

Assessment of the Water Purification Ability of Mangrove (*Sonneratia caseolaris*) in Mesocosm Tanks

Toru SHIMODA^{1*}, Yoshimi FUJIOKA², Tomoko SAKAMI^{2,4},
Chumpol SRITHONG³ and Chittima ARYUTHAKA³

¹ Marine Environment Section, Ishigaki Tropical Station, Seikai National Fisheries Research Institute, Fisheries Research Agency (Ishigaki, Okinawa 907–0451, Japan)

² Aquaculture System Division, National Research Institute of Aquaculture, Fisheries Research Agency (Minami-ise, Mie 516–0193, Japan)

³ Faculty of Fisheries, Kasetsart University (Jatujak, Bangkok 10900, Thailand)

Abstract

Model experiments using mangrove mesocosm tanks were performed two times at the campus of Kasetsart University, Thailand. Replicates of 13 mangrove (*Sonneratia caseolaris*) saplings were planted in 7 mesocosm tanks and water and soil samples were collected during the 2 experimental periods. The nitrogen removal rate showed 57 and 73 mgN/m²/day in the tanks where wastewater from shrimp aquaculture ponds was supplied and exchanged, and 164–425 mgN/m²/day in the tanks where the additional nutrients were provided. The phosphorus removal rate was 5.7 and 17 mgP/m²/day in tanks supplied and exchanged with wastewater, and 48 and 97 mgP/m²/day in the tanks provided with additional nutrients. Both nitrogen and phosphorus showed high removal rates in the mangrove mesocosm tanks. However, there was no marked increase in the bacterial community either in the water or surface soil, despite the organic matter-rich wastewater from shrimp aquaculture ponds or high concentrations of inorganic nutrients that were supplied.

Discipline: Aquaculture

Additional key words: bacteria, budget, nutrient, shrimp aquaculture

Introduction

Tropical coastal mangrove forests were once considered to be inhospitable insect-infested swamps, a pariah among ecosystems, but it was considered as a paradise for developers⁵. Therefore in South East Asian countries natural mangrove regions have diminished with urbanization and industrialization, and the development of shrimp culture ponds to acquire foreign currency. In Thailand, mangrove areas have decreased by more than half in 30 years⁴. In recent years, however, it has been recognized that mangrove trees and their associated ecosystem are biologically and ecologically, as well as economically and socially important in tropical and subtropical coastal areas¹². Mangrove forests contain a wide diversity of plant species and they are an important habitat for not only marine organisms but also mammals,

birds and reptiles. They provide forestry and fishery resources for the people who live close to mangrove regions. Moreover coastal mangrove forests can protect coastal areas from storms, tidal bores and strong winds⁶. Nowadays restoration of mangrove ecosystems has been carried out in many tropical and subtropical regions of the world⁶. In the Klong Khon District, Samut Songkhram Province near Bangkok, Thailand, there were many mangrove trees once, but they were cut down and converted into shrimp ponds, but now in Thailand a number of shrimp ponds are unproductive and lie idle³⁰.

Because mangrove trees do not produce a profit for the pond owners it is difficult to encourage the replanting of mangrove trees to their idle ponds. Whereas, in the tidal flat near shore in Klong Khon, *Sonneratia caseolaris*, a kind of mangrove, was planted from about ten years ago, and they have grown steadily. Although mangroves form a zonation, the division of mangrove species into

Present address:

⁴ Tohoku National Fisheries Research Institute, Fisheries Research Agency (Shiogama, Miyagi 985–0001, Japan).

*Corresponding author: e-mail t.shimoda@fra.affrc.go.jp

Received 22 October 2007; accepted 14 July 2008.

separate zones, *Sonneratia caseolaris* predominates at the outermost part of the mangrove forest¹⁷ and estuarine river banks, preferring low salinity areas with a freshwater input⁹. Therefore they are well suited to grow in the Klong Khon, Samut Songkhram.

Wastewater from the shrimp aquaculture pond contains a large amount of nutrients and organic substances, and therefore it leads to the deterioration of the surrounding coastal environment and has a bad influence on the fishery ground. There are many purposes for restoration of mangrove ecosystems, and one of them is that mangroves and their associated ecosystem improve the water quality. Mangroves are trees and absorb nutrients, and many kinds of organisms in the mangrove ecosystems incorporate nutrients and substances including detritus. Therefore, it is considered that mangrove ecosystems have a high potential for water purification.

The use of mesocosm tanks allows the control of the environment and more precise calculations of material budgets compared to field experiments^{26,27}. In this study, we used mangrove saplings and soil acquired from Klong Khon, Samut Songkhram, Thailand and mangrove mesocosm tank experiments were carried out to investigate the water purification ability of the mangrove ecosystem.

Materials and methods

Model experiments using mangrove mesocosm tanks were performed two times at the campus of Kasetsart University, Thailand. The experiments were carried out under the condition of natural sun light. Seven tanks 100 cm in diameter and 70 cm in height (each about 500 liters) made of fiber reinforced plastic (FRP) were prepared, and 13 mangrove (*Sonneratia caseolaris*) saplings were planted in each tank (Fig. 1). The mangrove sap-

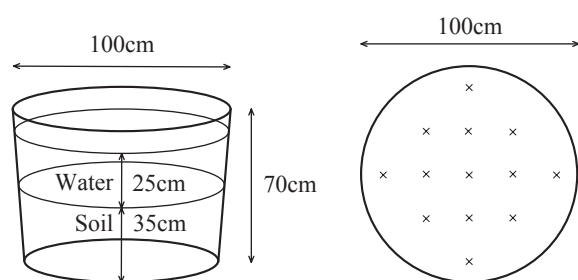


Fig. 1. Schematic diagram of the tanks used in this experiment

Mangrove saplings were planted at the X symbols in the diagram on the right.

lings and soil for the experiment were transplanted from the mangrove restoration region in Klong Khon district, Samut Songkhram Province, Thailand. The first experiment was conducted for a 35-day period from April 29 to June 3, 2002. An outline of the treatment of each tank is shown in Table 1. Tank 1 is the control, brackish water was supplied and water was not exchanged. Wastewater from intensive shrimp culture ponds was supplied to Tanks 2–4. In Tank 2, water was exchanged about every 3 days with a reservoir tank using a water pump. In Tank 3, water was transferred to a reservoir tank using a water pump as well as Tank 2 every 3 days and the dry and wet terms were repeated to simulate a natural mangrove region. After the wastewater was supplied, water was not exchanged or transferred in Tank 4. Artificial nutrients were supplied to the other three tanks every three or seven days throughout the experimental period. In Tank 5, about 200 liters of brackish water was supplied and only nitrogen (ammonium and nitrate) was provided to increase 100 μg at./L every 3 days, respectively. In Tank 6, phosphate in addition to ammonium and nitrate were provided as dissolved inorganic phosphorus (DIP). In Tank 7, large amounts of nutrients were provided. As they decreased sharply soon at the beginning of the first experiment, double the amounts of nutrients were provided only one time in the second experiment. The water quality was monitored every 3 to 24 hours during the first 2 days and every 2 to 4 days thereafter. Temperature, salinity, dissolved oxygen (DO), turbidity, and pH were measured with a WQC-20A water quality checker (DKK-TOA). However, during the second experiment, the pH probe of this water quality checker failed and its data was not acquired. Water samples of these tanks were directly taken in 300 mL or 500 mL plastic bottles. These samples were immediately filtered three times with Whatman GF/F for chlorophyll *a* and phaeopigment (Chl. *a* + Phaeo.) in water, and particulate nitrogen (PN) and phosphorus (PP). After the filters for Chl. *a* + Phaeo. analysis were homogenized in 90% acetone solvent, they were separated with a centrifuge, and the supernatant was analyzed using a spectrophotometer (Shimadzu UV-1201)¹¹. PN was measured with an elemental analyzer (Fisons EA 1108) and PP was analyzed using the method of Solorzano and Sharp²⁹. The filtrate was used to analyze concentrations of nutrients, total dissolved nitrogen and phosphorus. Ammonium concentration was measured using Sasaki and Sawada's method²⁴ immediately after filtration. Nitrate, nitrite and phosphate were analyzed with a spectrophotometer (Shimadzu UV-1201) using standard methods¹⁶. Using the methods of Solorzano and Sharp²⁸ for total dissolved nitrogen and of Menzel and Corwin¹³ for total dissolved phosphorus, digestion was carried out by

