Rice-soft shell turtle coculture effects on yield and its environment

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A B S T R A C T

Although traditional rice-fish farming (involving extensive aquaculture and low fish yields) can supply food and protect the environment, the economic viability and environmental effects are unknown for intensive rice-aquaculture systems that use high quantities of feed to produce high fish yields. Here, we studied an intensive, soft-shelled turtle (*Pelodiscus sinensis*) farm to determine whether an intensive rice-turtle system can produce high yields of turtle and rice without negatively affecting water and soil quality. Using a 6-year field survey and a 2-year field experiment, we compared the three production systems: rice monoculture (RM), rice-turtle coculture (RT), and turtle monoculture (TM). The field survey indicated that turtle

rice plants can use the unconsumed fish feed and because fish feces can serve as organic fertilizers (Frei and Becker, 2005a; Oehme et al., 2007). In addition, rice–fish farming can reduce some problems generated by freshwater aquaculture. For example, nutrients in the effluents generating by the raising of fish can be absorbed

2.2.5. Statistical anal sis

The general linear model (GLM) in SPSS (V.20.0) was used to perform two-way ANOVAs with year as a random factor, culture type (RM, RT, or TM) as a fixed factor, and rice yields, turtle yields, total-N applied, and total-P applied as dependent variables. For rice yield, the analysis concerned RM vs. RT. For turtle yield, the analysis concerned TM vs. RT. One-way ANOVA was used to assess changes in soil N and P with year as a fixed factor. Before the analysis, data were log-transformed to meet assumptions of normality and homogeneity.

2.3. Field e periment

2.3.1. E perimental design

We conducted a 2-year field experiment (2013–2014) to further assess rice and turtle yields, total N and P in field water and soil, and the balance of N and P in the three culture systems. The field for experiment was the rice monoculture field. The experiment had a randomized block design with three treatments and four replications or blocks. The three treatments were (1) rice monoculture (RM), (2) rice-turtle coculture (RT), and (3) turtle monoculture (TM). Each of the 12 plots (8 m × 10 m per plot) was separated by concrete ridges, and each had an independent water inlet and outlet. The three treatments were randomly assigned to the plots in the blocks.

Four weeks after germination, rice seedlings were transplanted into the RT and RM plots, with 30 cm between rows and 30 cm between hills (four seedlings per hill) within the rows. In RT and TM plots, the turtle population density was 6000 ha^{-1}, which is the standard density used in rice–turtle coculture farms in the area. Young turtles (150 g each) were added 5 days after rice was transplanted in RM and RT plots. All plots were irrigated at the time of transplanting and were then permanently flooded to 30–50 cm depth until harvest. No pesticide was applied for rice in either RM or RT plots. No fertilizer was used in RT plots, but 217.5 kg ha^{-1} of N and 67.5 kg ha^{-1} of P were applied for rice in RM plots. Turtles were fed with a formula feed containing 7.88% N and 2.26% P twice per day at about 07:00 and 17:00 throughout the experiment. The daily amount of turtle feed added was about 1.5% (0.5% at 7:00 and 1% at 17:00) of the turtle fresh body mass per plot, and this amount of course increased as the turtles grew. The same quantity of feed was added to RT and TM plots. By the end of the experiment, a total of 3.07 ton ha^{-1} of feed had been applied to the RT and TM plots. This mixed fish feed contained 7.88% N and 2.26% P, and thus the feed-N and feed-P inputs (totals for both years) were 242.31 kg $ha^{-1}$and 69.49 kg ha^{-1}, respectively, for RT and TM plots. In both years, the experiment was terminated 116 days after rice was transplanted.

2.3.2. Measurement of N and P in field water

At 30, 45, and 90 days after rice was transplanted in the second year of the experiment (2014), water samples were collected from the inlet, middle, and outlet of each plot. Water was collected at 09:00 with a 2-L water sampler. Water samples were passed through a 0.064-mm net to remove phytoplankton, zooplankton, and particulate organic matter. Total N was determined by alkaline potassium persulfate oxidation digestion and UV spectrophotometry, and total P was determined by potassium persulfate oxidation digestion and ammonium molybdate spectrophotometry (Fu and Zhang, 2013).

