Soil bacterial diversity under conservation agriculture-based cereal systems in Indo-Gangetic Plains

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Received: 21 November 2017 / Accepted: 18 June 2018 / Published online: 4 July 2018
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Abstract

In Indo-Gangetic plains (IGP) of India, natural resources (soil, water, and environment) are degrading under the conventional-till (CT)-based management practices in rice–wheat cropping system. A long-term field experiment was conducted to understand the soil bacterial diversity and abundance under different sets of management scenarios (Sc). The study comprised of four scenarios, namely, -Sc.I CT-based rice–wheat system (farmers' practice); Sc.II, partial conservation agriculture (CA) based in which rice is under CT—wheat and mungbean under zero-tillage (ZT); Sc.III, full CA-based in which rice–wheat–mungbean are under ZT and Sc.IV, where maize–wheat–mungbean are under ZT. These scenarios varied in cropping system, tillage, and crop residue management practices. Using Illumina MiSeq sequencing technology, the variable regions V3–V4 of 16S rRNA were sequenced and the obtained reads were analyzed to study the diversity patterns in the scenarios. Results showed the presence of 53 bacterial phyla across scenarios. The predominant phyla in all scenarios were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes* which accounted for more than 70% of the identified phyla. However, the rice-based systems (Sc.I, Sc.II, and Sc.III) were dominated by phylum *Proteobacteria*; however, maize-based system (Sc.IV) was dominated by *Acidobacteria*. The class *DA052* and *Acidobacteriia* of *Acidobacteria* and *Bacteroidetes* of *Bacteroidia* were exceptionally higher in Sc.IV. Shannon diversity index was 8.8% higher in Sc.I, 7.5% in Sc.II, and 2.7% in Sc.III compared to Sc.IV. The findings revealed that soil bacterial diversity and abundance are influenced by agricultural management practices as bacterial diversity under full CA-based management systems (Sc.III and Sc.IV) was lower when compared to farmer's practice (Sc.I) and partial CA (Sc.II) scenarios.

Keywords *Acidobacteria* · Bacterial diversity · Conservation agriculture · Metagenome · *Proteobacteria*

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13205-018-1317-9>) contains supplementary material, which is available to authorized users.

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Introduction

Agricultural practices have major role in deciding diversity of soil microorganisms in any agro-ecosystem. Soil quality (physical, chemical, and biological) parameters are significantly influenced by the crop management practices which, in turn, affect the abundance, diversity, and activity of microorganisms (Ceja-Navarro et al. 2010; Pascual et al. 2010). Not only soil, crop rotation, plant diversity, and residue management also affect soil microorganisms in different ways (Silva et al. 2013). Intensive-till/conventional agriculture and zero-till/conservation agriculture-based management practices have implications on soil moisture, thermal regime, and nutrient dynamics (Gathala et al. 2013; Indoria et al. 2017) that, in turn, influence distribution and abundance of microorganisms (Smith et al. 2016).

In the conventional-till (CT)-based rice–wheat cropping systems of North-West Indo-Gangetic Plains (IGP) of India, farmers preferred clean cultivation by repeated tillage operations and in-situ burning of residues (Lohan et al. 2018). The conventional agriculture management practices of cultivation has resulted in natural resource degradation (soil, water, and energy), low factor productivity, nutrient deficiencies, ground water depletion, and greenhouse gas emission through crop residue burning (Gathala et al. 2013; Jat et al. 2014). Tillage causes soil physical disturbance which, in turn, changes soil moisture, soil temperature, soil aeration, and placement of the crop residues within soil profile which influences biological properties of soil to a great extent (Kladivko 2001; Six et al. 2006). Intensive tillage operations are responsible for soil degradation through erosion (water and wind) and volatilization of fertilizer nutrients. Influence of tillage on soil properties and crop yield is controlled by soil type, climatic conditions, and cropping history (Halvorson et al. 2002). Adoption of CA-based cropping system management practices under different agro-ecologies can address the challenges of natural resource degradation, crises (energy, water, and labor), instability in crop yields, high production costs, low input (water and nutrient) use efficiency, and adverse effects of climate change (Hobbs et al. 2008; FAO 2014). CA embodies three inter-linked principles—minimum mechanical disturbance of soil (reduced or zero-tillage), maintaining a permanent cover on soil surface, diversified, and efficient crop rotations. CA practices in IGP are helpful in improving soil properties and nutrient availability (Jat et al. 2017). CA helps to build soil organic matter, conserves soil moisture and buffers against drought as well as temperature extremes by retaining the crop residues (Gathala et al. 2011) and, at the same time, arresting soil degradation, and provides environmental benefits through avoiding in-situ burning (Sharma et al. 2014).

