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Multi-functional pollution mitigation in a rehabilitated mangrove conservation area

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ABSTRACT

Many mangroves were forced to act as informal pollution mitigation zones and double up as conservation areas. Long-term data are presented for a high-profile mangrove reserve acting as such a mitigation zone in urban Thailand. Efficient mineralization of organic wastes by mangrove soil in a semi-engineered and hydraulically contained zone made it possible not to compromise the reserve's natural status. The data demonstrate that the treatment zone could process organic waste with an eight-fold efficiency in comparison to previous reports. Clones of microbial taxa critically novel for mangrove ecosystems were recovered (anammox bacteria and archaeal ammonia oxidizers), suggesting their significant presence. Community structures of nitrogen-cycling and other taxa of natural and hypernutrified soils did not differ substantially. It is suggested that waste nitrogen removal may have occurred through bacterial and archaeal nitrification, conventional denitrification and anammox process. The article addresses the issue of multi-functional use of ever-shrinking habitats available for wildlife conservation. Data on key microbial, floral and faunal communities demonstrate that the mangrove exhibited stability under the major nutrient load. Supply of additional nutrients correlated with an enhancement of mangrove growth and diversity of selected key invertebrates/vertebrates which increase conservation potential of the reserve. Serving to determine ecologically safe nutrification limits, the study suggests that a successful rehabilitation of an urban mangrove to its near-natural status is feasible.

1. Introduction

In the absence of modern treatment facilities in most parts of the tropics, coastal pollution requires a pragmatic approach that acknowledges the multi-functional value of mangroves. Properly managed wastes discharged into mangroves may not necessarily be detrimental (Hongsing and Aksornkoae, 2004). If pollution is locally contained and does not significantly disrupt natural processes of primary production and mineralization, it can even be of value in terms of nutrient addition to microbial, floral and faunal communities. To increase effectiveness of conservation, particularly in developing countries, there is a need to demonstrate the multi-functionality of mangroves combining pollution mitigation with nature education and conservation. Promising results have been achieved when mangroves were used as vegetative buffers for short-term pollution mitigation (Wong et al., 1997; Boonsong et al., 2003). However, it is still far from clear how mineralization occurs and what the long-term consequences of hypernutrification are. Therefore the first objective of the investigation was to elucidate if nutrients (C, N, P) accumulated in soil of the reserve due to a long-term discharge into the treatment zone.

Mangroves are among the most productive ecosystems with a high turnover of organic matter mediated by microbial processes (Alongi, 1994). But to be harnessed socio-economically, microbial activity should be understood. Despite recent interest (Beman and Francis, 2006; Yan et al., 2006; Liang et al., 2007) and prior research (reviewed by Holguin et al., 2001), our knowledge is still insufficient as to the involvement of various taxa in specific processes. Elucidation of microbial, particularly, N-cycling processes (bacterial and archaeal nitrification, denitrification and anaerobic ammonia oxidation), was the second objective.

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Less than 10% of original mangroves remain in the Bight of Bangkok, the situation typical of all urbanized coasts. Over the last decade mangrove rehabilitation has assumed a considerable importance, particularly due to its value for stabilization of erosion (Hongsing and Aksornkoae, 2004; Lee and Shih, 2004). However, few detailed scientific data are available on the success rate of rehabilitation projects, specifically in terms of attaining a natural status (Stevenson et al., 1999; Lewis, 2005). Since the present study was undertaken in such a rehabilitated mangrove, the third objective was to estimate its status attained over 20 years in comparison with the only two natural mangroves in the region.

The rehabilitation has occurred under a constant hypernutrification due to anthropogenic wastes (from pig farm, human settlement) and bird guano from ever increasing colonies. Hence the fourth objective was to evaluate a long-term impact of additional nutrients on key microbial, floral and faunal groups with a view to establishing ecologically safe limits for mangrove nutrification concomitant to pollution mitigation.

2. Materials and methods

2.1. Site description

The study area of the Bang Pu Nature Reserve, BNPR ($S = 1.1\ km^2$), N13°30′59.42″, E100°39′23.61″, is located within the boundaries of Greater Bangkok in the Samut Prakarn province, Thailand (Fig. 1). Under the aegis of the Royal Thai Army and WWF-Thailand as one of the last mangrove ecosystems in the Bight of Bangkok, it is situated within the Indochina Biodiversity Hotspot. It occupies the area of former shrimp ponds originally constructed in lieu of natural mangroves, but decommissioned in 1985 due to poor performance. Since then, residual mangroves have re-grown following the contours of nine ponds separated by earth dykes. These were breached by open water gates to allow for a free access of tidal water, hence the BPNR area experienced a near-natural tidal regime.

The area is covered by *Avicennia marina* and *Avicennia alba*, rarely interspersed with *Rhizophora apiculata* and, in the natural zone only, *Nypa fruticans*. It was officially declared a reserve in 2003 to be managed by the WWF Nature Education Centre welcoming thousands of visitors. The area supports a large number of birds (storks, herons, waders, gulls, etc.), including endangered, among at least 187 species (WWF-Thailand, 2007). Rainy season occurred from May to October 2006–2007; dry season: from November 2006 to April 2007. Average T_{water} was 24 °C (December) and 30 °C (April); T_{air}: 22 °C (December) and 32 °C (April). Mean annual rainfall in 2006–2007 was 1201 mm with 103 rainy days. In terms of additional nutrients received by the BPNR ecosystem, the overall site could be divided into four zones (Fig. 1): (1) hypernutrified treatment zone, fed with agricultural wastes (pig manure and urine); (2) nutrified enhancement zone, receiving attenuated nutrient-containing water from the treatment zone, as well as that from the inhabited zone; (3) inhabited zone, receiving domestic wastewater; (4) natural zone (control natural site No. 1) receiving guano nutrients from birds nesting and roosting in BPNR but feeding mainly outside.

Control sites. These areas served as reference sites for the comparison of ecological status of the BPNR natural zone which they closely resembled in terms of climate, hydrology, soil, vegetation, biodiversity, etc. Control natural site No. 2 ($S = 1.48\ km^2$), Royal Laem Phak Bia project (N13°03′06.22″, E100°05′35.52″) was situated in the Bight of Bangkok, 100 km south-west of BPNR. Part of the forest was subjected to an experimental feeding with a settled municipal sewage for 14 months in 1999–2000 at the following rate: TOC 0.67, TN 0.32, TP 0.1 $g\,d^{-1}\,m^{-2}$ (Boonsong et al., 2003). Another part ($S = 0.95\ km^2$) was permanently fed with a pre-treated effluent as follows: TOC 0.107, TN 0.007, TP 0.003 $g\,d^{-1}\,m^{-2}$, whose impact on mangrove ecology was evaluated and compared with the natural part. Control natural site No. 3 ($S = 0.57\ km^2$), the Samut Sakhon mangrove forest (N13°30′08.16″, E100°16′21.27″) was situated on the same coast, 50 km west of BPNR (Tribanyatkul, 2002; Hongsing and Aksornkoae, 2004).

