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Original Research Article

N_2-fixing cyanobacterial diversity and distribution in forest soils of Upper Assam, India

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Abstract: Cyanobacteria comprise a large group of structurally complex and ecologically significant gram-negative prokaryotes which flourish in almost all conceivable habitats. Some of the cyanobacterial taxa are capable of fixing the atmospheric nitrogen and their presence in various ecosystems is thought to maintain the nitrogen-level in the soil. The present study deals with the diversity of nitrogen fixing cyanobacteria in forest soils of Upper Assam (India). The soil samples were collected from five different reserve forests of the region. Altogether, 18 N_2-fixing cyanobacterial taxa belonging to 8 genera were isolated, of which 5 were heterocystous and 3 were non heterocystous genera. *Nostoc* and *Anabaena* were the most abundant genera (23%, 4 species), followed by *Oscillatoria* (17%, 3 species). Canonical Correspondence analysis (CCA) showed that the occurrence of cyanobacterial species was influenced mostly by pH, temperature, organic carbon and nitrogen.

Key words: Cyanobacteria, diversity, reserve forests, Upper Assam

Introduction

Cyanobacteria comprise a large group of structurally complex and ecologically significant gram-negative prokaryotes [1]. Their habitat may vary from vast oceanic areas to freshwater ponds, tropical and temperate soils to bare rocks and even extreme habitats like arid deserts, frigid lakes or hot springs. Morphologically members are simple unicellular/ multicellular to branched/ unbranched filamentous organisms. Their cell contents are not differentiated into membrane bound structures such as the nucleus, chloroplasts and mitochondria. The photosynthetic pigments inside the cells contain chlorophyll-a which gives green colour, along with the blue pigment, phycocyanin. A few among them possess the red pigment, phycoerythrin. The cells therefore often appear blue green in colour. The group cyanobacteria are divided into five orders namely, Chrococcales, Chaemo-siphonales, Pleurocapsales, Nostocales and Stigonematales [2]. Some of the cyanobacterial taxa belonging to the order Chrococcales, Nostocales and Stigonematales are capable of fixing the atmospheric nitrogen and hence maintain the nitrogen-level in soil where they grow and flourish. They could improve the physico-chemical characteristics of the soil too [3-5].

The soil borne nitrogen fixing cyanobacteria are popularly exploited as biofertilizer for augmenting the content of nitrogen, phosphorus and other growth promoting substances in their substratum along with some polysaccharide materials of the soil [6]. Considering potentialities of the group as a whole, lot of studies have been undertaken to find out the diversity and distribution of the native strains of cyanobacterial population throughout the globe [7]. As compared with that of fresh water and marine cyanobacteria, the knowledge on diversity and distribution of cyanobacteria in general and nitrogen fixing members of the group in particular in the forested areas is scanty [8-9].

North East India is well known for its exceptionally large floral and faunal diversity. Being a part of Indo-Burma biodiversity hot spot, the land mass is not only an abode of innumerable number of plants, animals and insects but also a home of diverse soil microorganisms [10] including nitrogen fixing cyanobacteria. Assam is one of the 8 states of the region which is lying between 89° 42′ E to 96°01' E longitude and 24° 8′ N to 28° 2′ N latitude with a geographical area of 78,438 sq. km. The state with its diverse climatic and topographic conditions supports many forests with a wide floral diversity. The entire state receives heavy rainfall annually

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from May to September and subsequently the valley areas experience flood. The average annual rainfall is around 1800 mm and that of humidity is 80 per cent. Since a little study has so far been done on diversity and abundance of indigenous N_2-fixing Cyanobacterial populations within the geographical boundary of Assam [11-14], the present investigation was undertaken to find out the edaphic N_2-fixing cyanobacterial diversity and their distribution in some selected reserve forests situated in the Upper Assam area of NE India in relation to varied soil physico-chemical characteristics.

