) were quantified with qPCR method employing 'Fast SYBR® Green dye in the Light Cycler® 96 Real-Time OCE System' (Roche Diagnostics crop, Indianapolis, Indiana, USA).

2.3. Statistical analysis, minimum data set (MDS) and biological soil health index (BSHI)

Duncan's multiple range test (DMRT) (Gomez and Gomez, 1984) was used to segregate the mean values for each (20.0) soil parameter. To assess the biological soil health index (BSHI), equivalent rice yield (ERY) was chosen as the management goal. The weighted additive indexing approach was utilized to carry out indicator selection, interpretation, and integration into the overall BSHI employing principal component analysis (PCA) (Andrews and Carroll, 2001; Andrews et al., 2002). The variables that best describe the system characteristics were believed to be the main components with high eigen values and factor loadings (Brejeda et al., 2000). After calculating the MDS indicators, each of the MDS variables was evaluated based on the performance of soil functions. The score functions for each variable were converted or normalised to a value between 0 (least favourable soil function) and 1 (most favourable soil function) (Andrews et al., 2002). To calculate the BSHI value, the MDS variables for each observation were weighted using the PCA scoring. Regression analysis was performed using R software to understand the relationship between selected indicators in BSHI and soil organic carbon and to validate the reliability of BSHI with ERY.

Weighted additive biological soil health index was calculated by using the following formula:

$$\mathrm{BSHI} = \sum_{i=I}^{n} W_i \times S_i$$

where S = indicator score, W = principal components weightage factor.

3. Results

3.1. Soil physico-chemical properties and pools of organic C

On average, a higher value of pH, and BD, but lower of MWD, and available N was observed under conventional tillage (TPR-CTW-CTMB); while the values of available P, K and S were maximised under triple ZT with residues (ZTDSR+MR–ZTW + RR–ZTMB+WR) treatment (Table 2). Under CA, an increase in various labile fractions of SOC was observed over the conventionally tilled (CT) system excepting the LOC fraction (Table 3). Triple zero tillage (ZT) with triple residue retention (MR-RR-WR) treatments generally maintained a higher amount of VLOC, KMOC, SOC, WSOC, HWEC, and MBC fractions; although MINC showed little variations among the treatments. However, the effect of triple residue (MR-RR-WR) management was not significant over the triple ZT.

3.2. Soil enzymatic activity, microbial population and function

The activity of most of the enzymes viz., DHA, FDA, ARS, BGA, ACP and ALKP was higher with CA practices; with increasing intensity (double/triple ZT with residues vs single/double ZT without residues) of CA practices, activities of the enzymes, in general, also increased. This was more so with triple ZT with residues than triple ZT without residues treatment, with a few exceptions; although maximisation of the activities of different enzymes occurred under different CA regimes (Table 4).

Like enzymatic activities, microbial population also responded positively and significantly with the intensity of CA practices. On average, double/triple zero tillage with and without residues had higher population of almost all the organisms viz., bacteria, fungi, actinomycetes, and CLB counted, with a few exceptions like PSB and NFB showed little responses. This was also true for nematodes attaining its highest under triple ZT with residue treatment (Table 5). However, like enzymes, population of different organisms was maximised at different regimes of CA. Of the two microbial functions assessed, potentially mineralizable nitrogen (PMN) attained the maximum value with triple ZT without residue (42.6 mg kg^{-1} 21 $days^{-1}$); while respiratory quotient (RQ) showed no significant difference among the treatments with a lowest value under triple ZT with residue (0.51), but highest with the CT (0.67) (Table 5).

3.3. Microbial gene abundance

Of the 7 microbial genes viz., *Archaeal* 16S rRNA, *Bacterial* 16S rRNA, *Alphaproteobacteria* 16S rRNA, *Betaproteobacteria*16S rRNA, *Bacteroidetes*16S rRNA, Bacterial *amoA*, and *nifH* gene copied by qPCR, majority of the genes were most abundant in soils under double/triple ZT with or without residues over the other treatments (Table 6); however, in most of the cases, the minimum abundance was observed with the CT treatment.

Table 2
Effect of conservation agriculture on physico-chemical properties of soil under rice-wheat cropping system.

Treatments	pH	BD (Mg/m^3)	MWD (mm)	Nitrogen (mg kg^{-1})	Phosphorus (mg kg^{-1})	Potassium (mg kg^{-1})	Sulphur (mg kg^{-1})
ZTDSR-ZTW	8.01c	1.52ab	1.03bc	79.0b	53.6b	185a	22.8b
ZTDSR + WR–ZTW + RR	8.08b	1.51ab	1.07bc	87.5ab	41.3c	179a	22.1b
ZTDSR+WR + SBM–ZTW + RR	8.10ab	1.50bc	1.12ab	92.9a	39.8c	158ab	22.2b
ZTDSR-ZTW-ZTMB	8.03c	1.49c	1.18a	98.7a	34.0c	163ab	24.6b
ZTDSR+MR–ZTW + RR–ZTMB WR	8.09b	1.47c	1.21a	96.0a	64.3a	190a	30.5a
TPR-CTW-CTMB	8.13a	1.54a	0.99c	75.9b	35.9c	134b	23.0b

BD, bulk density; MWD, mean weight diameter.
Values (mean) in each column (between the treatments) for particular soil parameter followed by different lower-case letters are significant according to Duncan's multiple range test at P = 0.05.

Table 3
Effect of conservation agriculture on labile fractions of soil organic carbon under rice-wheat cropping system.

Treatments	VLOC ($g{\cdot}kg^{-1}$)	LOC ($g{\cdot}kg^{-1}$)	KMOC ($g{\cdot}kg^{-1}$)	SOC ($g{\cdot}kg^{-1}$)	WSOC ($\mu g{\cdot}g^{-1}$)	HWEC ($\mu g{\cdot}g^{-1}$)	MINC (CO_2-C $\mu g{\cdot}g^{-1}{\cdot}60$ $days^{-1}$)	MBC ($\mu g{\cdot}g^{-1}$)
ZTDSR-ZTW	3.45b	1.32d	4.34a	6.57b	105ab	197ab	399a	716b
ZTDSR+WR–ZTW+ RR	4.20a	1.92cd	4.22ab	6.66b	97.0bc	189ab	413a	755b
ZTDSR+WR + SBM–ZTW + RR	2.40c	2.53abc	3.46c	6.64b	111ab	176b	433a	763b
ZTDSR-ZTW-ZTMB	3.30b	2.82ab	3.73bc	7.44a	112a	199ab	441a	844a
ZTDSR+MR–ZTW + RR–ZTMB WR	4.20a	2.37bc	4.44a	7.46a	117a	211a	431a	846a
TPR-CTW-CTMB	1.95c	3.27a	2.52d	5.43c	88.0c	110c	364a	548c

VLOC; very labile pool of soil organic carbon; LOC, labile pool of soil organic carbon; KMOC, $KMnO_4$-oxidizable organic carbon; SOC, soil organic carbon; WSOC, water soluble organic carbon; HWEC, hot water extractable organic carbon; MINC, mineralizable carbon; MBC, microbial biomass carbon.
Values (mean) in each column (between the treatments) for particular soil parameter followed by different lower-case letters are significant according to Duncan's multiple range test at P = 0.05.

