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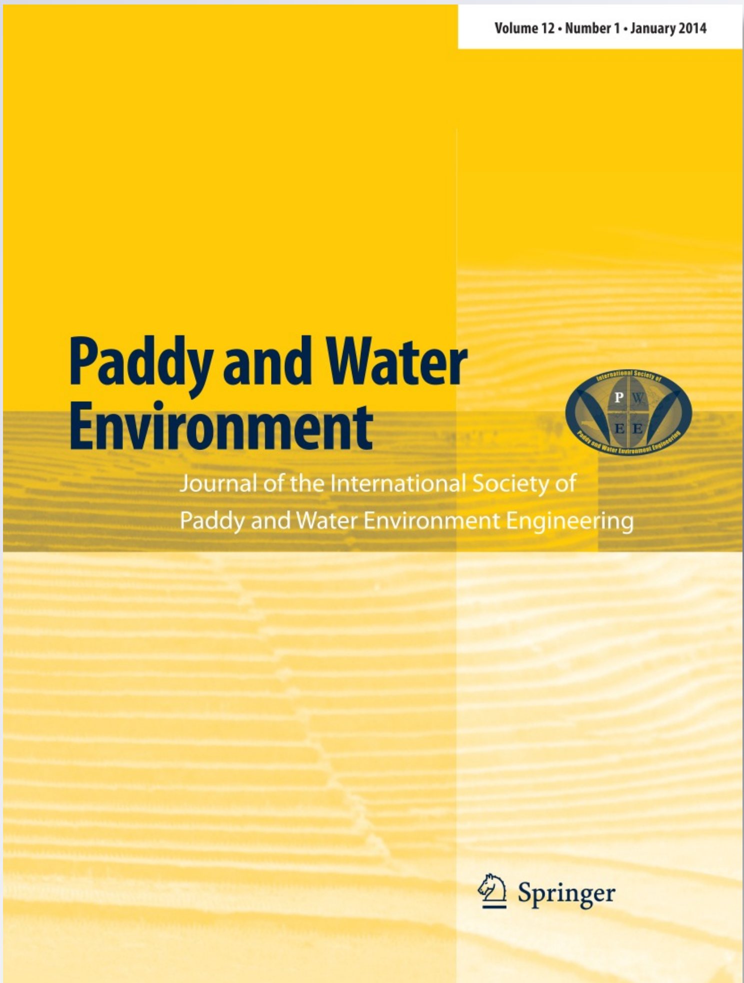

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ARTICLE

Reducing rice field algae and cyanobacteria abundance by altering phosphorus fertilizer applications

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Abstract In California's water-seeded rice systems, algal/cyanobacterial biomass can be a problem during rice establishment and can lead to yield reductions. Laboratory, enclosure, and field-scale experiments were established to evaluate the effects of fertilizer P management on algal/cyanobacterial growth. Two field-scale experiments evaluated the response of algal/cyanobacterial growth to three P management strategies: conventional surface applied, incorporated into the soil, and delaying P applications by 30 days. Results from these experiments indicated rice fields that received conventional surface-applied P fertilizer had 4–8 times more algal/cyanobacterial biomass and 3–11 times higher concentrations of soluble reactive phosphate (SRP) than those in which P fertilizer was incorporated or delayed. Laboratory experiments evaluated the ability of field water to support growth of *Nostoc spongiaeforme*. Results indicate that water from the incorporated or delayed P application fields was P limited for *N. spongiaeforme* growth. Water from the surface-applied fields was not P limited. Enclosure experiments evaluated the effects of delayed P applications on algal/cyanobacterial biomass and rice yields. Algal/cyanobacterial cover and biomass increased in enclosures which received added P. Soluble reactive phosphate concentrations were also significantly greater in these enclosures. Delaying the application by up to 28 days did not reduce rice yields in the enclosures. One management implication is that reducing SRP concentrations early in the season in rice field water will result in reduced algal/cyanobacterial biomass. Strategies to reduce water SRP include incorporating fertilizer P or delaying the P application by up to 30 days.

