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Studies on N_2-fixing cyanobacterial diversity and its seasonal variation in rice fields soils of Brahmaputra floodplain of Kamrup District Assam.

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Abstract

The present investigation was carried out to study the N_2-fixing cyanobacterial diversity in rice fields' soil of Kamrup, which is one of the oldest landmass of Brahmaputra floodplain of Assam. A total of 71 species was identified belonging to 20 genera under 9 families. The filamentous heterocystous (80%) cyanobacteria showed clear dominance over the unicellular/colonial (14%) and filamentous non-heterocystous (6%) forms. The well represented families were Nostocaceae (54%), followed by Chroococcaceae (14%) and Rivulariaceae (13%). At species level, Anabaena topped with 31% followed by Nostoc with 17% and Calothrix with 8% respectively. PCA analysis revealed that rice fields' soils of the district were dominated by heterocystous filamentous forms of N_2-fixing cyanobacteria from July to December and unicellular forms from January to June.

Keywords: Brahmaputra River, Cyanobacteria, Diversity, PCA ordination, Rice fields, Seasonality.

Introduction

Nitrogen is an essential and most limiting nutrient for plant growth in most of the world's agricultural soils, and hence, crop production worldwide relies heavily on its inputs. Plant mainly depends upon combined or fixed forms of nitrogen such as ammonia and nitrate. With exponential growth of human population, as well as subsequently increasing demands of rice lead to put pressure on farmers and the other stakeholders to produce and procure huge amount of rice. Some farmers hence, has started using chemical nitrogenous fertilizer to acquire more production without knowing the negative effects of those chemicals. Biological nitrogen fixation, on the other hand, offers a natural means of providing nitrogen for plants.

N_2-fixing cyanobacteria are one of the main components of the micro-biota in rice field soils (Ladha and Reddy, 2003) that make a valuable contribution to soil fertility by fixing atmospheric nitrogen (Tiwari *et al.*, 2001). Nitrogen fixed by this biological process was estimated to contribute about 60% of the nitrogen requirement of the living organism (Venkataraman, 1993). They are hence, considered as natural biofertilizer (Baftehchi *et al*., 2007). Different authors attributed cyanobacteria to be an important organism in controlling organic pollutants through biodegradation (Cerniglia *et al*., 1980 and Chaillan, 2006). They further enhance plant growth by synthesizing and liberating growth promoting substances (Pandey *et al*., 2005; Karthikeyan *et al*., 2007; Zulpa *et al*., 2008). Cyanobacteria excrete organic acids that render phosphorus solubilisation, making phosphorus available to plants (Fuller and Rogers 1952; Singh *et al.,* 1981). They also increase the humus content, improve soil structure and dissolve certain soil minerals. In addition, they also add substantial amount of organic matter to the soil. These organic matters thus gathers, acts as a storehouse of nutrients like nitrogen, phosphorus and micronutrients and take part in soil fertility and increase the water holding capacity (Goyal, 2002).

Though cyanobacteria are ubiquitous in nature, higher amount of them, comprising more than half the population of heterocystous are reported to be grown at or floating above the surface of water logged rice fields (Ladha and Reddy, 1995), as rice fields provide a very congenial condition for abundant growth of N_2-fixing cyanobacteria (Whitton, 2000; Nayak *et al*., 2001). Rice-fields are considered as one of the highly dynamic ecosystems. Changes in the physico-chemical properties of the rice fields' soil could be well monitored due to changes in seasons and cultivation cycle (Roger and Kulasooria, 1980; Fernández-Valiente and Quesada, 2005) and could be attributed to the variation in cyanobacterial diversity, distribution, density (Watanabe *et al*., 1978), biomass (Gupta, 1966) and contribution to the total nitrogen fixed in rice field soils (Watanabe and Cholitkal, 1979).

The present endeavour, therefore, was aimed to study the N_2-fixing cyanobacterial diversity along with their seasonal variation in rice fields' soils of Brahmaputra floodplain of Kamrup District, Assam.

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Material and Methods

Sites selection

The present study was conducted in the year 2011 in different rice fields of Kamrup district, Assam which lies between 25˚ 46' to 26˚ 49' N latitude and 90˚ 48' to 91˚ 50' E longitude and covers an area of ca. 4345 sq. km. A total of nine rice fields were selected for soil collection from three different sites situated on the lower Brahmaputra floodplain. Three rice fields were chosen on the south bank of the river, three on the north bank and three rice fields from southern bank of the almighty River Brahmaputra.

