ORIGINAL ARTICLE

Impacts of intensive shrimp cultivation on bacteria in the nitrogen cycle and physicochemical properties of sediments

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Abstract

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Bacterial numbers involved in the nitrogen cycle and some physicochemical properties in sediments of 4 intensive shrimp ponds, that had been cultivated continuously for 3 and 5 years, were studied between November 2004 to February 2005. The parameters were monitored as follows:, pH, electrical conductivity, organic matter, total Kjeldahl nitrogen (TKN), C/N ratio, ammonium, nitrite, and nitrate. The only significant differences ($p < 0.05$) found between the two types of ponds were ammonium (5.06-18.49 mg-N/kg dry weight). Comparisons among these shrimp ponds and control soils indicated that shrimp cultivation significantly increased the soil pH (5.66-7.57, $p < 0.05$) due to accumulation of ammonium in the sediments. Bacterial populations were determined in control soils and 2 levels of sediments (0-5 cm, > 5-10 cm) for both types of pond. Total numbers were in the order of heterotrophic plate count > azotobacteraceae >

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ammonifiers > denitrifiers > ammonium oxidizing bacteria > nitrite oxidizing bacteria.No significant difference was found between each bacterial group in the 2 levels of sediments from each sample set. In pond sediments from both sets of ponds organic matter was highly correlated with TKN (r = 0.783, p < 0.05,r = 0.883, p < 0.01) indicating that ammonia release and the increase pH in the sediments was to some extent determined by the deposit of organic matter.

Key words : impacts, intensive shrimp cultivation, bacteria in Nitrogen cycle, physicochemical properties, sediments

บทคัดย่อ

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ผลกระทบของการเลี้ยงกุ้งแบบพัฒนาต่อแบคทีเรียในวัฏจักรไนโตรเจนและสมบัติทางเคมี-กายภาพของตะกอนดินพื้นบ่อ

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การศึกษาแบคทีเรียในวัฏจักรไนโตรเจนและสมบัติทางเคมี-กายภาพของตะกอนดินบ่อเลี้ยงกุ้งแบบพัฒนาที่มีอายุการใช้งานระหว่าง 3 ปี จำนวน 2 บ่อ และ 5 ปี จำนวน 2 บ่อ ระหว่างเดือนพฤศจิกายน 2547 ถึงกุมภาพันธ์ 2548 พารามิเตอร์ที่ติดตามได้แก่ พีเอช การนำไฟฟ้า อินทรียวัตถุ ปริมาณไนโตรเจนทั้งหมด (Total Kjeldahl Nitrogen :TKN) C/N ratio แอมโมเนียม ไนไตรท์ และไนเตรท โดยพบว่าเกือบทุกพารามิเตอร์ที่ตรวจสอบของตะกอนดินบ่อกุ้งอายุ 3 ปี และ 5 ปี ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ (p > 0.05) ยกเว้นแอมโมเนียม (5.06-18.49 มก-ไนโตรเจน/กก น้ำหนักแห้ง) มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ (p < 0.05) และเมื่อเทียบกับดินชุดควบคุมพบว่าการเลี้ยงกุ้งทำให้พีเอชของดินเพิ่มขึ้น (5.66-7.57) อย่างมีนัยสำคัญทางสถิติ (p < 0.05) เนื่องจากมีการสะสมของแอมโมเนียม ปริมาณของแบคทีเรียกลุ่มต่าง ๆ ในวัฏจักรไนโตรเจนในตะกอนดินบ่อกุ้งทั้งอายุ 3 ปีและ 5 ปี รวมทั้งดินชุดควบคุม ซึ่งศึกษา 2 ระดับความลึก (0-5 ซม. และ > 5-10 ซม.) พบจำนวนประชากรเรียงจากมากไปน้อยดังนี้คือกลุ่ม heterotrophic plate count กลุ่ม azotobacteraceae กลุ่ม ammonifiers กลุ่ม denitrifiers กลุ่ม ammonium oxidizing bacteria และกลุ่ม nitrite oxidizing bacteria ไม่พบแบคทีเรียกลุ่มใดที่มีปริมาณแตกต่างกันทางสถิติ (p > 0.05) ระหว่าง 2 ระดับความลึกที่ศึกษา และพบว่าปริมาณอินทรียวัตถุในบ่อกุ้งที่มีอายุทั้ง 3 ปี และ 5 ปีต่างก็มีความสัมพันธ์ทางบวกในระดับสูงกับค่า TKN (r=0.783, p<0.05 และ r=0.883, p<0.01) แสดงว่าแอมโมเนียมที่เกิดขึ้นและการสะสมสารอินทรีย์ที่มาจากการเลี้ยงกุ้งส่งผลให้ตะกอนดินบ่อกุ้งมีพีเอชเพิ่มขึ้น