2.2. Biogeochemical and microbiological analyses

Sampling transect selection was governed by a perceived gradient of the impact of released additional nutrients on soil and its biota. Sampling (in triplicates) for water analyses along transects 1–4 was undertaken twice per month from February 2006 to September 2007. Sampling points along transects 1 and 2 were located at 20 m intervals, while those for transects 3 and 4 at intervals of 250 and 300 m, respectively. Samples for FISH and PCR were taken and pooled along transect 1 and 4 only (Fig. 1C and D). Characteristics of piggery waste, wastewater and mangrove interstitial water were analyzed according to the Standard Methods (APHA, 1998). Total organic carbon (TOC) was measured by Shimadzu 5000 TOC Analyzer. Portions of waste C, N, P removed through absorption by mangrove soil and plant tissues were determined by procedures outlined in Carter (1993) and Kalra (1998), respectively. Soil samples were collected in each sampling point, as water samples, at low tide at 1–10 cm depth using a soil corer; the same holes were used to collect interstitial water. Soil samples were taken to laboratory in airtight bags. Portions of waste C, N, P removed (retained) in the treatment zone were calculated as difference between loads applied and tidally exported to the enhancement zone through the inlet-outlet water gates 1–2. The soil-removed portions were calculated as difference between the overall removed (retained) and measured plant-absorbed portions. Flow through water gates was estimated twice monthly by a Price current meter over 24 h and during periods equally comprising different tidal phases. Load rates were measured weekly as follows. Treatment and natural zones: amount of pig (bird) wastes discharged per day and tidally spread over the zone area. Enhancement zone: fluctuating amounts of TOC, N, P exported from the treatment and inhabited zones via water gates were measured hourly to estimate composite concentrations as difference between outflow and inflow amounts.

FISH Fluorescence *in situ* hybridization (FISH) data was generated according to protocols by Amann et al. (1995). 14 Oligonucleotide probes were used (Loy et al., 2003): EUB338 (most bacteria); ALF1b (α-*Proteobacteria*); NIT3 (*Nitrobacter* spp., α-proteobacterial nitrifiers NOB); α-proteobacterial denitrifiers; GRb (*Rhodobacter* and *Roseobacter*) and PsMg (*Pseudomonas* spp.); Nitspa662 (*Nitrospira* spp., NOB); BET42a (β-*Proteobacteria*); Nso1225 (ammonia-oxidizing β-*Proteobacteria*, AOB); GAM42a (γ-*Proteobacteria*); PAR651 (γ-proteobacterial denitrifiers, *Paracoccus* spp.); CF319a (*Cytophaga-Flavobacterium*); HGC69a (*Actinobacteria*); LGC354a (*Firmicutes*); Pla46 (*Planctomycetales*, including anammox bacteria). Counts for specific probes were expressed as percentages of the EUB338 counts.

PCR DNA was extracted from soil according to Rotthauwe et al. (1997). Polymerase chain reaction (PCR) amplification was performed in a DNA thermocycler model 2400, PerkinElmer Cetus, CA from 30 to 80 ng μl^{-1} environmental DNA. *Ammonia-oxidizers* (β-*proteobacteria*): (Rotthauwe et al., 1997) primer set (*amoA*-1F and *amoA*-2R) generated 450–490 bp PCR product. *Ammonia-oxidizers* (γ-*proteobacteria*): (Ward et al., 2000) primer set (NOC1 25-45-F and NOC2 1168-1188-R). *Archaeal ammonia oxidizers* (Francis et al., 2005): *amoA* gene fragments (635 bp) were amplified with primer set (Arch-*amoA* F and Arch-*amoA* R). *Anammox bacteria* (Penton et al., 2006): primer set (Brod541F and Brod1260R)

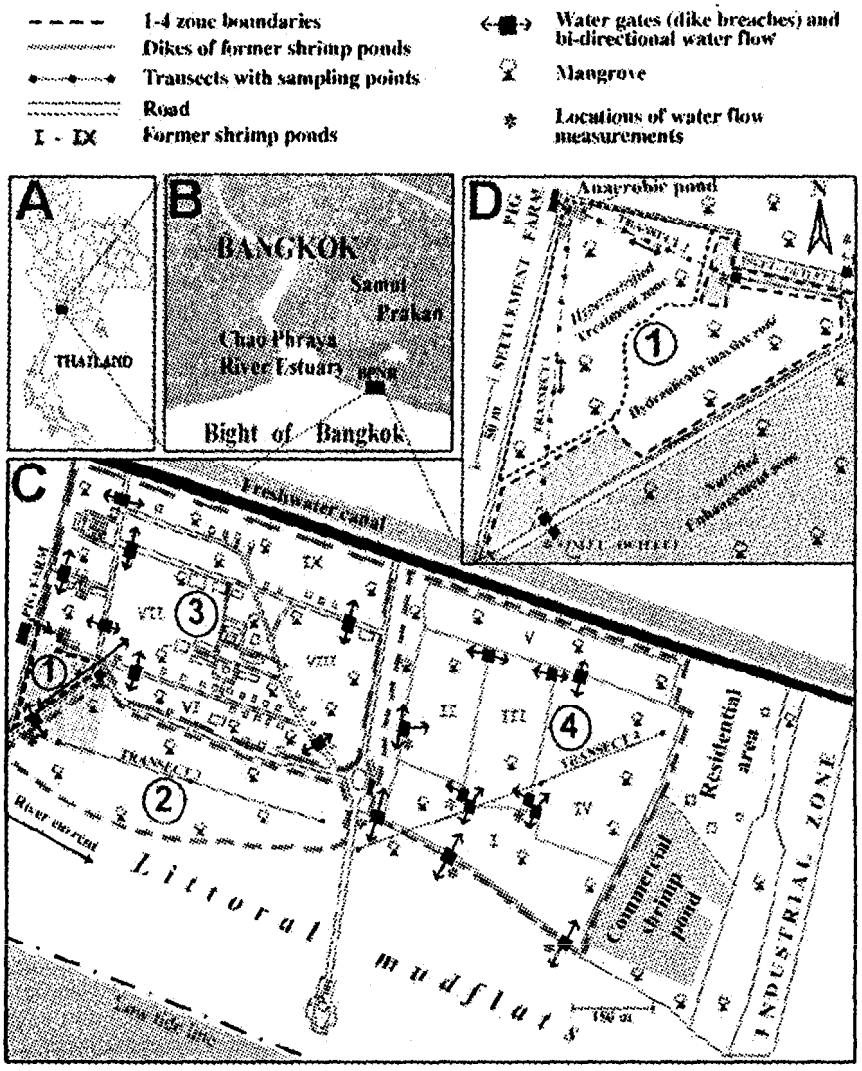

Fig. 1. Location of the study area, Bang Pu Nature Reserve,BPNR, Thailand (C), divided into zones. (1) Treatment zone, (2) enhancement zone, (3) inhabited zone, and (4) natural zone. (D) Detailed map of the treatment zone.

specific for "*Candidatus* Scalindua spp.". Triplicate PCR products were pooled and DGGE-purified. The products were cloned by using the TA Cloning kit (*Invitrogen Corporation, Carlsbad, CA*) and sequenced using the ABI Prism® 3100 system (Applied Biosystems). 15 Sequences were registered in GenBank under accession numbers EF68455–EF684595, AOB; EF684906–EF684910, Anammox; EF687846–EF687850, AOA.

