RESEARCH ARTICLE

Soil biological properties and fungal diversity under conservation agriculture in Indo-Gangetic Plains of India

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Abstract

A field experiment was undertaken to evaluate the effect of conservation agriculture (CA) based management on soil biological properties, and on fungal diversity and abundance after 5 years of continuous cultivation. Treatments included four crop managements *viz.*, conventional tillage (CT) rice-wheat (CT-RW; CT based), conventional tillage rice-zero tillage wheat and mungbean (CTR-ZTWMB; partially CA based), zero tillage rice-wheat-mungbean (ZT-RWMB; full CA based), and zero tillage maize-wheat-mungbean (ZT-MWMB; full CA based). Full rice, maize, and mungbean crop residue and anchored wheat residue were recycled in CA-based managements, while CT-based management was without any residue. Full CA-based management (ZT-MWMB) recorded 43% higher organic carbon, 56% microbial biomass carbon, 70% microbial biomass nitrogen, 73% phosphatase activity, and 40% β -glucosidase activity, than CT-RW management. *Ascomycota* (55-74%) was the dominant phylum followed by *Basidiomycota* and *Glomeromycota* (0 to 3%); abundance of these phyla varied amongst managements. *Ascomycota* abundance was in order of CT-RW < CTR-ZTWMB < ZT-RWMB < ZT-MWMB, however, *Basidiomycota* and *Glomeromycota* did not follow any trend. Diversity indices such as species richness, evenness and Shannon-Wiener diversity index were in the order: ZT-MWMB > ZT-RWMB > CTR-ZTWMB > CT-RW. This study clearly showed that CA with all three proven principles (no-tillage, residue retention and crop diversification) in maize-wheat-mungbean system resulted in higher microbial activities, fungal diversity and species richness compared to other cereal based management systems.

Keywords: Conservation agriculture, soil biological activities, fungal diversity and abundance, cereal based cropping systems.

1. Introduction

The rice-wheat (RW) cropping system is one of the world's largest agricultural production systems, covering an area of 26 million hectares spread over the Indo-Gangetic Plains (IGP) in South Asia and China (Sarkar, 2015). In South Asia, more than 85% of the RW system is practiced in the IGP. The sustainability of this important cropping system is at risk due to second-generation problems related to degradation of natural resources (soil health, air quality, and ground water depletion), and mono cropping (inadequate system diversity) under conventional tillage (farmer's practice/ 'business as usual') based management systems (Gathala *et al.*, 2013). Furthermore, this risk is intensified with *in-situ* burning of crop residues which hampered the soil biological activities and microbial diversity (Lohan *et al.*, 2017). To overcome formidable problems of the RW system in South Asia, conservation agriculture (CA) has emerged as an important alternative to conventional RW system. Higher yields of rice/maize were recorded with CA-based management practices over conventional management due to the improved soil quality indices (Choudhary *et al.*, 2018). The activity and diversity of soil microbial populations are directly affected by management practices (as tillage, crop establishment, residue disposal, water and nutrient management), and soil environment. Microbial biomasses which are sensitive indicators of changes in soil organic matter, are influenced by agriculture management practices (Madejón *et al.*, 2009). Microbial enzymes are involved in soil nutrient cycling, and are used to evaluate soil quality (Choudhary *et al.*, 2018).

Among soil microbes, fungi are critical components in soil system and are regarded as the primary decomposers in soils, as they secrete various enzymes that breakdown lignocelluloses (Choudhary *et al.*, 2016). Plenity of data reported on fungal communities in dif-