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Thailand is recognized as one of the world's leading countries for shrimp production. Recently, shrimp farmers have been faced with many problems such as shrimp diseases, negative environmental impacts and rejection of products from export markets. In order to solve these problems, the vision of a sustainable and friendly shrimp farming industry must be achieved and this will require more research. During intensive shrimp cultivation, nutrients and organic residues accumulate as sediments due to an absence of water exchange during the cultivation period. Too much the sediment can result in the deterioration of the pond system (Funge-Smith and Briggs,1998; Avnimelech and Ritvo, 2003). Shrimp feed has a high nitrogen content but most of it (80%) is not retained as shrimp biomass (Briggs and Funge-Smith,1994). As ammonia is the main excretory product of shrimp (Regnault,1987; Hargreaves,1998) and heterotrophic bacteria (ammonifiers) convert organic nitrogen including protein in the sediments to ammonium or ammonia depending on pH level in the environment. The occurrence of ammonia in alkaline condition i.e.pH 9.3, about 50% in form of ammonia (Hargreaves,1998) can become a major problem in shrimp pond. Consequently, nitrifiers (ammonium oxidizing bacteria: AOB and nitrite oxidizing bacteria: NOB) play an important role in the management of water quality by oxidizing ammonium to nitrite and nitrate, respectively (Jun *et al*.2000). Under anoxic conditions, denitrifiers use nitrate as an electron acceptor instead of oxygen for oxidizing organic compounds (Hargreaves, 1998). Some nitrogen in the ponds can also arise from nitrogen fixing bacteria such as azotobacteraceae, whose natural habitats is soil and water (Holt *et al*., 1994).

Research on shrimp ponds has concluded that there is a higher concentration of nutrients in sediments of 5-year ponds compared to 3-year ponds (Munsiri *et al*. 1996; Ritvo *et al*.1998). Nutritional elements from the soil are also released into the water column. The contact layer between the sediment surface and water column is the major source of nutrition (Sun and Zhaoyang1997). The quality of the sediment is therefore an important factor that influences the water quality and shrimp production (Shariff *et al*. 2001). From the above information, in order to sustain environmentally friendly shrimp cultivation, the impacts of intensive shrimp cultivation on the properties of the sediment must be further investigated. Hence, the aim in this study was focused on investigating the impacts of intensive shrimp cultivation in ponds with different periods of usage, the amounts of bacteria involved in the nitrogen cycle and also the physicochemical properties of the sediments.

Materials and Methods

1. Shrimp ponds and cultivation

Four ponds, in the Numnoy community, Hat-Yai district, Songkhla province, used continuously for the cultivation of shrimp (*Litopenaeus vannamai*; common name: white shrimp) for periods that ranged from 3 to 5 years, were used in this study. Ponds 3A and 3B (area = 0.56 ha/pond) had been cultivated for 3 years whereas ponds 5A and 5B (area = 0.48 ha/pond) had been cultivated for 5 years. Soil close to shrimp ponds, that had never been used for shrimp cultivation and had not received any water from shrimp ponds, was selected to use as control soils. These shrimp ponds and control soils are located in mangrove areas and the soil type is classified as an acid sulfate soil. The soil texture consists of sand, silt and clay and is strongly acidic (Thomas, 1996). Therefore, liming materials such as CaO had been applied in large amounts (1250 kg/ha) at each step of pond preparation. Water for filling the ponds and maintaining water levels was taken from the Songkhla lagoon. The density of shrimp stock was $100/m^2$ in 3-year ponds while in 5-year ponds it was $140/m^2$. Ponds were aerated mechanically with no water exchange. Shrimp was cultivated by experienced farmers who followed a good practice program for intensive shrimp farming as recommended by the Department of Fisheries. According to the pond owners, shrimp production usually was between 4460-5200 kg/ha. Following harvest, pond sediments were removed in the dry season, but in the rainy season the pond sediment was simply exposed to air for 2-4 weeks before refilling.