2.3. Primary productivity (microorganisms and trees)

Epipellic *Cyanobacteria* and microalgae were scraped off from a surface microbial mat at ten random spots, 1 ml of mat each, diluted in 100 ml dH_2O, (i) enumerated, specific cell counts expressed as percentages of total counts, and (ii) filtered through 0.45 μm GF filter to determine chlorophyll *a* (APHA, 1998). Data acquisition for vegetation was done by using the Point Centered Quarter Method (Mueller-Dombois and Ellenberg, 1974). 100 m^2 Square vegetation plots were marked out along transects where total number of tree species and individuals per species were determined. To evaluate the health of mangrove trees, a portable LAI-2000 Plant Canopy Analyzer (LI-COR, Inc., USA) was used to measure *in situ* Leaf Area Index (LAI).

2.4. Invertebrates and vertebrates

Transects, quadrats and visual count methods devised by Skov et al. (2002) were used to obtain representative samples of crustaceans (*Brachyura*). Transects were divided into 2 m × 2 m randomly selected plots. Observations took place in the morning (7:00–10:30) and were made through binoculars. Diversity Index, evenness, Simpson's dominance and abundance were calculated according to Magurran (2003). Abundance and diversity of amphibious *Oxudercinae* (mudskippers) were determined by the line transects and quadrats (4 m × 2 m) Clayton and Snowden (2000). Bird census for guano nutrient load estimates was undertaken monthly by authors while partial information was obtained from WWF-Thailand (2007).

2.5. Statistical analysis

One-way ANOVA tests were done to compare the difference in density, biomass, plant nutrient content and other vegetation characteristics of mangrove forests in three zones. Two-way ANOVA tests were conducted for wastewater nutrient content to determine the effect of sampling location and season. General linear model

was used to determine the difference in faunal composition among zones and sampling seasons. Sample location season and species number were considered as fixed factors in this analysis. Pair-wise comparisons for the species were done by using Bioferroni method at different confidence levels. Regressions were conducted, using faunal density versus nutrient concentrations in the wastewater, to determine whether wastewater nutrient levels influenced density of fauna.

3. Results and discussion

3.1. Hydraulic regime

Tidal amplitude varied from 1.4 m (lowest high, or neap tides) to 3.3 m (highest high, or spring tides) with one high and low tides per day, often asymmetrical. On average, the enhancement, inhabited and natural zones were exposed to neap and spring tides for 11 and 12 h, respectively. Average water volumes found to be received by these zones during the neap (*spring*) tides were measured at water gates (asterisks, Fig. 1C and D) as 74,435.2 (*141,426.5*), 22,672.9 (*36,276.6*) and 197,345.0 (*315,752.0*) m^3, respectively. High tide water covered soil for 11 (*12*) h, respectively. Treatment zone, due to its higher elevation, experienced a different regime: average 800 m^3 entered at spring tides only (15 days per month) to cover soil for 8 h per day). Average low tide duration was as follows: treatment zone (16 h), other zones (12 h). Salinity: seawater (November) 24,500–(June) 37,200 mg l^{-1}; residual mangrove water: (November) 8800–(June) 28,600 mg l^{-1}. Natural zone matched other zones in hydrology and vegetation.

3.2. Treatment of added nutrients

3.2.1. Hypernutrified treatment zone (No. 1)

This semi-engineered zone (active zone $S = 0.02\,km^2$), with boundaries defined by earth dykes, was directly adjacent to a pig farm containing up to 100 pigs (Fig. 1C). For over ten years, full wastes (manure and urine) were discharged into the zone at the C, N and P loading rates of 67.2 ± 0.1, 16.4 ± 0.6, $6.9 \pm 0.6\,kg\,d^{-1}$, respectively. At high tides, sea water entered and left the zone through water gates at outlets 1 and 2 at volume rates of 80 and 20%, respectively. The un-sequestered nutrients were thereby exported to the enhancement zone.

Manure characteristics (mg l^{-1}) were: TOC $24{,}074.1 \pm 690.0$; TKN 2567.5 ± 461.3; NH_4^+-N 662.4 ± 109.8; PO_4^{3-}-P 1093.0 ± 192.3; pH 7.3 ± 0.1; salinity 6.5 ± 1.3. Primary treatment occurred in an anaerobic pond (5 m × 4 m × 2 m deep) next to the farm, and then, the nutrient-rich water percolated through the soil and surface along transects 1 and 2 towards the outlets. Application of pig manure in the treatment zone proved to be a major load (C, N, P) exceeding by a factor of 5 the highest loads reportedly applied on mangroves (Boonsong et al., 2003; Wong et al., 1997). The previous reports dealt with a settled municipal wastewater, not the pig manure presenting a much greater challenge due to the higher content of N and P as compared to the C content. Load attenuation along transects 1 and 2 (TOC, N species and PO_4^{3-}-P) occurred within 50 m from the farm, while primary treatment in the anaerobic pond played a crucial role. Pond-treated wastewater at 1 m from the pond was as follows (mg l^{-1}): TOC 3676.7 ± 180.4 (303.7 mM); TKN 938.7 ± 156.3 (67.2 mM); NH_4^+-N 201.3 ± 51.2 (14.3 mM); NO_3^--N 2.0 ± 0.5 (0.13 mM); PO_4^{3-}-P 317.3 ± 101 (10 mM). No considerable accumulation of N- and P-containing matter was observed after over a decade-long operation in comparison to pristine mangroves (Table 1) suggesting an effective and tight coupling between mineralization and assimilation processes (Alongi, 1994; Rivera-Monroy et al., 1998). TN content in soil was 1.57–8.49 mg g^{-1} (natural area: 1.11–2.25), while TP content was 1.2–3.47 mg g^{-1} (natural area: 0.09–0.61), which only slightly exceeded values reported for pristine mangroves elsewhere (Table 1). *Avicennia* mangroves, in particular, tend to have higher natural soil TN of up to 3.7 mg g^{-1} (Khan et al., 2007). Soil pH was 7.2 ± 0.2. Interstitial water, however, contained highly elevated nutrient concentrations which were attenuated to near natural levels towards the enhancement zone. Up to 88.7% of TOC, 84.1% of TN and 72.4% of TP loads were removed, apparently by soil microbial consortia with the efficiency slightly higher during the rainy season. Only minor fraction was incorporated into vegetative biomass (Table 2). Residual nutrients were tidally exported to the enhancement zone, acting as an interface zone between the pig farm and natural mangrove with littoral mudflats. Both treatment and enhancement zones were characterized by soil silt content of 30 and 35%, respectively, i.e. lower than in the natural zone (40%) and control natural site No. 2 (33%), further indicating no organic matter accumulation. Specific treatment efficiency exceeded that of the highest previously achieved (Boonsong et al., 2003) by up to a factor of 8. This demonstrated a remarkable ability of an *Avicennia* mangrove to mineralize and assimilate nutrients.

3.2.2. Nutrified enhancement zone (No. 2)

The boundaries (Fig. 1D) were defined by the enhancement effect which the nutrients exported from the treatment and inhabited zones exerted on floral and faunal communities as discussed below. The inhabited area contributed only a small fraction of load (<3%). The cumulative nutrient load on the zone was an order of magnitude lower than that of the treatment zone (Table 2).

