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Nitrogen Uptake Dynamics in a Tropical Eutrophic Estuary (Cochin, India) and Adjacent Coastal Waters

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Abstract Quantification of nitrogen (N) transformation rates in tropical estuarine-coastal water coupled systems undergoing anthropogenic disturbances is scant. A thorough understanding of these metabolic rates is required to evolve a mitigation strategy to save such systems from further degradation. Here, we report the first measurements of ammonium (NH_4^+) and nitrate (NO_3^-) uptake along with N_2 fixation rates in the Cochin estuary, a tropical eutrophic ecosystem along the west coast of India, and two transects (off Cochin and off Mangalore) in the coastal Arabian Sea. In general, the Cochin estuary sustained higher uptake rates of NH_4^+ (0.32–0.91 μmol N l^{-1} h^{-1}) and NO_3^- (0.01–0.38 μmol N l^{-1} h^{-1}) compared to coastal waters. The N uptake in the nearshore waters of Cochin transect (NH_4^+:0.34 μmol N l^{-1} h^{-1} and NO_3^-:0.18 μmol N l^{-1} h^{-1}) was influenced more by estuarine discharge than was the Mangalore transect (NH_4^+:0.02 μmol N l^{-1} h^{-1} and NO_3^-:0.03 μmol N l^{-1} h^{-1}). Despite high dissolved inorganic nitrogen (DIN) concentrations, the Cochin estuary also showed higher N_2 fixation rates (0.59–1.31 nmol N l^{-1} h^{-1}) than the coastal waters (0.33–0.55 nmol N l^{-1} h^{-1}). NH_4^+ was the preferred substrate for phytoplankton growth, both in the Cochin estuary and coastal waters, indicating the significance of regenerative processes in primary production. A significant negative correlation between total nitrogen (TN): total phosphorus (TP), and NH_4^+ uptake (as well as N_2 fixation) rates in the estuary suggests that nutrient stoichiometry plays a major role in modulating N transformation rates in the Cochin estuary.

Keywords Estuary · Coast · Arabian Sea · Eutrophic · Nitrogen

Introduction

Estuaries and adjacent coastal regions are vulnerable ecosystems due to increased nutrient loading from anthropogenic activities. Excess nutrients enter the system as organic and inorganic nitrogen (N) and phosphorus (P) compounds through rivers and the atmosphere and threaten coastal ecosystems (Galloway et al. 2004). Anthropogenic inputs cause many estuarine-coastal systems to shift drastically from N limitation to an N surplus leading to eutrophication (Bricker et al. 1999), a major threat to most of the estuaries and coastal waters around the world (e.g., Hoch and Kirchman 1995; Gattuso et al. 1998; Middelburg and Nieuwenhuize 2000a, b; Mulholland et al. 2003). Intensive autotrophic and heterotrophic activities have been observed in such ecosystems leading to an increase in anaerobic processes such as denitrification, anammox, and methanogenesis (Fasham 2003).

At present, our knowledge of N and C cycling particularly N uptake dynamics and its dependence on N and P distribution in tropical estuaries and the adjacent coastal waters remains rudimentary. Information about the rates of transformation of N and C in such systems is needed to develop mitigation strategies to restore and save the estuarine-coastal coupled systems from eutrophication. Some studies related to N uptake dynamics in estuaries and the adjacent coastal regions in

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higher latitudes provide important insights into such coupled ecosystems (e.g., Middelburg and Nieuwenhuize 2000a, b; Mulholland et al. 2003). In India, research on coastal and estuarine studies have gained momentum with emphasis on C cycling (e.g., Sarma et al. 2001; Bouillon et al. 2003; Gupta et al. 2008, 2009; Shoji et al. 2008). However, most N biogeochemical cycling processes remain unstudied.

