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Assessment of community level physiological profiles and molecular diversity of soil bacteria under different cropping systems

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Abstract: Community level physiological profiles (CLPP) and molecular diversity of bacteria in soil under rice–rice (RR), rice–fallow (RF), rice–wheat (RW), legume (LG), mango orchard (MO), and grass land (GL) cropping systems occurring in South West Bengal, India (22°22′N latitude and 86°26′E longitude) were studied. The soils were mostly acidic (pH 5.4 to 6.85). The GL soil recorded the highest organic carbon (20.23 g kg^{-1}) and total nitrogen (1.96 g kg^{-1}). The RF soil was the most acidic and had the poorest nutrient contents. The CLPP, as studied by carbon source utilization in BIOLOG Ecoplates, revealed that the bacteria in soils under different cropping systems could differentially utilize all the groups of carbon sources viz. carbohydrate, amino acid, carboxylic acid, polymer, amine/amide, and phenolic compound. Carbohydrate was most utilized and amine/amide and phenolic compound were least utilized. Bacterial communities in RF soil utilized the highest amount of carbohydrate and carboxylic acid and also utilized a balanced amount of other individual carbon substrates. Molecular diversity was studied by polymerase chain reaction followed by amplified ribosomal DNA restriction analysis (ARDRA) of soil DNA. Variations in ARDRA banding pattern followed by cluster analysis and the resulting dendrogram indicated that the cropping systems induced changes in soil bacterial communities. The grouping of uncultivated soils (MO and GL) in a separate cluster clearly indicated the presence of different bacterial communities.

Key words: Amplified ribosomal DNA restriction analysis, BIOLOG, cluster analysis, community level physiological profile, cropping system, microbial communities

1. Introduction

Sustainable agriculture and soil quality are intimately connected. Soil quality does not depend just on physical, physicochemical, and chemical properties of soil, but is related to the soil microbial component (Elliott et al. 1996). Soil microbial activity and diversity have become fundamental aspects of sustainable agriculture (Anderson 2003; Schloter et al. 2003; Li et al. 2005).

Rhizosphere has been characterized as the zone of intense microbial activities with greater preponderance of microorganisms. This depends on plant species and soil type (Tate 2000). Changes in the activity and diversity of soil microbes may reflect changes in soil quality. The pattern of carbon sources utilization by the soil microorganisms and the change in the microbial community structure under the influence of plant is useful for better understanding of the soil functions and in the development of sustainable agroecosystems.

Larkin (2003) studied the substrate use profile of microbial communities under potato-based cropping systems using the BIOLOG GN2 plate. Differences in the utilization of individual carbon sources and substrate guilds, including carbohydrates, carboxylic acids, amines/amides, and amino acids under different crop rotations were evident. Yao et al. (2006) reported that continuous cucumber cropping and alternative rotations under protected cultivation considerably affected CLPP and DNA sequence diversity of the soil microbial community. Microbial community profiles of continuous cultivation soils were different from profiles of rotation soils. Rai et al. (2010) studied the dynamics of soil microbial community structure by ARDRA and activity during the cropping period of cotton. These authors did not find any correlation between the microbial activity and community structure. Chakraborty et al. (2011) studied the effect of the jute-rice-wheat cropping system under South Bengal condition on microbial substrate utilization pattern by BIOLOG Ecoplate system. The addition of organic supplements significantly increased microbial activity, but input of nutrient supplements only marginally affected the overall substrate utilization pattern of soil microorganisms.

Rice is the principal crop in tropical Asian countries including India. A large number of crops and cropping systems are grown and adopted by farmers in India. These

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have evolved from experiences of the farmers considering soil, water, and climate in their respective areas. Similarly, the farmers in south West Bengal have their own crops and cropping systems. These include rice, wheat, jute, legume, and fruit orchards. Tripathi et al. (2012) studied microbial and biochemical parameters of soil under undisturbed grass land, rice–fallow, rice–rice, rice–wheat, rice–legume, and mango orchards in south West Bengal, India. The authors observed that different land use caused distortion of lands to various degrees. A literature survey revealed that there is little information on the metabolic profile and molecular diversity of bacteria in tropical soils under the influence of different cropping in general, and in south West Bengal soils in particular.