Table 4
Effect of conservation agriculture on soil enzymatic activities under rice-wheat cropping system.

Treatments	DHA (μg TPF g^{-1} 24 h^{-1})	FDA (μg FL g^{-1} h^{-1})	ARS (μg PNP g^{-1} h^{-1})	BGA (μg PNP g^{-1} h^{-1})	ACP (μg PNP g^{-1} h^{-1})	ALKP (μg PNP g^{-1} h^{-1})	URS (mg NH_4^+ g^{-1} h^{-1})	AMDS (mg NH_4^+ g^{-1} h^{-1})
ZTDSR-ZTW	113bc	22.8b	48.9c	24.4d	31.2d	261a	68.0cd	80.0b
ZTDSR + WR–ZTW + RR	100cd	37.2a	73.7b	41.6c	47.2c	252a	119a	84.5b
ZTDSR+WR + SBM–ZTW + RR	123b	28.7ab	52.7c	43.8c	57.5b	257a	84.0b	75.8b
ZTDSR-ZTW-ZTMB	164a	30.5ab	128a	56.2b	47.0c	211a	76.3bc	101a
ZTDSR+MR–ZTW + RR–ZTMB WR	176a	37.4a	81.7b	63.3a	76.8a	265a	106a	112a
TPR-CTW-CTMB	83.0d	21.0b	32.8d	16.9e	28.2d	110b	56.7d	57.0c

DHA, dehydrogenase activity, μg TPF g^{-1} 24 h^{-1}; FDA, fluorescein di-acetate activity, μg fluorescein g^{-1} h^{-1}; ARS, arylsulphatase activity, μg PNP g^{-1} h^{-1}; ACP, acid phosphatase activity, μg PNP g^{-1} h^{-1}; ALKP, alkaline phosphatase activity, μg PNP g^{-1} h^{-1}; BGA, β-glucosidase activity, μg PNP g^{-1} h^{-1}; AMDS, amidase activity, mg NH_4^+ g^{-1} h^{-1}; URS, urease activity, mg NH_4^+ g^{-1} h^{-1}.
Values (mean) in each column (between the treatments) for particular soil parameter followed by different lower-case letters are significant according to Duncan's multiple range test at P = 0.05.

Table 5
Effect of conservation agriculture on microbial population, respiratory quotient and potentially mineralizable nitrogen under rice-wheat cropping system.

Treatments	Bacteria (CFU $\times10^5$ g^{-1})	Fungi (CFU $\times10^4$ g^{-1})	ACTNM (CFU $\times10^4 g^{-1}$)	PSB (CFU $\times10^4 g^{-1}$)	NFB (CFU $\times10^4 g^{-1}$)	CLB (CFU $\times10^4 g^{-1}$)	Nematode (200 g $soil^{-1}$)	RQ	PMN (mg kg^{-1} 21 $days^{-1}$)
ZTDSR-ZTW	1.67cd	1.33b	0.53d	1.77a	2.10a	1.57b	96.7d	0.56a	24.0c
ZTDSR + WR–ZTW + RR	2.40b	1.45b	0.90cd	2.43a	2.83a	1.85ab	157b	0.55a	25.7c
ZTDSR+WR + SBM–ZTW + RR	2.20bc	2.73a	1.87ab	2.02a	3.86a	1.66b	120c	0.57a	40.8a
ZTDSR-ZTW-ZTMB	3.12a	2.60a	2.27a	2.40a	3.55a	1.97ab	154b	0.52a	42.6a
ZTDSR+MR–ZTW + RR–ZTMB WR	3.17a	2.45a	1.87ab	2.49a	3.87a	2.03a	165a	0.51a	32.1b
TPR-CTW-CTMB	1.40d	1.07b	1.50bc	1.72a	1.60a	0.92c	52.7e	0.67a	22.5c

Bacteria, CFU $\times10^5$ g^{-1}; fungi, CFU $\times10^4$ g^{-1}; ACTNM, actinomycetes, CFU $\times10^4$ g^{-1}; NFB, N_2-fixing bacteria, CFU $\times10^4$ g^{-1}; PSB, phosphate solubilizing bacteria, CFU $\times10^4$ g^{-1}; CLB, cellulolytic bacteria, CFU $\times10^4$ g^{-1}; nematode, 200 g $soil^{-1}$; RQ, respiratory quotient; PMN, potentially mineralizable nitrogen, mg kg^{-1} 21 $days^{-1}$.
Values (mean) in each column (between the treatments) for particular soil parameter followed by different lower-case letters are significant according to Duncan's multiple range test at P = 0.05.

Table 6
Effect of conservation agriculture on abundance of microbial genes under rice-wheat cropping system.

Treatments	*Archaeal* 16S	*Bacterial* 16S	*Alphaproteobacteria*	*Betaproteobacteria*	*Bacteroidetes*	Bacterial *amoA*	*nifH*
ZTDSR-ZTW	1.73×10^{5}b	1.35×10^{10}c	1.76×10^{4}e	2.66×10^{8}d	6.60×10^{1}e	1.31×10^{5}d	5.16×10^{2}e
ZTDSR + WR–ZTW + RR	8.11×10^{4}e	4.56×10^{9}d	2.49×10^{5}c	9.54×10^{7}e	1.26×10^{2}c	7.18×10^{5}b	1.26×10^{3}d
ZTDSR+WR + SBM–ZTW + RR	1.62×10^{5}c	1.61×10^{10}b	3.60×10^{5}b	9.26×10^{8}b	9.17×10^{1}d	8.42×10^{5}a	6.67×10^{3}b
ZTDSR-ZTW-ZTMB	2.03×10^{5}a	1.42×10^{10}c	2.38×10^{4}e	5.37×10^{8}c	1.96×10^{2}b	2.33×10^{5}c	5.57×10^{3}c
ZTDSR+MR–ZTW + RR–ZTMB WR	1.27×10^{5}d	1.72×10^{10}a	5.62×10^{5}a	1.48×10^{9}a	3.17×10^{2}a	9.12×10^{5}a	7.49×10^{3}a
TPR-CTW-CTMB	1.62×10^{5}c	3.44×10^{8}e	1.37×10^{5}d	1.52×10^{7}e	2.25×10^{1}f	5.26×10^{4}e	2.43×10^{2}e

Microbial gene abundance; copy no. g^{-1} soil.
Values (mean) in each column (between the treatments) for particular soil parameter followed by different lower-case letters are significant according to Duncan's multiple range test at P = 0.05.

