Organic carbon and nitrogen availability determine bacterial community composition in paddy fields of the Indo-Gangetic plain

Arvind Kumar^{1,2} · Lal Chand Rai¹

Received: 20 December 2016 / Accepted: 17 April 2017 / Published online: 30 June 2017
© Springer-Verlag GmbH Germany 2017

Abstract Soil quality is an important factor and maintained by inhabited microorganisms. Soil physicochemical characteristics determine indigenous microbial population and rice provides food security to major population of the world. Therefore, this study aimed to assess the impact of physicochemical variables on bacterial community composition and diversity in conventional paddy fields which could reflect a real picture of the bacterial communities operating in the paddy agro-ecosystem. To fulfill the objective; soil physicochemical characterization, bacterial community composition and diversity analysis was carried out using culture-independent PCR-DGGE method from twenty soils distributed across eight districts. Bacterial communities were grouped into three clusters based on UPGMA cluster analysis of DGGE banding pattern. The linkage of measured physicochemical variables with bacterial community composition was analyzed by canonical correspondence analysis (CCA). CCA ordination biplot results were similar to UPGMA cluster analysis. High levels of species–environment correlations (0.989 and 0.959) were observed and the largest proportion of species data variability was explained by total organic carbon (TOC), available nitrogen, total nitrogen and pH. Thus,

results suggest that TOC and nitrogen are key regulators of bacterial community composition in the conventional paddy fields. Further, high diversity indices and evenness values demonstrated heterogeneity and co-abundance of the bacterial communities.

Keywords Bacterial diversity · 16S rRNA gene · PCR-DGGE · Paddy fields · Organic carbon

Introduction

Soil is a versatile dynamic system with highly complex and heterogeneous environment. It is a natural resource for the existence of life on our planet by providing various mankind services through complex interactions involving its biological, physical and chemical properties (Karlen et al. 1997). Food security is one of the most important services for human survival. Sustainability of the services depends on soil health and quality. Soil microorganisms are important constituents and play a major role in the maintenance of soil health and quality by microbial mediated nutrient transformations (nutrient cycling), litter decomposition, soil organic matter formation, energy flow and contaminant degradation (Fillip 2002; Liu et al. 2007).

Rice is an important cereal crop. It provides food to more than 50% world's population and also a source of income for the millions of households around the globe (FAO 2010; Liesack et al. 2000). Paddy fields remain flooded during the main stages of plant growth and result in the reduction of plow layer soil, leaching of the nutrients and microorganisms in the percolating water (Murase et al. 2005). Additionally, increased demand of productivity leads the development of high yielding varieties with shorter life span. It has increased the continuous cropping system

Electronic supplementary material The online version of this article (doi:10.1007/s13205-017-0810-x) contains supplementary material, which is available to authorized users.

✉ Arvind Kumar
arvindbhu7@gmail.com

¹ Molecular Biology Section, Laboratory of Algal Biology, Center of Advanced Study in Botany, Banaras Hindu University, Varanasi 221005, India

² Department of Biochemistry, Veer Bahadur Singh Purvanchal University, Jaunpur 222003, India

and resulted in the reduction of soil microorganism's diversity (Jing et al. 2010).

The study of microbial communities directly from soil samples became easier with the development of culture-independent techniques. Denaturing gradient gel electrophoresis (DGGE) is a type of culture-independent technique for microbial community analysis. It separates DNA fragments of same length on the basis of sequence heterogeneity (Muyzer et al. 1993). The separated bands may be re-amplified, sequenced and the obtained nucleotide sequences of marker genes used for the microbe identification. DGGE has been used for the monitoring of soil bacterial communities in constructed wetlands (Dong and Reddy 2010), and to assess the impact of long term fertilization (Shen et al. 2010), organic and conventional farming on bacterial communities (Lopes et al. 2011).

It is well known that rice is an important food crop and soil physiochemical characteristics play a key role in shaping bacterial communities (Rousk et al. 2010; Shen et al. 2010; Xu et al. 2009). Therefore, it is interesting to explore the impact of physicochemical variables on bacterial community composition and diversity in conventionally managed paddy fields. However, up to the best of my knowledge research has not been conducted in Indian paddy fields to prove that among many of the physicochemical variables which are contributing at large in determining bacterial community composition. Thus, the present study undertaken to assess the impact of soil physicochemical characteristics on bacterial community composition and diversity in conventionally managed paddy fields of eastern Uttar Pradesh and western Bihar region of India using PCR-DGGE.

Materials and methods

Study sites and soil sampling

The study sites were located in the eastern Uttar Pradesh and western Bihar region of Indo-Gangetic plain (Fig. 1). The climate of the selected region is warm and humid with an annual precipitation of 1000–1600 mm. The average temperature of the sites varied between 25–35 °C with approximately 90% humidity at the time of sampling. The sites were primarily used for paddy cultivation during the rainy season and follow a rice–wheat cropping system. The fields were planted with rice 30–35 days before sampling, and thus rice plants were at similar growth stage in all the fields during sample collection. Further, the samples were collected simultaneously from all the sites and at the time of sampling soils were either wet or partially water logged. In the first week of September, 2009; twenty bulk soil samples were collected from the different villages of the

eight districts namely Allahabad (81°51'E, 25°26'N), Ghazipur (83°34'E, 25°35'N), Arrah (84°40'E, 25°33'N), Chandauli (83°16'E, 25°15'N), Patna (85°08'E, 25°36'N), Mirzapur (82°33'E, 25°8'N), Ballia (84°08'E, 25°45'N) and Varanasi (82°58'E, 25°19'N). Five sub samples (4.0 cm diameter and 0–10 cm depth) were collected from each site, mixed and crop residues as well as rice roots were removed before analysis. The composite sample of each site was kept in the laboratory at 4 °C and processed soon for physicochemical characterization and metagenome extraction.