Materials and Methods

Description of study site

To perform the study, five different reserve forests viz; Lakhipothar, Nazirating, Jorajan, Upper Dehing and Pangeree were selected in and around Upper Assam region of North East India. The reserve forests are mostly evergreen and semi-evergreen type. The dominant forest species includes Hollong (*Dipterocarpus macrocarpus*), Bonsoom (*Phoebe goalparensis*), Sal (*Shorea robusta*), Arjun (*Terminalia arjuna*), Nahar (*Messua ferrea*), Kujithekera (*Garcinia cowa*), Azar (*Lagerstroemia flos-reginae*) and Kadam (*Neolamarckia cadamba*).

Three sampling sites from each forest were selected to collect the soil samples. Geographical locations of the different sampling sites as recorded using GPS (Garmin etrex) are given in Table 1.

Table1: Geographic details of the sampling locations

Forests	Study Sites / Location	Geo-coordinates	Dominant trees
Lakhipothar	LPRF-I	26^{0}12'12.1'' N 91^{0}47'55.2'' E	*Dipterocarpus marcocarpus* *Phoebe goalparensis*
	LPRF-II	26^{0}07'25.3'' N 91^{0}33'42.2'' E	*Dipterocarpus marcocarpus* *Shorea robusta*
	LPRF-III	26^{0}08'21.1'' N 91^{0}36'33.9'' E	*Shorea robusta* *Terminalia arjuna*
Nazirating	NRF-I	26^{0}07'11.9'' N 91^{0}53'11.4'' E	*Dipterocarpus marcocarpus* *Lagerstroemia speciosa*
	NRF-II	26^{0}07'07.1'' N 91^{0}53'30.1'' E	*Dipterocarpus marcocarpus* *Shorea robusta*
	NRF-III	26^{0}07'15.8'' N 91^{0}55'12.8'' E	*Dipterocarpus marcocarpus* *Messua ferrea*
Jorajan	JRF-I	26^{0}15'09.1'' N 91^{0}32'19.0'' E	*Dipterocarpus marcocarpus* *Garcinia cowa*
	JRF-II	26^{0}16'31.3'' N 91^{0}37'38.5'' E	*Neolamarckia cadamba* *Dipterocarpus marcocarpus*
	JRF-III	26^{0}16'08.2'' N 91^{0}37'39.8'' E	*Dipterocarpus marcocarpus* *Phoebe goalparensis*
Upper Dehing	UDRF-I	26^{0}13'10.2'' N 91^{0}37'31.6'' E	*Dipterocarpus marcocarpus* *Shorea robusta*
	UDRF-II	26^{0}14'38.1'' N 91^{0}31'34.1'' E	*Dipterocarpus marcocarpus* *Messua ferrea*
	UDRF-III	26^{0}13'18.5'' N 91^{0}37'25.6'' E	*Dipterocarpus marcocarpus* *Shorea robusta*
Pangeree	PRF-I	26^{0}16'56.0'' N 91^{0}39'09.0'' E	*Lagerstroemia speciosa* *Dipterocarpus marcocarpus*
	PRF-I	26^{0}09'12.7'' N 91^{0}39'38.4'' E	*Messua ferrea* *Dipterocarpus marcocarpus*
	PRF-I	26^{0}09'12.8'' N 91^{0}39'38.0'' E	*Dipterocarpus marcocarpus* *Messua ferrea*

Collection of soil samples, isolation and maintenance of cyanobacteria

Soil samples collected from each selected locations (Table 1) were utilized for culturing of cyanobacteria using BG-11 medium without nitrogen supplementation under optimal growth condition at 30^{0}C ± 2^{0}C temperatures in 2.3 Klux light intensity for 25-30 days at Ecology Laboratory of Department of Botany (GU). Enumeration of cyanobacterial populations was carried out by MPN technique [15]. The enrichment flasks and MPN tubes were regularly monitored for growth and the organisms were observed microscopically.

Identification of cyanobacterial strains

The cyanobacterial strains were viewed under a Magnus MLX*i* microscope and were identified based on their morphological features and cell structure following Desikachary [16], Stanier *et al.,* [17] and Anand [18].

