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Nutrient variability and its influence on nitrogen processes in a highly turbid tropical estuary (Bangpakong, Gulf of Thailand)

Adriano A. Bordalo[1,2,*], *Kashane Chalermwat*[3], *Catarina Teixeira*[1,2]

1. *Laboratory of Hydrobiology and Ecology, Institute of Biomedical Sciences, University of Porto, 4050-313 Porto, Portugal*
2. *Centre of Marine and Environmental Research, University of Porto, 4050-123 Porto, Portugal*
3. *Department of Aquatic Sciences, Burapha University, Chonburi, Thailand*

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ABSTRACT

Estuarine ecosystems in SE Asia have been poorly studied when compared to other tropical environments. Important gaps exist particularly in the understanding of their biogeochemical function and contribution to global change. In this work we looked into N-turnover in the water column and sediments of the Bangpakong estuary (13°N). A seasonal sampling program was performed along the salinity gradient covering different stretches of the estuary (68 km). Key physical and chemical characteristics were also monitored in order to unravel possible environmental controls. Results showed the occurrence of active denitrification in sediments (5.7–50.9 nmol N-N_2/(cm^3·hr)), and water column (3.5–1044 pmol N-N_2/(cm^3·hr)). No seasonal or spatial variability was detected for denitrification potential in sediment samples. However, in the water column, the denitrification activity peaked during the transition season in the downstream sites coinciding with high turbidity levels. Therefore, in that period of the year, the water column compartment may be an important contributor to nitrate reduction within the estuary. The rather low nitrification rates detected were not always measurable, probably due to the reduced oxygen content and high siltation. This study is one of the few dealing simultaneously with sediments and water column processes in a highly turbid tropical estuary. Therefore, it emerges as a valuable contribution for the understanding of the dynamics of the nitrogen cycle in tropical environments by exploring the role of estuarine N microbial activity in reducing the effects of increased nitrogen loads.

Introduction

Over the past several centuries, human nutrient over-enrichment (particularly nitrogen and phosphorus) associated with urban, agricultural and industrial development, has promoted accelerated rates of primary production, or eutrophication (Paerl and Paul, 2012). Therefore, as a widespread problem, eutrophication affects the structure, function, and services provided by estuarine and coastal ecosystems.

Estuaries are border areas where freshwater meets the sea, playing an important role as controls in exchanges between the continental and coastal environments. In the case of tropical estuaries, they are characterized by being warm, turbid and brackish (Harrison and Whitfield, 2006), where sediment dynamics are controlled by several key processes such as river discharge, morphology, salinity, erosion and deposition, setting of mud, physico-chemical processes and mangrove swamps (Capo et al., 2006).

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[*] *Corresponding author*. E-mail: bordalo@icbas.up.pt (Adriano A. Bordalo).

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Nitrogen is a key element controlling the species composition, diversity, dynamics, and functioning of terrestrial, freshwater, and marine ecosystems (Vitousek et al., 1997). Alterations in the N-cycle may have greater impacts on tropical aquatic ecosystems than those already observed in temperate zones, although N fluxes are less documented in the tropics (Downing et al., 1999). Tropical freshwaters are thought to be more sensitive to N-loading than temperate ones due to a faster turnover of the nutrient pools stimulated by the higher temperatures that characterize those systems (Søballe and Kimmel, 1987; Holmboe et al., 2001). Nitrate is a major eutrophication compound in estuaries and coastal waters (de Jonge et al., 2002), whereas denitrification is an anaerobic process that provides the natural removal of nitrate, and occurs mainly within the sediments (Seitzinger, 1988). The removal can be >500 mmol N/(m^3·hr) in temperate estuaries (Barnes and Owens, 1999), and as high as 2121 mmol N/(m^3·hr) in freshwater sediments (Seitzinger, 1990). Anaerobic ammonium oxidation (anammox) has also been recognized as a major sink for fixed inorganic nitrogen in anoxic waters of basins (Lam et al., 2009), and coastal marine sediments (Engström et al., 2005). Previous studies in estuarine sediments reported that anammox accounts for up to 30% of total N_2 production (*e.g.*, Nicholls and Trimmer, 2009; Teixeira et al., 2014; Hou et al., 2015).

The estimation of the magnitude of denitrification in estuaries, including tropical ones can give valuable information on the capability of a given estuary to cope with increasing nitrate loading from different origins (*e.g.*, autochthonous nitrification, input from land, atmospheric deposition). On the other hand, if the last stage of denitrification is blocked, then N_2O is released instead of N_2. Emissions of N_2O can contribute significantly to the accumulation of radiatively active trace gases in the atmosphere (Houghton et al., 1992), because N_2O is an efficient greenhouse gas, with a global warming potential more than 200 times that of carbon dioxide on a per mole basis (Dickinson and Cicerone, 1986). N_2O is also identified as the dominant ozone-depleting substance, and is projected to remain the largest throughout the 21st century (Ravishankara et al., 2009). Although the comprehension of the processes controlling fluxes of N_2O from coastal systems is imperative, they are still poorly understood and scarcely reported in the tropics (Harrison and Matson, 2003). Moreover, the nitrification process — a source of NO_3^- and NO_2^- from a sequential oxidation of NH_4^+, may be an important additional source of N_2O in aquatic systems (*e.g.*, Goreau et al., 1980; Santoro et al., 2011), even in low oxygen waters (Codispoti and Christensen, 1985), such as those typically existent in high turbidity tropical estuarine environments.

The Bangpakong estuary is the end-member of the largest watershed in Eastern Thailand. The area is strongly influenced by the wet Southeast monsoon from May to November, and by the dry Northwest monsoon from December to February. Thus, two distinct seasons emerge, a wet season in May–November and a dry season during the rest of the months (Bordalo et al., 2001). The estuary receives the influence of the neighboring Chao Phraya watershed that drains the Bangkok metropolitan area through an intricate network of canals, some of them dating back the XV century (Van Beek, 1995). The estuary is a source of dissolved inorganic phosphorous to the adjacent coastal waters, but is a sink for inorganic nitrogen (Buranapratheprat et al., 2002). Phytoplankton and water hyacinth blooms are common events in the estuarine and coastal areas (Sojisporn, 1995) especially at the onset of the wet season (Bordalo e al., 2001). In several stretches of the river, including the estuary, the water quality is not suitable for aquatic life or direct contact particularly during the dry season (Szuster and Flaherty, 2002)

The aim of this work was to study nutrient variability throughout seasons and evaluate its influence on the dynamics of key nitrogen processes such as denitrification, nitrification, and the potential release of N_2O from the tropical Bangpakong River estuarine system, in order to ascertain, for the first time, the role of sediments and water column microbial activity on nitrate turnover and their potential contribution to global warming.

