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Diversity and dynamics of N_2- fixing cyanobacterial population in soils of Brahmaputra floodplain in response to cropping systems and seasonality

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ABSTRACT

The N_2- fixing cyanobacteria are important agents in crop fields contributing to the primary productivity and nitrogen economy of the soil. In the present study, diversity and abundance of N_2- fixing cyanobacterial population were investigated in the rice ecosystems of the Brahmaputra floodplain in response to the soil physico chemical properties in four different seasons. Altogether 51 species of N_2- fixing cyanobacteria belonging to 15 genera under 7 families were recorded in two common rice cropping systems - double rice cultivation (RR) and rice rotated with mustard (RMR). Among the isolated genera, 10 were heterocystous and 6 were non heterocystous. The genera *Anabaena* (25%) and *Nostoc* (19%) comprised of the highest number of species followed by *Calothrix* (8%) and *Phormidium* (8%). Results of relative abundance also revealed the dominance of *Anabaena* and *Nostoc* in the rice ecosystems of the valley. Pearson's correlation analysis showed significant correlation between population number of N_2-fixing cyanobacteria and soil physico-chemical properties. Canonical component analysis (CCA) justified the seasonal pattern of population abundance along with the concomitant change of soil parameters like temperature, moisture, pH and available phosphate in the rice field soil of the valley.

Introduction

Cyanobacteria, an ancient group of photosynthetic prokaryotes [1] are significant micro inhabitants of all types of soils in the tropical and temperate region [2] including agricultural lands, forests[3], grasslands [4] and in hot and cold desert soils [5].They function as one of the primary producers in soil ecosystem growing on or below the soil surface[6]. Some of the cyanobacterial taxa are capable of fixing atmospheric nitrogen and are responsible for improving soil fertility and water holding capacity[7,8,9,10], thus playing an important role in the development and stabilization of soil ecosystems [11,12,13].The rice grown regions are the most favourable habitat for the luxuriant growth of cyanobacteria[14,15]. In crop fields, their growth is dependent on the change in seasons and subsequent stages of crop cultivation [7,16]. The physical and chemical environment of the soil along with the availability of essential soil nutrients greatly influence the population of N_2- fixing cyanobacteria in any cropping system[11,17,18].

Blessed with heavy monsoonal rains, the Brahmaputra Valley situated in the North Eastern region of India becomes a potential hub for cultivation of different varieties of rice. Rice is the principal crop cultivated throughout the year with an annual production of around 5.50 million tones. Along with rice, different crops like mustard, rapeseed, pea, lentil, black gram and other seasonal vegetables are cultivated in the region.The most prevalent rice cropping systems include the double rice or rice- rice (RR) and rice-mustard-rice (RMR) cropping system. In the RR system, summer rice is rotated with winter rice. In these fields, cultivation of summer varieties of rice starts at the onset of the monsoon season (May-June). After the harvest of summer rice in October-November, winter varieties of rice are sowed during January-February. The harvesting of winter rice is carried out in late April. In the RMR cropping system only summer rice was cultivated in rotation with mustard cultivation. In these fields, after the harvest of summer rice, the fields were ploughed for cultivation of mustard in winter.

Since cyanobacteria are an economically important group of soil organisms, it is of outmost importance to study the diversity of N_2- fixing cyanobacteria and their population characteristics in terms of abundance, diversity and dominance [18] in the rice as well as any agro ecosystems. Although a few attempts have been made to record the diversity of cyanobacteria from different rice fields of Assam [19, 20,21 ,22],there is scanty information on population ecology of N_2 fixing cyanobacteria in soils of the Brahmaputra Valley [23]. Since no work on the diversity of N_2 - fixing cyanobacteria in response to rice cropping systems have been carried out in the region, the present endeavor therefore aimed to study the population dynamics of these beneficial group of organisms in response to different cropping systems as well as with the seasons.

Materials and methods

Study Sites

Soil samples were collected from six different sites within the Lower Brahmaputra valley Agro climatic Zone. A group three sites each were selected from RR (RR- 1, RR- 2 and RR-3) and RMR (RMR-1, RMR-2 and RMR-3) cropping fields. This region experiences subtropical monsoon climate with an average annual rainfall of 1800 ± 200 mm and around 80% average humidity. The maximum temperature may rise up to

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38^0C in summer and fall up to 14^0 C in winter. The study was carried out from May 2012 to June 2014 at four different seasons - Pre monsoon (March to May), Monsoon (June-September), Post monsoon (September-November) and Winter (December-February).