The aim of the present work was to observe the impact of land use under different cropping systems on community level physiological profile (CLPP) and molecular diversity of soil bacterial communities in south West Bengal conditions, and also whether a change in molecular diversity was associated with a change in CLPP.

2. Materials and methods

2.1. Soil, its processing, and chemical parameters

Five replicated soil samples (0–15 cm) were collected from different cropping systems at the Agricultural Experimental Farm of Calcutta University (22°22′N latitude and 86°26′E longitude), Baruipur, 24-Parganas, West Bengal, India after crop harvest (except mango orchard and grass land) in the first week of January 2012. The cropping systems were: rice–rice (RR) with recommended dose of fertilizer for the last 15 years, monoculture of rice (RF) in monsoon season without any fertilizer for a long time, rice–wheat (RW) with recommended dose of fertilizers to both the crops, legume (LG) cultivated with recommended dose of fertilizers, 32-year-old mango orchard (MO) with application of fertilizer, and organic manures and no tilled grass land (GL). All the field moist soil samples, after removing plant debris and visible faunas, were divided into 2 parts. One part was stored at 4 °C for CLPP and molecular analysis, and the other part was air dried for determination of soil pH (1:2.5 soil-water suspension), soil organic carbon (Nelson and Sommers 1982), total nitrogen, and available phosphorous (Black 1965) and available potassium (Jackson 1967).

2.2. CLPP analysis and data processing

The CLPP analysis was carried out using BIOLOG Ecoplate system (Biolog Inc., Hayward, CA, USA) following the procedure of Chakraborty et al. (2011).

2.3. Extraction and purification of genomic DNA from soil samples

Genomic DNA from soil samples was isolated using the method of Tsai and Olson (1992), with some modifications as stated by Han et al. (2010). The only difference was that after phenol:chloroform treatment, the aqueous phase was treated with half-volume of polyethylene glycol (30% w/v)/NaCl (1.6 M) and incubated at room temperature for 2 h. The sample was centrifuged (10,000 × *g* for 20 min) and the partially purified nucleic acid pellet was resuspended in 0.5 mL of TE (10 mM Tris-HCl, 1 mM sodium EDTA, pH 8.0).

To prevent complication in PCR amplification by humic acid and other contaminations, DNA samples were purified by a Promega Gel Purification Kit using the manufacturer's protocol.

2.4. PCR amplification of 16S rDNA for ARDRA analysis

The PCR amplifications of 16S rDNA were performed (Das et al. 2011) in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) using the forward primer 515F (5′-GTGCCAGCAGCCGCGGTAA-3′) and the reverse primer 1492R (5′-TACGGYTACCTTGTTACGACTT-3′).

2.5. ARDRA analysis of the 16S rDNA samples

ARDRA was carried out by the following method (Poly et al. 2001): PCR product of 10 µL was used directly for restriction enzyme cleavage. The reaction enzyme mixture contained 1X restriction enzyme buffer and 1.25 U of restriction endonuclease. *Hae*III (Bangalore GENEI) was selected for its specificity for the amplified region of 16S rDNA, and was used as specified by the manufacturer. The PCR products were digested overnight at 37 °C. Digested DNA samples were analyzed by electrophoresis in a 2% agarose gel. The electrophoresis conditions were for 2 h at 75 V in 0.5X Tris-Borate-EDTA buffer, followed by 30 min of staining in ethidium bromide. The stained gel was photographed on a UV transilluminator.

2.6. Dendrogram analysis

The sequence divergence between the samples was estimated from the proportion of common PCR fragments. Presence–absence matrices were used to construct a dendrogram with cluster analysis based on Euclidean distances and Ward clustering (Blackwood et al. 2003).

