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Responses of few rice field cyanobacterial population of Brahmaputra flood plain to different fertilizer regimes

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Abstract

Gradual increase in the use of chemical fertilizers in agricultural activity around the Brahmaputra River Valley has emerged as a serious threat to the soil health and its inherent micro biota. In the present study, effect of five different fertilizer regimes on the soil physico chemical properties and abundance of cyanobacterial population were investigated in two rice cropping systems - double rice cultivation (DR) and rice rotated with mustard (RR) in four seasons. In each cropping system, plots laid down in randomized complete block design were subjected to five fertilizer treatments - T0: No fertilizer applied (control), T1: 200 kg/ha cow manure, T2: chemical fertilizers (NPK), T3: 50% NPK fertilizer + 200 kg/ha cow manure and T4: 30% NPK fertilizer + 200 kg/ha cow manure + crop straw. Results of multivariate analysis showed that both fertilizer treatment and season significantly regulated the soil characteristics and the most probable number (MPN) of cyanobacterial population as well. The T3 and T4 treated plots showed highest MPN of cyanobacteria and higher abundance of the dominant genera viz. *Nostoc, Anabaena, Weistellopsis, Calothrix, Scytonema and Phormidium.* The fertilizer regimes significantly ($P<0.05$) affected the soil organic carbon and soil nitrogen in RR fields whereas the later was unaffected in DR fields. Sampling time had a significant ($P<0.05$) effect on soil moisture in both the cropping systems. In DR fields, monsoon season recorded the highest abundance of cyanobacterial population, whereas in RR fields cyanobacterial population was highest in the post monsoon season.

Key words: Agriculture; rice cropping; fertilizer regime; relative abundance; crop straw

Introduction

The Brahmaputra River Valley, located in the North Eastern Region of Indian sub-continent is an extensive flood plain characterized by tropical monsoon climate. High rainfall during monsoon makes the valley suitable for rice cultivation and it is cultivated as a staple crop throughout the year in the entire valley. The prevalent crop sequences cultivated in the region are autumn rice - winter rice, autumn rice - winter rice - mustard, winter rice - wheat/mustard/pulses and jute - winter rice - vegetables, etc.

Population explosion from mid 1990 onwards and subsequent increase of high demand of food grains compelled the native farmers to opt for modern agricultural technologies including adoption of synthetic fertilizers to amplify crop yields (Singh and Sharma 2003; Santos *et al*. 2012). Though the use of commercially available fertilizers are neither ecofriendly nor economically viable (Cassman and Pingali 1994; Prasanna 2013) to the lower income group of farmers, it is very difficult for them to way out from using these chemicals. All these chemical fertilizer have some impact not only on the crops grown there but also on the soil microbiota including cyanobacteria of the soil systems. Cyanobacteria is a group of soil microorganisms seem to be a potential environmental friendly supportive for the soil which supplements N_2, organic carbon and different nutrients (Vaishampayan *et al*. 2001; Sinha *et al*. 2002; Choudhury and Kennedy 2005; Rai 2006). They buffer the soil pH, excrete growth promoting substances into the soil, increase the fertilizer use efficiency of crop plants (Mandal *et al*. 1998; Kaushik 2004; Karthikeyan *et al.* 2007), help in soil aggregation, water retention (Richert 2005; Maqubela *et al.* 2009) and improves the soil organic carbon content (Singh and Bisoyi 1989; Swarnalakshmi et al. 2007; Maqubela et al. 2009; Saadatnia and Riahi 2009) contributing to the growth and yield of rice (Ahmad 2001; Prasanna *et al.* 2009 a, b; Saadatnia and Riahi 2009).

The abundance of these beneficial micro inhabitants of soil depends upon a number of physical, chemical and biological factors of the soil (Irisarri *et al.* 2001). Indiscriminate supplement of chemical fertilizers is one such factor of serious concern as it affects the overall soil health by modifying soil properties as well as the activity and composition of the inherent cyanobacterial populations (Prosperi *et al.* 1992; Sinha and Hader 1996; Irrisari *et al.* 2001; Leganes *et al*. 2001). A few earlier workers reported significant differences in the cyanobacterial abundance and population number following application of different regimes comprising of organic-inorganic fertilizers (Yanni 1992; Yanni *et al*.1993; Leganes *et al.* 2001; Sharkawi *et al.* 2006; El-all *et al.* 2013; Spencer and Linguist 2013) in rice fields. Previous studies also indicated that cyanobacteria are affected by seasonal variations (Song *et al.* 2005; Queseda *et al*. 1995, 1997) and exhibit a crop specific and crop stage - specific diversity (Roger and Kulasooriya