2. Samples collection

Sediment samples were taken from shrimp ponds 4 times between November 2004 and February 2005. Sampling of sediments from pond 3A was conducted when the shrimp'age was 13, 42, 69 and 85 days , while for a pond 3B samples were taken at days 14, 43, 70 and 86. Samples from pond 5A were also collected at days 4, 31, 47 and 61, but only 3 times from pond 5B at days 31, 42 and 69 due to the shrimps being harvested early because of their poor health. Sediment was collected using an improvised sampler made from a PVC pipe (1 cm in diameter, 40 cm in length) at two levels: 0-5 cm depth (upper layer) and >5-10 cm depth (lower layer) for counting bacterial populations, whereas only one layer (0-10 cm depth) of sediments was collected for investigating the soil physicochemical properties. Each sample was kept in an ice box. Samples from 12 sites taken at the same time for each pond were later thoroughly mixed using aseptic techniques to obtain a composite sample. Control soil samples were also collected in parallel every time using the same protocol as for collecting the sediment samples.

3. Determination of physicochemical properties

The following parameters of the sediment samples were determined according to the methods described in Rayment and Higginson, (1992): $pH_{1:5}$ (soil in 1 M KCl), electrical conductivity: $EC_{1:5}$ (soil in water) and moisture after 105°C overnight. Ammonium was determined by a modified indophenol method following Sasaki and Sawada (1980). A diazotization method and a cadmium reduction column with diazotization were used to examine nitrite and nitrate,respectively (Bendschneider and Robinson, 1952). Organic carbon and organic matter was determined by the Walkley-Black method (Nelson and Sommers, 1998), where as total Kjeldahl nitrogen (TKN) was determined by the Kjeldahl method (Bremner, 1996).

4. Bacterial populations

Bacteria involved in the N cycle in each layer of sediments were counted following the method of Rodina (1972). A standard plate count, with plate count agar, was used to determine the total heterotroph bacterial count or heterotrophic plate count (HPC) and a nitrogen free medium for azotobacteraceae. Four tubes and three serial dilutions were used for MPN (most probable numbers) counts for ammonifiers (peptone broth), ammonium oxidizing bacteria (nitrite formation medium), nitrite oxidizing bacteria (nitrate formation medium) and denitrifiers (nitrate broth with Durham tube). All plates and tubes were incubated at 30°C for varying times depending on the cultures.

5. Statistical analysis

Independent sample t-tests was used to analyze bacterial populations in the two levels (0-5 cm, > 5-10 cm) of sediments from the shrimp ponds and control soils. Comparison of the physicochemical properties between sediments from ponds used continuously for 3 and 5 years and control soils was analyzed by one-way ANOVA. In addition, the relationships between physicochemical properties and bacterial populations were analyzed by Spearman rank correlation. All statistical analyses were carried out using the SPSS version 12 for Windows.

Results

1. Physicochemical properties of shrimp sediments

The Physicochemical properties of sediments collected from the white shrimp ponds, cultivated for 3 and 5 years, at various shrimp ages, were as follows:pH (5.66-7.57), EC (0.88-3.06 ms/cm), organic matters (1.86-14.61%), TKN (0.06-0.16%), C/N ratio (18-51), ammonium (5.06-18.49 mg-N/kg dry weight), nitrite (0.14-32.00 mg-N/kg dry weight), nitrate (2.59-55.23mg-N/kg dry weight) (see details in Table 1). No significant differences were found for almost all monitored parameters among shrimp ponds and control soils, except for pH and ammonium. Control soils had a pH in the range of 3.64-4.43 with an average of 4.00 and this was significantly different ($p<0.05$) from the shrimp ponds of different ages (both 3 and 5 years). Amongst the shrimp ponds, only the ammonium concentration showed significant differences ($p<0.05$) with higher concentration in the 3-year ponds (average: 10.76 mg-N/kg dry weight) than in the 5-year ponds (average: 5.88 mg-N/kg dry weight). However the one pond with more than 3 times (14.-61%) the average organic content (4.17%) was the one from which the shrimp were harvested early because of their poor health and that had been in use for 5 years.

2. Bacterial populations in the N cycle

In shrimp ponds used for different period of years (Table 2), the heterotrophic plate count (HPC) in the upper layer were between 6.2 x10^4 - 3.6 x10^6 CFU/g dry weight with an average of 1.2 x10^6 CFU/g dry weight (SD: ± 1.1 x10^6 CFU/g dry weight). In the lower layers HPC were in the range of 1.1 x 10^5 - 1.3 x 10^7 CFU/g dry weight with an average of 2.5 x10^6 CFU/g dry weight (SD: ± 3.8 x 10^6 CFU/g dry weight). Ammonifiers in the upper and lower

Table 1. Physicochemical properties of sediments collected from intensive shrimp ponds of different age.

