



Biodiversity of soil algae in a tropical endoaquept planted to rice under flooded condition under long-term application of chemical and organic-N sources

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ARTICLE INFO

Article history:

Received : 22 August 2015

Accepted : 5 October 2015

Keywords:

algal diversity
cyanophyta
green algae
diatoms
organic-N source

ABSTRACT

Organic N resources are being increasingly used to meet the N demand of the growing rice crop under organic cultivation which could be a win-win option for restoring soil health as well as reducing leakage of reactive N to the environment apart from impact on microbial diversity of rice soils. We studied the diversity of algae and cyanobacterial strains in a field experiment with 9 treatments involving chemical, organic and a combination of chemical and organic-N sources being used for growing rice crop for the last 15 years. A total of 66 species belonging to 33 genera under 4 classes of cyanobacteria, green algae, diatoms and euglenoids were recorded. Highest diversity of species was found for the cyanobacteria (30 species) followed by diatoms (22 species). Among the different treatments, the community structure of soil algae varied with highest diversity being recorded in field plots amended with chemical fertilizer (Shannon diversity index 3.22) and the lowest in the unamended field plots where no N source was added. Results indicate that anthropogenic activities in rice fields including application of organic residues can influence the diversity of soil algae

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1. Introduction

Variability in the activity and composition of soil microbial communities may have important implications for the whole gamut of microbially-mediated ecosystem functions upon which agricultural system depends. Soil organic matter is central to sustaining crop production and maintenance of soil health. Flooded rice ecosystem presents a unique microbial ecology that undergoes a cycling of flooded and non-flooded sequences and is characterized by several physicochemical and biological properties (Leisack *et al.*, 2001; Adhya and Rao, 2005). Considerable amounts of biomass are likely to be produced phototrophically in the floodwater (Roger, 1996) and also chemo-autotrophically in the presence of inorganic electron donors and CO₂ (Revsbech *et al.*, 1999).

Soil is the most important non-aqueous habitat for algae and cyanobacteria (Zenova *et al.*, 1995). Cyanobacteria

are ubiquitous in nature yet they show great diversity and abundance in rice field ecosystem, as it provides optimum conditions of light, temperature, water and nutrients for their growth and proliferation (Prasanna and Nayak, 2007). Climatic, soil and biotic factors influence the growth and colonization of cyanobacteria and other algae in rice fields that play an important role in C-turnover of this ecosystem. Cyanobacteria or blue green algae comprise a large group of photoautotrophic prokaryotes that contribute to the N-economy of the system, in view of the inherent potential of biological nitrogen fixation by the majority of the group. Soil fertility is generally improved by the organic matter produced by these organisms (Mishra and Pabbi, 2004) apart from the fact that they also secrete diverse growth-promoting substances such as hormones, vitamins, amino acids and organic acids affecting other organisms in many ways (Roger and Reynaud, 1982).

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In the rice-growing regions, application of chemical-N fertilizers is a very common practice to increase rice productivity (Balasubramanian et al., 1999) but is widely acknowledged to affect the soil microbial diversity. Earlier studies have shown that inorganic N-fertilization favours the growth of non-N fixing algae, which inhibit partial or total growth of N₂-fixing cyanobacteria, while N-deficient fields favour growth of N₂-fixing algae. Previous reports have also shown that incorporation of nitrogenous fertilizers in the form of organic and green manures have either inhibitory or favourable effects of algal growth in rice fields (Roger, 1996). In view of the harmful effect of the application of excessive fertilizer-N including release of reactive-N in the environment, application of organic N sources is getting credence. Those organic materials including compost and other organic residues, apart from serving as the source of N and other nutrients, also result in the sequestration of C and build-up of organic matter, apart from maintaining soil fertility and ecosystem resilience.

The objectives of the present research was (i) to evaluate the effects of organic, inorganic and combined nitrogen fertilizers on the diversity of algae and cyanobacterial strains in rice fields; and (ii) to identify the dominant algae and cyanobacterial strains (genera-wise) present through standard light microscopy techniques.

2. Materials and methods

2.1 Site description and field experiment

The study was conducted at the experimental farm of the Central Rice Research Institute, Cuttack, India (85°55'22" E, 20°25'22" N; elevation 24 m). Mean annual highest and lowest temperatures are 39.2 and 22.5°C, respectively, and the mean annual temperature is 27.7 °C. Annual precipitation is about ~1500 mm.yr⁻¹, of which 75–80% is received during June to September. The soil is an Aeric Endoaquept with sandy clay loam texture (26.3% clay, 21.4% silt, 52.3% sand), bulk density 1.40 Mgm⁻³, percolation rate <10 mmd⁻¹, pH (H₂O) 5.26, cation exchange capacity 15 mEq 100 g⁻¹, electrical conductivity 0.5 dSm⁻¹, total organic C 0.38% and total N 0.05%, exchangeable K 120 kg ha⁻¹.

