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Overall effect of rice biomass and fish on the aquatic ecology of experimental rice plots

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Abstract

The integration of rice and fish culture promises ecologically sound and economically successful management of floodwater ecosystems. Amongst other benefits, the stocking of fish in rice fields may contribute to the soil fertility of the rice field. We investigated the impact of the rice biomass and the stocking of fish (a polyculture of Nile tilapia and common carp) on ammonium, nitrate and *ortho*-phosphate levels in the field floodwater and the interstitial water of the oxidised soil layer. The collected data were submitted to principal component analysis (PCA) and ANOVA. The PCA solution contained three components, good for 66.21% of the total variability. Using the PCA components we were able to illustrate that the aquatic environment is largely determined by the growth of the rice crop. This is not surprising since the dry weight biomass per hectare for rice was more than 8000 kg as compared to an actual standing wet biomass of fish of 193.4 kg ha^{-1}. We suggest that the large difference in biomass means that effects on nutrients caused by the fish are obscured by rice driven processes. Still, the stocking of fish had a significant effect on 5 out of 12 variables under research. Chlorophyll-*a* levels nearly doubled, while oxygen levels were lower. The presence of fish also decreased the concentration of *ortho*-phosphate in the water and in the interstitial soil water. The floodwater and soil interstitial water patterns for nutrients were very similar.

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Keywords: Aquatic ecology; Rice; Fish; *Oreochromis niloticus*; *Cyprinus carpio*

1. Introduction

Rice fields have a unique limnology not mirrored by any natural aquatic habitat although they share some features of marshes, shallow lakes and ponds (Fernando, 1995). It is a temporary aquatic environment subject to large variations in insolation, temperature, pH, dissolved oxygen concentration, and nutrient status due to frequent disturbances such as the use of agrochemicals (Watanabe and Roger, 1985; Roger, 1989). Despite the eutrophic nature of lowland rice fields in tropical regions, frequent destruction of the environmental conditions by human activities inhibits its successional development towards a marsh (Watanabe and Roger, 1985). The extreme instability of the rice agro-ecosystem, the rapid fluctuations inherent to the crop cycle, and the artificial and temporary nature of the rice field renders it a difficult ecosystem to study as agrochemical use

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and frequent disturbances interrupt observations of community structure, population succession, and nutrient cycling (Watanabe and Roger, 1985; Grant et al., 1986).

The stocking of fish in rice fields is a 2000-year-old apparently successful practice. Fish are stocked in rice fields with the aim of increasing and diversifying the rice field productivity. According to Roger (1996) the integration of rice and fish is probably the most promising alternative to rice monocropping offering new opportunities to farmers. Rice–fish farming in many South and Southeast Asian countries is characterized by intensive rice-culture (two to three high yielding rice crops a year) and extensive culture of fish in a polyculture. Fish yields are usually very low, about 300 kg ha^{-1} (Lightfoot et al., 1992; Nhan et al., 1998). Nile tilapia and common carp are amongst the three most popular fish species for rice–fish culture in Vietnam's Mekong Delta (Rothuis et al., 1998c).

According to Lightfoot et al. (1993) stocking of fish in rice fields may contribute to the soil fertility of the rice field. The enhancing effect of fish on physico-chemical characteristics of soil (and floodwater) has been reported by a number of researchers, summarized by Cagauan (1995, 1999). Most of these studies, done in China, show positive outcomes but lack statistical validity (Cagauan, 1999). Cagauan (1995) hypothised that nutrients in the floodwater and oxidized surface soil can be influenced by fish in three ways: (1) additional nutrients from decomposing dead fish and from fish faeces, (2) fish perturbation of the soil–water interface which leads to release of fixed nutrients from soil to water and makes the soil porous for nutrients readily absorbed by the rice roots, and (3) fish grazing on the photosynthetic aquatic biomass and other components of the system which aids in nutrient recycling and decreases N losses. Halwart (1995) also added pH stabilization in the presence of fish as a possible process for increased nutrient availability.

While rice and fish production in rice–fish fields have often been studied (e.g. Little et al., 1996; Haroon and Pittman, 1997; Rothuis et al., 1998b, 1999; Vromant et al., 2002a,b,c, 2004), only a few studies have been conducted on the impact of fish stocking on the rice aquatic environment (Rothuis et al., 1998a; Vromant et al., 2001a,b). These studies found that the rice biomass has a large impact on the aquatic community and particular nutrients in the field floodwater. No peer-reviewed data on the impact of fish on soil nutrients in the field floodwater are available. In the present study, the main objective was to find out the impact of the rice biomass and the stocking of fish (a polyculture of Nile tilapia and common carp) on ammonium, nitrate and *ortho*-phosphate levels in the field floodwater and in the interstitial water of the oxidised soil layer just below the soil–water interface. Collection of data on rice and fish yield were not the main purpose of the experiment. Such data can be found in Rothuis et al. (1998b) and Vromant et al. (2002a,b,c, 2004).