Keywords Nostoc spongiaeforme · Algae/cyanobacteria management · Water-seeded rice · Phosphorus

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Introduction

California rice fields share many characteristics of California's native wetlands. They are used by more than 235 species of birds and other animals (California Rice Commission 2005) and may serve as model systems for the study of wetland processes (Lawler and Dritz 2005). More than 200,000 ha of rice (*Oryza sativa* L.) is grown annually in California with most of it grown in the Sacramento Valley (Hill et al. 2006). These shallow-water, relatively high-nutrient systems provide ideal conditions for growth of cyanobacteria and algae as well as rice. Biomass of mat-forming species of green algae (e.g., *Rhizoclonium* and *Hydrodictyon*) and N_2-fixing cyanobacteria (e.g., *Nostoc spongiaeforme*) may achieve up to 154 g m^{-2} dry weight (Spencer et al. 2006), which is among the higher values reported for rice fields worldwide (Roger and Kulasooriya 1980). Species of N_2-fixing cyanobacteria may be desirable in some rice culture systems (Vaishampayan et al. 2001), but may cause problems for the water-seeded rice production systems used in California. Especially during the first 30 days after initial flooding, rice seedlings become entangled with the algal/cyanobacterial mats and are subsequently uprooted by them when the mats dislodge from the soil surface. Managing algal/cyanobacterial growth in rice fields is a significant endeavor for growers. Traditional management approaches (copper sulfate applications) have become less effective, have raised environmental concerns (Hill et al. 2006), and are becoming less economical to apply. Thus, alternative management techniques which integrate management with features of the rice field environment are required. However, there is relatively little information about the ecological interactions and requirements of algae/cyanobacteria within the context of California rice fields (Spencer et al. 2006; Chapman et al. 1972) as most studies of rice field algae/cyanobacteria have been conducted in other rice-growing regions of the world (Whitton 2000; Roger and Kulasooriya 1980). Information on the specific environmental requirements of nuisance species of cyanobacteria and algae that grow in California rice fields would be useful in developing alternative management strategies for these systems.

The well-known relationships between nitrogen and phosphorus and the productivity of freshwater systems (Wetzel 2001; Kalf 2002; Istvanovics 2010) suggest that the high concentrations of these nutrients may be involved in abundant growth of cyanobacteria and algae in rice fields. Phosphorus may be especially important with respect to the cyanobacterium, *N. spongiaeforme*, because of its ability to fix nitrogen. Roger et al. (1987) reported that the abundance of cyanobacteria in 104 soil samples from rice fields in the Philippines, India, Malaysia, and Portugal was positively correlated with the level of available P in the soil and the soil pH. Another study of soils from 58 rice fields in Bangladesh reported that the concentration of available P in soil was significantly positively correlated with the abundance of heterocystous cyanobacteria (Mandal et al. 1993). Quesada and Fernandez-Valiente (1996) reported that water concentrations of soluble reactive phosphate (SRP) correlated positively with heterocystous cyanobacteria presence in rice fields in Valencia, Spain. A survey of rice fields in Hefeng County (China) by Qiu et al. (2002) found the total P content of water in fields where *N. sphaeroides* colonies were present was about 3–7 times more than in fields that did not have *N. sphaeroides* colonies. A survey of California rice fields indicated that there was also a significant positive relationship between SRP and the biomass of algae in fields (Spencer et al. 2006). Phosphorus fertilizer is typically added as a one-time application to the surface of California rice fields at various rates averaging 24 kg P ha^{-1} prior to flooding. The objective of our study was to develop alternative but economically viable P management strategies that reduce algal biomass while maintaining rice yields. These objectives were accomplished by testing two hypotheses: (1) that phosphorus fertilizer applied to the surface of California rice fields enhances growth of *N. spongiaeforme* and other mat-forming species and (2) that delaying fertilizer P applications by 4 weeks will not reduce rice yields. To accomplish our objectives, we performed two field-scale experiments, a laboratory experiment, and two enclosure experiments in production rice fields.

Materials and methods

This study consisted of a combination of experiments conducted in different growing seasons. Laboratory, enclosure, and field-level experiments were performed to evaluate the effects of fertilizer P management on algal/cyanobacterial growth in rice fields. There were two field-scale experiments which evaluated the response of algal/cyanobacterial growth to surface, incorporated, and delayed P applications. Additional laboratory growth experiments used water collected from these fields to determine whether or not the soluble reactive phosphorus levels in the water were sufficient for *N. spongiaeforme* growth or would limit growth of this troublesome species which occurs in California rice fields. Enclosure experiments conducted in two additional rice fields during 2010 further evaluated the effects of delayed P applications on algal/cyanobacterial biomass and rice yields.

