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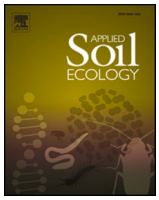

Organic farming induces changes in bacterial community and disease suppressiveness against fungal phytopathogens

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ABSTRACT

Due to higher microbial activity and diversity, organic farming serves as a sustainable alternative in preventing several soil-borne plant diseases. However, there are limited studies that have shown direct relationship between soil bacterial composition and its effect on disease suppressive potential under different farming systems. Thus, the objective of the study was to understand the effect of farming practices on disease suppressive ability of the soil using a long-term (managed since 18 years) field experiment under organic and conventional farming management. Amplicon sequencing revealed higher abundance of several biocontrol genera in organic field compared to conventional field. The diversity indices for bacterial communities were significantly higher in soil from organic field. Subsequently, the comparative disease suppressive potential of the two management practices was validated *in planta* against two model phytopathogens, *Rhizoctonia solani* and *Fusarium oxysporum*. The disease severity was less in plants treated with microbiome from organic field compared to that of conventional field. The study revealed the key taxa such as *Flavobacterium*, *Bacillus*, *Pseudomonas*, *Planctomycetes* etc. with potential to impart disease suppressiveness in organic field. This can serve as the basis for generation of synthetic microbial community to induce suppressiveness in otherwise conducive soil.

1. Introduction

Phytopathogens have threatened crop productivity globally and imposed serious challenges in providing food security to the ever-increasing world population. FAO reported that plant diseases result in a loss of US $220 billion to the global economy annually, with more detrimental consequences for developing countries (Klauser, 2018). Conventionally, to prevent the plants from being infested by pathogens, chemicals such as fungicides and pesticides, have been widely adopted (de Schutter, 2011). Such a practice of using synthetic amendments in agriculture is followed in conventional farming. Although it has a positive impact on the quality and productivity of crops, most of the management practices under conventional farming exert detrimental effects on soil health in terms of reducing microbial diversity (Suja et al., 2017). Microbial diversity, in turn, is directly correlated with soil fertility by performing several ecological functions in the soil (Singh and Gupta, 2018). Thus, there is a need to promote sustainable and eco-friendly agricultural practices that ensure the maintenance of good soil health and the preservation of soil biodiversity.

Organic farming practice, which is a sustainable alternative to conventional farming, is based on the use of natural amendments as a rich source of nutrients, without application of any chemical amendment. It employs crop rotation, manual weeding, and biological solutions to combat plant diseases (Rempelos et al., 2020). As per statistics from 2017, globally 69.8 million ha of land is under organic farming practice, with a significant proportion (about 1.78 million ha) in India (Lernoud and Willer, 2017). Organic farming is believed to preserve the integrity of the soil in terms of both biotic and abiotic components, thus maintaining soil fertility (Suja et al., 2017). Studies have indicated similar trends in crop productivity between conventional and organic farming (Bonanomi et al., 2016; Luo et al., 2018). However, there are other reports where a sharp decline in the yield parameters has been observed under organic farming compared to conventional farming (Niggli, 2015).

Soil microbes, the key players to maintain soil health, are directly influenced by diverse agri-management practices. Studies have revealed that higher microbial activity and diversity is present under organic farming practices (Lori et al., 2017; Lupatini et al., 2017; Liao et al.,

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2018; Cuartero et al., 2021). However, there is still no consensus with other reports stating no significant change or loss of microbial diversity under organic farming as compared to conventional farming (Bell et al., 2012).

An increase in activity of indigenous microbiome upon application of organic amendments has been correlated with suppression of a range of phytopathogens in the soil (Li et al., 2019). This leads to the phenomenon of "disease suppressiveness", which refers to the natural ability to inhibit the development of diseases even in the presence of actively growing plant pathogens. The property is attributed to the enrichment of several taxonomic groups having antagonistic activity against plant pathogens, which is mediated by production of antibiotics, antifungal compounds, hydrolytic enzyme activity, and competition for the available nutrients (de Corato, 2020). Soil properties like pH, calcium content, structural properties, and texture too have an impact on disease suppressiveness (Hoitink and Boehm, 1999). It has been found that the dominant bacterial phyla in disease suppressive soils mostly belong to *Acidobacteria*, *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidetes* (Mendes et al., 2011; Cha et al., 2016). Similarly, fungal microbiome also plays a critical role in determining disease suppressiveness in the soil. The dominant fungal genera associated with disease suppressive soils belongs to *Trichoderma*, *Mortierella*, *Penicillium*, *Cladorhinnum*, *Micrococcum, Westerdykella* etc. (Penton et al., 2014; Xiong et al., 2017). Thus, the presence of specific microbial taxa appears to be responsible for imparting disease suppressiveness in the agricultural systems (Mazzola, 2002). The selection of antagonistic microbes in the disease suppressive soil is favoured by various factors such as crop rotation, tillage system, type of nutrient amendment, and characteristic soil properties (Postma et al., 2008; Deltour et al., 2017; de Corato, 2020; Palojärvi et al., 2021). In most of the studies performed so far either animal manure or green manure has been used as a source of nutrients in organic farming, and their influence has been compared on soil microbial community (Sun et al., 2015; A. Das et al., 2017; S. Das et al., 2017; Zhang et al., 2017; Yang et al., 2019; Songjuan et al., 2021; Fu et al., 2021; LeBlanc, 2022). However, in this study, the integrated influence of green and animal manure was assessed on bacterial diversity, and the latter compared with the conventional soil microbiome.

To the best of our knowledge, there are no studies to date that have reported the impact of long-term organic farming practice on the bacterial diversity, based on high-throughput sequencing technology in Indian arable land. Further, little information is available as to how organic farming has influenced the disease suppressive potential of soils in such agro-ecosystems. Based on this background, we hypothesize that the long-term amendment of both green and animal manure as organic treatment changes the bacterial community of soil into a more disease suppressive bacterial community compared to conventional soil bacterial community. The objective of this study was to characterise the differences in bacterial community between long-term organic and conventional farming practices in the context of disease suppressive ability of the soil against broad host-range fungal pathogens. To accomplish this objective, special experimental fields managed for the last 18-year under organic and conventional practices were selected. In the selected organic field, no disease incidence was observed for last several years. However, in conventional production system in the last 2–3 years, the incidence of *Bakanae* disease was observed (seedborne fungal disease) during rice cropping season. The fungus infects plants through the roots or crowns. It then grows systemically within the plant. The characterization of soil and plant samples collected from fields under organic and conventional farming practices was done. The impact of these farming practices on bacterial communities, with special emphasis on the fractions pertaining to disease suppressive potential in soil, was deciphered by amplicon sequencing of 16S rRNA gene. The disease suppressive ability of organic and conventional fields was tested against two pathogens *in planta*. This study has attempted to correlate the structural changes in antagonistic bacterial composition and their functional validation of conferring disease suppression ability to the soil.

2. Materials and methods

2.1. Field experiment and soil sampling

An organic and conventional field experiment (followed since 2003), with a wheat variety HD 3086 and a rice variety of Pusa Basmati 1, grown in a cropping system mode, was chosen for the study. The experimental field was located at ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India. The location coordinates of the field were 28°38′-14.8″N; 77°08′-45.6″E and situated at 228.6 m above mean sea level. The field was randomly divided into organic and conventional plots of size 48 m^2, and each treatment was set up in triplicates. Since several years, the organic field showed better soil physical and chemical parameters such as soil pH, EC content, nitrogen, phosphorous and potassium content, and lower incidence of soil-borne plant pathogens in comparison to conventional field (Kaje et al., 2018). The ex-situ application of green manure comprised of *Leucaena* green leaf manure (LGLM) + farm yard manure (FYM) @ 10 t ha^{-1} + *Azotobacter* was applied to wheat and in-situ *Sesbania* green manure (SGM) + FYM @ 10 t ha^{-1} + blue green algae (BGA) @ 10 kg ha^{-1} was applied to rice which was organically managed under wheat-rice cropping system. In the organic field, no additional manuring was done. The conventional plots were amended with NPK fertilizers at the rate of 120 N kg ha^{-1}, 26.2 kg P ha^{-1}, and 41.7 kg K ha^{-1}, respectively in each crop in a cropping year. Potassium was applied in the form of muriate of potash and phosphorus as single super-phosphate at the time of sowing, while nitrogen in the form of urea was applied in 3 splits i.e., 1/3 at sowing/transplanting, 1/3 at 25 days after sowing/transplanting and remaining 1/3 at 55 days after sowing/transplanting. For organic plots, no additional application was made to control any disease, while for conventional plots, fungicides were used intermittently. From each plot, non-rhizospheric soil core was collected in triplicates at a depth of 0–30 cm using a soil corer during May 2019 after harvesting of wheat. The soil distant from both the conventional and organic plots, which was never managed with any farming practice served as control soil. In order to determine the physiochemical properties of the soil, the soil was stored at 4 °C. Another fraction of soil samples was snap frozen and stored at −20 °C for culture-independent analysis.