2. Materials and methods

2.1. Experimental setup and site

We conducted an experiment with two treatments (treatment 1: fish polyculture present, treatment 2: fish polyculture absent) in a completely randomized design with four replicates. The experiment was conducted in eight experimental rice–fish tanks at the Mekong Delta Farming Systems Research and Development Institute, Can Tho University, Can Tho, Vietnam in the wet season 1998.

All experimental rice–fish tanks measured 20 m^2 (5 m × 4 m) and were 60 cm deep. They had a separate water inlet (tap water) and two water outlets, one placed at soil surface level in order to drain the rice field, and the other (an overflow) placed at a level of 15 cm above soil surface level to maintain the water level at 15 cm. Each tank was divided in two unequal parts by building a 40-cm high dividing wall. The smaller part was 16% of the tank surface and was used as a trench. The larger part was 84% of the tank surface and was used as a rice field. The trench part of each tank was shaded by a partial shade cover to avoid extremely high water temperatures. The field part of each tank was filled to a depth of 40 cm with topsoil from a neighbouring rice field. The collected topsoil was homogenized by mixing before it was added to the tanks.

2.2. Experimental calendar

The experiment started on 28 August 1998 when the rice was transplanted. A full experimental calendar can be found in Table 1.

Table 1
Experimental and sampling calendar for the rice–fish experiment, Can Tho, Vietnam

Date	DAT	Activities	Note
01/07	−58	Harvest of pre-crop	
08/08	−20	Seeding rice in nursery	Rice variety: IR 62032
18/08	−10	Fertilisation of rice in nursery	Urea
19/08	−9	Killing off crabs in tanks	Product used: Pedan 5EC
26/08	−2	Weed control by hand	
27/08	−1	Fertilization: base dressing	Per tank: 53.2 g urea, 168 g DAP, 42 g KCl
28/08	0	Start of the experiment Rice transplanting Water level 2–3 cm	
31/08	3	Water level 5 cm Sampling[a]	
09/09	10	Fish stocking Sampling[a]	
12/09	15	Fertilization: 1st top dressing	Per tank: 53.2 g urea
14/09	17	Sampling[a]	
15/09	18	Water level 15 cm	
17/09	20	Weed control by hand	
19/01	22	Sampling rice[b]	
21/09	24	Sampling[a]	
28/09	31	Sampling[a]	
05/10	38	Sampling[a] Sampling rice[b]	
07/10	40	Fertilization: 2nd top dressing	Per tank: 53.2 g urea, 42 g KCl
12/10	45	Sampling[a]	
18/10	51	Sampling rice[b]	
19/10	52	Sampling[a]	
26/10	59	Sampling[a]	
01/11	65	Sampling rice[b]	
02/11	66	Sampling[a]	
18/11	82	Sampling[a]	
19/11	83	Fish sampling	
27/11	91	Sampling rice[b]	

All dates (dd/mm) in 1998. *Abbreviations:* DAT, days after transplanting; DAP, di-ammonium-phosphate; KCl, potassium-chloride.

[a] Floodwater and interstitial soil water sampling: Rhizon soil moisture sampling, pH (8 and 14 h), oxygen (8 and 14 h), chlorophyll-*a*, NH_4^+, NO_3^-, PO_4^{3-}.

[b] Sampling included: above-ground dry weight biomass and rice height.

2.3. Rice

The rice (IR 62032, cropping duration 95–100 days) was transplanted according to local practice (two to three seedlings per hill, spacing between hills 20 cm × 15 cm). Before the actual transplanting the soil was homogenised by flooding the soil surface with 3–5 cm water, and by repeated hoeing and levelling, followed by the planting of a rice pre-crop. Since the soil used for the tanks had not been cropped for a long time, elevated levels of available nutrients could be expected. The rice pre-crop was used to homogenise and deplete the soil. The pre-crop was removed on the 1st of July, before the start of the experiment. Between pre-crop removal and transplanting the plots remained flooded. A sub-optimal fertilisation rate was applied to the rice crop (Table 1): two applications (−1 and 15 days after transplanting (DAT)) and one application (40 DAT) to encourage rice panicle initiation. There were no pest control measures applied during the experiment.

2.4. Fish

Nile tilapia, *Oreochromis niloticus* (L.), and common carp, *Cyprinus carpio* L., fingerlings (both on average 10 g fish^{-1}) were obtained from local commercial nurseries. Both species were stocked at a stocking rate of 0.5 fish m^{-2} in four out of the eight available tanks (the other four tanks did not have fish). The resulting total stocking density was 1 fish m^{-2}. Fish were stocked at 10 DAT. Fish were not fed during the experiment. Towards the end of the experiment (83 DAT) Nile tilapia weighed on average 20.6 g fish^{-1}, while common carp weighed on average 31.9 g fish^{-1}. The survival rates were 60.0 and 82.5%, respectively.

2.5. Sampling of soil, water, rice biomass and chlorophyll-a

During the experiment, the people who collected the samples did not enter the plots but sampled while standing on the walls of the tanks, this to avoid frequent trampling of the muddy soil which would influence the quality of the collected data. The sampling equipment allowed us to sample at random over the entire plot surface.