ferent natural soil habitats (Marín *et al.*, 2017), but less is so far known about fungal communities in agricultural soils, and very limited in conservation agriculture practices (Wang *et al.*, 2016). An in-depth understanding of the fungal community in agricultural soils provides the path to study their roles in agro-ecosystems, which is not possible by traditional methods like culture-based methods and microscopic studies. With the advanced techniques of metagenomic analysis it is possible to decipher taxonomic and functional assemblages of natural indigenous communities, and their roles in the ecosystems. To date, this approach has been less used to evaluate the impact of agricultural practices on soil microbial communities (Wang *et al.*, 2016). Different agricultural practices like tillage, residue management, crop rotation, etc., affect the physical and chemical characteristics of the soil where microorganism live, thereby affecting their abundance, diversity, and activity (Govaerts *et al.*, 2007). In the North-West IGP of India most of farmers follow the rice-wheat cropping system with conventional-till (intensive tillage) management practices. In this situation the microbial activity is influenced largely by tillage practices as reported for the IGP (Choudhary *et al.*, 2018). In CA based management, retention of crop residue (> 12 t/ha every year) provides carbon and nutrients, and micro-climatic conditions for microbes therefore an increase in the microbial activity fungal diversity is expected. We hypothesize that fungal abundance would increase in CA based management practices compared to intensive tillage based system and pattern of abundance of different fungal class, orders, and genus will also influenced by management practices. Very scarce information is available on fungal diversity and species richness in CA managed systems (rice and maize) of IGP. In this study, efforts have been made to evaluate

the effects of CA based management practices (tillage, crop establishment, residue management, and cropping system intensification) on soil microbial activities, fungal diversity and fungal community structure in comparison to conventional-till RW systems of IGP.

2. Materials and Methods

2.1. Study site and experimental design

To study the impact of different agricultural practices on soil biological properties and microbial diversity, a field experiment with different management prac-

tices, including crop rotation, tillage and residue management was started in 2009 by the International Maize and Wheat Improvement Center (CIMMYT) at the Indian Council of Agricultural Research (ICAR) -Central Soil Salinity Research Institute (29.7057°N latitude, 76.9554°E longitude and at an elevation of 243 m.a.s.l.), Karnal, India (Figure 1). Climate of the region is semi-arid and sub-tropical with extreme weather conditions with hot and dry (April- June) to wet summers (July-September) and cold dry winters (October- March) with an average annual rainfall of 670 mm, 75– 80% of which is received during the monsoon season. The soil characteristics of the experimental site are given in Table 1.

Table 1. Initial soil characteristics (0-15 cm layer) of experimental site

Soil property	Value
Clay (%)	19.9 ± 0.50
Silt (%)	46.1 ± 0.76
Sand (%)	34.0 ± 0.77
Textural class	Loam
pH (1:2 soil:water)	8.00 ± 0.02
Electric conductivity (dS m ⁻¹) (1:2 soil:water)	0.37 ± 0.02
Total organic carbon (%)	0.56 ± 0.01
Total N (%)	0.06 ± 0.002
Olsen P (available P) (kg ha ⁻¹)	12.6 ± 0.29
1N NH ₄ OAc-extreactable K (available K) (kg ha ⁻¹)	130 ± 1.73

For all variables n = 3 ± standard error of mean.

Figure 1. Maps of (A) India, (B) Indo Gangetic Plains (IGP), (C) Haryana (D) Karnal (E) experimental site and lay out of treatments

The managements scenarios were designed keeping in view present as well as future drivers of agricultural changes in the region (Table 2). Treatments included four cereal-based cropping managements varying in cropping system, tillage, crop establishment methods, and crop residue management practices. Managements were organized in a randomized complete block design and replicated three times in production-scale plots, each one measuring 2000 m² (20 m x 100 m). The four managements viz., Rice {conventional tillage (CT)/transplanted rice (TPR)}-wheat (CT) rotation which is conventional-till based current

farmers' practice in NW-IGP of India (CT-RW); rice-wheat-mungbean {CT/TPR- ZT (zero-tillage)-ZT} with 100% residue retention/ incorporation (CTR-ZTWMb); rice-wheat-mungbean (ZT-ZT-ZT) with 100% residue retention (ZT-RWMb); maize-wheat-mungbean (ZT-ZT-ZT) with 65% of maize and 100% of wheat-mungbean residue retention (ZT-MWMb). Management 2 (CTR-ZTWMb) is partially CA whereas management 3 and 4 (ZT-RWMb and ZT-MWMb) are full CA based practices (Table 2) and other details of these managements can be obtained from a previous study (Gathala *et al.*, 2013).