Physicochemical characterization of soils

Soil samples were homogenously suspended in double distilled Milli-Q water in the ratio of 1:2.5 (w/v) and centrifuged at 2348 g for 5 min at 25 °C. Electrical conductivity (EC) and pH were measured from the clear supernatants using conductivity meter 306 and µpH SYSTEM 361, respectively, of Systronics, India.

Available phosphorus (AP) was extracted with addition of 200 mg activated charcoal at a soil/NaHCO₃ (0.5 M, pH 8.5) ratio of 1:20 (Olsen et al. 1954). Similarly, ammonium-N (NH₄⁺) was extracted from 10 g soil in 50 ml 2 M KCl with addition of 200 mg activated charcoal. The samples were shaken at 100 rpm in a temperature controlled incubator shaker at 15 °C for 1 h and filtered through Whatman No.1 filter paper. The extracted AP and NH₄⁺-N in the clear supernatants were determined by molybdophosphoric acid and phenate methods respectively (Eaton et al. 1995). Nitrate-N (NO₃⁻) extraction and measurement was carried as described by Jackson (Jackson 1967). Total nitrogen (TN) was estimated from the sieved samples following the Kjeldahl procedure (Eaton et al. 1995). Total organic carbon (TOC) was determined from air-dried, sieved soil samples (Kalembasa and Jenkinson 1973).

Exchangeable cations such as K⁺, Fe²⁺, Ca²⁺ and Mg²⁺ were extracted by shaking 5 g soil with 25 ml of 0.1 M EDTA solution. The extract was filtered through Whatman No.1 filter paper and appropriately diluted filtrate was subjected to atomic absorption spectroscopy (Perkin Elmer 2380).

Genomic DNA extraction, purification and 16S rRNA gene amplification

Genomic DNA was extracted from the collected soils in triplicate to minimize the chances of biased DNA extraction and pooled in one tube (Srivastava et al. 2007). The pooled DNA was passed through spin column containing Sepharose 4B (Sigma Chemical Co., USA) to remove humic acid and salts. Spin columns were prepared by

Fig. 1 Location of sampling sites showing the districts and villages (*in parentheses*) on the map of Indo-Gangetic plain

plugging 1 ml plastic syringe with glass wool and packing of Sepharose 4B. Thereafter, the columns were washed twice with TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and centrifuged (400 g for 5 min) to remove excess buffer. DNA samples were passed through the column under the same conditions as for column packing and washing. The eluent containing DNA sample was collected and checked on 0.8% agarose gel.

Bacterial 16S rRNA gene fragments (V3–V5 region) were amplified in an Icyler (Bio-Rad, USA) using the primers 341F GC: CGCCGCGCGCGCGGGCGGGCGG GGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG and 907R: CCGTCAATTCCTTTGAGTTT (Zaady et al. 2010). The reaction mixture (25 μ l) contained 30 ng genomic DNA, 2.5 μ l of 10 X PCR buffer with 15 mM $MgCl_2$, 0.5 μ l dNTPs mix (10 mM stock), 10 pmol of each primer and 0.6 U *Taq* DNA polymerase (Bangalore Genei, India). Thermal cycler profile was as follows: initial denaturation at

95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 60 s, annealing at 59 °C for 45 s and extension at 72 °C for 60 s. Final extension was carried out at 72 °C for 10 min. PCR amplification was ensured by resolving the products on 1.2% agarose gel containing ethidium bromide ($1.0 \mu\text{g ml}^{-1}$). All the amplification reactions were carried out in triplicate and PCR products were pooled in one tube.

Denaturing gradient gel electrophoresis (DGGE), 16S rRNA gene sequencing and diversity indices calculation

DGGE analysis was performed using DGGE-2001 system (C.B.S. Scientific Company, Inc., USA). The assembly containing 8% polyacrylamide (acrylamide/bisacrylamide, 38.93:1.07) gel with 40–60% linear denaturant (7 M urea and 40% (v/v) deionized formamide correspond to 100% denaturant) was placed in the DGGE running tank buffer

(1 × TAE). PCR products (300 ng) were loaded in the wells and run at 60 °C for 12 h at 85 volts. The gel was removed from running tank buffer, stained with ethidium bromide (1 µg ml⁻¹ in 1 × TAE buffer) for 15 min, visualized and photographed using GBOX HR gel documentation system (Syngene, UK). The separated DNA bands differing in their mobility were excised and eluted in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) for overnight at 4 °C. The eluted DNA (2 µl) was re-amplified using 341F: CCTACGGGAGGCAGCAG and 907R: CCGTCAATTCCTTTGAGTTT primers. The re-amplified products were resolved on 1.2% agarose gel and purified by Gene Elute Gel Extraction kit (Sigma Chemical Co., USA). Purified PCR products were sequenced on commercial basis from Macrogen, Inc. (Korea). The erroneous nucleotides were deleted from both the ends using online PINTAIL software and the trimmed sequences were subjected to BLASTN homology search of NCBI database. The sequences were submitted in the GenBank database with accession numbers JF910021–JF910090.

