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Abstract

On August 9th, 2007, two agriculture fields (rice and sorghum fields) were sampled for freshwater algae in St. Francis County. The purpose of this study was to document the algal species in the rice and sorghum fields and compare the similarities of species composition. There were a total of 53 species identified. Overall, diatoms and cyanobacteria were equally dominant with both represented by 21 species (39.6% of the total) and 11 green algal species (20.8%) were present. The sorghum field was dominated by *Chlorogloeopsis fritschii* and *Chroococcus limneticus*, while *Anabeana cylindrica* was abundant in the rice field.

Introduction

The United States provides a large portion of the world's grain (USDA 2008a). The world grain production is $20x10^{11}$ metric tons and USA is $3.4x10^{11}$ metric tons, which is 16.8% of the world's grain production (USDA 2008a).

The world's rice production is $4.2x10^{11}$ metric tons and sorghum is $64x10^{9}$ metric tons. USA produced $62x10^{9}$ metric tons of rice and $12.8x10^{9}$ metric tons of sorghum, which is 1.5% and 12.4% of the world production of rice and sorghum production (USDA 2008a).

Arkansas is the leading producer of rice in United States at $29.7x10^{9}$ metric tons (47.9% of USA's production) and $5.1x10^{8}$ metric tons of sorghum (USDA 2008b). The economic value is over 1 billion dollars for rice and 72 million dollars for sorghum (USDA 2008b).

There have been very few or no studies of algae associated to rice or sorghum fields in USA. There is some published information available for the algal species related to terrestrial agriculture soils in United States (Shimmel and Darley 1985; Fairchild and Willson 1967; Forest et al. 1959; Schlichting 1973) but most of the rice fields studies come from India (De 1939; Roger and Kulasooriya 1980; Nayak and Prasanna 2007) and China (Wassmann et al. 1993), which are typically dominated by cyanobacteria (Forest et al. 1959).

Rice throughout the world is mainly grown under irrigated conditions. This causes nitrogen fertilizer efficiency to be low because of large nitrogen losses from flooded soils (De Datta and Buresh 1989; Ghosh and Saha 1997). To maintain the soil nitrogen pool, it is primarily fertilized with agriculture fertilizer and through biological nitrogen fixation (Kundu and Ladha 1995; Cassman et al. 1998). Cyanobacteria are extremely important to fix atmospheric nitrogen in rice fields (Roger and Kulasooriya 1980; Roger and Ladha 1992). They can contribute to the natural fertility of the soils through nitrogen-fixation (De 1939) in their heterocysts. Cyanobacteria have been used as biofertilizers and used to inoculate rice fields (Irisarri 2006). Cyanobacteria can supply approximately 4 kg/N/ha from cyanobacteria biomass to the standing crop of rice (Roger 1991).

Most published data of inoculation with cyanobacteria refer to tropical rice fields, which are different in characteristics and agriculture land management from temperate ones. Biological nitrogen fixation is far more diverse and complex in the tropics than under temperate conditions (Balandreau and Roger 1996). Assays of cyanobacterial inoculation in temperate climates were performed in the USA (Reynaud and Metting 1988).

The purpose of the study reported herein was to document species of aquatic algae and cyanobacteria associated with rice and sorghum fields from St. Frances County, Arkansas (Figure 1 and 2). As the terms are used in this paper, algae are defined as any eukaryotic organisms containing chlorophyll "a" in the Kingdom *Protista* and cyanobacteria are prokaryotic organisms containing chlorophyll "a" in the Kingdom *Bacteria*.

Methods

Site Descriptions

Plankton and sediment samples were collected from one rice and one sorghum field on August 9th, 2007. There were 3 replicate samples collected from site. Both sites there were standing water in troughs averaging 40 cm deep. Samples were collected from side of the agriculture field, no wading was involved, and the outflow from the sorghum field was not

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observed. The air temperature was 43ºC. The particular sites which the samples were obtained are from St. Frances County, Arkansas (sorghum field-34°56'47.46"N, 91°0'20.70"W and rice field-34°57'12.44"N, 91°0'21.24"W) that are 0.76 km in distance apart (Figure 1).

