Environment and Ecology 40 (2A) : 528—535, April—June 2022
ISSN 0970-0420

Diversity of Culturable Soil Microorganisms in Different Rainfed Regions of India

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Received 3 November 2021, Accepted 10 March 2022, Published on 12 April 2022

ABSTRACT

Soil microorganisms perform various ecosystems functions. They are indispensable part of sustainable soil health and agricultural production. The aim of the present investigation was to ascertain the status of soil microbial diversity in selected rainfed regions of India. The soil samples were collected from four different locations viz., Ballowal Saunkhri (Punjab), Parbhani (Maharashtra), Ananthapuramu (Andhra Pradesh) and Vijayapura (Karnataka). Further, we have quantified the abundance of bacteria, fungi, actinomycetes, free living nitrogen fixing bacteria, *Pseudomonas* spp. and phosphorus solubilizing bacteria. Estimated Shannon diversity index (H), Simpson dominance index (C) and the ratio of fungi and bacteria (F: B). Our findings showed the significant differences among different microbial functional groups and F: B ratio across different rainfed regions of India. Highest Shannon diversity index was observed at Ballowal Saunkhri. Whereas, Simpson dominance index was maximum at Parbhani. A positive correlation was observed between different microbial functional groups with soil organic carbon. In conclusion, inceptisols of Ballowal Saunkhri with relatively higher soil organic carbon and slightly alkaline pH recorded maximum abundance and diversity indices of soil microorganisms vis-à-vis other rainfed regions.

Keywords Fungi, Bacteria, Rainfed, Soil microbial diversity, Shannon index.

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INTRODUCTION

The living soil is fundamental to sustain life on the planet earth (Schloter *et al.* 2018). Due to the presence of variety of micro organisms in soil, it is being

Table 1. Geographic, climatic and edaphic properties of the studied rainfed locations.

Particulars	Ballowal Saunkhri (Punjab)	Parbhani (Maharashtra)	Anantapur (Andhra Pradesh)	Vijayapura (Karnataka)
Geographic location (Latitude and longitude)	31°5′53.19″ N and 76°23′22.684″ E	19.2608 °N and 76.7748°E	14°41'218''N and 77040'115''E	160 46' 15.16" N and 750 44'53.78" E
Climate	Sub-humid (Hot dry)	Semi-arid (Hot moist)	Arid (Hot)	Semi-arid (Hot dry)
Soil type	Inceptisols	Vertisols	Alfisols	Vertisols
Organic carbon (%)	0.49	0.44	0.36	0.42
pH	7.6	7.85	6.51	8.02
Available N (kg ha^{-1})	117	189	143.5	198.5
Available P (kg ha^{-1})	45.6	17.93	76.5	13.2
Available K (kg ha^{-1})	168	432	345.5	389.8
Mean annual rainfall (mm)	1011	901	544	595
Mean annual temperature	40.8	33.01	34.10	41.81
Max and Min (°C)	2.30	18.22	21.76	7.6

termed as living and breathing skin of earth. There are about 5×10^{31} microbial cells, which constitute around 60 % of the total biomass (Rao 2007). Soil microorganisms are key drivers of several ecosystems functions such as bio-geochemical cycling of nutrients, removal of toxic pollutants, decomposition of organic matter, suppression of pest and diseases, improving plant growth and yield (Bargaz *et al.* 2018, Manjunath *et al.* 2016, 2018). The composition and abundance of soil microorganisms strongly influences soil health and also act as sensitive indicators (Wang *et al.* 2020, Schloter *et al.* 2018). Indiscriminate use of chemical fertilizers and pesticides in agricultural production is adversely affecting soil bio-diversity, soil health, productivity and also the environment (Walkiewicz *et al.* 2020, Cavicchioli *et al.* 2019). Beneficial microorganisms with their multiple functions like provisioning of nutrients and management of pest and diseases are integral part of sustainable agricultural production systems (Bakker *et al.* 2018, Wang *et al.* 2020). Bacterial and fungal abundance enhanced the wheat yield and nitrogen uptake under dryland conditions (Tautges *et al.* 2016). Chen *et al.* (2020) reported that, diversity of soil microorganisms is vital for sustainable plant productivity. Soils with higher magnitude of bio-diversity are more resilient to environmental changes (Jiao *et al.* 2019). Soil microbial diversity is affected by soil type, environment, crop and soil management practices (Srinivasa Rao *et al.* 2018). Decline of soil microbial diversity significantly impacts nutrient cycling (Philippot *et al.* 2013) and impairs ecosystem functioning in the long run (Hautier 2015). Hence, it is imperative to maintain the soil microbial diversity for sustainable ecosystem functioning. In view of the fact that, richer diversity of soil microorganisms provides better chances to different microorganisms carry out various ecosystem functions (Wagg *et al.* 2021). A very little information is available on the status of soil microbial diversity in different rainfed regions of India having different climate, soil type and rainfall. Hence, the objective of the present study was to estimate the current status of soil microbial diversity in selected rainfed regions viz., Ballowal Saunkhri (Punjab), Parbhani (Maharashtra), Ananthapuramu (Andhra Pradesh) and Vijayapura (Karnataka) representing sub-humid (hot dry), semi-arid (hot moist), arid (hot) and semi-arid (hot dry) climatic conditions, respectively.