In agro-systems, it is well known that bacterial diversity varies with wide range of indicators agro-system. The variation across crop production management practices, *viz.*, tillage, crop establishment, water, nutrient, and residue management, influences the soil environment which alters the microbial diversity (Ashworth et al. 2017; Ceja-Navarro et al. 2010; Smith et al. 2016). In CA, impact of systems management practices on soil microbes is poorly known for taxonomic composition. In-depth understanding of the microbial communities in agricultural soils provides the path to study their roles in agro-ecosystem which was not possible by the traditional methods like culture-based methods and microscopic study. These traditional methods can only reveal a fraction of microbial diversity. Efforts were made to study the indigenous soil microbial taxonomic diversity and composition by high-throughput sequencing technologies using Illumina sequencing of 16S rRNA genes. These technologies helped in better assessing the vast diversity

of soil microbial communities by generating thousands of sequences from a single-soil DNA sample (Roesch et al. 2007).

In cereal-based systems, overall diversity of microorganisms is affected by wide range of indicators like cropping system, tillage, crop residue, water, and nutrient management practices. Some researchers (Dong et al. 2017; Souza et al. 2016) studied the impact of tillage on soil bacterial diversity and found bacterial communities differed significantly between the different tillage treatments. However, information on bacterial diversity using metagenomics under CA-based systems with varied indicators is not available in IGP of India where 1.5 Mha (million hectares) area is under CA-based rice–wheat cropping system. Henceforth, need was realized to assess the impact of CA management practices on soil bacterial diversity in IGP to understand whether CA-based management may or may not have any significant effect on bacterial diversity. Under different climatic conditions, there have been reports that bacterial diversity increases with CA practices (Ceja-Navarro et al. 2010) and a few reports also shown its decrease (Lienhard et al. 2014) when compared to the conventional systems. Therefore, study was carried out after 6 years of continuous cultivation with the hypothesis that different management practices (CT vs CA) will differently influence soil bacterial diversity and abundance in cereal-based cropping system of IGP.

Materials and methods

Description of site and treatments

A field experiment was started at Indian Council of Agricultural Research-Central Soil Salinity Research Institute (ICAR-CSSRI) (29.42°N latitude, 76.57°E longitude, 243 msl elevation), Karnal, Haryana, India, in 2009. Climate of the region is semi-arid and sub-tropical with average annual rainfall of 670 mm, of which 75–80% received between June and September. The mean annual maximum temperature is 34 °C and minimum is 18 °C. However, in June, daily temperatures rise from 41 to 44 °C, and in January, temperatures go to a minimum of 0 to 4 °C. The relative humidity varies from 55 to 90% throughout the year. The treatments comprised of four cereal-based scenarios varying in cropping system, tillage, crop establishment methods, and residue management practices. Treatments were replicated thrice and organized in a randomized complete block design in production-scale plots, each measuring of 2000 m² (20 m × 100 m). The scenarios were designed keeping in view present as well as future drivers of agricultural changes in the region (Table 1) and their details can be obtained from earlier publication (Gathala et al. 2013).

Table 1 Different scenarios of conservation agriculture management practices

Scenario	Practices	Cropping system	Management system
Sc.I	Farmers' practice	Rice–wheat	Rice-puddling, wheat-conventional till; all residues removed
Sc.II	Integrated crop and resource management	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
Sc.III	Conservation agriculture-based systems	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
Sc.IV	Futuristic conservation agriculture-based systems	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

Soil sampling and processing

Soil samples were taken after harvesting of wheat after 6 years (May 2015) of continuous experiment with the same management practices. Crop residues were removed from surface and soil samples were taken from replicated plots. Four samples were randomly taken from each plot at 0–15 cm soil depth aseptically using an auger and thoroughly mixed to make a composite sample. After sampling, soil samples were immediately transferred to laboratory for chemical analysis and DNA extraction.