Situated at the interface of rivers, ocean, atmosphere, and human settlements, estuaries are subjected to a large number of natural and anthropogenic influences, which can lead to significant ecological and biogeochemical changes within estuaries (Cloern and Jassby 2012). The countering tides and river discharge cause variations in the availability and composition of nutrients and affect major biogeochemical cycles and species diversity in estuaries and adjoining coastal waters (Humborg et al. 1997; Rabalais et al. 2009). Although the supply of N to the estuarine-coastal ecosystems has increased significantly, P availability is the controlling factor for phytoplankton growth (Karl et al. 1995; Sanudo-Wilhelmy et al. 2001; Mills et al. 2004; Neill et al. 2005). Phosphorus is considered the limiting nutrient in the estuarine waters, whereas N may often limit productivity in coastal waters (Fisher et al. 1999; Neill et al. 2005; Paerl et al. 2014). Estuarine microbes are often sensitive to both N and P inputs (Ryther and Dunstan 1971; Schindler 1975; Rudek et al. 1991; Fisher et al. 1999; Boesch et al. 2001; Paerl and M. F. Piehler 2008; Smith and Schindler 2009; Paerl et al. 2014). Apart from nutrients, physical properties such as salinity (McLusky 1971), pH, turbidity, and light availability (Cloern 1987) also may regulate phytoplankton growth and distribution in estuaries.

With these perspectives as guidelines, we measured N transformation rates in the Cochin estuary (Fig. 1a) and the adjacent coastal Arabian Sea (Fig. 1b). The Cochin estuary, located along the southwest coast of India, is a tropical monsoonal estuary. Nitrogen loading to this estuary has increased recently due to extended aquaculture, agricultural practices, and aqua-tourism (Martin et al. 2010, 2013). Additionally, the estuary receives large volume of unprocessed industrial (104×10^3 m^3 day^{-1}) and domestic (0.26×10^3 m^3 day^{-1}) sewage from the city of Cochin and the remote areas through river runoff (KSPCB 1982, 1986; Balachandran et al. 2006). Together, these activities have altered the nutrient stoichiometry of this ecosystem leading to changes in microbial activity and biogeochemistry (Martin et al. 2008; Miranda et al. 2008; Shoji et al. 2008). Also, the eventual transfer of nutrients to the coastal Arabian Sea can potentially affect the metabolic activities of coastal phytoplankton, species diversity, and the microbial food web.

We conducted experiments to quantify nitrate (NO_3^-) and ammonium (NH_4^+) uptake rates (i.e., dissolved inorganic nitrogen (DIN) uptake) along with N_2 fixation rates in the Cochin estuary and the adjacent coastal Arabian Sea by using ^{15}N tracer techniques. We believe that this study is the first one to estimate DIN uptake rates in any Indian estuary. The central hypothesis of this study was that N_2 fixation is not significant in this estuarine system with high DIN concentrations. Our objectives were to (1) estimate DIN uptake and N_2 fixation rates in the Cochin estuary and coastal waters of the Arabian Sea, (2) understand the role of N:P stoichiometry in regulating N uptake and N_2 fixation rates, (3) understand the effect of anthropogenic activities on N uptake rates, (4) determine the role of hydrographic parameters in modulating N uptake rates under nutrient replete estuarine environment, and (5) investigate whether nutrient exchange between estuary and coastal waters affects N uptake rates.