Table 1. Tank treatment in the first and second experiments

Tank No.	Treatment
Tank 1	Control; Only brackish water was supplied.
Tank 2	Wastewater from shrimp pond was supplied. Water was exchanged about every 3 days.
Tank 3	Wastewater from shrimp pond was supplied. The dry and wet terms were repeated every 3 days.
Tank 4	Wastewater from shrimp pond was supplied. Water was not exchanged.
Tank 5	Ammonium and nitrate were added to increase 100 μ g at./L every 3 days, respectively.
Tank 6	Ammonium, nitrate and phosphate were added to increase 100 μ g at./L, 100 μ g at./L and 20 μ g at./L every 3 days, respectively.
Tank 7	Ammonium, nitrate and phosphate were added to increase 500 μ g at./L, 500 μ g at./L and 100 μ g at./L every 7 days, respectively in the first experiment and to increase 1mg at./L, 1mg at./L and 200 μ g at./L every 7 days, respectively in the second experiment.

autoclaving after $K_2S_2O_8$ was added to the samples, and nitrate and phosphate concentrations were measured, respectively. Soil samples from the surface layer down to a depth of 1 cm were collected using a syringe-type core sampler of 20 or 23 mm in diameter. The collected mud was dried, weighed and crushed with a mortar. The nitrogen content in the soil sample was measured with an elemental analyzer and the phosphorus content in soil was analyzed using Andersen's method². Moreover, height, diameter of stalks at the water surface, and the number of the leaves on each mangrove tree were measured at the beginning and the end of the first experiment.

Before starting the second experiment, about 50 liters of mud was collected from mangrove swamps at Klong Khon, Samut Songkhram Province and put in each tank. The second experiment was carried out to investigate microbial community and continued for an 8-day period from November 12 to 19, 2003. The mesocosm tank treatment and sampling procedure were the same as the first experiment except for Tank 7 (Table 1). In Tank 7, concentrations of ammonium, nitrate and phosphate were provided to increase 1 mg at./L, 1 mg at./L and 200 μ g at./L, respectively. Bacterial number in water and soil, and fungal biomass were examined in the second experiment. Water samples for bacterial number were collected directly in a glass tube. Soil samples were collected using a syringe-type core sampler of 23 mm in diameter, and the surface layer (0–1 cm) of the collected soil core was cut away and measured. The water and soil samples were fixed with glutaraldehyde (5% v/v) immediately after collection and kept under cool and dark con-

ditions. Bacterial counts were carried out by microscopic direct counting using fluorochrome DAPI¹⁸. The soil samples were sonicated in 0.05 M sodium pyrophosphate¹⁴ and analyzed in the same way as the water sample. Fungal biomass was estimated by ergosterol determination using high pressure liquid chromatography (HPLC)³¹. Four to 6 g of the surface soil sample was put into 10 mL of alcohol base (4% KOH in 95% methanol) and refluxed at 80°C for 30 min in a water bath. After refluxing, 10 mL of hexane was added to the sediment - alcohol base mixture and agitated well. Three mL of the hexane was removed after centrifugation. Hexane was evaporated under nitrogen gas and the dried residue was re-dissolved in 20 μ L of methanol. Ergosterol concentrations were determined following separation with HPLC by measuring absorbance at 282 nm.

Results

Table 2 shows the average height, number of leaves and diameter of stalks at the water surface for each mangrove sapling, *Sonneratia caseolaris*, at the beginning and the end of the first experiment. These values increased in all tanks, except for the number of leaves and diameter of stalks in Tank 1, which was the control, indicating that the mangrove trees grew well in tanks to which nutrients or wastewater were supplied.

Salinity was 7.6–8.8 in Tanks 1 and 5–7 supplied brackish water, and 3.9–4.2 in Tanks 2–4 provided wastewater at initial condition. Nutrients concentrations were low, and dissolved organic and particulate nitrogen and phosphorus concentrations were also low in Tanks 1 and

5–7 compared to wastewater from shrimp aquaculture ponds. Though nutrients concentrations were low, dissolved organic and particulate nitrogen and phosphorus concentrations were high in wastewater.

Fig. 2 shows the changes in temperature, salinity, dissolved oxygen (DO), pH, and turbidity during the first experimental period. Temperature ranged from 26.9 to 33.9°C and showed an almost identical pattern in all tanks. Salinity was 6.1–9.3 in the tanks to which nutrients were provided, and 3.7–5.3 in the tanks to which

wastewater was supplied, with only minor variations. Since *Sonneratia caseolaris* prefers low salinity with freshwater input⁹, the low salinity did not affect their growth. DO increased in all tanks immediately after the beginning of this experiment, especially in tanks to which nutrients were provided, exceeding 10 mg/L. It then decreased gradually afterwards and fell below the oxygen-poor 2 mg/L level several times. At the end of the experiment, DO in Tank 1 (control) was high, probably due to the thick seaweed growth. Very high turbidity was con-

Table 2. Average height, number of leaves and diameter of stalks at the water surface of mangrove saplings, *Sonneratia caseolaris*, at the beginning and the end of the first experiment

Tank No.	Height (cm)		Leaves (Number)		Stalk (mm)	
	Before	After	Before	After	Before	After
1	72.3	81.8	30.8	26.5	6.2	6.1
2	65.2	72.8	50.2	51.5	5.9	6.0
3	68.0	82.8	41.2	58.1	6.8	7.5
4	71.3	85.2	20.5	50.6	6.2	7.7
5	81.8	100.0	36.9	78.3	8.7	9.9
6	67.6	77.8	21.6	38.5	7.2	7.7
7	71.3	82.9	31.5	51.6	7.3	8.0