2.3.3. Determination of rice and turtle ields

Rice grain and straw, and gross turtle yields were determined by harvesting the rice and turtles from entire plots. Grain and straw yields were expressed as tons of air-dried grain or straw per ha. Gross turtle yield was expressed as tons of fresh turtle biomass per ha. Net turtle yield was calculated by subtracting mass before stocking from the total mass at harvest. Turtle yield was determined in accordance with the approved guidelines of the Zhejiang University Experimental Animal Management Committee.

2.3.4. Measurement of N and P in soil

At the start and at the end of the experiment, five surface soil samples (0–15 cm) were collected from each plot and combined to provide one soil sample per plot. Soil samples were air-dried. Soil N and P were analyzed as described in the field survey.

2.3.5. Estimations of N and P balances

Before rice and turtles were harvested in the second year of the experiment, five hills of rice and five turtles were randomly collected from each plot. Rice grain and straw were separated. Feed samples were collected during the application of turtle feed. Samples of rice grain, straw, turtles, and turtle feed were oven-dried at 65 °C and then ground. All samples were digested using the K_2SO_4-$CuSO_4$-Se method, and the total N and P were analyzed using a San^{++} Continuous Flow Analyzer (Skalar, Netherlands) (Lu, 1999).

Output of N or P in each harvest fraction was determined by multiplying the concentrations of N or P in the rice and turtle samples by dry biomass. The total quantities of N or P in harvested fractions (the output quantities) were subtracted from the input quantities (the N or P applied in turtle feed and rice fertilizer). A positive value following subtraction indicated that some portion of input N or P was not used by rice or turtles but had either remained in the plot (in soil, water, or other organisms) or had moved into the surrounding environment via volatilization, leaching, or drainage. A negative value following subtraction indicated that in addition to containing N or P applied in turtle feed or rice fertilizer, harvest fractions contained N or P from indigenous sources, i.e., from the soil, irrigation water, biological nitrogen fixation, or rain deposition. We did not attempt to identify these indigenous sources because we were primarily concerned with the net balance of N and P in the plots. We assumed that the effects of indigenous sources of N and P were similar across the plots.

2.3.6. Statistical anal sis

Total N and P concentrations in field water were compared among the three treatments (RM, RT, and TM) with repeated measures (sampling several times in a year) ANOVA. Data for rice grain yield, rice straw yield, total turtle yield, and net turtle yield were subjected to two-way ANOVAs with year as a random factor and treatment (RM, RT, or TM) as a fixed factor. For rice grain and straw yields, the comparison concerned RM vs. RT. For gross and net turtle yields, the comparison concerned TM vs. RT.

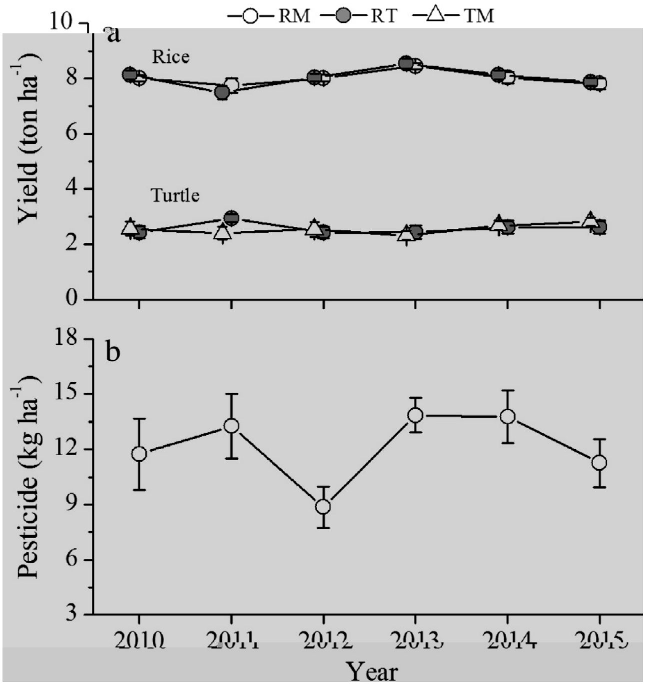
Paired *t*-tests were used to compare total N and P in soil at the start vs. the end of the field experiment; this was done separately for each treatment (RM, RT, and TM). Data for N and P in harvested fractions (rice grain, rice straw, turtle, and environment) were subjected to one-way ANOVAs using the general linear model (GLM) in SPSS (V.20.0). All data were log-transformed to meet the assumptions of normality and homogeneity before analysis. Means among the three treatments were compared by LSD at the 5% confidence level.