MATERIALS AND METHODS

Locations of soil sampling

The soil samples were collected during the year 2018 from 04 different rainfed regions of India differing in soil type, rainfall and climatic conditions. The locations were Ballowal Saunkhri (Punjab), Parbhani (Maharashtra), Anantapuramu (Andhra Pradesh) and

Table 2. Abundance of different functional groups of microorganisms across selected rainfed locations. Different alphabets within a column indicates significant difference at $p \leq 0.05$. *NFB – Nitrogen fixing bacteria, *PSB – Phosphorus solubilizing bacteria.

Rainfed regions	Bacteria (Log_{10} CFU g^{-1} soil)	Fungi (Log_{10} CFU g^{-1} soil)	Actinomycetes (Log_{10} CFU g^{-1} soil)	*Pseudomonas* spp. (Log_{10} CFU g^{-1} soil)	NFB* (Log_{10} CFU g^{-1} soil)	PSB* (Log_{10} CFU g^{-1} soil)
Ballowal (Sub-humid Hot dry)	8.57	4.38[a]	6.76[a]	7.22[a]	5.95[a]	4.11[a]
Parbhani (Semi-arid Hot moist)	8.60	2.89[b]	2.58[b]	5.16[b]	4.35[b]	2.12[bc]
Ananthapuramu (Arid hot)	8.40	3.12[b]	4.39[c]	5.17[b]	4.91[b]	2.61[abc]
Vijayapura (Semi-arid Hot dry)	8.62	1.75[c]	3.53[bc]	4.67[b]	5.16[ab]	1.70[c]
SEm	0.27	0.22	0.47	0.32	0.29	0.55
LSD ($p \leq 0.05$)	0.91	0.75	1.58	1.09	0.96	1.84

Vijayapura (Karnataka). The latitude, longitude, climate and soil type information has been presented in Table 1. Soil samples were taken from 0-15 cm (from the top surface) using an augur. Six random soil samples were collected from each location, they were hand crushed, passed through 2 mm sieves and immediately kept in cool boxes. The soil samples were brought to laboratory and kept at 4°C until analysis.

Soil chemical analyses

The available nitrogen (Subbiah and Asija 1956), available phosphorus (Olsen *et al.* 1954), available potassium (Jackson 1973), organic carbon (Walkley and Black 1934) and pH (soil : water : 1:2.5, Jackson 1973) of the samples were determined by following indicated established procedures.

Enumeration of culturable soil microbial population

The important groups of soil microorganisms viz., bacteria, actinomycetes, fungi, phosphorus solubilizing bacteria, free living nitrogen fixing bacteria and *Pseudomonas* spp. were quantified using different media such as nutrient agar (Seeley *et al.* 1991), Kenknight agar (Kenknight and Muncie 1939), Rose bengal agar (Martin 1950), Pikovskaya agar (Pikovskaya 1948), Jensen N-free agar (Jensen 1942) and King's B agar (King *et al.* 1954), respectively. The soil dilution spread plate technique was followed to enumerate the microorganisms. Briefly, ten grams of each soil sample was mixed in sterile water blanks (90 ml) for ten minutes in a shaker. After making required dilutions, 0.1 ml of the suspension was spread on the surface of the media plates. The plates were incubated at 28 ± 2˚C for different time periods. It was 2-3 days for bacteria, *Pseudomonas* spp. and free-living nitrogen fixing bacteria; 4-6 days for fungi, phosphorus solubilizing bacteria and fungi; 7-10 days for actinomycetes. After the incubation period, the colony forming units (CFU) were counted and expressed as CFU g^{-1} of soil. In case of Pikovskaya agar, formation of clear halo around the colonies was an indication of inorganic phosphate solubilization, such colonies were counted and expressed as colony forming units per gram of soil.