3.4. Selection of minimum data set (MDS) of biological soil health

Results of PCA analysis for selection of minimum data set of key biological soil health indicators cumulatively explained 91.3 % of the total variation among the data set (Table S1). Of the six PC's, PC1 had β-glucosidase, microbial biomass carbon, soil organic carbon and bacteria as highly weighted variables, and of these β-glucosidase was selected due to its highest factor loading and its significant correlation with other PC1 variables (Table S2). In PC2, the highly weighted variables were *Bacterial amoA*, *Betaproteobacteria* 16S and very labile organic carbon, and of these *Bacterial amoA* was retained following the same principle of PC1. Similarly, in PC3, PC4, PC5 and PC6, *Archaeal*16S, *Bacteroidetes*16S, *Bacterial* 16S, and mineralizable carbon, respectively were selected (Fig. 1). Subsequently, the selected indicators were transformed through linear scoring technique in a scale of 0 to 1.

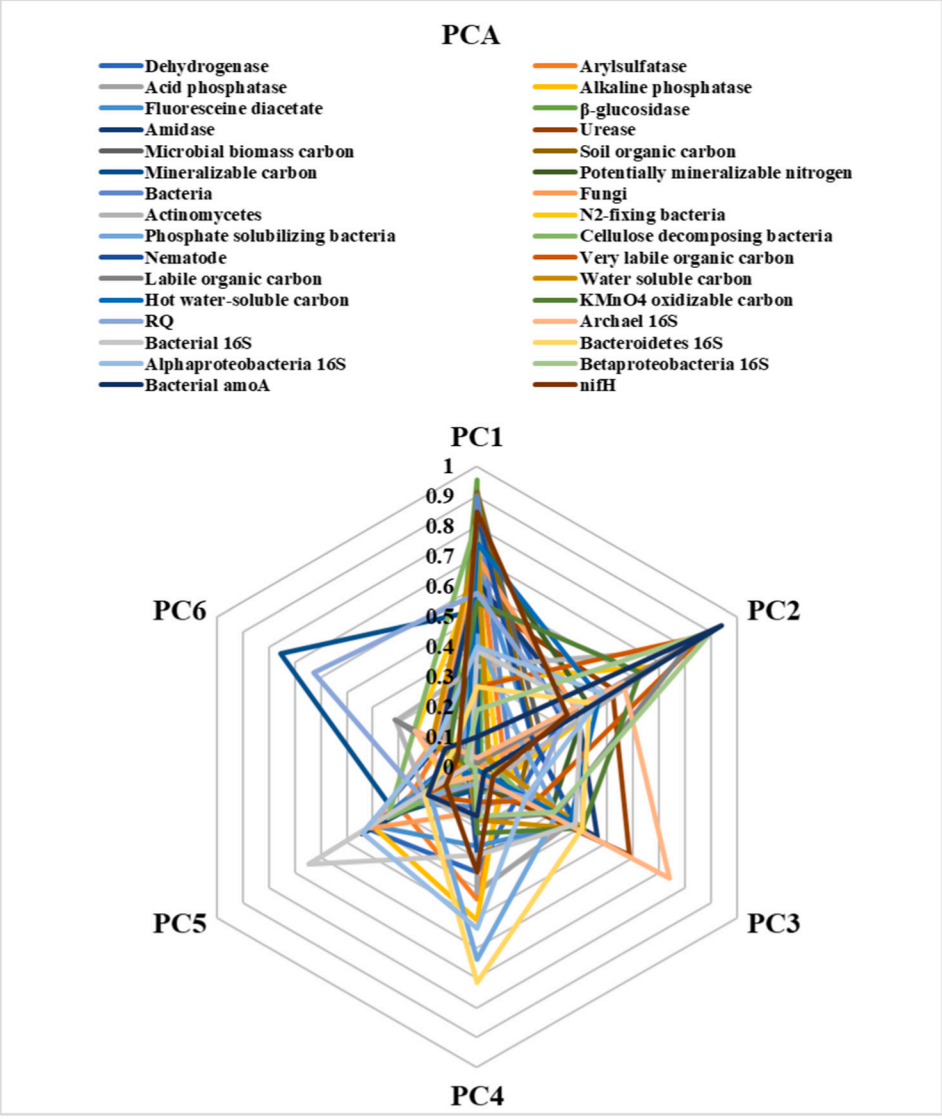


Fig. 1. Principal component analysis of biological soil health indicators.

3.5. Development of biological soil health index

Among the treatments, BSHI of triple ZT with residue retention (182 %) was significantly higher than that of the others viz., triple ZT without residue (155 %), double ZT with residue (168 %), double ZT without residues (120 %) over the CT treatment. Overall, the mean effect of ZT was 144 % higher over CT treatment. The relative contribution of the key indicators in the development of BSHI was as follow: β-glucosidase (43.7 %), *Bacterial amoA* (19 %), *Archaeal* 16S (6.4 %), *Bacteroidetes*16S (3.6 %), B*acterial* 16S (2.0 %) and mineralizable carbon (5.2 %) under triple ZT with residue treatment (Fig. 2).

3.6. Relationship between soil organic carbon and the key biological soil health indicators

To study the relationship between conventional and state of the art indicators, regression analysis was performed between the selected key biological soil health indicators and SOC. It was observed that the regression coefficient (%) of MINC (99), *Archaeal* 16S (93) and BGA (91) was above 80 % with SOC; whereas, some of the biological soil health indicators exhibited less regression coefficient values, such as *Bacterial* 16S (77), *Bacteroidetes* 16S (68), and *Bacterial amoA* (67) (Fig. 3).

3.7. Validation of biological soil health index

To validate the BSHI, simple regression analysis was performed between the BSHI and ERY of all the treatments. Result showed there was a good agreement (90 %) between biological soil health index and equivalent rice yield (Fig. 4).

4. Discussion

4.1. Pools of soil organic carbon

There was a higher content of labile fractions of SOC under CA with zero tillage and crop residue compared to the conventionally tilled practices due to a reduction in tillage intensity and higher C inputs. The reduced tillage caused lesser disturbance to soil aggregates and yielded a higher amount of physically protected SOC inside macroaggregates (Francaviglia et al., 2023). The permanganate oxidizable carbon (KMOC) consisting of labile humic materials and polysaccharides (Haynes, 2005) was higher with residue retention obviously for addition of a higher fresh residues Similarly, a higher amount of WSOC and HWEC representing plant litter, rhizo-depositions and soil humus etc. under triple ZT with residues treatment was due to the retention of residue (MR) with lower C:N which helped to proliferate the microbes resulting in an increased production of starch, proteins and other microbiological substrates (Chen et al., 2010). A non-significant difference in MINC between conventional tillage and zero tillage with residue treatment might be due to an excess tillage under the former treatment leading to less physically protected carbon and more CO_2 evolution (Zhang et al., 2018) like those with ZT with residues. Expectedly, the observed higher amount of MBC with residue retention was associated with the addition of ample source of substrate for microbial proliferation