Physical and chemical analyses of soil samples

Soil pH and electrical conductivity (EC) in soil: water ratio of 1:2 was determined by following standard methods (Jackson 1973). Soil bulk density (BD) was measured by core method collecting soil cores at 0–15 cm depth (Blake and Hartge 1986) after harvest of wheat in May 2015. The oxidizable organic carbon (OC) content of the soils was determined using method of Walkley and Black (1934). The available nitrogen (N) in soil was determined by alkaline permanganate method (Subbiah and Asija 1956).

DNA extraction and sequencing analysis

DNA was extracted from soil samples using PowerSoil® DNA isolation kit (MO BIO Laboratories Inc., Carlsbad, California, USA) following the manufacturer's protocol. Purity and quantity of DNA were verified using electrophoresis in 1% agarose gels and its quantity were adjusted to 50 ng/μL. The purity of the DNA was assessed using ND1000 nanodrop spectrophotometer (Thermo Scientific, MA, USA) at 260 and 280 nm. DNA of replicated samples of each treatment was pooled before sequencing.

Amplicon library construction

The library was constructed using the 16S Metagenomic Sequencing library preparation methodology (http://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html). Each sample with a gel-purified fragment (~4 ng) was used for amplifying V3–V4 region of 16S rRNA region with specific primers that has a tag sequence that are complementary to the Illumina sequence adapter and index primers from the Nextera XT Index kit V2 (Illumina, #FC-131-2002) which resulted ~430–480 bp amplicons. Amplification was verified on 1% agarose gel before proceeding for indexing PCR. In the next round of PCR (indexing PCR), Illumina sequencing adapters and dual-indexing barcodes were added using limited cycle PCR to generate a final product of ~570–620 bp. High Prep PCR post-PCR clean up system (MagBio Genomics, Inc., USA) was used to clean the libraries and quality control check of the library was performed using High Sensitivity Tape Station (Agilent Technologies, USA). PCR products with unique indices from each library were taken in equal (~4 ng) quantities and subjected to paired-end sequencing using Illumina sequencing platform.

Illumina adapter sequences:

5'AATGATACGGCGACCACCGAGATCTACAC [i5] TCGTCGGCAGCGTC 3'.

5'CAAGCAGAAGACGGCATACGAGAT [i7] GTCTCG TGGGCTCGG 3'.

[i5, i7]—unique dual index sequence to identify sample-specific sequencing data.

Sequencing and initial processing of sequence reads

The PCR products from each library having unique indices were taken in equal nanomolar quantities and were sequenced using Illumina MiSeq sequencer at Genotypic Technology Pvt. Ltd. (Bangalore, India). All the samples

were pooled in a single lane followed by image analysis and base calling using Illumina Analysis pipeline V 2.2. The generated paired-end raw reads were demultiplexed using bcl2fastq v 2.17 tool by allowing 1 bp mismatch in index1 and index2 barcodes, respectively. The reads from each sample were subjected to quality check using FastQC v 0.11.4. Reads having primer sequence and high-quality bases with phred score ($> Q20$) were processed further for analysis. Less than 2% of Ns were allowed in the sequence ends. Base quality $< Q20$ towards the end were removed from the sequence ends. Sequences were stitched by reverse complementing R2 reads and merging with R1 reads. These stitched reads were analyzed using QIIME-1.9.1 analysis workflow.

Data analysis

All sequences were processed using QIIME-1.9.1 software package. The reads were clustered at 97% identity against the chimera free Greengenes database (version 13.8) using UCLUST v1.2.22q. "Closed reference" approach was performed for this study where the reads that failed to match the reference sequences were discarded. Taxonomies were assigned from the reference database based upon the identity of the reference sequence, Operational taxonomic Unit (OTU) ids clustered against. The number of raw reads per sample were 165,936 (Sc.I), 208,264 (Sc.II), 178,895 (Sc.III), and 157,104 (Sc.IV) bps. The OTU table was rarefied to a sampling depth of 40,000 sequences per sample with step size of ten interactions. Alpha diversity indexes were determined based on the rarefied biome. The 97% OTUs phylogenetic tree supplied with Greengenes database was used for calculation of beta diversity using weighted and unweighted Unifrac distances. Supplementary S1 has software details on OTU picking analysis and graphical visualizations.