Table 1. Geographical coordinates of the study sites

Cropping systems	Sampling sites	Geo coordinates	Soil type
Rice- Rice (RR)	RR-1	N-$26^0 22^{/} 59.5^{//}$; E-$091^0 28^{/} 23.3^{//}$	Sandy loam
	RR-2	N-$26^0 28^{/} 21.4^{//}$; E-$091^0 23^{/} 30.9^{//}$	Clayey
	RR-3	N$26^0 21^{/} 53.5^{//}$; E$091^0 15^{/} 35.6^{//}$	Silty loam
Rice- Mustard-Rice (RMR)	RMR-1	N-$26^0 22^{/} 51.0^{//}$; E-$091^0 27^{/} 13.3^{//}$	Clay loam
	RMR-2	N-$26^0 26^{/} 22.5^{//}$; E-$091^0 22^{/} 27.4^{//}$	Sandy loam
	RMR-3	N-$26^0 22^{/} 11.4^{//}$; E-$091^0 15^{/} 18.2^{//}$	Clayey

Soil sampling and analysis

From each site, soil samples (from 0-20 cm depth) were collected from 10 randomly selected spots using a hand auger. The soil samples were mixed together in sterile plastic bags and transported to the Plant Ecology Laboratory of Department of Botany, Gauhati University. The composite samples were air dried, sieved (through 2 mm mesh size) and thoroughly homogenized. 1 gram soil from each composite samples were then inoculated in freshly prepared nitrogen free liquid BG-11 media [24] and maintained under aseptic laboratory condition under light intensity of 2500~3500 lux at 25^0C. MPN values were calculated based on tubes showing cyanobacterial growth within 21 days of innoculation. The isolated species were observed microscopically under a Magnus MLXi microscope and identified based on morphological characteristics following [25, 26, 27].

Physico-chemical analysis of soil

The soil pH and electrical conductivity (EC) was determined by using digital pH meter (Biochem PM 79) and conductivity meter (Systronics 304) respectively following Black [28]. Organic carbon (OC) was determined by Walkey and Black rapid titration method [29]. Soil moisture was calculated using Soil Survey Standard Test Method. The available phosphorus (P), potassium (K), Sodium(Na), soil nitrogen (N),Calcium (Ca), Magnesium (Mg) were estimated as per procedures described by Trivedi and Goel [30].

Data analysis

Relative abundance: The relative abundance of the dominant species observed were calculated using standard formula.

$$Relative\ abundance = \frac{Y}{X}\ X\,100$$

Where, X= total number of samples collected,
Y=number of samples from which a particular cyanobacteria type was isolated

Statistical analysis

To visualize the relationship between cyanobacterial population and environmental factors in different sampling time, a canonical corresponence analysis (CCA) was carried out using PAST software. For this purpose, the generic abundance of the isolated cyanobacterial taxa was taken into account. Pearson's correlation coefficient was also calculated between N_2-fixing cyanobacterial population and physico-chemical parameters of the soil at different seasons using SPSS software.

Results and Discussion

Cyanobacterial diversity and abundance

A total of 51 species of N_2- fixing cyanobacteria (Table-2) belonging to 15 genera under 7 families were identified from the two rice cropping systems of the Brahmaputra Valley. 9 genera (*Anabaena, Calothrix, Cylindospermum, Microchaete, Nostoc, Plectonema, Scytonema, Tolypothrix, and Westiellopsis*) belonging to 5 families (Nostocaceae, Stigonemataceae, Microchaetaceae, Rivulariaceae and Stigonemataceae) were heterocystous and 6 genera (*Aphanocapsa, Aphanothece, Chroococcus, Oscillatoria, Lyngbya, and Phormidium*) belonging to 2 families (Chroococcaceae and Oscillatoriaceae) were non-heterocystous.The filamentous heterocystous forms (69 %) were higher in number than the unicellular (12%) and non heterocystous forms (19%). The present findings are in consistent with Hazarika [31] and Dihingia and Barua [23] who also recorded dominance of heterocystous filamentous forms of cyanobacteria in the rice fields of Assam. With 25 species, Nostocaceae was the largest family with 3 genera *Anabaena* (13), *Cylindrospermum* (2), and *Nostoc* (10). The most dominant among all the N_2- fixing cyanobacterial genera was Anabaena with 25% of the species followed by *Nostoc* (19%), *Calothrix* (8%) and *Phormidium* (8%) respectively (Fig-1).