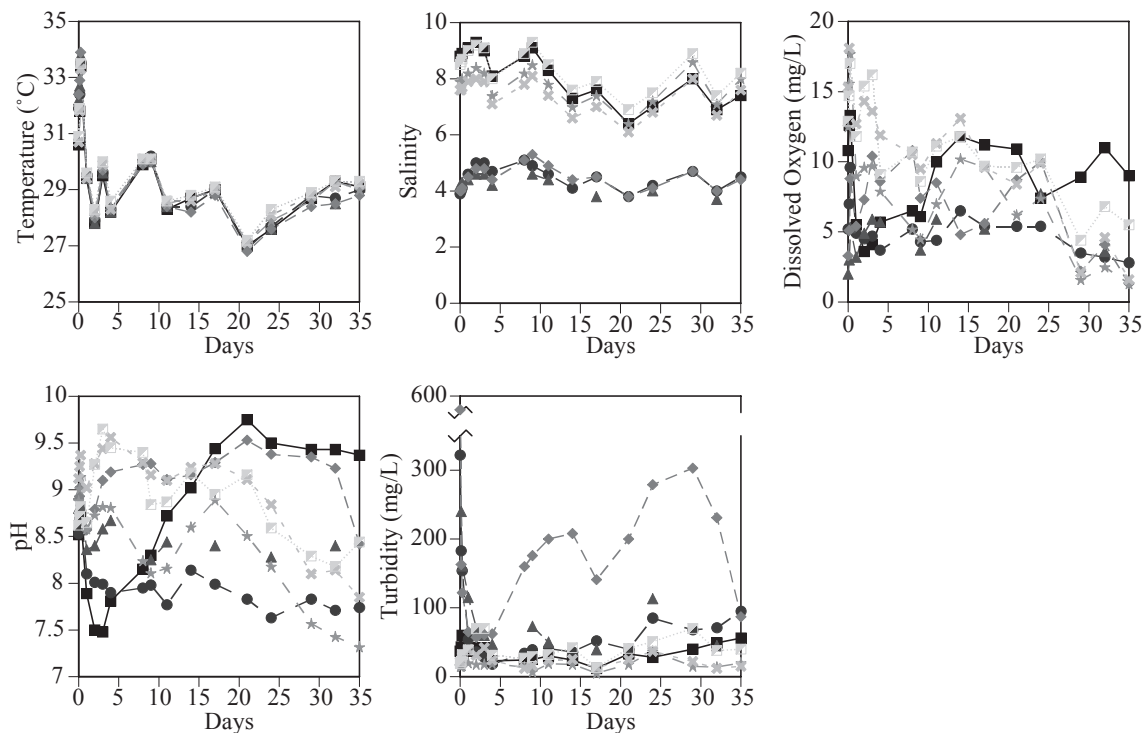


Fig. 2. Variations of water temperature, salinity, dissolved oxygen, pH, and turbidity during the first experiment

■ : Tank 1, ● : Tank 2, ▲ : Tank 3, ◆ : Tank 4, ★ : Tank 5, ✕ : Tank 6, ◻ : Tank 7

tinuously observed in Tank 4 supplied wastewater and not exchanged with the reservoir tank. Chl. *a* + Phaeo. concentration in water (Fig. 3) was always very high in Tank 4, and it ranged from 503 to 952 $\mu\text{g/L}$ during days 8–32. Comparatively high Chl. *a* + Phaeo. concentration in water was also observed in Tank 3 to which wastewater was supplied, and exchanged with the reservoir tank and the dry and wet terms were repeated, but was low in the other tanks. In the tanks to which nutrients were provided (Tanks 5–7), Chl. *a* + Phaeo. in soil fluctuated widely, and in tanks to which wastewater was supplied (Tanks 2–4), Chl. *a* + Phaeo. concentration in soil was the highest in Tank 4 as was the case for that in the water.

In Tanks 2 and 3, PN and PP were supplied from the reservoir tanks and they decreased sharply after 3 days, and dissolved organic nitrogen (DON) decreased gradually. Nitrogen and phosphorus contents in soil decreased in Tank 4, and DON, phosphate and DOP in water increased. In Tank 5 in which only nitrogen was provided, both ammonium and nitrate concentrations almost decreased by every 3 days after supplementation, and those concentrations before and after the 3 days interval of nutrients input did not increase until the end of this experiment. In Tank 6 where nitrogen and phosphorus were artificially provided, although nitrogen did not increase the same as in Tank 5, the phosphate concentration before and after the additions of nutrients increased gradually. Also in Tank 7 in which large amounts of nutrients were provided, dissolved inorganic nitrogen did not increase, but the phosphate concentration increased gradually before and after nutrients input. Nitrogen content in soil decreased in all tanks and the decrease was large except for the control tank (Fig. 4). The phosphorus content in soil showed a different tendency in each tank (Fig. 5). It decreased in Tanks 3 and 4. In contrast, it increased in Tanks 1, 6 and 7, and it kept the same level in Tanks 2 and 5.

The results for nitrogen and phosphorus budgets in each tank are shown in Tables 3 and 4. In the calculation for the nitrogen and phosphorus budgets, since only the surface soil was collected and the variation in the deep layer in the soil was not considered in this study, the budgets were calculated excluding the accumulation in the soil. Nitrogen decreased in Tanks 2 and 3 provided and exchanged with wastewater, and in Tanks 5–7 provided with additional nutrients. Only in Tank 4 where high Chl. *a* + Phaeo. concentration was observed in water, nitrogen increased in water. Removal rate in Tanks 2, 3 and 5–7 exceeded $50 \text{ mgN/m}^2/\text{day}$, especially in Tank 7 it was high; $425 \text{ mgN/m}^2/\text{day}$. Phosphorus also decreased in Tanks 2 and 3 provided and exchanged with wastewater and in Tanks 6–7 provided with additional nutrients

except for Tank 5 that was not provided with phosphate.

It is considered that the detritus food chain is important to decompose most leaf litter in mangrove areas²⁰. Therefore, in the second experiment, we examined the relationship between the bacterial community and the water and soil quality in the mesocosm tanks. Variations in water and soil quality in the second experiment are shown in Figs. 6–8. They showed similar trends as the first experiment, thereby it is considered that the experimental conditions for the bacterial community were almost the same as the first experiment. Variations in the bacterial number in water and soil, and fungal biomass in soil are shown in Fig. 9. The bacterial number in the control tank (Tank 1) became the maximum on the second day and decreased in the water and soil on the fourth and eighth days, respectively. Initial bacterial abundance was high in Tanks 2, 3 and 4 where wastewater from aquaculture ponds was supplied. However, it decreased on the first or third days to become similar to or less than that in the control tank (Tank 1). In the tanks supplied with nutrients (Tanks 5–7), the bacterial number was similar to or less than that in the control tank. Especially the bacterial abundance tended to decrease when only nitrogen was supplied (Tank 5). The bacterial number in the surface soil increased in the control tank. In the other tanks supplied with wastewater or nutrients, the bacterial abundances were less than that in the control tank, with the exception of Tank 4 where wastewater was supplied and kept unchanged during the experiment. The fungal biomass in surface soil also increased in the control tank markedly. In other nutrient treated tanks, the fungal biomass was less than that in the control tank. Only a relatively high fungal biomass was observed in Tank 3 where wastewater was supplied under the dry and wet condition.

Discussion

Thampanya et al.³² investigated the relative growth rate of height (RGR_H)⁷ of *Avicennia alba*, *Rhizophora mucronata* and *Sonneratia caseolaris* transplanted in Thailand, and the RGR_H of *Sonneratia caseolaris* was higher than those of the other two species of mangrove, and was $1.395\text{--}2.158 \text{ mm/cm/mo}$. The RGR_H of *Sonneratia caseolaris* in mangrove mesocosm tanks ranged from 1.012 to 1.901 mm/cm/mo , and they grew as well as ones under natural conditions. *Sonneratia caseolaris* is a major component widely distributed in tropical and subtropical regions, and prefers low salinity with freshwater input⁹. Neither water temperature nor salinity (Fig. 2) in the first experiment influenced growth markedly.