Graphical visualization and statistical analysis

Heatmap and stacked barcharts were generated at phylum and class level, respectively, with relative abundance values $\geq 1\%$ for all the samples from various scenarios in R 3.3.0 environment. Heatmap was plotted using NMF package with a heatmap function applying pairwise distance correlation method and stacked barcharts was generated using ggplot2. STAMP (Statistical Analysis of Metagenomic Profiles) tool was used for significance testing between two samples at order level and the corresponding significant orders ($P < 0.05$) were plotted as extended error barcharts for a pair of scenarios using Fisher's exact test with Storey-FDR correction and the difference between proportion was calculated using Newcombe–Wilson method (with confidence interval 95%) (Supplementary S2). Column barcharts generated

using R ggplot2 were compared across four scenarios at order level for a few classes of *Proteobacteria*.

Soil chemical and physical data were subjected to analysis of variance (ANOVA) and using the general linear model (GLM) procedure of the SPSS window version 17.0 (SPSS Inc., Chicago, USA). Treatment means were separated by Duncan Multiple Range Test at 5% level of significance ($P < 0.05$).

Nucleotide sequence accession number

Paired-end Illumina sequence data were submitted to the NCBI sequence Read Archive (SRA) under Bioproject accession number PRJNA348170 which consists of four biosample accession numbers SAMN05905538 (Sc.I), SAMN05905539 (Sc.II), SAMN05905540 (Sc.III), and SAMN05905541 (Sc.IV).

Results

Soil chemical parameters in different scenarios

In this study, scenario 1 (Sc.I) was based on farmers practice and scenario 2 (Sc.II) was based on partial CA-based, whereas scenarios 3 and 4 (Sc.III and Sc.IV) were based on full CA-based practices (Table 1). Results indicated that, after 6 years of continuous cultivation, EC was not affected by management practices as it did not differ significantly among scenarios. Full CA-based system recorded the lower pH and higher organic carbon and nitrogen than the partial CA and the conventional based system. Soil pH was recorded lower in Sc.IV (7.61) compared to Sc.I (7.89) and Sc.II (7.83), and it was followed by Sc.III (7.70). The CA-based scenarios (Sc.II to IV) recorded higher organic carbon (OC) and nitrogen (N) content averaged 0.78% and 178.17 kg/ha, respectively, compared to Sc.I (0.47% and 130.9 kg/ha). OC content was increased by 45, 77, and 79% in Sc.II, Sc.III, and Sc.IV, respectively, when compared to Sc.I (0.47%). Similar trend was recorded with N content where it was increased by 21, 48, and 49% in Sc.II, Sc.III, and Sc.IV, respectively, compared to Sc.I (130.9 kg/ha). Similarly, lower bulk density was recorded with CA-based scenarios (Sc.II to IV) averaged 1.56 Mg/m³ compared to Sc.I (1.63 Mg/m³) (Table 2).

Bacterial abundance/ community composition in different scenarios

A total of 710,199 raw reads were obtained from all four scenarios and 478,913 stitched reads were considered for analysis after processing. A total of ~42% of the stitched reads from all four samples after processing were as assigned into

Table 2 Soil chemical and physical parameters

Scenario	EC (dS/m)	pH	OC (%)	N (kg/ha)	BD (mg/m ³)
Sc.I	0.26 ± 0.03 ^a	7.89 ± 0.12 ^a	0.47 ± 0.04 ^c	130.9 ± 1.33 ^c	1.63 ± 0.01 ^a
Sc.II	0.23 ± 0.04 ^a	7.83 ± 0.13 ^a	0.68 ± 0.05 ^b	158.3 ± 2.06 ^b	1.57 ± 0.03 ^b
Sc.III	0.22 ± 0.05 ^a	7.70 ± 0.11 ^{ab}	0.83 ± 0.08 ^a	193.8 ± 1.09 ^a	1.56 ± 0.04 ^b
Sc.IV	0.22 ± 0.03 ^a	7.61 ± 0.18 ^b	0.84 ± 0.10 ^a	182.4 ± 2.01 ^a	1.55 ± 0.06 ^b