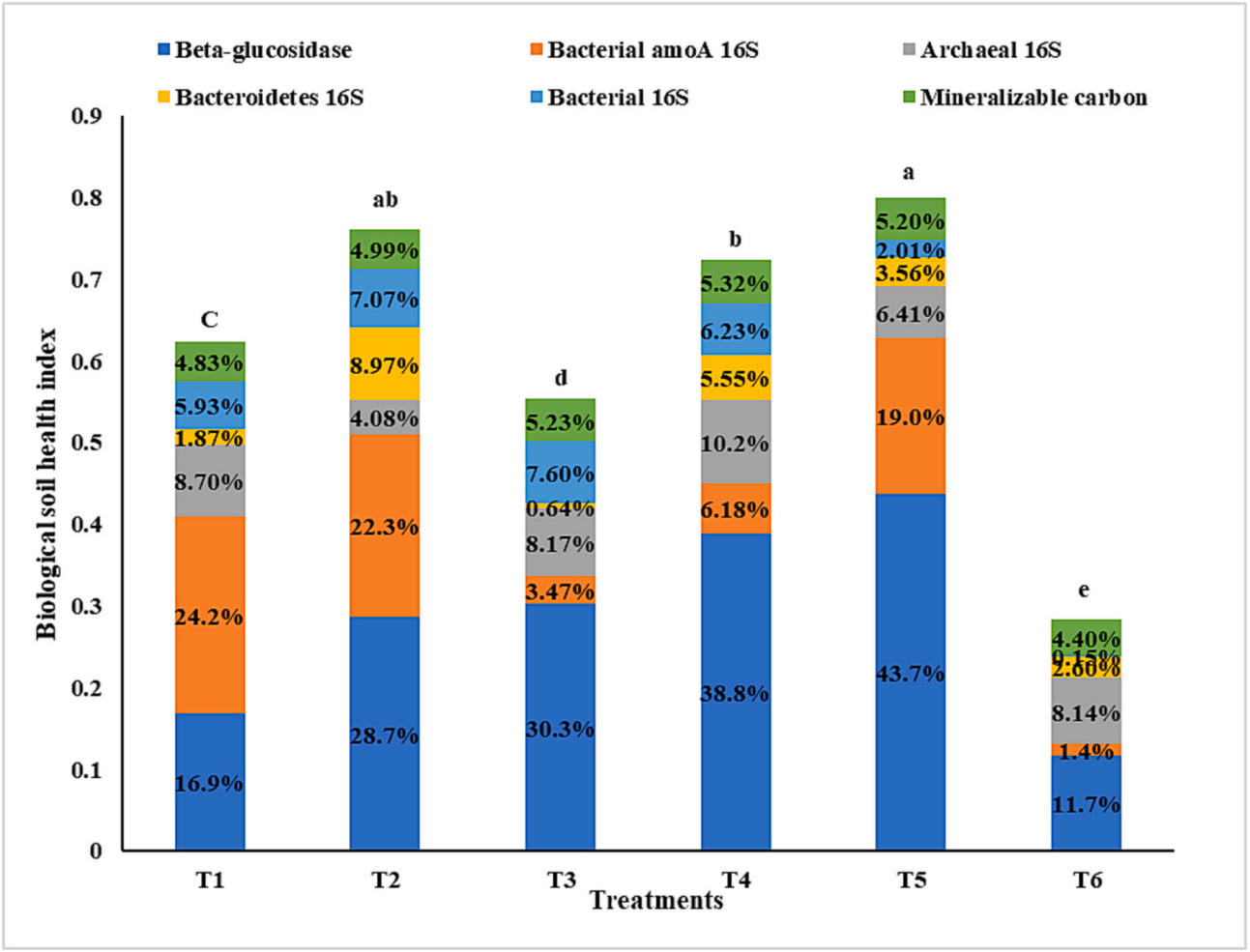


Fig. 2. Biological soil health index under CA based rice-wheat cropping system.