2.2. Soil physico-chemical parameters

For nutrient analysis of the soil, the samples were air-dried. The available organic carbon content of soil from both conventional and organic fields was determined using the protocol described by Walkley and Black (1934). The available nitrogen content was determined using Kjeldahl's method, phosphorous and potassium content was estimated as per the procedures described by Pooniya et al. (2012). Twenty grams of dried soil without any visible clumps from each treatment was dissolved in 20 mL deionized water and stirred for 30 min. The electrical conductivity (EC) and pH of this homogenous soil solution was measured using a benchtop pH/EC meter (Hanna, USA).

2.3. Biometric attributes and yield parameters of crop

Sampling of plants was performed in triplicates at the harvest stage of wheat from both agri-management practices, i.e., conventional and organic treatment. Plants were uprooted and their height was measured in terms of root length and shoot length in cm. Measurement of root length (measured from the stem end to the root tip in cm), shoot length (the above-ground plant height measured in cm), and plant height (sum of root length and shoot length) was performed for each crop. For measuring the crop biological yield (refers to the total dry matter produced per plant or per unit area including leaf, stem, grain, root etc.), after harvesting manually with sickle the crops were left for 3 days in the field for sun drying. After sun drying threshing was done with Pullman thresher for each plot separately. The grain yield and straw yields per

plot were recorded. The yield per plot was adjusted at 14 % moisture and finally expressed in terms of tonnes ha^{-1}. The straw yield was adjusted to oven-dry weight and expressed in terms of tonnes ha^{-1}.

2.4. Soil DNA extraction and PCR

DNeasy Power Soil Kit (Qiagen, Germany) was used for extraction of DNA from soil samples of each treatment following the recommendation of the manufacturer. DNA extraction was done for triplicate samples from conventional, organic and control soil. The concentration and quality of isolated DNA were assessed using a NanoDrop™ 2000 fluorospectrometer (Thermo Fisher Scientific, USA). The quality of isolated DNA was also determined by electrophoresis on 1 % agarose gel. The genomic DNA was stored at −20 °C till further analysis. For the amplification of hypervariable (V3-V4) region of 16S rRNA, the following universal set of primers: 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′- GACTACHVGGGTATCTAATCC -3′) were used (Bech et al., 2020). The following cycling condition was used for the preparation of 16S rRNA amplicons: initial denaturation at 95 °C for 15 min followed by 30 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, with final extension for 5 min at 72 °C (Bech et al., 2020). The PCR products were purified for sequencing using Agencourt AMPure XP (Beckman Coulter, USA) PCR purification kit.

2.5. Library preparation and 16S rRNA based amplicon sequencing

A total of 50 ng of the DNA from each sample was used for the preparation of libraries using NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, England) according to the manufacturer's protocol. The fragmentation of genomic DNA was done randomly by Bioruptor into 350 bp, then sample purification beads were used for narrow selection of DNA fragments. The full-length Illumina adaptors were ligated to the selected fragments after their end-polishing and A-tailing. After ligation, fragments were again filtered with the beads. The size distribution of the libraries was analysed with Agilent2100 Bioanalyzer and real-time PCR was done for their quantification. The sequencing of constructed libraries was done with pair-end sequencing approach (2 × 250 bp) with NovoSeq 6000 system (Illumina, USA).

2.6. Processing of sequencing reads, taxonomic annotation and statistical analysis

A total of 1,546,245 paired reads were obtained from Illumina NovoSeq 6000 paired-end sequencing technology. The median length for the forward and reverse sequencing reads was 242 bp. QIIME 2 version 2021.2.0, a freely available software, was used for processing the raw sequencing reads (Bolyen et al., 2019). Further, the demultiplexed reads were imported into QIIME 2. DADA2 plugin was used for denoising and clustering of sequences. The de-noising consisted of correcting marginal sequences error, filtering the noisy sequences, removing chimeric, primer sequences and singletons. The denoised paired-end reads were joined and dereplication of filtered sequences were done (Callahan et al., 2016). Overall, 974,237 paired sequences with 19,505 assigned features passed the DADA2 quality filtering. After filtering, an open-reference OTU picking process using QIIME was followed where reads were clustered against a reference sequence database (Green_genes_13_8_97) with 97 % similarity and unmapped reads were clustered by de novo method. Further OTUs were classified using an alignment-based method called classify-consensus-vsearch classifier. Overall, 11,198 OTUs were assigned to kingdom Bacteria, 331 to archaea with 68 remained unassigned. The raw sequence reads of the samples were submitted to SRA (Sequence read archive) database, NCBI with accession no. PRJNA624785.

All plots were generated in the R ggplot2 package (https://cran.r-project.org/package=ggplot2). Complex heatmap was used to create the heatmap (Gu et al., 2016). Diversity metrices, viz. Shannon, Simpson and Chao1 were calculated by QIIME diversity command with a minimum depth of 39,000 and a maximum depth of 208,000. Statistical analysis was done using the ggpubr R package (https://CRAN.R-project.org/package=ggpubr). Multiple groups p-value was calculated with one-way ANOVA and comparison between two groups was done with the Wilcoxon test. DEICODE (Robust Aitchison PCA) was used to link specific features to beta-diversity ordination (Martino et al., 2019). Mann-Whitney-Wilcoxon test was used to compare the difference in abundance of OTUs in different samples.

2.7. In planta assay for assessing disease suppressiveness of soil

To test the disease suppressive ability of soil under conventional and organic practices, the top layer of non-rhizospheric soil up to 5 cm was collected from each field (Martins et al., 2018). *Rhizoctonia solani* (MTCC No. 4633) and *Fusarium oxysporum* (MTCC No. 2087) were procured from Microbial Type Culture Collection and Gene Bank (MTCC), India, and used as model pathogens due to their wide host range pathogenicity. *R. solani* and *F. oxysporum* were grown on potato dextrose agar (HiMedia, India), which was supplemented with 300 mg L^{-1} of streptomycin sulphate and incubated for 7 days at 25 °C. In the case of *R. solani*, four to five mycelial plugs from the actively growing culture plates, each of 9 cm, were used to inoculate 200 mL of V8 Juice medium (HiMedia, India) supplemented with 3 g L^{-1} $CaCO_3$. The medium was incubated for 7 days at 150 rpm and 25 °C. Mycelium was collected by centrifugation and then crushed with the help of a blender. For *Rhizoctonia solani* the mycelial pellet was diluted to make an aqueous suspension of cell density of ~10^2 CFU mL^{-1} which was applied for infection to the plants (Martins et al., 2018). Spore suspension of *F. oxysporum* with a density of 10^6 spores mL^1 was used for infecting plants.

Disease suppressive ability of soil under different treatments was tested against the two pathogens using wheat as a model plant in a pot experiment. Wheat variety HD 3086 was procured from National Seeds Corporation Ltd., Pusa Complex, New Delhi, India. Before sowing the seeds, good quality seeds as observed by visual examination of shape, size and colour were selected and surface sterilized with 70 % ethanol for 30 s following sterilization with 0.01 % solution of sodium hypochlorite for 1 min. The seeds were further rinsed with autoclaved water and used for pot experiment. Plastic pots of capacity 250 g were used for the assay. Each pot was filled with 200 g of soil, and 2 seeds were placed at a depth of 2 cm per pot. A combination of soil from conventional and organic fields in equal proportion was autoclaved, and used as a sterile soil to assess the impact of sterilization on disease suppressive potential of the two soils. Five replicate pots were used for each treatment. The treatments were as follows: (1) sterile soil (S), (2) sterile soil + pathogen (SP), (3) soil from the conventional field (C), (4) soil from the conventional field + pathogen (CP), (5) soil from the organic field (O), and (6) soil from the organic field + pathogen (OP). The pots were watered regularly to their 50 % capacity. In total 30 pots (5 pots for each treatment) were used for studying the suppressiveness against each pathogen. Pots were incubated in a plant growth chamber maintained at 25 ± 2 °C temperature, 70–80 % humidity and 12 h photoperiod. The disease incidence, biometric parameters and stress-specific biochemical markers were observed after 25 days, at the plantlet stage of wheat.