The phylotype (band) richness, diversity and evenness of the bacterial communities were calculated as described earlier (Asakawa and Kimura 2008; Fromin et al. 2002).

Statistical analysis

The banding patterns of DGGE gel were analyzed using Quantity 1 software (Bio-Rad). The banding profile was scored as species presence–absence matrix. All the detected bands scored “1” and absences scored “0” were recorded in a spreadsheet. Similarities were calculated based on Pearson product moment correlation coefficient as it has been recommended for DGGE profiles analysis (Boon et al. 2002). Clustering of the bacterial communities was carried out employing unweighted pair-group method average (UPGMA) algorithm of Statistica 5.0 software.

Canonical correspondence analysis (CCA) was performed between DGGE band intensities and soil physicochemical characteristics to assess the impact of physicochemical variables on bacterial community composition using CANOCO 4.5 for Windows software. A Monte-Carlo unrestricted permutation test based on 499 random mutations was used for the analysis of the relationship between bacterial community composition and physicochemical variables. The resulting ordination biplot approximated the weighted average of each band with respect to environmental variables which were presented as arrows. The length of an individual arrow indicates the relative importance of physicochemical variable in explaining the bacterial community composition. Diversity results were analyzed by Duncan's test ($P \leq 0.05$) using SPSS software version 10.0 for significant differences.

Results

Physicochemical characteristics of the soils

Table 1 compiles the data of eleven important physicochemical parameters (pH, EC, AP, NO₃⁻, NH₄⁺, TN, TOC, K⁺, Mg²⁺, Ca²⁺ and Fe²⁺) of the soils. Based on the pH values, soils can be put into three classes, i.e., acidic (pH <6.50), slightly acidic (pH >6.50 and <7.00) and neutral to alkaline (pH ≥7.00). EC values ranged between 1.12 and 3.30 ds m⁻¹ depicting non-saline quality of the soils. The samples contained different amounts of AP, available nitrogen (NO₃⁻ and NH₄⁺), TN, TOC and other measured physicochemical variables. Thus, the collected soils were appropriate to assess the impact of physicochemical variables on bacterial community composition and diversity.

Bacterial community structure and diversity—DGGE fingerprinting

A comparison of the bacterial community structure was carried out by 16S rRNA gene profiling from the collected soils. It is clear from the differences in the banding pattern that samples harbored different bacterial community structure (Fig. 2). A total of 83 bands (marked with arrows) were excised and subjected to sequencing but only 70 bands produced good quality sequences that can be satisfactorily used in the BLASTN analysis. As a result thirteen bands marked with circles were not considered for identification.

UPGMA dendrogram derived from the presence–absence of DGGE bands formed three clusters based on the similarity matrix (Fig. 3). Cluster I contained bacterial profiles from neutral to alkaline soils with the exception being intermingling of Lilapur, while cluster II and III were presented by slightly acidic and acidic soils, respectively. CCA ordination biplot of DGGE band intensities and physicochemical variables also demonstrated the distinction of samples into three groups (Fig. 4). Interestingly, CCA grouping showed congruency with UPGMA cluster pattern. Axis 1 and 2 of the ordination biplot explained 34.6 and 22.8% variation in the bacterial communities, respectively (Table 2). Both axis demonstrated high species–environment correlation coefficient values (0.989 and 0.959) indicating a significant relationship between observed bacterial community composition and the selected physicochemical variables. Evaluation of the correlation of bacterial community composition with assigned environmental variables based on 499 random permutations of Monte-Carlo test indicated that both axis explained a significant proportion of the variation in the data