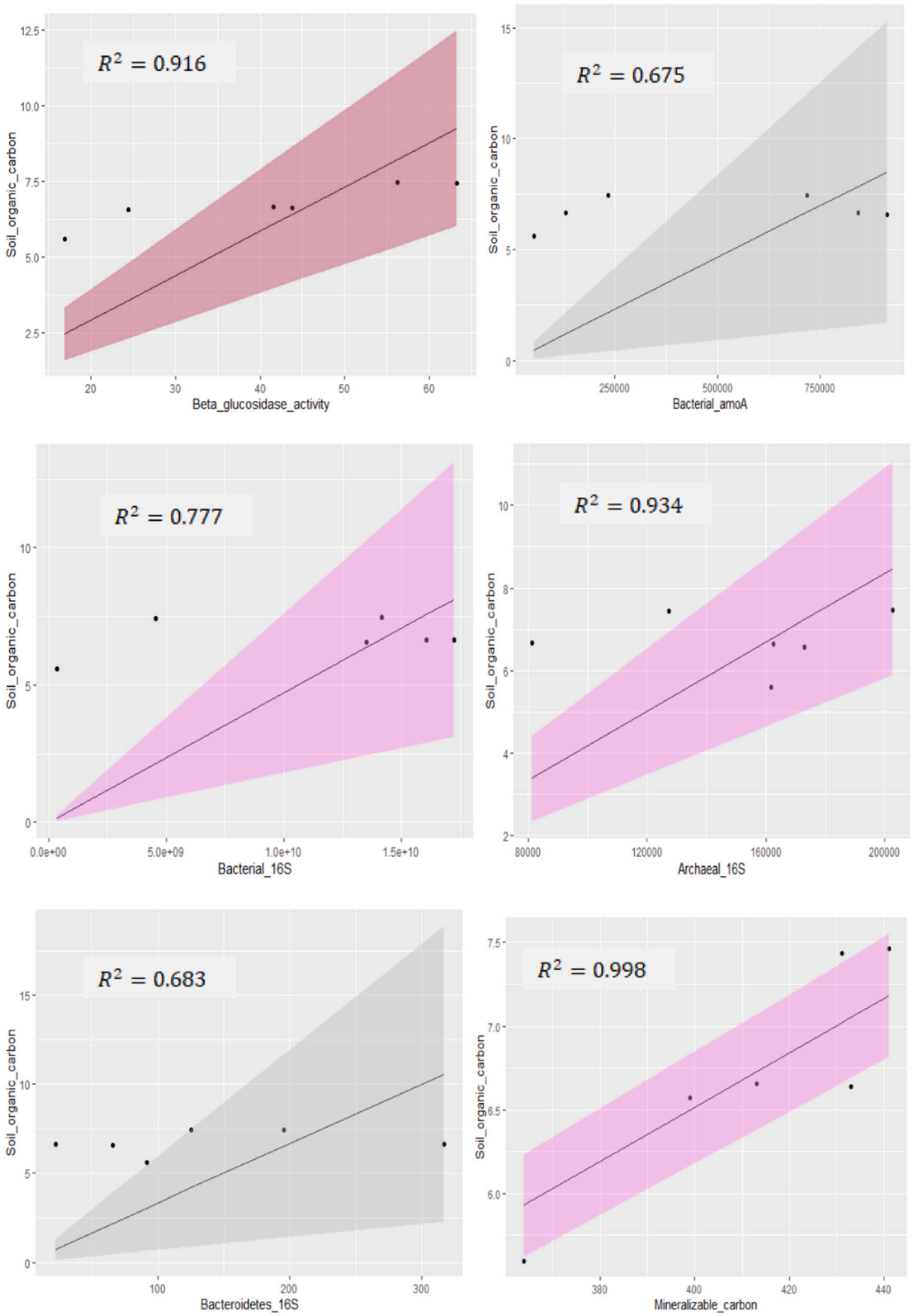


Fig. 3. Relationship between soil organic carbon and the key biological soil health indicators under CA based rice-wheat cropping system.

(Rehman et al., 2023).

4.2. *Soil enzymatic activity, microbial population and function*

On average, the enhanced activity of different enzymes under CA might be due to the presence of an ample amount of substrate as a source of nutrients for microbial proliferation and a less disturbance of soil. This provides a better environment for microbial survival. Of the enzyme activities, DHA, had much higher activity under triple ZT with residues and double ZT with sesbania brown manuring might be due to incorporation of leguminous residues (Kaur et al., 2023) or presence of root biomass of mungbean because of a lower C:N of leguminous

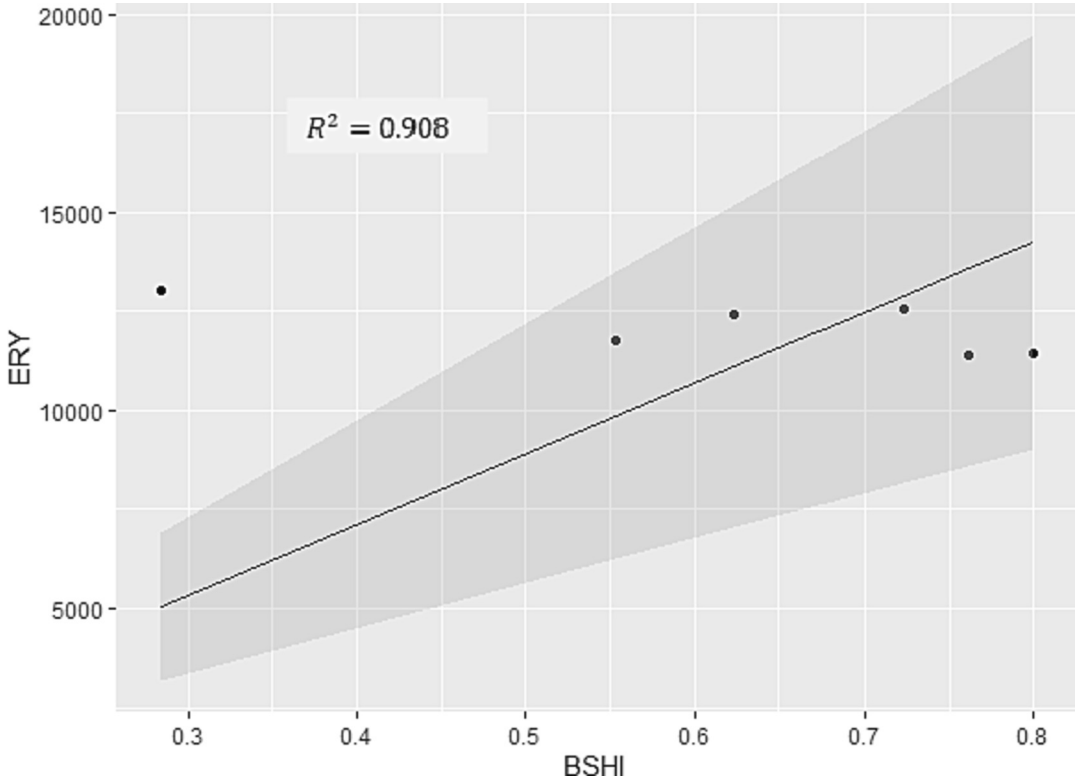


Fig. 4. Relationship between equivalent rice yield (ERY) and biological soil health index (BSHI) under CA based rice-wheat cropping system.

residues. Since, it facilitates microbial proliferation more vigorously (Singh et al., 2018). Arylsulphatase activity denotes the capacity of soil to mineralize the ester bound sulphate to make it available to plants. Its higher activity in triple ZT without residues treatment might be due to the less amount of sulphur in the treatment. β-glucosidase activity (BGA) responsible for organic matter decomposition, and URS and AMDS activities associated with N-cycling had higher values with CA as compared to the conventionally tilled treatment due to the presence of higher crop residues (Cárceles Rodríguez et al., 2022; Awale et al., 2017). Activities of both the ACP and ALKP were found higher in the plots under CA due to increasing intensity of no till (Munda et al., 2023) and the enhancing effect of residue retention (Biswas et al., 2021).

On average, the total microbial population count of bacteria, fungi and actinomycetes was higher in soils under CA-based system compared with those under conventionally tilled one. It was more pronounced in soils under double/triple ZT with residues. This was associated with a greater substrate availability and less disturbance of soil environment (Suman et al., 2022; Mhlanga et al., 2022). A higher population of PSB, NFB and CLB associated with a specific nutrient cycling in soils under CA might be due to creation of a better micro-environment in soils with ZT and residue retention for microbial proliferation (Li et al., 2020). Of these three, population of NFB was much higher under ZTDSR + MR-ZTW + RR-ZTMB + WR followed by ZTDSR + WR + SBM-ZTW + RR. This was possibly associated with a legume in the cropping system. Again, a higher nematode population with CA-based system might be associated with a greater supply of nutrients out of the residue retention (Sauvadet et al., 2016).

There was higher value of PMN in soils under CA-based system than the conventionally tilled one. Because of higher amount of substrate availability, microbial activity was increased and as a consequence PMN was higher in the former system than the latter. The effect was more pronounced where SBM with low C:N was added in the system. Contrarily, the values of respiratory quotient (RQ), an indicator of efficiency of microbes for substrate utilization, were lower in soils under CA-based system because of higher availability of readily decomposable substrate with fresh residue incorporation (Kaur et al., 2019). However, this value of RQ was higher in conventionally tilled system due to the deficiency of readily decomposable substrate (Benbi et al., 2015). A higher value of RQ in conventional system suggests a less efficient use of available C by the microbes there; whereas, its lower values in CA-based systems indicates their higher efficiency in preserving C in soils.