1. Material and methods

The Bangpakong watershed covers over 18,500 km^2, discharging 8.44 km^3 of freshwater a year into the Gulf of Thailand at 13.46°N; 100.96°E. The average freshwater discharge during the wet season is one order of magnitude higher than during the dry season, reaching 512 m^3/sec and 21 m^3/sec, respectively (Boonphakdee et al., 1999). Owing to the seasonal unbalanced river discharge, salt intrusion could reach 150 km upstream of the river mouth (Parkpoin et al., 2001) during the dry season, whereas in the wet season the estuarine system is dominated by freshwater or very low salinity water (Bordalo et al., 2002; Boonphakdee and Fujiwara, 2008).

In order to prevent salt intrusion into irrigated paddy fields along the flat alluvial plain, a dam was completed in March 2000, 68 km upstream the river mouth, thus establishing the present upper estuarine limit. Tides are microtidal (<2 m) to mesotidal (2–4 m) with neap tide range of 1 m and a spring tide range of 3.2 m. Tides are either semidiurnal or mixed. In the latter case, the inequality in tidal elevation leads to pronounced flood-ebb asymmetry that can reach 16 hr during flood. The average estuarine depth during low tide is 7 m, and the estuary is 440 m wide.

1.1. Sampling strategy

Sampling was performed during low tide covering the entire Bangpakong estuary, at five stations stretching from river mouth to the Diversion Dam (Fig. 1, Table 1). The locations were chosen to take advantage of bridges and pontoons, and the number of sampling sites was a compromise between distance and accessibility. Three surveys were organized: one on March 30, 2005 during the dry season (DS), the second on October 26, 2005 during the wet season (WS), and the third on May 10, 2007 during the transition period (TS). They were thought to represent of the dichotomy wet/dry season and the onset of the wet season, according to Bordalo et al. (2001).

Surface water was sampled with a Van-Dorn bottle, whereas triplicate sediment samples were retrieved with a Petit-Ponar grab (15 cm × 15 cm × 10 cm opening) (Wildco, USA). Water samples were placed in sterile plastic flasks and tubes, whereas sediments were placed in 500 mL sterile Whirl bags immediately upon collection. All samples were refrigerated in ice chests until processed. Samples for nitrification and denitrification experiments were kept on ice and processed not later than 24 hr from collection.

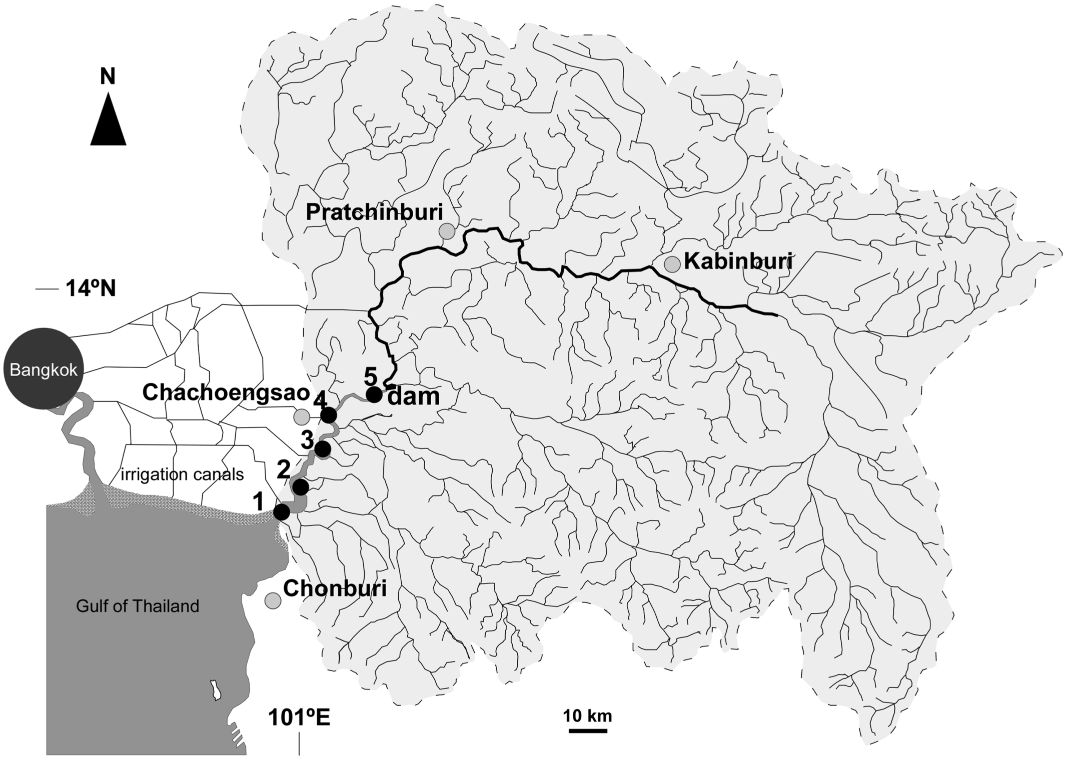

Fig. 1 – Bangpakong River watershed and location of the sampling stations within the estuary (Eastern Thailand).

1.2. *Field measurements*

At each location, water column vertical profiles of temperature, salinity, dissolved oxygen, pH and turbidity were measured by means of a YSI 6000 (YSI Inc., USA) series multiprobe analyzer, calibrated according to the specifications from the manufacturer. The exact position of each station was assessed with a Magellan model 315 GPS (WGS84) (Magellan, USA).