The field experiment on intensive rice cropping under different inorganic, organic, and combined fertilizers as N source was established in 1995 where rice is grown in a rice–rice–fallow sequence. Wet season (July–December) rice was grown under rainfed condition followed by the dry season (January–April) rice grown under irrigated condition. The field was ploughed thoroughly and flooded 2–3 days before transplanting for puddling and levelling. Rice plants (21-day old seedlings of cv. Gayatri during the wet season

and cv. Lalat during the dry season) were transplanted at a spacing of 15 cm x 15 cm with two seedlings per hill in the field plots (5m x 5m) well separated by levees. All the field plots remained continuously flooded to a water depth of 12 ± 7 cm during the entire period of crop growth and were drained 10 days before harvest. The crops were grown following recommended agronomic practices and harvested at maturity.

The experiment was laid out in a randomized block design with three replicates for all the treatments, including control. There were a total of 9 treatment combinations including (i) unamended control without any N addition, (ii) urea added as chemical fertilizer at the rate of 60 kg N.ha⁻¹, (iii) farm yard manure (FYM) at the rate of 60Kg N.ha⁻¹, (iv) Green manuring crop *Sesbania aculeata* at the rate of 60 Kg N.ha⁻¹ (v) FYM (30 Kg N.ha⁻¹) + *S. aculeata* (30 Kg N.ha⁻¹) (vi) FYM (30 Kg N.ha⁻¹) + *Azolla* (30 Kg N.ha⁻¹) (vii) *S. aculeata* (30 Kg N.ha⁻¹) + *Azolla* (30 Kg N.ha⁻¹), (viii) rice straw (30 Kg N.ha⁻¹) + *S. aculeata* (30 Kg N.ha⁻¹), (ix) rice straw (30 Kg N.ha⁻¹) + urea (30 Kg N.ha⁻¹). Organic residues like FYM, and rice straw at appropriate quantities were incorporated at the time of field preparation. Fresh biomass of *S. aculeata* and *Azolla* at appropriate quantity was incorporated at the time of puddling and the seedlings were planted 5 days later to allow decomposition of the biomass. Phosphorus (as P₂O₅) and potassium (as muriate of potash) were applied as basal at 40 kg.ha⁻¹ at the time of puddling.

2.2 Sampling and morphological observation

Algal samples were collected randomly from each plot in the maximum tillering stage (40 days after transplantation) of rice growth during the wet season of rice crop (rainfed condition). Water samples and soil cores of the top 0.5 cm were taken to determine planktonic and benthic algae and cyanobacteria. Samples were collected using forceps and needle and stored and preserved in pre-sterilized specimen bottles with formaldehyde (4% v/v). A part of each fixed soil suspension was boiled in 10% H₂O₂ solution to remove any organic material and was repeatedly rinsed with distilled water to obtain cleaned diatom frustules (Fujita and Ohtsuka, 2005). Cell measurement was carried out using ocular and stage micrometers and microphotograph of each specimen was taken using a Meiji ML-TH-05 Trinocular research microscope fitted with Nikon Coolpix 4500 digital camera. The organisms were identified following monographs for various algal groups (Desikachary 1959; Hoffman, 1989; Metting, 1981; Patrick and Reimer, 1975; Philipose 1967; Prescott, 1962; Ramanathan 1964; Randhawa 1959; Bhakta, Das and Adhikary, 2010).

2.3 Physicochemical properties of soil

Soil from each field plot was collected at the time of collection of algal samples and analyzed for total organic carbon (%), total N (%), pH and EC following standard protocol of soil analysis (Sparks, 1996) and the values of analysis are given in Table-I. The total organic carbon values were converted into organic matter by multiplying with 1.724 (Pribyl, 2010). Soil pH and electrical conductivity (EC) range were analysed (using soil: water = 1:2.5) using a portable pH meter (Philips model PW 9424) and by a mhos pH meter (Elico, Hyderabad, India) respectively. All the samples were analyzed in triplicate and the values provided are means of observations \pm S.D.