In both RR and RMR fields, *Anabaena* also showed the highest percentage of relative abundance of 57.69% and 63.46% followed by *Nostoc* with 52.5% and 57.5% and *Weistellopsis* with 50% and 50% respectively (Fig-2). Begum *et al*., [32] and Nayak and Prasanna, [11] also recorded more heterocystous forms comprising of *Nostoc* and *Anabaena* species while studying cyanobacterial abundance and diversity in rice grown areas of Bangladesh and India. The predominance of *Nostoc* and *Anabaena* in soil is due to their ability to withstand dessication [11, 33].

The number of N_2- fixing cyanobacterial population (MPN) varied in different seasons in both the rice cropping systems (Fig-2). In the RR fields, the cyanobacterial count was highest ($5.429 \pm 0.49 x 10^4$/g soil) during monsoon season, followed by pre monsoon ($5.216 \pm 0.66 x 10^4$/g soil) and that was lowest in the winter season (1.716 ± 0.25 $x10^4$/g soil). In the RMR fields, MPN was recorded highest ($7.4 \pm 0.50 x 10^4$/g soil) during post monsoon, followed by pre monsoon ($5.216 \pm 0.50 x 10^4$/g soil), and lowest in the winter season (1.496 ± 0.08 $x10^4$/g soil). The present findings are similar to the findings of Dihingia and Barua, [23]; Chunleuchanon *et al*., [34] who recorded highest number of cyanobacteria in the rainy seasons in rice cultivated fields. Song *et al*., [15] also observed the peak diversity of cyanobacteria during the rice growing season i.e particularly in monsoon.

Table 2. Occurrence of N_2- fixing cyanobacterial taxa in the two rice cropping systems in different seasons

Sl no	Cyanobacterial taxa	Cropping system							
		RR				RMR			
	Chroococcaceae	**Pre mon**	**Mon**	**Post mon**	**Win**	**Pre mon**	**Mon**	**Post mon**	**Win**
1	*Aphanocapsa endophytica*	-	+	-	-	-	-	+	-
2	*Aphanocapsa muscicola*	-	+	-	-	-	-	-	-
3	*Aphanocapsa biformis*	-	+	-	+	-	-	+	-
4	*Aphanothece virabilis*	+	-	-	-	-	-	-	-
5	*Aphanothece microscopica*	-	-	-	-	-	-	+	-
6	*Chroococcus minor*	-	-	-	+	-	+	-	-
	Nostocaceae								
7	*Cylindrospermum muscicola*	-	-	-	+	-	-	-	+
8	*Cylindrospermum stagnale*	+	+	-	-	-	-	+	-
9	*Nostoc commune*	+	+	-	+	-	+	+	-
10	*Nostoc piscinale*	-	-	+	-	-	-	+	+
11	*Nostoc muscorum*	-	+	+	-	+	+	+	-
12	*N.sphaericum*	+	+	+	-	+	-	+	+
13	*N.ellipsosporum*	-	-	+	+	-	-	+	-
14	*N.linckia*	-	+	-	-	-	+	-	-
15	*N. calcicola*	-	+	+	+	+	-	+	-
16	*N. carneum*	-	+	+	+	-	+	-	+
17	*N.hatei*	-	+	-	-	+	-	+	+
18	*N.spongiaeforme*	+	+	-	-	-	+	+	+
19	*Anabaena torulosa*	+	+	+	-	+	-	+	-
20	*Anabaena elenkinii*	-	+	+	-	-	+	+	-
21	*Anabaena oryzae*	+	-	+	+	+	-	+	+
22	*A.doliolum*	-	+	-	+	+	+	+	-
23	*A.fertilissima*	+	-	+	+	-	+	+	+
24	*A.aphanizomenoides*	-	+	-	+	+	-	+	-
25	*A.circinalis*	-	+	+	-	+	+	+	-
26	*A.virabilis*	+	-	+	-	-	+	+	+
27	*A.iyengarii*	-	+	+	-	+	-	+	-
28	*A.conctricta*	-	+	+	+	-	-	+	-
29	*A.circinalis*	-	-	+	-	+	-	+	+
30	*A.fuelliborni*	+	+	-	+	+	+	+	-
31	*A.naviculoides*	-	+	-	-	+	-	+	+
	Oscillatoriaceae								
32	*Oscillatoria limnetica*	-	-	-	+	-	-	+	-
33	*O.princeps*	-	+	-	-	-	+	+	+
34	*O.willei*	-	+	-	-	-	-	-	+
35	*Lyngbya contorta*	+	-	-	+	-	-	-	+
36	*Lyngbya willei*	-	+	-	-	-	+	+	+
37	*Lyngbya lagerheimii*	+	+	-	-	-	-	+	+

