



Functional diversity performs a key role in the isolation of nitrogen-fixing and phosphate-solubilizing bacteria from soil

Poulomi Chakraborty¹ · Prosun Tribedi¹

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Abstract

Functional diversity covers diverse functional traits of microorganisms in an ecosystem. Thus, we hypothesized that it could play an important role in the isolation of nitrogen-fixing and phosphate-solubilizing bacteria. These bacteria have been considered as biofertilizer for sustainable agriculture development. Soils were collected from different sites of agricultural field and performed several microbiological tests in which we observed considerable differences in heterotrophic microbial abundance and microbial activities among the microcosms. Functional diversity depends on both microbial richness and evenness. Based on the results of metabolic fingerprinting of the carbon sources of BiOLOG-ECO plates, richness and evenness was measured by determining Shannon diversity index and Gini coefficient, respectively. The results showed significant differences in both microbial richness and evenness, suggesting considerable variation of functional diversity among the microcosms. Thereafter, nitrogen-fixing and phosphate-solubilizing bacteria were isolated on Burk's and Pikovskaya media, respectively. The results revealed considerable variation of both types of bacterial abundance among the microcosms. Microcosm (T2) showing the highest functional diversity houses the maximum numbers of nitrogen-fixing and phosphate-solubilizing bacteria. Similarly, the microcosm (T5) exhibiting the lowest functional diversity houses the minimum numbers of nitrogen-fixing and phosphate-solubilizing bacteria. Thus, a strong positive correlation was observed between functional diversity and both types of bacterial abundance among the soil samples. Higher richness and evenness lead to the development of increased functional diversity that facilitates to accommodate substantial numbers of nitrogen-fixing and phosphate-solubilizing bacteria in soil. Taken together, the results demonstrated that functional diversity plays an important role in the isolation of nitrogen-fixing and phosphate-solubilizing bacteria from soil.

Introduction

Ecosystem is an interconnected system that gets stabilized by the balanced interactions between organisms and their environment. A little bit change in either of the two components brings a considerable difference in the dynamics of it. Determination in the efficiency of an ecological unit was addressed by species richness which is also considered as the principle index of biodiversity (Tribedi and Sil 2013). Higher biodiversity tends to develop an efficient and healthy ecology (Goswami et al. 2017). Functional diversity is an alternative classification to measure the ecological

grandness of species in a community (Laureto et al. 2015; Wolińska et al. 2017). It helps us to understand how biodiversity affects diverse functions of ecosystems. However, it generally concerns the range of activities that organisms do in communities and ecosystems. Functional diversity of a community can be estimated by functional richness and evenness. The number of species occupy a particular niche is termed as functional richness whereas functional evenness depicts how the species are being distributed evenly in the ecosystem (Mason et al. 2005; Clark et al. 2012; Goswami et al. 2017; Wolińska et al. 2017). Functional diversity provides enough information about the compositional distribution of the certain community rather describes simply species richness (i.e., the number of species present). Existing literature documented that it plays an important role in ecosystem functioning and overall productivity (Mason et al. 2005; Clark et al. 2012). Any imbalance of it has a negative impact on the ecosystem. Therefore, it appears that an ecological unit with higher and lower functional diversity shows an enriched and compromised ecosystem, respectively.

✉ Prosun Tribedi
tribedi.prosun@gmail.com
Poulomi Chakraborty
polochak12@gmail.com

¹ Department of Biotechnology, The Neotia University, Sarisha, West Bengal 743368, India

Biofertilizer comprises living microorganisms that enhance plant growth by increasing the supply of nutrients to the host plant (Bardi and Malusà 2012; Malusa and Vassilev 2014). They are often used to speed up the microbial processes that enhance the availability of nutrients to the plants (Mahanty et al. 2017). They increase the fertility of soil by executing several processes including fixation of atmospheric nitrogen and solubilization of insoluble phosphates (Mazid and Khan 2015). Biofertilizer application to soil happens to be a better option for the sustained agricultural practices as they are environment friendly, cost-effective, natural, and less toxic than the application of chemical fertilizers to soil (Mahanty et al. 2017). Thus, considerable efforts were given worldwide towards isolation of desired microorganisms having efficient biofertilizer-like potentialities. Soil happens to be a rich source of diverse types of microorganisms. In order to isolate strains of nitrogen-fixing and phosphate-solubilizing bacteria, selection of soil happens to be a major challenge as the soil microbial community is heterogeneous and discontinuous (Stotzky 2000; Nannipieri et al. 2017; Wolińska et al. 2017a). In this regard, in the current manuscript, efforts were given to establish a correlation if any between functional diversity of soil and abundance of nitrogen-fixing and phosphate-solubilizing bacteria in soil.

Materials and methods

Soil sampling and soil microcosm preparation

Two kilograms of soil samples (around 10 cm depth) were separately collected from five different sites of agricultural field of Canning, South 24 Parganas, West Bengal, India (20° 15' N, 80° 40' E) for the present work. The soil samples were immediately kept in a sealed and headspace minimized sterile plastic bags. Soil containers were cooled to 4 °C upon collection and kept under cold condition until analysis was initiated. No chemical preservatives were added during the preservation purpose. Containers carrying soil samples were protected and sealed in such a way that they did not deteriorate and did not lose any part of their contents during transport. During transportation, to maintain the temperature, the soil containers were kept in cool boxes and ice packs. The containers were kept away from light as well.