2.8. Scoring the disease severity of the plants

Disease severity of the plantlets was observed based on the percentage of yellow discolouration and wilting of the plants with a scoring scale from 0 to 4 (Wildermuth and McNamara, 1994; Song et al., 2004). Plants with no visible disease symptoms were scored 0, while score 1 to 4 represented increase in discoloration or wilting of plants. Score 1 was assigned to plants that were 0 to 25 % affected by the disease. Similarly scores 2, 3 and 4 were assigned to plants that were 26–50 %, 51–75 %, and 76–100 % affected by the pathogen infestation, respectively. The disease severity index (DI) under each treatment was calculated (Song

et al., 2004).

$$\text{DI}\ (\%) = \frac{\Sigma\text{Scale} \times \text{Number of plants infected}}{\text{Highest scale} \times \text{Total no. of plants}} \times 100$$

2.9. Measurement of biometric parameters of plants in pot experiment

Plant parameters such as root length, shoot length, fresh weight, was recorded at the plantlet stage of wheat for each treatment. The shoot length was measured in cm from the tip of stem to its base, similarly root length was also recorded. The fresh weight of the plantlets was measured (in mg) immediately after sampling.

2.10. Quantification of stress-specific biomarkers

For quantitative estimation of stress-specific biomarkers, plant material from each treatment was crushed into fine powder in liquid nitrogen using a mortar pestle. The following markers were measured: peroxidase enzyme activity, polyphenol oxidase enzyme activity, proline content and electrolytic leakage.

Peroxidase enzyme activity: A homogenous plant extract solution of concentration 20 mg mL^{-1} was prepared in ice-cold phosphate buffer. The enzyme activity of the plant material was measured by using 0.1 mL of homogenous plant extract solution, which was added to 3 mL of guaiacol (0.05 M). 0.1 M potassium phosphate buffer (pH 6.5) was used to prepare 0.05 M guaiacol. For initiation of the enzymatic reaction, 0.1 mL 0.8 M H_2O_2 was subsequently added to the reaction mixture. The absorbance of the reaction was observed at 470 nm for 3 min at a regular interval of 30 s. The reaction in which no plant material was added served as blank. The change in absorbance min^{-1} g^{-1} fresh weight was used to express the unit of enzyme activity (Meena et al., 2021).

Polyphenol oxidase enzyme activity: The enzyme activity of the plant material was measured by using 0.1 mL of homogenous plant extract solution which was added to 2.5 mL of catechol (0.1 M). A 0.1 M potassium phosphate buffer (pH 6.0) was used for the preparation of 0.1 M catechol. The change in absorbance of the reaction was observed at 495 nm for 3 min at a regular interval of 30 s. The change in absorbance min^{-1} g^{-1} fresh weight was used to express the unit of enzyme activity (Meena et al., 2021).

Proline content: The total proline content was measured by following the protocol of Bates et al. (1973). Proline was extracted with sulphosalicyclic acid and reacted with ninhydrin. The proline and ninhydrin complex was further extracted with toluene and observed at 520 nm. The proline content was measured in μ moles g^{-1} fresh weight of plant material.

Electrolytic leakage: Two grams of plant material was dissolved in 10 mL deionized water in sealed glass test tubes and incubated at 25 °C for 4 h. After this incubation, the electrical conductivity (first reading) was measured using an electrical conductivity meter (Hanna, USA). The tubes were then transferred to a water bath set at 90 °C for a period of 2 h. Further, the electrical conductivity (second reading) was recorded. The percentage of electrolytic leakage was calculated by dividing the first reading by the second reading and expressed in terms of percentage (Lima et al., 2002).

2.11. Statistical analysis for plant and soil parameters

The soil and plant parameters were assessed by principal coordinate analysis (PCoA) using R 4.1.0 (2021-05-18). The disease severity indices and plant biometric parameters *in planta* pot experiments were plotted using SPSS Statistics 23.0 (SPSS Inc. USA). The statistical significance between the treatments was analysed with One-way ANOVA where plant parameters were taken as dependent variables and treatments as independent variable. Tukey's HSD post-hoc test was used to compare the means with p valve ≤ 0.05 (Gomez and Gomez, 1984). The clustering of heatmaps was done with K-means clustering. The statistical difference in beta-diversity between the groups was calculated with permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity with 1000 permutations using R 4.1.0 (2021-05-18) (Clarke et al., 2006).

3. Results

3.1. Effect of agri-management practices on soil and plant parameters

The soil and the plant samples were collected from the conventional and organic field under management for 18 years. Organic farming had a statistically greater amount of organic carbon content, available nitrogen (N), phosphorus (P), and potassium (K) than conventional farming (Table S1). Electrical conductivity (EC) of soil from the organic field (0.36 dS m^{-1}) was higher compared to the soil from the conventional field (0.29 dS m^{-1}). In contrast, the bulk density was lower in the organic field (1.38 mg m^{-3}) compared to the conventional field (1.7 mg m^{-3}). Significant differences were found in grain yield, straw, and biological yields between conventional and organic farming. PCoA ordination showed that farming practices had a stronger effect on soil parameters, compared to plant biometric and yield parameters (Fig. 1). The PC1 axis accounted for 81.82 % of the overall variation, while the PC2 axis accounted for only 13.83 %. The samples from the organic field positively correlated with soil EC, organic carbon content, available N, P and K. The plant height, biological yield, and straw yield clustered together under both conventional and organic farming.

3.2. Composition of prokaryotic community under different agri-management practices

A total of 2349 bacterial OTUs were observed, where 605 OTUs (25.7 %) were exclusively present in soil from the organic field, while 195 (8.30 %) and 312 (13.2 %) OTUs were present in soil from the conventional field and control soil, respectively (Fig. 2a). There were 1038 (44.1 %) OTUs in common between soils from fields under organic and conventional management, and controls. Prokaryotic communities from organic and conventional fields had only 97 (4.1 %) OTUs in common, whereas 102 (4.3 %) OTUs were shared between conventional field and control soil. The diversity of the prokaryotic community at the taxonomic level was affected under different farming practices