2.7. Statistical analysis

Assigning soil as a treatment factor, analysis of variance (ANOVA) was carried out by a completely randomized design (CRD) using a STATISTICA 6.0 (StatSoft Inc., USA) package. The factor soil had 6 levels and the replicate had 5 levels. The least significance difference (LSD) test was applied to evaluate the significance of differences between the individual treatment factors. The treatment means were compared using Duncan's multiple range tests at $P < 0.05$.

3. Results

3.1. Physico-chemical properties of soil (Table)

The soils were mostly acidic (pH 5.4 to 6.85) and varied significantly. The GL soil (20.23 g kg^{-1}) recorded the

Table. Physicochemical properties of soil.

Soil code	pH	Organic carbon (g kg^{-1})	Total nitrogen (g kg^{-1})	Available phosphorous (kg ha^{-1})	Available potassium (kg ha^{-1})
RR*	6.10c**	16.64c	1.54b	88a	283a
RF	5.40d	9.92e	0.90d	27d	170e
RW	6.45b	11.29d	1.39c	55b	291a
LG	6.85a	9.85e	0.96d	58b	212b
MO	5.50d	18.51b	1.48b,c	84a	187d
GL	6.35b	20.23a	1.96a	45c	199c

* RR: Rice–Rice, RF: Rice–Fallow, RW: Rice–Wheat, LG: Legume, MO: Mango orchard, GL: Grass land.
** Values with the same letters are statistically similar at $P < 0.05$ limit.

highest organic carbon, while the LG soil (9.85 g kg^{-1}) recorded the lowest. The GL soil recorded the highest total nitrogen (1.96 g kg^{-1}) and the lowest was found in RF soil (0.9 g kg^{-1}), which was statistically similar to LG soil (0.96 g kg^{-1}). Other soils could be ranked as RR > MO > RW > LG. The available phosphorous level (kg ha^{-1}) was the highest in RR soil (88) and the lowest was in RF soil (27). The RR (88) and RW (55) soil recorded statistically similar values of available phosphorous to MO (84) and LG (58) soils respectively. The range of available potassium (kg ha^{-1}) of the studied soils varied between 170 (RF) and 291 (RW).

3.2. Community level physiological profile of bacterial community by BIOLOG technique

The CLPP of the bacterial communities was expressed as net areas for the single carbon source tested. The Ecoplate color development was gradually picked up, recording maximum absorbance values at 72 h. The bacterial communities under all the land use could utilize all the 31 carbon substrates in the form of carbohydrate, amino acid, carboxylic acid, polymer, amine/amide, and phenolic compound. Among these carbohydrate was best utilized, and amine/amide and phenolic compound were least utilized (Figure 1). Bacterial communities in RF soil utilized the highest amount of carbohydrate and carboxylic acid and they also utilized a balanced amount of different individual carbon substrates (Figure 2). Among the polymers, α-cyclodextrin and Tween 80 were best utilized by bacteria in MO soil (Figure 2). The utilization patterns of amines/amides and phenolic compounds did not vary much between the studied soils (Figure 2).

3.3. ARDRA and cluster analysis

The ARDRA patterns of the soil samples are shown in Figure 3. The ARDRA profiles of each soil were different, although some of the bands were common between the samples. The number of bands varied from 8 to 12 in the different soil samples. Although the ARDRA patterns of both RR and RF soils produced 10 bands and both RW

