Journal of
Food Chemistry & Nanotechnology

https://doi.org/10.17756/jfcn.2023-s1-077

Research Article

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Effect of Crop Rotation on Bacterial Diversity and Soil Quality under Organic and Conventional Farming System

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Received: September 19, 2023
Accepted: November 10, 2023
Published: November 15, 2023

Citation: Bhatt M, Mehta CM, Bhatt D, Sharma A, Singh DK, et al. 2023. Effect of Crop Rotation on Bacterial Diversity and Soil Quality under Organic and Conventional Farming System. *J Food Chem Nanotechnol* 9(S1): S612-S619.

Published by United Scientific Group

Abstract

Organic farming has played a major role in increasing soil organic matter (SOM) and improving soil quality. A comparative study of organic and conventional farming under field conditions was carried out to see the effect of farming practices on culturable and unculturable bacterial diversity and soil quality. Wheat, lentil, vegetable pea and brassica were taken in rotations along with basmati rice in four different plots of organic as well as conventional field. Green manure and vermicompost were used in organic fields while diammonium phosphate (DAP), urea and muriate potash (MOP) were used in conventional fields. Increase in yield was observed in plots under organic farming while yield was dropped in conventional farming along with time. Culturable bacterial diversity was observed higher in organic fields compared to conventional. Changes in rotation practices under organic farming, especially the rice-legume system induced a shift towards rich bacterial diversity, while no significant change in bacterial diversity was observed in conventional farming system. These results were best explained by Shannon weaver's diversity indices. Total bacterial diversity as assessed by denaturing gradient gel electrophoresis (DGGE) confirmed the results obtained by culturable techniques. Under organic farming, different clusters appeared in different crop rotations while in conventional farming, all crop rotations were placed closely in principal component analysis (PCA). Present findings indicated that the inclusion of crop rotations in organic farming practices improves soil quality and enhances rich bacterial diversity along with the sustainable increase in crop productivity over a period as compared to conventional farming.

Keywords

Conventional farming system, Organic farming, Crop rotation, Soil quality

Introduction

Conventional farming practices have improved the food production with the input of synthetic fertilizers and other chemicals, however many of the chemicals are hazardous to human health and may cause nutrient imbalance in soil [1], whereas organic farming is dependent on natural biological inputs which is found helpful in maintaining SOM, nutrient recycling, and microbial diversity [2, 3]. It is sustainable and can provide better yield without affecting ecological factors of soil [4].

The level of organic matter in soil is considered to be a good function of the net input of organic residues by the crop rotations [5, 6]. Rice-legume based cropping system is an effective system that results in rapid natural cycling of soil and plant nutrient which improves the organic carbon (OC) status of the soil [7]. Rice stands first among all the food grain crops of the world and is an important staple food of India. It has been estimated that half of the world's population subsists wholly or partially on rice [8]. Rice production includes frequent cycling between anaerobic and aerobic conditions, which enhances organic matter of the soil, thus in turn increases soil bulk density over the time which ultimately leads to hardening of paddy soil. This problem can be resolved through impregnation of the roots of legume plants in soil as they cause better aggregation than cereals due to increased SOM and root density [9]. Changes in rotation practices also induce major shifts in the number and composition of soil fauna and flora, including both pathogens and beneficial microorganisms [10, 11]. Many researchers have reported that organic farming practices affect soil flora, fauna, their processes, and metabolic activities in a positive way [12]. In the environment, various beneficial microorganisms are found in the form of complex and varied communities, helping in recycling of nutrients, modification of soil physical structure, maintenance of the SOM, suppression of pathogens and de-toxification of toxic chemicals [13]. Crop rotations and intercropping systems have proved to be beneficial in increasing microbial diversity and crop yield [14, 15]. However, the mechanism behind this is still not clear.

To observe the effect of different cropping systems and crop rotations on soil quality, soil enzymes are considered to be good indicators as they are easy to measure and show a rapid response of change in soil management practices [16]. Enzyme activity is usually associated with living microbial cells, their biomass, respiration rate and soil organic content [17]. Basically, soil quality is defined as a capacity of soil to function in order to sustain biological productivity and to uphold animal, plant and human health. Since, soil microorganisms can react rapidly towards environmental change and are, therefore, considered when soil status is monitored [18].