Sediment oxidation–reduction potential (ORP) within the top 5 cm depth was measured *in situ* using a platinum electrode (HI313B, Hanna Instruments, Italy). The electrode was regularly checked using a standard redox solution (HI7030, Hanna Instruments, Italy).

Table 1 – Location and main land use at the vicinity of sampling stations in the Bangpakong estuary.

Station	Coordinates	Distance from mouth (km)	Main land use
1	13.4752N; 100.9811E	3.4	Industry, oil and gas terminal, golf courses, shrimp farms, paddy fields
2	13.4852N; 100.0029E	9.7	Industry, power station, salterns, urban
3	13.5498N; 101.0003E	21.1	Paddy fields, shrimp farms, urban
4	13.6589N; 101.0641E	55.4	Shrimp farms, pig and poultry farms, orchards
5	13.7069N; 101.1352E	66.8	Shrimp farms, orchards

1.3. *Laboratory measurements*

Nutrient analyses were performed using 0.45 μm-filtered water. Orthophosphate (PO_4^{3-}), nitrite (NO_2^-), and ammonium (NH_4^+) were quantified using methods described in Grasshoff et al. (1983). Nitrate (NO_3^-) was assayed using an adaptation of the spongy cadmium reduction technique (Jones, 1984), with the nitrite value subtracted from the total. Detection limits were 0.03, 0.1, 0.02, and 0.3 μmol/L respectively for PO_4^{3-}, NH_4^+, NO_2^-, NO_3^-, respectively. The precision of all determinations was in the range 0.1% to 8% depending on the particular nutrient concentration. All determinations were performed in triplicate, and the values averaged.

For chlorophyll *a* determinations, between 300 and 500 mL of water sample were filtered onto cellulose acetate 0.45 μm membranes. Following a 24 hr extraction at 4°C in 90% (V/V) acetone and centrifugation at 4500 r/min for 15 min, the supernatant was assayed spectrophotometrically using standard equations (Parsons et al., 1984).

Total suspended solids were assessed by filtering 500 mL of estuarine water through a pre-weighted ignited GF/F filter, dried at 60°C until constant weight. The filters were then incinerated at 500°C for particulate organic matter assessment.

Sediment samples for organic matter content were dried to constant weight at 60°C, followed by ignition at 500°C for 4 hr, and reweighing.

1.4. *Potential denitrification and nitrification rate measurements*

Potential denitrification rates in sediments were measured using the acetylene inhibition technique (Sørensen, 1978) following the methodology described by Teixeira et al. (2010). Slurries were prepared by adding 10 mL of filtered (0.2 μm Sartorius) incubation water collected at each site to 50 mL serum bottles containing 5 mL of sediment. The weight of wet sediment was recorded for each serumbottle. Incubation water was amended with 300 μmol/L KNO_3 and 2 mmol/L glucose in order to avoid C and N limitation during the incubation. Serum bottles were hermetically sealed with butyl stoppers and aluminum crimp seals, and purged with helium to remove O_2. Triplicate samples with and without acetylene (20%, *V/V*) were run, and a separate set of time zero samples was sacrificed immediately after acetylene addition. All the remaining samples were incubated in the dark for 4 hr at *in situ* temperature and constant stirring (70 r/min). From a previous experiment with Bangpakong estuarine sediments (data not shown), it was found that during a 6 hr incubation the response was linear. Concomitantly, at time zero and 4 hr, 12 mL of headspace sample was collected from each serum bottle (after equilibration via vigorous shaking) by displacement with 12 mL of 3 mol/L NaCl solution (Joye et al., 1996). N_2O was quantified using a Varian gas chromatograph (CP-3800, Varian, USA) equipped with an electron-capture detector with two Hay Sep D columns at 100°C and an automatic back flush system to prevent C_2H_2 from passing the detector. The retention time of N_2O was 1.6 min, and its concentration was calculated using a standard curve generated from certified gas standards. N_2 produced via denitrification was calculated as the difference between the N_2O produced with and without acetylene.

Regarding the samples retrieved from the water column, the denitrification potential was assessed by filtering 250 mL of water unto an ashed GF/F glass fiber filter. Each filter was then placed inside a 50 mL serum bottle with 10 mL of incubation water, and further treated following the above-described protocol for sediment slurries.

Potential nitrification rates were measured in separate slurries by adding 25 mL of filtered (0.2 μm Sartorius) and oxygenated (air-saturated) water from each location to 50 mL serum bottles also containing 5 mL of homogenized sediment. Samples were run in triplicates, with and without difluoromethane (10%, *V/V*) according to Miller et al. (1998). All samples were incubated in the dark for 4 hr at *in situ* temperature and constant stirring (70 r/min). As in the case of denitrification, a 6 hr experiment was run in order to ascertain the linearity of the process, and a 4 hr incubation period was retained. At time zero and 4 hr, 11 mL of overlying water was collected. These samples were centrifuged, 0.2 μm-filtered and frozen (−20°C) for later quantification of NH_4^+. Nitrification rates were calculated as the difference between NH_4^+ production measured in treatments with and without difluoromethane.

1.5. *Data treatment*

Since discharge rates were not available for the surveyed period, calculations were made from data presented by Buranapratheprat et al. (2002) referred for the years 1994–1997. A curvilinear regression model was fitted to river discharge using salinity as the independent variable. The obtained equation ($y = 1239.1 - 89.042\text{SAL} + 1.5971\text{SAL}^2$) explained 87.4% of discharge variability ($p < 0.0001$).

Hydraulic residence time (RT) in days was calculated according to the equation $RT = V/Q$, where V (m^3) is the volume of the estuary, and Q (m^3/day) the calculated flow rate.

All data were tested for normality and homogeneity of the variances. Temporal and spatial differences were examined using analysis of variance (one-way ANOVA) followed by a post hoc Tukey honestly significant difference (HSD) multi-comparison test using the software STATISTICA 12.0 (StatSoft). Principal components analysis (PCA) was performed to ordinate the seasonal samples based on the values of environmental abiotic data. Data were $\log(x + 1)$ transformed and normalized prior to analysis to have comparable (dimensionless) scales. The relationships between N processes activities (as response variables), and the environmental variables were explored using redundancy analysis. Redundancy analysis is the direct extension of multiple regression to the modelling of multivariate response data (Legendre and Legendre, 1998). Highly correlated variables were examined using variance inflation factors and removed when proved to contribute little information to the ordination (ter Braak and Šmilauer, 2002). Intra-set correlations were used to examine the relative contribution of the environmental variables to the separate ordination axis. The significance of the generated ordination axes was determined using unrestricted Monte Carlo permutation tests (499 permutations). The significance level used for all tests was 0.05. Ordination analyses were performed using the software CANOCO for Windows 4.5 (Biometris).