Table 2. Different scenarios of conservation agriculture management practices

Scenarios	Cropping system	Management system
CT-RW	Rice-Wheat	Rice-puddling, Wheat-conventional till; All residue removed (current farmers' practice)
CTR-ZTWMB	Rice-Wheat-Mungbean	Rice-puddling, Wheat-zero-till, Mungbean-zero-till; Full (100%) rice residue retained; partial (30%) wheat residue (anchored); and full (100%) mungbean residues incorporated during puddling in rice season
ZT-RWMB	Rice-Wheat-Mungbean	Rice-zero-till, Wheat-zero-till, Mungbean-zero-till; Full rice and mungbean whereas partial (anchored) wheat residue retained on soil surface
ZT-MWMB	Maize-Wheat-Mungbean	Maize-zero-till, Wheat-zero-till, Mungbean-zero-till; Full maize and mungbean whereas partial (anchored) wheat residue retained on soil surface

CT-RW conventional tillage rice-wheat, *CTR-ZTWMB* conventional tillage rice-zero tillage wheat and mungbean, *ZT-RWMB* zero tillage rice-wheat-mungbean, *ZT-MWMB* zero tillage maize-wheat-mungbean

2.2. Soil sampling and processing

Soil samples were collected after the harvesting of the wheat crop during April-May 2014, from 0-15 cm soil depth using an auger (5 cm internal diameter). Each plot was divided into four 50 m x 10 m grids. Within each plot, sub-samples were collected from nine locations and then a composite sample of each plot was prepared. The samples were wet sieved (2-mm mesh) and a subsample was stored at -20 °C for DNA extraction. The remaining portion of the fresh soil samples was kept in a refrigerator at 4 °C for analysis of soil biological parameters. DNA extraction was done on the next day of sampling; other parameters were also analyzed within one month. Samples were pre-incubated at room temperature overnight before analyzing the microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and soil enzymes activities. All equipments like the auger and the sieve were wiped thoroughly with alcohol before every use.

2.3. Analysis of soil samples

Electrical conductivity (EC) and pH of soil samples in soil: water (1:2) dilution was determined by following standard methods (Jackson, 1973). Dehydrogenase activity (DHA) was tested from the conversion of 2,3,5-triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) over a 24-h period. Soil (20 g) was mixed with 0.2 g CaCO₃, and from this 6 g were placed in each of three test tubes. Aqueous solution of 3% TTC and 2.5 ml distilled water was added and incubated at 37 °C. After 24 h, 10 ml methanol was added. Intensity of reddish color filtrate (TPF) was measured by UV/VIS spectrophotometer (LABINDIA UV 3000+) at 485 nm wavelength (Dick, 1997). Alkaline phosphatase activity (APA) was analysed as described by Eivazi and Tabatabai (1977); briefly APA was determined by measuring the release of p-nitrophenol by incubating 1 g soil at 37 °C for 1 h with 0.2 ml toluene, 4 ml universal buffer (pH 11.0), and

1 ml 50 mmol p-nitrophenyl phosphate. Yellow color intensity of p-nitrophenol was measured at 400nm wavelength. Activity of β -glucosidase was analysed by the method of Alef and Nannipieri (1995). Microbial biomass carbon (MBC) and nitrogen (MBN) were estimated by chloroform fumigation method (Vance *et al.*, 1987). The values of KC and KN were taken 0.38 and 0.45, respectively for MBC and MBN.