Assessment of bacterial biomass and soil quality is an enumeration of total bacterial diversity of soil using both culturable and non-culturable techniques. Despite all attempts to measure bacterial population, soil and its microbiota remain a mystery. Culturable methods do not explore more than 0.1 to 1% soil bacterial diversity; hence research is now switched from biochemical and microbiological methods to molecular methods using soil DNA [19]. Amplification of 16S rRNA gene using PCR may give a rough overview of distant bacterial community. Amplified DNA can be cloned to vectors, sequenced, or can be resolved by DGGE or temperature gradient gel electrophoresis [20, 21] and non-denaturing gel electrophoresis [22]. For community analysis, DGGE has proved to be a very useful technique as it shows a clear picture of unique and minor change in bacterial community fingerprints [23, 24].

The aim of present study is to investigate the impact of organic farming practices along with crop rotation on soil bacterial diversity, total bacterial population, and their activity through assessing different functional enzymes present in the soil and its comparison with conventional farming practices was also done. The results were further confirmed through DGGE techniques.

Materials and Methods

Experimental details

An experimental trial on two farming practices with different sets of crop rotations was set up at the seed production center of G.B. Pant University of Agriculture and Technology. Different crop rotations followed every year and were arranged in a completely randomized block design with three replicates each (plot size, 14 × 7 m^2). Test crop rotations under organic farming system were basmati rice (*Oryzae sativa*) var. pusa basmati-1, wheat (*Triticum astivum*) var. PBW 343 (Co1), basmati rice-lentil (*Lens esculenta*) var. pant lentil 406 (Co2), basmati rice-veg. pea (*Pisum sativum*) var. arkel (Co3), basmati rice-brassica (*Brassica compestris*) var. GLS 1 (Co4). Under conventional farming, the same treatments and rotations were coded as Ci1, Ci2, Ci3, Ci4 (Figure 1). In conventional farming plots, DAP, urea and MOP were used in a standard ratio of 120:60:40 (N:P:K) while in organic farming plots, green manure in the form of sesbania aculeate and vermicompost were used @ 3.45 and 1 quintal (dry mass)/plot, respectively. Average N, P and K contents in sesbania green manure on dry weight basis as estimated by standard procedures were 2.23, 0.25 and 0.58%, respectively. Therefore, total nutrients added to soil through sesbania green manure were 76.94 kg N, 19.7 kg P_2O_5 and 24.2 kg K_2O per hectare which clearly signifies that approximately 80% of nutrient requirement especially nitrogen is fulfilled by sesbania green manure. The remaining 20 kg N was supplied through addition of vermicompost because recommendation of nitrogen for basmati rice under organic system is 100 kg N/ha (due to higher efficiency in organic sources). Neemex (Neem oil) was used for controlling the pest.

In order to investigate the effect of organic and conventional farming on bacterial diversity and soil quality, soil samples from the rhizosphere of basmati rice were taken. All three plots of all the treatments were sampled at the time of flowering of basmati rice. The soil of the experimental field

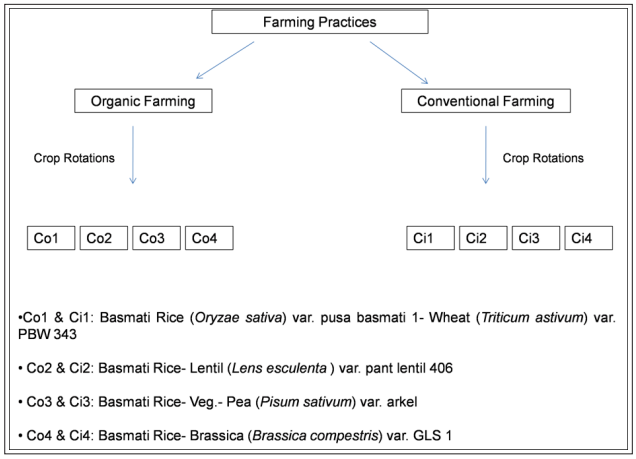

Figure 1: Diagrammatic flow chart showing experimental layout.

was sandy loam having pH 6.81; EC 0.3 ds/m; organic matter 0.81%, total P 12 kg/ha and total N 95 kg/ha.

Agronomic parameters

Crop was harvested in October month at maturity and data are presented as total grain yield (q/ha) after full duration of crop.