Parameter	Unit	Pond code/age of shrimp (days)																		
		3A				3B				5A				5B			Control			
		13	42	69	85	14	43	70	86	4	31	47	61	31	42	69	1	2	3	4
pH*	-	7.42	6.88	7.46	5.66	7.03	5.80	6.95	7.15	6.98	6.88	7.57	7.49	7.08	5.91	6.97	3.98	4.43	3.64	3.97
EC	ms/cm	1.81	0.88	1.66	1.96	2.96	0.89	1.24	2.87	1.84	1.84	2.52	2.85	2.12	2.33	3.06	1.60	1.70	1.42	3.97
Organic matter	%	3.44	2.52	2.48	2.61	3.29	6.24	3.78	4.33	2.48	3.59	3.85	1.87	1.86	3.21	14.61	6.9	26.68	3.64	1.86
TKN	%	0.07	0.06	0.06	0.05	0.10	0.15	0.10	0.09	0.07	0.08	0.09	0.05	0.06	0.06	0.16	0.16	0.77	0.07	0.06
C/N ratio	-	29	25	24	30	19	24	22	28	21	26	25	22	18	31	51	25	20	30	18
Ammonium*	mg-N/kg dry weight	17.87	8.48	5.05	9.85	18.48	7.77	6.95	11.59	5.39	6.00	9.03	5.87	5.02	3.96	5.95	0.03	0.10	0.03	0.03
Nitrite	mg-N/kg dry weight	0.57	0.65	1.01	9.47	0.39	1.38	1.45	64.50	0.83	1.25	1.34	0.14	3.98	12.59	1.16	0.29	0.19	0.19	0.29
Nitrate	mg-N/kg dry weight	14.81	8.48	6.44	4.60	14.18	8.67	3.88	55.23	6.53	8.46	6.95	34.52	2.59	10.72	8.76	24.94	22.53	21.15	25.76

***Significant difference ($p<0.05$) between sediments (3 and 5 year-old ponds) and control soils**

sediments were between 3.3×10^2 - 6.7×10^5 (average and SD: $1.2 \times 10^5 \pm 2.3 \times 10^5$) and 9.2×10^2 - 6.1×10^5 (average and SD: $6.6 \times 10^4 \pm 1.6 \times 10^5$) MPN/g dry weight, respectively. Aammonium oxidizers (AOB) in the upper and lower layers of sediments were similar but only between 0-240 MPN/g dry weight. Nitrite oxidizers (NOB) were detected only in the upper layer in small numbers (0-2.9 MPN/g dry weight). Denitrifiers were detected in the range of 0 - 670 MPN/g dry weight in the upper layer, and were a little higher, between 0 - 1271 MPN/g dry weight in the lower layer. Numbers of azotobacteraceae in the upper and lower layers of sediments were between 8.4×10^3 - 1.4×10^6 and 7.2×10^3 - 2.8×10^6 CFU/g dry weight respectively. Bacterial populations in each group of the nitrogen cycle both in the upper and lower layers of sediments from 3-year ponds or 5-year ponds were not significantly different ($p > 0.05$). Amounts of each bacterial group in the N cycle in the control soils were similar to those in the shrimp sediments and there was no significant difference between each of both the two layers and control soils (Table 2).

3. Statistical analyses

The correlations between the physicochemical properties and bacterial populations (Table 3) show that HPC in the upper sediments had a positive middle level correlation ($r = 0.558$, $p < 0.05$) with azotobacteraceae and also with nitrite ($r = 0.654$, $p < 0.01$). Ammonifiers were inversely correlated with pH ($r = -0.631$, $p < 0.05$). AOB were more closely correlated with NOB ($r = 0.673$, $p < 0.01$) than with denitrifiers ($r = 0.602$, $p < 0.05$). In the lower level of sediments (Table 4), HPC had a high correlation with azotobacteraceae ($r = 0.721$, $p < 0.01$) but they were also inversely correlated with denitrifiers ($r = -0.514$, $p < 0.05$).

Azotobacteraceae also had a positive correlation with the C/N ratio ($r = 0.571$, $p < 0.05$). Denitrifiers had a negative correlation with azotobacteraceae ($r = -0.768$, $p < 0.01$), organic matter ($r = - 0.545$, $p < 0.05$) and also TKN ($r = -0.668$, $p < 0.01$).

Table 2. Types and amounts of bacteria involved in the N cycle in sediments of shrimp ponds in use for different numbers of years.