3.2.3. Inhabited zone (No. 3)

It comprised houses and ponds only partially overgrown with mangroves. Of $S = 0.39\,km^2$ and permanently inhabited by 300 people, it generated domestic septic tank overflow and grey water at rates of TOC $0.02\,g\,d^{-1}\,m^{-2}$, TN $0.014\,g\,d^{-1}\,m^{-2}$, TP $0.001\,g\,d^{-1}\,m^{-2}$. The nutrients were tidally exported into the enhancement zone.

3.2.4. Natural zone (No. 4)

The least disturbed zone with $S = 0.42\,km^2$, it was hydraulically isolated from the inhabited zone and comprised five former ponds overgrown with rehabilitated forest. Characteristics were similar to those of the enhancement zone (Fig. 1D). Soil pH was 7.1 ± 0.1. An additional nutrient flow was incidentally established in the zone: guano introduced by birds collecting feed in the adjacent areas and releasing guano nutrients to the reserve area through the excretion of droppings (rich in N and P). Colonial birds are known to alter local nutrient flow of ecosystems (Smith and Johnson, 1995). Nutrient load was significant and increased year by year since the establishment of the reserve, which attracted birds by its relative security in comparison to surrounding residential and industrial areas. Average number of birds recorded in the rainy season was 13,350 (total weight 9200 kg) and 3500 (4200 kg) in the dry season. Calculation of nutrient load was based on assumptions used for colonial seabirds (Smith and Johnson, 1995).

Roosting, nesting or passing on migration, birds released guano on soil mainly in the area of ponds II–IV ($S = 0.23\,km^2$) at rates of $0.14\,g\,TOC\,d^{-1}\,m^{-2}$, $0.022\,g\,TN\,d^{-1}\,m^{-2}$, $0.004\,g\,TP\,d^{-1}\,m^{-2}$, i.e. considerably lower than the nutrient load in the enhancement zone (Table 2). Effective treatment by soil microbial consortia ensured that no C, N, P was accumulated in soil (Table 1). Residual nutrients were tidally exported to the downstream pond I area at rates of $0.029\,g\,TOC\,d^{-1}\,m^{-2}$; $0.004\,g\,TN\,d^{-1}\,m^{-2}$; $0.001\,g\,TP\,d^{-1}\,m^{-2}$. Ultimately, the mudflats received an insignificant load of $3.7\,mg\,TOC\,d^{-1}\,m^{-2}$; $0.71\,mg\,TN\,d^{-1}\,m^{-2}$; $0.26\,mg\,TP\,d^{-1}\,m^{-2}$.

Table 1
N- and P-species in mangrove interstitial water and soil (mg g^{-1} soil dry weight) in the BPNR zones as compared to those of pristine mangroves which range depending on a dominant mangrove species. Values are means along transects.

Parameter (μM)	Treatment zone	Enhancement zone	Natural zone	Pristine mangroves[a,b,c]
NH_4^+-N	381.0 ± 23.3	32.5 ± 2.4	13.5 ± 1.4	0.1–11.2
NO_3^--N	11.5 ± 2.8	1.0 ± 0.04	0.03 ± 0.001	0–1.75
TN	1202.8 ± 697.1	102.8 ± 52.4	90.5 ± 6.5	46.0–94.0
PO_4^{3-}-P	174.0 ± 90.3	24.9 ± 7.6	4.4 ± 0.4	0–5.3
TP	230.8 ± 119.7	32.9 ± 18.0	5.8 ± 0.1	0.7–10.5
TN_{soil} (mg g^{-1})	1.57–8.49	1.22–3.21	1.11–2.25	0.8–3.7
TP_{soil} (mg g^{-1})	1.2–3.47	0.73–1.68	0.09–0.61	0.1–0.6

[a] Robertson and Phillips (1995).
[b] Khan et al. (2007).
[c] Wong et al. (1997).

3.3. Impact of added nutrients on microbial diversity

FISH data Substantial presence of denitrifying and nitrifying bacteria was detected in the treatment zone soil (full data not shown). Denitrifiers *Pseudomonas* spp. (PsMg), *Rhodobacter/Roseobacter* spp. (GRb) and nitrifiers *Nitrobacter* spp. constituted the majority of α-*Proteobacteria*, namely, 14.5 ± 0.96 out of 20.2 ± 2.9%. Another prominent denitrifier, *Paracoccus* spp. (PAR651), was present in numbers of up to 4.0 ± 1.4%. Probed nitrifiers were represented by *Nitrosomonas* spp. (Nso1225, AOB), *Nitrobacter* spp. (NIT3, NOB) and *Nitrospira* spp. (Nitspa662, NOB), ranging from 1.2 ± 0.6 to 4.1 ± 0.6% of total bacterial counts, detected by the EUB338 probe. The microbial structure of the natural zone (data not shown) was very similar to that of the experimental zone though it featured lower numbers of the denitrifying and nitrifying taxa (up 0.9 ± 0.01 to 0.5 ± 0.01% of total bacterial counts, respectively). The data conform to the previous reports (Wong et al., 1997; Liang et al., 2007). Cumulative numbers of α-, β-, γ-*Proteobacteria* (Wong et al., 1997) were 42.6 ± 4.2% (11.5 ± 1.92, 2.0 ± 0.1, 29.1 ± 2.1%, respectively) in comparison to data for the natural zone in the present study: up to 37.9 ± 2.0% (12.0 ± 0.9, 15.5 ± 1.8, 10.4 ± 0.3%, respectively) and the treatment zone: up to 55.1 ± 5.3% (19.6 ± 1.9, 24.3 ± 3.0, 11.2 ± 1.3%, respectively). Number of *Planctomycetales* (probe Pla 46, including anammox bacteria) was relatively high (6.3 ± 1.1 to 8.1 ± 1.0%) for both zones. Microbial community structure (14 probes) at the treatment zone did not differ substantially from that of the natural zone (full data not shown).

PCR data Environmental sequence amplification carried out by Beman and Francis (2006) for the hypernutrified estuarine sediments was followed in search for archaeal and bacterial ammonia-oxidizers (AOA and AOB). Total of 34 AOA clones was recovered yielding 5 type sequences (Table 3). BLAST analysis showed 93–98% range of similarities. AOA sequences retrieved from both zones could not be grouped with any previously cultured archaea but clustered as 2 distinctly independent groups: Two type sequences could be associated with the cluster A within the *Crenarchaeota* group 1.1a (ubiquitous in most soils and other environments), while remaining 3 sequences—with the cluster B within the group 1.1b (retrieved from marine water and sediments). Though the type sequences from the treatment zone were the same as from natural zone, the number of clones retrieved was lower in the latter (34 vs 30), indicating a nutrient-related enhancement during the process of mineralization of the N-containing compounds derived from the wastes. This is the first report of the AOA recovery from a mangrove soil. Due to a novel nature of the *Crenarchaeota* clones and poorly developed taxonomy it is difficult to make more specific conclusions. High number and novel diversity of 16S rDNA clones of *Crenarchaeota* were recently recovered from mangrove soils using general archaea-specific, not amoA-specific primers, making it impossible to conclude if the retrieved sequences could be attributed to AOA. Numerical dominance of non-methanogenic archaea (*Crenarchaeota*) over methonogens was also noted for a *Kandelia* mangrove (Yan et al., 2006). The *Crenarchaeota* representatives were suggested to be possibly the most abundant ammonia-oxidizers in soil ecosystems on Earth (Leininger et al., 2006).