Ammonium, nitrate and nitrite were almost exhaust-

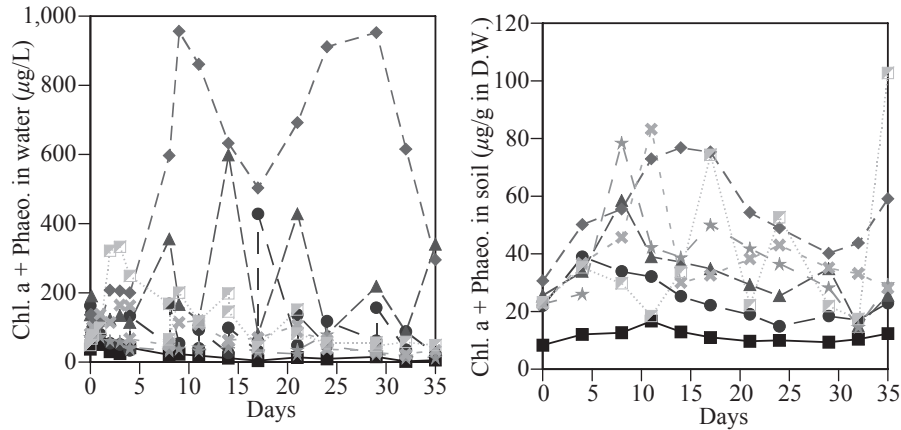


Fig. 3. Chlorophyll *a* (Chl. *a*) + phaeopigment (Phaeo.) concentrations in water (left) and in soil (right) in each tank
 Symbols indicating the number of each tank in the figures are given in Fig. 2.

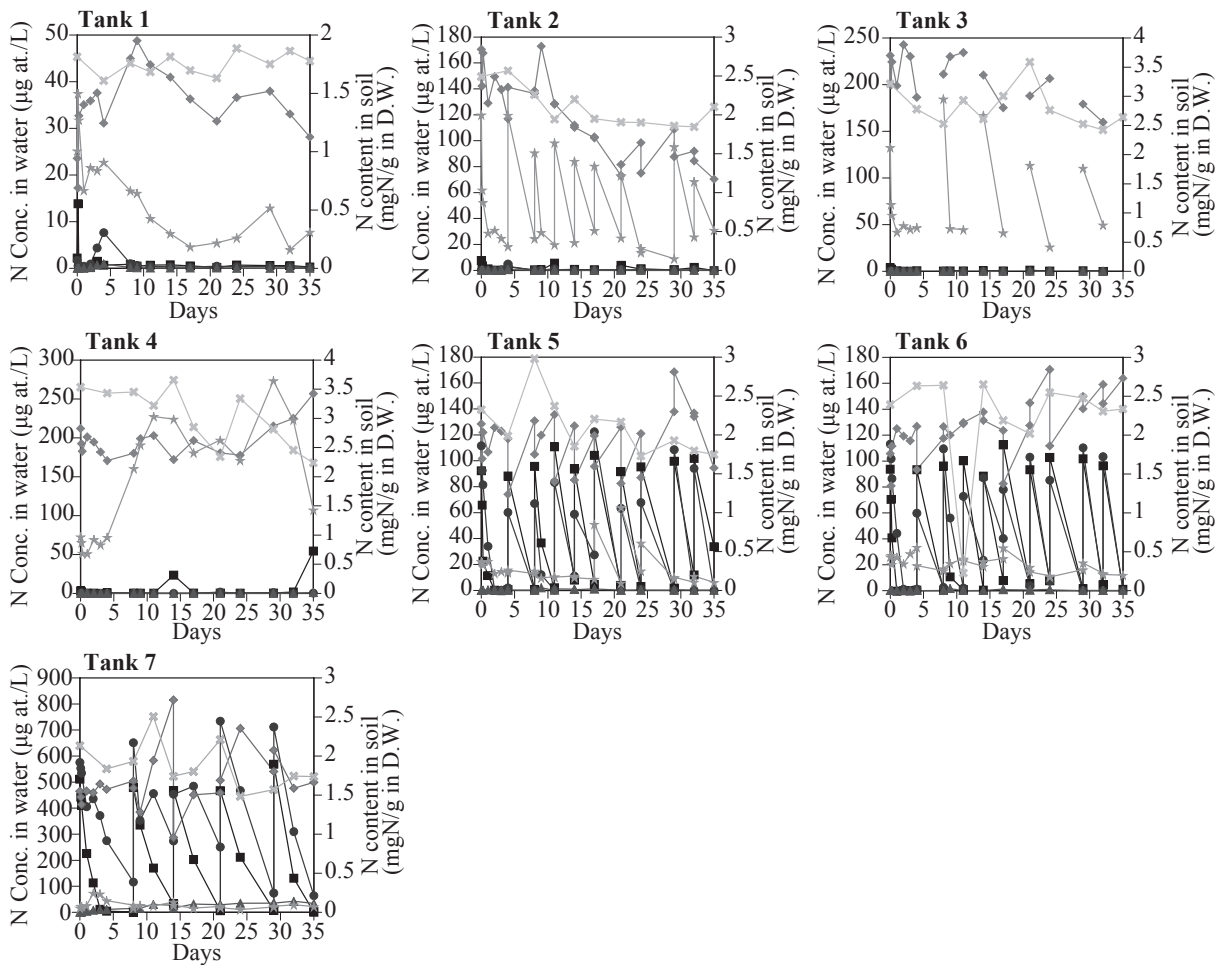


Fig. 4. Variations in ammonium, nitrate, nitrite, dissolved organic nitrogen (DON), particulate nitrogen (PN), and nitrogen contents in soil (N in soil) in each tank during the first experiment
 —■— : Ammonium, —●— : Nitrate, —▲— : Nitrite, —◆— : DON, —★— : PN, —×— : N in soil