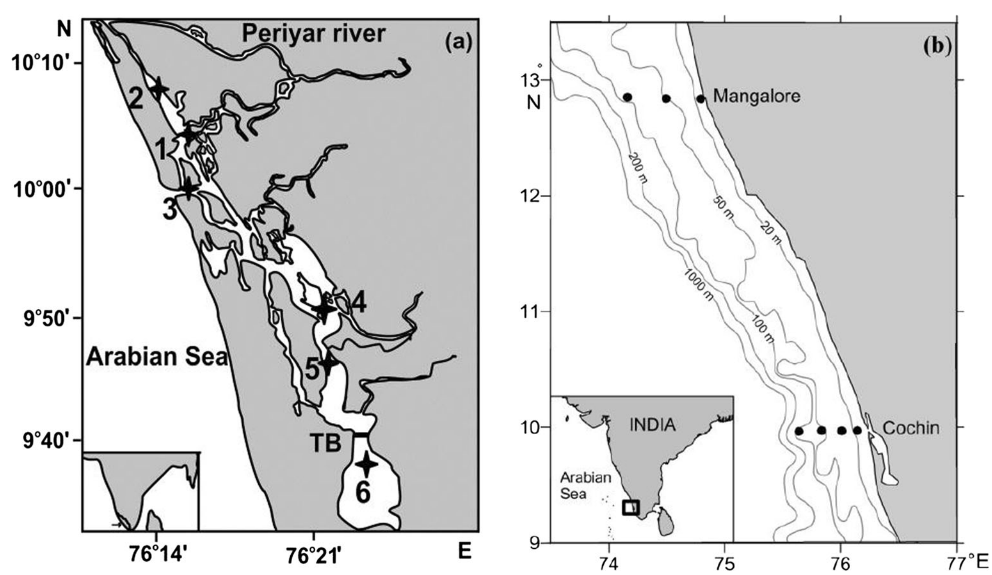


Fig. 1 Sampling locations in **a** Cochin estuary and **b** coastal Arabian Sea. *Numbers* in **a** indicate following stations: *1* Bolghatty-Periyar, *2* null zone, *3* bar mouth, *4* Arikutty, *5* Vaikom, and *6* Vembanad Lake. *TB* is Thannermukkam dam between Cochin estuary and Vembanad Lake. The *dots* in **b** indicate the coastal Arabian Sea stations

Materials and Methods

Nitrogen Uptake

Coastal Waters

A cruise was conducted onboard *FORV Sagar Sampada* (SS-299) during the pre-monsoon season (12–20 April 2012) to estimate DIN (NO_3^- and NH_4^+) uptake and N_2 fixation rates along two transects, viz. off Cochin and Mangalore in the coastal Arabian Sea along the west coast of India (Fig. 1b). The off Cochin region has direct interaction with the Cochin estuary (Gopalan et al. 1983; Gupta et al. 2009), while the off Mangalore region is influenced by the Nethravathi estuarine system, which is minor compared to the Cochin estuary.

Experiments for NO_3^- and NH_4^+ uptake rates along the Cochin transect were performed at stations (Table 1) with water column depths of 13 m (nearshore; surface), 30 m (inner shelf; 0, 10, and 25 m depth), 50 m (mid-shelf; 0, 10, 20, and 40 m depth), and 100 m (outer shelf; 0, 10, 20, 50, and 75 m depth). Similar sampling patterns were performed along the Mangalore transect at water column depths of 13 m (near shore; surface), 50 m (mid-shelf; 0, 10, 20, and 40 m depth), and 100 m (outer shelf; 0, 10, 20, 40, and 60 m depth). The same depth stations along the Cochin transect are farther from the coast compared to the Mangalore transect due to the gentle slope of the continental shelf near Cochin.

Cochin Estuary

The Cochin estuary is a eutrophic micro-tidal system (Fig. 1a), with two openings to the Arabian Sea (Bar mouth and Azhikkode) exchanging saline water and nutrients (Gupta et al. 2009). Total annual freshwater input to this estuary from various sources is about 2×10^{10} m^3, which makes it the largest wetland along the west coast of India (Srinivas et al. 2003). Freshwater inputs from six major rivers and tidal intrusion from the Arabian Sea regulate the salinity in the Cochin estuary (Madhupratap 1987; Srinivas et al. 2003). During southwest monsoon, the salinity of the major portion of the estuary approaches zero due to increased freshwater input, whereas reduced freshwater inflow causes higher salinity during rest of the year (Madhupratap 1987; Menon et al. 2000).

Table 1 Stations detail in the coastal Arabian Sea

Sample code	Geographical coordinates	Station depth (m)	Distance from the coast (km)	Sampling depth (m)
Cochin (CH)				
CH1-0	09.58° N, 76.08° E	13	10.69	0
CH2-0	09.58° N, 75.59° E	30	25.13	0
CH2-10				10
CH2-20				25
CH3-0	09.97° N, 75.84° E	50	44.29	0
CH3-10				10
CH3-20				20
CH3-40				40
CH4-0	09.58° N, 75.39° E	100	65.99	0
CH4-10				10
CH4-20				20
CH4-40				50
CH4-75				75
Mangalore (MR)				
MR1-0	12.50° N, 74.50° E	13	12.00	0
MR2-0	12.50° N, 74.29° E	50	36.59	0
MR2-10				10
MR2-20				20
MR2-40				40
MR3-0	12.85° N, 74.16° E	100	72.39	0
MR3-10				10
MR3-20				20
MR3-40				40
MR3-60				60