Analysis of soil Physico-chemical properties

The Electrical Conductivity (EC) and pH were analysed for all the soil samples (using soil: water = 1: 2.5) following the methodology outlined by Black [19]. Soil temperature was measured with a soil thermometer. Water holding capacity of soil was estimated following Jackson [20]. Determination of available phosphorus (P), potassium (K), exchangeable calcium (Ca) and exchangeable magnesium (Mg), organic carbon and total nitrogen (N) were done by following the standard protocols as described by Trivedy and Goel [21].

Statistical Analysis

The relative abundance of each cyanobacterial strain was calculated by employing the following formula:

Relative abundance = **Y/X** x **100**

Where,

X = total number of samples collected

Y= number of samples from which a particular cyanobacteria type was isolated.

The diversity indices (Shannon- Wiener) [22] were calculated using the following formula:

$$H = -\sum_{i=1}^{s} (Pi)\, ln\, (Pi)$$

Where,

H - Diversity in a sample of S species or kinds

S - The number of species in the sample

Pi - relative abundance of i^{th} species or kinds measures, = ni/N

N - total number of individuals of all kinds

ni - number of individuals of i^{th} species

ln - log to base 2

Similarity coefficient of cyanobacteria in different study sites were studied following Jaccard's index of community similarity (J) [23] as: $J = a\ (a + b + c)^{-1}$ Where, *a* represents the number of shared taxa in both samples, *b* is the number of taxa present in one sample, and *c* is the number of taxa in the second sample. The J index would equal one when all taxa are shared by samples, and zero when none are shared.

Canonical Correspondence Analysis (CCA) was employed in order to explain the correlation of species to specific environmental variables. For this purpose, the generic abundance was taken into account. CCA was performed using PAST Software. Soil temperature, pH, conductivity, water holding capacity, P, N, Organic C, Ca, K and Mg were the environmental variables included in the analysis.

Results

Soil physico-chemical properties

The physico-chemical properties of the soil viz; temperature, pH, conductivity, WHC, phosphate, carbon, calcium, magnesium, potassium and soil nitrogen varied with different sites (Table 2). The texture of the soil was loamy sand in all locations. The soil temperature ranged from 21.36 ± 0.27^{0}C to 38.24 ± 1.52 ^{0}C with the highest temperature (38.2^{0}C) recorded at Jorajan RF and the lowest (21.36^{0}C) at Pangeree RF. The pH of the soil varied from 4.92 ± 0.12 at Jorajan RF to 6.45± 0.05 at Lakhipothar RF. Soil Conductivity ranged from 61.6 µS ± 2.23 to 88.1 µS ± 2.59. The highest amount of available phosphate was recorded in Nazirating RF (7.26 mg/100g), against the lowest (3.84 mg/100g) in Jorajan RF. In contrast, highest percentage of organic carbon (2.13%) was recorded in Jorajan RF and that of lowest in Nazirating RF (0.45%). Potassium content varied between 16.22 mg/100g at Pangeree RF to 27.35 mg/100g at Jorajan RF. The nitrogen content of the soil was highest in Nazirating RF (2.3 mg/100g) and lowest in Jorajan RF (0.9 mg/100g). Calcium content varied from 34.9 mg/100g in Jorajan RF to 81.51 mg/100g in Upper Dehing RF. Lakhipothar RF had highest amount of soil magnesium content (127.53 mg/100g) and that was lowest in Jorajan RF (93.94 mg/100g).

Species composition, diversity and abundance

The details of cyanobacterial strains identified from each study site are given in Table 3. A total of 18 N_2-fixing cyanobacterial species belonging to 8 different genera were recorded. Out of these, 5 were heterocystous forms viz: *Nostoc* (4), *Anabaena* (4), *Calothrix* (2), *Rivularia* (1) *and Scytonema* (1), and 3 were non-heterocystous forms: *Oscillatoria* (3), *Lyngbya* (2) and *Phormidium* (1). *Nostoc* and *Anabaena* were the most abundant genera (23%, 4 species), followed

by *Oscillatoria* (17%, 3 species). The lowest abundant genera were *Rivularia* (5%), *Scytonema* (5%) and *Phormidium* (5%) with 1 species each. The overall generic diversity and % abundance is depicted in Fig. 1.