38	*Phormidium notatum*	-	-	+	+	-	-	+	-
39	*Phormidium tenue*	-	+	-	-	-	+	-	-
40	*P. fragile*	-	+	-	+	+	-	+	-
41	*P. foveolarum*	-	+	-	+	-	+	-	+
	Scytonemataceae								
42	*Scytonema bohnerii*	-	+	-	-	-	-	+	-
43	*Tolypothrix distorta*	-	-	+	-	+	-	+	-
44	*Plectonema indica*	-	+	-	-	-	-	+	-
	Microchaetaceae								
45	*Microchaete aequalis*	-	+	-	-	-	-	+	-
46	*M.uberrima*	+	+	-	+	-	-	+	-
	Rivulariaceae								
47	*Calothrix elenkinii*	+	-	+	-	-	-	+	-
48	*Calothrix fusca*	+	-	+	-	-	-	-	-
49	*C.javanica*	-	+	-	-	-	-	+	-
50	*C.marchica*	-	-	+	-	-	-	+	-
	Stigonemataceae								
51	*Westiellopsis prolifica*	-	-	+	+	-	+	+	-

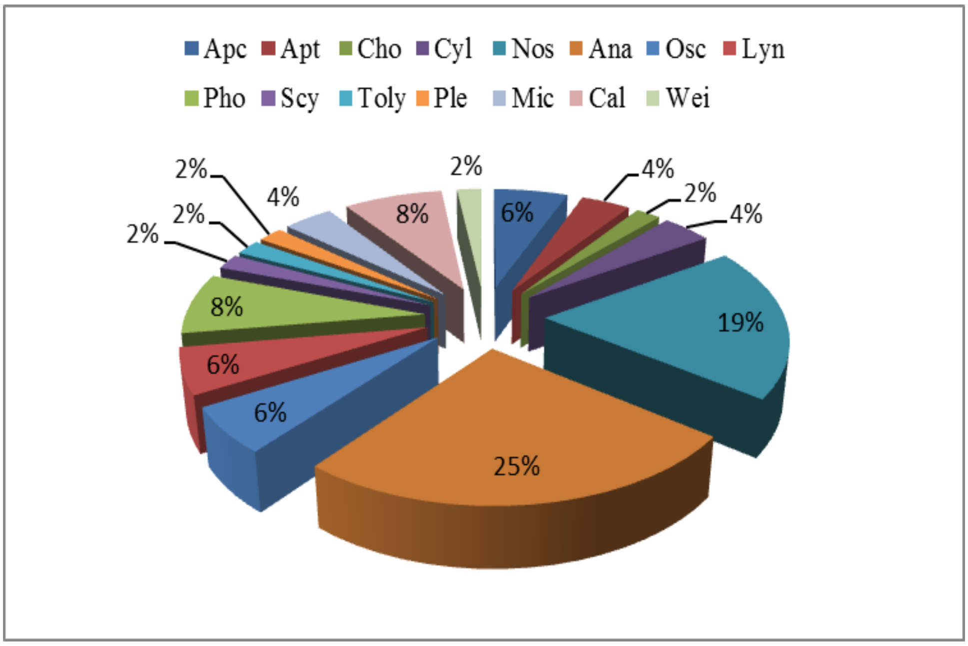

Figure 1. Percentage composition of N_2- fixing cyanobacterial genera in the two rice cropping systems of Brahmaputra valley, Apc-*Aphanocapsa* , Apt-*Aphanothece* , Cho-*Chroococcus* , Cyl-*Cylindrospermum* , Osc-*Oscillatoria* , Pho-*Phormidium* , Scy- *Scytonema*, Toly-*Tolypothrix* , Ple-*Plectonema* , Mic-*Microchaete* , Cal- *Calothrix*, Wei-*Weistellopsis* , Nos-*Nostoc* , Ana-*Anabaena* , Lyn-*Lyngbya*