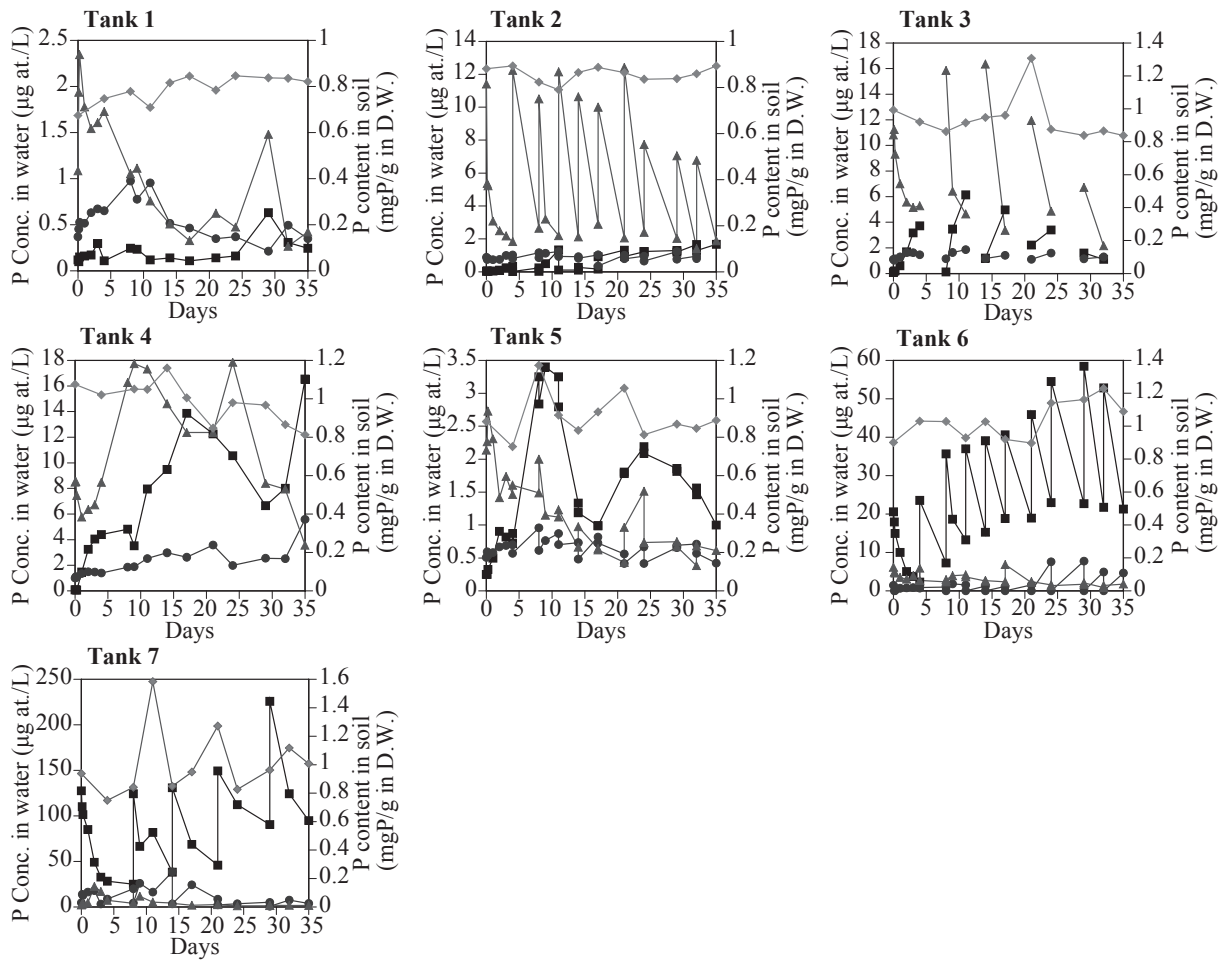


Fig. 5. Variations in phosphate, dissolved organic phosphorus (DOP), particulate phosphorus (PP), and phosphorus contents in soil (P in soil) in each tank during the first experiment
 —■— : Phosphate, —●— : DOP, —▲— : PP, —◆— : P in soil

Table 3. Nitrogen budget in each tank during the first experiment

Tank	Ammonia (mgN)	Nitrate (mgN)	Nitrite (mgN)	DON (mgN)	PN (mgN)	Amount of removal (mgN)	Removal rate (mgN/m ² /day)
1	-5.4	-4.3	-0.1	12.8	-48.8	45.8	1.7
2	-23.1	9.9	0.0	-230.6	-1,757.50	2,001.4	72.8
3	-14.5	-0.6	-0.9	-155.9	-1,400.40	1,572.4	57.2
4	143.5	0.7	2.6	125.5	96.9	-369.3	-13.4
5	-2,527.6	-2,224.0	-2.8	549.4	-410.6	4,615.6	168
6	-2,655.6	-2,355.1	1.8	545.8	-33.7	4,496.7	163.7
7	-6,845.4	-6,573.1	125.6	1,542.2	82.6	11,668.2	424.7

Positive values show an increase and negative values indicate a decrease in mesocosm tanks during the experiment period of ammonium, nitrate, nitrite, dissolved organic nitrogen (DON), particulate nitrogen (PN), and N contents in soil. Amount of removal is the sum total and the removal rate is the value per square meter per day.

Table 4. Phosphorus budget in each tank during the first experiment

Tank	Phosphate (mgP)	DOP (mgP)	PP (mgP)	Amount of removal (mgP)	Removal rate (mgP/m ² /day)
1	0.78	-0.13	-4.11	3.46	0.13
2	20.53	17.34	-492.46	454.59	16.55
3	87.58	12.05	-256.53	156.89	5.71
4	102.00	28.44	-30.50	-99.94	-3.64
5	4.65	-0.53	-9.45	5.33	0.19
6	-1,510.07	242.96	-41.98	1,309.08	47.65
7	-3,029.54	375.54	-2.69	2,656.69	96.69

Positive values show an increase and negative values indicate a decrease in mesocosm tanks during the experiment period of phosphate, dissolved organic phosphorus (DOP), particulate phosphorus (PP), and P contents in soil.

Amount of removal is the sum total and the removal rate is the value per square meter per day.

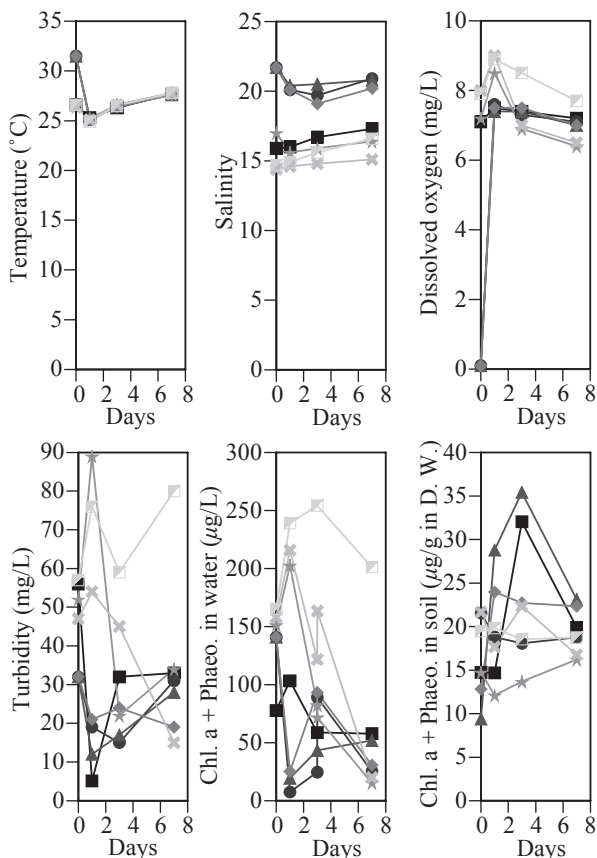


Fig. 6. Variations of water temperature, salinity, dissolved oxygen, pH, turbidity, and Chl. a + Phaeo. concentrations in water and in soil during the second experiment