2.4 Statistical analysis

All analyses were carried out using samples from replicated field plots for each treatment. Analysis of variance was done to determine the effects of treatment and their interaction using statistical package (IRRISTAT, version 3.1: International Rice Research Institute, Philippines). The indices of soil algal communities were estimated by Shannon species diversity index by $H = -\sum(p_i)(\log_2 p_i)$, where p_i is the proportion of the individual species (Shannon and Weaver, 1949). Differences were considered to be significant at $p < 0.05$ level.

3. Results and discussion

3.1 Algal biodiversity under different chemical and organic-N source amendments

Based on the morphology observed under the light microscope, a total of 66 species were identified in the soils sampled from rice field plots amended with different chemical and organic-N source amendments (Table 1). They belonged to cyanobacteria, green algae, diatoms and euglenoids in 4 classes and 33 genera. Highest diversity of species was found for the cyanobacteria (30 species) which belonged mostly to the genera *Anabaena*, *Oscillatoria* and *Phormidium*, followed by diatoms (22 species) (Tables 1 and 2, Plates 1-7), and a limited presence of green algae mostly predominated by filamentous species. The highest number of taxa was found for the genus *Navicula* (diatoms). Only one instance of euglenoids (*E. sanguinia*) was recorded from field plots amended with chemical N-fertilizer (urea).

Among the different treatments, the community structure of soil algae varied significantly from type to type, with Shannon diversity index ranging from 1.97 to 3.22. The highest index value was measured in the field plots amended with chemical fertilizer and the lowest in the unamended plots where no N source was added. Interestingly,

slowly decomposable organic-N sources like FYM and rice straw either in conjunction with green manuring *S. aculeata* or with urea, exhibited nearly similar magnitude of diversity. Algae belonging to cyanobacteria were recorded in a highly diverse pattern, both in the number of species as well as genera, from field plots amended with chemical fertilizer as compared to field plots amended with organic or combined forms of N-fertilizers. Algae belonging to the genera *Aulosira*, *Anabaena*, *Nostoc*, *Oscillatoria*, *Spirulina* and *Spirogyra* were dominant (>20%) in fields amended with chemical fertilizer. Interestingly, algae belonging to the group Bacillariophyta were larger in number in field plots amended with rice straw, especially in conjunction with urea, possibly because of larger supply of silica, from the rice straw, which is an integral part of diatom frustules.

The diversity of soil algal communities is the result of the complex influence of rice plants, soil properties, cropping practices and climatic conditions (Quesada et al. 1995). Occurrence of soil algae in rice paddies also varies with the growth stage of the rice crop (Roger and Reynaud, 1976). At the early growth stage, diatoms and unicellular algae dominate. As the biomass of soil algae increases, there is a shift to filamentous green algae and non-N fixing cyanobacteria just before panicle initiation (Choudhary, 2009), possibly due to crop growth and development of canopy and thus reduction in the available solar irradiation. In the present study, sampling was done at the maximum tillering stage – the most active growth stage of the rice crop and represented a high diversity in the algal community, mostly divided among cyanobacteria and diatoms.

Various fertilizers that are applied to rice crop at different stages of the crop growth can influence the nutrient dynamics for soil algae. Thus, the dynamics of algal diversity in rice fields are a result of a complex interaction of available solar irradiation, nutrients and crop growth. Earlier reports suggested that chemical N-fertilization favours the growth of non-N fixing algae due to partial or total inhibition of growth of N₂-fixing cyanobacteria. However, in the present study, field plot amended with chemical fertilizer had the highest cyanobacterial diversity with almost equal distribution of nitrogen and non-nitrogen fixers. Further, it is suggested that non-heterocystous cyanobacterial species might become dominant when fertilizers are applied (Jutono, 1973). In the present study, some N-fixing species including those of *Anabaena*, *Gleothoece* and *Nostoc* were found in the field plots together with larger proportion of non N-fixing species, such as *Microcystis*, *Oscillatoria* and *Spirulina*.

Table 1
Checklist of abundance of soil algae recorded in flooded field plots planted to rice and amended with inorganic and organic N sources