3.

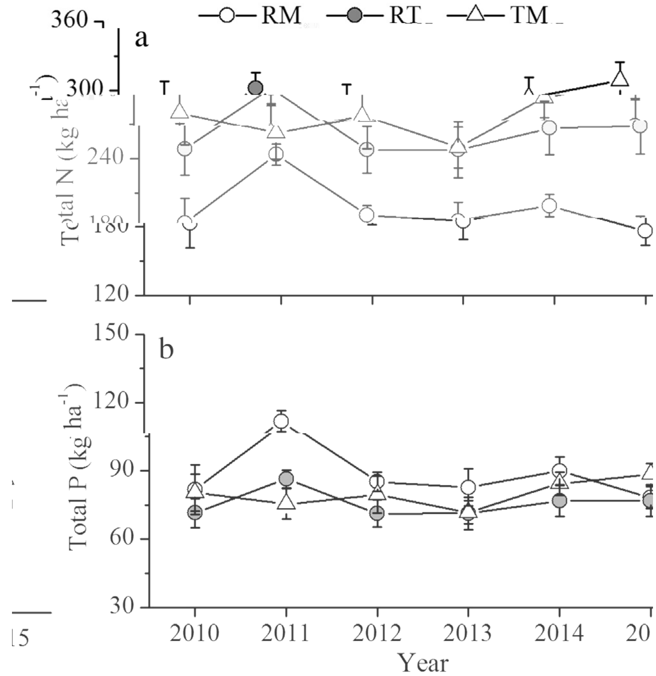
3.1. Field surve

3.1.1. Rice ields, turtle ields, and pesticide use

In the 6-year field survey, turtle yield was not lower ($F = 0.23$, $P = 0.651$) in rice–turtle coculture (RT) than in turtle monoculture (TM) (Fig. 1a). Rice yield also was not lower ($P > 0.05$) in RT than in



F . 1. Rice and turtle yields (a) and pesticide use (b) in a 6-year field survey of rice monoculture (RM), rice-turtle coculture (RT), and turtle monoculture (TM) in China. The turtle is *Pelodiscus sinensis*. Values are means• S.E.



F . 2. Total nitrogen input (a) and total phosphorous input (b) in a 6-year field survey of rice monoculture (RM), rice-turtle coculture (RT), and turtle monoculture (TM) in China. The turtle is *Pelodiscus sinensis*. Values are means• SE.

rice monoculture (RM) (Fig. 1a). For RT, no pesticide was applied for rice pest control, but the average yearly input of pesticides in RM was 12.12 a.i. kg ha^{-1}(Fig. 1b).

3.1.2. Input of N and P

In the farms surveyed, fertilizers were the sources of N and P for rice in RM, and turtle feed was the only source of N and P in RT and TM. No fertilizers were applied to RT and TM fields during the study. The total amount of N input significantly differed ($F = 25.288$, $P = 0.001$) among RM, RT, and TM fields (Fig. 2a). Nitrogen input was significantly higher ($P < 0.05$) in RT and TM than in RM fields, but did not differ ($P > 0.05$) between RT and TM fields. Total P did not significantly differ ($F = 1.153$, $P > 0.05$) among RM, RT, and TM fields (Fig. 2b).