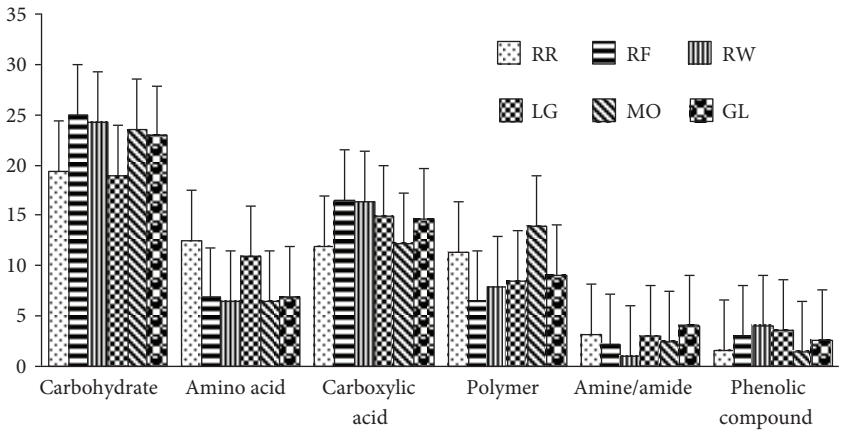

Figure 1. Net area (along y axis) under the major groups of substrate utilization curve of soils under different cropping systems. The error bars indicate the standard error of mean.