■ : Tank 1, ● : Tank 2, ▲ : Tank 3,
 ◆ : Tank 4, ★ : Tank 5, ☆ : Tank 6,
 □ : Tank 7

ed in the control tank and tanks supplied with wastewater from shrimp aquaculture ponds (Fig. 4). In Tank 4 where wastewater was supplied and stagnant water, the Chl. *a* + Phaeo. concentration was extremely high (Fig. 3). Although ammonium, nitrate and nitrite were almost exhausted, phosphate increased gradually in Tank 4 (Fig. 5). There was no disturbance by the water exchange, and the phosphorus content in the mud decreased at the end. The deep mud layer might become an anoxic state and phosphate supplied to the water^{8,33}. Therefore the bloom condition of phytoplankton resulted from the provision of phosphorus released from the mud due to the oxygen-poor conditions. Since the ammonium, nitrate and nitrite concentrations were low, it was suggested that nitrogen might be the limiting factor of phytoplankton growth in each mesocosm tank supplied with wastewater from the shrimp aquaculture ponds. Even in the tanks provided artificially with nutrients, ammonia and nitrate greatly decreased until the fresh additions of nutrients. On the other hand, the phosphate concentration before and after the additions of nutrients increased gradually in Tanks 6 and 7 where phosphate was supplied. It is reported that the denitrifying activity is high in mangrove regions¹⁹ and the denitrification is considered to relate to the removal of nitrogen, though phosphate accumulated by adsorbing to particles or the soil. PN and PP decreased greatly in Tanks 2 and 3 supplied wastewater from shrimp aquaculture ponds after exchanging or supplying wastewater every time. In Tanks 2, 3 and 4, because the content in nitrogen and phosphorus in soil decreased except for phosphorus in Tank 2, it is thought that the accumulated PN and PP into the surface soil also contributed to the water purification through the decomposition by bac-

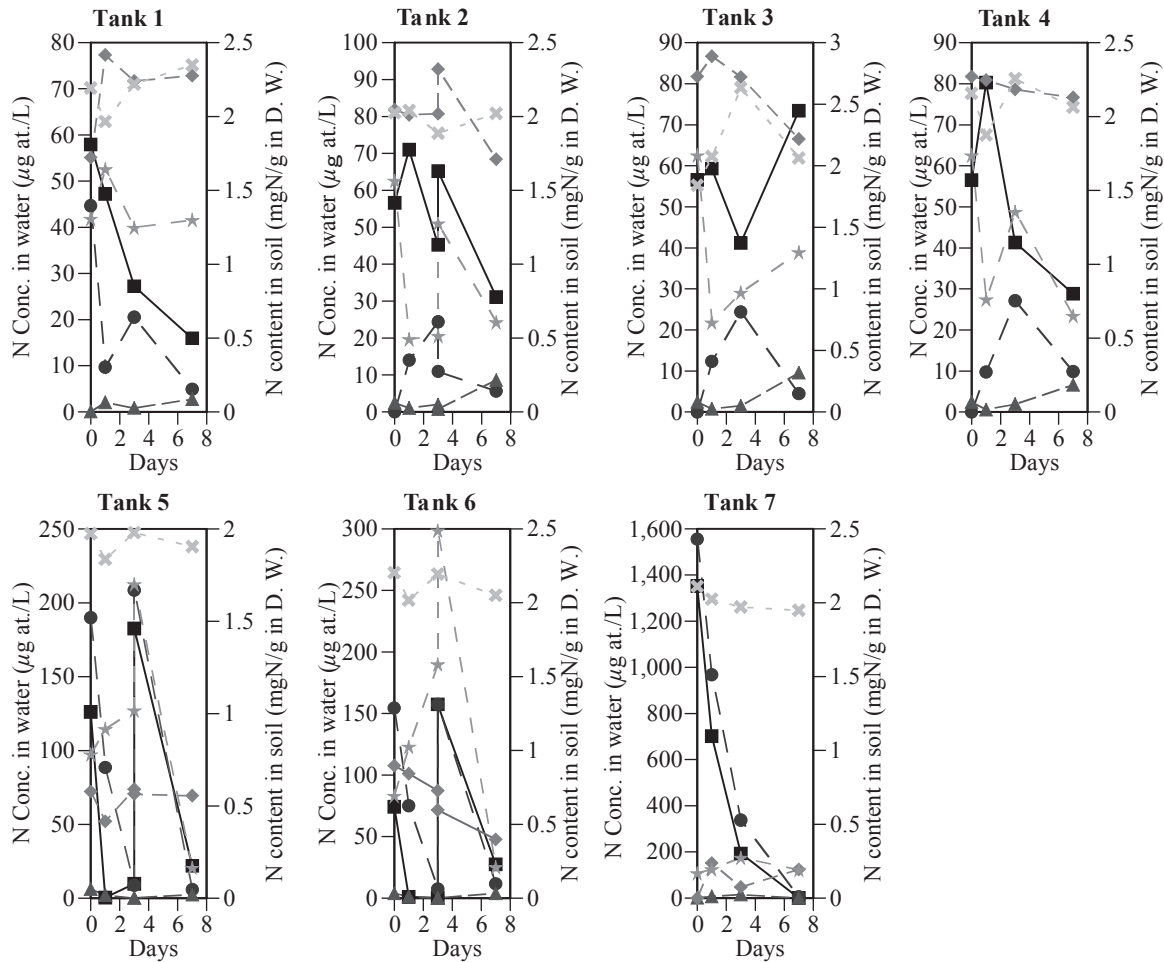


Fig. 7. Variations in ammonium, nitrate, nitrite, dissolved organic nitrogen (DON), particulate nitrogen (PN), and nitrogen contents in soil (N in soil) in each tank during the second experiment
 —■— : Ammonium, —●— : Nitrate, —▲— : Nitrite, —◆— : DON, —★— : PN, —✕— : N in soil

teria.

The nitrogen removal rate (Table 3) was 57 and 73 $\text{mgN/m}^2/\text{day}$ in the tanks where wastewater was supplied and circulated, and 164–425 $\text{mgN/m}^2/\text{day}$ in the tanks where the nutrients were provided. Robertson and Phillips²¹ calculated that the nutrients required for primary production in *Rhizophora*-dominated forests was 219 kg N/ha/year or 60 $\text{mgN/m}^2/\text{day}$. Rivera-Monroy et al.¹⁹ estimated that the reduction of dissolved inorganic nitrogen in shrimp pond effluent would be over 190 $\text{mgN/m}^2/\text{day}$ due to the high denitrifying activity. Excluding Tank 4 observed high Chl. *a* + Phaeo. concentration and Tank 7 supplied with large amounts of nutrients, the removal rate obtained in this experiment was comparable with their values. Although high concentrations of nutrients such as Tank 7 do not occur in actual mangrove regions, man-

groves potentially have a removal rate over about 400 $\text{mgN/m}^2/\text{day}$.