Microporous polymer tubes (Rhizon Soil Solution samplers (RSSS), Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) were used in situ to extract soil solution. RSSS are miniature porous cups with a 2.5-mm diameter and 10-cm long hydrophilic porous polymer tube, connected to the soil surface by a PVC tube. Soil water can be extracted from the soil surrounding the RSSS by creating a vacuum at the end of the PVC tube. RSSS are suited for measuring point dynamics or microvariation of nutrients in the root zone of rice plants (Lu et al., 2000; Abedin et al., 2002). The RSSS were inserted horizontally into the soil at a depth of 1 cm under the soil–water interface immediately after rice transplantation. The samplers were placed at this shallow depth because we wanted to find out whether fish have any effect on the soil component of the rice field system. As fish have contact with the soil surface layer it is probable that any fish effect is confined to the upper part of the soil. On a weekly basis (Table 1) 7–10 ml of soil solution was collected per sampler. Each tank had five rhizon samplers. The five samples collected in a tank were bulked together in a plastic bottle containing sufficient acid to bring the solution pH below 2. The soil solution was analysed for NH_4^+ (ppm), NO_3^- (ppm) and dissolved *ortho*-phosphate (ppm). In the remainder of the text we use the notation "NH_4^+ soil", "NO_3^- soil", and "*ortho*-phosphate soil" for the concentration of the respective nutrients in the interstitial water.

On every sampling day (Table 1), six sub-samples of one litre field floodwater per tank were collected, mixed together and homogenised. One litre of this was taken as the main sample for the analysis of chlorophyll-*a*, NH_4^+, NO_3^-, and *ortho*-phosphate in the field floodwater. Part of this 1 l sample (after filtration through a 55 μm mesh) was used to determine the nutrients in the floodwater. The unfiltered part of this 1 l sample was used for the chlorophyll-*a* analysis: two subsamples were filtrated over a 0.45 μm membrane filter and kept frozen until analysis. Chlorophyll-*a* was determined using the acetone extraction method (Strickland and Parsons, 1972). Oxygen (mg l^{-1}) and pH were measured regularly (see Table 1) in the morning (8:00 a.m.) and afternoon (2:00 p.m.), using portable electronic probes (Hanna Instruments HI9143).

To measure the above-ground dry weight biomass (in the remainder of the text abbreviated as rice biomass) three rice hills per tank were sampled occasionally (Table 1). The rice biomass data used in the statistical analysis were derived from third order regressions.

The same analysis methods were used for field floodwater and interstitial soil water. NH_4^+ was determined spectrophotometrically with the indophenol blue method (Solorzano, 1969), while NO_3^- was determined using the cadmium reduction method (APHA, 1992). For dissolved *ortho*-phosphate the acid ascorbic method was used (Murphy and Riley, 1962).

2.6. Statistical analysis

The data set contained 88 data cases (8 fields × 11 sampling dates). There were no missing values, except for the variable NO_3^- water, where there were no recorded data for the first day of sampling. The dataset was subjected to principal component analysis (PCA), and two-way analysis of variance (ANOVA). The variable NO_3^- water was not included in the PCA analysis but was used in the ANOVA analysis.

The PCA included 11 variables: rice biomass, ammonium water, ammonium soil, nitrate soil,

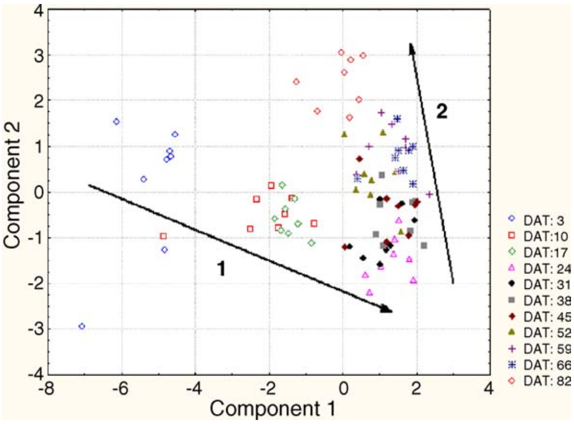

Fig. 1. Biplot showing the first two components of the principal component solution plotted against each other (Table 2). The biplot discloses a clear sequential path. From 3 to 24 days after rice transplanting (DAT), all changes happen along the component 1 gradient (arrow 1). From 24 DAT onwards, all changes occur nearly exclusively along the component 2 gradient (arrow 2).

inorganic phosphorous water, inorganic phosphorous soil, pH in the morning (pH a.m.) and afternoon (pH p.m.), dissolved oxygen in the morning (DO a.m.) and afternoon (DO p.m.), and chlorophyll-*a*. Where necessary, variables were ($\log_{10}$ +1)-transformed to comply with the PCA assumptions. Components with eigenvalues higher than 1.0 were retained. For the component solutions, variables with loading higher than 0.40 were retained. The first two components were plotted against each other in a bi-plot (Fig. 1).

ANOVA testing was done with time of sampling and fish presence as independent variables. Data for 3 and 10 days after transplanting were not retained for the analysis, as fish were not yet stocked at that time. Where necessary, variables were ($\log_{10}$ +1)-transformed to comply with the ANOVA assumptions. The main interest of the ANOVA testing was to find fish effects. Significant effects were further analysed with the Tukey Honest Significant Difference test. All significance testing was done at the 0.05 level.