Sample collection and culture

Fresh cyanobacterial samples were picked up carefully from the surface of the soil and collected in glass bottles. Slides were prepared and examined under microscope as soon as it was brought to the laboratory. Soil samples were also collected from 8-10 randomly selected spots to a depth of 10-15 cm. Sub -samples were then mixed thoroughly, air dried, powered and finally passed through a 0.1 mm sieve. Composite samples from each selected rice field were then stored in polythene bags separately for cyanobacterial culture. Collections of samples were done in two months interval time.

To culture the N_2-fixing cyanobacteria in laboratory conditions, soil samples weighed 0.5g was inoculated in sterilized nitrogen free BG-11 medium (Stanier *et al.,* 1971) at $30^0 \pm 2^0$C temperature in 2.3K lux light intensity for 20-25 days. To obtain a pure culture, isolation methods like **'**streak plate' and 'Spread plate' was performed in semi-solid media. Identification was done on morphological basis by following the keys given by Desikachary (1959).

Statistical analysis

Principal Component Analysis (PCA) was done using the statistical program XLSTAT.

Results and Discussion

Species diversity

A total of 71 species of N_2-fixing cyanobacteria was identified belonging to 20 genera under 9 families. Out of them, 57 species were filamentous heterocystous under 14 genera and 7 families. 10 species were unicellular under 5 genera and 1 family and 4 species belonging to single genus were filamentous non-heterocystous. Among filamentous heterocystous, Nostocaceae topped with 4 genera (*Anabaena*, *Anabaenopsis*, *Aulosira* and *Nostoc*), followed by Rivulariaceae with 3 genera (*Calothrix*, *Gloeotrichia*, *Rivularia*) and Scytonemataceae (*Scytonema*, *Tolypothrix*) and Stignemataceae (*Hapalosiphon, Westiellopsis*) with 2 genera. The rest of the filamentous heterocystous families had only 1 genus each and were belonged to Mastigocladaceae (*Mastigocladus*), Mastigocladopsidaceae (*Mastigocladopsis*) and Microchaetaceae (*Microchaete*). Oscillatoriaceae was the lone filamentous non-heterocystous family with the genus *Lyngbya*. Whereas, Chroococcaceae, the only recorded unicellular family had 5 genera (*Aphanocapsa*, *Aphanothece*, *Chroococcus*, *Gloeocapsa* and *Synechococcus*) (Table.1). Thus, the filamentous heterocystous (80%) cyanobacteria showed clear dominance over the unicellular/colonial (14%) and filamentous non-heterocystous (6%) forms. The dominance of filamentous heterocystous forms over other forms (unicellular and filamentous non-heterocystous) was also recorded in other rice field soils of India (Nayak and Prasanna, 2007). Hazarika (2007) reported 20.83% of unicellular/colonial and 30.56% of filamentous non-heterocystous form which was outnumbered by filamentous heterocystous cyanobacteria (48.61%) in soils of greater Guwahati.

Nostocaceae was the dominant family with 54% of species, followed by Chroococcaceae (14%), Rivulariaceae (13%), Scytonemataceae (7%), Oscillatoriaceae (6%), Stignemataceae (3%). Whereas, Mastigocladaceae, Mastigocladopsidaceae and Microchaetaceae contributed with only 1% of species each (Fig.1). Species of Nostocaceae, Chroococcaceae, Rivulariaceae, Oscillatoriaceae, Scytonemataceae and Mastigocladopsidaceae were reported from all the three sites of the district. Species of Stigonemataceae and Mastigocladaceae were reported in the rice fields of Southwest Kamrup and Microchaetaceae in rice fields of North Kamrup only.

Anabaena was reported to be the dominant genera with a total of 31% species. *Nostoc* (17%) and *Calothrix* (8%) were the second and third biggest genera followed by unicellular cyanobacteria *Aphanocapsa* (6%) (Fig.2). The predominance of *Anabaena* and *Nostoc* irrespective of chemical/biofertilizers supplementation and stage of crop growth was reported in different rice growing areas of India (Singh *et al*., 1996; Singh *et al*., 1997; Nayak *et al*., 2001, 2004). Thamizh and Sivakumar (2011) also reported *Anabaena* and *Nostoc* as the dominant genera among the heterocystous form of cyanobacteria in rice fields of Cuddalore district, Tamil Nadu.