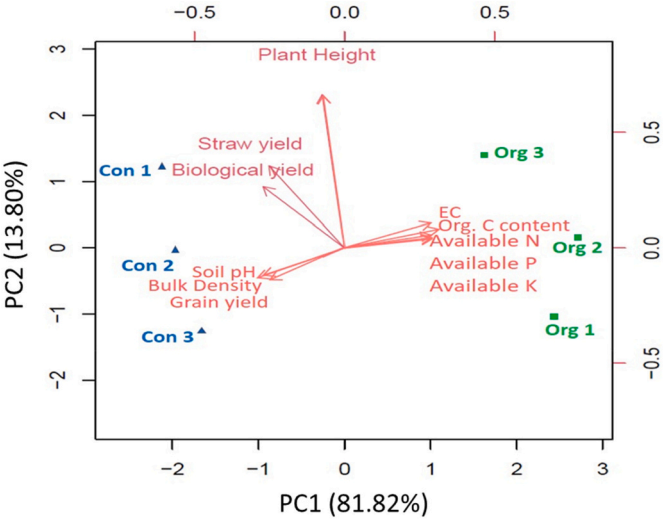
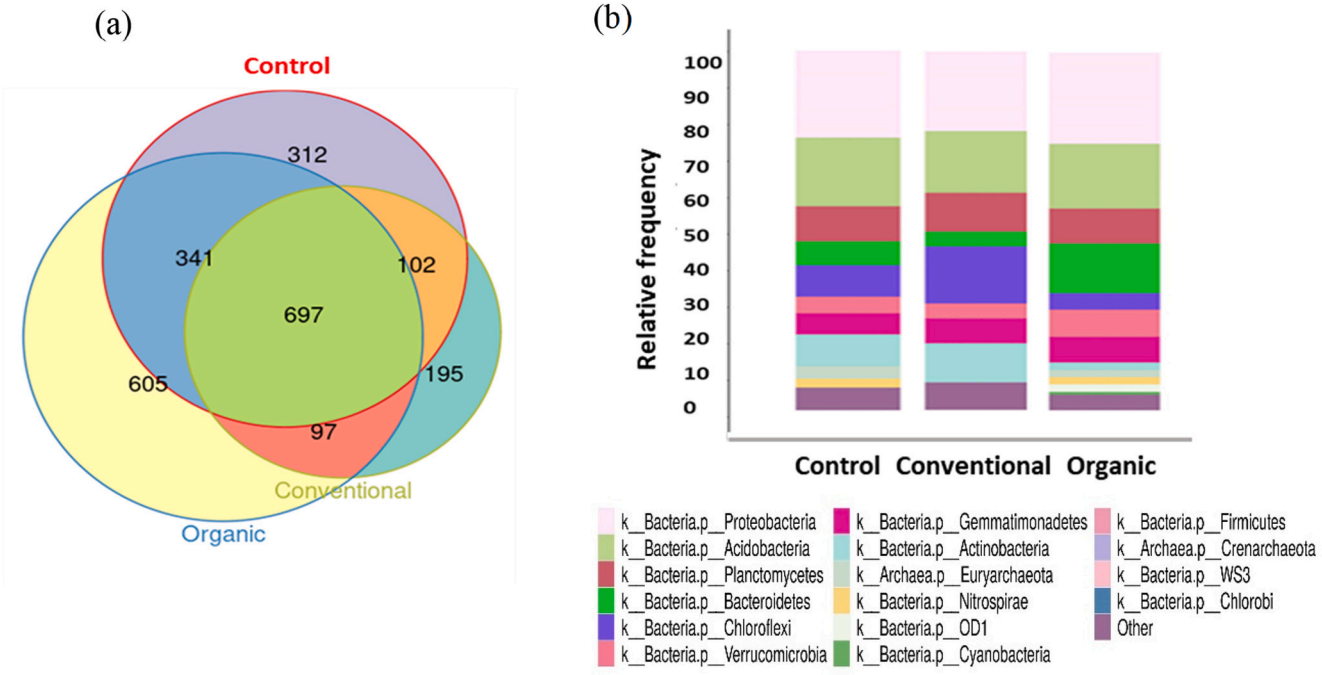
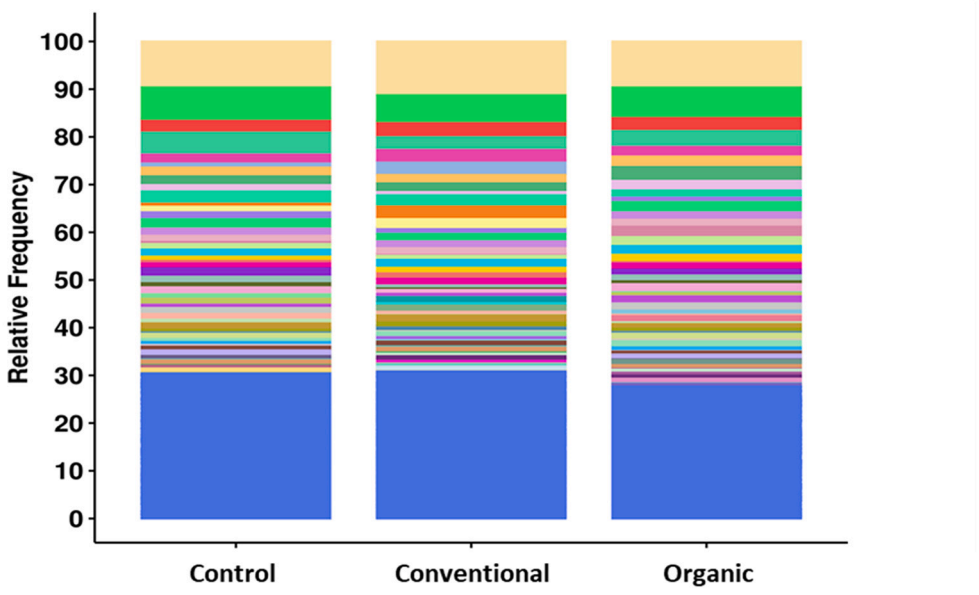


Fig. 1. Principal component analysis of soil and yield parameters under different farming systems. The two principal components PC1 and PC2 are shown along the x axis and y axis, respectively. The different agri-management practices (conventional and organic) are represented by PCA scores: green (organic), blue (conventional). Con - Conventional; Org - Organic ($n = 3$).





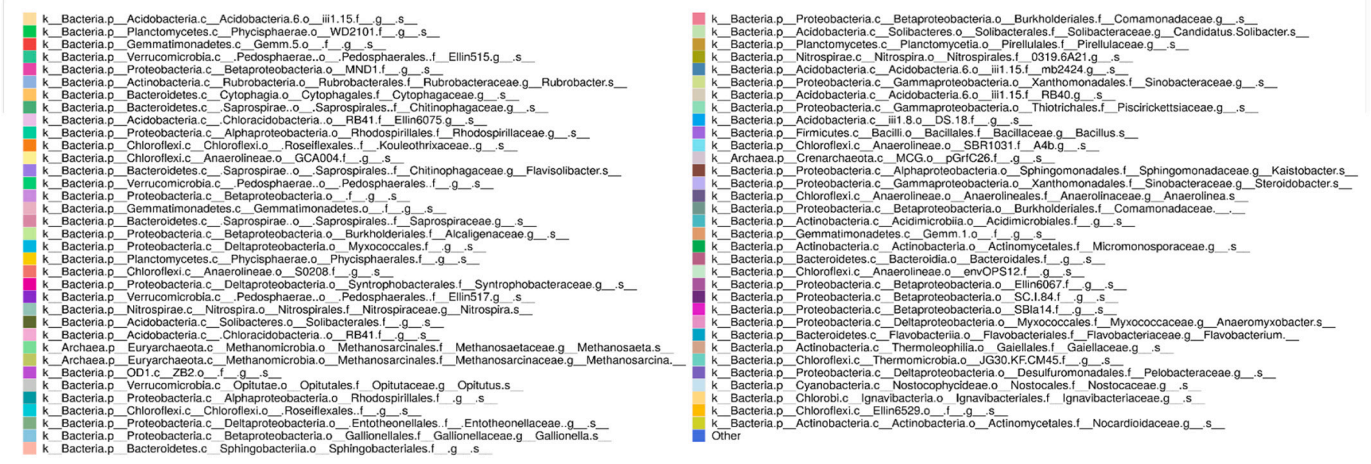


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Fig. 2. (a) Venn diagram depicting the total number of OTUs present in organic, conventional and control soil, (b) Stacked bar-plot based on relative abundance of several phyla in different treatments, phyla with relative abundance < 0.01 % were clustered and represented as "others", (c) Relative abundance plot showing taxa whose relative frequency > 0.5 ($n = 3$ for each treatment, average value of triplicates was used to prepare the plots).

(Supplementary Fig. S1). The most abundant phyla were *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Chloroflexi* and *Gemmatimonadetes* in all the samples (Fig. 2b). However, the abundance of *Acidobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Proteobacteria* and *Verrucomicrobia* was significantly higher in the case of the organic field compared to the conventional and control soil (Mann-Whitney-Wilcoxon test, $p < 0.05$). The abundance of *Acidobacteria* was 41 % and 19 % in soil from organic and conventional fields, respectively. However, the abundance of *Acidobacteria* was 40 % in case of control soil. Dominant phyla *Bacteroidetes* (56 %, 13 % and 31 %), *Gemmatimonadetes* (48 %, 20 % and 32 %), *Proteobacteria* (50 %, 19.3 % and 30.7 %) and *Verrucomicrobia* (48.7 %, 13.3 % and 38 %) also exhibited difference in abundance in the soil from organic, conventional practices and control, respectively. The abundance of several genera, viz. *Candidatus solibacter*, *Fluviicola*, *Pontibacter*, *Flavobacterium*, *Bacillus*, *Pseudomonas*, *Nitrospira*, *Pirellula*, *Planctomycetes*, *Bradyrhizobium*, *Xanthomonas*, *Kaistobacter*, *Azospira*, *Gallionella*, *Geobacter*, *Alcaligenes*, *Burkholderia*, *Cellvibrio*, *Steroidobacter*, *Spirochaeta*, *Luteolibacter* and