Field experiment

During the 2008 growing season, an experiment was carried out at two locations (site J, 39°27′38.83″N; 121°42′7.34″W and site C, 39°14′13.43″N; 121°42′28.41″W) in the Sacramento Valley, California. In this study, at least one field at each location received one of the following P fertilizer regiments: (1) conventional surface applied rolled (SUR), that is, liquid P applied directly to the surface followed by a roller implement; (2) spring applied (SA) incorporated, that is, P fertilizer applied in the spring and incorporated as part of seedbed preparation; and (3) thirty-day delay (30D), that is, P fertilizer applied 30 days after initial flooding of the field. At site J, the treatments, number of fields, and approximate field sizes treated were as follows: SUR, two fields (7.2 and 18.5 ha); SA, two fields (7.1 and 10.3 ha); and 30D, one field (6.7 ha). Similar information at site C is as follows: SUR, one field (3 ha); SA, one field (9.2 ha); and 30D, one field (5.6 ha). We collected 0.25 L water samples for soluble reactive phosphate (SRP) determination within 1 or 2 days following flooding (May 8 for site J and May 1 for site C). Samples were collected approximately 2 m from the edge of each field and at each point where water flowed into or out of the field. Samples were collected using an extendable aluminum pole with a stainless steel cup attached and placed into acid-rinsed plastic bottles. At site J, six samples were collected for each field. At site C, the number of samples per field varied from 15 to 17 due to the size and location of inlets and outlets within each field. Samples were placed on ice and returned to the laboratory where they were frozen. Subsequently, the samples were thawed, filtered through 47-mm glass fiber filters, and analyzed for the concentration of SRP using the molybdate–ascorbic acid–antimony method described by Wetzel and Likens (2000). Near the end of the initial 30-day period following flooding, we collected algal/cyanobacterial biomass using a 0.46-m-diameter by 0.5-m-tall section of PVC pipe. The pipe was randomly placed near the edge of a field at eight locations (2 per side of field), and all of the algae/cyanobacteria within it were collected using a 0.15-m-diameter stainless steel 1-mm-mesh strainer. The material was returned to the laboratory and dried at 65 C for 48 h and weighed. Biomass values were converted to grams dry weight per square meter.

For all treatments at all sites, the samples are considered as subsamples within the fields because there were not replicate fields. Statistical analysis in this case consisted of calculating the mean and standard error. Treatment effects were evaluated by comparing values for the 30D and SA treatments with the SUR treatment (which is the current practice used by more than 50 % of growers). This inferential method is similar to the "optimal impact studies" approach described by Hurlbert (1984) as being appropriate when it is not possible to employ replicate experimental units (i.e., fields). Hurlbert cites deforestation studies of nutrient cycling (Likens et al. 1970, 1977) and whole-lake fertilization experiments (Schindler et al. 1971; Schindler 1974) as examples where this approach has been informative. We used this approach because of the large sizes of fields (3–18.5 ha) employed in this study, as well as the fact that these fields were production rice fields. Growers were only willing to risk one or two fields for the experimental treatments which were new and untested in these systems prior to this work.

Laboratory experiment

The potential for water from fields that received each P fertilizer treatment (SUR, SA, 30D) to support growth of cyanobacterium, *N. spongiaeforme,* was determined in laboratory experiments. This was evaluated by collecting 3 L of water at a single site from each of the fields that received the above P fertilizer treatments within 1 or 2 days following flooding. The samples were returned to the laboratory and filtered using a glass fiber filter (47-mm glass fiber filters). Samples were frozen and later used in bioassay experiments. Experiments were conducted in growth chambers set to maintain 25 °C, 400 μM m^{-2} s^{-1}, and a 12:12-h light/dark cycle. *Nostoc spongiaeforme* used in this study had been originally isolated into "unialgal" cultures from a northern California rice field and maintained in culture at Purdue University (Spencer et al. 2011). Stock cultures were maintained in a growth chamber at the USDA ARS Exotic and Invasive Weed Research Unit (Davis, California) in 2.8-L Fernbach flasks containing 1 L of BG-11 medium (Stanier et al. 1971) without nitrate, at 25 °C, 400 μM m^{-2} s^{-1}, and a 12:12-h light/dark cycle. The filtered rice field water was adjusted to an initial pH of 6.8. *N. spongiaeforme* was transferred to fresh BG-11 medium free of PO_4-P and allowed to grow for 1 week prior to being used to inoculate experimental flasks. Flasks (0.5 L polycarbonate with 0.25 L of filtered field water) were inoculated with 2 mL of *N. spongiaeforme*. There were five flasks per P fertilizer treatment (SUR, SA, and 30D). Following inoculation, two 10-mL aliquots were withdrawn from each flask and filtered through a glass fiber filter. Chlorophyll content was determined following extraction with DMSO as described by Spencer and Ksander (1987). *Nostoc spongiaeforme* was allowed to grow for 7 days, and chlorophyll was determined on two additional 10-mL aliquots as described above. Logarithms (base 2) of initial and final chlorophyll concentrations were used to calculate *N. spongiaeforme* growth rates using linear regression of chlorophyll concentration versus sample day. Growth rates were compared across P fertilizer treatments using analysis of variance. All statistical analyses were performed using SAS (SAS Institute Inc 2004).