In laboratory, soil samples were separately searched to remove hazardous particles if any. Then, soils were then separately homogenized and filtered through 2-mm pore size sieve. After that, they were air-dried adequately at 37 °C. Physicochemical properties of soil samples collected from each microcosm were determined and presented in Table 1. For the current study, five soil microcosms (T1, T2, T3, T4, and T5) were prepared. For the preparation of each soil microcosm, 450 g dried soil samples were kept in different sterile

glass beaker. Furthermore, each microcosm was covered by perforated aluminum foil. All microcosms were then incubated at 30 °C for 10 days. Microcosms were kept moistened by adding sterile Milli-Q water.

Isolation of nitrogen-fixing and phosphate-solubilizing bacteria from soil

To obtain desired microorganisms from soil, 1 g soil was collected from each microcosm and suspended in 9 mL of sterile 0.85% NaCl. Thereafter, a series of dilution (10^{-1} to 10^{-6}) were accordingly prepared in sterile 0.85% saline solution. A 0.1 mL aliquot from 10^{-4} dilution was aseptically spread onto Burk's Agar media and Pikovskaya Agar medium to obtain nitrogen-fixing and phosphate-solubilizing bacteria, respectively.

Burk's media happens to be a selective media for the growth and isolation of nitrogen-fixing microorganisms (Park et al. 2005; Kayasth et al. 2014). Burk's media does not contain any nitrogen source. The only criterion for the growth of the organisms on Burk's media is to fix aerial nitrogen in the media to meet the nitrogen demands for the microorganisms. The composition of Burk's agar media (g/L) is as follows: $MgSO_4$ (0.2 g), K_2HPO_4 (0.8 g), KH_2PO_4 (0.2 g), $CaSO_4$ (0.13 g), $FeCl_3$ (0.00145 g), $NaMoO_3$ (0.000253 g), and sucrose (20 g). Agar (18 g/L) was added for making solid medium. The pH was adjusted to 7.0 before autoclaving. The plates were incubated at 30 °C for 7 days. The organisms that developed colony on the Burk's agar media after the required period of incubation indicated that they belong to nitrogen fixers.

Pikovskaya Agar media happens to be a popular media for the growth and isolation of phosphate-solubilizing microorganisms (Mehta and Nautiyal 2001; Panhwar et al. 2012). Pikovskaya Agar media contains calcium phosphate which is inaccessible to the microorganisms. Certain types of microorganisms can utilize calcium phosphate only when they release phosphorous from calcium phosphate. The composition of Pikovskaya Agar medium (g/L) is as follows: yeast extract (0.5 g), dextrose (10 g), $Ca_3(PO_4)_2$ (5 g), $(NH_4)_2SO_4$ (0.5 g), KCl (0.2 g), $Mg_3(PO_4)_2$ (0.1 g), $MgSO_4$ (0.0001 g), and $FeSO_4$ (0.0001 g). Agar (18 g/L) was added for making solid medium. The pH was adjusted to 7.0 before autoclaving. After the incubation at 30 °C for 10 days, the colonies that developed over the Pikovskaya growth media would be considered as phosphate-solubilizing organisms.

Determination of heterotrophic microorganisms of soil

One gram (1 g) of soil sample was collected from each soil microcosm and subsequently mixed with 9 mL of 0.85% NaCl solution. For isolating heterotrophic microorganisms, a

Table 1 Physicochemical properties of soil

Microcosm type	Organic carbon (%)	Total nitrogen (%)	Total phosphorous (%)	Electrical conductivity (EC) value (dS/m)	pH
T1	3.39 ± 0.06	0.25 ± 0.01	1.4 ± 0.04	8.15 ± 0.05	7.1 ± 0.11
T2	3.45 ± 0.05	0.29 ± 0.02	1.47 ± 0.03	8.19 ± 0.02	6.73 ± 0.09
T3	3.67 ± 0.03	0.32 ± 0.07	1.41 ± 0.06	8.53 ± 0.03	6.07 ± 0.05
T4	3.75 ± 0.06	0.39 ± 0.03	1.39 ± 0.03	8.5 ± 0.02	6.98 ± 0.08
T5	3.52 ± 0.04	0.30 ± 0.01	1.12 ± 0.04	8.13 ± 0.10	7.3 ± 0.12

series of dilution from 10^{-1} to 10^{-6} were prepared in 0.85% NaCl. A 0.1 mL aliquot from 10^{-6} dilution was spread aseptically onto sterile Luria Agar (LA) plates. Thereafter, LA plates were incubated at 30 °C for 2 days. The experiment was repeated three times to gain statistical confidence. The colonies that developed after the incubation on the LA plates would be considered as heterotrophic microorganisms.