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

EC electric conductivity, OC organic carbon, BD bulk density

Table 3 Distribution of dominant phyla in conservation agriculture scenarios

Scenario	Dominant phyla (%)			
Sc.I	<i>Proteobacteria</i> (33)	<i>Acidobacteria</i> (20)	<i>Actinobacteria</i> (11)	<i>Bacteroidetes</i> (7)
Sc.II	<i>Proteobacteria</i> (37)	<i>Acidobacteria</i> (22)	<i>Actinobacteria</i> (9)	<i>Bacteroidetes</i> (7)
Sc.III	<i>Proteobacteria</i> (31)	<i>Acidobacteria</i> (21)	<i>Bacteroidetes</i> (11)	<i>Actinobacteria</i> (7)
Sc.IV	<i>Acidobacteria</i> (32)	<i>Proteobacteria</i> (24)	<i>Bacteroidetes</i> (10)	<i>Actinobacteria</i> (7)

different OTUs with reference to the Greengenes database. The average read length for all the samples were ~410 bp. A total of 10,570 unique OTUs were detected from all the samples at 97% sequence similarity including the singletons. Rarefaction curve generated at a read depth of 40,000 displayed an asymptotic curve indicating the saturation profiling of the bacterial communities (Supplementary S3).

A total of 53 phyla were detected from all the samples; most of them were present in all scenarios. The predominant phyla in all scenarios were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes* which accounted for more than 70% of the identified phyla's, in each scenario, present in soil (Table 3). First three scenarios (Sc.I–III) were dominated by phylum *Proteobacteria* and it was followed by *Acidobacteria*. Conversely, in Sc.IV, dominating phylum was *Acidobacteria* followed by *Proteobacteria*. Three phyla of archaea—*Crenarchaeota*, *Euryarchaeota*, and *Parvarchaeota*—were also reported but their abundance was very low (<0.007%). To understand the clustering pattern for samples and their abundance profile across four scenarios, a biclustering heatmap (Fig. 1) was generated based on the relative abundance of the bacterial phyla contributing greater than 1%. This figure shows that bacterial community in Sc.I and Sc.II was closely clustered and distantly related to Sc.IV. Further stacked barchart was generated at class level (Fig. 2) to visualize the abundance percentage of the microbial communities residing across four scenarios. It was found that distribution of classes was similar in Sc.I, Sc.II, and Sc.III but differs in Sc.IV. Class *Alphaproteobacteria* of *Proteobacteria* was the most abundant class followed by *Acidobacteria-6* of *Acidobacteria* in all scenarios except in Sc.IV. The most abundant class in Sc.IV is *DA052* of *Acidobacteria* followed by *Alphaproteobacteria*. Apart from higher abundance of *DA052*, abundance of *Acidobacteriia* was also

Fig. 1 Phylum level biclustering heatmap and this is performed on the top 1% of the Phyla based on the relative abundance values. The heatmap was generated using NMF package with a heatmap function with pairwise distance correlation method. The color code scale represents the relative abundance values (<https://cran.r-project.org/web/packages/NMF/NMF.pdf>)

exceptionally higher in Sc.IV. As mentioned above the most dominating phylum is *Proteobacteria*, which comprises four major classes—*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria*. Further

Fig. 2 Stacked barchart generated at class level for samples across four scenarios with relative values $\geq 1\%$

comparative analysis was performed for these classes of *Proteobacteria* at order level (Fig. 3a–d). The class *Alphaproteobacteria* showed majority of reads of order *Rhizobiales*, *Rhodospirillales*, and *Sphingomonadales* order. *Deltaproteobacteria* was second most abundant class of *Proteobacteria* that was dominated by *Myxococcales* in all four scenarios and followed by *Syntrophobacterales*, *Desulfuromonadales*, and *Entotheonellales*. The order of *Betaproteobacteria* was dominated by *Burkholderiales* in all scenarios; MND1 was present in greater proportion in Sc.III, while *Neisseriales* showed more hits in Sc.IV compared to the others. *Gammaproteobacteria* had a higher proportion of *Xanthomonadales* followed by *Thiotrichales* and *Pseudomonadales*.