Enclosure experiment

The enclosure experiments were conducted during the 2010 growing season. Prior to flooding, twenty-four large metal rings (0.75 m diameter × 0.3 m tall) were installed in each of two rice fields (field R, 39°31′33.60″N; 121°48′52.44″W or M, 39°31′33.60″N; 121°48′52.44″W). The rings were driven into the soil to a depth of 0.1 m using a sledge hammer. Each ring enclosed an area of 0.44 m^2. Rings were randomly assigned as treated or untreated controls. The treatment consisted of adding 5.5 g of $Ca(H_2PO_4)_2$ per ring at each of three times: day 0 (i.e., prior to flooding), and 14 and 28 days after flooding (DAF). This treatment rate is equivalent to 24 kg P ha^{-1}, and it is the recommended P fertilizer application rate for these fields. Nitrogen- and potassium-containing fertilizers were also applied to all rings at the same rates used by growers to ensure that these nutrients did not limit rice growth. There were four replicate rings for each treatment combination. In addition, a Hobo Pendant temp/light data logger (Onset Computer Corp., Bourne, Massachusetts) was installed in one ring at the west end of the ring layout and one at the east end of the ring layout in each field. Daily mean water temperature was calculated and plotted versus date. Cameras (Moultre Game Spy D-55 IR digital game camera, EBSCO Industries Inc., Birmingham Alabama) were installed 2 m above the treated and untreated rings. The cameras recorded a digital photograph of the water surface within each ring once each hour for 14 days following the addition of the treatment. Photographs from days that biomass samples were collected (7 and 14 days after treatment) were chosen, and the area covered by floating algal mats was determined using an image analysis program (Sigma Scan Pro, SPSS, Inc., Chicago, Illinois, USA). Cover (%) of floating algal mat was estimated by dividing the area of the algal mat by the total area enclosed in the ring. Algal biomass was estimated by removing all algal material floating on the surface of the water 7 and 14 days after the treatment was applied using a 15-cm-diameter stainless steel 1-mm-mesh strainer. This method effectively collected floating colonies of the dominant *N. spongiaeforme* and other mat-forming cyanobacteria which are found in these rice fields (Spencer et al. 2006). The 7-day after-treatment samples in field R scheduled to be collected on June 16 were not collected. For this date, biomass was estimated from a linear regression equation relating mat area to biomass.

Flooding began at field M on May 13, 2010, and on May 26, 2010, in field R, and the crop was seeded 1–3 days later. We also collected 0.25 L water samples from each ring using clean plastic bottles which had been acid-rinsed. Water samples were analyzed for the concentration of soluble reactive phosphate (SRP) in the water using the molybdate–ascorbic acid–antimony method described by Wetzel and Likens (2000). At harvest, the grain yields were determined from the plants within each ring.

Algal biomass, cover data, and rice yield data were checked for homogeneity of variances and normality of error distributions prior to further analysis. When necessary to remove heterogeneity of variance, an appropriate power transformation was applied before performing analysis. Dry weights were transformed by raising them to the power 0.63 and cover to the power 0.69. Analysis of variance for algal biomass, algal cover, and water SRP was calculated using PROC GLM in SAS (SAS Institute, Inc. 2004), with P addition (+P), sample date (DAT), and treatment date (day treated) as the treatments. All appropriate interaction terms were included. For yield data in the enclosure experiments, there was no significant difference in yields for the untreated P controls. Therefore, the untreated controls were averaged across P addition times within each replication and data analyzed as a randomized complete block design.