3. Results

3.1. ANOVA for the dependent variables

Fig. 2 gives an overview of all the variables under research. Table 2 gives an overview of the ANOVA analysis. The stocking of fish had a significant effect on 5 out of the 12 variables under research (Table 2 and Fig. 2). The chlorophyll-*a* levels nearly doubled in the presence of fish (from an average of 30.9 to an average of 58.3 mg m^{-3}, $p < 0.001$), while oxygen levels were lower both in the morning (1.53 ppm versus 1.10 ppm, $p < 0.01$) and afternoon (4.47 ppm versus 2.72 ppm, $p < 0.001$). The presence of fish also decreased the concentration of *ortho*-phosphate in the water (0.10 versus 0.05, $p < 0.001$) and in the interstitial soil water (1.32 versus 0.97, $p < 0.001$).

For nearly all variables, values differed in time (Table 2; Fig. 2). The rice biomass increased throughout the experiment and measured 8172 kg DW ha^{-1} at 82 DAT. While chlorophyll-*a* levels were high at the onset of the experiment, they remained more or less stable from 24 DAT onwards. The dissolved oxygen (DO) levels showed a similar pattern. From 24 DAT onwards, DO a.m. levels remained under 2 ppm. The pH values dropped in the beginning of the experiment and increased once again towards the end. From 24 DAT onwards pH p.m. readings were often lower than pH a.m. readings. NH_4^+ levels in soil and water showed a sharp decline at the onset of the experiment until 24 DAT. Towards the end of the experiment NH_4^+ levels increased once again. The NO_3^- levels peaked around 17–24 DAT; from 31 DAT onwards they remained more or less stable. *ortho*-Phosphate levels were rather stable throughout the experiment.

For NH_4^+ soil and *ortho*-phosphate water interaction effects between time and fish stocking were observed (Table 2).

3.2. PCA

The PCA solution contains three components and accounts for 66.21% of the total variability (Table 3). Component 1, which explains 41.97% of the total variability, contains 10 out of the 11 variables and accounts for the processes taking place at the onset of the experiment (3–24 DAT, arrow 1 Fig. 1): an increasing rice biomass goes together with sharply decreasing chlorophyll-*a* values, dissolved oxygen concentrations, pH values and nutrient concentrations in both floodwater and interstitial soil water between 3 and 24 DAT (Fig. 2). Component 2, giving 13.54% of the total variability, explains the changes taking place