A total of 14 AOB clones was recovered, yielding 5 type sequences (Table 3). BLAST analysis (NCBI) of the sequences showed 93–97% range of similarities with sequences deposited in GenBank. AOB-specific primer set allowed retrieving a number of *Nitrosomonas*- and *Nitrosospira*-like amoA sequences from both zones. Four type sequences were closely homologous to *Nitrosomonas marina* and *Nitrosomonas aestuari* (97% similarity), while another sequence was distantly related to *Nitrosospira* sp. (93%). The findings corroborate FISH-related data showing substantial presence of AOB, particularly, in the hypernutrified treatment zone. Only three sequences were retrieved from the natural zone (as compared to five from the treatment zone), its lower clone number (5 vs 14) reflected a higher nutrient-related trophic level of the treatment zone. Notably, common marine γ-proteobacterial ammonia-oxidizers *Nitrosococcus* spp. could not be retrieved using a specific primer set (Ward et al., 2000) which indicated their absence.

AOA, AOB and NOB oxidize ammonia to nitrite and nitrate used by denitrifying and anammox bacteria as electron acceptors. Through the use of a primer set (Penton et al., 2006) amplifying 16SrRNA "*Candidatus* Scalindua spp." sequences, fragments close to

Table 2
Balance of waste C, N and P in the treatment and enhancement zones during dry season 2006–rainy season 2007 (rainy season in brackets).

Nutrient	C (g TOC d^{-1} m^{-2})	N (g TN d^{-1} m^{-2})	P (g TP d^{-1} m^{-2})
Load applied on treatment zone	3.36 ± 1.0 (3.2 ± 1.0)	0.82 ± 0.6 (0.8 ± 0.6)	0.21 ± 0.6 (0.19 ± 0.6)
Load removed, of which	2.98 ± 0.5 (2.71 ± 0.1)	0.69 ± 0.1 (0.62 ± 0.1)	0.15 ± 0.09 (0.16 ± 0.04)
Plant-absorbed	*0*	*0.1 ± 0.01 (0.1 ± 0.01)*	*0.08 ± 0.01 (0.085 ± 0.01)*
Soil-removed	*2.98 ± 0.4 (2.71 ± 0.3)*	*0.59 ± 0.01 (0.52 ± 0.1)*	*0.07 ± 0.01 (0.08 ± 0.01)*
Load removed (%)	88.7 (80.5)	84.1 (75.0)	71.0 (72.4)
Load applied on enhancement zone[a]	0.39 ± 0.01 (0.34 ± 0)	0.075 ± 0.01 (0.105 ± 0.01)	0.03 ± 0.01 (0.05 ± 0.02)

[a] Combined treatment zone effluent load and a minor anthropogenic effluent load from the inhabited zone.

Table 3
AmoA and 16SrDNA sequence similarities of N-cycling microbial clones obtained from mangrove soil in the experimental and-natural sites, compared to those retrieved from GenBank.

GenBank accession numbers	Number of clones[a]	Phylotype with highest sequence similarity accession numbers, source	Sequence similarity (%)
Ammonia-oxidizing bacteria, AOB (β-*Proteobacteria*)			
EF680455	2 (1)	Uncult. AOB clone 4-MX-Jan-1, DQ501177 Hyper-nutrified estuarine sediment	96
EF680456	4 (2)	*Nitrosomonas marina* clone pNM.1 AJ388586, Dutch freshwater sediments	97
EF680457	1 (0)	*N. aestuarii* clone Nm36 AJ298707, freshwater sediments	97
EF680458	1 (0)	Uncult. AOB clone P14-15 (*Nitrosomonas* sp.) AY702593, estuarine sediments	95
EF680459	6 (2)	*Nitrosospira* sp. Nsp62 AY123837, freshwater sediments	93
Anammox bacteria (*Planctomycetales*)			
EF684906	3 (2)	"*Candidatus* Scalindua brodae" clone BS 5 AY257181, Black sea sediment	98
EF684907	1 (1)	"*Ca.* S. brodae" clone BS 5 AY257181, same	93
EF684908	1 (1)	"*Ca.* S. brodae" clone BS 5 AY257181, same	87
EF684909	3 (2)	"*Ca.* S. wagneri" clone EN 5 AY254882, wastewater	92
EF684910	3 (1)	"*Ca.* S. wagneri" clone EN 5 AY254882, same	90
Ammonia-oxidizing archaea, AOA (*Crenarchaeota*)			
EF687846	12 (8)	Uncult. crenarchaeote clone MX 3 Jan 1 DQ500959, Hypernutrified estuarine sediment	97
EF687847	5 (6)	Uncult. crenarchaeote clone MX 2 Jan 19 DQ501132, same	97
EF687848	11 (10)	Uncult. crenarchaeote clone MX 5 Jan 11 DQ501101, same	93
EF687849	4 (3)	Uncult. crenarchaeote clone MX 3 Oct 10 DQ501044, same	98
EF687850	2 (3)	Uncult. crenarchaeote clone MX 2 Oct 25 DQ501170, same	98

[a] Natural area data in brackets.

"*Ca.* Scalindua brodae" and "*Ca.* Scalindua wagneri" were recovered type-wise from both zones equally. A total of 11 clones were recovered yielding 5 type sequences. BLAST analysis showed 87–98% range of similarities. The number of clones from the treatment zone exceeded that from the natural zone further indicating the nutrient-related enhancement (11 vs 7). The anammox process was recently reported from a mangrove ecosystem (Meyer et al., 2005) and the present work is the first recovery of anammox bacteria from the ecosystem. FISH technique detecting a broad taxon *Planctomycetales* (up to 8.1%), known to comprise anammox bacteria, indirectly corroborated the recovery.

The data do not indicate disruptions in community structures due to the waste-enhanced trophic level indicating adaptability of the ecosystem. Based on the presence of specific microbial groups, potential mechanisms for the N removal are suggested to be nitrification (both archaeal and bacterial), denitrification and anammox process. Novel microorganisms recovered ("*Ca.* Scalindua" and several novel crenarchaeotes) from natural and hypernutrified soils expand ecological boundaries of the environmentally significant groups: anammox bacteria and archaeal ammonia oxidizers. The anammox process has also been proposed to be responsible for up to a quarter of the N lost in estuarine sediments (Meyer et al., 2005), while AOA appear to be more important in soil nitrification than AOB (Leininger et al., 2006).