Results and discussion

Measurements from the 2008 field study comparing different phosphate fertilizer application methods (30D, SA, and SUR) indicate that SRP concentration was lower in fields that received either the SA or the 30D application (Table 1) with lowest SRP values for the 30D application. Algal/cyanobacterial biomass was also lowest for fields which received the 30D treatment (Table 1). These fields had from 72 to 88 % less algal/cyanobacterial biomass than fields which received the SUR phosphate application which had the highest levels of algal/cyanobacterial biomass. Algal/cyanobacterial biomass was greater for site J than for site C. Water temperature impacts *N. spongiaeforme* growth (Spencer et al. 2011), and this in addition to fluctuations in water depth may partly explain the differences between the locations.

Table 1 Soluble reactive phosphate (SRP) and algal dry weight from rice fields that received three P fertilizer application treatments (30D, P applied 30 days after initial flooding; SA, P applied in the spring and incorporated; and SUR, P applied directly to the soil surface prior to flooding)

Location	P fertilizer treatment	SRP (mg L^{-1})	Algal dry weight (g m^{-2})
C	30D	0.071 ± 0.008 (17)	1.92 ± 1.15 (8)
	SA	0.085 ± 0.006 (15)	3.54 ± 1.83 (8)
	SUR	0.216 ± 0.025 (16)	6.96 ± 3.67 (8)
J	30D	0.021 ± 0.003 (8)	5.07 ± 1.42 (6)
	SA	0.057 ± 0.011 (16)	17.51 ± 5.61 (13)
	SUR	0.230 ± 0.063 (16)	41.07 ± 9.57 (17)

Values are the mean ± standard error. The number of subsamples is indicated by the number in parenthesis

Nostoc spongiaeforme growth rates were higher in water from the SUR fields than in water from the SA and 30D fields (Table 2). Growth rates from SA and 30D fields were stimulated to near those of the 30D treated fields by the addition of P. This indicates that P was limiting growth for water from the SA and 30D treatments. These results agree with the algal/cyanobacterial biomass collected after 30 days and the SRP measurements from the fields shortly after initial flooding.

For the ring (enclosure) experiments, mean daily water temperature varied over time (Fig. 1). This was especially obvious for field M, which flooded earlier and had low water temperatures during the 2-week period immediately after flooding (Fig. 1). In contrast, water temperatures in field R generally increased over time and did not display an initial low period after flooding. Algal cover and biomass increased in rings which received added P when compared to untreated control rings (Table 3). Analysis of variance for the cover and biomass data from field R indicates that adding P fertilizer significantly increased biomass of cyanobacteria and algae present in the rings (Table 4). A similar significant response to P addition was observed for the analysis of the data from field M (Table 4). However, in field M there were additional significant effects.

Table 2 *Nostoc spongiaeforme* growth rates in water collected from rice fields that received three P fertilizer application treatments (30D, P applied 30 days after initial flooding; SA, P applied in the spring and incorporated; and SUR, P applied directly to the soil surface prior to flooding)

Site	P fertilizer treatment	Growth rate (doublings day^{-1})	
		Filtered water	Filtered water ± P
C	30D	0.017 ± 0.003	0.147 ± 0.024
	SA	0.024 ± 0.002	0.101 ± 0.005
	SUR	0.117 ± 0.010	0.134 ± 0.010
J	30D	0.033 ± 0.006	0.158 ± 0.029
	SA	0.070 ± 0.023	0.125 ± 0.026
	SUR	0.176 ± 0.011	0.166 ± 0.010

The heading "filtered water" indicates growth rates for water from a field that received a particular treatment and that was filtered only. The heading "filtered water ± P" indicates that the filtered water was supplemented with PO_4-P equivalent to 22 mg L^{-1}. Values are the mean ± standard error based on five replications. Analysis of variance results indicate significant differences due to P fertilizer treatment method (C, $P < 0.0001$; J, $P < 0.0001$), P addition (C, $P < 0.0001$; J, $P = 0.0002$), and the interaction between these main effects (C, $P = 0.0013$; J, $P = 0.0005$)

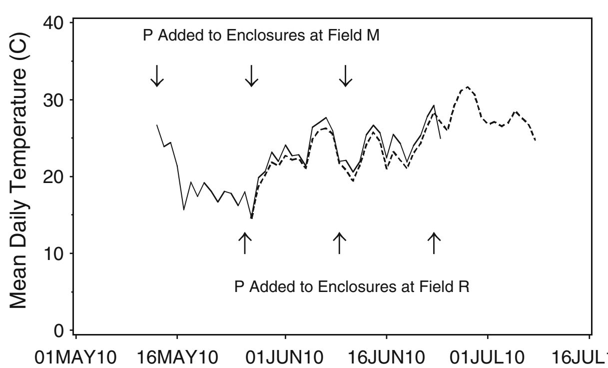

Fig. 1 Mean daily water temperature (C) in metal rings used in the enclosure experiments at two sites. The *solid line* presents mean water temperature for field M and the *dashed line* for field R. Values are the daily means of readings taken every 0.5 h by two data loggers. *Arrows* indicate the timing of the addition of P fertilizer into the rings

The significant effect of day treated and the interaction between day treated and day sampled reflect the lack of algal/cyanobacterial mats in the rings which received the P fertilizer treatment immediately after the field was flooded. This was likely due to the lower water temperatures in the rings during this period (Fig. 1).