Among the study sites, Lakhipothar RF recorded the highest number of species (15), where members belonging to *Nostoc* (4) and *Anabaena* (4) were dominant. Nazirating RF was represented by 14 cyanobacterial species, where *Nostoc and Oscillatoria* were two dominant genera with 3 species each.

Upper Dehing RF recorded 12 species and the dominant genera being *Anabaena* (4) and *Nostoc* (3). Jorajan RF and Pangeree RF recorded lowest species richness (11), which were dominated by species of *Anabaena* (3) and *Nostoc* (4) respectively (Table 3).

Table 2: Physico- chemical properties of soil samples from different Reserve forests

Soil Properties	Study Sites				
	Lakhipothar RF	Nazirating RF	Jorajan RF	Upper Dehing RF	Pangeree RF
Temp (^{0}C)	27.5±0.54	29.36±0.27	38.2±1.52	34.8±0.8	21.3±1.22
pH	6.4 ± 0.05	5.9 ± 0.09	4.9 ± 0.22	5.8 ± 0.15	5.3 ± 0.13
Cond. (µS)	61.6 ± 0.54	73.03 ± 0.23	88.1 ± 0.43	62.8 ± 0.62	77.8 ± 0.32
WHC (%)	40 ± 0.5	40.5 ± 3.5	48 ± 2.5	39.5 ± 2	43 ± 1.5
Texture	Loamy sand	Loamy sand	Loamy sand	Loamy sand	Loamy sand
P (mg/100g)	6.07 ± 0.03	7.26 ± 0.15	3.84 ± 0.09	6.63 ± 0.02	5.47 ± 0.24
Organic C (%)	0.78 ± 0.05	0.45 ± 0.01	2.13 ± 0.17	0.81 ± 0.14	0.68 ± 0.05
K (mg/100g)	17.13 ± 0.18	19.76 ± 0.06	27.35± 0.41	21.15 ± 0.23	16.22 ± 0.07
Soil N (mg/100g)	1.7 ± 0.14	2.3 ± 0.09	0.9 ± 0.29	1.3 ± 0.32	1.5 ± 0.12
Ca (mg/100g)	47.4 ± 0.07	59.63 ± 0.26	34.9 ± 0.04	81.51 ± 0.18	63.4 ± 0.12
Mg (mg/100g)	127.53 ±0.11	102.36 ±0.16	93.94 ±0.28	108.7 ±0.35	118.65±0.45

Values are expressed as the mean ±S.D; n=9

Table 3: Occurrence and abundance of N_2-fixing cyanobacterial taxa at different locations

Sl. No	Cyanobacterial taxa	Study Sites					Relative Abundance (%)
		Lakhipothar RF	Nazirating RF	Jorajan RF	Upper Dehing RF	Pangeree RF	
1	*Nostoc linckia* (Roth) Bornet ex	+	-	+	+	+	33.33
2	*N. commune* Vaucher ex Born.et Flah. (FH)	+	+	+	+	+	66.66
3	*N. punctiforme* (Kütz.) Hariot	+	+	+	-	+	33.33
4	*N. sphaericum* Vaucher ex Born.et Flah	+	+	-	+	+	26.66
5	*Anabaena virabilis* Fritsch.	+	+	+	+	-	26.66
6	*A. circinalis* Rabenhorst ex Born.et Flah	+	+	+	+	+	53.33
7	*A. oryzae* Fritsch	+	-	+	+	-	33.33
8	*A. fertilissima* Rao, C.B.	+	-	-	+	+	20
9	*Calothrix braunii* (A.Br.) Bornet et Flahault	+	-	+	+	-	26.66
10	*C. marchica* Lemmermann	+	+	-	+	+	26.66
11	*Rivularia sp.*	+	+	-	-	-	13.33
12	*Lyngbya contorta* Lemm	-	+	-	+	+	26.66
13	*L. willei* Lemm	-	+	-	-	+	13.33
14	*Phormidium tenue* (Menegh.)Gomont	+	+	+	+	-	33.33
15	*Oscillatoria princeps* Vaucher ex Gomont (F)	+	+	+	-	-	26.66
16	*O. tenuis* C. Agardh ex Gomont (F)	+	+	-	-	-	20
17	*O. willei* Gardner em.Drouet	+	+	+	+	+	40
18	*Scytonema rivulare* Borzi	-	+	+	-	+	20