Bacterial group*	Pond code/age of shrimp (days)																		
	3A				3B				5A				5B			Control			
	13	42	69	85	14	43	70	86	4	31	47	61	13	42	69	1	2	3	4
Heterotrophic count	**CFU/g dry weight**				**CFU/g dry weight**				**CFU/g dry weight**				**CFU/g dry weight**			**CFU/g dry weight**			
Upper layer (0 - 5 cm)	3.4×10^{5}	6.2×10^{4}	1.1×10^{6}	1.7×10^{6}	2.9×10^{5}	1.8×10^{6}	2.8×10^{6}	1.6×10^{6}	3.4×10^{5}	1.5×10^{6}	3.6×10^{6}	5.4×10^{5}	8.9×10^{5}	7.7×10^{5}	8.7×10^{4}	1.2×10^{5}	2.2×10^{6}	3.1×10^{3}	3.3×10^{4}
Lower layer (>5 - 10 cm)	8.1×10^{5}	3.8×10^{5}	1.5×10^{5}	2.6×10^{5}	3.9×10^{5}	3.8×10^{6}	8.3×10^{6}	7.4×10^{5}	1.1×10^{5}	4.9×10^{6}	1.5×10^{6}	5.1×10^{5}	2.2×10^{6}	1.3×10^{7}	6.2×10^{5}	7.0×10^{5}	1.8×10^{6}	3.7×10^{3}	4.9×10^{3}
Ammonifiers	**MPN/g dry weight**				**MPN/g dry weight**				**MPN/g dry weight**				**MPN/g dry weight**			**MPN/g dry weight**			
Upper layer (0 - 5 cm)	2.4×10^{4}	1.1×10^{4}	3.3×10^{2}	6.7×10^{5}	2.4×10^{4}	1.3×10^{5}	1.4×10^{4}	8.6×10^{2}	1.3×10^{5}	1.5×10^{4}	8.2×10^{3}	2.3×10^{4}	3.4×10^{2}	6.6×10^{5}	3.3×10^{4}	3.2×10^{4}	1.4×10^{6}	2.5×10^{3}	1.7×10^{4}
Lower layer t (>5 - 10 cm)	1.3×10^{5}	2.8×10^{3}	2.4×10^{4}	2.2×10^{4}	1.9×10^{4}	1.1×10^{4}	2.3×10^{3}	1.3×10^{5}	5.6×10^{3}	9.2×10^{2}	1.1×10^{3}	1.5×10^{3}	3.3×10^{3}	6.1×10^{5}	2.3×10^{4}	3.4×10^{3}	1.4×10^{4}	2.5×10^{3}	2.0×10^{2}
Ammonium oxidizers	**MPN/g dry weight**				**MPN/g dry weight**				**MPN/g dry weight**				**MPN/g dry weight**			**MPN/g dry weight**			
Upper layer (0 - 5 cm)	0	240	0	29	0.28	35	160	140	2.27	0	150	23	0	24	0	20	0	60	0
Lower layer (>5 - 10 cm)	0.2	240	15.1	23.9	23.2	0.5	10.5	33.2	0	0	0	18.1	0	5.6	0	0	0	110	30
Nitrite oxidizer	**MPN/g dry weight**				**MPN/g dry weight**				**MPN/g dry weight**				**MPN/g dry weight**			**MPN/g dry weight**			
Upper layer (0 - 5 cm)	0	2.9	0	0	0	2.9	0	0	0	2.9	0	0	0	2.9	0	0	0	0	0
Lower layer (>5 - 10 cm)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Denitrifiers	**MPN/g dry weight**				**MPN/g dry weight**				**MPN/g dry weight**				**MPN/g dry weight**			**MPN/g dry weight**			
Upper layer (0 - 5 cm)	7.6	170	2.9	670	0	0	2.9	0.28	370	0	640	8.2	5	15	1.6	30	0	0	0
Lower layer (>5 - 10 cm)	3	163.2	0	238.5	0	0	0	0	1,271	0	0	238.5	0	7.5	0	50	0	0	0
Azotobacteraceae	**CFU/g dry weight**				**CFU/g dry weight**				**CFU/g dry weight**				**CFU/g dry weight**			**CFU/g dry weight**			
Upper layer (0 - 5 cm)	7.6×10^{4}	3.4×10^{4}	3.1×10^{5}	1.4×10^{6}	2.7×10^{5}	2.8×10^{5}	9.8×10^{4}	2.8×10^{5}	8.4×10^{3}	5.5×10^{5}	3.6×10^{5}	1.4×10^{5}	9.5×10^{4}	3.3×10^{5}	8.7×10^{4}	3.1×10^{4}	4.2×10^{4}	2.5×10^{2}	9.7×10^{3}
Lower layer (>5 - 10 cm)	3.3×10^{4}	3.4×10^{5}	5.1×10^{4}	4.2×10^{4}	1.8×10^{5}	9.9×10^{5}	2.8×10^{6}	3.8×10^{5}	2.1×10^{4}	2.6×10^{6}	3.6×10^{5}	7.2×10^{3}	1.3×10^{6}	1.7×10^{5}	3.7×10^{5}	5.7×10^{4}	5.9×10^{5}	2.5×10^{3}	2.1×10^{4}

