Research article

Soil microbial diversity of peri-urban agricultural field and riverbank along Yamuna river in Delhi, India

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Abstract

Soil microbes play an important role in the earth's ecosystem; variations in any environmental factor would have a great impact on microbial function and diversity. Yamuna river is regarded as one of the most polluted rivers in India. It is a major source of water for household and agricultural practices in Delhi and its national capital territory regions. In the present work, we studied the physico-chemical properties and soil microbial diversity in riverbanks and agricultural field along the Yamuna river. Physicochemical properties indicate the soil quality of the two distinct soil sites and correlation analysis showed that these are the key factors responsible for shift in soil bacterial community. Characterization of soil microbial community was done. Sequence analysis showed that both riverbank soil and field soil harbor microbial community with variation in their relative abundance. *Proteobacteria* is the dominating bacterial community in both riverbanks (70.03%) and agricultural field soil (56.41%) while *Bacteroidetes* (38.39%) in riverbank soil and *Actinobacteria* (11.3%) in agricultural field soil. Field soil also harbors some specific group of bacteria, i.e., *Actinomycetes* and *Cyanobacteria*. Bioinformatics and statistical analysis showed that bacterial community was found to be significantly different in two soil environments and mainly driven by climatic conditions and concentration of pollutant. Our work provides insight into soil quality, microbial community, and phylogenetic turnover under different soil conditions in polluted areas.

Keywords Yamuna river · Agricultural Fields · 16S rRNA gene · T-RFLP · Microbial diversity

1 Introduction

Microbes are one of the most important constituents of a soil ecosystem. They are essential for various biogeochemical and nutrient cycles in the environment. Among the soil microbes, bacteria are the most abundant and thoroughly studied groups. They are present in the entire ecological niche from deep-ocean to high mountain ranges. However, the microbial populations found in the extremely different ecosystem vary from one another. Microbes are highly dynamic in nature and are sensitive toward changes in the surrounding ecosystem, viz. nutrients, toxicants, temperature, pH, and moisture. This property of soil

microbes makes them a suitable candidate as bioindicator of changing environmental conditions. Many studies have been done on soil bacterial community and show the dynamic nature of bacterial groups in correlation with various environmental factors [1–3].

Yamuna river is regarded as a lifeline for many big cities comes along its journey from Himalaya to Uttar Pradesh. It is a major source of water for people residing near its bank. With the help of various dams, river water is used for household purposes as well as for agriculture practices. In Delhi, 70 percent of water supplies come from Yamuna river. It enters in Delhi from Palla village at Delhi-Haryana border and exit from Basantpur at Delhi-Uttar Pradesh

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border and constitute about 2% of the total stretch of the Yamuna river. There are 22 major drains in and around Delhi, which dump their treated and untreated waste directly into the river and responsible for about 80% of the river water pollution [4, 5].

Various physicochemical parameters of river water have been studied which shows that the BOD, COD, and DO level of Yamuna river become very poor due to the contamination of heavy metals [6] and pesticides [7, 8]. Various studies showed the presence of heavy metal in the Yamuna river. Heavy metals are not readily degraded in the environment. They remain in the animal and plants body and cause various harmful effects. The concentration of heavy metals and pesticides present in the Yamuna river was found to vary seasonally [8–10]. Heavy metals affect the structural and biological functioning of biomolecules [11]. They interfere with synthesis and metabolism of the hormones [12–14]. Various water quality models had been proposed to improve the quality of Yamuna river [15, 16]. Hence, it is important to understand the ecological dynamics in the bacterial community present in riverbank soil and agricultural field soil.

Soil microbial diversity can be studied through various cultural and uncultural techniques. Since, less than 1% of the total bacterial population is cultural, so cultural techniques would not encompass the whole picture of soil microbial community [17]. Therefore, in the present study, microbial population of Yamuna river soil was identified by using Terminal Restriction Fragment Length Polymorphism (T-RFLP) of 16S rRNA gene. 16S rRNA gene is highly conserved gene and hence, can be easily amplified by a universal set of primers. Large number of database and softwares are easily available on Internet for analysis of T-RFLP of 16S gene. T-RFLP helps in reproducible analysis of a large number of samples, also of those containing high diversity. On the basis of the different peak size and peak area, we performed *in silico* approach and correlate these patterns with the environmental factors [18].