S. No.	Species	Control	Urea-N	FYM	S. <i>aculeata</i>	FYM + S. <i>aculeata</i>	FYM + Azolla	FYM + S. <i>aculeata</i> + Azolla	Rice straw+ S. <i>aculeata</i>	Rice straw + Urea
Cyanophyta/ Cyanoprokaryota										
1	<i>Anabaena ambigua</i>	D	D	D	C	C	-	-	-	-
2	<i>A. constreta</i>	-	C	R	-	R	R	R	-	-
3	<i>A. fertilissima</i>	-	C	-	-	-	-	-	-	C
4	<i>A. torulosa</i>	D	D	D	-	C	C	-	-	-
5	<i>Aphanothece bullosa</i>	D	-	D	-	D	D	C	-	-
6	<i>A. gelatenosa</i>	D	-	C	C	D	C	C	-	-
7	<i>Aulosira prolifica</i>	-	D	D	D	-	-	D	C	-
8	<i>Cylindrospermum catenatum</i>	-	C	-	-	-	-	-	D	-
9	<i>C. muscicola</i>	-	C	-	-	-	-	-	D	D
10	<i>Glaucocystis duplex</i>	-	C	C	C	D	D	C	-	-
11	<i>Gloeothece rupestris</i>	-	-	-	-	-	-	-	-	D
12	<i>Gloeothece natans</i>	-	-	-	-	-	-	-	-	C
13	<i>Microchate tenera</i>	-	R	-	-	-	-	-	-	-
14	<i>Microcoleus chthonoplastes</i>	-	-	-	D	D	-	-	C	-
15	<i>Microcystis wesenbergi</i>	-	-	D	D	D	D	C	C	-
16	<i>Myxosarcina spectabilis</i>	C	-	-	-	-	-	-	-	-
17	<i>Nostoc patulosum</i>	C	C	C	D	D	-	-	C	C
18	<i>Oscillatoria anguina</i>	-	D	C	-	-	C	C	-	-
19	<i>O. anguistissima</i>	-	D	C	-	-	D	C	-	-
20	<i>O. chlorina</i>	-	D	C	-	-	D	C	-	-
21	<i>O. tenuis</i>	-	D	D	-	-	C	C	-	-
22	<i>O. terebriforme</i>	-	-	-	C	C	-	-	D	-
23	<i>Phormidium boryanum</i>	D	D	C	-	-	D	C	-	-
24	<i>P. muscicola</i>	-	D	D	-	-	-	-	-	-
25	<i>P. orientalis</i>	-	C	C	-	-	C	D	-	-
26	<i>P. rotheanum</i>	-	-	-	C	-	-	-	D	D
27	<i>P. tenue</i>	-	C	-	D	C	-	-	C	-

57	<i>P. gibba</i>	-	C	-	-	-	-	-	-	-	-	D
58	<i>P. obscura</i>	-	-	-	-	-	-	-	-	-	D	D
59	<i>P. subsimilis</i>	C	-	-	-	-	-	-	-	-	-	-
60	<i>Pleurosigma normanii</i>	C	-	-	-	-	-	-	-	-	-	-
61	<i>Stauroneis platystoma</i>	-	-	-	-	-	-	-	-	-	D	D
62	<i>Surirella tenera</i> var. <i>nervosa</i>	-	-	-	-	-	-	-	-	-	C	C
63	<i>Synedra crystallina</i>	-	C	C	-	-	-	-	R	-	-	-
64	<i>S. mesolepta</i>	-	-	-	-	-	-	-	-	-	R	R
65	<i>S. tabulata</i>	-	C	-	-	-	-	-	-	-	-	R
Euglenoids												
66	<i>Euglena sanguinia</i>	-	R	-	-	-	-	-	-	-	-	-

Abundance: D = Dominant (>20%); C = Common (10-20%); R = Rare (<10%); - = Not found

Table 2.

Summary of the total species (genus) numbers of soil algae revealed in flooded field plots planted to rice and amended with inorganic and organic N sources

Group	Control	Urea-N	FYM	<i>S. aculeata</i>	FYM + <i>S. aculeata</i>	FYM + <i>Azolla</i>	<i>S. aculeata</i> + <i>Azolla</i>	Rice straw + <i>S. aculeata</i>	Rice straw + Urea
Cyanobacteria	7 (5)	19 (10)	16 (9)	10 (9)	11 (8)	13 (7)	13 (8)	10 (8)	7 (7)
Chlorophytes	2 (1)	5 (3)	3 (2)	5 (4)	3 (2)	3 (2)	3 (2)	5 (5)	3 (1)
Diatoms	5 (3)	11 (7)	3 (2)	2 (2)	2 (1)	2 (2)	2 (2)	6 (6)	13 (10)
Euglenoids	-	1 (1)	-	-	-	-	-	-	-
Total	14 (9)	36 (21)	22 (13)	17 (15)	16 (11)	18 (11)	18 (12)	21 (19)	23 (18)

Table 3.