3.1.3. Soil N and P

Soil N and P significantly declined ($P < 0.05$) 2 years after the TM system had been changed into an RT system, but soil N and P in TM fields remained at the same level ($P > 0.05$) (Fig. 3). At the end of the survey (2015), soil N and P did not significantly differ between RM and RT fields ($P > 0.05$), but soil N and P were significantly higher ($P < 0.05$) in TM than in RM or RT fields (Fig. 3).

3.2. Field e periment

3.2.1. Rice and turtle ields

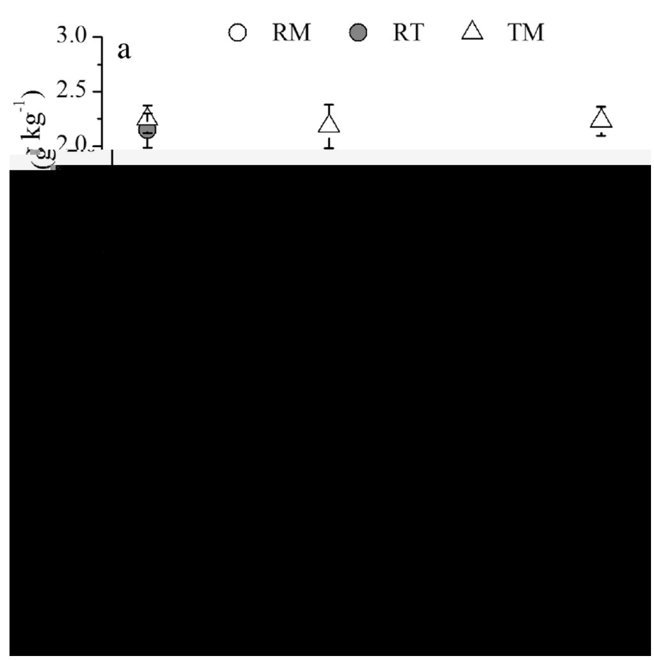
Rice grain yield did not significantly differ between RT and RM ($F = 0.517$, $P = 0.504$, Table 1), but rice straw yield was significantly greater in RT than in RM ($F = 11.358$, $P = 0.02$, Table 1). Gross and net yields also did not differ between RT and TM plots (for gross yield, $F = 3.625$, $P = 0.106$; for net yield, $F = 3.024$, $P = 0.115$) (Table 1).

3.2.2. N and P in field water

Both total N and P in field water significantly differed among the culture systems (for total N, $F = 7.411$, $P = 0.013$; for total P, $F = 4.838$, $P = 0.042$). Total N and P in field water was significantly greater ($P > 0.05$) in TM than in RM or RT plots but did not significantly differ ($P > 0.05$) between RM and RT plots (Fig. 4).

3.2.3. Changes in soil N and P

Total soil N did not significantly change ($P > 0.05$) during the field experiment (start values vs. end values) in RM or RT plots but significantly increased ($P < 0.05$) in TM plots (Fig. 5a). Total P in soil



F . 3. Soil nitrogen (a) and soil phosphorous (b) in a 6-year field survey of rice monoculture (RM), rice-turtle coculture (RT), and turtle monoculture (TM) in China. The turtle is *Pelodiscus sinensis*. Values are means• SE.

Table 1
Turtle and rice yields in a 2-year field experiment with rice monoculture (RM), rice-turtle coculture (RT), and turtle monoculture (TM) in China. The turtle is *Pelodiscus sinensis*.

	Treatment		
Yields	RM	RT	TM
Rice (ton ha^{-1})			
Grain yield	7.67 ± 0.15[a]	7.46 ± 0.26[a]	
Straw yield	4.67 ± 0.04[b]	4.98 ± 0.09[a]	
Turtle (ton ha^{-1})			
Gross yield		1.56 ± 0.01[a]	1.48 ± 0.03[a]
Net yield		1.15 ± 0.01[a]	1.09 ± 0.03[a]

Values are means ± SE ($n = 4$). Means in a row followed by different letters are significantly different ($P < 0.05$).

significantly increased ($P < 0.05$) in RM and TM plots but did not change ($P > 0.05$) in RT plots (Fig. 5b).