3.4. Impact of added nutrients on primary productivity

3.4.1. Epipellic cyanobacteria and microalgae

The data obtained for the photosynthesizing communities of the soil surface conform to less elaborate findings on a similar mangrove of the Bight of Bangkok coast (control natural site No. 2, Tribanyatkul, 2002). Seasonal fluctuation (cell number, chlorophyll *a*) of 62 species closely correlated with the amount of supplied nutrients (Table 4). The supply quantitatively impinged on phytoplankton of the treatment and enhancement zones; however, only very few taxa disappeared or appeared in comparison to the natural zone. Diversity Index did not change, or even increased slightly due to a boost to cyanobacteria, particularly in the rainy season. Observed cyanobacteria (*Oscillatoria*, *Anabaena*, *Spirulina*, *Phytoconis*, *Merismopedia* and *Sphaerocystis*) were neither harmful, nor potentially problematic species. Chlorophyll-wise these increased over ten-fold in the enhancement zone as compared to the natural zone. Periodic blooms were natural phenomena in marine environments for millennia (McGowan et al., 1999).

Table 4
Cell numbers, chlorophyll *a* and diversity (H') of epipellic microalgae and *Cyanobacteria* in zones (dry season 2006–rainy season 2007). Values are mean cell count percentages along transects 1–4 (rainy season in brackets).

Group	Species	Treatment zone (%)	Enhancement zone (%)	Natural zone (%)
Cyanobacteria	9	31.7 (26.3)	27.8 (42.2)	6.6 (5.2)
Chromophyta	28	50.2 (45.4)	66.7 (45.2)	68.3 (64.7)
Chlorophyta	20	17.0 (26.9)	14.1 (12.6)	25.1 (30.1)
Dynophyta	5	1.8 (1.4)	1.4 (0)	0
Chlorophyll *a* ($\mu g\,ml^{-1}$ of microbial mat)		0.45 ± 0.1 (0.38 ± 0.08)	1.57 ± 0.2 (1.66 ± 0.11)	0.16 ± 0.2 (0.14 ± 0.01)
Diversity index (H')		2.2 ± 0.02 (2.7 ± 0.02)	2.6 ± 0.02 (2.5 ± 0.04)	2.4 ± 0.02 (2.0 ± 0.02)

Reported bloom impacts at present times are always site-specific and not necessarily detrimental. Some were reported to be even beneficial, particularly, in the tropics where turnover rates are high (Böttger-Schnack and Schnack, 1989; Rivera-Monroy et al., 1998; Pittman and Pittman, 2005). Nagarkar et al. (2004) noted that cyanobacteria-dominated biofilms are of a great importance for intertidal grazers due to nutritional value. Cyanobacteria, through complex interactions (diazotrophy, production of plant growth factors) with mangrove aerial roots, may facilitate establishment of new plants, while enhancing resilience and stability of mature tree growth (Holguin et al., 2001). Mangrove soil also provides a harbour for meiobenthos (oligochaetes, juvenile crustaceans, etc.) which graze on diatoms, cyanobacteria and are critically important for ecosystem stability.

3.4.2. Mangrove tree growth

Relatively minor amount of nutrients was incorporated into vegetative biomass in the treatment zone (Table 2). The N leaf content was considerably elevated as compared to that of the natural zone trees, potentially providing a higher nutritional feed value to herbivorous invertebrates (insects, crabs, etc.) and enhancing the abundance. Leaf Area Index, an indicator of the physiological state of mangrove trees, was lower in the treatment zone indicating a stressful condition, while that for the enhancement zone was considerably higher, approaching the range typical of pristine *Avicennia* mangroves (Steinke et al., 1995) and the natural zone mangroves (Table 5). Woody biomass distribution was significantly higher ($p = 0.007$) in the enhancement zone as compared to other zones. If the N content and LAI could have reflected a short-term enhancement, the increase in woody biomass would indicate a longer-term impact on the enhancement zone exerted by nutrients tidally exported over a decade from the treatment zone. A similar phenomenon was described by Onuf et al. (1977) for mangrove leaves grown under hypernutrified conditions and suggested to be beneficial to the adjacent estuarine ecosystem.

3.5. Mangrove rehabilitation success

Comparison of vegetation parameters of BPNR with those of control natural sites No. 2 and 3 showed similarity. As Table 5 shows, the tree diameter at breast height (DBH), canopy height, tree density were similar in all BPNR zones (except in the stressed treatment zone: DBH 10.5 ± 2.7 cm). Control sites 2 and 3 were similarly characterized by DBH 18.1 ± 4.8 cm, canopy height 500.4 ± 148.5 cm and tree density 22.1 ± 8.4 trees per 100 m^2. The floral similarity of the urban mangrove with the undisturbed natural mangroves of the coast of the Bight of Bangkok suggests a successful rehabilitation of the mangrove. The results are in line with previous rehabilitation studies (Stevenson et al., 1999). Another study on rehabilitated *Kandelia candel* mangroves showed that as judged by macro-bentic faunal community, the mangrove reached maturity in 20 years (Chen et al., 2007), corroborating the presented findings on the rehabilitated *Avicennia* mangrove of the same age. The work paves the way towards the rehabilitation of much larger areas of the seriously degraded coast. Although full-scale trials on municipal waste treatment by mangroves demonstrated that mangroves could successfully recycle considerable amounts of C, N and P, the use of remnant mangroves in areas where they are scarce should not necessarily be encouraged. Mangrove rehabilitation and restoration projects need to encompass the use of mangroves as multi-functional treatment facilities.

3.6. Impact of added nutrients on key faunal groups

3.6.1. Invertebrates

Selected key groups significant for conservation and nature education were monitored in terms of diversity and abundance. Up to 17 mangrove crab species (*Brachyura*) were recorded in a mature Andaman coast mangrove (Macintosh et al., 2002), which compares well with the BPNR data, 15 species, even considering the fact that the Gulf of Thailand mangrove diversity is generally poorer as a result of lower tidal amplitude.

Direct impact of pig wastes clearly explains the fact that the treatment zone was inhabited by three species only (Table 6). Low presence of the leaf-eating crabs led to accumulation of leaf litter (15.5 $g\,m^{-2}$) which increased towards the more stressed farm area. Ecosystem stabilized over the long-term impact period and the enhancement zone got discriminated from the natural zone by a considerably higher diversity and abundance despite the similar habitat heterogeneity. As a result, these two zones accumulated less leaf litter (2.6 $g\,m^{-2}$). Many species (e.g. *Uca urvilla*, *Uca lacetea*, *Metaplax elegans*, etc.) appeared to have migrated seasonally to the enhancement zone from the natural zone. The latter may have been potentially attractive for leaf-eaters due to the higher N leaf content and better digestibility (Onuf et al., 1977). The enhancement zone experienced a boost in diversity and abundance and regained natural status lost in the treatment zone. Moreover, the status which it achieved exceeded that of the natural zone. The enhancement zone supported additional five species (15 vs 10). As for the abundance, it was also considerably higher. An apparent explanation for the behaviour of crabs was the higher primary productivity (phytoplankton, leaves) and, presumably, better quality of undigested and digested leaf litter available for ocypodid (mainly detritivores) and grapsid, mainly herbivorous, crabs (Table 6). Natural status of the enhancement zone was also suggested by the equal species number of these groups. Predominance of ocypodids over grapsids is considered to be indicative of habitat degradation (Macintosh et al., 2002). Notably, another study, albeit much less elaborate (Hongsing and Aksornkoae, 2004), yielded similar findings indicating that additional nutrients did not negatively impact natural mangroves. Li and Lee (1998) found more crab species in a nutrified area than in a natural area. However, no further conclusions (abundance, enhancement, etc.) could be inferred due to the fragmentary data. Data on insects, mollusks and polycheates (also incomplete) pointed in the same direction. Of particular significance, as a corroborating evidence, was a short-term ecological research (1.7 year) undertaken by Yu et al. (1997) on the Hong Kong mangroves when waste load, lower by an order of magnitude than

Table 5
Parameters characterizing mangrove vegetative growth in zones. Significant differences between zones for each variable as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, superscript numbers (1, 2, 3) indicate the zone with which comparison was done.