Culturable soil microbial diversity and dominance indices

The culturable soil microbial diversity index was calculated by following one of the most widely used diversity index i.e., Shannon diversity index (H). The following equation (Shannon 1948) ;

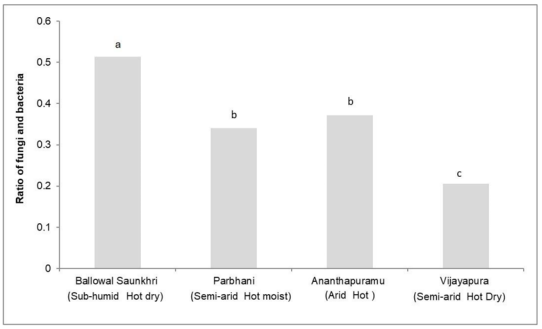

Fig. 1. Ratio of fungi and bacteria across analyzed rainfed locations of India.

$$H = -\sum_{i=1}^{S} (Pi.\ln Pi)$$

was used for the estimation of culturable soil microbial diversity index. Wherein, H - Shannon diversity index for culturable soil microbial diversity; Pi = portion of the whole population made up of microbial genus/group i, s = numbers of microbial genus/group observed, ∑ = sum from species 1 to species s, ln - natural logarithm.

Similarly, culturable soil microbial dominance index (C) was estimated by following the Simpson dominance index equation (Simpson 1949) ;

$$C = -\sum_{i=1}^{S} Pi^2$$

Wherein; C- culturable soil microbial dominance index, Pi = portion of the whole population made up of microbial genus/group i, s = numbers of microbial genus/group observed, ∑ = sum from species 1 to species s.

Statistical analyses

The data was analyzed by means of analyses of variance (ANOVA) using Statistical Packages for Social Sciences (2016). To find out statistical significance between treatment means, LSD test at 95% probability level was done. To compare the treatment means Duncan's multiple range test (DMRT) was used. Simple correlation coefficients were computed to find out the relationships between the variables using SPSS (2016).

RESULTS AND DISCUSSION

Soil parameters such as organic carbon, pH, soil nutrients and weather parameters like rainfall and temperature affect structure of soil microbial communities (Cao *et al.* 2016). A summary of soil chemical properties of all the locations is given in Table 1. Soil organic carbon content ranged from 0.36 -0.49%. Highest organic carbon content was recorded at Ballowal Saunkhri. The available N, P and K ranged from 117-98.5, 13.2-76.5, 168-432 kg/ha, respectively. The highest available N, P and K was observed at Vijayapura, Ananthapuramu and Parbhani, respectively. The pH of the soils ranged from 6.51-8.02, the highest and lowest pH was recorded at Vijayapura and Ananthapuramu, respectively.

Significant differences among different microbial functional groups such as fungi, actinomycetes, *Pseudomonas* spp., nitrogen fixing bacteria and phosphorus solubilizing bacteria were observed across different rainfed regions of India. Highest fungal, actinomycetes, *Pseudomonas* spp., nitrogen fixing bacteria and phosphorus solubilizing bacterial population was recorded at Ballowal Saunkhri. The lowest fungal, *Pseudomonas* spp. and phosphorus solubilizing bacterial population was noticed at Vijayapura. Similarly, Parbhani samples recorded lowest actinomycetes and nitrogen fixing bacteria in comparison with other rainfed regions (Tables 2, 3). Soil organic carbon which acts as a major energy source to microorganisms, plays a significant role in shaping the abundance and functional diversity of soil microorganisms (Hao *et al.* 2008, Luo *et al.* 2015). The relatively higher soil organic carbon content of Ballowal Saunkhri as compared to other regions might have influenced the abundance of fungal, actinomycetes, *Pseudomonas* spp., nitrogen fixing bacteria and phosphorus solubilizing bacterial population. Further, a positive correlation between different groups of microorganisms and soil organic carbon was also recorded in the present investigation (Fig. 3). Soil pH is another important parameter which determines the composition of soil microor-