Determination of soil enzyme activity

Fresh rhizospheric soil samples of rice crop in three replications were collected in sterile bags at the time of flowering and kept immediately in dry ice till the collection. After harvesting, all the soil samples were sieved through a 20 - mesh sieve. 200 - 300 g of soil was stored at 4 °C for bacterial count and enzyme assays. 100 g soil was stored at -20 °C for DNA extraction.

Soil quality was determined by estimating soil enzymes. Activity of following enzymes was detected: Urease activity was measured according to the method of Kandeler and Gerber [25]. 5 g soil was mixed with 2.5 ml urea (80 mm) and 20 ml borate buffer (75 mm) having a pH of 10.0. Mixture was incubated in a shaker for 4 h at 20 °C and 180 rpm. In control urea was replaced by 2.5 ml water. After incubation, ammonia was extracted with acidified 2 M KCl solution and was determined by a modified Bertholet reaction at 690 nm using UV-Vis spectrophotometer (UV-1800, make-shimadzu). The modified method of Thalmann [26], was used to detect dehydrogenase activity. 1 g soil was mixed with triphenyl tetrazolium chloride solution and incubated in a shaker (20 °C and 180 rpm) for 16 h. Extraction of produced triphenyl formazan was done with acetone and measured at 546 nm in UV-Vis spectrophotometer (UV-1800, make-shimadzu). Activity of acid and alkaline phosphomonoesterase was determined according to the method of Tabatabai and Bremner [27]. 10 g soil was mixed with 50 ml 0.05 M acetate buffer (pH 9.5 for alkaline phosphomonoesterase and 6.5 for acid phosphomonoesterase). Sodium 4-Nitrophenyl phosphate was used as a substrate. The mixture was incubated at 37 °C for 1 h and extinction of pNP (p-Nitro phenol) was measured by UV-Vis spectrophotometer (UV-1800, make-shimadzu) at 410 nm wavelength. Samples were stored at -20 °C till their analysis.

Evaluation of culturable bacterial diversity

Bacterial population was determined in rhizospheric soil samples of rice by dilution method. One gram soil was suspended in 10 ml sterile saline water (0.8%) and mixed thoroughly. 1 ml of soil suspension was serially diluted (1:10) in saline water and pour plated on nutrient agar. Total number of bacterial colonies (cfu) was counted after incubating the plates at 28 °C for 24 h. Different colonies were selected on the basis of morphology. Bacterial colonies were tested for their functional properties viz. lipase, amylase, phosphate-solubilization and siderophore production. For estimation of lipase and amylase, minimal media was amended with 0.1% Tween 80 and 0.4% soluble starch respectively. Phosphate solubilizer were enumerated on Pikovaskya's medium [28] whereas siderophore producers were tested on nutrient agar plates amended with Chrome-azeurol S dye [29].

Isolation of DNA from soil

Soil samples were passed through a 20-mesh sieve in order to separate plant roots, animal debris and other debris. Community DNA of soil was extracted according to manufacturer's protocol of fast DNA spin kit (Bio 101, Vista, USA) using a bead beater (fast-prep, model FP 120, Bio 101). In brief, 500 mg soil samples were added to a lysing matrix tube (matrix is made up of glass beads). In this tube, 978 µl sodium phosphate buffer and 122 µl MT buffer (which was kit specific) were added and sample were homogenized in a vortex mixer and further this mixture was centrifuged at 14000 × g for 5 min. Supernatant was separated in a clean tube and 250 µl protein precipitating solution was added and mixed well by shaking manually. Again, centrifugation was done as earlier, and supernatant was taken in a clean tube. The binding matrix was resuspended in 1 ml supernatant and tube was inverted for binding the DNA. 500 µl supernatant was discarded and remaining amount was transferred to spin filter and centrifuged at 14000 × g for 1 min. The catch tube mixture was discarded, and filter was washed again with SEWS-M and previous step was repeated and catch tube was discarded. Binding matrix was resuspended in 50 µl DNase and centrifugation was done to elute pure DNA into a new catch tube. The spin filter was discarded and extracted DNA was stored at -20 °C till analysis. Yield and quality of extracted DNA was assessed using 0.8% agarose gel electrophoresis and staining with ethidium bromide (0.5 µg/ml).