Table 1 Physicochemical characteristics of the soil samples

Districts	Villages/sites	Physiochemical parameters										
		pH	EC (ds m ⁻¹)	AP (μg g ⁻¹)	NO ₃ ⁻ (μg g ⁻¹)	NH ₄ ⁺ (μg g ⁻¹)	TN (mg g ⁻¹)	TOC (mg g ⁻¹)	K ⁺ (μg g ⁻¹)	Mg ²⁺ (μg g ⁻¹)	Ca ²⁺ (μg g ⁻¹)	Fe ²⁺ (μg g ⁻¹)
Allahabad	Phoolpur	6.44 ± 0.03	3.30 ± 0.03	28.06 ± 2.16	5.64 ± 0.11	2.04 ± 0.08	0.73 ± 0.11	6.81 ± 0.17	180 ± 6.66	157 ± 9.45	1414 ± 38	190 ± 04
	Chainpur	5.48 ± 0.04	2.46 ± 0.15	20.91 ± 2.66	4.95 ± 0.12	1.59 ± 0.04	0.67 ± 0.02	6.29 ± 0.05	202 ± 6.66	148 ± 8.18	1157 ± 35	217 ± 14
	Baburhipura	5.86 ± 0.03	2.28 ± 0.06	26.74 ± 2.51	4.17 ± 0.06	3.92 ± 0.07	0.58 ± 0.01	5.63 ± 0.09	230 ± 6.24	141 ± 12.22	1195 ± 26	217 ± 07
Ghazipur	Sultanpur	4.98 ± 0.13	1.33 ± 0.06	24.88 ± 1.33	5.18 ± 0.06	2.38 ± 0.12	0.73 ± 0.02	5.78 ± 0.04	244 ± 9.60	57 ± 7.09	744 ± 20	279 ± 05
	Sherpur	5.36 ± 0.04	1.71 ± 0.06	22.04 ± 3.05	4.94 ± 0.10	1.98 ± 0.11	0.69 ± 0.02	6.28 ± 0.08	164 ± 9.71	92 ± 8.54	694 ± 20	252 ± 14
Arrah	Dhangaon	5.97 ± 0.02	1.89 ± 0.07	17.20 ± 0.67	5.28 ± 0.06	1.42 ± 0.07	0.66 ± 0.01	6.61 ± 0.10	172 ± 7.50	114 ± 6.51	853 ± 22	215 ± 18
	Bhojpur	6.13 ± 0.06	1.44 ± 0.22	35.57 ± 2.40	5.67 ± 0.14	2.75 ± 0.12	0.87 ± 0.01	7.72 ± 0.17	220 ± 10.06	69 ± 7.55	631 ± 07	228 ± 06
Chandauli	Devria	7.05 ± 0.07	2.53 ± 0.06	10.32 ± 1.60	7.11 ± 0.16	2.92 ± 0.16	1.03 ± 0.04	9.34 ± 0.08	220 ± 21.79	137 ± 9.29	915 ± 30	202 ± 06
	Kundalia	7.82 ± 0.12	2.88 ± 0.34	27.81 ± 4.49	9.86 ± 0.22	2.75 ± 0.09	0.84 ± 0.01	10.02 ± 0.17	259 ± 22.05	209 ± 13.00	1488 ± 21	338 ± 16
	Lilapur	6.20 ± 0.04	2.89 ± 0.12	21.97 ± 1.33	8.66 ± 0.14	4.09 ± 0.13	0.87 ± 0.02	8.76 ± 0.08	167 ± 12.12	179 ± 7.2	1393 ± 30	275 ± 10
Patna	Amraha	7.15 ± 0.18	2.67 ± 0.10	35.33 ± 2.16	7.05 ± 0.17	3.29 ± 0.13	0.81 ± 0.02	8.91 ± 0.07	100 ± 10.69	132 ± 8.02	1170 ± 26	166 ± 18
	Bihtia	7.24 ± 0.10	1.98 ± 0.23	35.13 ± 2.67	5.75 ± 0.18	2.39 ± 0.14	0.98 ± 0.04	8.67 ± 0.05	136 ± 6.24	87 ± 6.51	944 ± 08	152 ± 10
	Vikram	7.72 ± 0.15	3.04 ± 0.26	30.44 ± 4.69	9.22 ± 0.12	2.77 ± 0.12	0.93 ± 0.03	9.83 ± 0.13	186 ± 6.80	212 ± 11.79	1266 ± 17	186 ± 06
Mirzapur	Sarsa	6.92 ± 0.04	1.63 ± 0.26	22.51 ± 1.01	4.23 ± 0.08	1.58 ± 0.21	0.49 ± 0.01	3.70 ± 0.07	135 ± 23.15	130 ± 12.22	1493 ± 29	305 ± 16
	Aharora	6.72 ± 0.03	1.59 ± 0.09	16.26 ± 2.12	5.10 ± 0.13	2.57 ± 0.10	0.48 ± 0.01	3.65 ± 0.07	137 ± 8.08	156 ± 9.45	1230 ± 25	340 ± 12
Ballia	Balegi	6.62 ± 0.03	1.51 ± 0.05	17.17 ± 0.47	3.41 ± 0.08	1.03 ± 0.15	0.53 ± 0.02	3.89 ± 0.06	127 ± 12.16	127 ± 6.56	862 ± 20	232 ± 13
	Fafna	6.52 ± 0.05	1.56 ± 0.21	9.34 ± 1.81	4.76 ± 0.07	1.57 ± 0.11	0.22 ± 0.01	2.08 ± 0.09	122 ± 11.79	85 ± 6.65	761 ± 27	221 ± 13
	Mahrev	6.58 ± 0.07	1.12 ± 0.1	9.54 ± 2.50	2.96 ± 0.05	1.63 ± 0.08	0.18 ± 0.01	1.94 ± 0.04	166 ± 11.13	122 ± 7.50	660 ± 22	278 ± 21
Varanasi	Jivnathur	6.35 ± 0.05	1.42 ± 0.21	18.24 ± 4.58	3.92 ± 0.05	1.25 ± 0.06	0.49 ± 0.01	3.83 ± 0.04	136 ± 8.50	134 ± 17.43	769 ± 30	272 ± 10
	Ramnagar	6.78 ± 0.04	2.10 ± 0.08	21.59 ± 2.64	3.56 ± 0.11	1.58 ± 0.14	0.18 ± 0.01	4.36 ± 0.12	141 ± 21.50	146 ± 28.74	1008 ± 49	182 ± 10