One other aspect of the algal/cyanobacterial biomass results that deserves consideration concerns the similarity in biomass for the 14-day samples in field R for the latest day of treatment, June 23 (Table 3). Between June 17 and June 20 while this experiment was still in progress, the water level in the field (and thus the rings) dropped to the point that bare soil was exposed. This would have altered

Table 3 Dry weight (g) and cover (%) for floating mats of cyanobacteria and algae present in rings that received added phosphorus (Yes) or not (No) on three dates and sampled 7 or 14 days after the P was added

Field	Date added	Days sampled	+P	Dry weight (g) Mean ± SE	Cover % Mean ± SE
M	5/13	7	No	0 ± 0	0 ± 0
			Yes	0 ± 0	0 ± 0
		14	No	0 ± 0	0 ± 0
			Yes	0 ± 0	0 ± 0
	5/27	7	No	1.0 ± 0.4	4 ± 3
			Yes	2.6 ± 1.8	14 ± 12
		14	No	1.5 ± 0.6	7 ± 4
			Yes	4.3 ± 3.5	26 ± 23
	6/10	7	No	2.9 ± 2.7	17 ± 18
			Yes	7.4 ± 4.1	46 ± 27
		14	No	11.4 ± 2.2	72 ± 15
			Yes	11.3 ± 1.0	72 ± 7
R	5/26	7	No	0.5 ± 0.1	1 ± 1
			Yes	2.2 ± 3.1	16 ± 22
		14	No	1.6 ± 0.5	8 ± 3
			Yes	7.9 ± 6.0	67 ± 23
	6/09	7	No	1.5 ± 1.3	7 ± 8
			Yes	5.5 ± 6.6	34 ± 43
		14	No	5.2 ± 7.0	32 ± 46
			Yes	5.6 ± 5.6	34 ± 37
	6/23	7	No	0.2 ± 0.3	0 ± 0
			Yes	2.1 ± 1.6	11 ± 10
		14	No	2.4 ± 1.5	14 ± 10
			Yes	2.7 ± 1.5	15 ± 10

Values are the mean and standard error (SE) based on four replications per treatment combination. The dates were 0, 14, or 28 days after the field was initially flooded. Note that for field R the water level dropped to zero depth on 6/18 and 6/19. Refer to Table 5 for statistical analysis

both algal growth and phosphate availability and may account for the similarity in biomass. This information was available to us because of the cameras that had been installed above the rings and which recorded a photograph every hour. In future experiments it may be worthwhile to install this type of monitoring system especially in rice fields which often undergo "unpredictable" water-level fluctuations.

Water SRP concentrations in water samples from the P-added rings were significantly greater (16–45 times) than those in untreated rings in samples collected the same day that the fertilizer was added (Tables 5 and 6). Mean SRP concentrations decreased markedly after 1 week. This was likely due to uptake by algae which grew during the period and adsorption of P to soil surfaces. The analysis of variance results indicated more significant interaction terms for

Table 4 Results of analysis of variance for dry weight (g m^{-2}) and cover (%) of floating mats of cyanobacteria and algae versus P addition (+P, 0 added P or 24 kg ha^{-1}), days after treatment that the sample was collected (DAT, 7 or 14), and the day treated (day treated, 0, 14, or 28 days after flooding) for two northern California rice fields (M and R)