Fluorescein diacetate hydrolysis assay

To determine the soil microbial activity, we had performed fluorescein diacetate hydrolysis (FDAH) test of each soil microcosm as FDAH happens to be widely used methods to determine the soil microbial activity (Green et al. 2006; Teng et al. 2010; Goswami et al. 2017a; Dey and Tribedi 2018). Fluorescein diacetate (3,6-diacetyl-fluorescein) (FDA) is a colorless compound where fluorescein is bound with two acetate radicals. Both membrane-bound enzymes (e.g., lipases, protease, esterase) and cell-secreted enzymes (exo-enzymes) can break the ester bond of FDA that resulted in the release of fluorescein which can be quantified through Spectrophotometer (JASCO V-630, serial number C394661148, made in Japan).

To measure the FDAH assay, 5 g soil was separately collected from each soil microcosm and mixed with sterile 15 mL of 60 mM sodium phosphate buffer (pH 7.6) in 50-mL Erlenmeyer flask. FDA solution (1 mg/mL) was prepared by dissolving 10 mg FDA into 10 mL of reagent-grade acetone in a 50-mL sterile glass volumetric flask. Thereafter, 0.2 mL of FDA solution was separately added to each tube. All the tubes were incubated at 32 °C for 20 min. The generated fluorescein was then extracted with 15 mL of chloroform-methanol (2:1) solvent mixture. Finally, the amount of generated fluorescein was measured by recording absorbance at 490 nm using a spectrophotometer (JASCO V-630, serial number C394661148, made in Japan).

Soil dehydrogenase assay

The dehydrogenase activity of each microcosm was assessed by a modification of the protocol described by Kaczynska et al. (2015). In brief, 6 g soil sample was collected from each

microcosm and was placed in a 50-mL sterile polypropylene tube and subsequently mixed with 1 mL of 3% aqueous solution of 1,3,5-triphenyltetrazolium chloride (TTC) solution. In each tube, 2.5 mL of demineralized sterile water was added and mixed properly. Tubes were then incubated at 37 °C for 24 h in dark. After the incubation, 25 mL methanol was added to each tube to extract the triphenylformazan (TPF) produced by reduction of TTC. The concentration of generated TPF was then determined spectrophotometrically by measuring the absorbance of TPF at 485 nm, and the results were expressed as micrograms of TPF produced per gram of soil.

Determination of soil microbial functional diversity

Existing literature reported that BiOLOG ECO plates could be used frequently to determine the soil microbial functional diversity (Choi and Dobbs 1999; Tribedi and Sil 2013; Tribedi and Sil 2013a; Wolińska et al. 2017). In order to measure that, 1 g soil was separately collected from each soil microcosm and was subsequently dissolved in 9 mL of sterile 0.85% saline water. Heterotrophic microorganisms were then independently determined in it. Then, 150 µL of soil suspension (having almost 1000 colony-forming units (CFUs)) was separately added to BiOLOG ECO plates. After that, all the plates were incubated at 30 °C for 48 h. After the incubation got over, the absorbance of each well of BiOLOG ECO plates was recorded at 590 nm. From the obtained absorbance value, the functional richness (Shannon diversity index (H)), and functional evenness (Gini coefficient (G)) was subsequently determined. Shannon diversity index was calculated by using the equation: $H = -\sum p_i \ln p_i$, where p_i is the ratio of the extent of utilization of each substrate (Absorbance_{*i*}) to the sum of the extent of utilization of all substrates (\sum Absorbance_{*i*}) (Tribedi et al. 2015). Gini coefficient (G), a measure of evenness or inequality was measured by using the following equation:

$$G = 1 - 2 \int_0^1 L dF$$

where L is the Lorenz curve and F is the standardized cumulative distribution of the standardized population (Tribedi et al. 2015).

Contour plot analysis

A contour plot happens to be a graphical technique for representing a three-dimensional surface by plotting constant z slices, called contours, on a two-dimensional arrangement (Chakraborty et al. 2018). Contour plot was generated among three variables namely Shannon diversity index, Gini coefficient, and nitrogen-fixing or phosphate-solubilizing bacteria using the software MINITAB 16.

Statistical analysis

Experimental results were subjected to statistical analysis of one-way analysis of variance (ANOVA). Mean values were compared at the 5% level using MINITAB 16. Each experiment was repeated three times to get statistical confidence.

Results and discussion

Variation in the abundance of microbial population and microbial activity among different microcosms

Since soil is heterogeneous in nature and a discontinuous system, the microbial communities of soil are irregularly distributed in it. They are present in soil in discrete microhabitats (Stotzky 2000; Nannipieri et al. 2017). Thus, in the current manuscript, we had examined the abundance of heterotrophic microbial population in soil samples collected from different soil microcosms. The result revealed that there is a significant difference in the numbers of heterotrophic microbial population among the tested soil microcosms (Fig. 1a). We observed that the microcosm T4 and T1 showed the highest and the lowest numbers of heterotrophic bacteria out of all the tested soil microcosms, respectively (Fig. 1a). Since soil microbial activity often reflects soil microbial health (Green et al. 2006; Goswami et al. 2017a; Dey and Tribedi 2018), in the current study, FDAH had been adopted to assess the soil microbial activity among different soil microcosms. The soil microcosms showed significant variation in the microbial activity wherein the microcosm T4 showed the highest and T1 showed the lowest level of microbial activity, respectively (Fig. 1b). The pattern of variation in heterotrophic microbial population exhibited considerable similarity with the pattern of variation in microbial activity among the tested soil microcosms (compare Fig. 1a, b). The correlation coefficient value (r) had been determined between the heterotrophic microbial population and soil microbial activity of the soil microcosms where the value of the same (r) was found to be 0.9851 (Fig. 2). This high value of correlation coefficient demonstrated that soil microbial activity is positively correlated with the abundance of heterotrophic microbial population (Fig. 2). The result indicated that the microbial activity gets changed with