2.4. DNA extraction and sequencing

The metagenomic DNA was extracted from 0.25 g soil, using PowerSoil® DNA isolation kit (MO BIO Laboratories Inc., Carlsbad, California, USA) following the manufacturer's protocol. The DNA was quantified, and its purity was checked using Nano Drop spectrophotometer (Thermo Fisher Scientific, USA) at the wavelengths of 260 nm and 280 nm. The DNA purity and quantity were also verified by electrophoresis in 1% agarose gel and samples were adjusted to 50 ng/ μ L. The DNA was amplified for the fungal ITS region using universal forward ITS1-F primer CTTGGTCATTTAGAGGAAGTAA and the reverse ITS1Univ-R primer GCTGCGTTCTTCATC-GATGC. The amplifications were performed in 25 μ l reactions with Qiagen HotStar Taq master mix (Qiagen Inc, New Delhi), 1 μ l of each 5 μ M primer, and 1 μ l of template. The reactions were performed on ABI Veritithermocyclers (Applied Biosystems, UK) under the following thermal profile: 95 °C for 5 min, then 35 cycles of 94 °C for 30 sec, 54 °C for 40 sec, 72 °C for 1 min, followed by one cycle of 72 °C for 10 min, and 4 °C hold. The amplification products were visualized with eGels (Life Technologies, Grand Island, New York, USA). The products were then equimolarly pooled and each pool was size-selected in two rounds using Agencourt AMPure XP (BeckmanCoulter, Indianapolis, Indiana, USA) in a 0.7 ratio for both rounds. The size selected pools were then quantified

using the Qubit 2.0 fluorometer (Life Technologies, Grand Island, New York, USA) and loaded on an Illumina MiSeq (Illumina, Inc. San Diego, California, USA) 2x300 flow cell at 10pM.

2.5. Bioinformatic and statistical analyses

The data analysis was carried out in two stages denoising and chimera detection, followed by microbial diversity analysis. The paired-end reads were demultiplexed by Illumina supplied CASAVA. The primer binding sequences were filtered and its quality was checked using FastQC v 0.11.4 (Andrews *et al.*, 2010). The low quality reads trimming and paired end reads merging was performed using PEAR v 2.1 (Zhang *et al.*, 2013) The reads with base quality less than 25 were trimmed in order to retain high quality reads. Dereplication is performed using USEARCH 7 (Edgar, 2010) prefix-based algorithm that groups reads into clusters that shows 100% match to the centroid sequence. The sequences with a length greater than 100 bp were retained. Clustering at 4% divergence (consensus sequences) is performed using the USEARCH clustering algorithm where the singleton clusters (<2 member sequences) were filtered. Operational Taxonomic Unit (OTU) selection was performed using the UPARSE algorithm to classify the large number of clusters into OTUs. Chimera checking was performed on the selected OTUs using the UCHIME (Edgar *et al.*, 2011) chimera detection software executed in de-novo mode. Each clustered centroid was then mapped to their corresponding OTUs and chimeric sequences were then removed. Reads were then mapped to their corresponding non-chimeric cluster and taxonomies were assigned using the RDP classifier algorithm (Cole *et al.*, 2008) against UNITE database to form a OTU table. For each taxonomic level (phylum to species), the sequences and its corresponding percentages per full taxonomic match per sample were calculated, and the diversity indices,

i.e Shannon-Wiener diversity index (H), species richness (S), evenness (E) were calculated by OTUs using this formula:

$$H = -\sum [(p_i) * \ln(p_i)]$$

$$E = H/H_{\max}$$

Where, SUM = Summation, p_i = Number of individuals of species i /total number of samples S = Number of species or species richness, $H_{\max}$ = Maximum diversity possible, E = Evenness Soil chemical and biological data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of the SPSS software version 17.0 (SPSS Inc., Chicago, USA). The treatment means were separated by the Duncan Multiple Range Test at 5% level of significance ($P < 0.05$). The sequences of this study have been submitted to NCBI under BioProject Accession: PRJNA260467, BioSample: SAMN03023598.

2.6. Crop yield

The wheat samples were collected from four locations from each plot in April 2014. Crop was harvested manually from randomly selected $4 \times 5 \text{ m}^2$ quadrants for grain yield. Grain yield of crops was recorded at 14% moisture basis.