Community fingerprinting by PCR-DGGE

Amplification of 550 bp 16S rRNA gene including the variable V3 region from soil DNA was performed by PCR using MF341-GC as a forward primer and MR907 as a reverse primer according to Muyzer et al. [30]. An additional 40 nucleotide GC rich clamp was added to the 5' end of primer 341f. Reaction mixture contained 2 µl of soil DNA, 5 µl 10X buffer, 1 µl of dNTPs (10 mM), 1 µl of each primer (10 µM), 0.5 µl of BSA (20 mg/ml) and 0.5 µl of Taq DNA polymerase (2 U/µl). Sterile water was added to make up the final volume 50 µl. PCR was performed in bio-rad thermal cycler (DNA Engine) using the amplification program: 94°C for 5 min, 33 cycles, denaturation at 94 °C for 20 s, annealing at 57 °C for 20 s and extension at 72 °C for 30 s, followed by a final extension at 72 °C for 5 min, hold at 11 °C for 20 s and storage at 4 °C. Amplicons were checked on 1% agarose gel.

DGGE was performed in scie-plas system according to the instruction manual. PCR samples were applied directly onto 9% (w/v) acryl-bisacrylamide gel (37.5:1) with 30 - 60% urea/formamide denaturing gradient (100% denaturant corresponds to 7 M urea and 40% formamide). PCR product (30 µl) was electrophoresed in 1 X TAE buffer at 200 V, 60 °C for 30 min followed by 80 V, 60 °C for 17 h. Gel was stained in dark, using Cyber Gold Dye (1:10,000 i.e., 5.0 µl in 50 ml dH_2O) for 30 min. The gel was photographed with multi-analyst package from bio-rad.

Data analysis

A binary matrix was generated on the basis of banding

pattern obtained in DGGE gel. The presence or absence of a band in a lane was written as 1 and 0, respectively. UPGMA dendrogram was constructed as per Jaccard's similarity coefficient produced by DGGE profile.

Statistical analysis

Data was analyzed using one way ANOVA for bacterial counts. Shannon Weaver's diversity index was calculated for culturable bacterial diversity. PCA was used for analyzing bacterial diversity on the basis of DGGE.

Results

Agronomic parameters

In general, total grain yield was increased under organic farming over a period of time. An increase of 27.21% and decrease of 1.47% of rice crop yield was recorded under organic and conventional farming respectively. Significantly maximum yield was recorded in rice-lentil rotation (Co2) followed by rice-veg pea (Co_3) in organic farming system (Figure 2).

Soil enzyme activities

Soil quality was determined by comparing different soil enzymes in organic and conventional farming systems. Activity of all the four enzymes (i.e., urease, dehydrogenase, acid and alkaline phosphomonoesterase) was high in the organic farming system as compared to conventional farming (Figure 3). Activity of phosphomonoesterase was highest among all the soil enzymes tested under both the farming systems (Figures 3C and 3D). A significant increase of 307.5% was recorded in urease activity in rice-lentil crop rotation (Co_2) under organic farming as compared to conventional farming system (Figure 3A). Dehydrogenase activity varied significantly in all the crop rotations under organic farming as compared to conventional farming system with a maximum increase of 76.6% in rice-lentil crop rotation (Co_2) (Figure 3B).

Among all the crop rotation under organic farming rice-lentil (Co_2) and rice-veg pea (Co_3) recorded significantly increased concentration of all four enzymes.

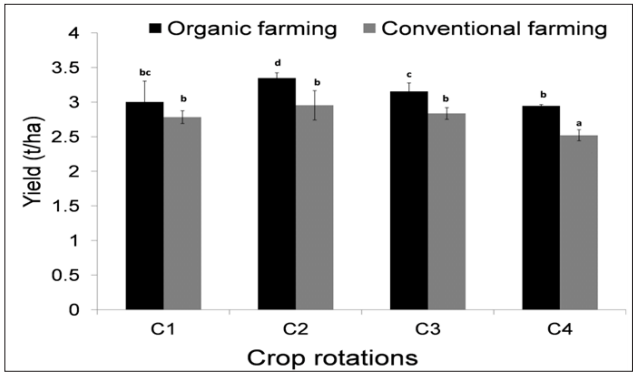

Figure 2: Effect of organic and conventional farming on yield of basmati rice. Different crop rotations used as a treatment were: C1 - basmati rice - wheat rotation; C2 - basmati rice - lentil rotation; C3 - basmati rice - veg pea; C4 - basmati rice - brassica. Values are the means of three replicates. Columns with different letters (lowercase superscripts) are significantly different ($p < 0.05$). Error bars indicate standard deviation.