Physicochemical properties* of soils planted to rice and maintained under different treatments

Treatments	pH (soil:water::1:1.25)	EC	Total organic C (%)	Total N (%)	Organic matter (%)
Control	5.3 ± 0.50	0.173 ± 0.02	0.382 ± 0.01	0.053 ± 0.01	0.658 ± 0.04
Urea (60 Kg N. ha ⁻¹)	5.1 ± 0.20	0.148 ± 0.02	0.581 ± 0.01	0.075 ± 0.02	1.002 ± 0.02
FYM (60 Kg N.ha ⁻¹)	5.1 ± 0.15	0.167 ± 0.02	0.772 ± 0.03	0.544 ± 0.04	1.331 ± 0.02
<i>Sesbania aculeata</i> (60 Kg N.ha ⁻¹)	5.3 ± 0.13	0.224 ± 0.02	0.575 ± 0.03	0.470 ± 0.03	0.991 ± 0.02
FYM (30 Kg N.ha ⁻¹)+ <i>S.a</i> (30 Kg N.ha ⁻¹)	5.1 ± 0.16	0.287 ± 0.01	0.738 ± 0.03	0.506 ± 0.04	1.272 ± 0.02
FYM (30 Kg N.ha ⁻¹)+ <i>Azolla</i> (30 Kg N.ha ⁻¹)	4.9 ± 0.18	0.201 ± 0.03	0.668 ± 0.03	0.460 ± 0.02	1.152 ± 0.01
<i>S.a</i> (30 Kg N.ha ⁻¹)+ <i>Azolla</i> (30 Kg N.ha ⁻¹)	5.2 ± 0.18	0.188 ± 0.02	0.659 ± 0.03	0.450 ± 0.04	1.136 ± 0.02
RS (30 Kg N.ha ⁻¹) + <i>S.a</i> (30 Kg N.ha ⁻¹)	5.1 ± 0.16	0.311 ± 0.03	0.786 ± 0.03	0.468 ± 0.05	1.355 ± 0.01
RS (30 Kg N.ha ⁻¹) + Urea (30 Kg N.ha ⁻¹)	5.4 ± 0.16	0.172 ± 0.02	0.750 ± 0.03	0.064 ± 0.01	1.293 ± 0.02

* Mean of three replicate values ± standard deviation

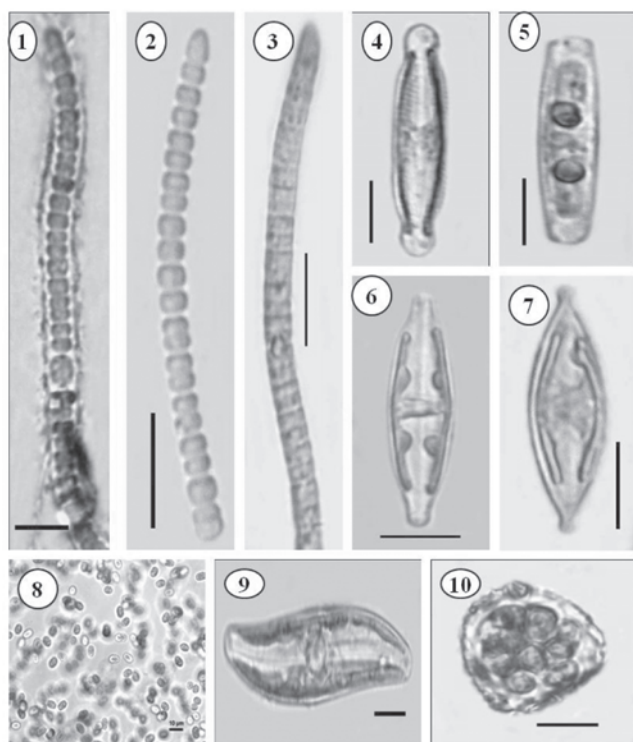
FYM = Farm yard manure; *S.a* = *Sesbania aculeata*; RS = rice straw

PLATE-1 (Control Field)

Figs. 1-10: 1- *Anabaena ambigua*, 2- *Anabaena torulosa*, 3- *Phormidium boryanum*, 4- *Aphanothece gelatenosa*, 5- *Pinnularia acrosphaeria*, 6- *Myxosarcina spectabilis*, 7- *Navicula microspora*, 8- *Pinnularia subsimilis*, 9- *Pleurosigma normanii*, 10- *Navicula cryptocephala*. Scale = 10µm