2. Results

2.1. *Environmental conditions*

Calculated freshwater discharge rates from the river mouth ranged from 1178 m^3/sec during the WS survey to a mere 15 m^3/sec during the DS survey, a 79-fold difference. At the onset of the wet season the calculated discharge rate yielded 280 m^3/sec. Residence time was in the range of 0.2–10.8 days, with lower values for the WS and higher for the DS, whereas in the TS the residence time reached 0.6 days *i.e.*, slightly higher than a tidal cycle in the case of semidiurnal tides. During the DS, brackish water with salinities in the polyhaline range (19.1–24.6) was found within the entire estuary (Table 2). In contrast, during the WS, freshwater was found throughout the estuary, except near the mouth where it reached 2.6. In the TS, intermediate salinities were found in the oligohaline (near the dam) to mesohaline ranges (near the river mouth) (Fig. 2).

Temperature also presented a temporal pattern, with lower values found in the WS and higher values in the DS ($p < 0.001$; Fig. 2), although the spatial variability was similar in the three surveys. Indeed, values increased systematically in the last 20 km stretch of the estuary, particularly at station 2 located 11 km downstream the Bangpakong Combined Cycle Power Plant. In the WS and DS, the estuarine water was well oxygenated with values >80% saturation especially in the lower stretch (stations 1–3) (Fig. 2). However, in the TS oxygen concentrations decreased significantly ($p < 0.001$), being the oxygen saturation <70% throughout most of the estuary, denoting oxygen removal processes. Similarly, the lowest pH values were found during the TS (Table 2), and no temporal

Table 2 – Average, minimum, maximum, and standard error (SE) for key environmental variables and processes during the dry season (DS), transition season (TS), and wet season (WS) surveys in the Bangpakong estuary.

Variable	Survey	Average	Minimum	Maximum	SE
Salinity	DS	22.9	19.1	24.6	1.1
	TS	7.7	0.8	14.6	2.8
	WS	0.7	0.2	2.6	0.5
Temperature (°C)	DS	33.4	31.7	35.4	0.7
	TS	31.0	30.6	31.3	0.1
	WS	28.9	28.6	29.5	0.2
Dissolved oxygen (% sat)	DS	80	71	85	2.4
	TS	67	58	73	2.5
	WS	87	78	99	4.3
pH	DS	7.4	7.1	7.7	0.1
	TS	6.8	6.7	7.0	0.0
	WS	7.2	6.9	7.5	0.1
Turbidity (NTU)	DS	80	40	120	13
	TS	206	106	333	42
	WS	70	36	113	14
Total suspended solids (mg/L)	DS	158	109	203	19
	TS	304	105	497	65
	WS	70	48	104	10
Particulate organic matter (mg/L)	DS	15.1	12.3	17.7	1.1
	TS	167	62.0	368	59.7
	WS	4.6	2.4	8.4	1.1
Nitrate (μmol/L)	DS	55.8	39.6	71.4	5.8
	TS	47.4	17.2	77.1	13.1
	WS	16.2	8.2	27.6	3.6
Nitrite (μmol/L)	DS	2.0	0.8	4.5	0.6
	TS	1.6	0.7	2.7	0.4
	WS	2.0	0.9	3.4	0.5
Ammonium (μmol/L)	DS	3.4	0.1	6.5	1.0
	TS	4.1	1.2	7.1	1.1
	WS	2.2	0.4	5.0	1.1
Phosphate (μmol/L)	DS	3.5	0.4	4.3	0.4
	TS	1.9	0.9	3.0	0.4
	WS	0.7	0.1	1.7	0.3
N:P ratio	DS	19.5	11.2	29.3	4.0
	TS	27.2	20.2	30.9	1.9
	WS	46.9	20.8	80.3	11.9
Chlorophyll *a* (mg/m^3)	DS	12.3	8.5	18.5	1.9
	TS	32.8	9.6	48.9	6.7
	WS	7.5	6.0	10.3	0.8
Denitrification — water column (pmol N/(cm^3·hr))	DS	106.5	44.7	247.8	36.8
	TS	608.3	33.0	1043.8	165.1
	WS	55.8	3.5	165.0	31.5
Denitrification — sediments (nmol N/(cm^3·hr))	DS	25.1	5.7	36.3	5.4
	TS	32.0	17.2	50.9	5.9
	WS	23.5	10.1	35.7	4.1
N_2O production — sediments (nmol N/(cm^3·hr))	DS	2.0	0.2	7.5	1.4
	TS	5.2	0.7	9.2	1.7
	WS	1.5	0.3	2.6	0.4
N_2O:N_2 ratio (%)	DS	6.5	0.7	19.7	3.5
	TS	16.3	1.4	25.6	5.3
	WS	6.6	1.3	12.1	1.8
Nitrification — sediments (nmol N/(cm^3·hr))	DS	2.7	n.d.	6.4	1.4
	TS	10.5	n.d.	19.5	3.8
	WS	1.3	n.d.	2.0	0.4
Sediment redox potential (ORP) (mV)	DS	−85	−182	42	42
	TS	−128	−156	−96	10
	WS	n.a.	n.a.	n.a.	n.a.
Sediment organic matter (mg/g)	DS	75.5	46.9	94.3	8.1
	TS	51.2	37.3	59.5	3.8
	WS	60.0	38.0	95.4	9.6

n.a.: non-available data; n.d.: non-detected.