Parameter	Treatment zone [1]	Enhancement zone[2]	Natural zone[3]
Nitrogen in leaves (%dry wt)	2.11 ± 1.0^{**2,3}	1.64 ± 0.5^{*3}	1.08 ± 0.64
Phosphorus in leaves (%dry wt)	1.47 ± 0.3^{**2,3}	0.72 ± 0.4	0.78 ± 0.53
Leaf Area Index, LAI	2.92 ± 0.39	4.18 ± 0.47^{***1}	4.5 ± 0.81^{***1,*2}
Woody biomass (kg m^{-2})	16.5 ± 12.1	20.4 ± 14.1^{**1,*3}	18.8 ± 6.8^{*1}
Height (cm)	496.5 ± 133.1	602.4 ± 139.5^{**1,*3}	567.1 ± 154.8^{*1}
DBH (cm)	10.5 ± 2.7	20.2 ± 7.4^{**1,3}	15.5 ± 5.5^{*2}
Density (100 m^{2})	26.2 ± 17.9	28.8 ± 21.2^{*1}	32.3 ± 11.3^{*2}

in the present study, was applied. As a result the mean biomass of total benthos (mollusks, crustaceans, polychaetes) increased over six-fold within one year in a waste-fed site in comparison to a natural control. Notably, faunal community structures of the sites did not change.

3.6.2. Vertebrates

Significant differences in terms of abundance and diversity of amphibious mudskippers (*Oxudercinae*) were found in three zones (Table 7). The treatment zone was clearly under stress (one species with a low diversity), particularly due to an elevated concentration of ammonium originating from pig wastes. The only species found in the zone, even in the anaerobic pond with an enormous NH_4^+-N concentration (201 mg l^{-1}), was an ammonium-tolerant *Periophthalmodon schlosseri*, known to physiologically transport ammonium against the gradient. The mudskipper population got a boost by additional detritus, leaf litter and invertebrates in the enhancement zone receiving attenuated nutrients from the treatment zone. In contrast to the natural zone featuring only three species, the enhancement zone was characterized by the higher diversity and abundance: four out of nine amphibious species reported for Thailand. Species number was also higher than in the natural control sites No. 2 (2 spp.) and 3 (3 spp.). *B. boddarti* tended to move from the natural area to the more abundant enhancement zone during the stressful dry season. *P. septemradiatus* was found exclusively in the natural area due to its association with *Nypa* palms present in this zone only. Selective predation may be another important factor determining the observed zonal distribution. Such smaller species as *P. chrisospilos* and *P. septemradiatus* (6 cm in length) were more vulnerable to predation by birds and water snakes than the 25 cm long *B. boddarti* and *P. schlosseri*. Additional nutrients in soil translated into a stable season-independent food supply for mudskippers. As in the case of crustaceans, the response of mudskippers was in line with their ecology. The ecological features apparently determined different responses of the species to additional nutrients, of which N was the most critical. The crucially important invertebrates and fish link primary producers to higher trophic level consumers such as birds.

3.7. Nutrification and enhancement of the ecosystem

Avian fauna is the biggest asset for the reserve's nature education run by the WWF Education Centre. Additional production of biomass (phytoplankton, leaves, invertebrates) most certainly would constitute a trophic boost for resident and migratory birds. Due to a high mobility of birds and relatively small area of the studied zones, it was impossible to evaluate the enhancement effect of nutrients on their diversity and abundance. Nevertheless, one should take into account the fact that a significant avian diversity and abundance has been achieved in the reserve bordering residential and industrial areas on three sides. At least 187 species, including endangered species, were recorded in 2007, which compares well with the avian diversity found in the control natural sites

Table 6
Abundance, evenness, Simpson Index and diversity (H') of crustaceans (*Brachyura*) as a function of nutrient availability in zones (dry season 2006–rainy season 2007). Significant differences between zones for each variable as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, superscript numbers (1, 2, 3) indicate the zone with which comparison was done (rainy season in brackets).

Species	Abundance, individuals 100 m^{-2}		
	Treatment zone[1]	Enhancement zone[2]	Natural zone
Chiromantes eumolpe[a]	0.6 ± 0.1 (0.6 ± 0.1)	0.9 ± 0.1 (0.9 ± 0.06)	2.0 ± 0.1^{**1,2} (2.7 ± 0.3)^{***1,2}
Episesarma versicolor[a]	1.0 ± 0.2 (1.2 ± 0.1)	4.4 ± 0.6^{**1} (3.3 ± 0.2)^{**1,3}	5.5 ± 0.4^{**1,2} (6.0 ± 0.6)^{***1,2}
Metopograpsus sp.[a]	0 (0)	1.7 ± 0.2 (1.0 ± 0.06)	4.0 ± 0.3^{**2} (2.0 ± 0.3)
Ilyogynis sp.[a]	0 (0)	3.2 ± 0.1 (4.0 ± 0.1)	6.5 ± 0.7^{**2} (5.0 ± 0.1)^{**2}
Metaplax elegans[a]	0 (0)	1.0 ± 0.1 (1.1 ± 0.2)	0 (0)
Uca annulipes[b]	0 (0)	4.0 ± 0.7 (3.8 ± 0.9)^{**1,3}	3.8 ± 0.6 (2.0 ± 0.2)
U. forcipata[b]	0 (0)	1.8 ± 0.2^{*3} (3.0 ± 0.2)	1.0 ± 0.1 (2.7 ± 0.4)
U. vocans[b]	0 (0)	2.8 ± 0.4^{**3} (4.0 ± 0.6)^{**3}	1.4 ± 0.3 (2.0 ± 0.3)
U. lacetea[b]	0 (0)	0 (1.67 ± 0.2)	0 (0)
U. paradussumieri[b]	0 (0)	1.7 ± 0.3 (2.0 ± 0.5)^{**3}	1.6 ± 0.2 (1.2 ± 0.2)
U. urvilla[b]	0 (0)	0.3 ± 0.1 (0.3 ± 0.04)	0 (0)
Scylla serrata	2.2 ± 0.3 (2.0 ± 0.4)	1.0 ± 0.1 (0.7 ± 0.09)	1.8 ± 0.2 (1.0 ± 0.1)
S. paramamosain	0 (0)	0.03 ± 0.01 (0.04 ± 0.02)	0 (0)
Clibanarius sp.	0	0.03 ± 0.01 (0)	0
Limulus polyphemus	0	0.5 ± 0.01 (0)	0.2 (0)
Total abundance individuals 100 m^{-2}	3.8 ± 0.7 (2.0 ± 0.5)	6.6 ± 0.2^{***1,3} (6.3 ± 0.2)^{***1,3}	5.4 ± 0.2 (5.5 ± 0.2)
Diversity Index (H')	0.86 ± 0.02 (0.83 ± 0.02)	2.84 ± 0.2^{***1,3} (2.49 ± 0.2)^{***1,3}	1.85 ± 0.1 (1.98 ± 0.1)
Simpson's dominance (D)	0.42 (0.4)	0.13 (0.18)	0.17 (0.13)
Evenness (J)	0.9 (1.0)	0.84 (0.85)	0.83 (0.8)

[a] Grapsid crabs.
[b] Ocypodid crabs.