1980; Nayak *et al.* 2001). Hence the shifts of cyanobacterial populations which act as a natural biofertilizer in rice field soil must be understood well in relation to fertilizer applications and season in order to suggest a proper agricultural management practice. There is scanty information regarding the population and abundance of cyanobacteria in the flood plains of Brahmaputra river and no work has been carried out to study the interrelation of the beneficial group of organisms with fertilizers and seasonal impact. The present endeavor was therefore aimed to evaluate the effect of different fertilizer regimes on cyanobacterial population and soil physico chemical properties in relation to season and cropping practices prevalent in the Brahmaputra flood plains.

Materials and methods

Field description and experimental design

The experimental site was located in the Borbhag ($26^0$22’55.5” N latitudes and $91^0$28’23.3” E longitudes, 89 m above mean sea level) of Nalbari district (Assam) lying in the Lower Brahmaputra Valley Agro Climatic Zone .The fertilization experiments were carried out in two typical rice cropping systems practiced in the region viz. autumn rice - winter rice or double rice cultivation (DR) and winter rice rotated with mustard (RR). In the DR system, ploughing process starts with the onset of monsoon (May) each year for sowing of autumn rice and harvesting is done during October- November. Subsequently, winter rice cultivation starts in the same field from January onwards and harvest in late April. In the RR system, after harvesting of summer rice, the fields are ploughed and tilled well to sow mustard in the month of November and the crop is harvested by the end of March every year.

Field plots of size 6m x 6m were laid out following a complete randomised block design. Five fertilizer treatments were applied each having three replicates. The regimes were T0: No fertilizer applied (control), T1: 200 kg/ha cow manure, T2: chemical (NPK) fertilizers, T3: 50% NPK fertilizer +200 kg/ha cow manure, T4: 30% NPK fertilizer + 200 kg/ha cow manure + crop straw.

Soil sampling and analysis

Soil sampling was carried out at four seasons, i.e. S1-pre monsoon (Feb-April), S2- monsoon (May-September), S3-post monsoon (October-November) and S4-winter (December-January). Ten replicates of soil samples were collected from the plough layer (0–20 cm) of each plot in sterile plastic bag and transported to the laboratory. The composite samples were dried, sieved (through 2 mm mesh size) and thoroughly homogenized for physico chemical analysis. Soil samples were inoculated in freshly prepared liquid BG-11± N media (Rippka *et al.* 1979) and kept under aseptic laboratory condition with light intensity of 2500~3500 lux at 25^0C. MPN of cyanobacterial population was enumerated following Singh et al. (2002) and isolated species were identified using Desikachary (1959) and Komarek and Anagnostidis (2005). The relative abundance of the species were calculated using the following formula:

$$\textbf{Relative abundance} = \frac{Y}{X} \times 100$$

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

Physico-chemical properties of soil

The soil analysis of each sample was performed in the Department of Botany, Gauhati University. The soil pH and electrical conductivity (EC) was measured using digital pH meter (Biochem PM 79) and conductivity meter (Systronics 304) respectively following Black (1992). Soil moisture was calculated using Soil Survey Standard Test Method. The soil organic carbon (OC), available phosphorus (P), potassium (K) and soil nitrogen (N) were estimated as per procedures described by Trivedi and Goel (1986).

Fertilizer and manure application

Single super phosphate, muriate of potassium and urea were applied as fertilizer to the concerned plots at the recommended dose of N (120 kg/ha) P_2O_5 (80 kg/ha): K_2O (60 kg/ha) in both the rice cropping systems. Doses of P, K and manure were applied as basal fertilizers before transplanting of rice at the time of ploughing, whereas N fertilizer (urea) was used not only as basal fertilizer prior transplanting but also supplemented during tillering as well as in the panicle initiation stage of the rice sapling. Cow manure was applied in the T1 treated plots at the time of ploughing only. In the RR fields, NPK fertilizers were also added before sowing of mustard seeds i.e. during October - November.