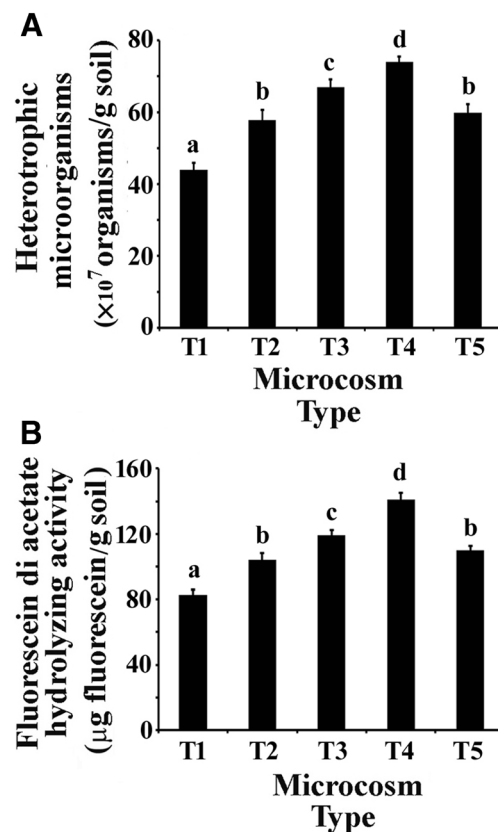


Fig. 1 Microcosm T4 showed the maximum abundances of heterotrophic microorganisms and microbial activity among the tested microcosms. **a** Heterotrophic microorganisms were collected from five different microcosms and subsequently counted on LA growth media. Three replicates were used for each type of microcosm, and the result represented the average of these three replicates. Error bars indicated standard deviation (\pm SD). Statistical significance among the results was evaluated by ANOVA at 5% level. Mean values with different letters are significantly different among the tested soil microcosms. **b** Microbial metabolic activity of the soil microcosms was measured by fluorescein diacetate hydrolysis assay. Three replicates were used for each type of microcosm, and the result represented the average of these three replicates. Error bars indicated standard deviation (\pm SD). Statistical significance among the results was evaluated by ANOVA at 5% level. Mean values with different letters are significantly different among the tested soil microcosms

the alteration in the abundance of heterotrophic microbial population in soil. The coefficient of determination (R^2) was also measured between soil microbial activity and heterotrophic microbial abundance, and the value of the same was found to be 0.9704 (Fig. 2). R^2 is a statistical measure that demonstrates how closely the regression predictions match with the real data points. The high value of R^2 , i.e., 0.9704, indicated that the regression prediction closely matches with the observed data.

Microorganisms produce several types of hydrolytic enzymes such as esterase, protease and hydrolase which can cleave the ester bond in FDA that resulted in the production of fluorescein. The generated fluorescein was then quantified by measuring the absorbance through spectrophotometer.

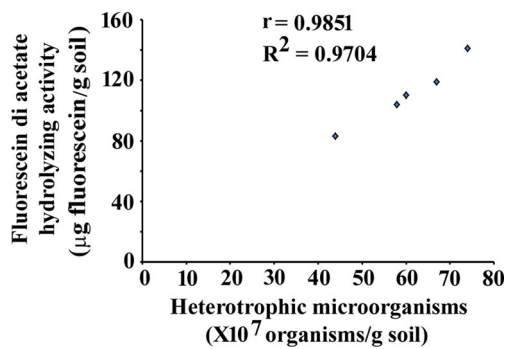


Fig. 2 Strong positive correlation coefficient was observed between heterotrophic microbial abundance and soil microbial activity

Higher microbial population results in the release of more hydrolytic enzymes that can be correlated by the production of increased fluorescein. Thus, the result revealed that enriched microbial population displayed higher microbial activity. To gain further confidence, dehydrogenases activity was measured among the tested soil microcosms as dehydrogenases are often used as an indicator of overall soil microbial activity (Gu et al. 2009; Salazar et al. 2011). We observed a significant variation in the dehydrogenase activity among the soil microcosms wherein the microcosm T4 and T1 showed the highest and the lowest level of dehydrogenase activity, respectively (Fig. 3). Dehydrogenase happens to be an intracellular enzyme, and only the metabolically active viable cells possess the enzyme for oxidation–reduction purpose. We observed the similar pattern of results between FDA hydrolysis and dehydrogenase activity among the tested microcosms (see Figs. 1b and 3). Therefore, the results indicated that soil