*** No significant difference ($p > 0.05$) in each bacterial group between two layers of sediments in each treatment (3-year ponds, 5-year ponds and control soil)**

In sediments of both 3- and 5-year ponds (Tables 5 and 6) the organic matter was highly correlated with TKN ($r = 0.783$, $p < 0.05$; $r = 0.883$, $p < 0.01$). In addition, the ponds in use for 5 years had a close correlation with the C/N ratio ($r = 0.821$, $p < 0.05$).

Table 3. Correlation between (i) different bacterial groups; and (ii) bacterial groups and physicochemical properties in an upper layer of shrimp sediments.

	Upper layer of shrimp sediments (0 - 5 cm)					
Parameter	**Heterotrophic plate count**		**Ammonifiers**		**Ammonium oxidizers**	
	r	**p**	**r**	**p**	**r**	**p**
Ammonifiers	-0.003	0.992	-	-	-	-
Ammonium oxidizers	0.211	0.450	0.020	0.944	-	-
Nitrite oxidizers	0.169	0.548	-0.368	0.177	0.673**	0.006
Denitrifiers	-0.059	0.834	0.195	0.486	0.602*	0.017
Azotobacteraceae	0.558*	0.031	0.038	0.894	0.078	0.782
pH	-0.286	0.301	-0.631*	0.012	-0.222	0.426
EC	-0.273	0.324	0.009	0.975	-0.255	0.360
Organic matter	0.165	0.556	0.225	0.419	0.226	0.419
TKN	0.009	0.974	0.123	0.662	0.057	0.840
C/N ratio	-0.005	0.985	0.329	0.231	0.033	0.908
Ammonium	-0.071	0.800	0.080	0.776	0.302	0.274
Nitrite	0.654**	0.008	-0.002	0.995	0.291	0.293
Nitrate	-0.329	0.231	0.198	0.478	-0.007	0.979

r = Correlation; r >0.70 (high correlation), r = 0.40-0.69 (medium correlation), r = 0.20-0.39 (low correlation)
p = Probability-value;* Significant difference (p < 0.05), ** Significant difference (p < 0.01)

Table 4. Correlation between (i) different bacterial groups; and (ii) bacterial groups and physicochemical properties in a lower layer of shrimp sediments.

	Lower layer of sediment (> 5 - 10 cm)					
Parameter	**Denitrifiers**		**Azotobacteraceae**		**Nitrite**	
	r	**p**	**r**	**p**	**r**	**p**
Denitrifiers	-	-	-0.768**	0.001	-0.258	0.353
HPC	-0.514*	0.040	0.721**	0.002	0.568*	0.027
Organic matter	-0.545*	0.036	0.509	0.052	-	-
TKN	-0.668**	0.007	0.509	0.052	-	-
C/N ratio	0.836	0.058	0.571*	0.026	-	-

r = Correlation; r >0.70 (high correlation), r = 0.40-0.69 (medium correlation), r = 0.20-0.39 (low correlation)
p = Probability-value;* Significant difference (p < 0.05), ** Significant difference (p < 0.01)

Table 5. Correlation between physicochemical properties in the sediments of 3- year ponds.

Parameter	pH		EC		OM		TKN		C:N ratio		Ammonium		Nitrite	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p
EC	0.286	0.589	-	-	-	-	-	-	-	-	-	-	-	-
OM	-0.214	0.645	0.393	0.383	-	-	-	-	-	-	-	-	-	-
Total N (TKN)	0.072	0.878	0.162	0.728	0.783**	0.022	-	-	-	-	-	-	-	-
C/N ratio	-0.571	0.180	0.500	0.253	-0.084	0.844	-0.661	0.075	-	-	-	-	-	-
Ammonium	0.393	0.383	0.250	0.589	0.643	0.119	0.060	0.887	0.216	0.608	-	-	-	-
Nitrite	-0.321	0.482	-0.143	0.760	0.036	0.939	-0.193	0.647	0.419	0.301	-0.357	0.385	-	-
Nitrate	-0.250	0.589	0.607	0.148	0.214	0.645	0.265	0.526	0.168	0.691	0.690	0.058	-0.231	0.658

r = Correlation; r >0.70 (high correlation), r = 0.40-0.69 (medium correlation), r = 0.20-0.39 (low correlation)
p = Probability-value;* Significant difference (p < 0.05), ** Significant difference (p < 0.01)

Table 6. Correlation between physicochemical properties in the sediments of 5-year ponds.