2 Materials and methods

2.1 Site description and soil sampling

In order to compare the soil microbial diversity along the Yamuna river, sampling was performed thrice in a year from riverbank and agricultural field. Soil samples were collected from ten different sites between (28°48'48.2"N to 077°12'38.4"E) and (28°31'39.5"N to 077°20'01.4"E) in three different seasons (July, November, and March) for three consecutive years (2010–11, 2011–12, 2012–13) (Table S1) (Figure S1). Meteorological data of the study area was given in Table S2. There are two major drains,

i.e., Shahdara and Najafgarh drain, dump directly in the Yamuna river at the Wazirabad bridge and Noida bridge. On the basis of drainage system dumped in the Yamuna river, these ten sampling points were divided into three streams, i.e., upstream (Palla village, Christian Ashram, Jagatpur village, Sonia Vihar), middle stream (Wazirabad bridge, Shastri Park, I.P. Power station), and lower stream (Okhla, Noida bridge and Basantpur). Sampling was done randomly from 1 to 15 cm depth using the rectangular sampler (5 × 5 × 10). Five subsamples were collected from each field and mixed well into one composite sample for each site. The collected soil samples were kept in ice cool boxes for transportation in the laboratory and then homogenized and passed through a 2 mm mesh sized sieve. Subsamples for microbial community analysis were stored at – 20 °C until further analyzed.

2.2 Soil physicochemical analysis

Physicochemical properties except soil moisture content were performed with air-dried and sieved (< 2 mm) soil samples according to their respective standard techniques. Total heavy metal content was determined using AAS (Sensa AAS Dual, GBC, Australia) by EPA's acid digestion procedure [19]. Soil texture was determined by using a sieving–separation method [20] and analyzed using soil texture triangle [21]. Soil moisture content and soil respiration were determined using R. Ohlinger method [22]. Ratio of total soil carbon and nitrogen content was analyzed using a C.H.N.S.O analyzer (Elementar Analysensysteme GmbH Vario EL V3.00) from the University Science Instrument Center, Delhi University. Soil pH was measured by using two different buffers having pH 4.0 and pH 7.0 using Corning pH meter.

3 Soil microbial community analysis

3.1 Soil DNA isolation and quantification

Soil DNA was isolated from 1 g of soil using Ultra Clean™ Soil DNA isolation kit, (Mo Bio Laboratories, CA, USA) according to the manufacturer's instructions. Isolated DNA was visualized on 0.8% agarose gel and quantified at 260/280 nm on NanoDrop (ND-1000, Spectrophotometer, Thermo Fisher Scientific, DE, USA).

3.2 Amplification and T-RFLP of 16S rRNA gene

16S rRNA gene amplification was done from 50 ng of extracted soil DNA on PCR Thermocycler (BIO RAD, USA) with a total volume of 50 µl by using 1 µM concentrations of the universal primers, i.e., FAM™ labeled 6f (5'AGAGTTTGA

TCCTGGCTCAG3') and 928R (5'CCGTC AATTCCTTTGAGTTT-3') (Sigma). Each 50 µl of PCR reaction mixture contains 200 mM of dNTP (Genei™, Bangalore), 2 Units of DNA polymerase and 1×PCR buffer (Genei™, Bangalore). The PCR conditions are as follows: 5 min at 94 °C with 30 cycles of, 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C, plus an additional 15 min cycle at 72 °C. Amplified products were visualized on 0.8% agarose gel in the gel documentation system (Gel Doc™ XR + BIO RAD, USA). Bands were excised and eluted by PCR cleanup kit (Wizard® SV Gel and PCR Cleanup System, Promega, Madison WI, USA). 100 ng of eluted DNA samples were digested with 2.5 U of three different restriction enzymes, i.e., AluI [AG[^]CT], MspI [C[^]CGG], and HaeIII [GG[^]CC] at 37 °C for 12 h. The digested products were sequenced from SCL genome laboratories, Cochin, India. T-RFLP profiles were visualized using GeneMapper® software (version 4.0. Applied Biosystems).