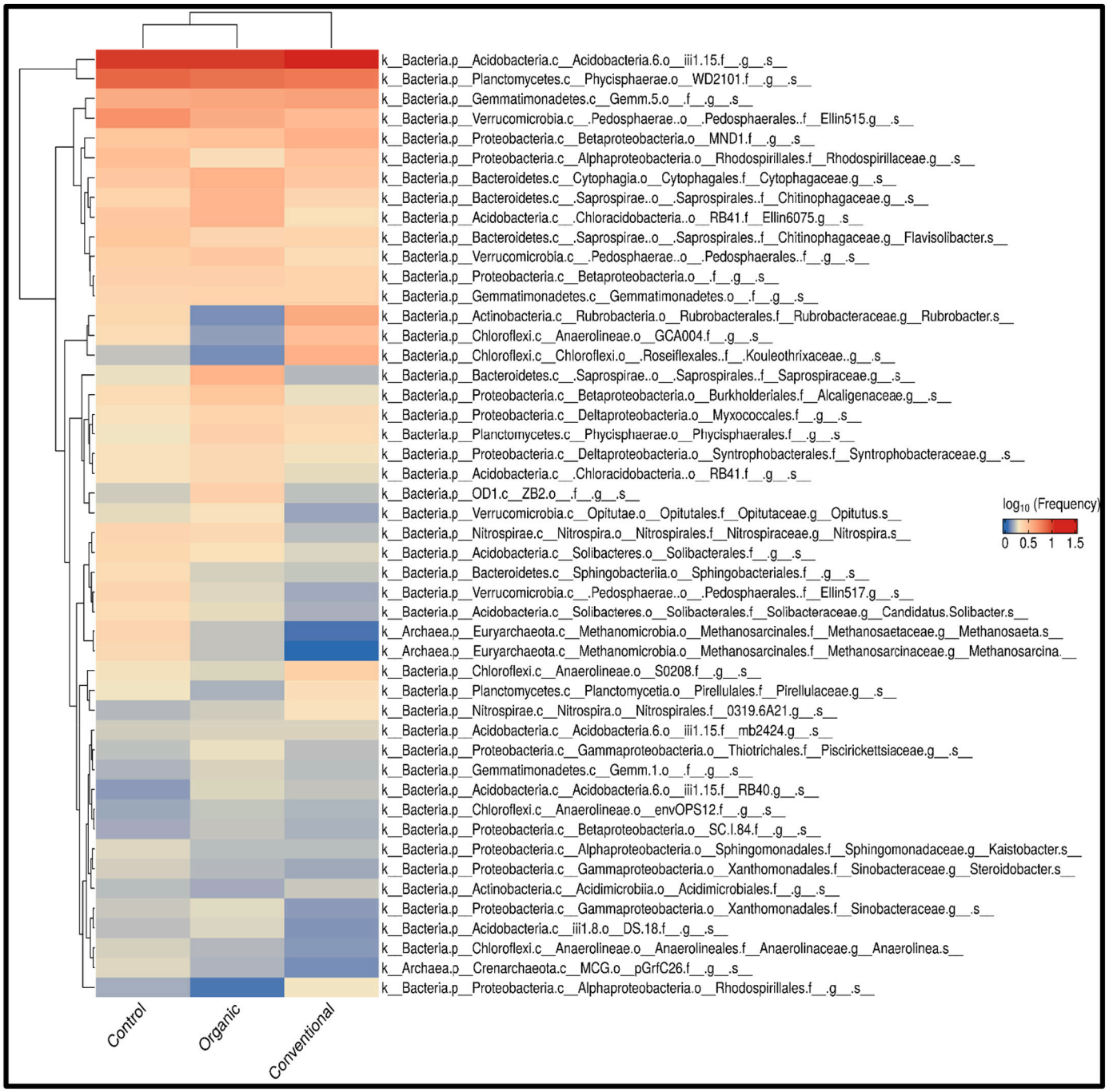

Fig. 3. Heat map of top 50 most abundant OTUs present at genera level under different farming practices. Values were transformed and plotted on scale of $\log_{10}$.

Optitutus, was found to be significantly higher in soil from organic field compared to the conventional field (Fig. 2c). Several unidentified genera of phyla *Actinobacteria*, alpha, beta, delta and gamma *Proteobacteria*, *Bacteroidetes*, *Planctomycetes*, *Gemmatimonadetes*, *Verrucomicrobia* and OD1 were observed to be enhanced in organic field soil in comparison to conventional farm soil. The top 50 dominant OTUs in conventional and organic field soil have been represented as a heatmap based on their relative abundance (Fig. 3). Based on abundance of OTUs, significant differences were found between soil from organic and conventional fields. Higher abundance of *Burkholderia*, *Pedosphaerales*, *Saprospirales*, *Cyclophagales*, *Nitrospirales*, Solibacterales, *Xanthomonadales* etc. was found in organic field soil compared to conventional and control soil.

Alpha diversity was measured with different indices such as total observed OTUs, Shannon, Simpson and Chao1. The rarefaction curves of the soil from the organic field indicated maximum richness in comparison to the soil from conventional field and control soil samples (Supplementary Fig. S2). The rarefaction curve of all three soil samples reached a clear asymptote suggesting a good representation of species diversity. Shannon values were also significantly different between the prokaryotic community in organic and conventional fields, with less richness and diversity of the prokaryotic community in the conventional field (Fig. 4). The Simpson index, on the other hand, did not change significantly between soil from conventional and organic fields. There was no significant difference in Shannon and Simpson values between control and the organic field soil. However, Simpson diversity of control soil was higher than the conventional field soil. The Chao1 index value was significantly higher for the organic field compared to the conventional field indicating the presence of several rare taxa in the organic field. PCoA revealed that farming practices had a significant effect on prokaryotic diversity, which was calculated based on total and relative abundance of OTUs present under different farming practices (Fig. 5). The prokaryotic community in soil from organic field clustered apart from both conventional and control soils. Beta diversity was higher in the organic field than in conventional and control soil. The PERMANOVA analysis confirmed a significant difference in the beta diversity between conventional and organic field soil ($p < 0.05$) (Table S2).

3.3. Plant assay to assess disease suppressiveness

The organic soil significantly reduced the development of disease compared to the soil from the conventional field and sterile soil (Fig. 6a, b). In the case of *Rhizoctonia solani*, disease severity (%) was highest in sterile soil + pathogen (SP), followed by conventional soil + pathogen (CP), and then organic soil + pathogen (OP) (Fig. 6c). Similarly, in the case of *Fusarium oxysporum*, the plants of SP treatment were severely impact followed by CP and OP (Fig. 6d). Plant physical parameters such as root length, shoot length, fresh weight were impacted by the pathogen's infestation. There was a significant difference in the fresh weight in the case of *R. solani* between CP and OP, OP and SP as well as CP and SP (Fig. 6e). *F. oxysporum* also impacted the fresh weight of the plantlets with significantly lower fresh weight in CP compared to treatment OP. A similar trend for the length of root and shoot was observed for both pathogens. Significantly higher root length and shoot length were observed for OP compared to CP for each pathogen. However, there was no significant difference in the shoot length between CP and SP when the plant was infected with *Fusarium oxysporum* (Fig. 6f).