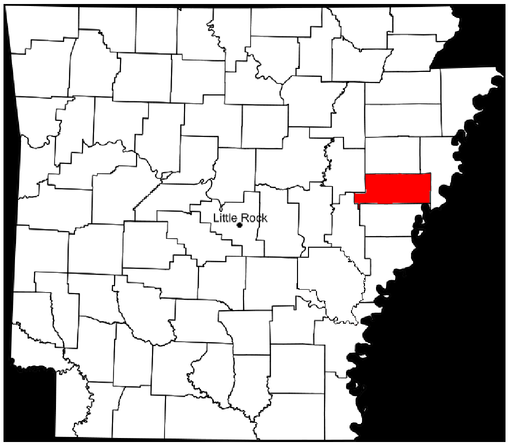

Figure 1. Locations of the two agriculture fields in St. Francis County, Arkansas.

The rice field was 22 hectares in size (Figure 2). The water temperature was 30ºC. It was disked and leveled and planted the second week of April. On May 19th, 2007, the field was fertilized with nitrogen and flooded for 2 weeks and then drained. After two weeks, the field was fertilized and flooded again and remained flooded. The field was finally drained on August 23rd and harvest started the middle of September. The yield averaged 7500 kg/hectare.

The sorghum field was 49 hectares in size (Figure 2) and the water temperature was 40ºC. It was planted on April 30 and again on May 1, 2007. Once the sorghum stalk head had bloomed, the field was irrigated in the furrows and fertilized in June. The irrigation method used followed this schedule: turn on the wells, wait until the water reached the other end of the sorghum field and stop irrigation. The field was allowed to dry out and irrigation was then repeated. They harvested in October. The yield averaged 7000 kg/hectare.

Samples were taken for plankton and sediment for identification of algal and cyanobacteria species. Plankton was collected using a Fieldmaster Mini Net 80 µm mesh from the water column and surface. Sediment was scrapped from the top 1 cm of the benthic region. The samples were collected in a sterile Whirl-pak® bag and placed in a cooler on ice (0ºC) until they were stored in the laboratory freezer. In the laboratory, algae were preserved with M3 (American Public Health Association 1992).

Figure 2. The two agriculture fields in St. Francis County, Arkansas (Google Earth).

Plankton samples were allowed to settle for concentration, while sediment samples were homogenized and mixed for slide preparation. Semipermanent slides were prepared with distilled water and sealed with epoxy (Smith, 2003). A Nikon BH-2 microscope was used at 1000X to identify algal and cyanobacteria species. Nomenclature, descriptions and keys follow Ettl & Gänter (1995), Desikachary (1959), Dillard (1989a, 1989b, 1990, 1991a, 1991b, 1993), Komárek and Anagnostidis (1999, 2005), Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b, 2000), Tilden (1910), and Uherkovich (1966).

Results and Discussion

Fifty-three species were identified from the two sampling sites (Table 1 and 2). Diatoms and cyanobacteria were equally dominant, represented by both having twenty-one species each (39.6% of the reported species) and green algae included eleven identified species (20.8%).

From the rice field, there were forty-three algal and cyanobacteria species identified, which is 81.1% of the total species identified. Diatoms were still dominant with twenty species identified (48.4% of the reported species), cyanobacteria had twelve species (27.9%) and green algae had eleven species (25.6%). Two of the twenty diatom species were planktonic and the other eighteen were benthic species, while seven green algal species were planktonic and only four were benthic.

Table 1. Annotated taxonomic list of the species of cyanobacteria and *Chlorophyta* recovered from samples collected from rice and sorghum fields in St. Francis County, Arkansas.