Table.1. Diversity of N_2-fixing cyanobacteria enumerated in all the rice fields of Kamrup district

Sp. No.	Species Name	Family	South west			North			South east		
			1	2	3	4	5	6	7	8	9
1	*Aphanocapsa biformis* A.Braun	Chroococcaceae	-	-	-	-	+	-	-	-	-
2	*Aphanocapsa littoralis* Hansgirg	Chroococcaceae	-	+	+	-	-	-	-	-	-
3	*Aphanocapsa pulchra* (Kützing) Rabenhorst	Chroococcaceae	-	-	+	-	-	+	-	-	-
4	*Aphanocapsa roeseana* De Bary	Chroococcaceae	-	+	-	-	-	+	-	-	-
5	*Aphanothece microscopica* Nägeli	Chroococcaceae	-	-	-	-	-	-	+	+	-
6	*Aphanothece naegelii* Wartmann	Chroococcaceae	-	-	-	-	-	-	+	+	+
7	*Chroococcus montanus* Hansgirg	Chroococcaceae	+	-	-	-	-	-	+	+	-
8	*Gloeocapsa decorticans* (A.Braun) P.Richter	Chroococcaceae	-	-	+	+	-	+	-	-	-
9	*Gloeocapsa quaternata* Kützing	Chroococcaceae	-	-	+	-	-	-	-	-	-
10	*Synechococcus aeruginosus* Nägeli	Chroococcaceae	+		+	-	-	-	-	-	-
11	*Mastigocladus laminosus* Cohn ex Kirchner	Mastigocladaceae	+	+	-	-	-	-	-	-	-
12	*Mastigocladopsis jogensis* Iyengar & Desikachary	Mastigocladopsidaceae	-	+	+	+	-	-	-	-	-
13	*Microchaete aequalis* (Frémy) Desikachary	Microchaetaceae	-	-	-	+	-	-	-	-	-
14	*Anabaena ambigua* C.B.Rao	Nostocaceae	-	-	-	+	-	-	+	+	-
15	*Anabaena anomala* F.E.Fritsch	Nostocaceae	-	+	+	+	+	-	+	+	-
16	*Anabaena circinalis* Rabenhorst ex Bornet & Flahault	Nostocaceae	+	-	+	+	+	+	-	-	-
17	*Anabaena constricta* (Szafer) Geitler	Nostocaceae	-	+	-	-	-	-	+	+	+
18	*Anabaena doliolum* Bharadwaja	Nostocaceae	-	-	+	+	+	-	-	-	-
19	*Anabaena fertilissima* C.B.Rao	Nostocaceae		+	+	+	+	+	-	-	-
20	*Anabaena flos-aquae* Brébisson ex Bornet & Flauhault	Nostocaceae	-	-	-	+	-	-	-	-	-
21	*Anabaena fuellebornii* Schmidle	Nostocaceae	+	-	-	-	-	-	-	-	-
22	*Anabaena gelatinicola* Ghose	Nostocaceae	-	-	-	-	+	-	-	-	-
23	*Anabaena iyengari* Bharadwaja	Nostocaceae	-	-	-	-	-	-	-	-	+
24	*Anabaena orientalis* S.C.Dixit	Nostocaceae	-	-	+	-	-	-	-	-	-
25	*Anabaena oryzae* F.E.Fritsch	Nostocaceae	+	+	+	-	+	+	-	-	-
26	*Anabaena oscillarioides* Bory de Saint-Vincent ex Bornet & Flahault	Nostocaceae	-	-	-	-	-	-	+	+	-
27	*Anabaena smithii* (Komárek) M.Watanabe	Nostocaceae	-	-	-	+	-	-	-	-	-
28	*Anabaena sphaerica* Bornet & Flahault	Nostocaceae	-	+	-	-	-	-	-	-	+
29	*Anabaena spiroides* Klebahn	Nostocaceae	+	+	+	+	-	+	-	-	-
30	*Anabaena torulosa* Lagerheim ex Bornet & Flahault	Nostocaceae	-	-	-	-	-	+	-	-	-
31	*Anabaena unispora* N.L.Gardner	Nostocaceae	+	-	-	-	-	-	-	-	-
32	*Anabaena vaginicola* F.E.Fritsch & Rich	Nostocaceae	-	-	+	-	+	+	-	-	-