Bacterial diversity in different scenarios

Different alpha diversity indices such as Shannon diversity index, chao1, PD_whole tree, and observed species

were measured. Phylogenetic diversity of the individual samples was calculated for species richness (Chao1) and Faith's diversity index (PD_whole tree). Converging trend in the alpha diversity values of chao1, PD_whole tree, and observed species were recorded. Shannon diversity index was 8.7% higher in Sc.I, 7.3% in Sc.II, and 2.6% in Sc.III compared to Sc.IV. The Chao1 diversity was also higher by 27.09% in Sc.I, 17.84% in Sc.II and almost similar in Sc.III as compared to Sc.IV (Table 4).

Discussion

Soil chemical parameters in different scenarios

In full CA-based scenarios (Sc.III and Sc.IV), lower pH was observed which attributed to more accumulation of organic matter in the upper surface layer (0–15 cm) under

Fig. 3 Column barcharts showing the relative abundance of top orders identified in **a** Alphaproteobacteria; **b** Betaproteobacteria; **c** Gammaproteobacteria; **d** Delta proteobacteria

Table 4 Alpha diversity of bacteria in soils of conservation agriculture scenarios

Scenario	Shannon diversity index	Chao1	PD_whole_tree	Observed species
Sc.I	11.133	8690.763	249.123	6000
Sc.II	10.990	8058.105	239.627	5675
Sc.III	10.507	6898.574	217.194	4737
Sc.IV	10.240	6838.160	207.602	4650

CA-based management which causes increase in electrolytes concentration and reduction in pH. Similarly Rahman et al. (2008) also reported lower value of pH with no tillage compared to the conventional tillage. Higher content of OC and N was recorded with CA-based management

scenarios due to zero-tillage coupled with huge residue retention of 64.3 t/ha in Sc.II, 64.8 t/ha in Sc.III and 80.4 t/ha in Sc.IV, respectively, when compared to Sc.I (no residue retention) in the last 5 years. Residue retention helped in increasing the soil physico-chemical and biological properties (Jat et al. 2015; Choudhary et al. 2018a, b) which results in improved OC and N content in soil. Similar findings of increased SOC and N were also reported by other researchers (Jat et al. 2017; Mu et al. 2016). The conventional tillage practices deteriorate soil structure resulted in lower SOC and total nitrogen due to higher mineralization and leaching rate (During et al. 2002). In CA-based practices, lower bulk density might be attributed to increase in soil organic matter, increase in activities of soil micro- and macroorganisms, and improved aggregation compared to the conventional

tillage practices (Blanco-Canqui et al. 2005; Choudhary et al. 2018b; Gupta et al. 2000; Singh and Sidhu 2014).

Abundance pattern of bacterial phyla in scenarios

The cropping systems, management practices, and recycled residues affected the dominance of bacterial phyla. *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes* were among the most abundant phyla in the all samples, this result is consistent with the previous studies (Navarro-Noya et al. 2013; Wang et al. 2016). *Proteobacteria* was the dominating phyla in rice-based cropping systems (Sc.I, Sc.II and Sc.III) where lower amount of residue recycled. However, *Acidobacteria* was found dominating in maize-based cropping system (Sc.IV) where higher amount of residue recycled. *Proteobacteria* is the largest and phenotypically most diverse division amongst prokaryotes (Gupta 2000), and found in many different shapes which reflect enormous morphological and physiological diversity within this phylum (Stackebrandt et al. 1988). *Acidobacteria* is one of the most distributed bacterial groups which is abundantly present in the environment (Hugenholtz et al. 1998), but its abundance is usually reported lower than *Proteobacteria* (Zhao et al. 2014). In the study of Meisinger et al. (2007), it was found that some members of *Acidobacteria* were always associated with *Gammaproteobacteria*. This association is the result of ecological relationship between *Proteobacteria* and *Acidobacteria* which may influence each other's position in the community (Kielak et al. 2016) as, in our study, both were found in dominance. These bacteria may be an important contributor to agriculture ecosystems, as they are particularly abundant within soils. Although high abundance and diversity of *Acidobacteria* were reported, but relatively less information about the activities and ecology of the members of this phylum are available, because majority of these are difficult to cultivate and lack in bacterial culture collections (Eichorst et al. 2011; Navarrete et al. 2013). *Acidobacteria* and *Bacteroidetes* tend to inhabit eco-niches supplied with plant-derived organic matter, where these are specializing in their degradation (Naumoff and Dedysh 2012) as it was evident from the presence of exceptionally higher abundance of members of *Acidobacteria* (*DA052* and *Acidobacteriia*) and *Bacteroidetes* (*Bacteroidia*) in Sc.IV (Fig. 2). In the Sc.IV (maize-based systems), large quantities of residue were recycled which might be responsible for making soil environment more acidic than other scenarios (Jat et al. 2017). The lower pH may favor the occurrence of some groups of *Acidobacteria* (*DA052* and *Acidobacteriia*) (Shen et al. 2013; Fierer et al. 2007; Navarrete et al. 2013). Apart from amount of residues, SOC was also higher in Sc.IV that might be resulted in higher abundance of *Acidobacteria* (Table 3), and theses are in line of work done by Navarrete et al. (2013) and Liu et al. (2016). A decline