3.3 T-RFLP analysis of 16S rRNA gene

T-RFLP profile data of 16S gene consist of size (length) in base pair, peak height, peak area, and data point for each T-RF in each sample. For each sample, peaks over a threshold of 25U above background fluorescence were analyzed by manually aligning fragments to the size standard. To avoid detection of primers and uncertainties of size determination, terminal fragments less than 25 bp were excluded. Percentage relative abundance of each T-RF in the respective samples was calculated by the ratio of peak area of each peak to the total peak area of a sample. T-RFs with an area of less than 1% of the total area were excluded from further analysis. Each restriction enzyme data was prepared separately. Fragment Sorter 5.0 (FragSort 5.0, Ohio State University, USA) was used to potentially identify specific organisms in a community based on their T-RFs length..csv files of T-RFs data were loaded in the software. T-RFs data were entered according to T-RFs size and relative percentage abundance of peak area. This downloadable window program compares TRF'S obtained from microbial community DNA amplified with a labeled primer, and digested with multiple restriction enzymes. The output result is a list of microorganisms and T-RF sizes that correlate with multiple experimentally generated T-RFLP profiles. The organisms are initially listed in descending order from the greatest to the least normalized peak area. The output was sorted based on organism name. Fragment sizing error was adjusted to constant sizing value of one [23].

4 Statistical analysis

Level of significance in all the above studied parameters was assessed using two way analysis of variance (ANOVA) followed by the Tukey's test ($P < 0.001$, Sigma plot version 13.0). Two-way ANOVA was performed in order to observe the impact of heavy metal concentration as well as climatic conditions on the soil microbial diversity. Pearson's correlation coefficient was calculated between physico and chemical parameter of soil and bacterial community.

To compare species diversity among the samples, Shannon–Wiener diversity index was calculated as $H' = -\sum (N_i/N) \times \ln (N_i/N)$. N_i = number of individuals of species "i" or % peak area of species "i" and N = total number of individuals of all species or total sum of the peak area of individual sample. The diversity index helps to compare the relative complexity among communities and to estimate the completeness of sampling [24]. Rarefaction curve was also constructed on the basis of OTUs obtained in both the soil samples. This allows the calculation of species richness for a given number of individual samples.

Microbial community similarity analyses were conducted by UPGMA and PCA clustering using MVSP 3.1 statistical package. UPGMA clustering is one of the most important statistical methods to group samples whose T-RFLP profiles are similar to one another using the Jaccard measure of similarity [25]. While PCA basically calculates new synthetic variables (principal component), which are linear combination of the original variables [26]. These statistical analyses help to determine the relationship between microbial OTUs (operational taxonomic units) distribution and community composition as a function of the seasons, geographic origin, or habitat structure [27]. UPGMA and PCA graphs were constructed on the basis of number of OTUs obtained in the riverbank soil and agricultural field soil along the Yamuna river.

5 Results

5.1 Physicochemical properties

Physicochemical properties of riverbank soil and peri-urban agricultural field soil were performed by their respective methods. Soil texture of the riverbank soil belongs to silty loam group with average moisture content 13.69% in July to 25.54% in November. On the other hand, agriculture field soil belongs to silty clay

loam with average moisture content 22.49% in July to 29.43% in November. Average C:N ratio of river soil was significantly higher (19.05%) as compared to field soil (2.31%). pH of both types of soil, i.e., from riverbank and agricultural field remains alkaline and ranging from pH 7.4 to 7.7 throughout the study period. Whereas, in case of soil respiration agriculture field soil showed significantly ($P < 0.001$) higher value (22.11 ± 0.3) as compared to riverbank soil (18.43 ± 0.1).

Total heavy metal content of riverbank soil and peri-urban field soil was observed and tabulated in Table 1. Except Pb, Cr, Ni, Cu, and Mg concentrations of each observed heavy metal in riverbank soil and field soil become higher than their prescribed safety limit as recommended by WHO. Metal salt, i.e., Na, K, Mg, and Ca showed significant ($P < 0.001$) increase in their concentration as compared to other heavy metals.