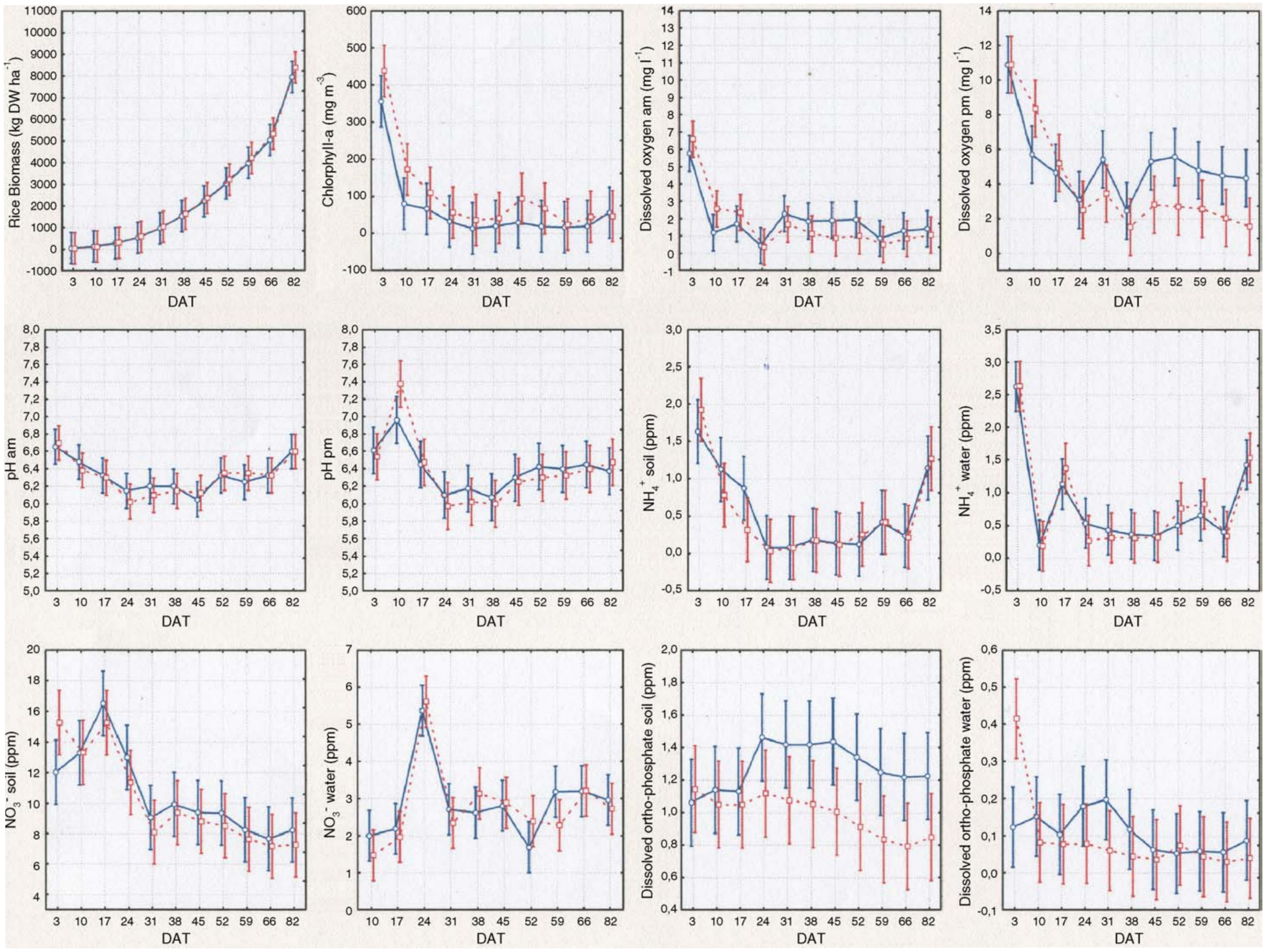

Fig. 2. An overview of the changes in aquatic variables with increasing rice biomass and with fish or no fish. The dotted lines represent the fish-treatment, the full line represents the rice monoculture treatment. Fish were stocked at 10 days after rice transplanting (DAT).

Table 2
ANOVA and mean comparisons of different aquatic variables of rice–fish systems between 17 and 82 days after transplanting (DAT), in the presence or absence of stocked fish

Variable	Unit	ANOVA			Fish treatment		Days after transplanting								
		DAT	Fish	DAT-fish^{+}	No-fish	Fish	17	24	31	38	45	52	59	66	82
Rice biomass$^{\#}$	kg DW ha^{-1}	***	ns	ns	2838^{a}	3011^{a}	294^{a}	559^{b}	1008^{c}	1585^{cd}	2292de	3128^{ef}	4093^{fg}	5187^{gh}	8172^{h}
Chlorophyll-*a*$^{\#}$	mg m^{-3}	***	***	ns	30.9^{a}	58.3^{b}	88.4^{c}	45.0^{ab}	25.6^{ab}	31.3^{ab}	62.2^{bc}	43.6^{ab}	21.0^{a}	32.8^{ab}	51.6^{bc}
Oxygen a.m.$^{\#}$	mg l^{-1}	***	**	ns	1.53^{b}	1.10^{a}	2.03^{c}	0.41^{a}	1.99^{c}	1.50^{bc}	1.39^{bc}	1.49^{bc}	0.69^{ab}	1.08^{abc}	1.24^{bc}
Oxygen p.m.	mg l^{-1}	**	***	ns	4.47^{b}	2.72^{a}	4.93^{b}	2.80^{ab}	4.45^{b}	2.00^{ab}	4.07^{ab}	4.14^{ab}	3.69^{ab}	3.28^{ab}	2.95^{ab}
pH a.m.		***	ns	ns	6.27^{a}	6.26^{a}	6.31^{ab}	6.09^{a}	6.15^{a}	6.17^{a}	6.09^{a}	6.34^{ab}	6.30^{ab}	6.32^{ab}	6.60^{b}
pH p.m.		***	ns	ns	6.30^{a}	6.25^{a}	6.46^{c}	6.04^{a}	6.10^{ab}	6.04^{a}	6.28^{abc}	6.36^{abc}	6.36^{abc}	6.42^{bc}	6.42^{bc}
NO_3^- soil$^{\#}$	ppm	***	ns	ns	10.2^{a}	9.3^{a}	15.9^{c}	12.2^{bc}	8.6^{a}	9.7^{ab}	9.1^{a}	8.9^{a}	7.9^{a}	7.4^{a}	7.7^{a}
NO_3^- water$^{\#}$	ppm	***	ns	ns	2.97^{a}	2.95^{a}	2.08^{ab}	5.49^{c}	2.53^{ab}	2.88^{ab}	2.85^{ab}	2.04^{a}	2.73^{ab}	3.21^{b}	2.85^{ab}
NH_4^+ soil$^{\#}$	ppm	***	ns	***	0.37^{a}	0.33^{a}	0.60^{c}	0.06^{a}	0.08^{ab}	0.18^{ab}	0.13^{ab}	0.19^{ab}	0.42^{c}	0.23^{b}	1.20^{d}
NH_4^+ water	ppm	***	ns	ns	0.65^{a}	0.68^{a}	1.26^{c}	0.41^{ab}	0.38^{ab}	0.34^{a}	0.34^{a}	0.64^{ab}	0.74^{b}	0.38^{ab}	1.49^{c}
ortho-Phospate soil	ppm	ns	***	ns	1.32^{b}	0.97^{a}	1.09^{a}	1.29^{a}	1.25^{a}	1.24^{a}	1.22^{a}	1.13^{a}	1.04^{a}	1.00^{a}	1.04^{a}
ortho-phosphate water$^{\#}$	ppm	***	***	**	0.10^{b}	0.05^{a}	0.09^{ab}	0.13^{b}	0.13^{b}	0.08^{ab}	0.05^{a}	0.06^{a}	0.05^{a}	0.04^{a}	0.06^{a}

ANOVA significance levels: ns (not significant), **($p < 0.01$), ***($p < 0.001$). For mean comparison: for each treatment, indices within a row with the same superscript (a–h) are not significantly different at the 0.05 level.