TN, TOC, AP, K⁺, Mg²⁺, Ca²⁺ and Fe²⁺ represent total nitrogen, total organic carbon, available phosphorus, exchangeable potassium, magnesium, calcium and iron respectively. Values are mean ± SD of three replicates

Fig. 2 Bacterial community profiling of the paddy soils using DGGE. The lane names are given using *initial three letters* of the village names; **a** *PHO* Phoolpur, *CHA* Chainpur, *BAB* Baburahipura, *SUL* Sultanpur, *SHE* Sherpur, *DHA* Dhangaon, *BHO* Bhojpur; **b** *DEV* Devria, *KUN* Kundalia, *LIL* Lilapur, *AMR* Amraha, *BIH* Bihta, *VIK*

Vikram; **c** *SAR* Sarsa, *AHA* Aharora, *BAL* Balegi, *FAF* Fafna, *MAH* Mahrev, *JIV* Jivnathpur, *RAM* Ramnagar. Bands marked with *arrows* were excised, re-amplified and sequenced. Chimeric sequences were observed for the bands marked with *circles*

Fig. 3 UPGMA dendrogram of the bacterial community structure based on DGGE banding patterns. Village names in the dendrogram were abbreviated using *initial three letters* such as *KUN* Kundalia, *VIK* Vikram, *BIH* Bihta, *AMR* Amraha, *LIL* Lilapur, *DEV* Devria, *RAM* Ramnagar, *JIV* Jivnathpur, *MAH* Mahrev, *FAF* Fafna, *BAL*

Balegi, *AHA* Aharora, *SAR* Sarsa, *BAB* Baburahipura, *SUL* Sultanpur, *CHA* Chainpur, *BHO* Bhojpur, *DHA* Dhangaon, *SHE* Sherpur, *PHO* Phoolpur. Values in the *parenthesis* indicate pH of the respective soil samples

($P = 0.04$). CCA axis 1 was found to be strongly correlated with TOC, NO_3^- , NH_4^+ , EC and pH, while axis 2 correlated only with K^+ .

Indices for phylotype richness, diversity and evenness of the bacterial communities were presented in Table 3. Number of phylotypes (DGGE bands) ranged from 12 to

34. The highest phylotype richness was observed in the village Kundalia and lowest in Mahrev. Similarly, maximum Shannon diversity was observed in Kundalia, Vikram, Lilapur and Devria, while lowest in the soils of Mahrev, Balegi, Ramnagar and Sarsa. Simpson diversity did not show much variation between the different samples but followed the pattern of Shannon's diversity index. Its highest value was 0.96 and lowest being 0.83. Evenness results also suggested that bacterial communities were homogenously distributed in all the samples except for Mahrev, Ramnagar and Balegi.

Identification of DGGE bands

Bacterial communities analyzed from different samples displayed close homology with cultured and uncultured Firmicutes, Alphaproteobacteria, Betaproteobacteria Deltaproteobacteria and Epsilonproteobacteria, Actinobacteria, Crenarchaeota and Euryarchaeota (Table S1). Many of the sequences from all the sites showed close similarity with uncultured bacterial sequences. Therefore, sequence analysis was also performed with CLASSIFIER program of the ribosomal database project to classify them up to different taxonomic levels (Table S1).

Fig. 4 CCA ordination biplot of DGGE band intensities with physicochemical variables. Soil samples were clustered in three groups and marked with symbols star (group I), circle (II) and X-mark (III). Village names were abbreviated using initial three letters as in Fig. 3. TOC total organic carbon, P available phosphorus, pH soil pH, NO_3^- nitrate nitrogen, NH_4^+ ammonium nitrogen, TN total nitrogen, EC electrical conductivity and exchangeable cations, K potassium, Ca calcium, Mg magnesium, Fe iron

Discussion

PCR-DGGE is a widely acclaimed powerful technique for microbial community composition and diversity analysis (Lopes et al. 2011; Muyzer et al. 1993; Shen et al. 2010). However, certain chimeric sequences may be observed due to biased PCR amplification, heteroduplex DNA fragments, co-migration or dispersion of the dominant band sequences in the gel (Ferris et al. 1996; Kisand and Wikner 2003; Nikolsauz et al. 2005).