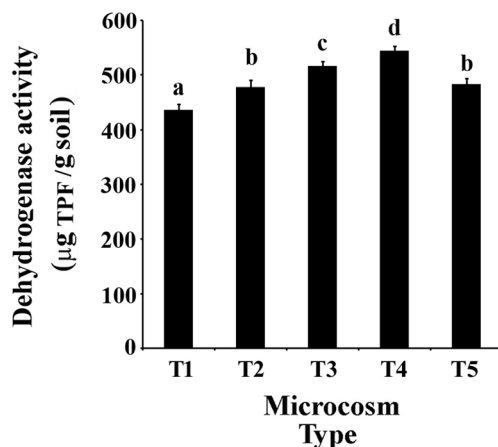


Fig. 3 Microcosm T4 showed the maximum level of dehydrogenase activity among the tested microcosms. Soil samples were separately taken out from each microcosm and microbial activity was subsequently measured in each microcosm by performing dehydrogenase assay as described in “Materials and methods” section. Three replicates were used for each type of microcosm, and the result represented the average of three replicates. Error bars represented standard deviation (\pm SD). Statistical significance among the results was evaluated by ANOVA at 5% level. Mean values with different letters are significantly dissimilar among the microcosms

microbial activity is a function of heterotrophic microbial abundance.

Measurement of functional diversity among soil microcosms

Microbial functional diversity reveals the metabolic potentialities of microorganisms that help the organisms to execute wide range of functions in an ecosystem (Goswami et al. 2017). Previous literature documented that the ecosystem productivity and microbial functional diversity is robustly correlated (Goswami et al. 2017; Wolińska et al. 2017; Dey and Tribedi 2018). Thus, in the present study, we had put our efforts in measuring the functional diversity of soil microcosms and tried to establish the role of functional diversity if any on the abundance of nitrogen-fixing and phosphate-solubilizing bacterial population in soil. It was reported that functional diversity depends on both richness and evenness (Sarkar et al. 2017; Dey and Tribedi 2018). Shannon diversity index has been used in literature to determine the metabolic richness of an ecosystem (Tribedi et al. 2015). Thus, in the present study, we had determined Shannon diversity index for the measurement of functional richness in each soil microcosm. Our results proved that there was a considerable variation in Shannon diversity index among the tested soil microcosms wherein the microcosm T2 and T5 showed the maximum and the minimum Shannon diversity index, respectively (Fig. 4). The results showed that the maximum richness was observed in microcosm T2 and the minimum richness was observed in microcosm T5 (Fig. 4). Richness represents the

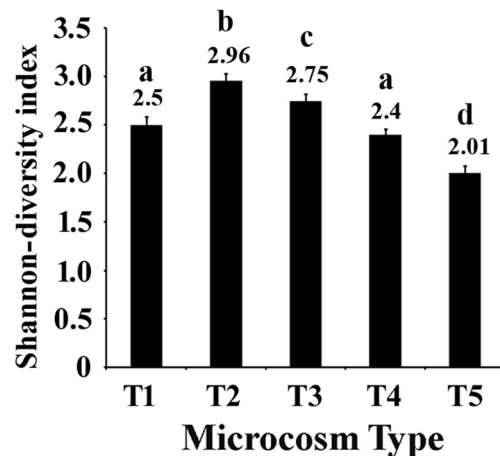


Fig. 4 Microcosm T2 showed the maximum level of functional diversity among the tested microcosms. Functional diversity of each microcosm was measured by determining the Shannon diversity index. Shannon diversity index was determined by considering the ability of the microcosms to utilize different carbon sources present in BiOLOG ECO plates. Three replicates were used for every independent microcosm, and the result represented the average of these three replicates. Error bars indicated standard deviation (\pm SD). Mean values with different letters are significantly different among the tested soil microcosms. Statistical significance among the results was evaluated by ANOVA at 5% level

availability of microbial species in an ecosystem. Higher microbial richness in microcosm T2 revealed more variation of microbial species in it that resulted in the exhibition of diverse metabolic functions. However, lower microbial richness in microcosm T5 indicated less variation of the microbial species in it that lead to the exhibition of compromised metabolic functions. In an ecosystem, the distribution of species is very important for ecological stability. Microbial species that are nearly evenly distributed in the ecosystem could resist the stress in a better way compared to the microbial species that are unevenly distributed in the ecosystem. There is always a fair chance of getting lost from the ecosystem for those species having less richness (Goswami et al. 2017). Thus, in the present report, we had measured the functional evenness of each soil microcosm. It was reported that Gini coefficient (G) could be used to determine the evenness of an ecosystem (Tribedi and Sil 2013; Dey and Tribedi 2018). Therefore, we had determined the Gini coefficient (G) from the dataset obtained from the carbon source utilization spectrum of BiOLOG ECO plates. Its value ranges from 0 to 1 wherein the value of 0 and 1 explains the maximum and the minimum extent of evenness of microbial species, respectively (Tribedi et al. 2015). In the current study, we had determined Gini coefficient for each soil microcosms wherein the soil microcosm T2 and T5 showed the lowest and the highest value of Gini coefficient, respectively (Fig. 5). Thus, the results indicated that microcosm T2 developed a healthy ecosystem as it showed the highest richness and maximum evenness (see Figs. 4 and 5). On the contrary, we also noticed that microcosm T5 developed a vulnerable or compromised ecosystem as it showed the lowest value of richness and the highest level of unevenness