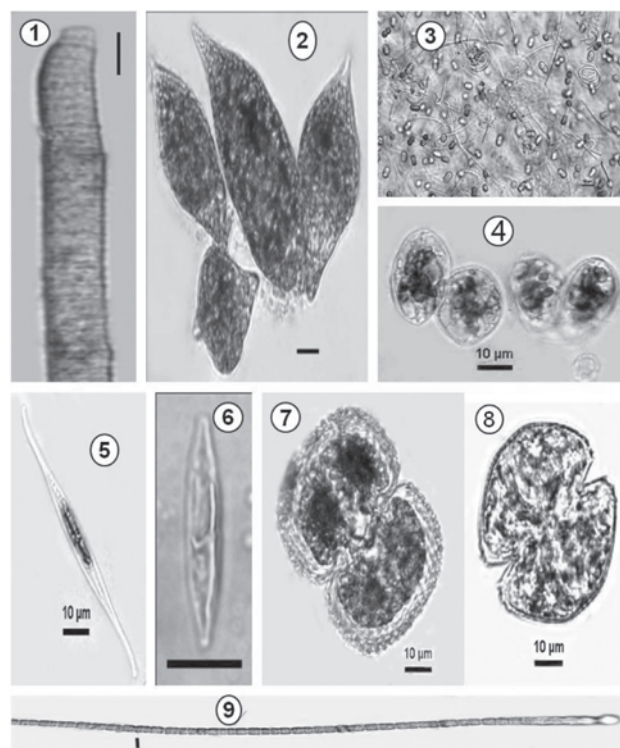


PLATE-2 [Inorganic nitrogen fertilizer (Urea)]

Figs. 1-9: 1- *Oscillatoria anguina*, 2- *Euglena sanguinia*, 3- *Phormidium muscicola*, 4- *Glaucocystis duplex*, 5- *Nitzschia closterium*, 6- *Microchate tenera*, 7- *Cosmarium pseudocoronatum*, 8- *Cosmarium auriculatum*, 9- *Navicula notha*. Scale = 10µm

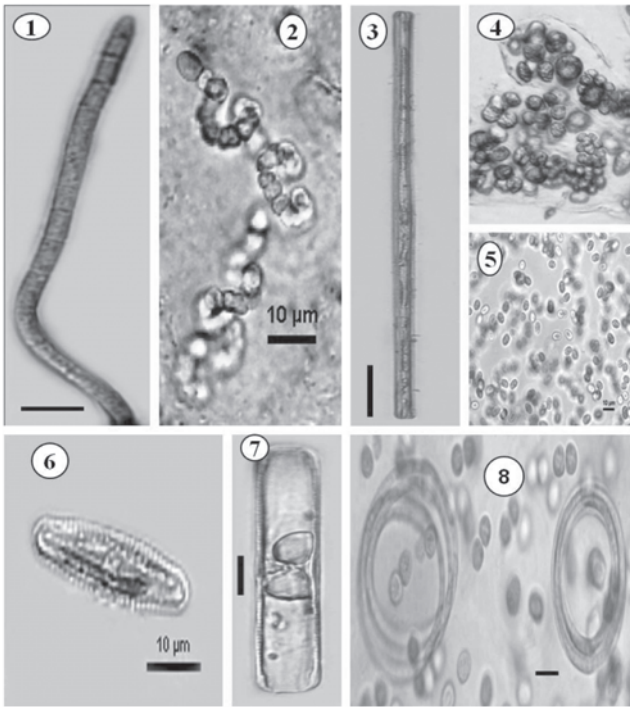


Plate-3 (Farm Yard Manure)

Figs. 1-8: 1- *Oscillatoria chlorina*, 2- *Nostoc paludosum*, 3- *Synedra crystallina*, 4- *Microcystis wesenbergi*, 5- *Aphanothece gelatinosa*, 6- *Navicula protracta*, 7- *Navicula jurgensii*, 8- *Aphanothece bullosa* + *Phormidium orientalis*. Scale = 10µm