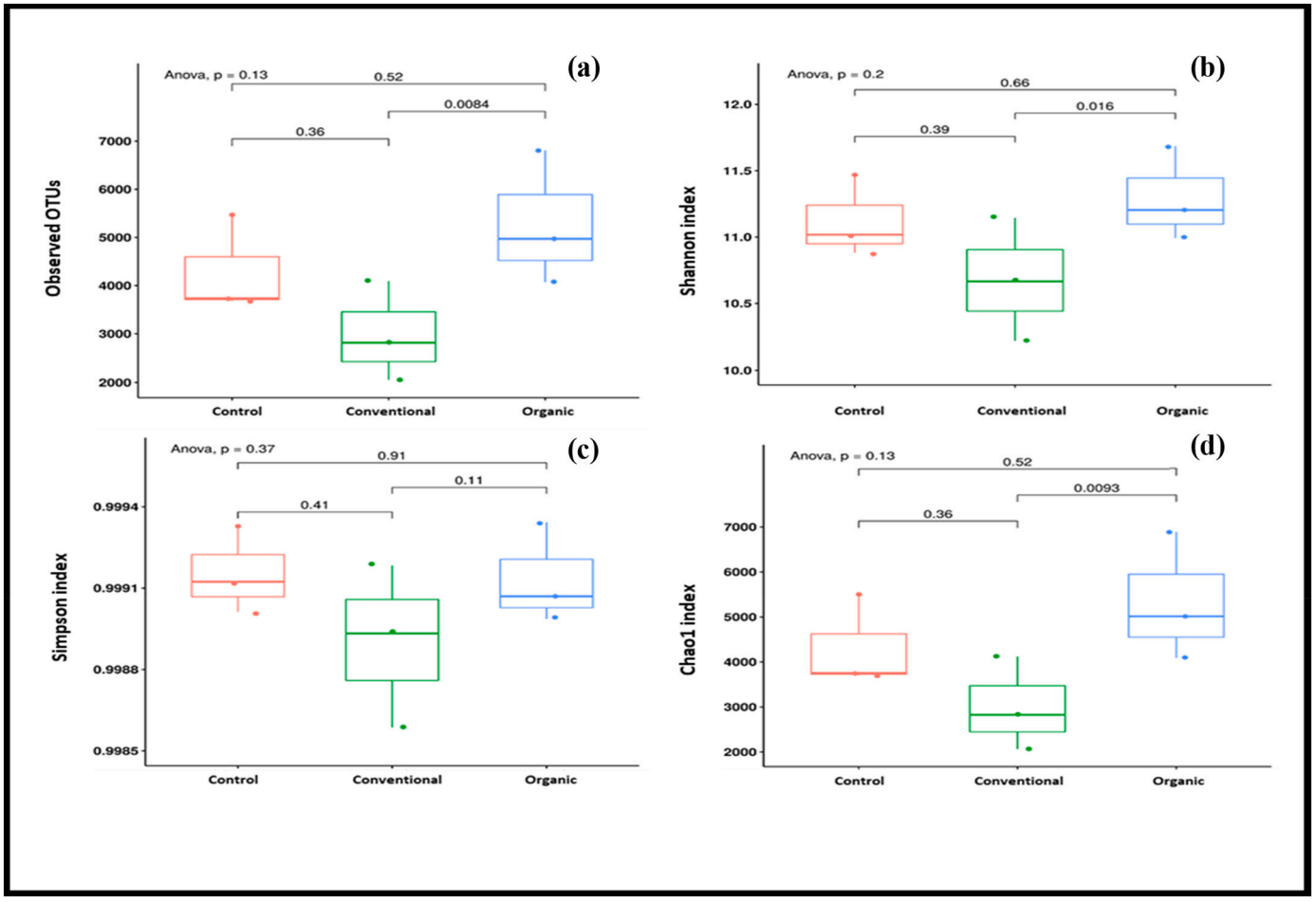

Fig. 4. Box plots displaying different prokaryotic diversity indices in soil samples from conventional and organic farming ($n = 3$ for each agri-management practice, $p < 0.05$). (a) Number of observed OTUs, (b) Shannon diversity index, (c) Simpson diversity index, and (d) Chao1 diversity index.

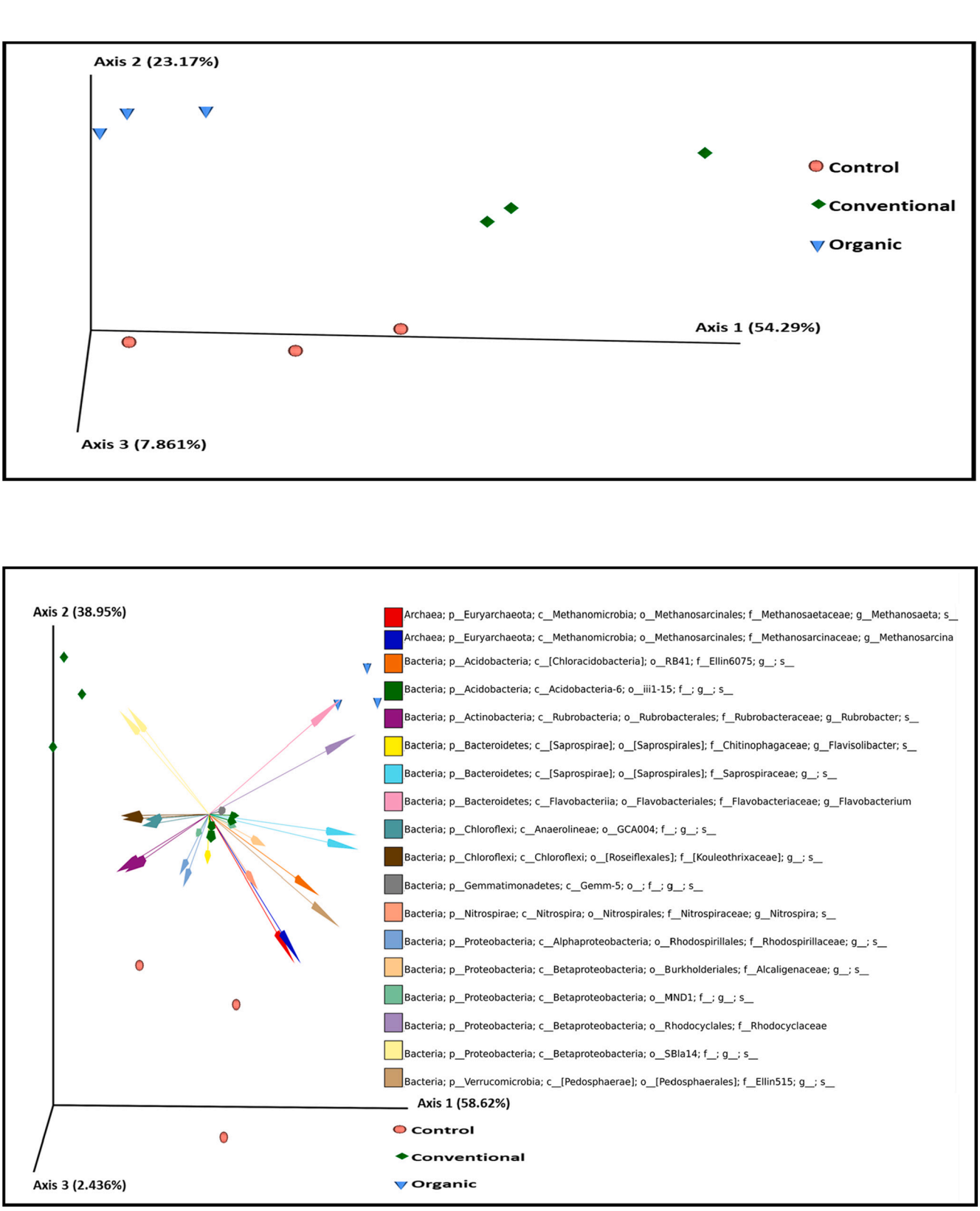

Fig. 5. Principal coordinate analysis using weighted Unifrac distances revealed beta-diversity between soil samples from different farming systems ($n = 3$ for each treatment). (a) PCoA based on total OTUs present in different samples, and (b) PCoA based on relative abundance of different genera in different samples.