5.2 T-RFLP analysis of 16S rRNA gene

In the present study, microbial diversity and their structural dynamics were observed by using 16S rRNA gene in two different soil types, i.e., riverbank and peri-urban agricultural field with the help of T-RFLP technique. FragSort analysis of sequencing result was done and percentage relative abundance was calculated. On the basis of FragSort analysis, twelve different bacterial phyla were observed in riverbank soil while fourteen in agricultural field soil (Fig. 1). Out of 149,551 sequences of 16 s gene

retrieved during the whole study period, 85,906 OTUs belonged to river soil and 64,455 OTUs from agricultural field soil. In river soil, out of 85,906 OTUs 8451 were belonged to *Proteobacteria*, 2024 for *Bacteroidetes*, and 120 for *Actinobacteria* while in agricultural field soil, out of 64,455, *Proteobacteria* contribute 5251 OTUs and *Bacteroidetes* and *Actinobacteria* were 1958 and 584 OTUs, respectively.

Similarly, the percentage relative abundance of *Proteobacteria* was dominated in both riverbank soil and agricultural field soil with 70.03% and 56.41%, respectively. In case of *Bacteroidetes*, riverbank soil (21.03%) showed higher abundance as compared to agricultural field soil (16.77%) while *Actinobacteria* showed higher abundance in agricultural field soil (6.37%) than the riverbank soil (0.9%). Agricultural field soil also harbors some specific group of bacteria, i.e., *Cyanobacteria* (1.28%), *Rothia* (1.08%), and *Actinomycetes* (0.09%). Other bacterial groups were also found in these soil samples, but with very low relative abundance. Majority of bacterial groups presents in riverbank soil and peri-urban agricultural field soil belongs to the phylum *Proteobacteria* followed by *Bacteroidetes* and *Actinobacteria*.

Seasonally, riverbank soil and peri-urban agriculture field soil showed a higher number of bacterial population in winter season (7022 and 4681 OTUs) followed by rainy season (2572 and 3675 OTUs) and summer season (2473 and 952 OTUs). Similarly, the relative abundance was higher during the winter season (58.19% and

Table 1 Heavy metal concentration (ppm) in riverbank soil and peri-urban agricultural field soil along the Yamuna river

	Pb	As	Cr	Co	Ni	Cu	Zn	Cd	Fe	Al	Sn	V	Mg	Li	Na	K	Ca
Riverbank soil	0.1	0.02	0.07	0.2	0.04	0.05	1.6	0.1	28.72	3.5	3.2	0.01	36.5	3.07	484.7	14.74	17.51
Agricultural field soil	0.04	0.02	0.1	0.03	0.02	0.02	1.2	0.0009	25.58	1.2	0.03	0.01	36.02	3.7	392.06	13.76	29.24
Safe limit (ppm)	0.2	0.02	0.4	0.001	0.07	0.1	0.3	0.003	0.002	0.005	NA	NA	325	1.6	NA	NA	NA

ppm- Part per million

Fig. 1 Relative abundance (%) of dominating bacterial phyla in riverbank soil and peri-urban agricultural field soil on the basis of T-RFLP profiles. Values were calculated as mean of the entire soil samples during the study period

50.29%) followed by rainy season (21.31% and 39.48%) and summer season (20.49% and 10.22%).

Diversity index (H') of the 16S rDNA gene showed significantly higher value in peri-urban agricultural field soil (4.74 ± 0.01) as compared to riverbank soil (3.67 ± 0.01). Seasonal variation in the diversity index of these two soil types was also observed. Among the different seasons, winter season (River Soil: 3.20 ± 0.02 , Field Soil: 4.25 ± 0.02) showed the higher values of diversity index followed by rainy season (River Soil: 3.48 ± 0.01 , Field Soil: 4.49 ± 0.01) and winter season (River Soil: 4.34 ± 0.01 , Field Soil: 5.4 ± 0.01). Rarefaction curve constructed on the basis of number of OTUs obtained in both the soil samples also showed variation in the species richness of two different soil environment. As the variation increases in the number of species richness, the distance between two plateaus become increases (Fig. 2). Agriculture field

soil was found to have higher species richness as compared to river soil.