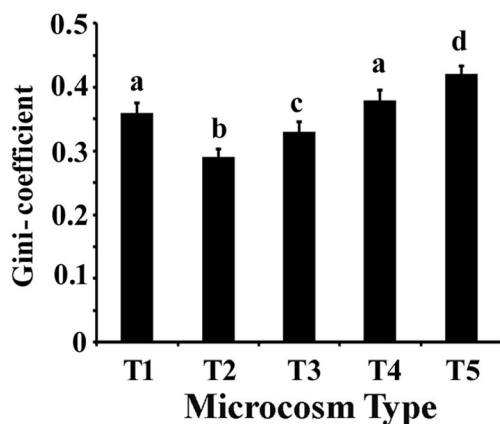


Fig. 5 Microcosm T2 showed the lowest value of Gini coefficient among the tested microcosms. Gini coefficient of each microcosm was determined to address the functional evenness based on the ability of each microcosm to utilize different carbon sources available in BiOLOG ECO plates. Three replicates were taken for every independent microcosm, and the result represented the average of these three replicates. Error bars indicated standard deviation (\pm SD). Mean values with different letters are significantly different among the tested soil microcosms. Statistical significance among the results was evaluated by ANOVA at 5% level

(see Figs. 4 and 5). Taken together, the results demonstrated that higher richness and evenness tends to increase the functional diversity that plays an important role in ecosystem functioning.

Variation in the abundance of nitrogen-fixing and phosphate-solubilizing bacteria among the soil microcosms

Nitrogen is required for the synthesis of chlorophyll, amino acids, nucleic acids, and ATP that are required for the growth and survival of plants. Though nitrogen happens to be the most abundant elements in earth, plants cannot utilize the nitrogen gas directly (Mahanty et al. 2017). Plants can use the reduced form of nitrogen either in the form of ammonia or nitrate. A specialized group of bacteria called “diazotrophs” can convert nitrogen (N_2) to ammonia (NH_3) using “nitrogenase” enzyme complex (Hoffman et al. 2009; Smith et al. 2013). This ammonia can be further oxidized to nitrate by the action of nitrifying bacteria. Since plants do not have the nitrogenase enzyme, they are unable to synthesize ammonia from aerial nitrogen on their own (Mahanty et al. 2017). Thus, plants use microbially fixed ammonia to produce the aforementioned nitrogenous biomolecules for their growth and survival. In the current manuscript, efforts were given to determine the abundance of nitrogen-fixing bacteria in the tested soil microcosms and examined the correlation if any between soil microbial functional diversity and nitrogen-fixing bacterial abundance in soil. In this connection, we observed that the tested soil microcosms showed considerable variation in the abundance of nitrogen-fixing microorganisms (Fig. 6). The result confirmed that the maximum abundance of nitrogen fixers

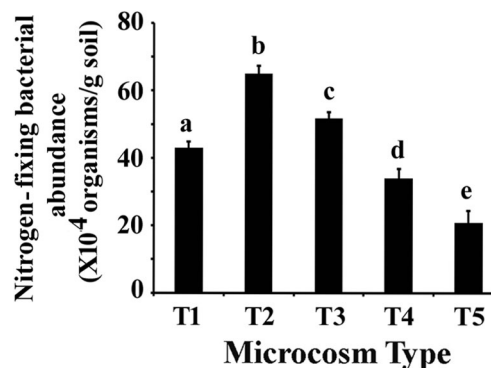


Fig. 6 Microcosm T2 showed the highest abundance of nitrogen-fixing bacteria among the tested soil microcosms. Nitrogen-fixing bacteria were screened on Burk’s agar media. Three replicates were used for every independent microcosm, and the result represented the average of these three replicates. Error bars indicated standard deviation (\pm SD). Mean values with different letters are significantly different among the tested soil microcosms. Statistical significance among the results was evaluated by ANOVA at 5% level

were present in microcosm T2 (Fig. 6). On the contrary, the least numbers of nitrogen fixers were available in microcosm T5 (Fig. 6). Soil microcosm (T5) that showed the lowest functional diversity exhibited poor abundance of nitrogen-fixing organisms and microcosm (T2) that revealed the highest functional diversity confirmed maximum abundance of nitrogen fixers in soil (see Figs. 4 and 6). Thus, the results demonstrated that the microbial functional diversity and the abundance of nitrogen fixers in soil followed the same trend wherein the correlation coefficient was found to be 0.9926 between them (see Fig. 7). This high value of correlation established a strong positive dependence between functional diversity and the abundance of nitrogen fixers in soil. The result indicated that the increase in microbial functional diversity leads to raise the abundance of nitrogen-fixing organisms in soil. Microbial functional diversity represents the wide range of functional traits of microorganisms in an ecosystem. Nitrogen-fixing capability is a special type of functional traits exhibited by specific types of microorganisms. Thus, it is expected that if an ecosystem exhibits higher functional diversity, the possibility of isolating organisms having nitrogen-fixing property would be more. In order to gain confidence, we also determined the R^2 value between them as R^2 value indicates the coefficient of determination between two variables. We noticed a high value (0.9853) of R^2 between microbial functional diversity and the abundance of nitrogen-fixing organisms in soil (Fig. 7). Thus, the result demonstrates a high degree of dependence and association between them.