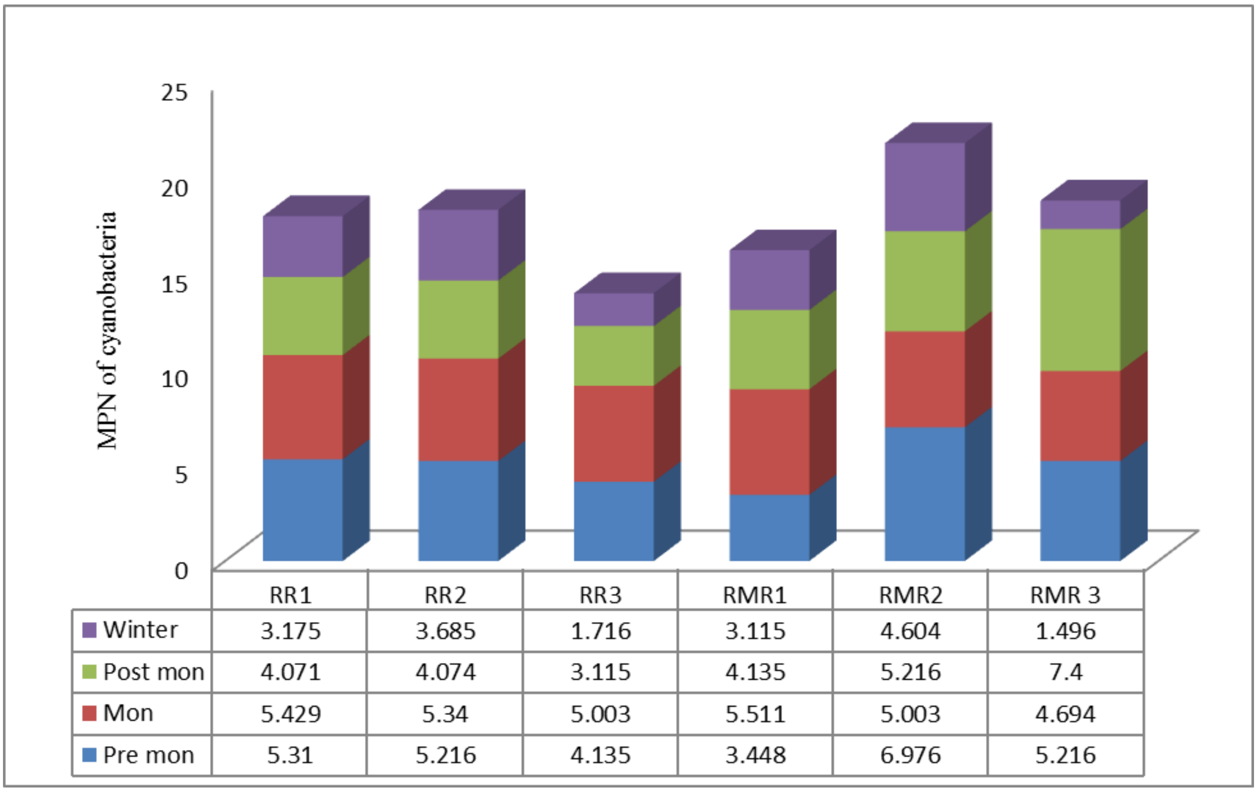

Figure 2. Cyanobacterial counts (MPN x10^4) in different seasons in RR and RMR fields. Mon-Monsoon, Premon-Premonsoon season, Post mon- post monsoon.

Table 3. Physico chemical properties of the soil in RR cropping system

Soil properties	Pre monsoon	Monsoon	Post monsoon	Winter
Temperature(^{0}C)	24.24± 0.38	34.54±0.57	32.22 ± 0.81	15.16± 0.55
pH	5.79± 0.10	7.57± 0.04	7.29± 0.01	6.01± 0.16
Conductivity(µS)	174.6± 2.50	36.6± 1.67	38.4± 2.08	168.4± 9.12
Soil Moisture(SM %)	8.09±1.88	29.76± 3.74	24.31±4.17	5.89±2.29
Organic carbon (OC %)	0.58±0.40	0.36±0.13	0.23±0.16	0.51±0.49
N(mg/100g)	1.14±0.05	0.31±0.33	0.24±0.11	0.31±0.07
P(mg/100g)	6.30 ± 0.01	8.39±0.01	6.26± 0.01	3.4 ± 0.12
K(mg/100g)	26.54±0.14	12.02±0.03	21.22±0.06	23.21±0.01
Na(mg/100g)	16.17± 0.09	8.51± 0.01	9.74± 0.01	11.87± 0.08
Ca(meq/100g)	4.32 ± 0.01	4.02 ± 0.06	4.11± 0.01	4.87± 0.008
Mg(meq/100g)	0.73±0.89	1.73 ± 0.87	0.47± 0.004	1.34± 0.02