Parameter	pH		EC		OM		TKN		C:N ratio		Ammonium		Nitrite	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p
EC	0.250	0.589	-	-	-	-	-	-	-	-	-	-	-	-
OM	-0.214	0.645	0.393	0.383	-	-	-	-	-	-	-	-	-	-
Total N (TKN)	0.072	0.878	0.162	0.728	0.883**	0.008	-	-	-	-	-	-	-	-
C/N ratio	-0.571	0.180	0.500	0.253	0.821*	0.023	0.523	0.229	-	-	-	-	-	-
Ammonium	0.393	0.383	0.250	0.589	0.643	0.119	0.667	0.102	0.216	0.608	-	-	-	-
Nitrite	-0.321	0.482	-0.143	0.760	0.036	0.939	0.018	0.969	0.419	0.301	-0.250	0.589	-	-
Nitrate	-0.250	0.589	0.607	0.148	0.214	0.645	- 0.234	0.613	0.168	0.691	0.357	0.191	-0.286	0.302

r = Correlation; r >0.70 (high correlation), r = 0.40-0.69 (medium correlation), r = 0.20-0.39 (low correlation)
p = Probability-value;* Significant difference (p < 0.05), ** Significant difference (p < 0.01)

Discussion

Significantly increasesd of pH in the sediments of shrimp ponds were correlated to the accumulation of ammonium in the sediments (Table 1). Accumulation of ammonium in the ponds used for 3 years was higher than that in ponds used for 5 years and this seemed to be related to the time of shrimp cultivation rather than to the years of cultivation. The higher amounts of ammonium in the ponds used for 3 years (average amount 10.75 mg-N/kg dry weight) than in those used for 5 years (average amount 5.88 mg-N/kg dry weight) was mainly caused by the amounts of ammonium detected at the first sampling time (average amounts: 18.18 mg-N/kg dry and 5.20 mg-N/kg dry weight, respectively). The time for shrimp cultivation was 13-14 days in ponds used for 3 years but 4 and 31 days in the ponds used for 5 years. Different amounts of ammonium at that time may be related to a lower density of shrimp (100/m^2) in the ponds in use for 3 years than those (140/m^2) used for 5 years. Other results have indicated that shrimp cultivation increased soil pH from strongly acidic (3.64-4.43) to be slightly acidic and neutral (5.66-7.57) (Thomas, 1996). However, Das *et al*. (2004) reported that shrimp cultivation in semi-intensive ponds decreased the soil pH and Ritvo *et al*.(1997)

also summarized that soil pH decreased markedly after one growing cycle, but then stabilized. The increase of soil pH in this study also was affected by another factor as no nitrification process occurred in sediments because only small numbers of nitrifiers were detected. It is realized that the nitrification process lowers the pH of the environment due to production of hydrogen ion, nitrite and nitrate (Hargreaves,1998). There could be 3 main reasons for the low amounts of nitrifiers both AOB and NOB in this study. Firstly, the sediment pH (5.66-7.59) was not in the optimal growth range (7.5-8.0) for these bacteria (Bitton, 1994). Secondly, high levels of organic matter (1.86-14.61) in the sediments under a wide range of C/N ratios (18-51) promoted proliferation of HPC, ammonifiers and azotobacteraceae to be the dominant populations (Table 2). Consequently, available oxygen was probably not enough to support the growth of nitrifiers because the nitrifiers require a good oxygen supply for grow (Bitton, 1994; Hargreaves, 1998). The C/N ratio in the range of 10-15 in aquaculture ponds is suitable for supporting the nitrification process (Adhikari, 2003). However, high levels of ammonium and nitrate in the 3-year ponds at the first sampling time (Table 1) indicate that ammonification and nitrification processes actively occurred. That means nitrifiers must be detected in high numbers at that time because high levels of nitrate depends on nitrifiers, but they were detected in small numbers (Table 2). Therefore, the method used to count the nitrifiers may not determine the correct numbers in this case, in which that sediments contained high amounts of organic matter (Table 1), and some method involving DNA probes might be more useful.