4.3. Microbial gene abundance

Microbial and functional gene abundance assessed by qPCR targeting different phylogenetic groups viz. *Archaeal* 16S rRNA, *Bacterial* 16S rRNA, *Bacteroidetes* 16S rRNA, *Alphaproteobacteria* 16S rRNA, *Betaproteobacteria* 16SrRNA, and other two viz., *Bacterial amoA* and *nifH* gene involved in N cycling showed that *Archaeal* 16S gene was abundant in soils under triple/double ZT without residues treatment over CT. This might be due to the oligotrophic nature of *Archaea* which prevents them to grow in high nutritious environment (Ortiz-Cornejo et al., 2017). On the other hand, the abundance of *Bacterial* 16S gene in soils under CA-based system over the conventionally tilled one was associated with its increased TOC. When TOC levels rise, the overall number of bacteria also rise. Zero or reduced tillage enhanced microbial biomass in various agroecosystems, such as rice (*Oryza sativa* L.) and soybean (*Glycine max* (L.)). One probable reason is that a lack of tillage could delay the breakdown of organic materials (Zotarelli et al., 2007) since no tilled soils had a physical protection and storage of organic C due to soil aggregation preventing organic matter decomposition (Van Groenigen et al., 2010). Again, the *Proteobacteria* and *Bacteroidetes* are copiotrophs by nature (Fierer et al., 2007) and survive efficiently utilizing the labile carbon sources and thus found abundant in soils under systems with higher amount of labile carbon (Bei et al., 2018). Further, the gene coded for ammonium oxidizing bacteria (*Bacterial amoA*) and N-fixation (*nifH* gene) were higher under triple ZT with triple residues and double ZT with double residues and sesbania brown manuring treatments. This happened due to incorporation of sesbania brown manuring (SBM) and mungbean residues (MR) with corresponding increased number of

ammonium oxidizing bacteria and nitrogen fixing microorganisms in the soils (Regan et al., 2017).

4.4. Relationship between labile fractions of soil organic carbon and key biological soil health indicators

The biological parameters were mainly governed by labile source of carbon as it provides easily usable substrate for microbial proliferation which, in turn, helps in enhancing extra-cellular enzymatic activities or improving other microbiological parameters (Alves de Castro Lopes et al., 2013). Such enhancement of light fractions of organic carbon and KMOC/SOC ratios are linked with an abundance of certain taxonomic groupings at the genus level. This may explain why *Bacteroidetes* (*Adhaeribacter*, *Flavisolibacter*, and *Niastella*), *Proteobacteria* (*Skermanella*, *Ramlibacter*, and *Sphingomonas*), and *Archaea* (*Thaumarchaeota*) were dominant groups when there was high KMOC/SOC and low light fractions of organic matter content; however, the microbial taxa responded differently to both labile C fraction types (Ramírez et al., 2020).

4.5. Biological soil health index and indicators

Among the indicators selected, β-glucosidase activity had the highest contribution to the biological soil health index computed. It is an important enzyme involves in terrestrial C cycling producing glucose which is the main energy source of microbes. Systems with ZT and residue retention enhanced microfloral population and thereby increased the BGA activity (Singh et al., 2018). Selection of BGA is thus justified for CA-based system as this enzyme denotes the organic matter decomposition potential of the field. Reports of BGA as an indicator of soil health under rice-based cropping system are not rare (Biswas et al., 2017), however, its selection as a key indicator of BSH in CA-based system is not known. Similarly, the selection of MINC as a key indicator in our CA-based system with lot of residues is also justified because of its usefulness in motoring organic matter decomposition in soils under a system (Anderson, 1982). We also came across a new set of key indicators viz., *Bacterial amoA*, *Archaeal* 16S rRNA, *Bacteroidetes*16S rRNA, *Bacterial* 16S rRNA selected for indexing biological soil health in our study. Fierer et al. (2021) reported microbial gene abundance determined by qPCR analysis or the community structure and also the functional genes can be used to assess the soil biological health on the ground of changes in the microbial community in soils under different management practices and systems. Information through qPCR analysis regarding existence of diverse communities is useful for assessing the chosen system as to its resilience and resistant to degradation/diseases and also the possibility of having a higher rate of nutrient cycling. Use of PLFA-based microbial community composition to assess the soil biological health has also been advocated (Mann et al., 2019). We observed that the highest BSHI with ZTDSR + MR-ZTW + RR- ZTMB + WR treatment wherein a leguminous crop is incorporated in the system along with residue management and zero tillage. Similar findings of a higher value of biological soil quality index (BSQI) were also reported with minimum tillage treatment than with the conventional tillage one (Sharma et al., 2016). Biswas et al. (2021) reported that the highest value of biological soil quality index (BSQI) was found in triple ZT with rice, mustard and mungbean residues treatment under CA based rice-mustard system. This new set of key biological soil health indicators showed good agreement with conventional key indicator, SOC, reported by many researchers across the world. To validate the BSHI values thus obtained, regression analysis was performed and result showed a good agreement (90 %) between ERY and BSHI.

5. Conclusions

We report a new set of biological soil health indicators such as *Bacterial amoA*, *Archaeal* 16S rRNA, *Bacteroidetes* 16S rRNA and *Bacterial* 16S rRNA with higher sensitivity under CA-based rice-wheat system for prediction of biological soil health index (BSHI). Of different variants of CA practices, triple ZT with residues or double ZT with sesbania or moonbean residues always maintained higher labile pools of carbon, enzymatic activities, microbial population, and functions and the BSHI and therefore, be upscaled in the Indo-Gangetic plains.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors are grateful to the ICAR-Indian Agricultural Research Institute, New Delhi for providing necessary facilities to conduct the research. Authors are thankful to Prof. Biswapati Mandal, Ex-Pro Vice Chancellor, BCKV for his valuable inputs for preparation of the manuscript. First author is highly indebted to Indian Council of Agricultural Research (ICAR), New Delhi for extending financial assistance in terms of Post Graduate Fellowship towards completion this research work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.105128.

References

Alves de Castro Lopes, A., Gomes de Sousa, D.M., Chaer, G.M., dos Reis, Bueno, Junior, F., Goedert, W.J., de Carvalho Mendes, I., 2013. Interpretation of microbial soil indicators as a function of crop yield and organic carbon. Soil Sci. Soc. Am. J. 77, 461–472.

Anderson, J.E.E., 1982. Soil respiration. In: Page, A.L. (Ed.), Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties. American Society of Agronomy, Madison, Wisconsin, pp. 831–871.

Andrews, S.S., Carroll, C.R., 2001. Designing a soil quality assessment tool for sustainable agro-ecosystem management. Ecol. Appl. 11, 1573–1585.

Andrews, S.S., Karlen, D.L., Mitchell, J.P., 2002. A comparison of soil quality indexing methods for vegetable systems in Northern California. Agric. Ecosyst. Environ. 90, 25–45.