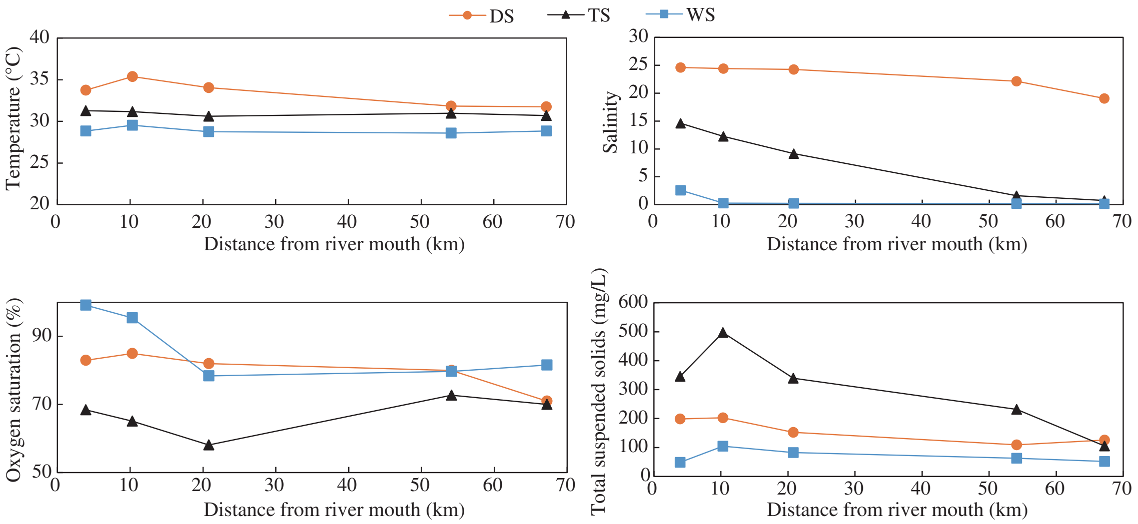

Fig. 2 – Spatial and seasonal variability of temperature, salinity, dissolved oxygen saturation and total suspended solids the Bangpakong estuary (DS: dry season; TS: transition season; WS: wet season).

differences were found ($p > 0.1$) between WS and DS. On a spatial basis, pH tended to increase downstream following the temperature pattern. It should be noted that pH values where always below 7 in the TS.

At the onset of the wet season, the runoff from the watershed was appreciable, and fostered the increase of the total suspended solids (TSS) load to 304 ± 65 mg/L (Table 2), nearly twice the amount of the concentration in the DS or a 4.3× increase over the WS. As in the case of temperature, a sharp increase was noticed at station 2 as a result of sediment resuspension due to the power plant operation (Fig. 2). The organic fraction of the TSS was also higher during the TS, reaching an average of 53% ± 10%, whereas in the other two surveys the values where <10% (Table 2).

Nutrient concentrations in the WS were systematically lower than during the DS, with the exception of nitrite at sites 2 and 3 (Fig. 3). Nitrate was the major inorganic nitrogen form and showed in average a 3.5 fold increase during the DS survey, when compared to WS (Fig. 3), representing 82% of all inorganic nitrogen forms, whereas phosphate increased in average 4.9 fold. A similar seasonal trend for phosphate was found, with values in the DS significantly higher ($p < 0.001$) than in the WS. Generally, intermediate values of inorganic nutrient concentrations were registered at the TS survey (Fig. 3). While the N:P during the DS approached the Redfield value (16:1) in the first stretch of the estuary, the N:P average for WS and TS surveys was 46.9 and 27.2, respectively (Table 2). Interestingly, the higher estuarine water turbidity observed during the TS survey was followed by higher phytoplankton biomass (mean Chl *a* of 32.8 mg/m^3; Table 2).

Sediment sample organic content ranged from 46.9 to 94.3 mg/g during the WS and 38.0 to 95.4 mg/g during the DS. Surprisingly, the concentration of particulate organic matter and sediments in percentage terms was similar in the WS, 6.2% and 6.0%, respectively.

PCA was applied to investigate further the relationship between the observed spatial distribution and temporal variation

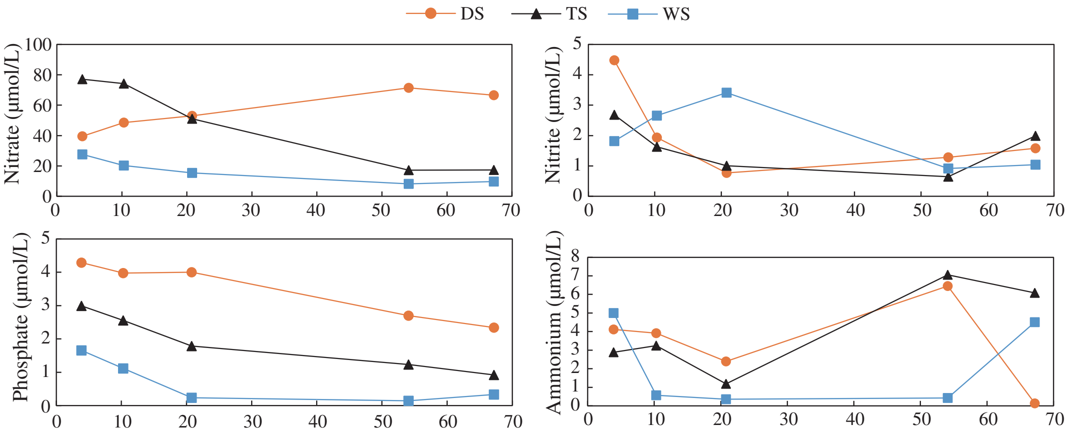

Fig. 3 – Spatial and seasonal variability of nutrients in the Bangpakong estuary. The SD of three replicates was always smaller than the size of the symbol.