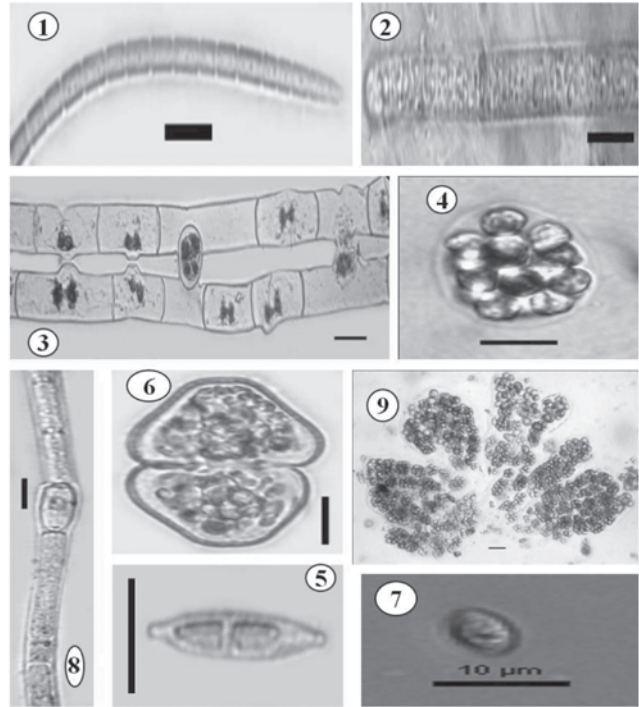


Plate-4 (*Sesbania aculeata*)

Figs. 1-9: 1- *Phormidium tenue*, 2- *Phormidium rotheanum*, 3- *Zygnema giganteum*, 4- *Oocystis elliptica*, 5- *Nitzschia vasnii*, 6- *Cosmarium supergranatum*, 7- *Scenedesmus obtusus*, 8- *Aulosira prolifica*, 9- *Microcystis wesenbergi*. Scale = 10µm

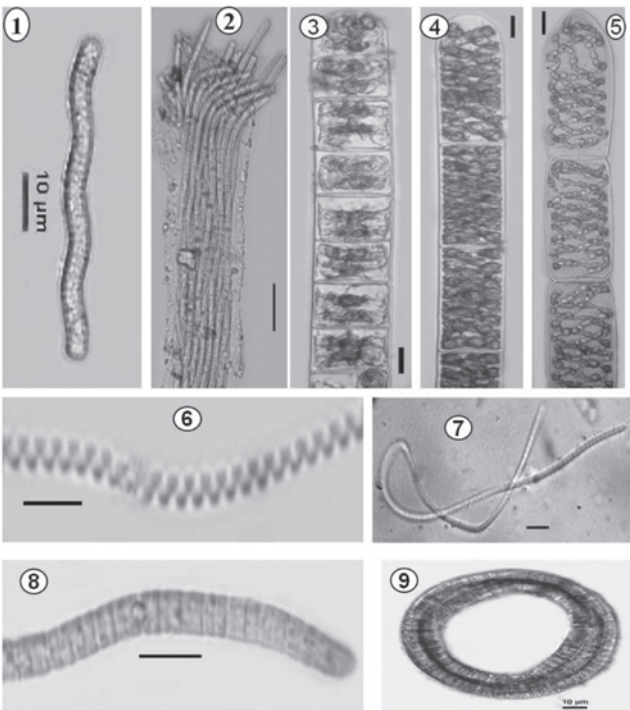


Plate 5 (Farm Yard Manure + *Sesbania aculeata*)

Figs. 1-5: 1- *Oscillatoria terebriforme*, 2- *Microcoleus chthonoplastes*, 3- *Zygnema czurdae*, 4- *Spirogyra condensata*, 5- *Spirogyra ghosei*. Scale = 10µm

Plate 5 (Farm Yard Manure + *Azolla*)

Figs. 6-9 : 6- *Spirulina subtilissima*, 7- *Oscillatoria angustissima*, 8- *Phormidium boryanum*, 9- *Oscillatoria tenuis*. Scale = 10µm

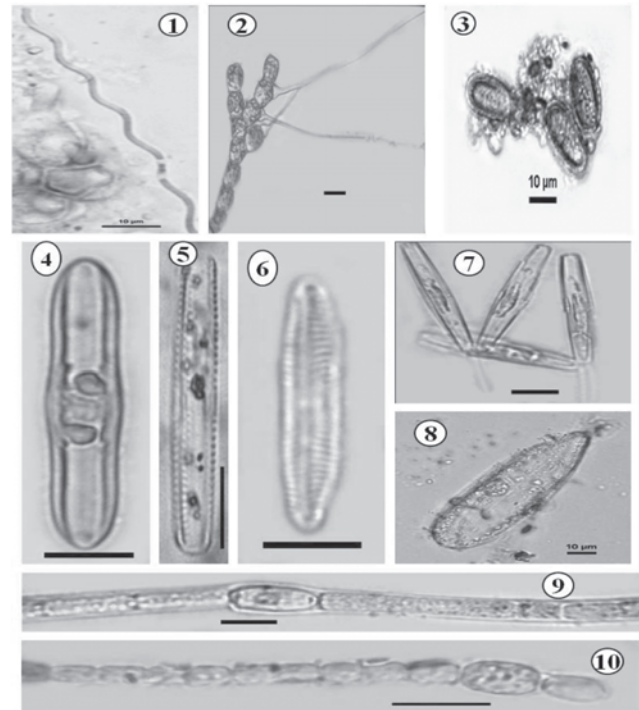


Plate-6 (Rice straw + *Sesbania aculeata*)