No significant differences were found in each bacterial group between the upper (0-5 cm) and lower layers (>5-10 cm) because this is an acid sulfate soil containing large amounts of sand that provides for a good distribution of nutrients. Correlations between nitrifiers and denitrifiers were found although they were detected in low numbers. The correlations between AOB and NOB ($r = 0.673, p < 0.01$) were higher than AOB and denitrifiers ($r = 0.602, p < 0.05$). This is expected as the AOB provide the substrate, nitrite, for NOB and NOB produce nitrate for the denitrifiers. The numbers of ammonifiers in the sediments collected from shrimp ponds was inversely correlated with pH ($r = -0.631, p < 0.05$) even though these bacteria are one source of ammonium (Table 3). The result could be explained if the ammonium produced was rapidly utilized for the growth of other heterotrophs and they produced organic acids and CO_2 lowering the pH. The populations of HPC and azotobacteraceae were much higher than the ammonifers (Table 2). Numbers of HPC in the upper and lower layers with averages of 1.2×10^6-2.5×10^6 CFU/g dry weight (Table 2) were similar with to those reported by Abraham *et al*. (2004) where the average number of HPC in shrimp ponds in West Bengal, India, was 10^6 CFU/g dry weight.

In both layers of sediments the amounts of HPC positively correlated with the amounts of azotobacteraceae and the latter bacteria were also negatively correlated with denitrifiers (Table 3 and 4). The results indicate that the azotobacteraceae were also part of the HPC count. This is also expected because the azotobacteraceae are normal constituents of soil and water and they prefer to use ammonium instead of fixing nitrogen gas when growing in an environment enriched with a nitrogen source (Holt *et al*. 1994). In addition, some of them are ammonifiers because they can utilize peptone and release ammonium (Tchan, 1984). Competition for organic matter and TKN among heterotrophs is well recognized, thereby and a negative correlation between azotobacteraceae and denitrifiers was found in this study. Denitrifiers are facultative anaerobice bacteria that use nitrate as a final electron acceptor when oxygen is depleted and most of them are heterotrophic (Focht and Verstraete, 1977; Jana and Patel, 1985). Therefore competition for oxygen and nutrients between HPC such as azotobacteraceae and denitrifiers would occur in the sediments of the shrimp ponds. However, only small numbers of denitrifiers were detected in this study. A possible reason to explain the result was the positive correlation between azotobacteraceae and C/N ratio (Table 4). It could mean that the sediment with high C/N ratio liberates less available nitrogen so azotobacteraceae have to fix nitrogen gas, but denitrifiers cannot fix nitrogen gas. Consequently, azotobacteraceae out-competed denitrifiers in this study. Analyses of the physico-chemical properties of sediments from shrimp ponds with different age years of use show a high correlation between organic matter and TKN. This means that organic matter was the main source of TKN in the sediments. Boyd *et al*. (1994) and Hopkins *et al*. (1994) have reported that as accumulated sediments in shrimp ponds are highly enriched in nitrogen and phosphorus. In addition, the soil quality in the shrimp ponds was similar to that from Tilapia ponds in Thailand with acid sulfate soils that have had been used continuously for semi-intensive production of Tilapia, and presumably other species, for many years (3-39 years) without serious deterioration of bottom soil quality (Thunjai *et al*. 2004).

According to the data there was very little difference in the measured parameters among the 4 shrimp pond systems. However, it was claimed that pond 5B was terminated early because the shrimp were unhealthy. The parameters of this pond were significantly different in 3 ways. The organic matter at 69 days was more than 3 times higher than any other pond at about this time. The C/Nratio was double that of any other indicating that material with more C was being deposited than in any of the other ponds. The HPC in the top layer was significantly less than in any other pond. Perhaps the deposited material was less degradable and it may be just shrimp residue from dead shrimps, perhaps the result of some infection, maybe virus or *Vibrio* sp. Another possible reason in that some residues of antibiotics used in the past such as oxytetracycline and norfloxacin accumulated in the pond 5B and affected some HPC. Therefore, there is perhaps more chance that infections accumulate in 5-year ponds indicating that cleaning out the debris and avoiding the use of antibiotics may be important steps for sustainable and friendly shrimp farming.

The pH of the shrimp pond sediment soil increased due to the liberation of ammonium whose conversion to nitrate was limited by its utilization for the growth of heterotrophs and limited access to oxygen and low pH resulting in low numbers of nitrifying bacteria. The increase in pH over time and accumulation of nutrients as organic matter in the sediments may have a positive impact on shrimp culture. Hence it is not clear what physiochemical factors, if any, may contribute to ponds, that have been under intensive cultivation for 5 years, being less conducive to producing healthy shrimps than ponds in use for 3 years.

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