Awale, R., Emeson, M.A., Machado, S., 2017. Soil organic carbon pools as early indicators for soil organic matter stock changes under different tillage practices in Inland Pacific Northwest. Front. Ecol. Evol. 5, 96.

Bei, S., Zhang, Y., Li, T., Christie, P., Li, X., Zhang, J., 2018. Response of the soil microbial community to different fertilizer inputs in a wheat-maize rotation on a calcareous soil. Agric. Ecosyst. Environ. 260, 58–69.

Benbi, D.K., Kiranvir, B.R.A.R., Sharma, S., 2015. Sensitivity of labile soil organic carbon pools to long-term fertilizer, straw and manure management in rice-wheat system. Pedosphere. 25, 534–545.

Bhatt, R., Kukal, S.S., Busari, M.A., Arora, S., Yadav, M., 2016. Sustainability issues on rice-wheat cropping system. Int. Soil Water Conserv. Res. 4, 64–74.

Bhattacharyya, R., Ghosh, B.N., Mishra, P.K., Mandal, B., Srinivasa Rao, C.H., Sarkar, D., Das, K., Anil, K.S., Lalitha, M., Hati, K.M., Franzluebbers, A.J., 2015. Soil degradation in India: challenges and potential solutions. Sustainability. 7, 3528–3570.

Biswas, S., Hazra, G.C., Purakayastha, T.J., Saha, N., Mitran, T., Roy, S.S., Basak, N., Mandal, B., 2017. Establishment of critical limits of indicators and indices of soil quality in rice-rice cropping systems under different soil orders. Geoderma. 292, 34–48.

Biswas, S., Das, T.K., Bhattacharyya, R., Das, S., Dwivedi, B., 2021. Biological soil quality and seasonal variation on enzyme activities under conservation agriculture-based rice-mustard system in the Indo-Gangetic Plains. Soil Res. 60 (6).

Blake, G.R., Hartge, K.H., 1986. Bulk density. Methods of soil analysis: part 1 physical and mineralogical methods, 5, 363–375.

Brejeda, J.J., Moorman, T.B., Karlen, D.L., Dao, T.H., 2000. Identification of regional soil quality factors and indicators. I. Central and southern high plains. Soil Sci. Soc. Am. J. 64, 2115–2124.

Cárceles Rodríguez, B., Durán-Zuazo, V.H., Soriano Rodríguez, M., García-Tejero, I.F., Gálvez Ruiz, B., Cuadros Tavira, S., 2022. Conservation agriculture as a sustainable system for soil health: a review. Soil Syst. 6 (4), 87.