3. Results

3.1. Soil chemical and biological properties

The soil EC was significantly not different among the managements and varied from 0.22 to 0.26 dS/m (Table 3). Similarly, pH ranged from 7.84 to 7.88 (Table 3), and the lowest pH (7.60) was recorded in the CTR-ZTWMB management. Organic carbon (OC) was significantly higher in partial CA (CTR-ZTWMB) and full CA (ZT-MWMB and ZT-RWMB) based managements by 30%, 43%, and 47%, respectively compared

to conventional agriculture (CT-RW) based management (Table 3). Microbial biomass carbon (MBC) was highest ($265.92 \mu\text{g g}^{-1}$ dry soil) in ZT-MWMB followed by ZT-RWMB ($231.97 \mu\text{g g}^{-1}$ dry soil); In full CA-based managements (ZT-RWMB and ZT-MWMB), MBC was increased by 36% and 56%, and microbial biomass nitrogen (MBN) by 66% and 70%, respectively compared to conventional agriculture (CT-RW) (Table 3). Higher phosphatase activity (APA) was observed under ZT-MWMB ($59.23 \mu\text{g p-NP g soil}^{-1} \text{ h}^{-1}$) and ZT-RWMB ($55.76 \mu\text{g p-NP g soil}^{-1} \text{ h}^{-1}$), being 73% and 63% higher than CT-RW, respectively. Dehydrogenase activity (DHA) was significantly higher under ZT-MWMB ($62.88 \mu\text{g TPF g soil}^{-1} \text{ h}^{-1}$) and ZT-RWMB ($63.16 \mu\text{g TPF g soil}^{-1} \text{ h}^{-1}$) compared to CTR-ZTWMB and CT-RW ($51.14 \mu\text{g TPF g soil}^{-1} \text{ h}^{-1}$ and $37.26 \mu\text{g TPF g soil}^{-1} \text{ h}^{-1}$) (Table 3). Activity of β -glucosidase was statistically similar in ZT-MWMB and ZT-RWMB, however it was significantly higher compared to CT-RW (Table 3). ZT-MWMB and ZT-RWMB had 40% and 41% higher β -glucosidase activity compared to CT-RW (Table 3).

3.2. Fungal community abundance

From the four managements, a total of 2,32,024 quality-filtered sequences were obtained from extracted DNA. After the taxonomy based analysis of these sequences, it was found that at the phylum level, soils of all the four managements were dominated by Ascomycota followed by Basidiomycota and Glomeromycota. The relative abundance of Ascomycota was ranged from 55 to 74%, with the highest dominance found in ZT-MWMB (74%) followed by ZT-RWMB (71%), CTR-ZTWMB (68%), and CT-RW (55%), Basidiomycota and Glomeromycota did not follow any trend as both of these phyla were found in an abundance between 0 to 3% (Figure 2).

In total, 11 well defined classes were observed out of which Sordariomycetes had the highest abundance

and it was found in all managements, this class was followed in abundance by Dothideomycetes and

Eurotiomycetes (Figure 3a). Some classes were not evenly distributed among managements.

Table 3. Soil chemical biological properties and crop yield

Scenarios	EC (dS/m)	pH	OC	MBC (µg/g dry soil)	MBN (µg/g dry soil)	APA (µg p-NP/ g soil/ h)	DHA (µg TPF /g soil/ 24 h)	β- Glucosidase (µg p-NP/ g soil/ h)	Wheat yield (Mg ha ⁻¹)
CT-RW	0.26 ± 0.03 ^a	7.88 ± 0.08 ^a	0.47 ± 0.03 ^b	170.0±8.66 ^c	58.8±4.36 ^c	34.18±4.71 ^c	37.26±4.56 ^b	36.23±2.49 ^b	5.01±0.36 ^a
CTR-ZTWMb	0.23 ± 0.04 ^a	7.60 ± 0.11 ^b	0.61 ± 0.04 ^a	207.0±12.74 ^b	79.9±4.81 ^b	52.34±5.37 ^a	51.14±4.82 ^{ab}	43.12±1.79 ^{ab}	5.38±0.20 ^a
ZT-RWMb	0.23 ± 0.03 ^a	7.87 ± 0.13 ^a	0.69 ± 0.05 ^a	232.0±10.99 ^b	97.7±4.30 ^a	55.76±4.16 ^a	63.16±7.09 ^a	51.24±1.92 ^a	5.38±0.20 ^a
ZT-MWMb	0.22 ± 0.05 ^a	7.84 ± 0.10 ^a	0.67 ± 0.02 ^a	265.9±13.81 ^a	99.7±5.10 ^a	59.23±4.05 ^a	62.88±4.84 ^a	50.83±2.68 ^a	5.41±0.31 ^a