Table 4. Physico chemical properties of the soil in RMR cropping system

Soil properties	Pre monsoon	Monsoon	Post monsoon	Winter
Temperature(0C)	25.6 ± 0.19	30± 0.71	31± 0.38	14.56 ± 0.38
pH	7.31± 0.008	7.88 ± 0.07	7.36 ± 0.19	5.31 ± 0.88
Conductivity(µS)	150± 1.21	174.80± 3.56	45.40± 0.89	173 ± 2.03
Soil Moisture(SM %)	8.94±3.66	29.72±3.22	25.91±3.76	9.5±2.71
Organic carbon (OC %)	0.52±0.44	0.42±0.14	0.27±0.13	0.55±0.47
N(mg/100g)	0.19±0.08	0.19±0.06	0.20±0.05	0.27±0.06
P(mg/100g)	4.99± 0.06	6.06± 0.03	6.75± 0.04	6.70± 0.046
K(mg/100g)	16.55± 0.12	9.646 ± 0.02	13.34± 0.06	16.71 ± 0.11
Na(mg/100g)	18.36± 0.08	8.57± 0.005	9.68 ± 0.01	45.36 ± 0.02
Ca(meq/100g)	4.33± 0.013	4.21 ±0.02	2.13 ± 0.01	6.16 ± 0.21
Mg(meq/100g)	1.16± 0.01	0.54± 0.02	0.664 ± 0.01	0.74 ± 0.09

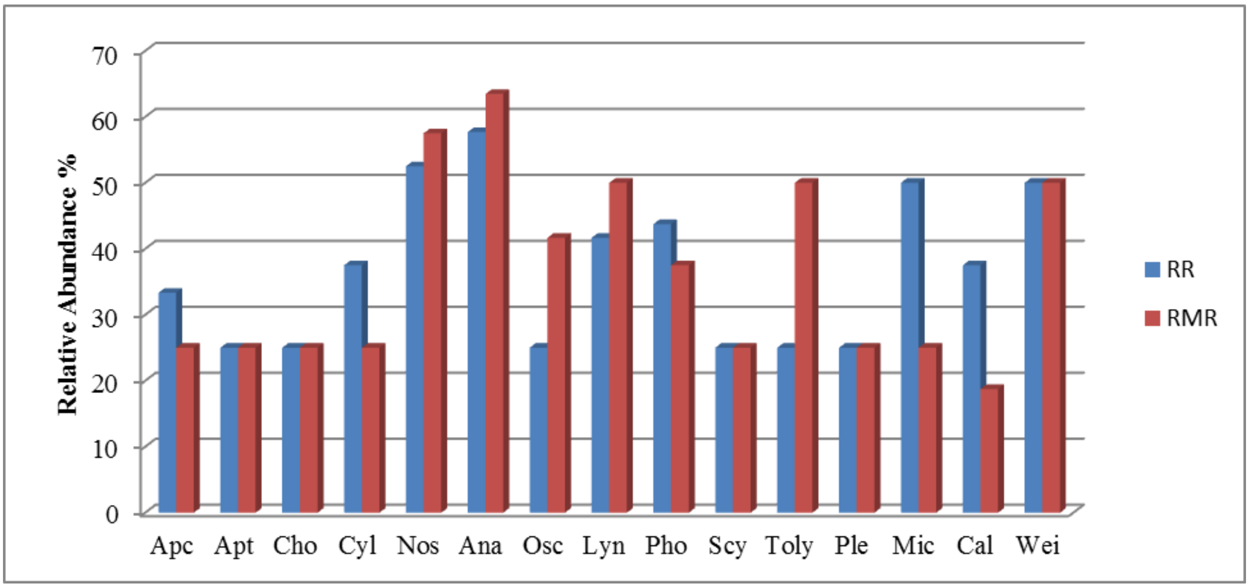

Figure 3. Relative abundance of the N_2- fixing cyanobacterial genera the RR and RMR cropping systems. Apc-*Aphanocapsa* , Apt-*Aphanothece* , Cho-*Chroococcus* , Cyl-*Cylindrospermum* , Osc-*Oscillatoria* , Pho-*Phormidium* , Scy-*Scytonema*, Toly-*Tolypothrix* , Ple-*Plectonema* , Mic-*Microchaete* , Cal- *Calothrix,* Wei-*Weistellopsis* , Nos-*Nostoc* , Ana-*Anabaena* , Lyn-*Lyngbya*

Table 5. Pearson's correlation coefficient between population number (MPN) of N_2-fixing cyanobacteria and soil physico-chemical in the two cropping systems