Figs. 1-10: 1- *Spirulina laxa*, 2- *Bulbochate basispora*, 3- *Cylindrospermum muscicola*, 4- *Synedra mesolepta*, 5- *Stauroneis platystoma*, 6- *Pinnularia obscura*, 7- *Gomphonema geminata*, 8- *Surirella tenera* var. *nervosa*, 9- *Aulosira prolifica*, 10- *Cylindrospermum catenatum*. Scale = 10µm

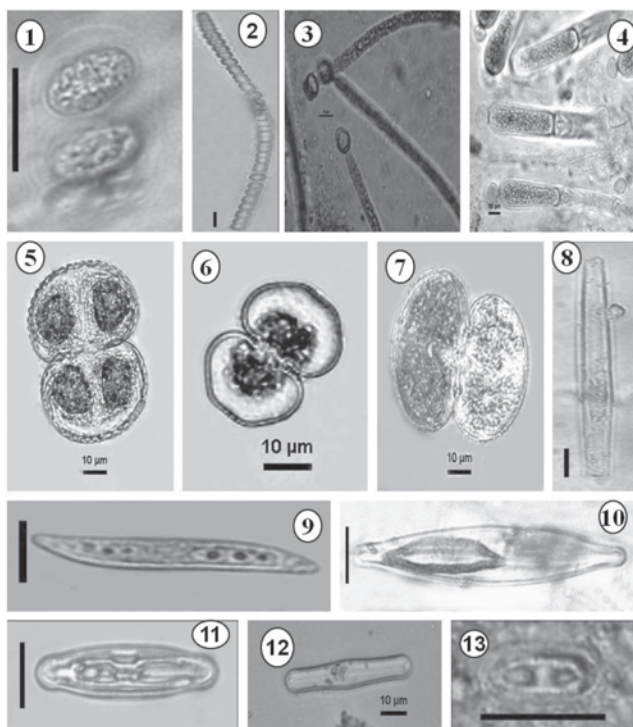


Plate-7 (Rice Straw + Urea)

Figs. 1-13 : 1- *Gloeotheca rupestris*, 3- *Anabaena fertilissima*, 3- *Rivularia aquatica*, 4- *Gloeotrichia natans*, 5- *Cosmarium crenatum*, 6- *Cosmarium nitidulum*, 7- *Cosmarium pachydermum*, 8- *Synedra tabulata*, 9- *Nitzschia nana*, 10- *Frustulia rhomboides*, 11- *Navicula ignota*, 12- *Pinnularia gibba*, 13- *Achnanthes hungarica*. Scale = 10µm

3.2 Physicochemical properties of soil

The field plots indicated a marginal or almost no change in the soil pH and salinity under the influence of different types of fertilization (Table 3). The pH of the soils remained slightly acidic ranging from 4.9 to 5.4. As expected, application of organic residues resulted in an increase in the organic C content, especially in field plots amended with FYM either alone or in combination with other organic sources, as FYM is considered to be a slow degrading organic residue. FYM amended plots also show large diversity next only to urea-N amended plots.

4. Conclusion

Rice fields are places of intense farming activities including tillage and field preparation, application of fertilizers and other agrochemicals including pesticides and herbicides. Such activities are known to influence the diversity of soil microflora including that of algae. Nitrogen is the major nutrient limiting rice crop growth and amendment of fertilizer-N is a standard practice to increase the crop yield. However, intensive application of chemical fertilizer to the crop are reported to have several adverse impact on ecosystem functioning. Currently, there are efforts

to grow crops without the influence of agrochemicals, the so called organic agriculture. Organic agriculture practices insist on using organic resources for plant nutrition. Our studies reveal that application of organic residues apart from resulting into increase in organic-C content of the soil also influences the algal flora.

Acknowledgements

This work was supported in part by the ICAR Networking project, "Application of Microorganisms in Agriculture and allied Sciences (AMAAS) – theme Nutrient management, PGPR and Biocontrol" by the Indian Council of Agricultural Research, New Delhi. We are grateful to Prof. S.P. Adhikary, Vice-chancellor, F.M. University, Balasore, Odisha for scientific advice and technical help during the course of this investigation.

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