33	*Anabaena variabilis* Kützing ex Bornet & Flahault	Nostocaceae	+	+	+	+	-	-	+	+	+
34	*Anabaena variabilis* var. *ellipsospora* F.E.Fritsch	Nostocaceae	-	-	+	-	+	-	-	-	+
35	*Anabaena volzii* Lemmermann	Nostocaceae	+	-	-	-	-	-	-	-	-
36	*Anabaenopsis tanganyikae* (G.S.West) Woloszynska & V.V.Miller	Nostocaceae	-	-	-	-	-	-	-	+	-
37	*Aulosira aenigmatica* Frémy	Nostocaceae	-	-	-	-	-	-	-	-	+
38	*Aulosira bombayensis* Gonzalves	Nostocaceae	-	-	-	-	-	-	+	-	-
39	*Aulosira prolifica* Bharadwaja	Nostocaceae	-	+	+	-	-	-	-	-	-
40	*Nostoc calcicola* Brébisson ex Bornet & Flahault	Nostocaceae	-	-	+	+	-	-	-	-	-
41	*Nostoc carneum* C.Agardh ex Bornet & Flahault	Nostocaceae	-	+	+	+	-	-	-	-	-
42	*Nostoc commune* Vaucher ex Bornet & Flahault	Nostocaceae	+	-	-	-	-	-	-	-	-
43	*Nostoc ellipsosporum* Rabenhorst ex Bornet & Flahault	Nostocaceae	+	-	+	-	-	+	-	-	-
44	*Nostoc hatei* S.C.Dixit	Nostocaceae	-	+	-	-	-	-	-	+	+
45	*Nostoc linckia* Bornet ex Bornet & Flahault	Nostocaceae	+	+	+	+	+	+	-	-	+
46	*Nostoc muscorum* C.Agardh ex Bornet & Flahault	Nostocaceae	-	+	+	-	-	-	+	+	-
47	*Nostoc passerinianum* Bornet & Thuret ex Bornet & Flahault	Nostocaceae	-	+	+	-	-	-	-	-	-
48	*Nostoc paludosum* Kützing ex Bornet & Flahault	Nostocaceae	-	-	+	+	+	-	+	+	+
49	*Nostoc piscinale* Kützing ex Bornet & Flahault	Nostocaceae	+	+	+	+	+	+	-	-	+
50	*Nostoc punctiforme* Hariot	Nostocaceae	+	+	+	-	-	-	+	+	-
51	*Nostoc spongiaeforme* C.Agardh ex Bornet & Flahault	Nostocaceae	-	-	-	+	+	+	-	+	-
52	*Lyngbya allorgei* Frémy	Oscillatoriaceae	-	-	-	-	-	-	+	+	-
53	*Lyngbya palmarum* Martens ex Brühl & Biswas	Oscillatoriaceae	-	-	-	+	+	+	+	-	-
54	*Lyngbya rubida* Frémy	Oscillatoriaceae	+	+	-	+	-	-	-	+	-
55	*Lyngbya perelegans* Lemmermann	Oscillatoriaceae	+	-	+	-	-	-	-	-	-
56	*Calothrix brevissima* G.S.West	Rivulariaceae	+	-	-	+		+	-	-	-
57	*Calothrix clavatoides* Ghose	Rivulariaceae	+	+	-	-	-	-	-	-	-
58	*Calothrix marchica* Lemmermann	Rivulariaceae	+	+	-	+	+	+	+	+	+
59	*Calothrix membranacea* Schmidle	Rivulariaceae	-	-	+	-	+	+	-	-	-
60	*Calothrix scytonemicola* Tilden	Rivulariaceae	+	-	+	-	-	-	-	-	-
61	*Calothrix weberi* Schmidle	Rivulariaceae	-	+	+	-	-	-	+	-	+
62	*Gloeotrichia longicauda* Schmidle	Rivulariaceae	+	+	-	-	-	-	-	+	+
63	*Gloeotrichia pilgeri* Schmidle	Rivulariaceae	+	-	-	-	-	-	-	-	-
64	*Rivularia hansgirgi* Schmidle	Rivulariaceae	-	-	+	+	+	-	-	-	-
65	*Scytonema fritschii* S.L.Ghose	Scytonematacea e	-	+	+	-	-	-	-	-	-
66	*Scytonema hofmannii* C.Agardh ex Bornet & Flahault	Scytonematacea e	-	-	-	+	-	-	-	-	-
67	*Scytonema simplex* Bharadwaja	Scytonematacea e	-	+	-	+	+	-	-	-	-
68	*Tolypothrix nodosa* Bharadwaja	Scytonematacea e	-	+	+	-	-	-	-	-	-

69	*Tolypothrix tenuis* Kützing ex Bornet & Flahault	Scytonematacea e	-	-	-	-	-	-	+	+	-
70	*Hapalosiphon welwitschii* West & G.S.West	Stigonemataceae	+	+	+	-	-	-	-	-	-
71	*Westiellopsis prolifica* Janet	Stigonemataceae	-	+	+	-	-	-	-	-	-

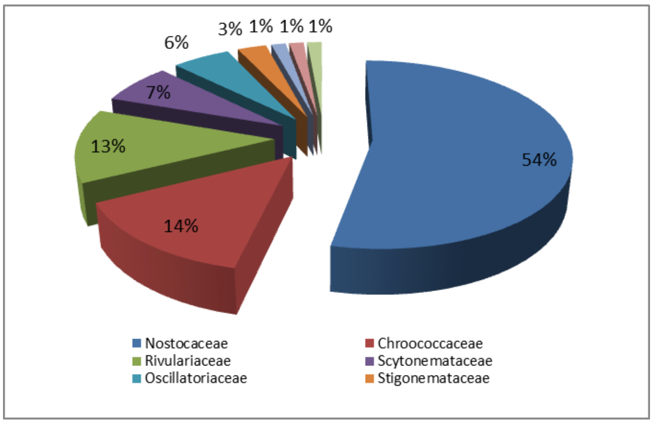

Figure.1. Percentage composition of N_2-fixing cyanobacterial families in rice fields' soil of Kamrup.