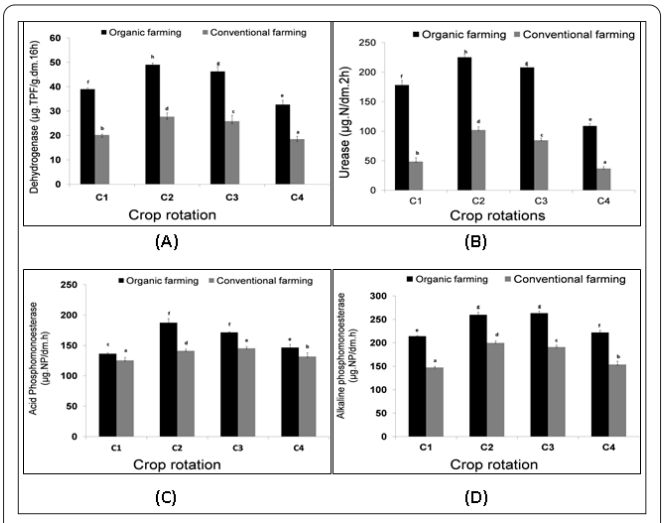

Figure 3: Effect of different crop rotations on enzyme activity in organic and conventional farming system **(A)** urease activity; **(B)** dehydrogenase activity; **(C)** acid phosphomonoesterase activity; and **(D)** alkaline phosphomonoesterase activity. Abbreviations for crop rotations are similar as in figure 1. Values are the means of three replicates. Columns with different letters (lowercase superscripts) are significantly different ($p < 0.05$). Error bars indicate standard deviation.

Culturable bacterial diversity in soil

A clear difference in culturable bacterial diversity between organic and conventional farming systems was observed (Figure 4). In rice-veg pea (C3) crop rotations, population of bacteria was significantly high in organic farming as compared to conventional farming system. Both the farming practices showed the highest population of The population of other producers was varying, and no consistent results were obtained.

Shannon's diversity index varied significantly in all the crop rotations in both the systems. Rice-lentil (Co_2) and rice-veg. pea (Co_3) had significantly maximum diversity under organic farming; however, rice-wheat rotation (Ci1) under conventional farming showed significantly minimum value of Shannon diversity index among different rotations in both the farming. The rest of the rotations under both the farming had no significant effect on bacterial diversity (Figures 5A and 5B).

Determination of bacterial community by DGGE

Bacterial community in different cropping systems was also analyzed by DGGE fingerprinting. A striking difference

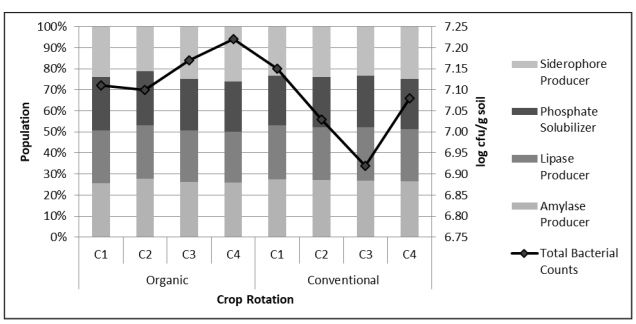

Figure 4: Total bacterial count (log cfu/g of soil) and functional assay of culturable bacterial population in conventional and organic farming under different crop rotations. Log values of total bacterial count and functional diversity for bacteria as the means of three replicates.

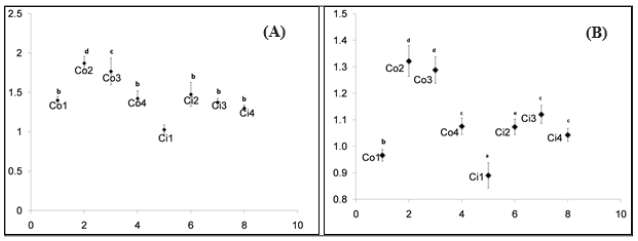

Figure 5: Shannon's index of diversity amongst: **(A)** total bacterial population and **(B)** functionally active bacterial population. Different crop rotations used as treatments were: In organic farming: Co1 - basmati rice - wheat rotation; Co2 - basmati rice- lentil rotation; Co3 - basmati rice - veg pea; Co4 - basmati rice - brassica. In conventional farming: Ci1 - basmati rice - wheat rotation; Ci2 - basmati rice - lentil rotation; Ci3 - basmati rice - veg. pea; Ci4 - basmati rice - brassica.