For all variables $n = 3 \pm$ standard error of mean. Different small letters within the same column show the significant difference at $P = 0.05$ according to Duncan Multiple Range Test for separation of mean

CT-RW conventional tillage rice-wheat, *CTR-ZTWMb* conventional tillage rice- zero tillage wheat and mungbean, *ZT-RWMb* zero tillage rice-wheat-mungbean, *ZT-MWMb* zero tillage maize-wheat-mungbean, *EC* electric conductivity, *OC* organic carbon, *MBC* microbial biomass carbon, *MBN* microbial biomass nitrogen, *APA* Phosphatase activity, *DHA* Dehydrogenase activity

The presence of Leotiomyces was recorded only in ZT-RWMb and ZT-MWMb, whereas Pezizomyces was found only in CTR-ZTWMb. Agaricomycetes was found only in CT-RW and CTR-ZTWMb. Three OTUs of Wallemiomycetes were found exclusively in ZT-MWMb. The dominating orders in all four managements were: Sordariales, Hypocreales and Pleosporales of the Ascomycota phylum (Figure 3b). The pattern of abundance of these three orders was similar in all four managements as Sordariales was dominating (17-40%), followed by Hypocreales, and Pleosporales. In CT-RW (Figure 4a), *Alternaria* was the most abundant genus (13.52%), followed by *Stachybotrys* (10%), whereas in CTR-ZTWMb (Figure 4b) *Podospora* (15.08%) was the most abundant genus, followed by *Alternaria* (10.08%). In ZT-RWMb (Figure 4c), *Epicoccum* (19.67%) was the most abundant genus, followed by *Cercophora* (7.98%), and in ZT-MWMb (Figure 4d), an unclassified genus of the

Sordariaceae family (10.50%) was the most abundant, followed by an unclassified genus of the Nectriaceae family (10.12%). Different diversity indices were calculated: the maximum number of fungal species was found in ZT-MWMb (95), followed by CTR-ZTWMb (91), ZT-RWMb (85), and CT-RW (54) (Table 4). The same trend was observed for the Shannon-Wiener diversity index, as it was higher in ZT-MWMb, CTR-ZTWMb and ZT-RWMb by 10%, 9%, and 35%, respectively, when compared to CT-RW (Table 4).

3.3. Crop yield

The wheat yield was recorded higher in ZT-MWMb (5.41 Mg ha⁻¹) but significantly at par with other management practices (Table 3). The wheat grain yields were recorded higher by 8%, 7% and 5% for ZT-RWMb and CTR-ZTWMb, respectively, when compared to CT-RW (5.01 Mg h⁻¹)

Figure 2. Distribution of different Phyla in the four managements

CT-RW: conventional tillage rice-wheat; *CTR –ZTWmb*: conventional tillage rice- zero tillage wheat and mungbean; *ZT-RWmb*: zero tillage rice-wheat-mungbean; *ZT-MWmb*: zero tillage maize-wheat-mungbean

(a)

Figure 3. Relative abundance of (a) Classes and (b) Orders in four different managements

CT-RW: conventional tillage rice-wheat; *CTR –ZTWmb*: conventional tillage rice- zero tillage wheat and mungbean; *ZT-RWmb*: zero tillage rice-wheat-mungbean; *ZT-MWmb*: zero tillage maize-wheat-mungbean

Figure 4. Distribution of different genera in *CT-RW* (a), *CTR-ZTWmb* (b), *ZT-RWmb* (c), and *ZT-MWmb* (d)