Field	Source	Pr. > F
Dry weight (g m^{-2})		
M	+P	0.01
	DAT	0.0003
	+P × DAT	0.34
	Day treated	<0.0001
	+P × day treated	0.17
	DAT × day treated	0.0008
	+P × DAT × day treated	0.14
R	+P	0.02
	DAT	0.02
	+P × DAT	0.75
	Day treated	0.18
	+P × day treated	0.79
	DAT × day treated	0.79
	+P × DAT × day treated	0.35
Cover (%)		
M	+P	0.01
	DAT	0.0003
	+P × DAT	0.33
	Day treated	<0.0001
	+P × day treated	0.16
	DAT × day treated	0.0007
	+P × DAT × day treated	0.14
R	+P	0.017
	DAT	0.02
	+P × DAT	0.77
	Day treated	0.19
	+P × day treated	0.66
	DAT × day treated	0.75
	+P × DAT × day treated	0.36

field M. This is most likely due to the data from the May 27 P application where there were no differences in SRP due to the addition of P (Table 5). This is in contrast to data from the two other treatment dates from field M and all three dates in field R, which showed significant differences when P was added. There are at least two possible explanations for this discrepancy. One is that the water samples were collected prior to the addition of the P, and the second is that the samples may have been collected too soon after the P addition for the P to have completely dissolved. This may have happened because two separate groups of people were responsible for adding the P fertilizer and collecting the water samples. Despite this, for five of six application dates the differences in SRP shortly after P application were marked and consistent.

Table 5 Concentrations of soluble reactive phosphate (SRP) in water samples from rings treated with triple superphosphate ($Ca(H_2PO_4)_2$) on various date and measured on 0, 7, or 14 days after the triple superphosphate was applied

Field	Date applied	Day sampled	P fertilizer applied	SRP (mg L^{-1})
M	May 13	0	No	0.019 ± 0.003
			Yes	0.687 ± 0.199
		7	No	0.015 ± 0.001
			Yes	0.019 ± 0.002
		14	No	0.013 ± 0.001
			Yes	0.017 ± 0.001
	May 27	0	No	0.014 ± 0.001
			Yes	0.019 ± 0.004
		7	No	–
			Yes	–
		14	No	0.016 ± 0.002
			Yes	0.017 ± 0.001
	June 10	0	No	0.017 ± 0.003
			Yes	0.760 ± 0.092
		7	No	0.017 ± 0.001
			Yes	0.055 ± 0.010
		14	No	0.017 ± 0.003
			Yes	0.025 ± 0.002
R	May 26	0	No	0.027 ± 0.004
			Yes	0.430 ± 0.230
		7	No	0.017 ± 0.003
			Yes	0.017 ± 0.001
		14	No	0.018 ± 0.004
			Yes	0.014 ± 0.000
	June 10	0	No	0.016 ± 0.001
			Yes	0.284 ± 0.072
		7	No	0.020 ± 0.001
			Yes	0.089 ± 0.014
		14	No	0.015 ± 0.002
			Yes	0.032 ± 0.009
	June 24	0	No	0.016 ± 0.001
			Yes	0.503 ± 0.154
		7	No	0.023 ± 0.003
			Yes	0.045 ± 0.003
		14	No	0.015 ± 0.001
			Yes	0.021 ± 0.003

Values are mean ± standard error, $N = 4$. Data are not available for field M on day 7. Refer to Table 7 for statistical analysis

P was applied as 5.5 g triple superphosphate per ring which corresponds to 2.14 g of PO_4-P. Assuming that the water depth within the rings was 10–15 cm, the volume within each ring was 44–67 liters. Dividing the amount of PO_4-P applied by these volumes shows that if all of the

Table 6 Results of analysis of variance for soluble reactive phosphate (SRP) versus P addition (+P, 0 added P or 24 kg ha^{-1}), days after treatment that the sample was collected (DAT, 0, 7, or 14), and the day treated (day treated, 0, 14, or 28 days after flooding) for two northern California rice fields (M and R)

Field	Source	Pr. > F
SRP (mg L^{-1})		
M	+P	<0.0001
	DAT	<0.0001
	+P × DAT	<0.0001
	Day treated	<0.0001
	+P × day treated	<0.0001
	DAT × day treated	<0.0001
	+P × DAT × day treated	<0.0001
R	+P	<0.0001
	DAT	<0.0001
	+P × DAT	<0.0001
	Day treated	0.78
	+P × day treated	0.79
	DAT × day treated	0.64
	+P × DAT × day treated	0.66

PO_4-P dissolved in the water, the SRP concentration would be in the range of 32–48 mg L^{-1}. Since the mean value measured just after application was 0.49 mg L^{-1}, then on average between 2 and 3 % of the applied PO_4-P was actually measured in the water as SRP and available to support algal/cyanobacterial growth.

The rice yield data from the 2010 study show a positive response to fertilizer P application at both locations (Fig. 2). Furthermore, P applications at flooding and 14 and 28 DAF resulted in the same yields. This supports other work by Lundy et al. (2012) showing that delaying fertilizer P applications by up to 28 DAF does not negatively impact yields.