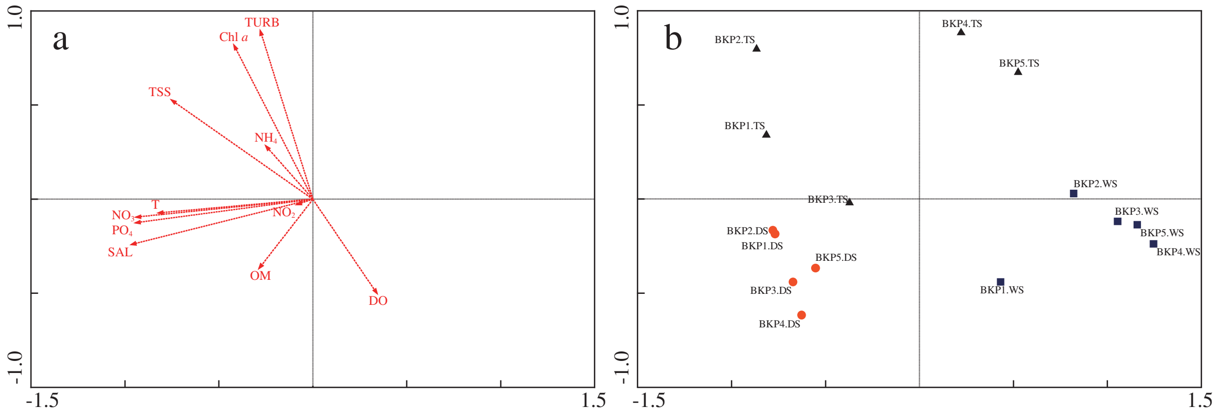

Fig. 4 – Principal components analysis (PCA) ordination plot of measured environmental variables (a) and sample distribution along the same PCA axes (b).

of physical and chemical characteristics (Fig. 4). The analysis showed that the first principal component axe (PC1, horizontal), mainly related to salinity, nitrate, and phosphate, explained alone 60.6% of the variability. The second axe (PC2, vertical), characterized by turbidity and chlorophyll *a*, accounted for an additional 20.3% of the variability. Thus, both axes explained 80.9% of total variance. Sample distribution on the same PCA plot revealed a clear pattern over seasons. Separation of DS and WS was noted along the PC1 axis of variability, related to salinity variation and nutrient concentrations, while the more turbid TS samples were distributed along the positive side of PC2 gradient.

2.2. Potential denitrification and nitrification activities

The potential denitrification activity in sediments showed similar averaged values in all surveys (25.1 ± 5.4, 32.0 ± 5.9, 23.5 ± 4.1 nmol N-N_2/(cm^3·hr), respectively for DS, TS and WS) (Table 2, Fig. 5). However, the spatial trend of denitrification was diverse according to the seasonal survey. In the DS, denitrification was more active in the upper sites, whereas in the WS and TS higher rates were encountered downstream. The higher rates of denitrification in the three surveys coincided with high NO_3^- availability. Actually, denitrification was found to be significantly correlated with the water column NO_3^- concentrations ($R^2 = 0.54$, $p < 0.05$, $n = 15$).

Denitrification activity associated with suspended particles in the water column showed a spatial trend of higher denitrification rates in the downstream sites (Fig. 5). Indeed, water column denitrification potential was found to be one order of magnitude higher during the TS season, when lower oxygen and higher turbidity values were recorded (Table 2).

No significant ($p > 0.05$) seasonal variation of N_2O production in anaerobic slurries was detected (Table 2, Fig. 5). In percentage, the measured N_2O accounted for an average of 6.5%, 16.3% and 6.6% of the N_2 produced in the slurries in DS, TS and WS, respectively (Table 2).

The obtained potential rates of nitrification process in sediments for all surveys are presented in Table 2 and Fig. 5. Values were significantly lower in the WS whereas higher activities were measured in the TS ($p < 0.05$). It should be noted

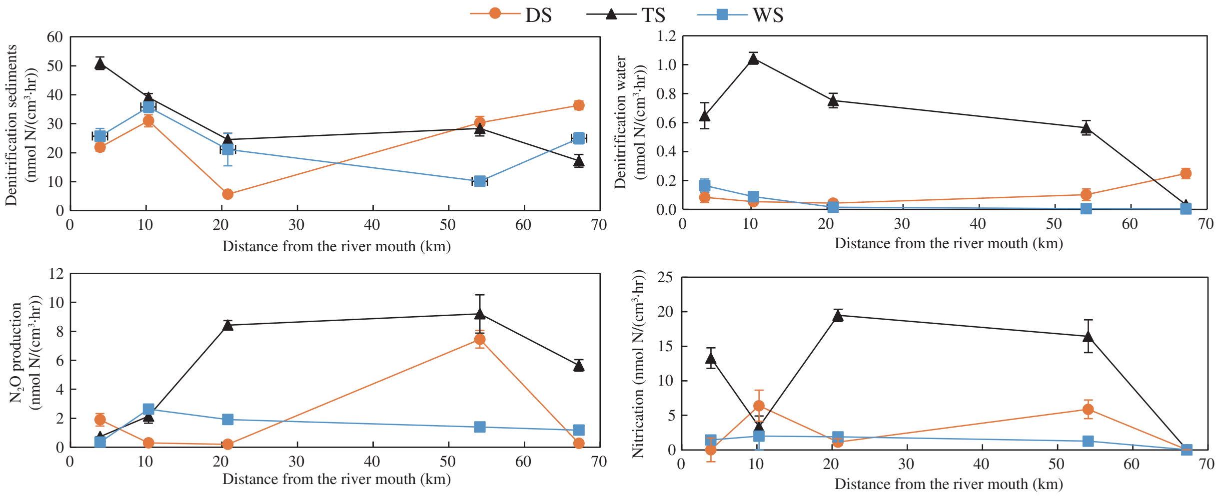

Fig. 5 – Spatial and seasonal variability of potential denitrification activities in sediment and in the water column, and potential N_2O production and nitrification activity in sediments in the Bangpakong estuary. Vertical bars represent the SE of three replicates.

that at the upper estuarine end-member, nitrification was totally inactivated in the three surveys (Fig. 5), and a release of NH_4^+ from the sediments was observed. The redox potential at this location was negative, denoting anaerobic conditions.

The relationship between N cycle processes and measured environmental variables were examined using redundancy analysis and the results presented in the triplot ordination diagram of Fig. 6. Correlations between the two sets of variables were high for Axis 1 (0.954, horizontal), and for Axis 2 (0.689, vertical), and the response variables were significantly related to both canonical axes ($p = 0.006$). The first axis accounted for most of the variability (88.5%) of the data, being TSS, NO_3^-, and DO the best explanatory variables. Therefore, measured processes in Bangpakong estuary seemed to be related to suspended solids/turbidity and NO_3^- levels, while negatively influenced by dissolved oxygen in the water column (Fig. 6).