3.2.4. Balance of N and P

N and P in rice grains did not significantly differ between RM and RT plots (for grain-N, $F = 0.452$, $P = 0.769$; for grain-P, $F = 0.517$, $P = 0.504$) (Fig. 6). However, N and P in straw were significantly greater in RT than in RM plots (for straw-N, $F = 11.358$, $P = 0.010$; for straw-P, $F = 11.358$, $P = 0.020$) (Fig. 6). N and P in turtles (based on net yield) did not significantly differ between TM and RT plots (for turtle-N, $F = 3.626$, $P = 0.106$; for turtle-P $F = 3.625$, $P = 0.106$) (Fig. 6). Apparent N and P remaining in the environment (total input minus total output) significantly differed among the three treatments (for environmental-N, $F = 788.512$, $P = 0.000$; for environmental-P, $F = 1673$, $P = 0.000$). According to calculations, 79.6% of feed-N and 77.2% of feed-P were lost to the environment in TM plots, whereas no feed-N was lost to the environment and only 25.6% of feed-P was lost to the environment in RT plots (Fig. 6). For RM, no input fertilizer-N was lost, but 47.5% of fertilizer-P was lost to the environment (Fig. 6).

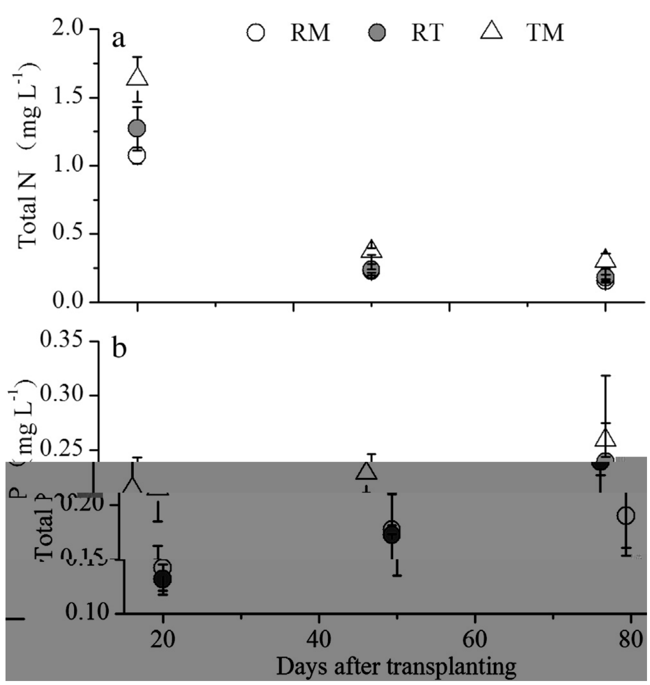


Fig. 4. Total nitrogen (a) and total phosphorous (b) in field water during the growing season in a field experiment in China. RM: rice monoculture, RT: rice-turtle coculture; TM: turtle monoculture. The turtle is *Pelodiscus sinensis*. Values are means ± SE.

4. Discussion

Our field survey and field experiment showed that turtle yield did not decrease relative to turtle monoculture when turtles were cocultured with rice. Moreover, coculture produced 8.3 ± 0.17 t ha^{-1} of rice each year (Fig. 1a). Turtle yield may not have decreased in coculture with rice because rice plants may improve the environment for turtles. The shading provided by rice plants, for example, can reduce the water temperature and light intensity at the water surface (Xie et al., 2011), which could lower thermal stress and thus greatly benefit turtles on hot summer days. Rice plants can also improve the water quality for turtles by reducing N and P concentrations (Fig. 4). Decreased ammonia levels might also reduce toxic stress to turtles (Rangel-Mendoza et al., 2014). Although ammonia-N levels in water were not measured in the current study, a previous study found that ammonia levels were significantly lower in fish coculture with rice rather than in fish monoculture (Xie et al., 2011).