Although phosphorus decreased in the mesocosm tanks provided and exchanged with wastewater from shrimp ponds and artificial phosphate (Table 4), phosphorus was not removed and increased in Tank 4. Although the phosphorus content in the soil was measured in the surface 1 cm of soil, it was thought that phosphorus was probably desorbed from the soil below 1 cm depth and it was absorbed by seaweed or phytoplankton in the water. The amount of removal was very low in Tank 5, to which only nitrogen was provided. It is considered that the desorption and uptake of phosphorus were balanced closely to each other in this tank. Phosphorus was removed in the other tanks. The phosphorus removal rate was 5.7 and 17 $\text{mgP/m}^2/\text{day}$ in Tanks 2 and 3 supplied and

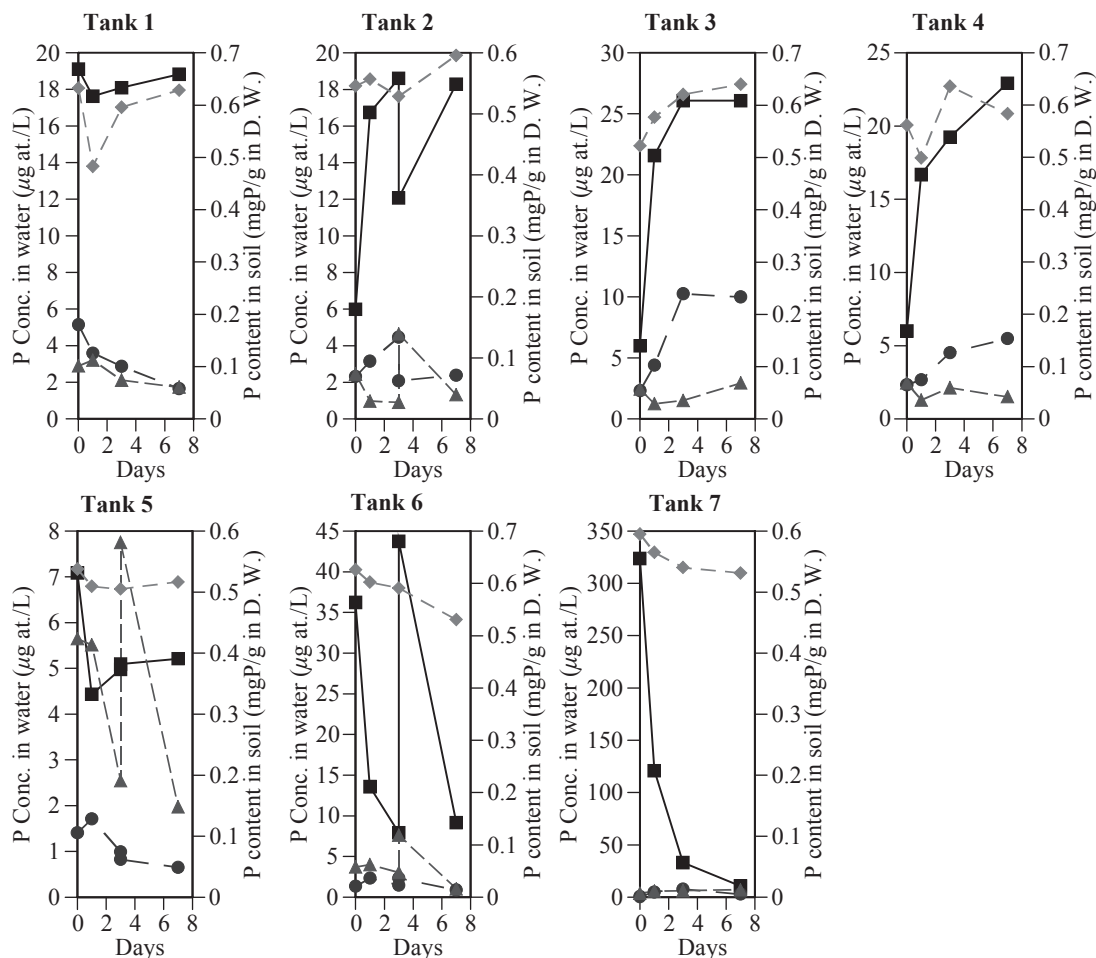


Fig. 8. Variations in phosphate, dissolved organic phosphorus (DOP), particulate phosphorus (PP), and phosphorus contents in soil (P in soil) in each tank during the second experiment
 —■— : Phosphate, —●— : DOP, —▲— : PP, —◆— : P in soil

exchanged with wastewater from shrimp aquaculture ponds, and 48 and 97 mgP/m²/day in the Tanks 6 and 7 provided with additional nutrients. Phosphorus required for primary production in *Rhizophora*-dominated forests was estimated as 20 kg P/ha/year, 5.5 mgP/m²/day²¹. The removal rate of phosphorus by *Sonneratia caseolaris* which grows faster than *Rhizophora*³², and their related ecosystems exceeded that of the latter. However, the phosphate concentration increased gradually in the tanks supplied with nutrients before and after the additions of nutrients implying that mangroves could not process everything and it might have accumulated in the deep layer of mud.

In mangrove ecosystems, microbial communities have important roles in organic matter cycling, not only as degraders but also as secondary producers of the detri-

tus food web system based on mangrove litter falls²³. This detritus food web system is considered to be the prevailing one in mangrove areas³. In the second experiment, however, there was no marked increase in bacterial densities either in the water or the surface soil in the nutrients supplied tanks (Tanks 5–7). This result indicates that the removal of nitrogen and phosphorus in the mesocosm tanks was not directly related to the microbial biomass accumulation. Generally microbial activities are enhanced in shrimp aquaculture ponds¹. Some mesocosm studies also have shown that enrichment of nutrients activates microbial communities in water and/or soil^{10,25}. Though the standing stocks did not change in the mesocosm tanks, microbial productivity and/or the turnover rate might have changed by the nutrient supply. Fungi are also important decomposers as well as bacteria in man-

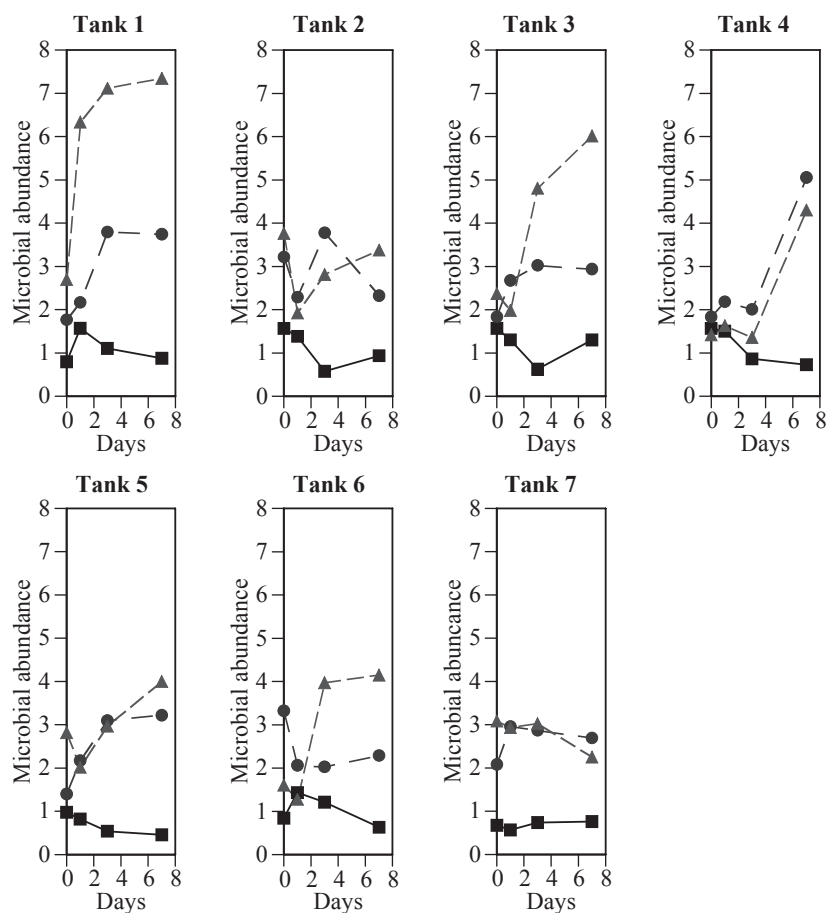


Fig. 9. Variations in bacterial number in water and in soil, and fungal biomass in soil in each tank during the second experiment

—■— : Bacterial number in water ($\times 10^7$ cells/cm³), —●— : Bacterial number in soil ($\times 10^9$ cells/cm³), —▲— : Fungal biomass in soil ($\times 10^{-1}$ mg ergosterol/cm³)

grove ecosystems¹⁵. The fungal biomass was always less in the nutrient enriched mesocosms. We also have indicated that fungal biomass in a shrimp culture pond was much less than that in a mangrove forest²². These results suggest that nutrient loads to mangrove areas may inhibit fungal activity and change the features of the microbial community.