To study the correlation between physicochemical parameters and bacterial diversity of agriculture field soil and river soil, Pearson correlation coefficient was performed using statistical software MVSP v3.1 (Table S3). To understand the temporal variation among the different bacterial groups found in riverbank soil and peri-urban agricultural field soil, hierarchically clustered UPGMA (Unweighted Pair Group Method with Arithmetic Mean) was performed by using the Jaccard similarity index. The output result is in the form of dendrogram which indicates the percentage of similarity among the different microbial groups present in agriculture field soil and riverbank soil. Bacterial groups showed similar behavior toward the environmental conditions are grouped together while those bacterial groups showed dissimilar

Fig. 2 Rarefaction curve of OTUs present in peri-urban agriculture field soil and riverbank soil. Values were calculated as a means of the entire soil samples during the study period

Fig. 3 UPGMA dendrogram showing coefficient of similarity between different bacterial phyla obtained in riverbank soil and peri-urban agricultural field soil. Similarity coefficient was calculated by using the Jaccard's index on the basis of their percentage relative abundance

behavior were grouped apart [28]. All the bacterial phyla present in both the soil types are grouped in a single cluster and showed linear relationship with similar value of Jaccard similarity coefficient except *Actinomycetes* and *Cyanobacteria* (Fig. 3). These two microbial groups present only in agriculture field soil and showed 0.5% of the similarity index with other bacterial groups.

Principal component analysis was performed to display the unevenness among the relative abundance of microbial groups present at riverbank soil and agricultural field soil. In Fig. 4, PCA graph showed that *Proteobacteria* is the most abundant bacterial group present in both the soil types and accounts 99.79% of the total discrepancy with higher value of PCA score (3.32) along the axis PC1. This suggests entirely different behavior of *Proteobacteria* toward two different types of soil environment as comparison to other bacterial groups. *Bacteroidetes* and *Actinobacteria* account only 0.02% of the total variation with 1.1 and 2.3 PCA score, respectively, along axis PC2. Overall, both the principal component,

i.e., PC1 and PC2 were involved in 99.81% of the total discrepancy of the different microbial community.

The principal component analysis was also implemented on two different soil environment with respect to the microbial diversity and their physicochemical properties (Fig. 5). In this graph, both riverbank soil and agricultural field soil are at the two opposite sides of the central axis. Riverbank on the right side of the center axis with PCA score 18.379 and agriculture field soil on the left side of the central axis with PCA score – 18.739.

6 Discussion

6.1 Microbial community and covaried environmental factors

T-RFLP analysis of 16S rRNA gene was used to analyze the difference between soil microbial diversity present in riverbank and agricultural field along the Yamuna river. Riverbank soil and agricultural field soil showed significant

Fig. 4 Principal component analysis graph showing percentage of variance among different bacterial phyla present in riverbank soil and peri-urban agricultural field soil on the basis of their percentage relative abundance

Fig. 5 Principal component analysis graph showing percentage of variance between riverbank soil and peri-urban agricultural field soil on the basis of percentage relative abundance of bacterial phyla present

variation in the abundance of their soil microbial community. *Proteobacteria* was the most prominent group found in both types of soil followed by *Bacteroidetes* and *Actinobacteria*, this might be because of their ecological importance in degrading various organic and inorganic waste present in riverbank soil and agriculture field soil [20–31]. Higher relative abundance of these three groups explains their resistance toward an increase in heavy metal concentration. These bacterial groups are widely distributed in the environment including soil, sediments, seawater, and also well-known decomposer of various organic and inorganic materials present in soil [32].

In case of *Proteobacteria*, metal resistant strains, i.e., *Ralstonia* Sp., *Salmonella* Sp., *Vibrio* Sp., *Helicobacter* Sp., *Escherichia* Sp., and *Yersinia* Sp. were observed. The presence of these groups also indicates the contamination level of riverbank soil as well as of agricultural field soil [30, 33, 34]. *Desulfovibrio* Sp. the member of *Delta-proteobacteria*, a sulfate reducing bacteria was also found in agricultural field soil. Sood et al. [1] studied the bacterial diversity of the Gangetic river system in Uttarakhand and found a similar kind of bacterial groups as the present study with *Proteobacteria*, the most abundant group.

Relative abundance of *Bacteroidetes* showed a higher percentage in riverbank soil as compared to *Actinobacteria* in agricultural field soil. In case of *Bacteroidetes*, abundance of *Flavobacterium* Sp., *Polaribacter* Sp., *Cellulophaga* Sp., *Gatebulibacter* Sp., *sphingobacterium* Sp. and *Winogradskyella* Sp. were observed in riverbank soil. These are metal resistant group of bacteria and also might be responsible to increase the richness of *Bacteroidetes* in riverbank soil [35–37]. Similar results had been shown by Guillermina Hernandez-Raquet et al. [38] while studying the molecular diversity of bacterial communities in oil polluted site.