$^{+}$ Interaction term.

$^{\#}$ For the ANOVA analysis and the mean comparison (Tukey Honest Significance Difference), the data were log transformed. In the table we present the non-log-transformed data.

from 24 to 82 DAT (arrow 2 Fig. 1). An increasing rice biomass goes together with increased pH a.m. and NH_4^+ soil and water values and decreased NO_3^- soil and *ortho*-phosphate values. Component 3, good for 10.70% of the total variability, suggests a link between pH p.m. and *ortho*-phosphate soil.

4. Discussion

4.1. Rice biomass and the aquatic environment

In littoral zones of lakes or in marshes, it is well known that emergent macrophytes impact on subsurface light levels and reduce algal growth (Payne, 1986; de Haan et al., 1993). Similarly, shading by the growing rice biomass has been identified as one of the most important factors limiting aquatic photosynthesis in rice fields (Kurasawa, 1956; Saito and Watanabe, 1978; Heckman, 1979; Watanabe and Roger, 1985) and in rice–fish fields (Vromant et al., 2001a; Mustow, 2002). Besides the competition for light, rice also competes with the field floodwater's photosynthetic active biomass (PAB) for available nutrients (Heckman, 1979), especially N, the most limiting nutrient in irrigated rice fields (De Datta et al., 1988; Kropff et al., 1993). Component 1 (Table 3; Fig. 1) confirms these patterns: with increasing rice biomass, the chlorophyll-*a* concentration, NH_4^+ water, oxygen a.m., oxygen p.m., pH a.m., and pH p.m. decreased, clear indications for a reduced aquatic photosynthesis.

Table 3
PCA component loadings for aquatic variables of the field flood water of rice fish fields

	Component 1	Component 2	Component 3
Log_{10} biomass	0.78	0.44	
Log_{10} chlorophyll-*a*	−0.75		
Log_{10} oxygen a.m.	−0.74		
Oxygen p.m.	−0.81		
pH a.m.	−0.61	0.52	
pH p.m.	−0.59		−0.52
NO_3^- soil	−0.61	−0.50	
Log_{10} NH_4^+ soil	−0.75	0.45	
Log_{10} NH_4^+ water	−0.60	0.46	
PO_4 soil			−0.76
Log_{10} PO_4 water	−0.47	−0.47	
Eigenvalue	4.61	1.48	1.17
Total (%)	41.97	13.54	10.70

Loadings >0.40 were withheld. Total explained variation 66.21%; 88 active cases.

Furthermore, component 1 also shows that NO_3^- soil, and NH_4^+ soil decreased with the rapid increase of the rice biomass at the onset of the experiment. According to Yoshida (1981), the roots of the rice plant are able to take up both NO_3^- and NH_4^+, with a preference for the latter.

The sequential pattern found in Fig. 1 is quite revealing regarding the main ecological changes in the experimental rice plots. At the onset of the experiment, the high pH values, together with the high dissolved oxygen values and chlorophyll-*a* values, suggest that the autotrophic pathway was dominant within the aquatic phase of the experimental rice fields (high readings for component 1, Fig. 1). However, as the rice biomass increased, the decreasing pH values, together with the low dissolved oxygen values and chlorophyll-*a* values, suggest that the autotrophic pathway lost importance (low readings for component 1, Fig. 1). From about 24 DAT the component 1 readings remained low and changes in the ecosystem happened along the component 2 axis (see discussion under the next headings). Only towards the end of experiment did we once again observe increased component 1 readings.

We did not measure productivity and light levels in the aquatic phase of the experimental rice-fields, but based our discussion on the interpretation of the PCA components. Still, our findings agree with the observations made by Mustow (2002) in transplanted Bangladeshi rice–fish fields. He found that the gross phytoplankton productivity decreased markedly during the experiment and attributed this to increased shading from the growing rice plant. Towards the end of his experiment, Mustow (2002) also observed that the adverse effect of rice on primary production was relatively limited. He concluded that nutrient limitation and light limitation are key factors limiting aquatic primary production in rice–fish fields. While Watanabe and Roger (1985) state that the floodwater in an irrigated rice field is an oxic–photic environment, the data presented by Mustow (2002) and our data indicate that oxygen levels and light availability are low for the larger part of the cropping period.

The decreased importance of the autotrophic pathway should result in harsh conditions for plankton feeding fish. It has indeed been observed that, with increasing rice biomass, Nile tilapia, stocked in rice–fish fields, gradually switched from feeding on the algal biomass to a diet primarily composed of detritus (Chapman and Fernando, 1994; Rothuis et al., 1998a), a process which results in interspecific competition with the common carp (Vromant et al., 2002b, 2004).