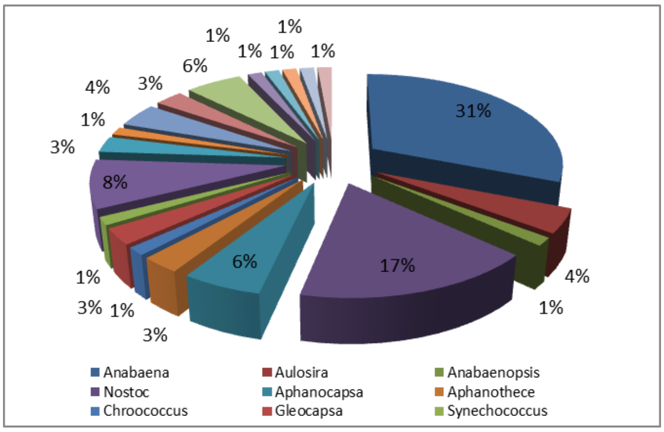

Figure.2. Percentage composition of N_2-fixing cyanobacterial genera in rice fields' soil of Kamrup.

Anabaena, *Calothrix* and *Nostoc* were recorded with maximum number of species in rice grown areas of Tripura (Singh *et al*., 1996). Singh *et al*. (1997) also recorded highest number of species belonging to genera *Anabaena* and *Nostoc* in rice fields of Nagaland. In the rice field of Sonitpur district of Assam, *Nostoc, Anabaena, Aulosira, Calothrix, Westiellopsis and Aphanocapsa* were dominant (Dasgupta and Ahmed, 2013). *Nostoc* and *Anabaena* can be considered as one of the most versatile and highly competitive genera observed in all types of environments, that have the capacity to colonize as floating assemblages or as edaphic forms in rice fields soil (Singh *et al*., 1996; Singh *et al*., 1997a; Nayak *et al*., 2001, 2004; Prasanna and Nayak, 2007; Thamizh and Sivakumar, 2011).

Seasonal variations

To study the seasonal variation of N_2-fixing cyanobacteria, Principal Component Analysis (PCA) was carried out. The PCA ordination Map (Figure.3. to 5.) indicated close relationship between the species of cyanobacteria and the months during which the species were mostly dominant. PCA (Figure.3.) showed that majority of cyanobacterial species reported from rice fields of Southwest Kamrup, were dominant during July-August. These species were *Chroococcus montanus*, *Anabaena anomala, A. fertilissima, A. vaginicola, A. variabilis, Aulosira prolifica, N. ellipsosporum, N. punctiforme, Lyngbya rubida, Rivularia hansgirgi* (sp7, sp15, sp19, sp32, sp33, sp39, sp43, sp50, sp54, sp64). September-October was favourable for *Lyngbya perelegans, Calothrix* *brevissima, Nostoc passerinianum, Gloeotrichia Pilgeri and Sytonema simplex* (sp55, sp56, sp47, sp63, sp64) and November-December was favourable for *Anabaena constricta, A. oryzae, Nostoc paludosum, Calothrix*