Soil properties	**MPN x10^4 / g of soil**	
	RR	**RMR**
Temperature(0C)	0.546*	0.693***
pH	0.244	0.531*
Conductivity(µS)	-0.213	-0.440*
Soil Moisture(SM %)	0.389	0.463*
Organic carbon (OC %)	0.469*	-0.198
N(mg/100g)	-0.182	-0.433
P(mg/100g)	0.760**	-0.220
K(mg/100g)	-0.315	-0.346
Na(mg/100g)	0.092	-0.691***
Ca(meq/100g)	-0.646**	-0.664
Mg(meq/100g)	0.1588	0.039

*, **and*** denote significance level of 0.05, 0.01 and 0.001 respectively

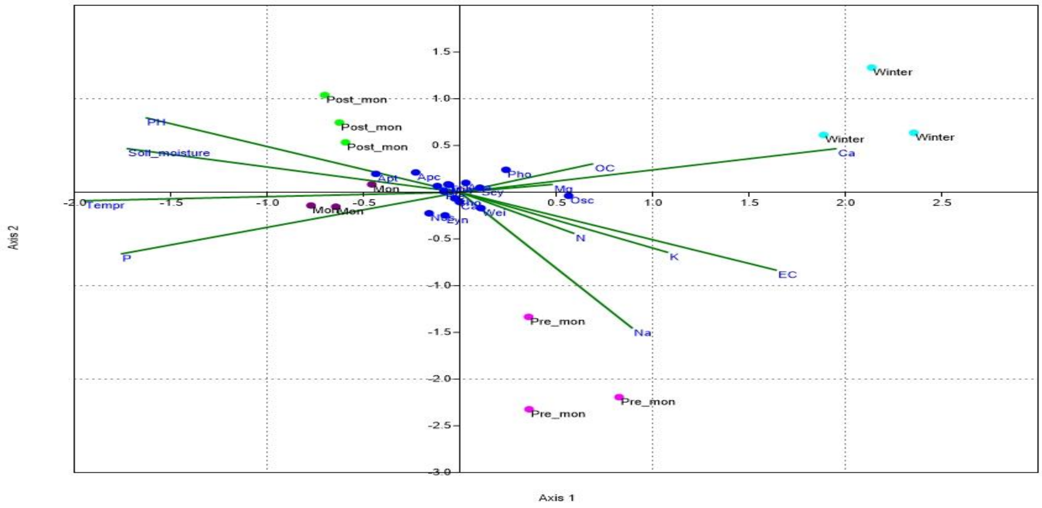

Figure 4. CCA (Canonical correspondence analysis) ordination diagram correlating relative abundance of cyanobacterial taxa and MPN values with soil physico chemical characteristics in RR sites in different seasons. Pre mon- Pre monsoon, Mon-Monsoon, Post mon- Post monsoon, Win-Winter, Apc-*Aphanocapsa* , Apt-*Aphanothece* , Cho-*Chroococcus* , Cyl-*Cylindrospermum* , Osc-*Oscillatoria* , Pho-*Phormidium* , Scy- *Scytonema*, Toly-*Tolypothrix* , Ple-*Plectonema* , Mic-*Microchaete* , Cal- *Calothrix,* Wei-*Weistellopsis* , Nos-*Nostoc* , Ana-*Anabaena* , Lyn-*Lyngbya*