Statistical analysis

A multiple analysis of variance (MANOVA) using SPSS16 was used to determine the effects of fertilizer treatment and sampling time on the dependent variables

Results

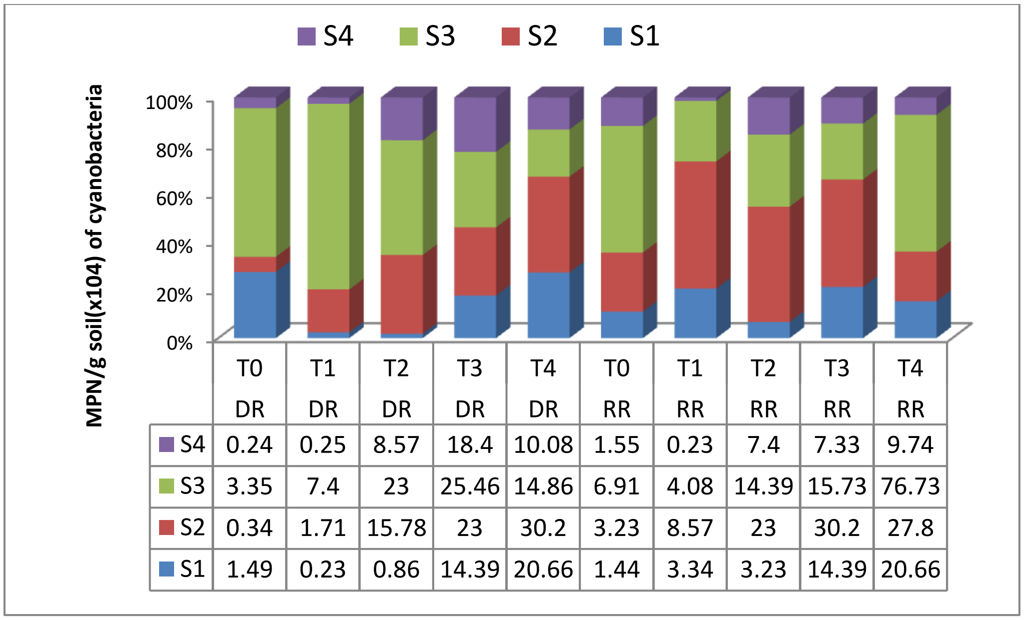
Soil physico-chemical characteristics

The soil pH, EC, P and K were significantly ($P<0.05$) affected by treatment, sampling time and treatment X sampling time interaction in both DR and RR fields (Table 1 and 2). In DR field, the soil OC was significantly ($P<0.05$) affected by different fertilizer treatments. OC was recorded highest (0.90%) in T4 treatment and lowest (0.32%) in T2 treatment (Table-1). While in RR field, both soil OC and N were significantly ($P<0.05$) effected by the fertilizer treatment (Table-2). Soil OC was highest (0.86%) in T4 and lowest (0.38%) in T0 treatment and N was highest (0.25mg/100g) in T2 and lowest (0.13 mg/100g) in T0 treatment in RR field (Table 2). Sampling time had a significant ($P<0.05$) effect on SM in both the DR and RR fields. It was found to be highest in S2 (26.55%, 26.78%) and lowest in S4 (6.58%, 9.00%) in DR and RR fields respectively (Table 1and 2).

Cyanobacterial number and abundance

Results showed that the most probable number (MPN) of cyanobacterial population per gram of soil varied in relation to sampling time and fertilizer treatments in the studied rice cropping systems. The most abundant growth of the cyanobacterial taxa were recorded during S2 and S3 seasons in both the DR and RR fields respectively (Fig-1). In the DR fields, the cyanobacterial count was highest (30.2×10^4) in the T4 treatment during S2, and lowest in the T1 treatment (0.23×10^4) in S1 (Fig-1). Analysis of variance tests revealed that, fertilizer treatment and sampling time both significantly ($P<0.05$) affected the MPN of cyanobacterial population in DR fields (Table - 1). Whereas in the RR fields, where mustard crop was cultivated with rice, cyanobacterial count was highest (76.73×10^4) in S3 in the T4 treatment and lowest (1.44×10^4) in S1 in the T0 treatment (Fig-1). In these fields fertilizer treatment, sampling time and fertilizer treatment X sampling time all had significant effect ($P<0.05$) on the total cyanobacterial counts (Table - 2).