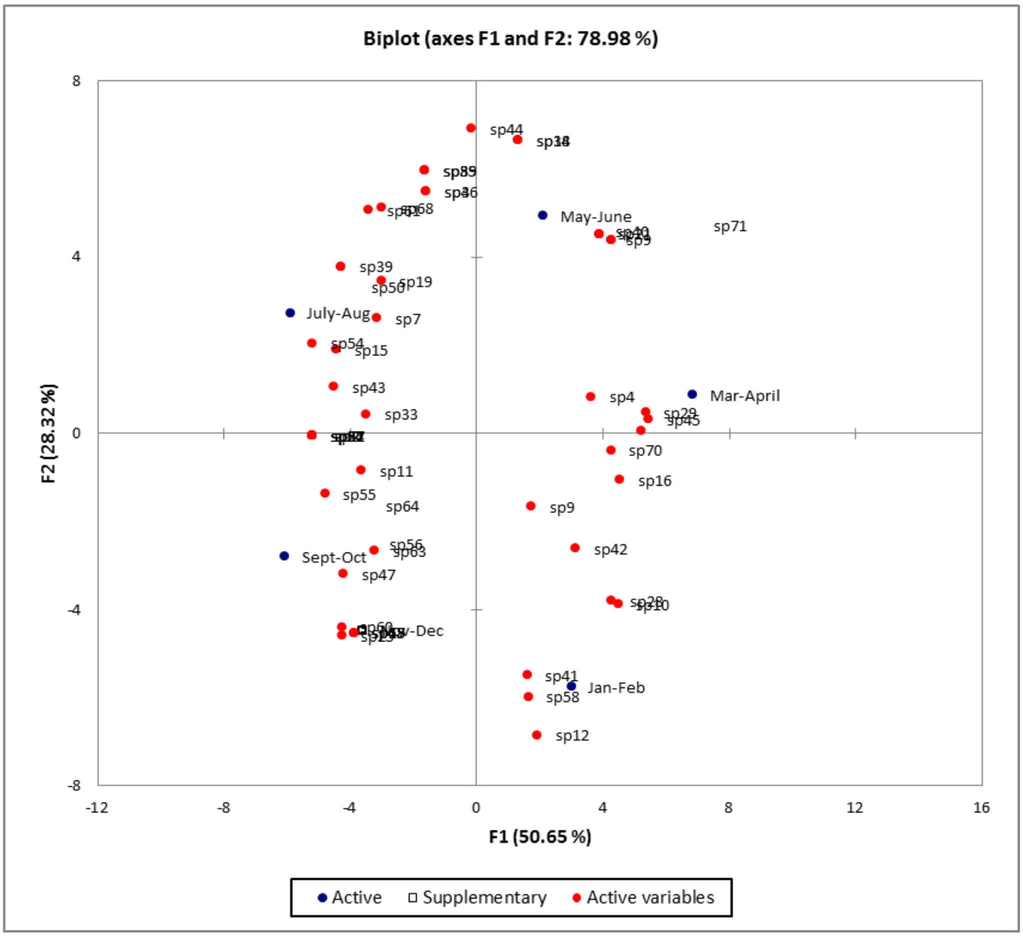

Figure.3. PCA ordination analysis for rice fields' soil of Southwest Kamrup.

Calothrix scytonemicola, and *Scytonema fritschii* (sp17, sp25, sp48, sp60, sp65). *Synechococcus aeruginosus, Mastigocladopsis jogensis, Anabaena sphaerica, Nostoc carneum* and *Calothrix marchica* (sp10, sp12, sp28, sp41 and sp58) were the dominant species recorded during January-February, whereras *Aphanocapsa roeseana, Anabaena spiroides, Nostoc linckia* (sp4, sp29, sp45 and sp50) and *Anabaena fuellebornii, Nostoc calcicola, Gloeocapsa quaternata* and *Westiellopsis prolifica* (sp21, sp40, sp9, sp71) were the dominant species in March-April and May-June respectively.

In the rice fields of North Kamrup, *Microchaete aequalis, Anabaena anomala, Anabaena variabilis, Nostoc doliolum* and *Scytonema simplex* (sp13, sp15, sp18, sp40, and sp67) were the dominant species during July-August. *Nostoc calcicola, Lyngbya palmarum, Lyngbya rubida, Calothrix brevissima Calothrix marchica, Calothrix membranacea, Rivularia hansgirgi* (sp33, sp53, sp54, sp56, sp58, sp59, sp64) were the dominant species during September-October and *Mastigocladopsis jogensis, Anabaena fertilissima, A. oryzae, A. torulosa* and *Nostoc linckia* (sp12, sp19, sp25, sp30 and sp45) during November-December. During January-February, *Aphanocapsa pulchra*,