in banding pattern was observed among the bacterial community of both the farming systems. Community fingerprinting of bacterial 16S rDNA by DGGE showed some common dominating bands in all the samples regardless of cropping systems. Along with these dominating bands, some faint bands also appeared in higher number in the samples of organic plots (Co1, Co2, Co3 and Co4), which were considered in the cluster analysis. Cluster analysis using UPGMA of DGGE profile showed a distinct pattern of bacterial community in both the farming systems (Figures 6A and 6B). It is deduced from figure 6A that rice-wheat (Ci1), rice-lentil (Ci2), rice-veg.pea (Ci3) crop rotations of conventional farming had similar type of bacterial communities as they formed a single cluster. The bacterial community of rice-brassica (Ci4) crop rotation lied distantly. Under organic farming, Co2 and Co3 treatments had similar bacterial community and were placed in a single cluster (Figure 6A).

A complex bacterial diversity was found in the soil samples of organic farms. PCA was also performed for statistical analysis of banding pattern of DGGE (Figure 6B). The first two principal components accounted for 86.36% of the total variance and effectively captured the main pattern of variation in the original variables. Samples separated into distinct groups in the PCA ordination revealed the connection of farming system and bacterial fingerprints. The total variance of the data explained by 1 and 2 axes was 68.15% and 18.21%,

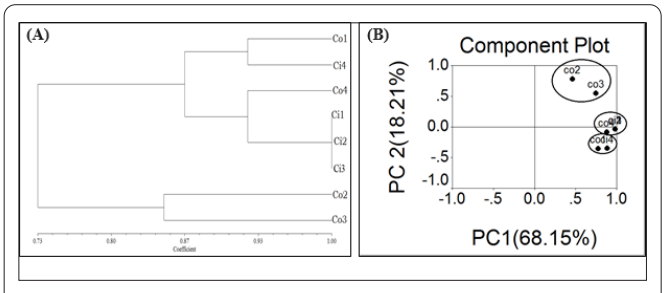

Figure 6: Bacterial diversity generated by PCR-DGGE with specific primers for the domain bacteria. **(A)** UPGMA cluster analysis (Shannon's coefficient of similarity) of molecular banding patterns. Scale bar represents percent similarity and **(B)** two-dimensional plot of the principal component analysis (PCA) generated from DGGE profile of both organic and conventional farming. Only the first principal component (PC1) and second principal component (PC2) were shown. Each crop rotation in the figure is defined as a dot. Abbreviations for crop rotations in both the farming systems are similar as in figure 3.

respectively in the PCA ordination analysis of DGGE band patterns. The results obtained by the PCA were comparable with the result of DGGE.

Discussion

In the present study, we have evaluated the changes in crop yield, soil quality and bacterial community due to crop rotations under organic and conventional farming. Different agricultural management practices have a significant impact on crop productivity and soil health [31, 32]. A year wise increase in crop production was observed in the plots under organic farming, on the other hand, conventional farming showed a gradual decline in the crop yield. These results can be predicted as an organic farming system not only supports the soil quality, but also affect sustainable crop production. Better crop productivity and yield stability can be achieved by a more complex bacterial diversity [33-35]. The bacterial population of the soil is also directly affected by farming practices and crop rotation. Many authors have reviewed that soil microbial communities [36-38] and soil health [39-41] are influenced by crop rotation.

Bacterial diversity was found more complex in rice-legume crop rotation under organic farming, whereas crop rotations under conventional farming did not influence the diversity in a major way. This observation is best explained by Shannon's diversity index by which it is clearly depicted that total bacterial population as well as functional bacterial population was higher in organic farming plots as compared to conventional one (Figure 5A and 5B). Higher bacterial population in organic farming practices could be related to the amendment of green manure and vermicompost in the field, which are a good source of OC and available minerals and enhance the population of beneficial microorganisms and bacterial biomass [42, 24]. Increase in total OC content results in enhancement of bacterial biomass [43, 44]. According to Gunapala and Scow [45], the amount of C entering in the systems is mainly responsible for differentiating the bacterial activities in different farming systems.