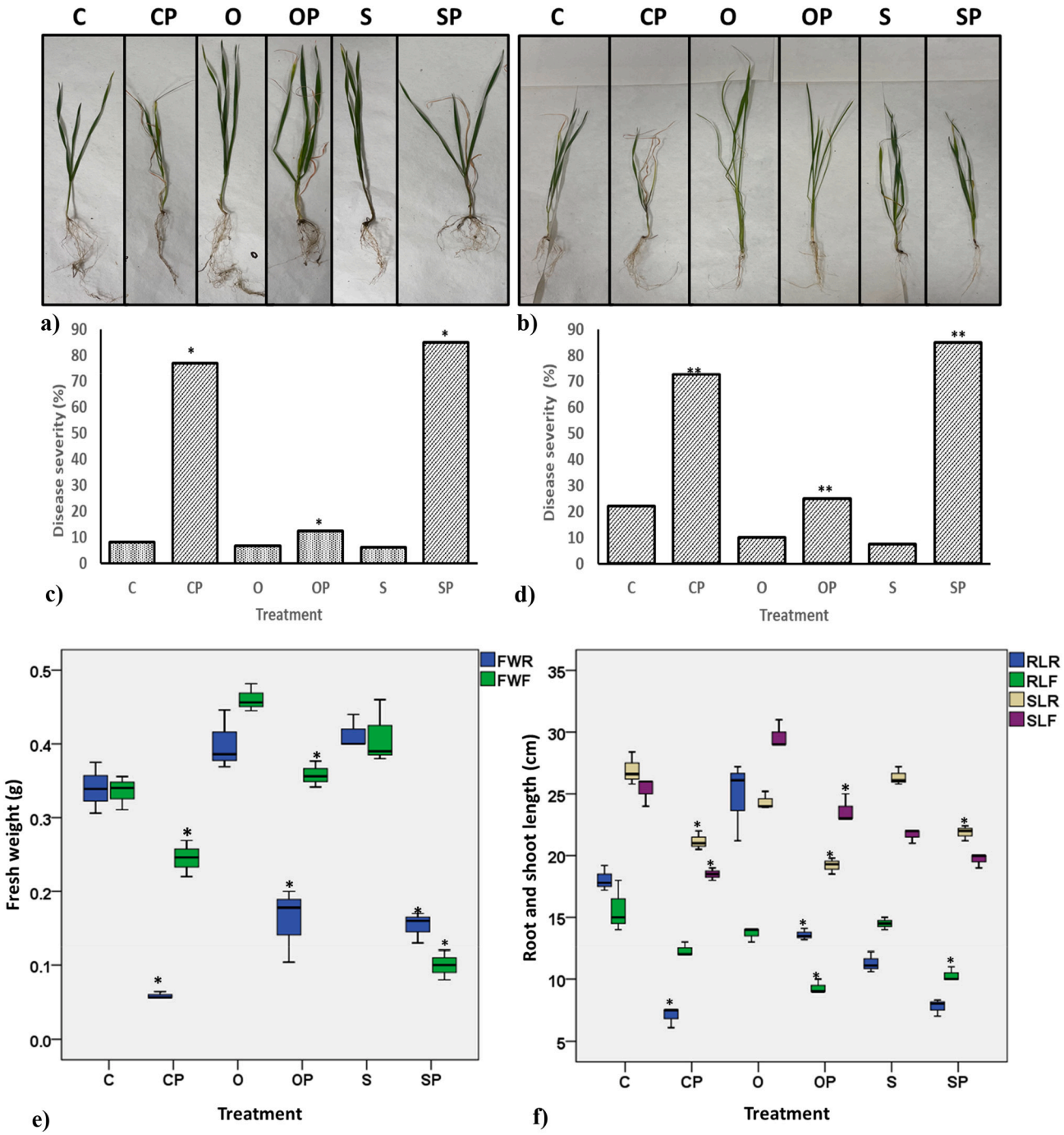

Fig. 6. Disease suppressive potential of organic and conventional field soil. Visual impact of pathogens on wheat growth under different treatments: *Rhizoctonia solani* (a), and *Fusarium oxysporum* (b). Bar plot showing disease severity (%) of wheat infested with *R. solani* (c) and *F. oxysporum* (d). Boxplot showing the effect of *R. solani* and *F. oxysporum* on fresh weight (e), and on root length and shoot length (f). RLR and SLR- root length and shoot length for *R. solani*, respectively; FWR- fresh weight for *R. solani*; FWF- fresh weight for *F. oxysporum*, RLF and SLF- root length and shoot length for *F. oxysporum*, respectively, S: sterile soil, SP- sterile soil + pathogen; C: soil from conventional field, CP: soil from conventional field + pathogen; O: soil from organic field, OP: soil from organic field soil + pathogen. *Significant difference between the treatments for both *R. solani* and *F. oxysporum* ($n = 10$ for each treatment).

Several stress-specific biomarkers associated with plants grown in soil from organic and conventional fields were quantified upon treatment with both pathogens. A higher level of electrolytic leakage, polyphenol oxidase activity, peroxidase activity, proline content was observed for treatment CP in comparison to OP for the two pathogens (Fig. 7). In *F. oxysporum* infested plants polyphenol oxidase activity was highest for treatment C, followed by SP and CP, while peroxidase activity was observed higher in SP compared to CP and OP. There was no significant difference in electrical leakage between SP and CP treatments, while the lowest electrolytic leakage was observed for treatment OP. In *R. solani* infested plants, peroxidase activity, and polyphenol oxidase activity were highest for treatment CP, contrary to lowest levels observed in treatment O. Electrolytic leakage was higher for treatments SP and CP compared to the other treatments. A similar trend for proline content was observed in the case of each pathogen, where the highest values were observed for SP and CP compared to other treatments.

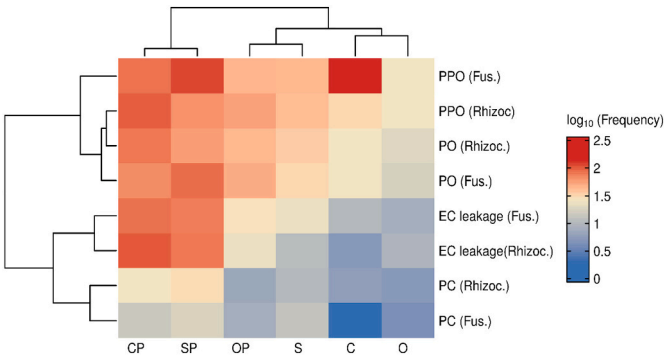

Fig. 7. Heatmap showing variation in disease specific markers levels in different treatments for both the pathogens. Values were transformed and plotted on scale of $\log_{10}$. PPO: polyphenol oxidase activity, PO: peroxidase activity, EC: electrolytic leakage, PC: proline content, Fus: *Fusarium oxysporum*, Rhizoc: *Rhizoctonia solani* ($n = 5$ for each treatment).

4. Discussion

The long-term effect of organic farming and its role in disease suppressiveness can be delineated by specifically targeting the changes in the composition of the bacterial community. The type of amendments in agriculture has a significant impact on bacterial diversity, but how these changes alter the disease suppressive ability of the soil is not well understood. The mapping of structural changes in the prokaryotic community, especially in identifying the key bacterial taxa having biocontrol activity, can provide the underlying mechanism of disease suppressiveness influenced by farming practices. In the present study, a long-term (managed since 2003) conventional and organic field experiment was selected to determine the effect of agri-management practices on soil prokaryotic community and its effect on disease suppression. The soil type and climatic conditions were similar for both conventional and organic fields. Hence, the overall changes in soil physio-chemical properties and bacterial diversity under organic farming and the development of disease suppressive potential can be attributed to the consistent soil management with green manure and animal manure.

Several changes in the soil properties such as pH, bulk density, and nutrient content (C, N, P and K) were observed between the organic and conventional farming practices. The slightly lower pH of the organic field might be due to the accumulation of hydrogen ions released from the organic anions present in the organic matter in the soil from the organic field compared to the soil from the conventional field (Himelick and Watson, 1990; Cuartero et al., 2021). A significant increase in the available carbon content, N, P, and K contents, was observed under organic farming compared to conventional farming. It has been reported that the decomposition of plant material causes an increase in the nutrient content of the soil (A. Das et al., 2017; S. Das et al., 2017; Lori et al., 2017; Peigné et al., 2018). Lesser bulk density of soil was observed in the organic field compared to the conventional field, which accounts for better soil health and higher microbial activity (Black et al., 2008; Lark et al., 2014). Similar to previous studies (Marinari et al., 2006; Melero et al., 2006), the electrical conductivity of soil from the organic field was higher as compared to the conventional field. The high EC of the soil from the organic field results due to the release of salts and ions from the decomposed organic matter added to the soil (Carmo et al., 2016). There were significant differences in the plant parameters between conventional and organic farming. However, only a slight difference was observed in the yield parameters between conventional and organic farming. These results are consistent with the studies that have reported that due to the stability of the soil parameters, long-term organic farming practice reduces the yield gap between the two treatments (de Ponti et al., 2012; Schrama et al., 2018).