in proteobacterial population was observed in organically managed soils compared to the conventional managed soils (Aparna et al. 2014; Chinnadurai et al. 2014). Smit et al. (2001) hypothesized that *Proteobacteria* and *Acidobacteria* (P/A) ratio may provide insight into the general soils nutrient status; high and low P/A ratio would be indicative of copiotrophic and oligotrophic soils, respectively. In our study, low P/A ratio was observed in maize-based system (Sc.IV). In Sc.IV, higher amount of maize residue were present than any other crop residue. Maize residues (maize stalks) are not easily decomposes because of wide C/N ratio and less contact with surface compared to rice residue, and this may create oligotrophic conditions as it was evident by the presence of exceptionally higher abundance of *Ellin6513* and *Acidobacteriales* which are orders under *Acidobacteria* in Sc.IV (Supplementary S2f). *Acidobacteriales* was dominated by *Koribacteraceae*. *Ellin6513* and *Koribacteraceae* are considered as oligotrophs by some researchers (Fierer et al. 2007). Negative correlation between abundance of *Acidobacteria* and carbon (C) mineralization rates, and positive correlation of β -*Proteobacteria* and *Bacteroidetes* abundances with C mineralization were reported by Fierer et al. (2007). Similarly, in our study, positive correlation of C mineralization and β -*Proteobacteria* was found in Sc.II where the highest C mineralization rate and highest abundance of β -*Proteobacteria* (Fig. 3b) were recorded. However, no correlation was found between abundance of *Acidobacteria* and C mineralization rates in other scenarios. Highest abundance of *Acidobacteria* (Table 3) was recorded with Sc.IV, while the lowest C mineralization was recorded with Sc.I ($61.4 \mu\text{g C g}^{-1}$) compared to other scenarios (Sc.II- $91.5 \mu\text{g C g}^{-1}$, Sc.III- $70.74 \mu\text{g C g}^{-1}$ and Sc.IV- $83.64 \mu\text{g C g}^{-1}$), while the highest C mineralization was recorded in Sc.II which has the highest abundance of β -*Proteobacteria* (Fig. 3b).

Bacterial community composition at class and lower level

Soil bacterial community composition was influenced by CA-based management practices. In this study, it was observed that, within phylum *Proteobacteria*, the most abundant class was *Alphaproteobacteria* which was followed by *Deltaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* in all scenarios. Similar trend of abundance of classes was also reported by Spain et al. (2009) in their crop fields. The *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria* were dominated by the members of *Rhizobiales*, *Burkholderiales*, *Xanthomonadales*, and *Myxococcales*, respectively. Castro et al. (2010) also reported the same trend of dominance except for *Gammaproteobacteria*, which was dominated by *Chromatiales* and *Enterobacteriales* in their study. *Alphaproteobacteria* are one of the most studied bacterial classes (Ettema and