Table 7
Abundance, evenness, Simpson Index and diversity (H') of mudskippers (*Oxudercinae*) as a function of nutrient availability in zones (dry season 2006–rainy season 2007). Significant differences between zones for each variable as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, superscript numbers (1, 2, 3) indicate the zone with which comparison was done (rainy season in brackets).

Species	Treatment zone[1]	Enhancement zone[2]	Natural zone[3]
Species abundance individuals 100 m^{-2}			
Boleophthalmus boddarti	0 (0)	7.8 ± 1.4 (3.7 ± 0.8)	0 (3.5 ± 0.4)
Periophthalmodon schlosseri	2.5 ± 0.3 (1.3 ± 0.1)	$8.7 \pm 0.9^{***1,3}$ $(5.7 \pm 0.7)^{***1,3}$	$5.9 \pm 0.8^{***1}$ $(2.7 \pm 0.2)^{***1}$
P. septemradiatus	0	0	0.6 (0)
Periophthalmus chrysospilos	0 (0)	3.0 ± 0.4 (1.2 ± 0.2)	0 (0)
P. novemradiatus	0 (0)	2.7 ± 0.4 (2.5 ± 0.5)	0 (0)
Total abundance individuals 100 m^{-2}	2.5 ± 0.3 (1.3 ± 0.1)	$29.7 \pm 4.7^{***1,3}$ $(18.0 \pm 2.5)^{***1,3}$	$8.0 \pm 0.9^{***1}$ $(3.0 \pm 0.4)^{***1}$
Diversity Index (H')	0 (0)	$1.26 \pm 0.2^{***1,3}$ $(1.09 \pm 0.1)^{***1,3}$	0.35 ± 0.02 (0.3 ± 0.04)
Evenness (J)	1.0	0.9 (0.8)	1.0
Simpson's dominance (D)	0	0.19 (0.39)	0.15 (0)

No. 2 and 3. On the other hand, the abundance by far exceeds that of the natural sites (WWF-Thailand and authors' observations).

Ecologically sustainable pollution enhancing nutrient status of mangrove mudflats was reported from China (Li and Lee, 1998), where the mangroves served as feeding ground for shorebirds. 50% of nutrients thought to originate from the polluting stream were channeled through the food web to invertebrates as feed for birds. More than 20,000 tons of pollutant carbon entered the Deep Bay yearly through wastewater discharge from two main rivers making it a major source of carbon budget for the ecosystem. What appeared to be a major pollution problem from the viewpoint of water quality management was actually a blessing to the conservation value of this mangrove-fringed embayment. Additional nutrients appeared to stimulate ecosystem, which was estimated to be near carrying capacity for birds. This allowed avoiding displacement of the populations to alternative feeding grounds. However, in contrast to the present study, the nutrification was not managed.

The observed impacts of nutrification as reviewed in literature were: (i) frequently increased primary productivity, (ii) reduction in numbers of species, and (iii) dominance by less desirable species leading to the elimination of some due to an interspecific competition (Pearson and Rosenberg, 1978). Nevertheless, the evidence collected over the last two decades showed, at least in case of mangroves, a positive rather than negative outcome of *managed* human interventions (Onuf et al., 1977; Li and Lee, 1998; Rivera-Monroy et al., 1998). However, more work needs to be done to firmly establish ecologically safe limits for such nutrification-mediated enhancement. One issue deserves particular attention: that of the impact of anthropogenic nutrients on biodiversity and a fuzzy divide between the un-intentional unmanaged eutrophication and intentional managed waste-induced wetland productivity enhancement without the loss of biodiversity. This is practically important since anthropogenic nutrient levels will continue to rise globally in the foreseeable future. Robust constructed ecosystems which harbour bio-diverse communities despite the pollution may be encouraged in situations where natural ecosystems fail. Such ecologically engineered systems could play an increasingly important role. A nutrient supply which is (i) evenly spread over an extended area and (ii) sufficient to ensure the diversity/abundance enhancement, but (iii) not exceeding ecologically safe limits, can be engineered through multiple outlets from a main treatment zone.

From the socio-economic perspective the enhancement would substantially increase conservation and livelihood-supporting potential of urban and peri-urban mangroves. Appropriate facilities mitigating pollution in the developing countries will take a long time to materialize. In the interim period, natural treatment systems including mangroves could perform this function. Many impoverished and ever-dwindling residual mangroves in the urban areas are unlikely to be turned into recognized conservation zones and will be ear-marked for destruction through property development. Sequestration of a small portion of a mangrove ecosystem for waste management appears to be a viable option from both socio-economic and conservational points of views. The approach will keep the environmental pollutants in the most of ecosystem at lower levels while accentuating multi-functionality of mangroves and facilitating sustainable conservation and nature education.

4. Conclusions

Presented data demonstrated that a mangrove can act as a vegetative buffer zone on a long-term basis and at significantly higher waste treatment efficiency than was previously reported. Contained, the treatment zone could perform pollution mitigation even in a conservation area. Nitrogen removal mechanisms in soil were proposed based on detected major presence of bacterial nitrifiers and denitrifiers, as well as still poorly known archaeal nitrifiers and anammox bacteria. Two latter groups were only recently recognized as major players in global mineralization of organic nitrogen. The study reports their presence in mangrove soil for the first time. Similar range of representatives of taxa novel for mangroves were recovered from both natural and hypernutrified soils. The number of clones and FISH-generated microbial community structure of N-cycling species and other taxa did not vary substantially between hypernutrified and natural soils indicating robustness of the mangrove soil microbial consortia.

Urban expansion and agricultural development encroach on many mangroves. Human-induced pollution impinges even on the ecological integrity of reserved ecosystems. However, mangroves appear to be able to mitigate pollution without compromising natural status. Successful mangrove rehabilitation over two decades shows that re-created mangrove habitat acting multi-functionally can harbour natural diversity and abundance of wildlife while mitigating pollution. When managed and locally contained, the enhanced primary productivity may prove beneficial for an ecosystem's education and conservation potential, as well as for livelihoods of local population.

Crabs, mudskippers and, indirectly studied birds, as critically important groups, exhibited stability in terms of abundance and diversity under a major long-term pollution pressure. The diversity and abundance of these highly visible components were even found to increase, thereby enhancing conservation potential of the reserve. With only a fraction of mangroves remaining in the Bight of Bangkok, such environments of artificially enhanced biodiversity attain an important status in terms of education and conservation. Engineered mangrove systems could not only save a larger environment from eutrophication but would compensate for wildlife habitats lost to human development. Strengthening multi-functionality of mangroves, the study lays a foundation for

the monitoring of ecologically sustainable anthropogenic pressures through the search for ecological indicators of habitat change in pristine and engineered mangroves.

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