Phosphorus (P) is the world's second largest nutritional supplement for the production of crops after nitrogen (Adnan et al. 2017). Phosphorous is available in soil in the two forms, organic and inorganic. The free inorganic phosphorous is soluble in nature, and therefore, plants use it comfortably. However, generally the organic phosphorous seems to be insoluble in nature, and thus plants, cannot use it directly (Adnan et al. 2017). In some cases, the mineral phosphorous

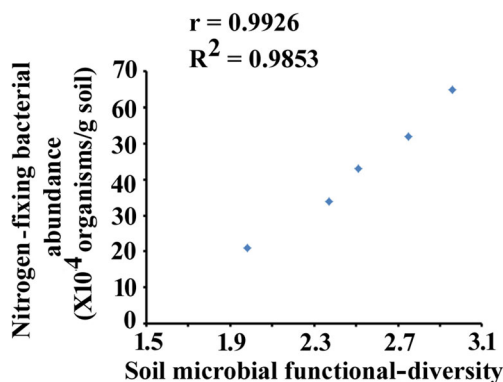


Fig. 7 Strong positive correlation coefficient was determined between the abundance of nitrogen-fixing bacterial population and soil microbial functional diversity

gets inaccessible due to precipitation reactions with cations such as Mg-P and Ca-P in alkaline soil or Al-P and Fe-P in acidic soil (Adnan et al. 2017). Nearly soil contains 400–1000 mg kg⁻¹ of total P, of which merely 1.00–2.50% is accessible to plants because of certain group of microbial functions (Adnan et al. 2017). It was reported that phosphate-solubilizing bacteria represents 1–50% and fungi constitutes 0.1–0.5% of the total respective population in soil (Zaidi et al. 2009). Microorganisms often show the two mechanisms for phosphate solubilization. The soluble phosphorous would be then utilized by plants for its growth and survival. In the first case, microorganism releases organic acids that carry lots of negative charges. This negative charge binds with the cations and frees inorganic phosphorous (Satyaprakash et al. 2017). Secondly, some microorganisms secrete phosphatases enzyme that cleave the bond between organic molecules and phosphorous that results in the release of free inorganic phosphorous (Bhattarai and Mandal 2017). In the current manuscript, we had targeted our efforts to determine the numbers of phosphate-solubilizing bacteria in soil by plating soil microorganisms to Pikovskaya media as Pikovskaya media happens to be a selective media for the isolation of phosphate-solubilizing bacteria. We noticed that the tested microcosms exhibited considerable variation in the abundance of phosphate-solubilizing bacteria (Fig. 8). The highest and the lowest numbers of phosphate-solubilizing bacteria were isolated from the microcosms T2 and T5, respectively (Fig. 8). The microcosm (T2) that exhibited the highest functional diversity houses the highest numbers of phosphate-solubilizing bacteria and the microcosm (T5) that showed the lowest functional diversity houses the least numbers of phosphate solubilizers (see Figs. 4 and 8). All the tested microcosms followed the similar pattern of variation between functional diversity and the abundance of phosphate

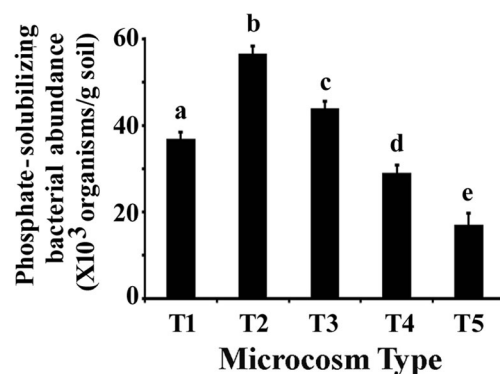


Fig. 8 Microcosm T2 showed the highest abundance of phosphate-solubilizing bacteria among the tested soil microcosms. Phosphate-solubilizing bacteria were screened on Pikovskaya agar media. Three replicates were used for every independent microcosm, and the result represented the average of these three replicates. Error bars indicated standard deviation (\pm SD). Mean values with different letters are significantly different among the tested soil microcosms. Statistical significance among the results was evaluated by ANOVA at 5% level