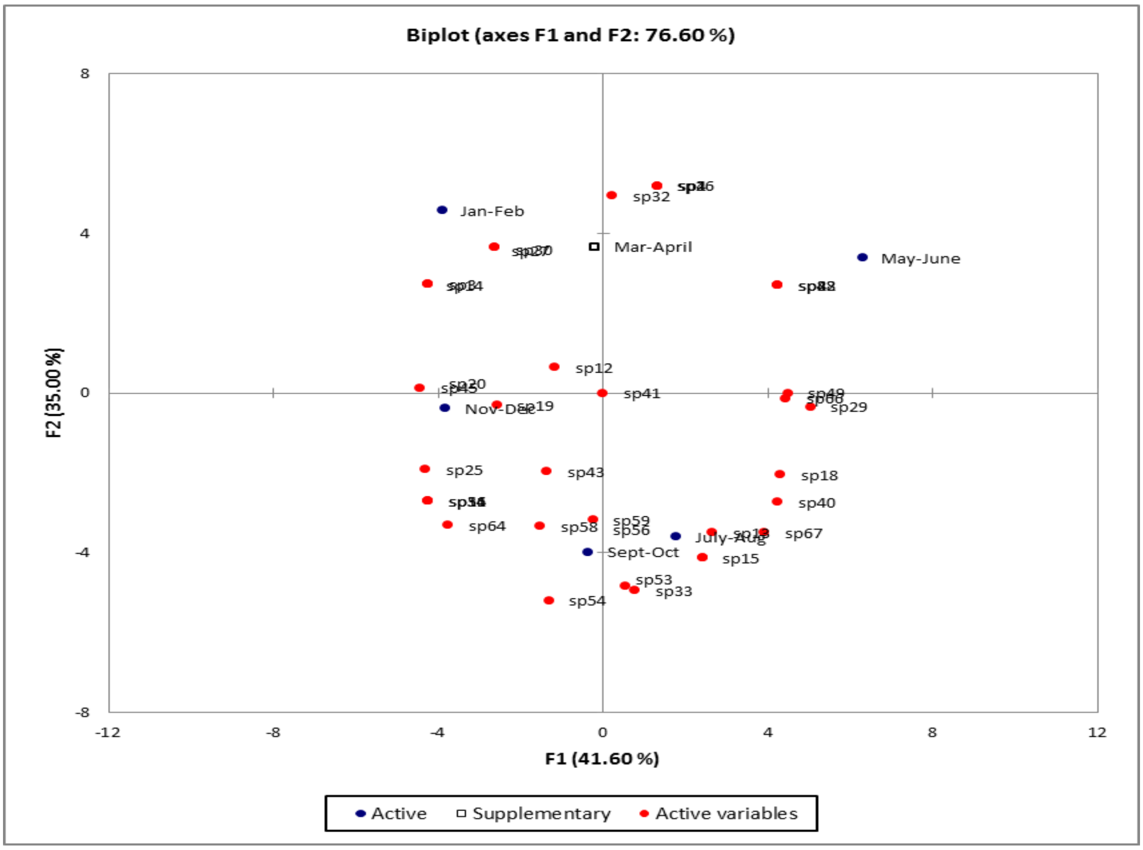

Figure.4. PCA ordination analysis for rice fields' soil of North Kamrup.

Anabaena ambigua, A. flos-aquae, A. smithii (sp3, sp14, sp20, sp27), during March-April were *Aphanocapsa biformis, Aphanocapsa roeseana, Anabaena oscillarioides, A. vaginicola* (sp1, sp4, sp26, sp32) and during May-June *Gloeocapsa decorticans, Anabaena gelatinicola* and *Nostoc paludosum* (sp8, sp22, sp48)were the dominant species occurring in the rice fields of North Kamrup (Fig.4).

In the rice fields of Southeast Kamrup, *Anabaena anomala, A. sphaerica, A. variabilis, Nostoc linckia, N. muscorum, N. piscinale,* (sp15, sp28, sp33, sp45, sp49, sp46) were the dominant species during July-August. *Anabaena variabilis* var. *ellipsospora,*