Table 4. Diversity indices of fungi in scenarios

Diversity indices		CT- RW	CTR- ZTWmb	ZT- RWmb	ZT- MWmb
Species richness S		54	91	85	95
Shannon-Wiener Index (H)	Diversity	3.22	3.49	3.34	3.54
Evenness (E) = H/Hmax		0.807	0.773	0.751	0.777

CT-RW conventional tillage rice-wheat, *CTR-ZTWmb* conventional tillage rice- zero tillage wheat and mung-bean, *ZT-RWmb* zero tillage rice-wheat-mungbean, *ZT-MWmb* zero tillage maize-wheat-mungbean

4. Discussion

4.1. Chemical and biological soil properties

Conservation agriculture management practices where zero-tillage coupled with residue retention revealed higher OC content in soils compared to conventional agriculture (Jat *et al.*, 2017). As crop residues decompose slowly under zero-tillage, and, organic matter thus gradually accumulates in the soil, which might have caused higher OC contents under CA-based managements (Dikgwatlhe *et al.*, 2014). Soil organic matter serves as a good source of energy for soil microbes, positively affecting microbial growth and hence influences their distribution (Wang *et al.*, 2016). Conservation agriculture based practices increases soil microbial biomass and activity viz., MBC, MBN, phosphatase, DHA, and β -glucosidase in full CA-based managements (ZT-RWmb and ZT-MWmb), because of a minimum disturbance of the soil system. Conservation agriculture system favored increased levels of microbial biomass carbon/ nitrogen, when compared to conventional agriculture; this might be due to increased associated C inputs, residue retention, and reduced tillage (Wang *et al.*, 2012). Cropping systems also affected MBC and MBN as these were found in higher amounts in multi cropping systems, when compared to one or two crop rotations (Moore *et al.*, 2000). Diverse type of crops secretes diverse root exudates, and also provide diverse types of crop residues in the soil, which affects microbial diversity, density, and growth (Venter *et al.*, 2016).

4.2. Enzyme activities

Conservation agriculture based managements reflected the higher soil enzymes activities (Table 3), compared to CT management. In CA, residue recycling, zero tillage, and crop rotation play a significant

role in increasing higher soil enzyme activities. High concentration of residues along with roots of previous crops in the surface soil, affects its microbial activity to a wider extent under undisturbed soil. The beneficial effect of crop roots, i.e. “the rhizosphere effect”, probably contributes significantly towards higher enzyme activities under CA management compared to CT systems (Bandick and Dick, 1999). In CT systems, soil moisture holding capacity is comparatively lower than CA systems because of crop residues retention and this influences moisture availability period which ultimately influences the enzyme activities under CT systems (Jin *et al.*, 2009). The phosphatase activities are highly influenced by agricultural practices, tillage, cropping systems, and crop residues managements. No-till with residue retention on the soil surface increases the total soil P and phosphatases activity (Wang *et al.*, 2011), as it increases the supply of readily available substrates, such as carbohydrates for microorganisms. Dehydrogenase activity (DHA) is highly influenced by tillage operations as it decreases with intensive tillage (Rolden *et al.*, 2005). β -glucosidase was found higher with CA based cereal (R/W) systems compared to conventional farmers practice (CT-RW). β -glucosidase plays an important role in the C cycle, as it involves in the microbial degradation of cellulose to glucose (Eivazi and Tabatabai, 1990).

4.3. Fungal diversity

Apart from SOC and enzyme activities, agriculture management practices also influences microbial diversity (Govaerts *et al.*, 2007). Higher microbial diversity was observed under full CA-based management practices due to the continuous presence of the crop residues on soil surface throughout the year. Reduced disturbances in ZT systems may favor fungal growth and activity due to enhanced establishment and maintenance of extensive hyphal networks.