Andersson 2009) which have different roles in ecosystem like- plant-growth promoter (*Azospirillum*), plant symbiont (*Rhizobium*, *Azorhizobium*, *Mesorhizobium*, and *Sinorhizobium*), phototrophs (*Rhodobacter*), and pathogens (*Agrobacterium*, *Brucella*, and *Rickettsia*). The *Betaproteobacteria* dominating by *Burkholderia* plays different roles in soils like biological control of pathogens (Parke and Gurian-Sherman 2001), plant-growth promotion (Nehra and Choudhary 2015), and nitrogen fixation (Wong-Villarreal and Caballero-Mellado 2010). Chemolithotrophic genera related to nitrification, including *Nitrosomonas* and *Nitrospira*, are also important members of the *Betaproteobacteria* (Schmidt and Bock 1997; Shaw et al. 2006). The member of *Deltaproteobacteria* may also plays important role in the availability of micronutrients for plants and soil microbiota, as they reported to be involved in the reduction of iron and sulphate (Foti et al. 2007; Hori et al. 2010). The *Gammaproteobacteria* class comprises several important bacteria like *Xanthomonas* and *Pseudomonas* spp. *Pseudomonas* is one of the most commonly described genera possessing plant-growth promoting activities (Nehra and Choudhary 2015), whereas *Xanthomonas campestris* is widely known as pathogenic bacterium of soil (Boch and Bonas 2010). Although, by the high-throughput sequencing methods, we are able to study more bacterial groups than the traditional cultural methods but very limited information is available on these bacterial groups, because it is very often that many of these sequences do not match with known sequences of bacterial species. Only fractions of the soil bacterial species are known, and hence, it is a major challenge for researchers to identify and culture them to study their role in their eco-niches.

Diversity of bacteria in scenarios

Soil microbial diversity is considered to be critical to the integrity, function, and long-term sustainability of soil ecosystems (Kennedy and Smith 1995). Bacterial diversity was more in the conventional practices (Sc.I) compared to CA-based scenarios (Sc.II to IV). Degruene et al. (2016) and Lienhard et al. (2014) also reported similar trend as in the conventional practices bacterial richness and diversity increases, whereas some reported inverse of this trend that they found highest levels of bacterial diversity and richness in soils under CA (zero-tillage with crop residue retention)-based system (Constancias et al. 2014; Ceja-Navarro et al. 2010). In full CA-based scenarios (Sc.III and IV), high stability of aggregates was observed after 5 years of continuous cultivation (Choudhury et al. 2014) which may provide the stable environment to the microbes. Once the soil stabilizes a particular type of bacterial communities, this, in turn, may dominate any other variety of communities which may narrow down the bacterial diversity. Higher diversity in the conventional till soil may be due to

the disturbance and exposure of soil to the different climatic conditions which causes release of organic matter, making it available for bacterial activities (Degruene et al. 2015).

Conclusion

Conservation agriculture (CA)-based management practices in cereal-based cropping system influenced the bacterial diversity and abundance. Higher bacterial diversity was recorded with farmers' practice compared to CA-based practices. Most abundant phylum in rice and maize-based systems were *Proteobacteria* and *Acidobacteria*, respectively. The high abundance of *Acidobacteria* in Sc.IV was governed by tillage, crop rotation, and amount of crop residues recycled. At class level, *Proteobacteria* was dominated by *Alphaproteobacteria*, and it was closely followed by *Deltaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*. In this study, a number of reads belongs to many bacterial classes were recorded, but very little is known about their individual functions and role in the soil, and hence, more research is required to understand the influence of layering of crop management practices on bacterial species.

Acknowledgements We acknowledge that this research was undertaken in collaboration with International Maize and Wheat Improvement Centre (CIMMYT) under Cereal Systems Initiative for South Asia (CSISA) project supported by Bill and Melinda Gates Foundation (BMGF), USAID and CGIAR Research Programs on Climate Change, Agriculture and Food Security (CCAFS), and Wheat Agri-food Systems (WHEAT). We also acknowledge the support received from the Director, ICAR-CSSRI, Karnal.

Author contributions MC, PS, and HS designed the study; AM, ML, and HS gave the idea of study and supported the design; MC conducted the research; AD and BR analyzed the data; MC wrote the manuscript and HS assisted with revising the draft manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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