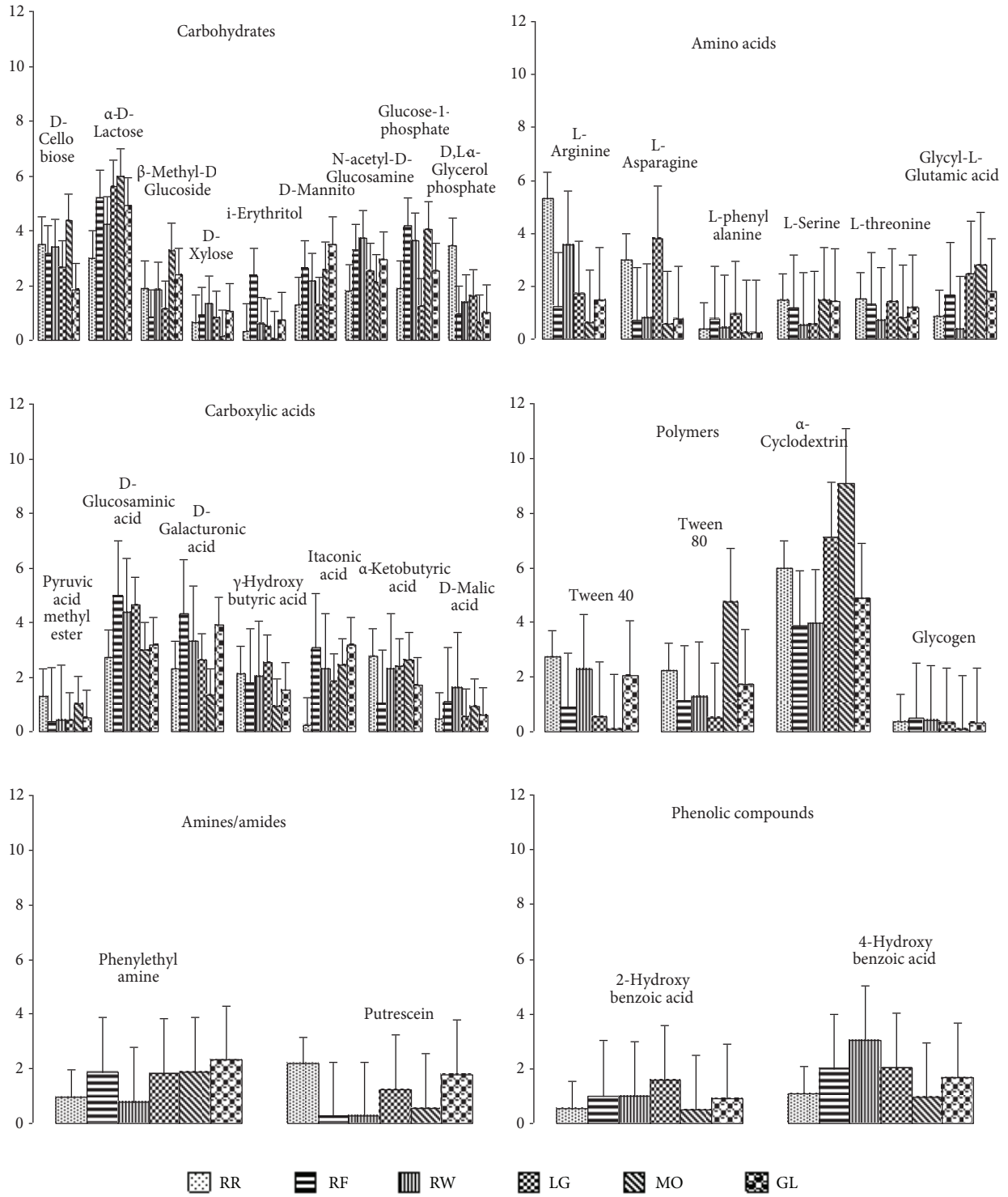

Figure 2. Net area (along y axis) under the individual substrate utilization curve of soils under different cropping systems. The error bars indicate the standard error of mean.

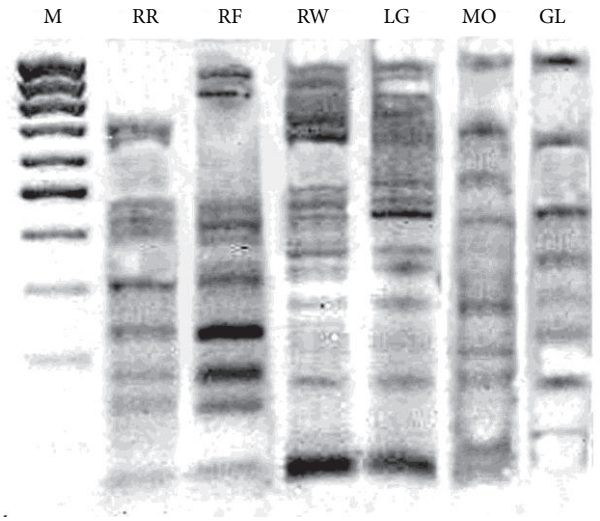

Figure 3. ARDRA pattern of *Hae III* digested 16S rDNA samples; M: Marker, RR: Rice–Rice, RF: Rice–Fallow, RW: Rice–Wheat, LG: Legume, MO: Mango orchard, GL: Grass land.

and LG soils produced 12 bands, the positions of all the bands and their intensities were not similar in both cases. The dendrogram constructed with the presence–absence matrix of the ARDRA profiles of studied soil samples (Figure 4) may be grouped into 3 clusters, viz. cluster I (RR and RF), cluster II (RW and LG), and cluster III (MO and GL). The weights of the 3 clusters are the same, with 2 components each. Cluster I has the highest compactness, followed by cluster II and cluster III. Cluster I is also fairly distinct from cluster II and cluster III.

4. Discussion

The highest organic carbon status of GL soil could be explained as the whole land was a rhizosphere due to the network of grass roots and their rhizodeposition. The higher organic carbon status of MO soil seems to be related to the litter deposition from the tree, as well as the undisturbed perennial root systems producing enormous rhizodeposition. Agricultural soils viz. RR, RF, RW, and LG are regularly tilled and receive little organic deposition compared to the previous 2 soils. As a result, soil organic carbon status of such soils is comparatively poor.

Among the soils studied, RF soil was most acidic and poorest with respect to nutrient content. The poorest nutrient condition of RF soil resulted from long-term cultivation without any input.

BIOLOG technique is basically culture dependant. Thus, only a fraction of total microbial community is detected (Kirk et al. 2004). Slow growth of microorganisms at the initial phase represents their adaptation to an artificial nutritional environment. Once adapted, the organisms could utilize the substrates in differential ways.