The field survey and field experiment also showed that rice grain yield was not lower in rice–turtle coculture than in rice monoculture, even though no chemical fertilizer or pesticide was applied to plots with rice–turtle co-culture (Figs. 1 and 2). Some researchers have argued that integrating rice culture with aquaculture may reduce rice yield because some field space is required for animal refuges (Lightfoot et al., 1992). Some studies reported that integrating aquaculture in rice fields did reduce rice yield (Rothuis et al., 1998; Dwiyana and Mendoza, 2008). In many other studies, however, the culturing of fish with rice did not significantly decrease rice yield and even increased riceyield (Vromant et al., 2002a; Mohantyet al., 2004; Frei and Becker, 2005b; You, 2006; Wu et al., 2010; Hu et al., 2013; Ren et al., 2014; Tsuruta et al., 2011). In an experiment in India, for example, rice yield was 4.9–8.6% greater with rice–fish coculture than with rice monoculture (Mohanty et al., 2004). A meta-analysis by Ren et al. (2014) found that rice–fish farming increased rice yield. Fish farming may increase rice yield because rice plants are healthier in fields with fish than in fields without fish (Vromant et al., 2002a; Mohanty et al., 2004). In the current study, the culturing of turtles with rice did not reduce grain yield and increased straw yield (Table 1), even though 10% of the field was used for turtle refuges.

N and P are the two main pollutants produced by intensive aquaculture (Schneider et al., 2005; Wu et al., 2014). Because feed is incompletely used by aquatic animals, eutrophication usually occurs in intensive aquaculture systems and in the surrounding areas (Abimorad et al., 2009; Lucas et al., 2010; Qin et al., 2007). In our field experiment, only 20.4% of feed-N and 22.8% of feed-P were converted into turtle body mass in turtle monoculture, resulting in large quantities of N and P remaining in the environment (Fig. 6). In our field survey, soil N and P levels were high with turtle monoculture (Fig. 3), even though sludge was removed every 2 years.

In the rice-aquaculture system, however, N and P in the unconsumed feed can be used by rice plants (Xie et al., 2011; Hu et al., 2013). Besides producing fecal matter, turtles excrete excess N in the form of ammonia and urea that can be directly used by rice (Ip et al., 2012; Lee et al., 2007). This use of N and P by rice plants may result in low N and P accumulation in the environment. Our field survey showed that levels of soil N and P were significantly lower with rice–turtle coculture than with turtle monoculture (Fig. 3). Our field experiment also showed that levels of N and P in field water and soil were significantly lower with rice–turtle coculture than with turtle monoculture (Figs. 4 and 5). Although we cannot determine exactly how much feed-N and -P were used by rice plants in rice–turtle coculture, our calculations in the field experiment indicate that rice plants take up substantial quantities of N and P in the rice–turtle coculture system (Fig. 6). As a consequence, integrating rice culture with turtle culture can reduce N and P accumulation in field water and soil, and thereby reduce the chance of eutrophication (Hu et al., 2013; Ding et al., 2013).

Freshwater aquaculture is an important source of aquatic protein for humans, especially in inland areas (Cressey, 2009; Garaway et al., 2013). As the global population continues to increase, however, freshwater and land available for aquaculture are becoming scarce. Freshwater aquaculture now faces the challenge of satisfying the demand for aquatic protein despite scarce water and land resources. Although intensive freshwater aquaculture can greatly increase aquaculture yields, it generates environmental problems (Broughton and Walker, 2010). The results of our study indicate that the intensive culturing of turtles with rice can produce large yields while reducing environmental problems. With the integration of intensive aquaculture and rice production, freshwater aquaculture should be able to expand in spite of limitations in the availability of land and water.

5. C

Integrating intensive turtle culture with rice culture can produce substantial turtle yields and stable rice yields. Moreover, rice–turtle coculture can reduce the quantities of feed-N and feed-P that accumulate in the environment and can thus reduce the potential for environmental pollution resulting from intensive turtle monoculture.

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