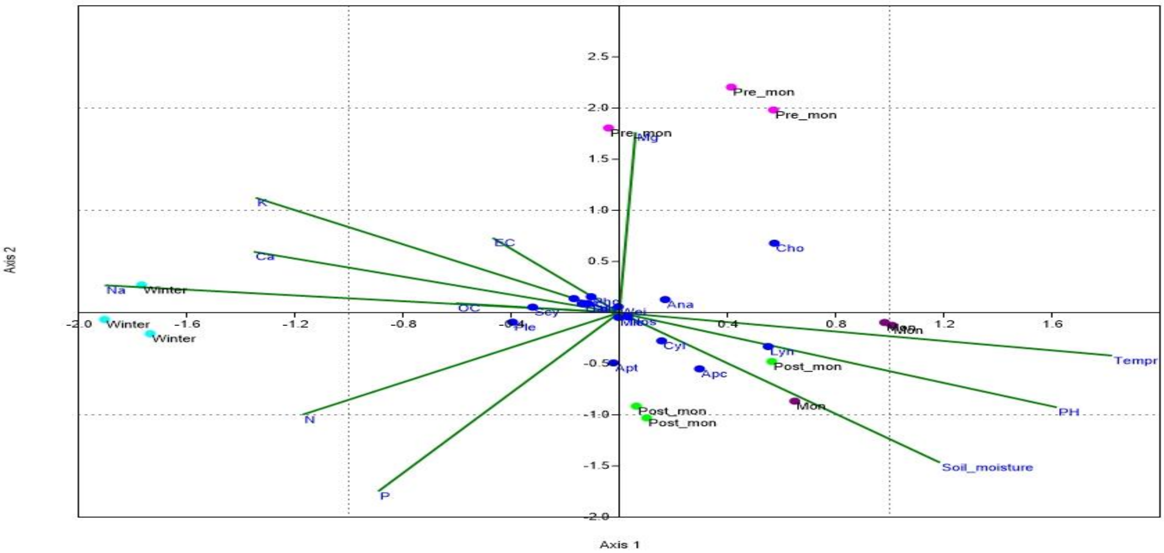

Figure 5. CCA (Canonical correspondence analysis) ordination diagram correlating relative abundance of cyanobacterial taxa and MPN values with soil physico chemical characteristics in RMR sites in different seasons. Pre mon- Pre monsoon, Mon-Monsoon, Post mon- Post monsoon, Win-Winter, Apc-*Aphanocapsa* , Apt-*Aphanothece* , Cho-*Chroococcus* , Cyl-*Cylindrospermum* , Osc-*Oscillatoria* , Pho-*Phormidium* , Scy- *Scytonema*, Toly-*Tolypothrix* , Ple-*Plectonema* , Mic-*Microchaete* , Cal- *Calothrix,* Wei-*Weistellopsis* , Nos-*Nostoc* , Ana-*Anabaena* , Lyn-*Lyngbya*

Relationship between cyanobacterial population and soil physico chemical properties

Results of Pearson's correlation analysis (Table- 5), revealed significant positive as well as negative correlation between the MPN of N_2-fixing cyanobacteria and soil properties. In RR fields, cyanobacterial population showed significant positive correlation with soil temperature, organic carbon (OC) and Phosphate (P) and showed significantly negatively correlattion with Calcium (Ca). In the RMR fields, MPN showed significant positive correlation with soil Temperature, pH and soil moisture (SM) and a significant negative correlation with conductivity (EC) and Sodium (Na). Similiar results were observed by Bisoyi and Singh [35]; Castenholtz and Waterbury, [36]; Whitton *et al*.,[37].

In order to understand the influence of season and soil properties on the abundance of the N_2-fixing cyanobacterial genera, CCA was also carried out on the two cropping systems. CCA based on the multivariate data yielded 0.043 and 0.060 eigen values showing 45% and 41% variability in generic abundance data in the RR and RMR fields. The ordination diagram (Fig-3) indicated that in RR fields soil temperature, pH, SM, and P were higher in the monsoon and post monsoon seasons. Simultaneously, Higher abundance of *Aphanocapsa*, *Aphanothece*, *Chroococcus*,*Cylindrospermum*, *Tolypothrix* ,*Plectonema*, *Microchaete*, *Calothrix*, *Nostoc*, *Anabaena*, *Lyngbya* were recorded in these seasons in comparison to winter and the Premonsoon season. Lesser abundance N_2-fixing cyanobacterial genera in winter and premonsoon season could be attributed to the higher content of Ca, EC, Na, K in the soil (Tab-3). The abundance of non heterocystous *Oscillatoria* and *Phormidium* genera were also associated with high values of Mg, N and OC contents of the soil.

In RMR fields, CCA analysis revealed that the cyanobacterial genera *Lyngbya*, *Aphanocapsa*, *Aphanothece*, *Phormidium*, *Cylindrospermum*, *Nostoc and Weistellopsis* were highly abundant in the monsoon and Post monsoon seasons (Fig-4). In these seasons, higher values of soil temperature, pH, SM, P and N were recorded in the crop fields. In contrast, the genera *Plectonema*, *Scytonema Phormidium and Calothrix* were found to be abundant in the winter season where values of OC, EC, Ca, Na, K were higher in the soils (Tab-4). *Chroococcus and Anabaena* spp. were abundant only in the premonsoon season in the RMR fields.

Our study showed that the diversity and composition of cyanobacteria is influenced by the soil physico chemical properties like pH, temperature, soil moisture, organic carbon, available nitrogen, phosphate, etc. The results were in conformity with Irisarri *et al.*,[18]; Nayak and Prasanna, [11]. Similar results were also obtained by Chunleuchanon *et al.*,[34]; Song *et al*., [15]; Saminathan *et al.*,[38] who observed that cyanobacterial population varied with the soil qualities in different seasons.

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