3. Discussion

The Bangpakong River receives freshwater inputs from hundreds of small waterways, particularly vulnerable to discharges from the evolving land. Indeed, irrigated paddy fields, plantations, and orchards, together with inland aquaculture of shrimp as well as pig, cattle, and poultry farms represent almost 75% of the total land use of the lower part of the watershed (PCD, 1998). Besides soil particles, sludge discharges from animal and shrimp farms are dumped into the canals, and eventually reach the estuary (Szuster and Flaherty, 2002).

It was found that, independently of the season, turbidity was always high. Surprisingly, suspended load was higher during the TS, when residence time increased but the freshwater influx into the estuary was rather low and, concomitantly, salt intrusion was present within the entire estuary. During the WS, a massive export of freshwater and dissolved and particulate matter of fluvial and terrestrial origin occurs downstream reaching the Gulf of Thailand. Sedimentation rates within the estuary can be as high as 0.72 cm/year (Cheevaporn et al., 1994). Thus, siltation is a major problem in the Gulf (Boonphakdee et al., 1999), and probably some of the processes reported herein occur in sediments and in the water column well beyond the estuarine physical boundary. Since the vast mangrove swamps almost disappeared, and are confined to a very narrow strip within the river mouth, their role in controlling sediment dynamics is residual. During the dry season, the tidal force is strong enough to mix the entire water column that combined with very low river flow prevents vertical stratification to occur (Boonphakdee and Fujiwara, 2008). The strong tidal currents during the DS may impel unconsolidated coastal and river mouth sediments upstream, provoking the high turbidity found.

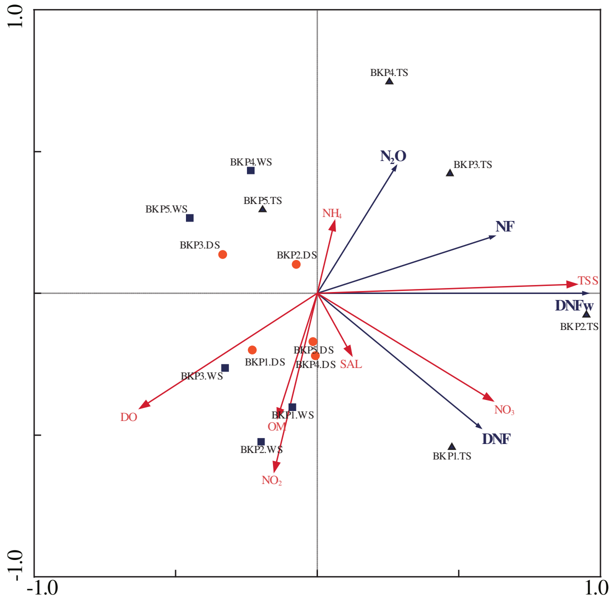

Fig. 6 – Redundancy analysis ordination plot showing the relationship of potential denitrification rates in the water column (DNFw); potential denitrification (DNF), potential nitrification (NF), and potential N_2O production in sediments, and measured environmental variables (TSS — total suspended solids; SAL — salinity; DO — dissolved oxygen; OM — sediment organic matter.

Water column nutrients are in accordance with earlier studies (Smith et al., 2000; Bordalo et al., 2001; Buranapratheprat et al., 2002), and followed the expected seasonal variability *i.e.*, values are higher in the DS. Due to the land use within the watershed, one would expect strong nutrient seasonal fluctuations, with an increase during the WS, when surrounding fields are flooded. This process may promote the export to the estuary of fertilizers (paddy fields and orchards), sludge (shrimp and animal farms), and urban runoff. However, the huge increase in river flow may also be able to generate enough dilution power fostering a low nutrient signal, explaining the obtained results.

The Bangpakong estuarine sediments act as traps for inorganic nitrogen (Buranapratheprat et al., 2002). It is known that dissimilatory nitrate reduction to ammonium (DNRA) promotes the storage of N in sediments as NH_4^+ (Herbert, 1999). If this process occurred during part of the year, namely in WS then, due to resuspension as a result of tidal and wind forcing in the DS, NH_4^+ could be release into the water column. In fact, a 4-fold increase of ammonium was found during the driest period of the year. Once released and in the presence of water column dissolved oxygen (>5 mg/L), coupled with a rather high residence time (>7 days), the nitrification rates almost triplicated, thus explaining the 3-fold increase in NO_3 during the DS comparing with the WS. It was also found that the progressive increase in salinity that occurred during the DS had no deleterious effect on the nitrification rates, contrarily to the trends in some temperate estuaries (Rysgaard et al., 1999). Moreover, in the upper estuary where the lowest salinity was found, no nitrification activity was noticed at all. Not only the water column oxygen content was the lowest, which influences negatively nitrification activity (Rysgaard, 1994), but also the eventual production of H_2S inferred from the dark color of sediments and from the recognized smell which is not only able to consume oxygen but also inhibits the enzyme responsible for nitrification (Joye and Hollibaugh, 1995). Indeed, Cheevaporn et al. (1995) recorded concentrations of H_2S in top sediments higher than 1 μmol/L that remained spatially stable along the salinity gradient. On the other hand, high sulfide concentrations not only inhibit nitrification and denitrification, but may also enhance DNRA by providing an electron donor (An and Gardner, 2002).

Another possible explanation is the absence of a suitable bacterial community able to perform nitrification under the particular environmental conditions of the upper estuary, which watershed is dominated by shrimp farms and orchards. Besides N, the availability of P should also be considered. If the N:P Redfield ratios of receiving waters are high (>16), then the potential impact of enhanced N input into the system is limited because of low P availability (Downing et al., 1999). In this study, spatial and seasonal variations of N:P were found. The averaged ratio during the WS was high and similar to those found in tropical forests (Loehr, 1974), whereas in the DS the ratio decreased to values commonly found in sewage effluent (Downing et al., 1999). Incidentally, in the urban portion of the estuary, the ratios were <16, denoting urban runoff with phosphorous accretion. On the other hand, the observed low N:P ratios may also suggest that denitrification was operating as an efficient N removal process (Tyrrell and Law, 1997).