Chan, K., Bowman, A., Oates, A., 2001. Oxidizible organic carbon fractions and soil quality changes in an oxic paleustalf under different pasture leys. Soil Sci. 166, 61–67.
Chen, H.L., Zhou, J.M., Xiao, B.H., 2010. Characterization of dissolved organic matter derived from rice straw at different stages of decay. J. Soils Sediments 10, 915–922.
Chesnin, L., Yien, C.H., 1951. Turbidimetric determination of available sulfates. Soil Sci. Soc. Am. J. 15, 149–151.
Cobb, N.A., 1918. Estimating the Nema Population of Soil, With Special Reference to the Sugar-beet and Root-gall Nemas, Heteroderaschachtii Schmidt and Heteroderaradicicola (Greef) Müller: and With a Description of Tylencholaimusaequalis n. sp, vol. 1. US Government Printing Office.
Das, T.K., Bhattacharyya, R., Sudhishri, S., Sharma, A.R., Saharawat, Y.S., Bandyopadhyay, K.K., Seema, S., Bana, R.S., Aggarwal, P., Sharma, R.K., Bhatia, A., Singh, G., Datta, S.P., Kar, A., Singh, B., Singh, P., Pathak, H., Vyas, A.K., Jat, M.L., 2014. Conservation agriculture in an irrigated cotton-wheat system of the western Indo-Gangetic Plains: crop and water productivity and economic profitability. Field Crop Res. 158, 24–33.
Dick, R.P., Breakwell, D.P., Turco, R.F., 1996. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In: Doran, J.W., Jones, A.J. (Eds.), Methods for Assessing Soil Quality. SSSA Inc. Madison, Wisconsin, USA, pp. 247–272.
Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. 71, 4117–4120.
Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology. 88, 1354–1356.
Fierer, N., Wood, S.A., de Mesquita, C.P.B., 2021. How microbes can, and cannot, be used to assess soil health. Soil Biol. Biochem. 153, 108–111.
Francaviglia, R., Almagro, M., Vicente-Vicente, J.L., 2023. Conservation agriculture and soil organic carbon: principles, processes, practices and policy options. Soil Syst. 7 (1), 17.
Franzluebbers, A.J., Arshad, M.A., 1996. Soil organic matter pools during early adoption of conservation tillage in northwestern Canada. Soil Sci. Soc. Am. J. 60 (5), 1422–1427.
Ghani, A., Dexter, M., Perrott, K., 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biol. Biochem. 35, 1231–1243.
Ghorai, P.S., Biswas, S., Purakayastha, T.J., Ahmed, N., Das, T.K., Prasanna, R., Das, S., 2023. Indicators of soil quality and crop productivity assessment at a long-term experiment site in the lower Indo-Gangetic plains. Soil Use Manag. 39 (1), 503–520.
Gomez, K.A., Gomez, A.A., 1984. Statistical Procedures for Agricultural Research. John Wiley and Sons, New York, USA.
Hanway, J.J., Heidel, H., 1952. Soil analysis methods as used in Iowa state college soil testing laboratory. Iowa Agric. 57, 1–31.
Haynes, R.J., 2005. Labile organic matter fractions as central components of the quality of agricultural soils: an overview. Adv. Agron. 5, 221–268.
Himedia Manual, 2009. HiMedia Manual for Microbiology Laboratory Practice. HiMedia Laboratories Pvt, Ltd, India.
Jensen, H.L., 1930. Azotobacteriaceae. Bacteriol. Rev. 189, 195–214.
Kaur, S., Dheri, G.S., Benbi, D.K., 2019. Effect of long-term fertilization in maize-wheat cropping system on carbon mineralization in soil. Carbon Manag. 10, 523–532.
Kaur, P., Jain, D., Singh Bhullar, M., 2023. Effect of conventional and conservation agriculture practices on dissipation of metribuzin and clodinafop-propargyl, soil enzymatic and microbial activities in wheat in a rice-wheat system. Soil Sediment Contam. Int. J. 1–21.
Ladha, J.K., Kumar, V., Alam, M.M., Sharma, S., Gathala, M.K., Chandna, P., Saharawat, Y.S., Balasubramanian, V., 2009. Integrating crop and resource management technologies for enhanced productivity, profitability and sustainability of the rice–wheat system in South Asia. In: Ladha, J.K. (Ed.), Integrated Crop and Resource Management in the Rice–Wheat System of South Asia. IRRI, Los Banos, Philippines, pp. 69–108.
Li, Y., Zhang, Q., Cai, Y., Yang, Q., Chang, S.X., 2020. Minimum tillage and residue retention increase soil microbial population size and diversity: implications for conservation tillage. Sci. Total Environ. 716, 137–164.
Mann, C., Lynch, D., Fillmore, S., Mills, A., 2019. Relationships between field management, soil health, and microbial community composition. Appl. Soil Ecol. 144, 12–21.
Martin, J.P., 1950. Use of acid, rosebengal and Streptomycin in the plate method for estimating soil fungi. Soil Sci. 69, 215–232.
McGill, W.B., Cannon, K.R., Robertson, J.A., Cook, F.D., 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. Can. J. Soil Sci. 66, 1–19.
Mhlanga, B., Pellegrino, E., Thierfelder, C., Ercoli, L., 2022. Conservation agriculture practices drive maize yield by regulating soil nutrient availability, arbuscular mycorrhizas, and plant nutrient uptake. Field Crop Res. 277, 108403.
Munda, S., Khanam, R., Nayak, A.K., Guru, P.K., Shahid, M., Kumar, A., Mohapatra, S.D., 2023. Conservation agriculture for enhancing crop productivity, energy use efficiency, carbon stock, soil health and reducing GHG emissions. Commun. Soil Sci. Plant Anal. 54 (8), 1134–1150.
Olsen, S.R., 1954. Estimation of Available Phosphorus in Soils by Extraction With Sodium Bicarbonate (No. 939). US Department of Agriculture.
Ortiz-Cornejo, N.L., Romero-Salas, E.A., Navarro-Noya, Y.E., González-Zúñiga, J.C., Ramirez-Villanueva, D.A., Vásquez-Murrieta, M.S., Verhulst, N., Govaerts, B., Dendooven, L., Luna-Guido, M., 2017. Incorporation of bean plant residue in soil with different agricultural practices and its effect on the soil bacteria. Appl. Soil Ecol. 119, 417–427.
Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), 1982. Methods of Soil Analysis; 2. Chemical and Microbiological Properties, 2. Aufl. 1184 S. American Soc. of Agronomy (Publ.), Madison, Wisconsin, USA.
Pikovskaia, R.L., 1948. Mobilization of phosphate in soil in connection with the vital activities of some microbial species. Mikrobiologia. 17, 362–370.
Ramírez, P.B., Fuentes-Alburquenque, S., Díez, B., Vargas, I., Bonilla, C.A., 2020. Soil microbial community responses to labile organic carbon fractions in relation to soil type and land use along a climate gradient. Soil Biol. Biochem. 141, 107–692.
Regan, K., Stempfhuber, B., Schloter, M., Rasche, F., Prati, D., Philippot, L., Marhan, S., 2017. Spatial and temporal dynamics of nitrogen fixing, nitrifying and denitrifying microbes in an unfertilized grassland soil. Soil Biol. Biochem. 109, 214–226.
Rehman, S., Ijaz, S.S., Raza, M.A., Din, A.M.U., Khan, K.S., Fatima, S., Ansar, M., 2023. Soil organic carbon sequestration and modeling under conservation tillage and cropping systems in a rainfed agriculture. Eur. J. Agron. 147, 126840.
Sauvadet, M., Chauvat, M., Fanin, N., Coulibaly, S., Bertrand, I., 2016. Comparing the effects of litter quantity and quality on soil biota structure and functioning: application to a cultivated soil in Northern France. Appl. Soil Ecol. 107, 261–271.
Schollenberger, C.J., 1927. A rapid approximate method for determining soil organic matter. Soil Sci. 24, 65–68.
Sharma, K.L., Rao, C.S., Chandrika, D.S., Nandini, N., Munnalal, Reddy, K.S., Kumar, T. S., 2016. Assessment of GMean biological soil quality indices under conservation agriculture practices in rainfed Alfisol soils. Curr. Sci. 111, 1383–1387.
Singh, G., Bhattacharyya, R., Das, T.K., Sharma, A.R., Ghosh, A., Das, S., Jha, P., 2018. Crop rotation and residue management effects on soil enzyme activities, glomalin and aggregate stability under zero tillage in the Indo-Gangetic Plains. Soil Tillage Res. 184, 291–300.
Subbiah, B.V., Asija, G.L., 1956. A rapid method for the estimation of nitrogen in soil. Curr. Sci. 26, 259–260.
Suman, J., Rakshit, A., Ogireddy, S.D., Singh, S., Gupta, C., Chandrakala, J., 2022. Microbiome as a key player in sustainable agriculture and human health. Front. Soil Sci. 2, 821589.
Tabatabai, M.A., Bremner, J.M., 1969. Use of *p*-nitrophenylphosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307.
Tabatabai, M.A., Bremner, J.M., 1970. Factors affecting soil arylsulfatase activity. Soil Sci. Soc. Am. Proc. 34, 225–229.
Tirol-Padre, A., Ladha, J.K., 2004. Assessing the reliability of permanganate-oxidizable carbon as an index of soil labile carbon. Soil Sci. Soc. Am. J. 68, 969–978.
Van Groenigen, K.J., Bloem, J., Bååth, E., Boeckx, P., Rousk, J., Bodé, S., Forristal, D., Jones, M.B., 2010. Abundance, production and stabilization of microbial biomass under conventional and reduced tillage. Soil Biol. Biochem. 42, 48–55.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. Microbial biomass measurements in forest soils: the use of the chloroform fumigation-incubation method in strongly acid soils. Soil Biol. Biochem. 19, 697–702.
Walkley, A., Black, I.A., 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29–37.
Wirth, S., Ulrich, A., 2002. Cellulose-degrading potentials and phylogenetic classification of carboxymethyl-cellulose decomposing bacteria isolated from soil. Syst. Appl. Microbiol. 25, 584.
Yoder, R.E., 1936. A direct method of aggregate analysis of soils and a study of the physical nature of erosion losses 1. J. Agron. 28, 337–351.
Zhang, Y., Li, X.J., Gregorich, E.G., McLaughlin, N.B., Zhang, X.P., Guo, Y.F., Liang, A.Z., Fan, R.Q., Sun, B.J., 2018. No-tillage with continuous maize cropping enhances soil aggregation and organic carbon storage in Northeast China. Geoderma. 330, 204–211.
Zotarelli, L., Alves, B.J.R., Urquiaga, S., Boddey, R.M., Six, J., 2007. Impact of tillage and crop rotation on light fraction and intra-aggregate soil organic matter in two oxisols. Soil Tillage Res. 95, 196–206.
Zuberer, D.A., 1994. Recovery and enumeration of viable bacteria. In: Methods of Soil Analysis: Part 2 - Microbiological and Biochemical Properties, vol. 5, pp. 119–144.