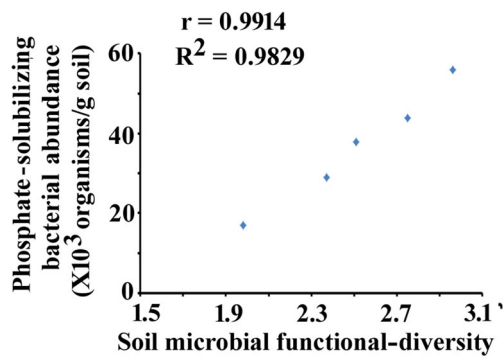


Fig. 9 Strong positive correlation coefficient was measured between the abundance of phosphate-solubilizing bacteria population and soil microbial functional diversity

solubilizers as well (see Figs. 4 and 8). Thus, the results showed that there is a strong positive correlation ($r = 0.9914$) exists between functional diversity of soil and the availability of phosphate-solubilizing bacteria in soil (Fig. 9). The coefficient of determination (R^2) was also determined between these two variables, and the value of the same was found to be 0.9829 (Fig. 9). Thus, the results demonstrated that soil microbial functional diversity showed a strong positive linkage with the abundance of phosphate-solubilizing bacteria in soil. It was reported that higher functional diversity of a microbial ecosystem displayed wide range of functional attributes such as polythene remediation (Tribedi et al. 2015), polycyclic aromatic hydrocarbon (Teng et al. 2010), and poly-hydroxyl butyrate degradation (Dey and Tribedi 2018). Thus, in accordance with the previously published observations, in the current study, we observed that the highest level of functional diversity in microcosm T2 confirmed the maximum abundance of nitrogen-fixing and phosphate-solubilizing bacteria (see Figs. 4, 6, and 8). Similarly, the lowest level of functional diversity in microcosm T5 ensured the least abundance of nitrogen-fixing and phosphate-solubilizing bacteria among the tested

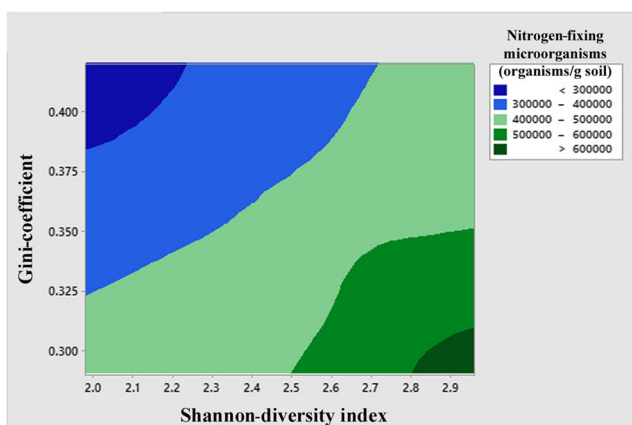


Fig. 10 High degree of relation exists among Shannon diversity index, Gini coefficient, and the abundance of nitrogen-fixing bacterial population through the constriction of contour plot

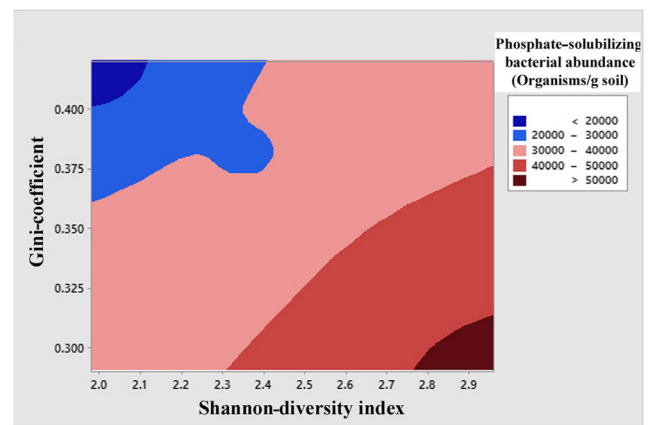


Fig. 11 High degree of relation exists among Shannon diversity index, Gini coefficient, and the abundance of phosphate-solubilizing bacterial population through the constriction of contour plot

microcosms (see Figs. 4, 6, and 8). In the present work, we had determined the physicochemical properties of each soil microcosms (Table 1). The result showed that most of the tested physicochemical properties of soil remained almost similar in microcosms T2 and T5 (Table 1). However, we had experienced a considerable difference in functional diversity between the microcosms T2 and T5. The difference in microbial functional diversity between them (T2 and T5) could be attributed to the variation in microbial community structure. In this context, previous literature also revealed that the alteration in microbial community structure brings a change in microbial functional diversity (Tribedi and Sil 2013). Thus, the difference in the abundance of nitrogen-fixing and phosphate-solubilizing bacterial population between the microcosm T2 and T5 could be attributed to the variation of functional diversity as functional diversity represents a range of functional traits of microorganisms in an ecosystem. To gain further confidence, we had constructed contour plot to establish the correlation among Shannon diversity index, Gini coefficient, and the abundance of nitrogen-fixing and phosphate-solubilizing organisms in soil. The result showed that the increase in functional richness and evenness in soil microcosms display rich exhibition of nitrogen-fixing and phosphate-solubilizing bacteria in soil microcosms (Figs. 10 and 11). Higher functional richness and evenness efficiently contributes and stabilizes the microbial functional diversity that leads to the formation of a healthy and metabolically diverse ecosystem that displayed the proficient abundance of nitrogen-fixing and phosphate-solubilizing bacteria in soil. Considering all the results, it can be stated that microbial functional diversity appears as an efficient tool to select soil samples having preferred numbers of nitrogen-fixing and phosphate-solubilizing microorganisms. Since microbial function is not limited to soil fertility enhancement alone, it could be exploited in wide range of other functions as well in which microbial activities are linked.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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