The increase in algal/cyanobacterial biomass in both field and enclosure studies with added P occurred across soil types. The soils at each location were moderately acidic and contained relatively high amounts of clay which are typical of many California rice soils (Table 7). Based on soil Olsen P values, sites J and C had sufficient P levels and sites M and R were low and required P fertilizer for optimal rice growth (Linquist and Ruark 2011). The increase in algal/cyanobacterial biomass with added P agrees with previous data from California rice fields on the relationship between algal/cyanobacterial biomass and SRP water concentration in rice field water samples (Spencer et al. 2006). Results also agree with reports on the response of cyanobacteria to P additions. Elwood et al. (1981) reported that *Nostoc* abundance was significantly greater in portions of a Tennessee woodland stream which received added P than in sections which did not. Mateo et al. (2010) measured phosphatase activities (an indicator of P limitation) associated with four species of cyanobacteria (including *N. verrucosum)*, growing in the River Muga (Spain), and concluded that their growth was limited by available P. These results also contribute to an improved understanding of data from rice fields in other parts of the world (Rogers et al. 1987; Mandal et al. 1993; Quesada and Fernandez-Valiente 1996).

The high levels of SRP in treated rings were similar to previously reported SRP levels in California rice fields (Spencer et al. 2006). Taken together, the experimental results for the effect of SRP on algal/cyanobacterial biomass and the data on SRP in P-added rings are strong evidence that the well-known relationship between P and the productivity of aquatic systems applies to rice fields as well as other freshwater habitats (Wetzel 2001; Kalf 2002).

The management implication from these experimental results is that reducing SRP concentrations in rice field water will result in reduced algal biomass. This reduction can be accomplished by either incorporating P fertilizer

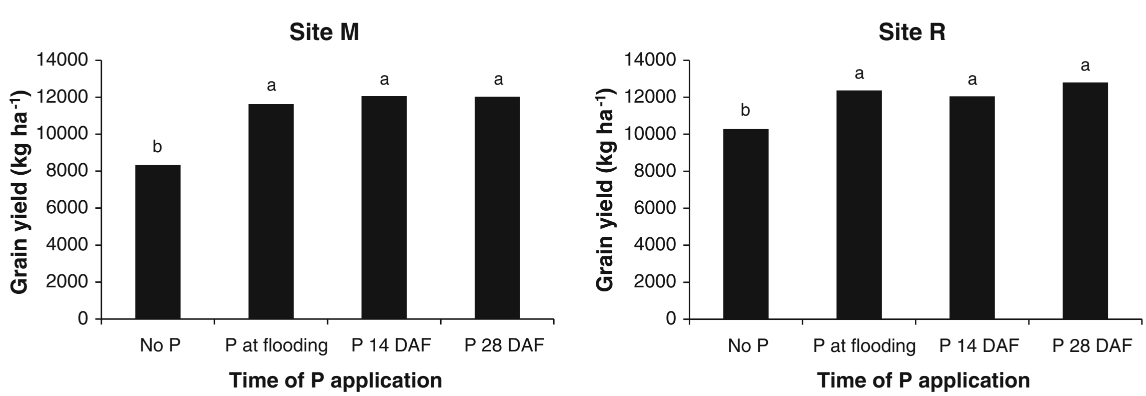

Fig. 2 The effect of fertilizer P and the timing of application on rice yields at two locations. Yields are adjusted to 14 % moisture. Different *letters* above each *bar* for each location indicate that the yields are significantly different ($P < 0.05$). DAF refers to days after flooding the field in preparation for planting

Table 7 Soil properties of the four study locations

Site	pH	Olsen P mg kg^{-1}	CEC meq 100 g^{-1}	Sand %	Silt %	Clay %
J	5.1	10.0	28	27	40	33
C	5.6	10.4	23	43	29	28
M	5.1	1.5	47	17	24	59
R	5.7	2.2	45	24	24	52

into the soil or delaying its application until rice plants are well above the water surface (usually within two to 3 weeks after planting). Certainly, growers need to consider the economic impacts of these decisions. Incorporation of P into soils is likely to have limited economic impact as the timing of P fertilizer applications can be changed and applied earlier—before the last tillage operation. Delaying the P applications can be more costly, and many growers also apply a top-dress N application by aircraft around this time; this P fertilizer could be applied at that time, resulting in little extra cost.

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