However, in conventional farming, the use of chemical fertilizers influenced the growth of microorganisms in a negative way. The positive effect of crop rotation under organic farming on the presence of beneficial microorganisms was also reported by Larkin and Honeycutt [46]. Among all the crop rotations, rice-veg pea and rice-lentil systems had shown increased bacterial diversity while rice-wheat and rice-brassica system did not support the bacterial population. This can be understood by the fact that the rice-wheat cropping system is an exhaustive system in terms of nutrition. More than 650 kg ha^{-1} of N, P, K, and 0.5 - 1.0 kg ha^{-1} Zn, 2 - 3 kg ha^{-1} Fe and 3.0 - 3.5 kg ha^{-1} Mn are required annually for rice-wheat cropping systems [47]. In the rice-brassica system, brassica being a non-host plant for mycorrhizal fungi, can impart a negative effect on interaction of beneficial bacteria with plant and lower down the bacterial diversity. Many authors have hypothesized that mycorrhiza regulates the behavior of plant composition, bacterial diversity, and succession of plant communities [48-52]. Amount of soil N and OC is greatly improved by inclusion of legumes in cereal based cropping systems [53, 54].

In the present study, the activity of all the four enzymes increased in organic farming as compared to conventional farming which could be related to enhanced bacterial population. In organically managed soil, increase in activity of phosphatase [55, 56], urease [57], and dehydrogenase [58] has also been reported. Enhanced enzymatic activity is a result of stimulation of bacterial activity in soil amended with organic residues [59, 60]. Phosphatase seems to be produced by microbes because higher plants are devoid of this enzyme [61]. Application of manure and vermicompost in soil enhances phosphatase activity which is an indicative of the effect of increased OC on metabolic activities of soil bacterial population [62]. Activity of urease and dehydrogenase was also strongly correlated with enhanced OC [57, 63]. Reduction in enzyme activity under conventional farming may be due to reduction in nutrient recycling in soil [64]. Organic farming practices may lead to increased soil microbial biomass, enzymatic activity, microbial functionality, and diversity in comparison to conventional farming [65-68].

Result of bacterial community analysis using DGGE revealed the presence of different banding pattern in both the farming systems. Crop rotations altered the bacterial population thus the community of residing microflora changed which is clearly depicted by PCA. Crop rotation and bacterial community structure from organic and conventional farming soils were included in a single PCA to access the interaction between them and to select the variables that better discriminate between these two farming systems. Rice-wheat (Co_1) and rice-brassica (Co_4) rotation made a different cluster while rice-lentil (Co_2) and rice-veg. Pea (Co_3) rotation appeared in a same cluster under organic farming while in conventional farming Ci1, Ci2 ,Ci3 were closely placed and Ci4 appeared with rice-wheat rotation (Co_1) of organic farming. It is evident from cluster analysis that rice-wheat and rice-brassica rotation do not enhance the bacterial diversity, hence lie in a same cluster.

By the cluster analysis we can predict that in conventional farming, within the crop rotations there were very slight changes in the bacterial diversity while in organic farming, the diversity became different and much more complex among different crop rotations. This may be understood by the fact that nature of the root exudates differ with plant species and results in species specific shifts in bacterial community under a particular soil ecosystem [69, 70]. DGGE reflected the shift in genetic structure of soil bacterial communities as a result of different crop rotations and farming practices, which is consistent with other studies [71-73]. In summary, it was observed that bacterial population of rice field, subjected to different crop rotations under organic farming system presented enhanced bacterial diversity with variation on enzymatic activity and unculturable bacterial community as compared to conventional farming. The rice-legume system was proved to be beneficial over rice-wheat or rice-brassica system as it has increased bacterial diversity and in turn increased the soil organic fertility.

Conclusion

Based on the outcome, it can be predicted that organic farming is helpful in maintaining soil quality and bacterial diversity. Organic farming resulted in better soil quality and comparable yield with conventional farming. Changes in rotation practices under organic farming, especially the rice-legume system induces a shift towards rich bacterial diversity, while in conventional farming system no significant change in bacterial diversity was observed. The study also shows that the Shannon diversity index and molecular techniques like DGGE could be a beneficial tool for depicting a clear picture of changes in rhizospheric bacterial diversity.

Acknowledgements

Authors are thankful to Indo-Swiss Collaboration in Biotechnology program for financial support and university for providing research facility to carry out this work.

Conflict of Interest

None.

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