Shannon's diversity index of N_2-fixing cyanobacteria in all the selected reserve forests were analysed (Fig 4). Nazirating RF was found to be rich in cyanobacterial population with the highest diversity index (1.864), followed by Lakhipothar RF (1.632), Upper Dehing RF (1.426) and Pangeree RF (1.325). Jorajan RF showed lowest diversity index (1.068) among the selected forests. Similarity coefficients between different reserve forests were calculated using Jaccard's similarity index (Table 4). Higher values of similarity index indicate the occurrence of more number of common species between two locations. Jaccard's indices indicated that Lakhipothar RF and Upper Dehing RF were most similar (J=0.687) in relation to N_2-fixing cyanobacterial species composition. Jorajan RF and Pangeree RF showed least similarity (J=0.375).

Table 4: Value of Jaccard's similarity indices between different Reserve forests

Location	Total Isolates	Similarity indices				
		Lakhipothar RF	Nazirating RF	Jorajan RF	Upper Dehing RF	Pangeree RF
Lakhipothar RF	15	-	0.610	0.526	0.687	0.440
Nazirating RF	14	-	-	0.470	0.440	0.562
Jorajan RF	11	-	-	-	0.533	0.375
Upper Dehing RF	12	-	-	-	-	0.533
Pangeree RF	11	-	-	-	-	-

Canonical Correspondence Analysis (CCA) of species abundance data with environmental variables produced an ordination where first two axes were statistically significant ($P < 0.05$) with Eigen values 0.11 and 0.07 for Axis 1 and Axis 2 respectively. The two axes alone explained a large proportion of variance in cyanobacterial species composition environment relationship (74.12%), hence axis 1 and axis 2 were considered for constructing the CCA biplot [24] (Figure 4). The CCA biplot showed that the cyanobacterial genera are well separated along both the axes, indicating different physico-chemical demands for different species. The environmental variables may be ranked in terms of their influence in the order of temperature, pH, soil N, organic C, calcium, phosphate, conductivity, WHC, potassium and magnesium respectively. Species of *Oscillatoria, Phormidium* and *Rivularia* were abundant in Lakhipothar RF where pH and phosphate content was relatively high. Jorajan and Upper Dehing reserve forest experienced high temperature along with high carbon and magnesium content which favored the occurrence of species of *Nostoc* and *Calothrix*. Most of the organisms preferred to grow in high pH and low nitrogen zones. Species belonging to *Lyngbya* and *Scytonema* favored the sites with high phosphate and calcium content and that of *Nostoc* and *Anabaena* showed preference to the sites with low nitrogen content and high temperature.

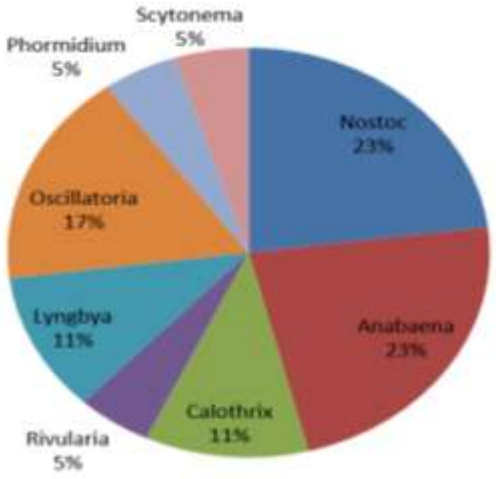

Figure 1: Total percent abundance of N_2-fixing Cyanobacterial Genera (summed up over all locations).