Similar to the soil and yield parameters, the changes in the bacterial community under long-term conventional and organic field experiments, especially in context to antagonistic bacterial taxa were observed. *Acidobacteria*, *Proteobacteria*, *Bacteroidetes*, *Verrucomicrobia* and *Gemmatimonadetes* were the phyla whose abundance was found to be higher in soil from the organic field as compared to the conventional field. In correlation with other studies (Mazzola et al., 2004; Hu et al., 2016), the overall increase in the abundance of different bacterial species in soil under organic treatment has also been observed in this study. The abundantly present bacterial species can compete with the pathogens for nutrient resources, hence providing general disease suppression against soil-borne pathogens (Mazzola et al., 2004; Hu et al., 2016). The dominance of bacterial genera such as *Acidobacterium*, *Terriglobus*, *Edaphobacter*, *Granulicella*, *Bryobacter*, belonging to the phylum *Acidobacteria*, in soil from organic field can be used to predict the disease suppressive ability of soil under organic farming, as there are several studies that have reported their abundance in disease suppressive soils against *Fusarium* wilt, *Rhizoctonia solani* etc. (Shen et al., 2015; Liu et al., 2016; Ossowicki et al., 2020). However, the functional role contributed by *Acidobacteria* in disease suppressive soils is not very clear. Similarly, the higher abundance of genera *Pseudomonas*, *Kaistobacter*, *Lysobacter*, *Xanthomonas*, *Burkholderia* etc. belonging to *Proteobacteria* is highly correlated with disease suppressive ability of soils against *Rhizoctonia solani*, *Ralstonia solanacearum* and *Fusarium oxysporum* (Mendes et al., 2011; Rosenzweig et al., 2012; Liu et al., 2016). Also, the genera *Flavobacterium*, *Flavisolibacter*, *Pontibacter*, *Sphingobacteria*, and *Chitinophaga* belonging to *Bacteroidetes* were found in higher abundance in disease suppressive soils against *Pythium irregulare* and *Streptomyces scabies* (Kopecky et al., 2019; Ros et al., 2020). The genera belonging to these two phyla are known for the production of different types of secondary metabolites, which have a direct role in inhibiting the fungal and bacterial pathogens (Sang and Kim, 2012; Caulier et al., 2018; Ghazy and El-Nahrawy, 2021). The genera *Luteolibacter* and *Optitutus* of phyla *Verrucomicrobia* and *Gemmatimonadetes*, respectively were also found to be significantly higher in soil from organic field as compared to the soil from conventional field. The abundance of these two phyla was also reported to be higher in soils that showed natural suppression against *Rhizoctonia solani* and *Fusarium oxysporum* (Yin et al., 2013; Hong et al., 2020).

The taxonomic annotation of 16S rRNA gene sequences till the genera level revealed that there were several dominant taxa, which were exclusively abundant in soil from the organic field as compared to the soil from the conventional field such as *Flavobacterium*, *Bacillus*, *Pseudomonas*, *Bradyrhizobium*, *Xanthomonas*, *Kaistobacter*, *Alcaligenes*, *Burkholderia* etc. These genera have been reported to exhibit biocontrol properties and have been associated with disease suppressive soil (Liu et al., 2016; Gómez Expósito et al., 2017; Xiong et al., 2017). The biocontrol activities imparted by these genera might be due to the production of wide range of antifungal and antibacterial agents such as siderophores, NRPS (non-ribosomal peptide synthetases), volatile compounds and direct competition for nutrients (Gómez Expósito et al., 2017). Role of *Candidatus solibacter* as a biocontrol agent against root rot was identified but the mechanism responsible for this inhibition is not yet known (Shu et al., 2019). The abundance of these genera has also been found in soil with high organic content, which might be due to its role in the degradation of organic carbon (Pearce et al., 2012). An abundance of *Fluviicola* has not been reported in disease suppressive soils, although it is resistant to bacterial plant pathogen, *Ralstonia solanacearum* (Sahu et al., 2020). The abundance of *Pontibacter*, *Bacillus* and *Pseudomonas*, in soil from the organic field was found to be associated with disease suppressiveness against *Pythium irregulare* and other fungal pathogens (Weller et al., 2002; Mendes et al., 2011; Ros et al., 2020). Similarly, the role of these genera has been established in plant growth promotion and biocontrol (Michelsen et al., 2015; Liu et al., 2016; Köberl et al., 2017; Jin et al., 2019; Barelli et al., 2020).

The alpha diversity indices such as observed OTUs, and Chao1 index, were found to be significantly higher in soil from the organic field

compared to the conventional field and control soil. However, there was no significant change observed in the Simpson values between soil from conventional and organic field. A significantly higher Shannon diversity was observed in soil from the organic field compared to the conventional field, which can be directly correlated with an earlier study, where significant decrease in the bacterial diversity was observed in organic farms as compared to conventional farms (Bonanomi et al., 2016). A similar positive effect of long-term organic farming practice on beta diversity was observed in other studies which have compared the effect of conventional and organic farming on microbial communities (Hartmann et al., 2015; Lupatini et al., 2017).

Based on the results obtained by amplicon sequencing, soil from the organic field showed significant changes in bacterial community, which is believed to be responsible for imparting disease suppressiveness to the soil against broad host-range plant pathogens. To confirm the inhibitory activity of soil from organic field, *in planta* assays were set up with *Rhizoctonia solani* and *Fusarium oxysporum* using wheat as the model crop for this study. Wheat, which was chosen as a model plant for the study, is a staple and economical crop of India. The reason for selecting *Rhizoctonia solani* and *Fusarium oxysporum* as model pathogens in this study was their wide host range to several plant species including wheat (Hane et al., 2014; Li et al., 2021). The plants grown in soil from the conventional field along with pathogen infestation (CP), were observed to be more diseased compared to the plants grown in soil from the organic field with pathogens (OP). The pathogen infestation also had an impact on the growth attributes of plants. The root length, shoot length and fresh weight of plants under treatment CP was lower compared to the plants under treatment OP. These results signified the impact of organic farming in controlling the pathogenesis of plant disease, and consequently prevented the development of severe symptoms. The biochemical markers selected to map the stress response of the plants due to pathogen infestation were also found to be significantly different between the treatments. The higher level of electrolytic leakage in treatment CP indicated that more damage was caused at the cellular level due to the pathogen's infestation as compared to the treatment OP, which is in accordance with a recent study (Hafez et al., 2020). The proline content was found to be higher in plants under treatment CP compared to OP, further indicating the suppressive effect of the soil microbiome of the organic field against the selected plant pathogens. A higher proline content in diseased plants is due to severe stress caused as a result of pathogen infestation (Ahmad et al., 2019; Siddiqui et al., 2019). Similarly, higher polyphenol oxidase and peroxidase activity in plants under treatment CP was observed as compared to the treatment OP. Plants produce these enzymes as a defence mechanism against biotic and abiotic stress factors (Seleim et al., 2014; Hafez et al., 2020). In the treatment OP, reduced effect of stress was found in the plants, which reflects upon the enhanced disease suppressive potential of organic soil, which might nullify the severe effects of the pathogen.

This study highlights the impact of farming practices on bacterial diversity, and revealed a higher abundance of disease suppressive bacterial community in soil from organic field compared to conventional field. The plant bioassay also showed positive correlation of organic farming to disease suppressive microbiome. However, the disease suppressive ability of soil observed in plant bioassay could be the cumulative effect of both fungal and bacterial diversity, thus studying the fungal community under different farming practices will provide enhanced understanding of how farming practices influence microbial communities of soil in context to disease suppressiveness. The selective isolation of disease suppressive bacteria from organic field can be used for the development of synthetic microbial community (SMC). The generated SMC can then effectively transform the conducive soil to otherwise suppressive soil which will be the most suitable sustainable alternative to prevent plant diseases.

5. Conclusions

Overall, significant differences in bacterial community structure of soils in organic and conventional fields were observed. The change in the abundance of bacterial taxa having known antagonistic activities was observed between the two-farming system. These structural changes in the bacterial community have been correlated to disease suppressiveness against model pathogens *in planta*. The detailed analysis of the diversity of bacterial species exhibiting potent biocontrol properties enhance our understanding of their role in contributing to disease suppressiveness in soils. Also, such a correlation can be harnessed to isolate specific biocontrol strains and establish their mechanistic contribution to suppressiveness.

Availability of data and material

Not applicable.

Code availability

Not applicable.

CRediT authorship contribution statement

LJ and SS conceptualized, supervised and administered the study; SK and SD conducted the experiments; YSS coordinated the field experiment; SK, LJ and SS analysed the data; SK wrote the original draft: YSS, LJ and SS acquired the funding and reviewed the manuscript; all co-authors approved the manuscript.

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

I have shared the accession number for my data in the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2022.104658.

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