Bailey *et al.* (2002) reported a shift towards fungal dominated microbial communities under zero-tillage, which are important for residue decomposition and nutrient cycling processes, and bear the closest resemblance to natural ecosystems. In our study, Ascomycota was the dominating phylum, followed by Basidiomycota in all the four managements. A similar dominance of Ascomycota, followed by Basidiomycota, was also reported by Miura *et al.*, 2015 with no-tillage. The maximum crop residue biomass in ZT-MWMb might be responsible for the highest (74%) abundance of Ascomycota (Figure S1) as these fungi are mainly responsible for residue degradation. Moreover, an intrinsic quality of the substrate may also influence abundance of Ascomycota, as in ZT-MWMb the quality of retained crop residues is different than other three managements. In ZT-MWMb residue of maize was retained but in rest three managements rice residue was retained. Classes of Ascomycota viz., Sordariomycetes, Dothideomycetes, and Eurotiomycetes were the dominating classes in all four managements which is in accordance with a previous study (Wang *et al.*, 2016). Members of Sordariomycetes are found in residue-rich soils, as these fungi have the potential to produce cellulolytic enzymes (Phosri *et al.*, 2012). The Dothideomycetes also represents a dominant class in the phylum Ascomycota, which are found as endophytes or epiphytes on living plants, and act as saprobes degrading cellulose and other complex carbohydrates in dead or partially digested plant organic matter (Hyde *et al.*, 2013). In our study, Sordariales followed by Hypocreales were the dominating Ascomycetous orders; similar observations were also reported by Klaubauf *et al.*, (2010), with both molecular and traditional cultivation approaches in agricultural soils. : In this study it was found that dominating phyla, classes and orders were similar in all managements but distribution of genera varied. It may be because of different practices (tillage, crop

establishment and residue recycling) under these managements did not much influence Ascomycota at higher level of classification (phylum, class and order) but affects at genera level (Wang *et al.*, 2016). Unclassified genera of Sordariaceae and Nectriaceae were dominant only under maize based systems, however other unclassified genera were dominant in rice based managements. This might be due to difference in accessible C sources and N availability in the diverse (maize and rice) crop residues (Miura *et al.*, 2015) in different managements. Several genera constituting less than 1% of abundance are not shown in figures (Figure4 a-d) but these taxa may contribute to the compositional uniqueness of the soil.

4.4. Diversity indices

When comparing diversity indices, ZT-MWMb showed higher diversity compared to other managements which indicates more stability of that management system (Giller *et al.*, 1997). Tillage plays a major role in influencing the microbial densities under agricultural soils (Wang *et al.*, 2016). Similarly observations of higher levels of bacterial diversity with no-till compared to conventional-till system was reported by Ceja-Navarro *et al.*, (2010). Under CA-based management systems, not only zero tillage but crop residues also play major roles in determining fungal dominance, as residues serve a continuous energy source for microorganisms, and retention of these crop residues on the soil surface increases their abundance by providing improved conditions for reproduction (Salinas-Garcia *et al.*, 2002). The highest species richness in ZT-MWMb can be correlated with the presence of maize in crop rotation system which provide greater amounts of plant residue with more decomposability that would support greater microbial populations and diversity compared to rice residues (Moore *et al.*, 2000).

4.5. Crop yield

Result of our study clearly showed the effect of ZT, crop establishment and residue retention/incorporation on wheat crops yields. Higher yields of wheat with CA-based management practices might be due to the compound effects of many factors like better soil quality (Choudhary *et al.*, 2018), improved soil physical and chemical properties (Jat *et al.*, 2017), and improved micro-climatic conditions that results in a lesser terminal heat effect (Gathala *et al.*, 2013).

5. Conclusions

This study showed that soil organic carbon and biological properties are influenced by CA based management practices. Organic carbon, MBC, MBN, APA, DHA and β -glucosidase were found higher under CA-based systems, compared to the conventional system (CT-RW), all of which resulted in higher wheat yields. The soil fungal communities at the phylum, class, and order levels varied among managements. All the four managements were dominated by Ascomycota, followed by Basidiomycota and Glomeromycota, but the abundance of these phyla varied with management systems. The CA based maize-wheat-mungbean system (ZT-MWMB) showed more fungal diversity than the rice based systems (CT-RW, CTR-ZTWMB and ZT-RWMB). Zero tillage with residue retention under efficient crop sequence increases the soil microbial properties, and thus increases soil fungal diversity and richness.

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