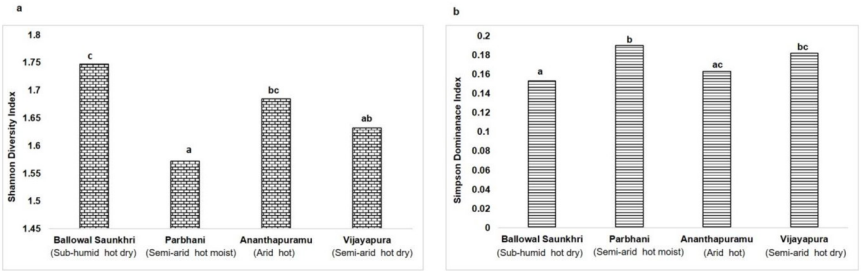

Fig. 2. Soil microbial diversity indices (a) Shannon Index , (b) Simpson Index of the analyzed rainfed regions of India. Different alphabets above the bars indicates significant difference at $p \leq 0.05$.

ganisms, as it strongly influences solubilization and availability of nutrients (Kemmitt *et al.* 2006, Yang *et al.* 2017). In our study, significant positive correlation was observed between pH and bacteria. Whereas, a negative correlation was noticed between fungi, actinomycetes, *Pseudomonas* spp. and phosphorus solubilizing bacteria with the soil pH. Rousk *et al.* (2009) observed the increase of fungal growth and decrease of bacterial growth at lower pH. Similarly, relatively higher soil pH recorded at Vijayapura and Parbhani might be the reason for low fungal counts. Actinomycetes generally grow abundantly in neutral or slightly alkaline soils (Cavaletti *et al.* 2006). In the present study, slightly alkaline pH of Ballowal Saunkhri favored the growth of actinomycetes and recorded higher population as compared to other regions. Chemical fertilizers particularly nitrogen decreases soil microbial diversity (Nie *et al.* 2018, Nemergut *et al.* 2008). Negative correlation obtained between soil available nitrogen and fungi, actinomycetes, *Pseudomonas* spp. nitrogen fixing bacteria and phosphorus solubilizing bacteria in the present study also confirms previous reports. Soil phosphorus had a positive influence on soil microorganisms (Allison *et al.* 2005). Similarly, our results showed a positive correlation between soil phosphorus and

Table 3. Comparative analysis of variance (ANOVA) for different functional groups of microorganisms. *NFB – Nitrogen fixing bacteria, *PSB – Phosphorus solubilizing bacteria.

Source of variance	Dependent variable	Type III Sum of squares	df	Mean square	F	Sig
		0.186	0.138	0.138	0.138	0.936
	Fungi	21.05	3	22.89	22.89	0.000
	Actinomycetes	57.67	3	14.15	14.15	0.000
	Pseudomonas spp.	23.21	3	11.96	11.96	0.000
	NFB	7.93	3	5.23	5.23	0.008
	PSB	20.01	3	3.65	3.65	0.030
Error	Bacteria	9.02	20	0.451		
	Fungi	6.13	20	0.307		
	Actinomycetes	27.16	20	1.35		
	Pseudomonas spp.	12.93	20	0.647		
	NFB	10.10	20	0.505		
	PSB	36.55	20	1.828		
Total	Bacteria	1764.52	24			
	Fungi	249.04	24			
	Actinomycetes	532.39	24			
	Pseudomonas spp.	777.73	24			
	NFB	641.66	24			
	PSB	223.56	24			

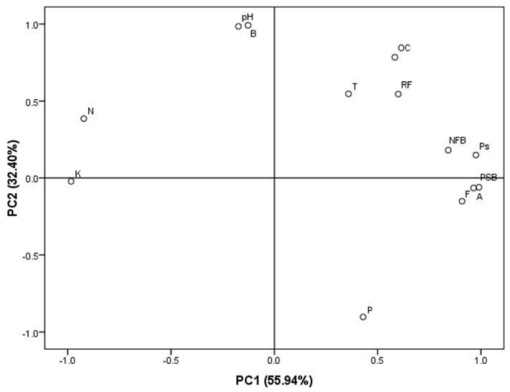

Fig. 3. Principal component analysis of soil microbial communities, soil chemical and weather parameters. B-bacteria, A-actinomycetes, F-fungi, PSB-phosphorus solubilizing bacteria, NFB-nitrogen fixing bacteria, Ps-*Pseudomonas* spp.; OC-organic carbon, RF-rainfall, T-temperature, N-nitrogen, P-phosphorus and K-potassium.