4.2. Nutrients

The floodwater and soil interstitial water patterns of nutrient fluxes are very similar (Fig. 2), which is not surprising as the rice field floodwater and the soil form a continuum (Watanabe and Furusaka, 1980).

The NH_4^+ and NO_3^- concentrations at the beginning of the experiment were high as a result of fertilizer applications at 3 and 15 DAT (Fig. 2). The fertilizer application at 40 DAT was not reflected in the data (Fig. 2), probably due to an increased N uptake by rice for panicle initiation, an N-demanding process. Halwart (1991) noted that ammonium in rice fields does not often exceed 1 ppm. However, after fertilizer application, peaks may occur of up to 4.6 ppm; they usually decrease 6–10 days after application (De Datta et al., 1983). Our data and the data presented by Vromant et al. (2001a) show that NH_4^+ values higher than 1 ppm are not that rare, especially in the beginning of the cropping season when fertilizer applications are frequent.

The NO_3^- concentration in the interstitial soil water was higher than the NO_3^- concentration in the field floodwater and than the NH_4^+ concentration in both soil and field floodwater (Fig. 2). The second fertilization (15 DAT) increased the NO_3^- concentration, clearly suggesting strong nitrification processes in the upper layer of the soil. It is indeed well known that continuously flooded rice fields are characterised by an oxidized surface layer (De Datta, 1981). In such layers in lakes and streams, nitrate concentrations in the interstitial water of the soil are also higher than those in the overlying water (Horne and Goldman, 1994) due to nitrification processes that are stimulated with increasing dissolved oxygen concentrations in the overlying water (Jensen et al., 1993; Rysgaard et al., 1994). At 17 DAT, dissolved oxygen levels in the afternoon are still high.

Unlike component 1, component 2 explains part of the observed variability from 24 DAT onwards (Fig. 1): an increasing rice biomass coupled with an increase in pH a.m. and NH_4^+ in superstitial water and field floodwater and a decreasing *ortho*-phosphate water and NO_3^- water. It is thought that this relationship is mainly caused by the deteriorating oxygenation of the water and sediment (as expressed by component 1) and its impact on the nitrification process. Nitrification stops when dissolved oxygen levels drop below 2.24–0.96 mg l^{-1} (Cooke and White, 1987). A continued, though slow ammonification would then result in a steady increase in NH_4^+. A lower nitrification rate would reduce denitrification levels and the associated pH reduction.

4.3. Fish and the aquatic environment

Just like Vromant et al. (2001a), we found the *ortho*-phosphate concentrations in the field floodwater to be lower in the presence of stocked fish. Additionally, *ortho*-phosphate concentrations in the interstitial soil water (not measured by Vromant et al., 2001a), were also lower. For shallow lakes and ponds, turbulence acting on the surficial layers of sediments – e.g. through resuspension of the sediment by fish – is known to increase the phosphorus concentration in the water phase (Böstrom et al., 1988; Havens, 1993; Jana and Sahu, 1993; Breukelaar et al., 1994; Cline et al., 1994; Riise and Roos, 1997), while decreasing phosphorus concentrations in the sediment (Jana and Sahu, 1993). Both of the fish species used in our experiment are known to be predominantly detritus feeders in rice–fish fields (Chapman and Fernando, 1994; Rothuis et al., 1998a). Common carp is also known to bring mineral and organic matter from the sediments into suspension through its feeding activities. This results in (1) increased water turbidity in the rice fields (Vromant et al., 2001b), (2) aeration of the rice field soils (Heckman, 1979), (3) P release from the sediment (Breukelaar et al., 1994), and (4) establishment of a contact between the benthic and pelagic compartments, which are otherwise fully separated (Richardson et al., 1990). However, our data did not show any increase in *ortho*-phosphate concentration in the presence of stocked fish, on the contrary, increases in *ortho*-phosphate were only observed in fields without fish (Fig. 2 and Table 2). The collected data do not allow a thorough explanation of the processes responsible for the higher *ortho*-phosphate levels in the absence of fish. However, a few processes can be excluded. Firstly, we do not think that translocation processes from field floodwater to trench water play a role. Vromant et al. (2001a,b) found similar losses of P in both trench and field in the presence of fish. Secondly, we think that it is unlikely that increased aeration of the sediment as suggested by Heckman (1979) and associated lower release rate of phosphorus by the sediments (Rippey and Jewson, 1982) could explain the process, as dissolved oxygen levels in our plots with fish were significantly lower, and as NH_4^+ and NO_3^- concentrations did not differ in plots with and without fish. One plausible explanation could be the presence of an enlarged sink in plots of fish. This sink could be the fish, the increased phytoplankton community, the rice plant or the sediment. The hypothesis that fish perturbation of the soil–water interface might make the soil porous for nutrients readily absorbed by the rice roots, postulated by Cagauan (1995) might be plausible. More research is needed to verify this.