Acknowledgments

Appreciation is expressed to Dr. Tokuda for his help with measuring ergosterol. This paper reports the results obtained by the Japan International Research Center for Agricultural Sciences (JIRCAS) Research Project "Studies on Sustainable Production Systems of Aquatic Animals in Brackish Mangrove Areas" and "Development of

environmental management technology for sustainable crop production in tropical and subtropical islands".

References

1. Allan, G. L., Moriarty D. J. W. & Maguire, G. B. (1995) Effects of pond preparation and feeding rate on production of *Penaeus monodon* Fabricius, water quality, bacteria and benthos in model farming ponds. *Aquaculture*, **130**, 329–349.
2. Andersen, J. M. (1976) An ignition method for determination of total phosphorus in lake sediment. *Water Res.*, **10**, 329–331.
3. Canfield, D. E. Thamdrup, B. & Kristensen, E. (2005) Microbial ecosystems. In *Aquatic geomicrobiology, advances in marine biology* vol. 48, eds. Southward, A. J., Tyler P. A., Young, C. M. & Fuiman, L. A., Elsevier, London, 465–506.

4. Clough, B. F. (1993) Status and value of mangrove forests. *In* The economic and environmental values of mangrove forests and their present stage of conservation in the South-East Asia/Pacific region, ed. Clough, B. F., ISME/ITTO/JIAM, International Society for Mangrove Ecosystems, International Timber Organization, and Japan International Association for Mangroves, 1–10.
5. Dodd, D. S. (1999) Diversity and function in mangrove ecosystems. Kluwer Academic Publishers, Dordrecht, Netherlands, pp.142.
6. Field, C. D. (1996) Restoration of mangrove ecosystems. International Society for Mangrove Ecosystems, Okinawa, Japan, pp.250.
7. Hunt, R. (1982) Plant growth curves: the functional approach to plant growth analysis. London: Edward Arnold, pp.248.
8. Jensen, H. S. et al. (1995) Phosphorus cycling in a coastal marine sediment, Aarhus Bay, Denmark. *Limnol. Oceanogr.*, **40**, 908–917.
9. Kitamura, S. et al. (1997) Handbook of mangroves in Indonesia –Bali & Lombok–. JICA and ISME, Tokyo, Japan, pp.119.
10. Lebaron, P. et al. (1999) Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. *Aquat. Microb. Ecol.*, **19**, 255–267.
11. Lorenzen, C. J. (1967) Determination of chlorophyll and phaeopigments: spectrophotometric equations. *Limnol. Oceanogr.*, **12**, 343–346.
12. Mastaller, M. (1997) Mangroves. Tropical Press SDN. BHD., Kuala Lumpur, Malaysia, pp.189.
13. Menzel, D. W. & Corwin, N. (1965) The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulphate oxidation. *Limnol. Oceanogr.*, **10**, 280–282.
14. Mille-Lindblom, C. & Tranvik, L. J. (2003) Antagonism between bacteria and fungi on decomposing aquatic plant litter. *Microb. Ecol.*, **45**, 173–182.
15. Newell, S. Y. (1996) Established and potential impacts of eukaryotic mycelial decomposers in marine/terrestrial ecotones. *J. Exp. Mar. Biol. Ecol.*, **200**, 187–206.
16. Parsons, T. R., Maita, Y. & Lalli, C. M. (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford, pp.173.
17. Piyakarnchana, T. (1980) South China Sea fisheries development and coordinating programme. The present state of mangrove ecosystems in Southeast Asia and the impact of pollution, Thailand. FAO and UNEP, pp.138.
18. Porter, K. G. & Feig, Y. S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, **25**, 943–948.
19. Rivera-Monroy, V. H., Torres, L. A. & Bahamon, N. (1999) The potential use of mangrove forests as nitrogen sinks of shrimp aquaculture pond effluents: the role of denitrification. *J. World Aquac. Soc.*, **30**, 12–25.
20. Robertson, A. I. and Alongi, D. M. (1992) Tropical mangrove ecosystems. American Geophysical Union, Washington, DC, USA, pp.329.
21. Robertson, A. I. & Phillips, M. J. (1995) Mangroves as filters of shrimp pond effluent: prediction and biogeochemical research needs. *Hydrobiologia*, **295**, 311–321.
22. Sakami, T. et al. (2007) Microbial abundances and community structure in shrimp culture ponds and natural mangrove areas in Thailand. *In* Sustainable production system of aquatic animals in blackish mangrove areas (2005), JIRCAS Working Report No. 56, ed. Nakamura, K., Japan International Research Center for Agricultural Science, Tsukuba, 43–49.
23. Sakami, T., Fujioka, Y. & Shimoda, T. (2008) Comparison of microbial community structure in intensive, extensive shrimp culture ponds and a mangrove area in Thailand. *Fish. Sci.*, **74**, 889–898.
24. Sasaki, K. & Sawada, Y. (1980) Determination of ammonia in estuary. *Bull. Jpn. Soc. Sci. Fish.*, **46**(3), 319–321.
25. Schafer, H. et al. (2001) Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in the genetic diversity of bacterial populations. *FEMS Microb. Ecol.*, **34**, 243–253.
26. Shimoda, et al. (2005) Phosphorus budget in shrimp aquaculture pond with mangrove enclosure and aquaculture performance. *Fish. Sci.*, **71**, 1249–1255.
27. Shimoda, et al. (2007) Effect of water exchange with mangrove enclosures based on nitrogen budget in *Penaeus monodon* aquaculture ponds. *Fish. Sci.*, **73**, 221–226.
28. Solorzano, L. & Sharp, J. H. (1980) Determination of total dissolved nitrogen in natural waters. *Limnol. Oceanogr.*, **25**, 751–754.
29. Solorzano, L. & Sharp, J. H. (1980) Determination of total dissolved phosphorus and particulate phosphorus in natural waters. *Limnol. Oceanogr.*, **25**, 754–758.
30. Stevenson, N. J. (1997) Disused shrimp ponds: Options for redevelopment of mangrove. *Coast. Manag.*, **25**, 423–425.
31. Suberkropp, K. & Weyers, H. (1996) Application of fungal and bacterial production methodologies to decomposing leaves in streams. *Appl. Environ. Microbiol.*, **62**, 1610–1615.
32. Thampanya, U., Vermaat, J. E. & Duarte, C. M. (2002) Colonization success of common Thai mangrove species as function of shelter from water movement. *Mar. Ecol. Prog. Ser.*, **237**, 111–120.
33. Watanabe, Y. & Tsunogai, S. (1984) Adsorption-desorption control of phosphate in anoxic sediment of a coastal sea, Funka Bay, Japan. *Mar. Chem.*, **15**, 71–83.