fungi, actinomycetes, *Pseudomonas* spp. nitrogen fixing bacteria and phosphorus solubilizing bacteria. Precipitation enhances soil microbial biomass as it helps in movement of various substrates and also the motility of microorganisms (Saetre *et al.* 2005, Blazewicz *et al.* 2014). A positive correlation of rainfall with the different soil microbial communities in the present investigation corroborates the previous studies. Temperature strongly influence the metabolic activity of microorganisms (Zaidi and Imam 2008). Pietikainen *et al.* (2005) reported that, microbial activity increased with increasing temperature in the agricultural soil. Similarly, our results also showed a positive correlation between temperature and soil microbial communities.

The ratio of fungi and bacteria is a key parameter of soil functioning which is strongly related to carbon storage in soils (Ananyeva *et al.* 2006). In the present investigation, highest value was observed at Ballowal Saunkhri vis-vis other analyzed rainfed regions. The dominance of fungi in the community of soil microorganisms improves carbon sequestration and soil aggregation (Six *et al.* 2006). Significant differences in F:B were observed between different rainfed regions. Ballowal Saunkhri recorded significantly highest value as compared to other rainfed regions. The Parbhani and Ananthapuramu recorded statistically at par values. The lowest F : B ratio was noticed at Vijayapura (Fig. 1).

Shannon diversity index (H) is one of the most commonly used diversity index to determine the richness and evenness of culturable soil microorganisms since it gives uniform weight age to common and rare groups of microorganisms (Shannon 1948, Mahanta *et al.* 2017, Manjunath *et al.* 2018). Higher value of H index implies higher species diversity and more evenness of species in a community. In the present study, maximum and minimum Shannon diversity index (H) was recorded at Ballowal Saunkhri and Parbhani respectively (Fig. 2a). However, there was no significant difference with Ananthapuramu. The lowest Shannon diversity index was observed at Parbhani (Fig. 2a). The Simpson dominance index provides information about the most common species in the community. The value of Simpson index is more means a few species are predominant in the community (Simpson 1949, Mahanta *et al.* 2017). In our study, higher value was observed at Parbhani indicating the dominance of very few species as compared to Ballowal Saunkhri, which recorded lowest value signifying the diversity of different species (Fig. 2b).

The correlations between soil microbial communities, soil chemical and weather parameters were analyzed using principal component analysis (Fig. 3). The first and second axes explained 55.94 % and 32.40 % of the total variation respectively. Soil organic carbon, rainfall and temperature showed a positive correlation with the different soil microbial communities. Significant correlation coefficient (r) was observed between bacteria and pH. Negative correlation was recorded between fungi, actinomycetes, *Pseudomonas* spp. and phosphorus solubilizing bacteria with the soil pH. Except for bacteria a negative correlation was recorded between nitrogen, potassium content of soil and different functional groups of microorganisms.

CONCLUSION

Present research findings revealed the significant differences in soil microbial diversity at different rainfed

locations of India viz., Ballowal Saunkhri (Punjab), Parbhani (Maharashtra), Anantapuramu (Andhra Pradesh) and Vijayapura (Karnataka). A positive correlation was observed between different microbial functional groups with soil organic carbon, rainfall and temperature. In future, soil metagenomics studies can be conducted to get in sights of unculturable soil microorganisms.

ACKNOWLEDGEMENT

We thank the authorities of ICAR-CRIDA, AICRPDA, Hyderabad; ICAR Network project on National Innovations in Climate Resilient Agriculture (NICRA); AICRPDA center, PAU, Ballowal Saunkhri; AICRPDA center, UAS (D), Vijayapura; AICRPDA center, VNMKV, Parbhani and AICRPDA center, ANGRAU, Ananthapuramu for providing necessary facilities towards undertaking this study.

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