Frankia belongs to the phylum *Actinobacteria*, is a nitrogen fixing bacteria, and found to be present only in field soil. Presence of *Frankia* might be responsible to increase the relative abundance of *Actinobacteria* in field soil as compared to the riverbank soil. Agriculture field soil also harbors some specific groups of bacteria, i.e., *Actinomycetes* and *Cyanobacteria* might be due to significantly different soil properties. Both bacterial groups are the efficient members of the nitrogen fixing group of bacteria and also reported their role in bioremediation and biotransformation of heavy metals [39, 40].

In our study, it is observed that bacterial community covaried with physicochemical properties of soil. Pearson correlation coefficient was calculated using statistical software (MVSP 3.1) between pH, C/N ration, soil moisture content, soil respiration, and bacterial community. Of all the environmental variables, soil respiration ($r = 0.891$, $P = 0.05$) showed highest correlation with the bacterial community while Soil moisture content ($r = 0.326$, $P = 0.05$)

showed significantly less correlation. The increase in soil respiration agriculture field soil gave higher number of OTUs as compared to river soil in case of soil moisture content, increase in water content decrease the soil respiration which might be responsible for less number of OUT's in river soil as compared to field soil. Difference in moisture conditions has been responsible for causing variations in microbial community structure [41–44]. C/N ratio ($r = 0.139$, $P = 0.05$) was also showed significant correlation with bacterial community [45, 46]. C/N ratio is regarded as the indicator of soil nutrient and increase in carbon content increases the C/N ratio. Increase in C/N ratio in river soil is significantly correlated with the presence of significantly higher number of pathogenic and methanogen bacterial species belongs to the *Proteobacteria* group in river soil [47]. Soil pH was also showed correlation ($r = 0.024$) with bacterial community at river soil and agricultural field soil but not statistically significant.

Shannon–Wiener diversity index and rarefaction curve constructed on the basis of number of OTUs also showed higher species richness in agriculture field soil. Index value higher in agriculture field soil confirms the higher species richness. Similarly, in rarefaction curve, we also observed that as the variation increases in the number of species richness, the distance between two plateaus becomes increases. Agriculture field soil was found to have higher species richness as compared to river soil.

PCA and Jaccard similarity index also showed that riverbank soil and peri-urban agricultural field soil are different in the composition and abundance of their microbial community. As we observed that PCA done on the basis of study site showed that both the soil environment are differ in their property and nutrient composition thus harbors diverse microbial community. Might be due to the presence of household waste and heavy metal in Yamuna river, only the existence of those bacterial community which are able to degrade these waste get flourish. In the current study, diversity index of agriculture field soil was significantly higher than riverbank soil as the phylogenetic analysis showed the presence of *Actinomycetes* and *Cyanobacteria* in agricultural field soil only. These results confirmed that microbial community of peri-urban agricultural field soil is more diverse than riverbank soil.

It is very difficult to vindicate a broad statement about the difference in the composition of microbial communities and its abundance present in riverbank soil and peri-urban agricultural field soil. Difference in microbial diversity may occur due to various factors. Key factors like physicochemical properties of soil and the presence of heavy metals may shape the soil microbial community [48–50]. Variation in soil properties and the presence of heavy metals could select some specific bacterial groups more over another because of their ability to survive using

organic and inorganic pollutant as a source of energy. Different bacterial groups are specific in their activity and their habitat. They are very sensitive toward the changes occurring in the environment, and therefore, any kind of environmental stress results in a variation in the microbial community structure in the environment [51]. Our results are capable to explain the behavior of microbial community under different environmental stress conditions.

7 Conclusion

This study provides a detail information about the microbial community pattern in different soil habitat. Riverbank soil and agricultural field soil differ in their physicochemical properties and heavy metal concentration. These two study sites are significantly dominated by three groups of bacteria *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* and also harbor some specific bacterial phyla like *Actinomycetes* and *Cyanobacteria* present in agricultural field soil only. Phylogenetic analysis and statistical analysis suggest that the bacterial community present in riverbank soil and agricultural field soil are similar in their richness except *Actinomycetes* and *Cyanobacteria* but differ significantly in their abundance, indicating the strong environmental stress on microbial community of two different habitats.

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Compliance with ethical standards

Conflict of interest We have not any financial/commercial conflicts of interest.

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