Altogether 49 cyanobacterial taxa belonging to 15 genera (*Aphanocapsa, Aphanothece* , *Chroococcus*, *Oscillatoria*, *Phormidium*, *Lyngbya, Cylindospermum, Nostoc, Anabaena, Calothrix, Microchaete, Plectonema, Scytonema, Tolypothrix*, and *Westiellopsis)* under 7 families (Chroococcaceae, *Oscillatoriaceae, Nostocaceae, Stigonemataceae, Microchaetaceae, Rivulariaceae and Stigonemataceae*) were isolated from the studied plots. Based on their relative abundance values, *Nostoc, Anabaena, Weistellopsis*, *Calothrix, Scytonema and Phormidium* were found to be the most abundant genera (Figure-2).



	T0 DR	T1 DR	T2 DR	T3 DR	T4 DR	T0 RR	T1 RR	T2 RR	T3 RR	T4 RR
🟪 S4	0.24	0.25	8.57	18.4	10.08	1.55	0.23	7.4	7.33	9.74
🟩 S3	3.35	7.4	23	25.46	14.86	6.91	4.08	14.39	15.73	76.73
🟥 S2	0.34	1.71	15.78	23	30.2	3.23	8.57	23	30.2	27.8
🟦 S1	1.49	0.23	0.86	14.39	20.66	1.44	3.34	3.23	14.39	20.66

Figure-1: Cyanobacterial counts (MPN x10^4) in different seasons and fertilizer treatments in DR-Double Rice field, RR-Rice rotated field, S1-Premonsoon season, S2-Monsoon, S3-Post monsoon, S4-Winter, T0, T1, T2, T3, T4 are the fertilizer treatments as mentioned earlier

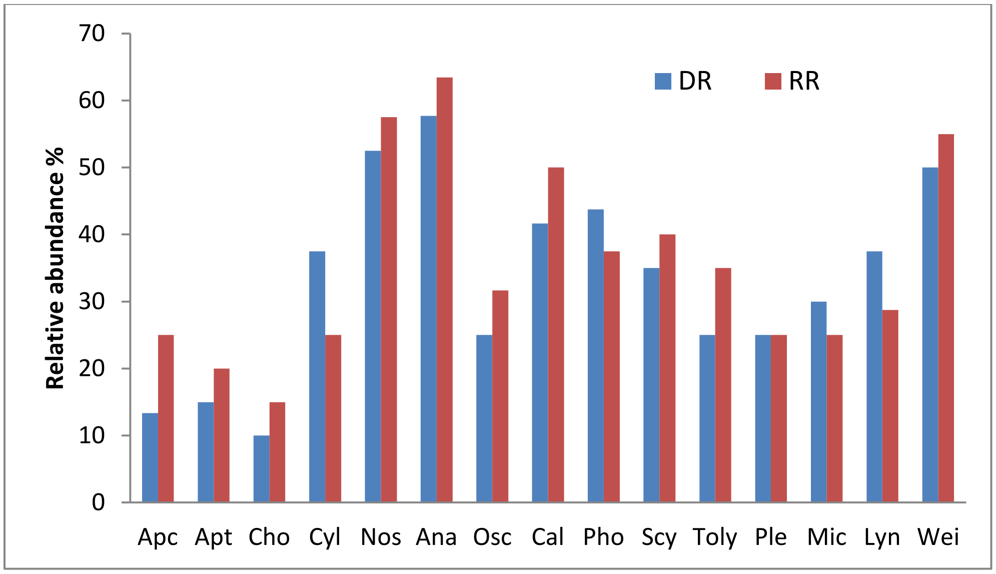


Figure-2 Relative abundance of the cyanobacterial genera the RR and RMR cropping systems. Apc-*Aphanocapsa* , Apt-*Aphanothece* , Cho-*Chroococcus* , Cyl-*Cylindrospermum* , Nos-*Nostoc*, Ana-*Anabaena,* Osc-*Oscillatoria* , Cal-*Calothrix*, Pho-*Phormidium* , Scy- *Scytonema*, Toly-*Tolypothrix* , Ple-*Plectonema* , Mic-*Microchaete* , Lyn- *Lyngbya,* Wei- *Weistellopsis,*

Table 1 Effect of fertilizer regime, sampling time and the interaction between them on the soil physico chemical properties and MPN of cyanobacteria in DR fields