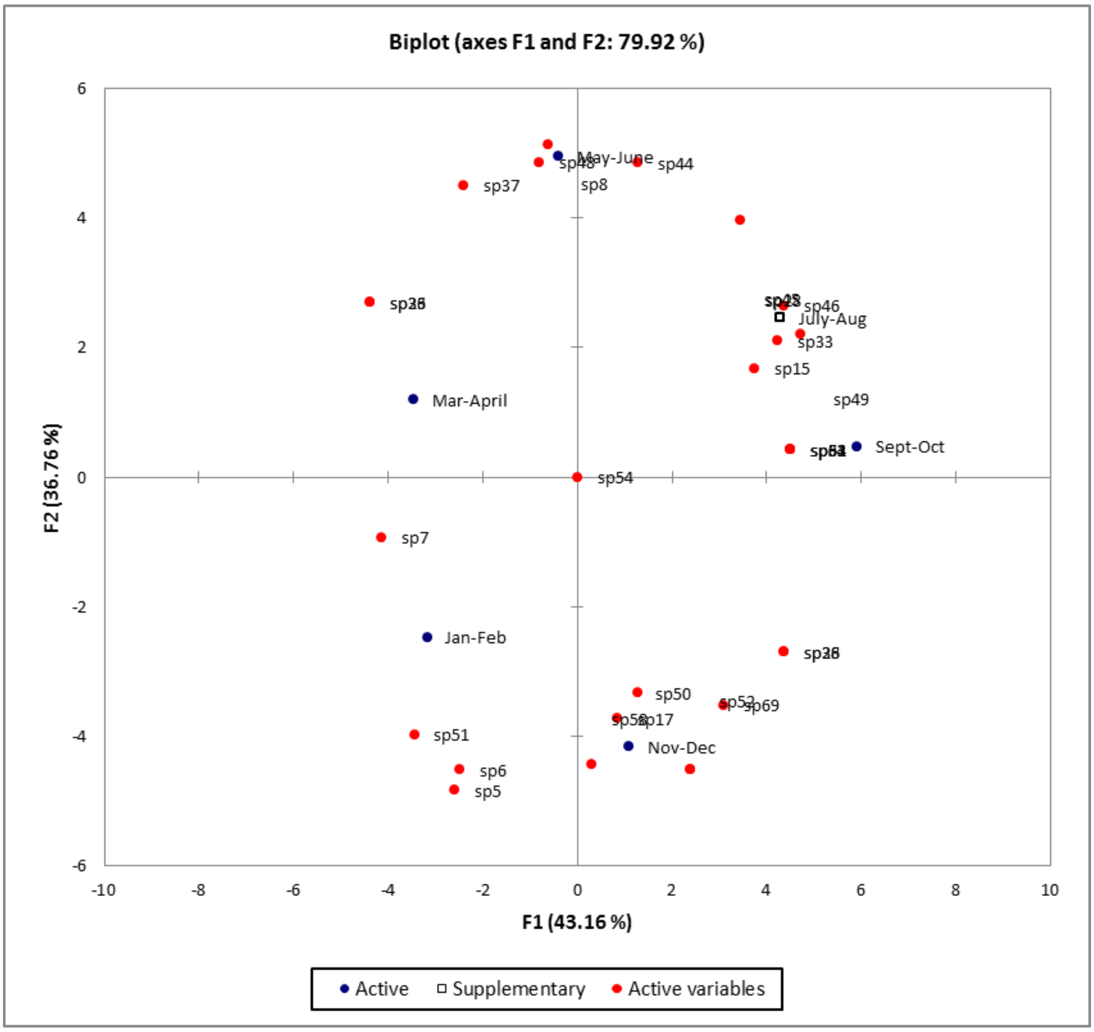

Figure.5. PCA ordination analysis for rice fields' soil of Southeast Kamrup.

Lyngbya palmarum and *Calothrix weberi* and *Gloeotrichia longicauda* (sp34, sp53, sp61, and sp62) were dominant during September-October and *Anabaena constricta, N. punctiforme, Lyngbya allorgei* and *Calothrix marchica* and *Tolypothrix tenuis* (sp17, sp50, sp52, sp58 and sp69) during November-December. During January-February the dominant species were *Aphanothece microscopic, A. naegelii, Chroococcus montanus* and *Nostoc spongiaeforme* (sp5, sp6, sp7 and sp51). *Anabaena iyengarii* and *Anabaenopsis tanganyikae* (sp23 and sp36) were the only dominant species recorded during March-April. The dominant genera in May-June were *Gleocapsa decorticans, Aulosira aenigmatica, Nostoc hatei* and *N. paludosum* (sp8, sp37, sp44 and sp48) (Fig.5).

PCA analysis thus revealed that rice fields' soil of Kamrup district were dominated by heterocystous filamentous forms of N_2-fixing cyanobacteria from July to December and unicellular forms from January to June. PCA too revealed that *Anabaena* and *Nostoc* were the most dominant genera among the heterocystous filamentous forms. Species of these two genera were observed in entire sampling period but their optimum growth recorded from July-December. *Calothrix, Lyngbya*, *Gloeothrichia*, *Scytonema*, *Tolypothrix* were the prominent genera next to *Anabaena* and *Nostoc* which mostly flourished during September to December. In contrast, the unicellular forms restricted themselves to a particular season. *Aphanocapsa* was the dominant genus recorded during January to April and *Gloeocapsa* during May-June. The changes in the environment or the seasonal variation might be the reason that affect particular species and induced the dominance of other species, which led to the succession of several species in a course of time (Muthukumar *et al*., 2007).

Conclusion

The rice fields' soils of Kamrup harbour a rich population (71 species) of N_2-fixing cyanobacteria. *Anabaena* (31%) and *Nostoc* (17%), that belongs to the family Nostocaceae were the most dominant genera recorded therein. Their presence was also observed throughout the whole sampling period in all the rice fields. So, strains of these two potent nitrogen fixing genera which are already blessed with the favourable local environment could be used as indigenous biofertilizer to maintain a sustainable agro ecosystem.

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