The averaged NO_3^- concentrations during the DS and TS were above the 50 μmol/L threshold usually used as the boundary between high and low nitrate sets for denitrification (Piña-Ochoa and Álvarez-Cobelas, 2006). In the WS, the concentrations were well under this value (Table 2). However, no seasonal differences in the sediment denitrification rates were found, although the values were slightly higher during the DS. This is a surprise since it is well known that the magnitude of N loss through denitrification is a function of water residence (Nixon et al., 1996). The denitrification values obtained in this study were within the range reported in other tropical and temperate systems (Table 3). Sediment denitrification in the Bangpakong estuary was mainly related to NO_3^- concentration in the water column in accordance with earlier studies (*e.g.*, Ogilvie et al., 1997; Cornwell et al., 1999; Teixeira et al., 2010).

In the water column, the denitrification activity associated with suspended particles was much higher in the TS compared with the WS, when the water turbidity increased dramatically and the lowest O_2 concentrations were recorded. Moreover, when roughly estimating the potential N turnover by denitrification in the water column and sediment compartments by volume (assuming maximal activity in sediments within the top 5 cm depth), it was found that the water column may be able to reduce twice as much NO_3^- as sediments during the transition period. Denitrification is expected to occur mainly within sediments where more favorable conditions are found (Seitzinger, 1988), and was reported to be residual in the water column (Ogilvie et al., 1997). While this remains true for most described temperate systems, the contribution of the water column to nitrate reduction in tropical low oxygen and turbid coastal waters has been somehow neglected in the literature. The presented findings emphasize the importance of denitrification processes occurring in the water column of the Bangpakong estuary, leading to the maintenance of non-harmful levels of inorganic N in a highly turbid tropical system.

In temperate estuaries, nitrification plays a major role in reducing the effects of increased nitrogen loading from human activity, with much of the denitrification resulting from close coupling to nitrification (Seitzinger, 1988; Nixon et al., 1996). In contrast, nitrification in tropical coastal sediments appears to be more severely constrained than in comparative temperate environments (Morell and Corredor, 1993). In the case of Bangpakong estuary, potential nitrification in sediments was detected, although values were rather modest probably due to the low oxygen contents in the overlaying water column, and the above mentioned presence of hydrogen sulfide, which is not only able to consume oxygen, but also directly inhibits the nitrification process (Joye and Hollibaugh, 1995).

The production of N_2O is linked to the microbial turnover of inorganic nitrogen by nitrifying and denitrifying organisms (Poth and Focht, 1985; Wrage et al. 2001; Casciotti and Ward, 2005). In this study, reported N_2O production was assessed in anaerobic incubations, and therefore it is assumed that nitrification or coupled nitrification–denitrification would not be the source of N_2O. Additionally, nitrification was found to be a minor process in the system as mentioned above. The highest N_2O production, observed in TS, did not coincide with the highest denitrification rates resulting in N_2O/N_2 ratios higher than 20%. High N_2O/N_2 ratios may be indicative of reduced efficiency of the denitrification process and may be related to higher NO_3^- availability, lower O_2 concentrations, and/

Table 3 – Potential denitrification activity rates (DNF) measured in sediment slurries from different locations.

Location	DNF	Reference
Bangpakong estuary, Thailand (subtidal)	5.7–50.9 nmol N/(cm^3·hr) 3.6–37.9 nmol N/(g ww·hr) 5.4–68.6 nmol N/(g dw·hr)	This study
Rivers, England (benthic)	0.005–260 nmol N/(g dw·hr)	Garcia-Ruiz et al., 1998
Amazonian lake, Brazil (benthic)	<32 nmol N/(g dw·hr)	Esteves et al., 2001
Headwater streams, USA (benthic)	<1.1 nmol N/(g dw·hr)	Martin and Mulholland, 2001
Pelorus Sound, New Zealand (benthic)	0.9–59 nmol N/(g ww·hr)	Gibbs et al., 2002
Headwater streams, USA (benthic)	<50 nmol N/(g dw·hr)	Inwood et al., 2005
Douro estuary, Portugal (intertidal)	<18 nmol N/(g ww·hr)	Magalhães et al., 2005
Reservoir, USA (benthic)	0–123 nmol N/(g dw·hr)	Wall et al., 2005
Scheldt estuary, Belgium (intertidal marsh)	662–2400 nmol/(cm^3·hr)	Laverman et al., 2006
Agricultural streams, USA (benthic)	8.6–790 nmol N/(g dw·hr)	Arango et al., 2007
Yangtze estuary, China (intertidal)	0.10–0.48 nmol N/(g ww·hr)	Hou et al., 2007
Douro estuary, Portugal (subtidal)	0.4–38 nmol N/(g ww·hr)	Teixeira et al., 2010
Cape Fear estuary, USA (subtidal)	2.5–44.2 nmol N/(g ww·hr)	Lisa et al., 2015
Yangtze estuary, China (subtidal)	0.06–4.51 nmol N/(g ww·hr)	Deng et al., 2015

dw: dry weight; ww: wet wright.

or the H_2S inhibition of N_2O reduction to N_2 during denitrification (Magalhães et al., 2005; Seitzinger, 1988; Senga et al., 2006). Additionally, DNRA processes (not studied) may play a role in N cycling in Bangpakong sediments which may also be an additional source of N_2O under oxygen limited conditions (Smith and Zimmerman, 1981). Indeed, Dong et al. (2011) observed a predominance of DNRA over denitrification in tropical estuaries probably due to higher affinity for nitrate by the nitrate ammonifiers.

4. Conclusions

The presented data provide a snapshot of water quality and key nitrogen processes in water and sediments during the dry, transition and wet season surveys that are thought to be seasonally representative. The results must be interpreted with caution but give valuable indications on the spatial and temporal dynamics of those processes. A more comprehensive monitoring should be carried out in the future to allow a better insight not only on sediment transport within the estuary, but also on biogeochemical processes and their controls, including the potential effect of high salinity, temperature and organic matter on the bioavailability of the different forms of inorganic N. In this vein, the water column compartment should not be neglected as it may have an effective contribution to the nitrogen turnover in turbid tropical estuaries and, in the end, to the eutrophication process.

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