Soil parameters	**pH**	**EC(µS)**	**S M (%)**	**O C (%)**	**Available N(mg/100g)**	**Available P(mg/100 g)**	**Available K(mg/100 g)**	**MPN /g soil (x$10^{4)}$**
Fertilizer treatment(FT)								
T0	5.86 ± 0.77 a	56.66 ± 29.06 a	12.98 ± 7.35 a	0.33 ± 0.30 a	0.07 ± 0.04 a	2.34 ± 1.40 a	15.15 ± 5.71 ac	1.35 ± 1.31a
T1	6.45 ± 0.68 b	38.36 ± 22.98 b	16.16 ± 9.49 ab	0.45 ± 0.32a b	0.20 ± 0.32 ab	2.74 ± 0.92 b	23.32 ± 8.38 ab	2.40 ±1.08 a
T2	6.10 ± 0.84 c	61.33 ± 30.84 c	17.36 ± 10.21 b	0.32 ± 0.30 a	0.26 ± 0.09 b	3.19 ± 1.52 c	24.72 ± 12.09 a	13.71± 6.67 b
T3	6.14 ± 0.35 dc	64.90 ± 25.60 dc	18.77 ± 10.79 cb	0.79 ± 0.45 bc	0.33 ± 0.33 ab	6.71 ± 1.50 de	32.16 ± 9.72 b	20.33± 5.96 c
T4	6.94 ± 0.65 e	54.30 ± 17.71 a	16.06 ± 8.33 ab	0.90 ± 0.56 c	0.25 ± 0.22 ab	6.79 ± 0.98 e	49.86 ± 36.90 c	18.95± 8.30dc
Sample Time(ST)								
S1	5.84 ± 0.36 a	26.66 ± 9.38 a	10.79 ± 4.50 a	059 ± 0.35 a	0.27 ± 0.36 a	3.84 ± 1.58 a	30.98 ± 9.31 a	8.83 ±8.21a
S2	7.17 ± 0.73 b	38.44 ± 10.63 b	25.32 ± 4.65 b	0.52 ± 0.41 a	0.12 ± 0.04 a	5.56 ± 1.65 b	31.58 ± 14.62 a	13.77± 11.8 b
S3	6.11 ± 0.75 c	72.57 ± 18.06 c	22.38 ± 6.79 b	0.41 ± 0.74 a	0.24 ± 0.21 a	3.97 ± 2.47 ac	36.77 ± 16.59 a	15.28± 9.66 b
S4	6.16 ± 0.51 c	82.77 ± 10.35 d	6.57 ± 2.91 c	0.72 ± 0.55 a	0.25 ± 0.21 a	4.04 ± 3.12 c	16.83 ± 7.36 b	7.51 ±7.56a
Anova P-values								
FT	0.000*	0.000*	0.079	0.002*	0.128	0.000*	0.000*	0.000*
ST	0.000*	0.000*	0.000*	0.291	0.332	0.000*	0.004*	0.000*
FT x ST	0.000*	0.000*	0.851	0.767	0.670	0.000*	0.005*	0.000*

Values are means ± standard deviation (n=12), P- values followed by asterisks are significant ($P<0.05$), means followed by the same letter in the same column do not differ significantly

Table 2 Effect of fertilizer treatment, sampling time and the interaction between them on the soil physico chemical properties and MPN of cyanobacteria in the RR fields

Soil parameters	**pH**	**EC(µS)**	**S M (%)**	**O C (%)**	**Available N(mg/100g)**	**Available P(mg/100g)**	**Available K(mg/100g)**	**MPN /g soil (x$10^{4)}$**
Fertilizer treatment(FT)								
T0	5.76 ± 1.12 a	34.17 ± 12.87 a	18.61 ± 7.07 a	0.38 ± 0.13 a	0.13 ± 0.06 a	1.76 ± 0.40 a	16.67 ± 10.13 a	3.28 ± 2.31 a
T1	6.13 ± 0.34 b	53.30 ± 14.34bc	16.20 ± 10.00 a	0.43 ± 0.16 a	0.23 ± 0.13 b	2.47 ± 0.81 b	22.10 ± 14.18 a	4.05 ± 3.38 a
T2	6.14 ± 0.86 bc	56.50 ± 23.89 b	15.56 ± 6.74 a	0.46 ± 0.31 a	0.25 ± 0.07 b	2.5 ± 0.62 b	28.66 ± 7.27 a	12.00 ± 7.88 b
T3	6.38 ± 0.71 c	48.92 ± 25.61 c	19.30 ± 10.61 a	0.45± 0.31 a	0.22 ± 0.06 b	3.43 ± 0.93 c	32.80 ± 17.86 ac	16.91 ±8.73 cb
T4	6.73 ± 0.63 d	58.03 ± 13.50 b	17.90 ± 9.67 a	0.86 ± 0.39 b	0.20 ± 0.08 ab	4.02 ± 0.53 d	69.23 ± 66.36 b	33.73 ±31.97 d