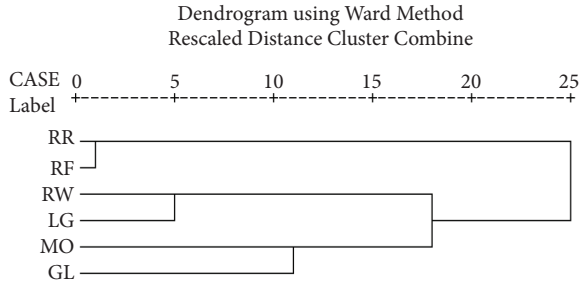

Figure 4. Dendrogram analysis of different soil microbial communities (RR: Rice–Rice, RF: Rice–Fallow, RW: Rice–Wheat, LG: Legume, MO: Mango orchard, GL: Grass land).

Irrespective of cropping systems, the highest utilization of carbohydrates could be due to these substances being a well-known energy source, as well as a carbon source for cell protoplasm (Alexander, 1972). Higher utilization of carbohydrate and carboxylic acid in RF soil compared to RR soil (Figure 1) could be explained by the bacterial communities of RF soil being stressed due to soil acidity and poor nutrient status. This possibly provoked them to utilize higher amount of carbohydrates as carbon and energy sources. Again, the carboxylic acids used in BIOLOG plates (Figure 2) are mainly the products of carbohydrate metabolism or modified forms of monosaccharides. Hence, the bacteria in RF soil jump upon those readily utilizable substrates. The bacteria in RR soil are in a more nutrient-rich environment as the soil regularly received fertilizers. The amino acids are not the primary carbon sources and the bacterial communities in RR soil are better adapted to amino acids than those occurring in RF soil (Figure 2). The better utilization of α-cyclodextrin and Tween 80 in MO soil suggests that the bacterial community of such soil possibly receives polymeric substances from rhizodeposition, as well as the decomposition products of mango leaves on soil surfaces. The bacterial community of such soil is well adapted to these sorts of compounds.

The ARDRA analysis can be used for a quick assessment of genotypic changes in the community over time, or to compare communities subject to different environmental conditions (Gich et al. 2000). The variations in the banding pattern between the soil samples revealed the presence of different bacterial communities. Cluster analysis has been used as a search for a natural grouping of microbial communities depending on the similarity in banding patterns (Ampe and Miambi 2000; Gafan et al. 2005; Qi et al. 2009). The cluster analysis and the resulting dendrogram clearly revealed that the cultivation practices caused changes in soil bacterial communities, and the grouping of uncultivated soils (MO and GL) in a separate cluster clearly indicated the presence of different bacterial communities.

Specific crops grown can greatly affect soil microorganisms (Curl and Truelove 1986). There are relatively few studies that document the effects of specific crop rotations on changes in microbial communities in bulk soil, which may better reflect potential effects on subsequent crops (Larkin 2003). Larkin and Honeycutt (2006) proposed that cropping systems resulted in distinct differences in soil microbial communities directly associated with specific rotation of crops and cropping sequences. Both the specific rotation of crops and the cropping sequence were important in shaping the soil microbial characteristics. Crops have a strong influence on soil microorganisms, activity, and processes.

Decomposition of organic matter is one of the most important functions of soil microorganisms. Decomposed products are utilized for their cell synthesis as well as for an energy source. Utilization of different carbon substrates by soil microorganisms is a manifestation of their activities. Carbohydrate, amino acid, carboxylic acid, polymer, amine/amide, and phenolic compound were utilized similarly but there were differences in the utilization of individual carbon substrates in certain instances. However, molecular study clearly demonstrated variation in bacterial diversity under different cropping systems, which was not reflected in the metabolic profiles of bacteria of soils. Rai et al. (2010) observed that there was no correlation between microbial diversity and microbial activity. Nannipieri et al. (2003) stated that no relation exists between microbial diversity and decomposition of organic matter, and a reduction in any group of species has little effect on overall soil process because the surviving microorganisms can carry out the decomposition of organic matter. Similarly the alteration in microbial diversity due to crop effect did not impact on overall CLPP of bacterial community. Another explanation could be that in BIOLOG technique only viable microorganisms are considered, whereas molecular diversity encompasses total microorganisms in soil. Therefore, logically these 2 may not correspond to each other.

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