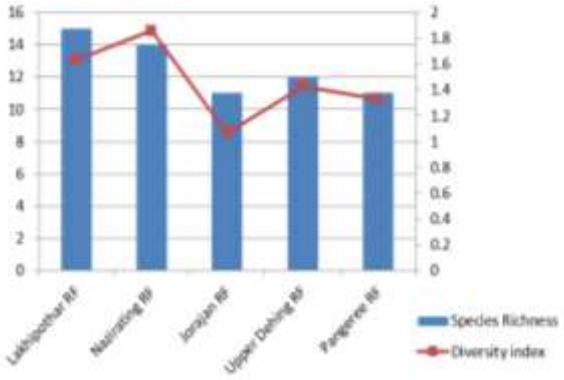

Figure 2: Diversity indices of N_2-fixing Cyanobacteria at different Reserve forests

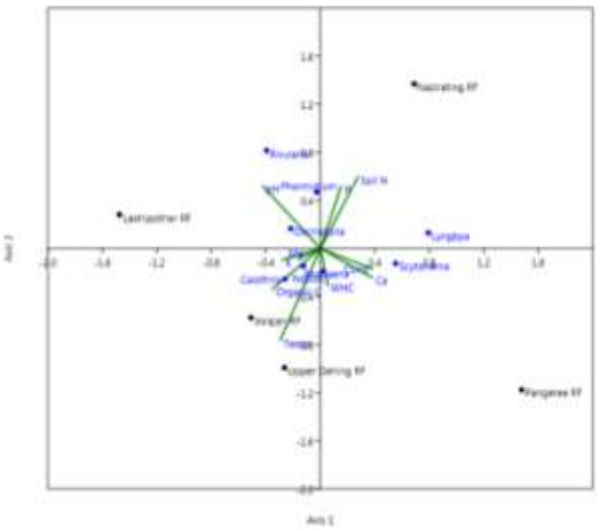

Figure 3: CCA (canonical correspondence analysis) ordination diagram of selected environmental variables Temp: Temperature, pH, Cond: Conductivity, WHC: water holding capacity, P: soil phosphate, Organic C: organic carbon, Ca: calcium, Soil N: soil nitrogen, K: potassium, Mg: magnesium correlated with cyanobacterial assemblages in different Reserve forests.

Discussion

The present investigation showed the occurrence of 18 N_2-fixing cyanobacterial taxa from different forest soils of Upper Assam. The abundance of heterocystous forms was more as compared to the non heterocystous forms. The differences in ecological parameters prevailing in the sampling areas play a vital role in determining the distribution of cyanobacterial population. Among different physico-chemical properties, pH is important in determining growth, establishment and diversity of cyanobacterial flora [25]. In the present study, richness of N_2-fixing cyanobacterial species was found to vary with respect to pH of the concerned soil. Although all the forest areas of the present study recorded acidic pH, higher diversity was seen in the forests that showed pH close to neutral. The present result support pH as a regulating factor increasing the number of cyanobacterial population which was in conformity with the findings of Nayak and Prasanna [26]. Besides pH, temperature also influences the distribution of N_2-fixing cyanobacteria in the forest soil. The diversity and species richness was higher in Nazirating and Lakhipothar reserve forest where the temperature was high. The soil elements like calcium, potassium, available phosphate, magnesium and mainly the nitrogen content of soil too play an important role in the distribution of cyanobacteria. Prevalence of the members of *Nostoc* and *Anabaena* with total percent abundance around 23% each (Fig 3), in all forests indicated their resilience and high adaptability in varied types of ecosystems.

Conclusion

Role of N_2-fixing cyanobacteria in improving soil health and fertility is well known. To exploit the full potential of these cyanobacteria in management of soil quality and fertility, region-specific biodiversity study is important. Knowledge of their diversity in the region may help in selecting appropriate cyanobacterial inocula to be applied as biofertilizer as well as in finding suitable strains with other biotechnological potential leading to improve quality and fertility of acidic soils of the region.

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