UPGMA grouping of slightly acidic and acidic samples close to each other indicates that bacterial communities in these samples are similar and corroborates from earlier study where significant differences were not observed in the bacterial community composition between the pH ranges of 6.82 and 8.02 (Rousk et al. 2010). However, separation of the samples into two clusters raised the question of pH being the sole factor responsible for bacterial community structuring. Additionally, grouping of one acidic sample (Lilapur) in the cluster I suggested that in addition to pH other environmental variables may also be actively involved in shaping bacterial communities. Thus, CCA analysis was carried out to understand the relationship between bacterial community composition and environmental variables in a better way (Ramatte 2007). Among the different variables tested, EC may not be considered because it displayed high inflation factor (Shen et al. 2010). Therefore, TOC, available nitrogen ($NO_3^- + NH_4^+$), TN and pH emerged prominent determinants of bacterial community composition in the study sites. The highest contribution of TOC in determining bacterial community composition can be discussed on the basis of the fact that organic carbon provides energy for bacterial growth and proliferation (Wallis et al. 2010). The above finding also gets support from the results of a recent study where microbial community composition was primarily determined by the below ground carbon and nitrogen inputs (Huang et al. 2014). Consequently, UPGMA results are also harmonious with TOC results, i.e., soils of cluster I had higher, III moderate and II lowest carbon contents. Nitrogen is also an important factor in determining bacterial community composition and synthetic N-fertilizer application in the paddy field increased growth of cultivable bacterial population (Lopes et al. 2011). In a similarly way, NH_4^+ and NO_3^- concentration also influence bacterial species distribution and community composition (Dong and Reddy 2010; Sheng et al. 2013). Therefore, our observation of available nitrogen as a second most important bacterial community composition determinant appears justified. Impact of pH in shaping bacterial communities in the agricultural field has already been demonstrated (Rousk et al. 2010) and it also emerged

Table 2 Summary of canonical correspondence analysis results

	Axis 1	Axis 2
Eigen values	0.355	0.234
Species–environment correlations	0.989	0.959
Cumulative percentage variance of species data	24.7	41.0
Cumulative percentage variance of species–environment relation	34.6	57.4
Weighted correlation		
pH	0.518	−0.455
P	0.326	0.373
TN	0.554	0.245
TOC	0.773	0.115
NH ₄ ⁺	0.588	0.309
K ⁺	0.099	0.523
Mg ²⁺	0.005	−0.217
Ca ²⁺	0.253	−0.126
Fe ²⁺	−0.299	−0.359
NO ₃ [−]	0.692	0.407
EC	0.619	0.296

as a major determinant of bacterial community composition along elevation on a mountain (Shen et al. 2013).

Diversity indices revealed heterogeneity and co-abundance of bacterial communities in the analyzed soils. The

highest diversity values observed from the soils of Kundalia, Vikram, Lilapur and Devria may be due to higher TOC and available nitrogen contents which favor growth and survival of diverse bacteria (Lopes et al. 2011). The observed diversity results are in agreement with previous studies (Asakawa and Kimura 2008; Girvan et al. 2005). Further, evenness values are in accordance with the values observed from conventionally managed paddy fields (Lopes et al. 2011). Low phylotype richness, diversity and evenness results of Mahrev, Balegi, Ramnagar and Sarsa may be linked to scarce availability of TOC and nitrogen. The observed high values of diversity may provide stability to these paddy fields in the event of environmental perturbations because multiple species may perform similar functions (Girvan et al. 2005). Thus, under circumstances functional role of one species may be compensated by increase in the population of another species.

DGGE band identification revealed the presence of Firmicutes, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria and Actinobacteria. All the six bacterial phyla have been reported from soil environments (Asakawa and Kimura 2008; Janssen 2006). The observation of highest numbers of Firmicutes may be due to their robust nature. In Indian scenario, paddy fields were left exposed to sunny summer

Table 3 Phylotype richness, diversity and evenness of the bacterial communities in the analyzed soils

Districts	Villages	Richness (<i>R</i>)*	Shannon diversity index (<i>H</i>)	Simpson diversity index (<i>D'</i>)	Evenness (<i>E</i>)
Allahabad	Phoolpur	22	2.99 ± 0.08 ^{h,i}	0.96 ± 0.01 ^{d,e}	0.97 ± 0.02 ^e
	Chainpur	18	2.77 ± 0.05 ^{f,g}	0.93 ± 0.01 ^{a,b,c,d}	0.96 ± 0.02 ^{d,e}
	Baburahipura	20	2.90 ± 0.03 ^{g,h}	0.94 ± 0.02 ^{b,c,d,e}	0.95 ± 0.01 ^e
Ghazipur	Sultanpur	19	2.83 ± 0.12 ^{f,g}	0.94 ± 0.01 ^{b,c,d,e}	0.96 ± 0.04 ^{d,e}
	Sherpur	17	2.56 ± 0.13 ^{b,c,d}	0.91 ± 0.02 ^{a,b}	0.91 ± 0.04 ^{b,c,d}
Arrah	Dhangaon	17	2.70 ± 0.04 ^{e,f}	0.94 ± 0.02 ^{b,c,d,e}	0.95 ± 0.01 ^{d,e}
	Bhojpur	31	3.05 ± 0.03 ⁱ	0.94 ± 0.02 ^{b,c,d,e}	0.89 ± 0.01 ^{a,b}
Chandauli	Devria	18	3.10 ± 0.09 ^{i,j}	0.95 ± 0.01 ^{c,d,e}	0.93 ± 0.03 ^{c,d,e}
	Kundalia	34	3.32 ± 0.05 ^k	0.96 ± 0.01 ^{d,e}	0.94 ± 0.01 ^{c,d,e}
	Lilapur	28	3.19 ± 0.02 ^{j,k}	0.96 ± 0.01 ^e	0.96 ± 0.01 ^{d,e}
Patna	Amraha	24	3.03 ± 0.03 ⁱ	0.95 ± 0.03 ^{c,d,e}	0.95 ± 0.01 ^{d,e}
	Bihta	21	2.96 ± 0.14 ^{h,i}	0.95 ± 0.01 ^{c,d,e}	0.97 ± 0.04 ^e
	Vikram	31	3.28 ± 0.04 ^k	0.96 ± 0.01 ^{c,d,e}	0.95 ± 0.01 ^{d,e}
Mirzapur	Sarsa	16	2.48 ± 0.01 ^{b,c}	0.90 ± 0.02 ^{a,b}	0.89 ± 0.00 ^{a,b,c}
	Aharora	19	2.76 ± 0.04 ^{f,g}	0.92 ± 0.01 ^{a,b,c}	0.94 ± 0.01 ^{c,d,e}
Ballia	Balegi	19	2.44 ± 0.04 ^b	0.92 ± 0.01 ^{a,b}	0.83 ± 0.01 ^a
	Fafna	17	2.63 ± 0.10 ^{d,e}	0.92 ± 0.02 ^{a,b}	0.93 ± 0.04 ^{b,c,d,e}
	Mahrev	12	2.10 ± 0.16 ^a	0.89 ± 0.02 ^a	0.85 ± 0.06 ^a
Varanasi	Jivnathpur	15	2.60 ± 0.07 ^{c,d,e}	0.92 ± 0.03 ^{a,b}	0.96 ± 0.02 ^{d,e}
	Ramnagar	19	2.44 ± 0.07 ^b	0.91 ± 0.02 ^{a,b}	0.83 ± 0.03 ^a