The DO and chlorophyll-*a* levels were, respectively, significantly lower and higher in the presence of fish (Fig. 2 and Table 2), confirming observations made by Rothuis et al. (1999) and Vromant et al. (2001a). Cagauan et al. (1993) found higher photosynthetic productivities in the presence of fish and attributed this to improved cycling and distribution of nutrients. On the contrary, we did not find any increase in nutrients in the presence of fish. The higher chlorophyll-*a* concentration found in the rice fields with fish can most probably be explained by top–down trophic cascading interactions through fish feeding on zooplankton. Indeed, Rothuis et al. (1999) and Vromant et al. (2001a) found lower macrozooplankton densities in rice fields with fish. Higher algal densities in an environment lacking in light are known to increase algal respiration (Smith and Piedrahita, 1988), while an increase in mineral turbidity in the water results in a decrease of primary production, entailing a decrease in dissolved oxygen, which in some cases may result in oxygen depletion (Szumiec, 1989). This and the presence of the fish biomass could explain the lower DO levels in the treatment with fish.

Fish are said to contribute to nitrogen accumulation in rice fields through their faeces (Lightfoot et al., 1993). Our data did not show any clear impact of fish on nitrogen. Some researchers (Nakashima and Leggett, 1980; Hudson et al., 1999) argue that large animals only play a minor role in supplying nutrients as compared to smaller animals. Vanni (2002) notes that this may seem logical as (1) large animals excrete nutrients at lower mass-specific rates, and as (2) they often have a lower population biomass than smaller organisms. Many researchers (summarized by Vanni, 2002), however, found that fish could play an important role in nutrient related processes. In rice–fish fields, standing biomasses of fish are typically low as farmers stock with small fish fingerlings (typically 2–10 g fish^{-1}) at rates varying from 0.5 to 3.5 fish m^{-2} (Rothuis et al., 1998c). At high stocking densities, fish mortalities can be extremely high (up to 95%). Higher stocking rates also result in lower specific growth rates and increased intraspecific and interspecific fish competition as a result of low primary production in the field floodwater due to shading by the rice crop (Vromant et al., 2002b,c, 2004). In our experiment, the actual standing wet biomass of fish at fish harvest was 193.4 kg ha^{-1} as compared to more than 8000 kg dry weight biomass ha^{-1} for rice. We suggest that the large difference in biomass means that effects on nutrients caused by the fish are obscured by rice driven processes (such as nutrient uptake processes). It is therefore even more remarkable that differences were found for *ortho*-phosphate between plots with and without fish. On the other hand, rice mainly needs phosphorus during its early vegetative stages (De Datta, 1981), as there is a high mobility of phosphorus from old leaves to young ones (Chang, 1976).

4.4. Experimental tanks versus fields

The physico-chemical parameters recorded in the experimental tanks reflected those found in experimental fields and farmer fields in the Mekong Delta (compare with Rothuis et al., 1998a, 1999; Vromant et al., 2001a,b). The weed and arthropod community did not differ from the surrounding rice fields. The tanks allowed us to exert control on the water level and on the fish community. In experimental fields and farmers' fields, it is not always easy to prevent wild fish species from entering the fields. The experimental tanks allowed us fully control the variable "fish presence". As we wanted to find out the effect of fish on the aquatic ecology it was also important to keep the water level equal in both treatments. This implicates that the results presented for the treatment "no-fish" do not correspond with the rice monoculture environment found in farmers' fields. Neither does the "fish" treatment correspond with the rice–fish system conditions in farmers' fields. Rice–fish farmers often let the water levels drop in their field as they are afraid that high water levels might negatively affect the rice yield (Vromant et al., 2002a).

5. Conclusions

The aquatic environment in rice fields is largely influenced by the growth of the rice crop. The stocking of fish does have an effect on some of the floodwater and interstitial soil water characteristics, though the impact on nutrients is limited. This undermines the supposition by Lightfoot et al. (1993) that fish may contribute to the soil fertility of rice fields. Scrutinizing the three hypotheses by Cagauan (1995) regarding the effect of fish on nutrients (see Section 1) two may not be discarded. Firstly, additional nutrients from decomposing dead fish and from fish faeces might contribute to the nutrient cycling in the rice field environment, but most probably only when the fish population biomass is large, which is unlikely in extensive rice–fish systems as food availability through primary production is low. In farmers' fields, the fish population biomass can also be low due to high mortality rates as a result of high water temperature, predation, variable water levels, theft and fish escape during floodings (Rothuis et al., 1998b,c; Vromant et al., 2002b,c, 2004). Secondly, fish perturbation of the soil–water interface might make the soil porous for nutrients readily absorbed by the rice roots, though more research is needed to investigate the validity of this statement. We did not find proof for the hypothesis that fish perturbation of the soil–water leads to the release of nutrients. We doubt whether fish can increase nutrient cycling and decrease N-losses through feeding on the photosynthetic aquatic biomass (PAB) (third hypothesis by Cagauan, 1995) as the PAB is very low and as fish are known to feed on lower trophic levels (detritus) with increasing rice biomass. That the PAB is low can be concluded from the low chlorophyll-*a* readings from 31 DAT onwards (Table 2). In fertilized pond aquaculture, chlorophyll-*a* readings of more than 100 mg m^{-3} are no exception (Jamu et al., 1999; Brummett, 2000).

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