Taxa	Rice	Sorghum
Cyanobacteria		
Anabaena cylindrica Lemmermann	X	
Aphanocapsa fusco-lutea Hansgirg	X	
Aphanocapsa incerta (Lemmermann) Cronberg & Komárek	X	X
Aphanothece bullosa (Meneghini) Rabenhorst	X	
Aulosira fertilissima Ghose	X	
Calothrix confervicola (Dillwyn) C. Agardh	X	
Chlorogloeopsis fritschii (A. K. Mitra) A. K. Mitra et D. C. Pandey		X
Chroococcus disperses (Keissler) Lemmermann	X	
Chroococcus limneticus Lemmermann		X
Cylindrospermum marchicum Lemmermann	X	
Jaaginema geminatum (Meneghini ex Gomont) Anagnostidis & Komárek		X
Lyngbya aestuarii var. *arbustiva* Brühl & Biswas	X	
Microcystis natans Lemmermann		X
Nodularia spumigena Mertens	X	
Nostoc carneum C. Agardh	X	
Nostoc calcicola Brébisson ex Bornet & Flahault	X	
Nostoc piscinale Kützing ex Bornet & Flahault		X
Oscillatoria minnesotensis Tilden		X
Oscillatoria simplicissima (Gomont) Anagnostidis & Komárek		X
Phormidium aerugineo-coeruleum (Gomont) Anagnostidis & Komárek		X
Pseudanabaena limnetica (Lemmermann) Komárek		X
Chlorophyta		
Chara sp. 1 Linnaeus	X	
Coelastrum probiscideum Bohlin in Wittrock & Nordstedt	X	
Cosmarium granatum var. *concavum* Lagerheim	X	
Cosmarium rectangulare Grunow	X	
Cosmarium vexatum W. West	X	
Microspora stagnorum (Kützing) Lagerheim	X	
Microspora tumidula Hazen	X	
Oedogonium sp1. Link	X	
Oocystis solitaria Wittrock	X	
Scenedesmus ecornis (Ehrenberg) Chodat	X	
Scenedesmus lefevrei Deflandre	X	

There were seven heterocyst cyanobacteria species (58.3% of the cyanobacteria species identified) from the rice field samples. *Anabaena cylindrica* was abundant in the rice field, which may account for the abundance of free floating akinetes in the sample. Akinetes are resting spores to withstand adverse environmental conditions. Vegetative growth occurs from germinating akinetes (Wildman et al. 1975) as well has heterocysts (Tischer 1975). This might account for the high numbers of heterocyst species observed in the community composition. Their recruitment might come from soil akinetes, which needs to be studied further.

The sorghum field had eleven cyanobacteria and algal species identified (18.9% of the total species identified). Cyanobacteria were now dominant with ten species (90.9%) and only one diatom species (9.1%). Filamentous algae was the dominant form comprised of five species (50%) while the dominant observed species were coccoid species (*Chlorogloeopsis fritschii* and *Chroococcus limneticus*). There was only one heterocyst species found in the samples.

There was only one species (*Aphanocapsa incerta*) that was found in both agriculture fields. When ANOVA was used to compare the species similarities on the presence/absence species data, it was not surprising that the p-value was highly significant ($p=2.4x10^{-11}$). When Correspondence Analysis (CA) was run on the species data, 100% of the variation was explained by the first axes and species data separated out into distinct points (Figure 3).

Table 2. Annotated taxonomic list of the species of *Bacillariophyta* recovered from samples collected from rice and sorghum fields in St. Francis County, Arkansas.