Results were statistically analyzed using Duncan's test ($p \leq 0.05$) and different letters (a to k) in the superscripts indicate significant differences within the same column

* Average values from three independent DGGE gels

after many times of tillage and also facing problems of multiple wetting and drying because of intermittent irrigation either through canals or by rain. The above observation also finds support from the view that Firmicutes are widely distributed in the paddy fields irrespective of the cultivated rice variety (Hardoim et al. 2011). Alpha- and Betaproteobacteria forms association with plant roots and frequently observed from soil environments (Tilak et al. 2005). Rhizobia (Alpha- and Betaproteobacteria) promote paddy growth by enhancing nitrogen uptake, phosphorus, potassium and iron as well as production of indole acetic acid (Hardoim et al. 2011). Actinobacteria are other important typical inhabitants of paddy soils known to mobilize insoluble phosphate and actively degrade organic matter (Hardoim et al. 2011; Hayakawa and Nonomura 1987; Janssen 2006). Thus, phylogenetically diverse bacterial taxa observed in the study sites may maintain sustainable functioning of the paddy soils.

Although, soil is a very heterogeneous and dynamic system; studied paddy soils harbored Firmicutes, Betaproteobacteria and Actinobacteria abundantly. Observed diversity values indicate that conventional paddy fields remain microbiologically sustainable and may bear out any ecological disturbance by adjusting the population of similar role playing pre-existing bacterial taxa. Among the various physicochemical variables TOC and available nitrogen are key regulators of bacterial community composition in conventional paddy fields.

Acknowledgements L. C. Rai thank to ICAR and DST, New Delhi for grant in the form of network project and J. C. Bose National Fellowship, respectively. Research facilities extended by CAS in Botany at Banaras Hindu University, Varanasi were highly acknowledged. Arvind Kumar is grateful to CSIR for senior research fellowship. Thanks to Mr. Gullu and Anurag Rai who helped in the sample collection. Funding was provided by National Bureau of Agriculturally Important Microorganisms (Grant Number P-27/115).

Compliance with ethical standards

Conflict of interest Authors declare that there is no conflict of interest between the persons of the same organization or any other organization/institution.