S1	5.44 ± 0.77 a	29.07 ± 8.67 a	12.87 ± 5.99 a	0.59 ± 0.30 a	0.25± 0.15 a	3.01 ± 0.92 a	39.66± 15.44 a	38.66 ±7.43a
S2	7.15 ± 0.46 b	42.09 ± 11.71 b	26.78 ± 3.29 a	0.55 ± 0.34 a	0.17± 0.03 b	2.50 ± 0.06 b	15.14 ± 12.75 b	54.66 ±5.16b
S3	6.30 ± 0.46 c	64.48 ± 9.32 c	21.41 ± 6.87 b	0.34 ± 0.16 a	0.15± 0.04a	3.23 ± 1.08 ab	28.89 ± 13.09 c	67.33 ±14.86 b
S4	6.02 ± 0.44 d	65.10 ± 20.29 c	9.00 ± 4.62 a	0.59 ± 0.43 a	0.23 ± 0.07 b	2.64 ± 1.00 a	51.86 ± 17.19a c	37.33 ±7.03a
Anova P-values								
FT	0.000*	0.000*	0.290	0.000*	0.026*	0.000*	0.000*	0.000*
ST	0.000*	0.000*	0.000*	0.057	0.498	0.000*	0.000*	0.000*
FT x ST	0.000*	0.000*	0.054	0.697	0.806	0.000*	0.000*	0.000*

Values are means ± standard deviation (n=12), P-values followed by asterisks are significant (P<0.05), means followed by the same letter in the same column do not differ significantly

Discussion

Our present study on two popularly practiced rice cropping systems of Brahmaputra flood plain revealed that both fertilizer treatment and sampling time had an impact on the soil physico chemical characteristics and abundance of cyanobacterial population in rice fields. It was observed that mixed fertilizer treatment (T3 and T4) consisting of organic fertilizers + crop residues + chemical fertilizers significantly modified the soil physico chemical properties in comparison to the unfertilized plots (T0) and the plots fertilized only by organic manures (T1) or chemical fertilizers (T2). The soil pH though was lower in the control treatment (T0) showed significant increase with the subsequent treatments. T4 treatment recorded the highest pH in both the fields (Tab-1and 2). The enhancement of the soil pH may be due to the reduction in the quantity of chemical fertilizers which are commonly known to acidify the soil (Van Diepeningen *et al*. 2006, Zhao *et al.* 2014). Slightly alkaline pH encouraged greater abundance of cyanobacterial species in the T3 and T4 plots which was similar as reported by Nayak and Prasanna (2007) from different parts of India. Along with pH, high moisture in the said treatments during monsoon and post monsoon seasons helped cyanobacterial population to flourish as they prefer to grow in the wet environment (Maqubela *et al.* 2008). The T4 treatment had the highest concentration of soil OC in both the rice cropping systems as application of organic fertilizer such as crop straw along with inorganic fertilizer in the fields can slowly release nutrients into the soil that is in conformity with Liu *et al*. (2014).

The soil N however varied in different treatments and seasons in both the cropping systems. In DR field, N was highest in T3 field and lowest in T_2 field. Seasonal variations were also observed in the N content being higher in S2 and lower in S3. This variation may be due to the difference in quantity and time of application of NPK fertilizers. Irrisari *et al.* (2001) reported that nitrogen fertilization may be inhibitory to the diversity and population density of N_2 fixing cyanobacteria. Our study also revealed that the treatment plots with low nitrogen showed higher abundance of cyanobacterial population. The soil P and K content also increased significantly due to fertilizer treatment and varied with respect to seasons affecting the cyanobacterial abundance. The results of soil characteristics thus indicated that different fertilizer regimes along with the seasonal influence gradually changed the soil attributes which supposed to activate the soil borne cyanobacterial population. Incorporation of crop residues in fertilizer doses helps to balance the agricultural productivity (Kushwaha *et al.* 2001; Singh *et al.* 2007) by maintaining soil nutrients. Based on the results of our study, a consortium of mixed fertilizers constituting organic, inorganic and composted crop residues could be recommended to be used for sustainable rice production in the Brahmaputra flood plain.

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