Taxa	Rice	Sorghum
Bacillariophyta		
Achnanthes hauckiana Grunow	X	
Caloneis schumanniana (Grunov) Cleve	X	
Cocconeis placentula Ehrenberg	X	
Encyonema minutum (Hilse) D.G. Mann	X	
Fragilaria tenera (W. Smith) Lange-Bertalot	X	
Fragilaria ulna var. *acus* (Kützing) Lange-Bertalot	X	
Gomphonema augur Ehrenberg	X	
Gomphonema gracile Ehrenberg	X	
Hantzschia amphioxys (Ehrenberg) Grunow		X
Navicula cryptocephala Kützing	X	
Navicula molestiformi Hustedt	X	
Navicula subminuscula Manguin	X	
Navicula veneta Kützing	X	
Nitzschia amphibia Grunow	X	
Nitzschia fonticola (Grunow) Grunow in Van Heurck	X	
Nitzschia hantzschiana Rabenhorst	X	
Nitzschia intermedia Hantzsch	X	
Nitzschia palea (Kützing) W. Smith	X	
Nitzschia tryblionella var. *victoriae* (Grunow) Grunow	X	
Pinnularia microstauron (Ehrenberg) Cleve	X	
Sellaphora pupula (Kützing) Mereschkovsky	X	

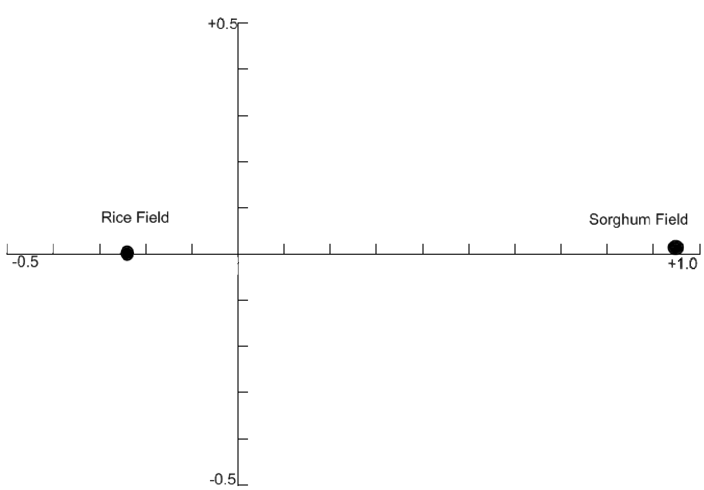

Figure 3. Correspondence Analysis (CA) conducted on the species data from the Rice and Sorghum Fields.

As a general observation, the overall study sites were diverse with respect to algal species richness, especially as a one-time sampling method. With respect to specific agriculture field assemblages of species present, the rice field was very diverse while the sorghum field was not. It was first thought both field would have similar species composition because of the close proximity of the sampling sites.

There was a 10-degree water temperature and utilization of different irrigation techniques between the two fields. This is likely the cause of the significant difference between the species assemblages. The rice field was flooded from May to August creating a more stable and homogenous and presumably less stressful environment. This in turn could allow ample time for algal colonization and increasing species diversity. The sorghum field, on the other hand, was flooded periodically, allowed to dry with higher temperatures and when needed flooded again. This may have created a higher disturbance not allowing many species to get established causing a lower species richness and lower community diversity. This follows the Intermediate Disturbance Hypothesis model proposed by Connell (1978).

The heterocyst cyanobacteria species (*Anabaena cylindrical, Aulosira fertilissima, Calothrix confervicola, Cylindrospermum marchicum Nostoc carneum, Nostoc calcicola*) made up a large portion of the algal community in the rice field. Future research needs to be accomplished to determine nutrient (nitrogen and phosphorus) concentrations of the water throughout the growing season. In addition, other environmental factors need to be investigated to determine their importance of limiting rice (Isisarri et al 2006) and sorghum growth.

It would be of further interest to understand the environmental conditions, which promotes the colonization of heterocyst species and their significance to the rice field community. Cyanobacteria heterocyst species inoculums can be applied to agriculture fields, which need to studied, as is done other countries. The inoculums of natural biological nitrogen-fixers have the potential of increasing soil nitrogen and thus crop yield (Roger and Kulasooriya 1980) and cutting the amount of agriculture nitrogen fertilizers, thereby reducing agricultural costs.

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