References

- Asakawa S, Kimura M (2008) Comparison of bacterial community structures at main habitats in paddy field ecosystem based on DGGE analysis. *Soil Biol Biochem* 40:1322–1329
- Boon N, Windt W, Verstraete W, Top EM (2002) Evaluation of nested PCR-DGGE (denaturing gel electrophoresis) with group specific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants. *FEMS Microbiol Ecol* 39:101–112
- Dong X, Reddy GB (2010) Soil bacterial communities in constructed wetlands treated with swine wastewater using PCR-DGGE technique. *Bioresour Technol* 101:1175–1182
- Eaton AD, Clesceri LS, Greenber AE (1995) Standard methods for examination of water and waste water. American Public Health Association, Washington D.C
- FAO (2010) Food outlook—global market analysis. Available online at <http://www.fao.org/docrep/012/ak349e/ak349e00.pdf>. Accessed 10 Feb 2014
- Ferris MJ, Muyzer G, Ward DM (1996) Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined population inhabiting a hot spring microbial mat community. *Appl Environ Microbiol* 62:340–346
- Fillip Z (2002) International approach to assessing soil quality by ecologically related biological parameters. *Agri Ecosys Environ* 88:169–174
- Fromin N, Hamelin J, Tarnawski S, Roesti D, Jourdain-Miserez K, Forestier N, Teyssier-Cuvelles S, Gillet F, Aragno M, Rossi P (2002) Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. *Environ Microbiol* 4:634–643
- Girvan MS, Campbell CD, Killham K, Prosser JI, Glover LA (2005) Bacterial diversity promotes community stability and functional resilience after perturbation. *Environ Microbiol* 7:301–313
- Hardoim PR, Andreote FD, Reinhold-Hurek B, Sessitsch A, van Overbeek LS, van Elsas JD (2011) Rice root-associated bacteria: insights into community structures across 10 cultivars. *FEMS Microbiol Ecol* 77:154–164
- Hayakawa M, Nonomura H (1987) Humic acid-vitamin agar, a new medium for the selective isolation of soil Actinomycetes. *J Ferment Technol* 65:501–509
- Huang X, Liu S, Wang H, Hu Z, Li Z, You Y (2014) Changes of soil microbial biomass carbon and community composition through mixing nitrogen-fixing species with *Eucalyptus urophylla* in subtropical China. *Soil Biol Biochem* 73:42–48
- Jackson ML (1967) Soil chemical analysis. Prentice-Hall of India Pvt. Ltd., New Delhi
- Janssen PH (2006) Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl Environ Microbiol* 72:1719–1728
- Jing L, Feng-zhi W, Yang Y (2010) Effects of cinnamic acid on bacterial community diversity in rhizosphere soil of cucumber seedlings under salt stress. *Agr Sci China* 9:266–274
- Kalembasa SJ, Jenkinson DS (1973) A comparative study of titrimetric and gravimetric methods for the determination of organic carbon in soil. *J Sci Food Agric* 24:1085–1090
- Karlen DL, Mausbach MJ, Doran JW, Cline RG, Harris RF, Scuman GE (1997) Soil quality: a concept, definition, and framework for evaluation. *Soil Sci Soc Am J* 61:4–10
- Kisand V, Wikner J (2003) Limited resolution of 16S rDNA DGGE caused by melting properties and closely related DNA sequences. *J Microbiol Methods* 54:183–191
- Liesack W, Schnell S, Revsbech NP (2000) Microbiology of flooded rice paddies. *FEMS Microbiol Rev* 24:625–645
- Liu B, Tu C, Hu S, Gumpertz M, Ristaino JB (2007) Effect of organic, sustainable and conventional management studies in grower fields on soil physical, chemical and biological factors and the incidence of Southern blight. *Appl Soil Ecol* 37:202–214
- Lopes AR, Faria C, Prieto-Fernández A, Trasar-Cepeda C, Manaia CM, Nunes OC (2011) Comparative study of microbial diversity of bulk paddy soil of two rice fields subjected to organic and conventional farming. *Soil Biol Biochem* 43:115–125
- Murase J, Shimizu M, Hayashi M, Matsuya K, Kimura M (2005) Vertical changes in bacterial communities in percolating water of a Japanese paddy field as revealed by PCR-DGGE. *Soil Sci Plant Nutr* 51:83–90
- Muyzer G, Waal ECD, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* 59:695–700

- Nikolausz M, Sipos R, Révész S, Székely A, Márialigeti K (2005) Observation of bias associated with re-amplification of DNA isolated from denaturing gradient gels. *FEMS Microbiol Lett* 244:385–390
- Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular, US Government Printing Office, Washington D.C.
- Ramette A (2007) Multivariate analysis in microbial ecology. *FEMS Microbiol Ecol* 62:142–160
- Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* 4:1340–1351
- Shen J-P, Zhang L-M, Guo J-F, Ray JL, He J-Z (2010) Impact of long term fertilization practices on the abundance and composition of soil bacterial communities in Northeast China. *Appl Soil Ecol* 46:119–124
- Shen C, Xiong J, Zhang H, Feng Y, Lin X, Li X, Liang W, Chu H (2013) Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. *Soil Biol Biochem* 57:204–211
- Sheng R, Meng D, Wu M, Di H, Qin H, Wei W (2013) Effect of agricultural land use change on community composition of bacteria and ammonia oxidizers. *J Soils Sediments* 13:1246–1256
- Srivastava AK, Ara A, Bhargava P, Mishra Y, Rai SP, Rai LC (2007) A rapid and cost-effective method of genomic DNA isolation from cyanobacterial culture, mat and soil suitable for genomic fingerprinting and community analysis. *J Appl Phycol* 19:373–382
- Tilak KVBR, Ranganayaki N, Pal KK, De R, Saxena AK, Nautiyal CS, Mittal S, Tripathi AK, Johri BN (2005) Diversity of plant growth and soil health supporting bacteria. *Curr Sci* 89:136–149
- Wallis PD, Haynes RJ, Hunter CH, Morris CD (2010) Effect of land use and management on soil bacterial biodiversity as measured by PCR-DGGE. *Appl Soil Ecol* 46:147–150
- Xu Y, Wang G, Jin J, Liu J, Zhang Q, Liu X (2009) Bacterial communities in response to soil type, soybean genotype, and their growth stage. *Soil Biol Biochem* 41:919–925
- Zaady E, Ben-David EA, Sher Y, Tzirkin R, Nejdat A (2010) Inferring biological soil crust successional stage using combined PLFA, DGGE, physical and biophysiological analyses. *Soil Biol Biochem* 42:842–849