Immediately after the coastal cruise (26 April–1 May 2012), experiments were conducted on samples from five stations across the salinity gradient to measure NO_3^- and NH_4^+ uptake rates in the surface waters of the Cochin estuary (Fig. 1a). Similar experiments were performed at one station (station 6; Fig. 1a) in the freshwater Vembanad Lake, located upstream of the estuary and separated by a dam. Stations were selected based on the importance of surrounding features and anthropogenic influences. Station 1 (Bolghatty-Periyar) is situated at the mouth of the Periyar River and receives nutrients from many small and large industries located upstream and untreated sewage from the human settlements around it. Station 2 (null zone) is a region where saline streams from both the inlets meet in opposite direction during high tide resulting in weak dispersion of nutrients (Ramamirtham and Muthusamy 1986; Balachandran et al. 2008). This part of the Cochin estuary is dominated by fertilized aquaculture ponds, which provide nutrients to the estuary. Station 3 (Barmouth, main inlet to the estuary) signifies confluence of the estuary and coastal water and is affected by the Cochin harbor activities. It is polluted by ship ballast and sewage exhaust from the city of Cochin. Station 4 (Arikutty) is located at the meeting point of the Muvattupuzha river with the main estuary. Station 5 (Vaikom) is located at the end point of the estuary and is separated from the Vembanad Lake by a dam. This dam prevents saltwater intrusion into the lake. Station 6 is located in the Vembanad Lake, which is influenced by surrounding tourism and agricultural activities. The dam was closed and the exchange between the estuary and lake was insignificant during the study period. Only stations 1–5 are considered as estuarine stations as station 6 falls in the freshwater region.

Methodology

The data for temperature and salinity during the study period were collected using a Sea-Bird CTD profiler (model 911 plus for coastal and model SBE 25 for the estuary). Inorganic nutrients, viz. NH_4^+, NO_3^-, dissolved inorganic phosphate (DIP), and silicate (Si), were analyzed by spectrophotometry using standard methods (Grasshoff et al. 1999). Total phosphorus (TP) and total nitrogen (TN) were estimated using persulfate oxidation (Grasshoff et al. 1999). Dissolved organic phosphorus (DOP) and nitrogen (DON) were calculated as difference between TP, TN, and their respective total inorganic fractions. The analytical precisions for NH_4^+, NO_3^-, DIP, Si, TN, and TP were ±0.07, ±0.05, ±0.02, ±0.03, ±0.11, and ±0.06 μM, respectively. The pH was measured using Potentiometric Titrator (907 Titrando, Metrohm) with a precision of ±0.005 and ±0.008 for coastal and estuarine samples, respectively. Chlorophyll *a* (Chl *a*) was measured using 90 % acetone extraction followed by fluorometric analysis (Grasshoff et al. 1999).

Duplicate water samples (coastal: 1 L and estuary: 0.5 L), for DIN (NO_3^- and NH_4^+) uptake experiments at all stations and depths, were collected in acid-washed polycarbonate Nalgene bottles. Incubation bottles were covered immediately with black cloth to avoid light shock to phytoplankton. Samples were spiked with 99 atom% enriched $^{15}NH_4Cl$ and $Na^{15}NO_3$ to trace NH_4^+ and NO_3^- uptake rates, respectively. Tracer additions were less than 10 % of the ambient concentrations. Immediately after the tracer addition, sample bottles were deployed for in situ incubation for 4 h, approximately symmetrical to the local noon. Post-incubation samples were filtered onto pre-combusted (4 h at 400 °C) 47 mm Whatman GF/F filters and stored for the mass spectrometric analysis after drying at 50 °C overnight.

Mass spectrometric analyses of post-incubation samples were performed using a continuous flow stable isotope ratio mass spectrometer (Delta V Plus) connected to an elemental analyzer (Flash EA 2000), where ^{15}N atom% and particulate organic N (PON) content were measured. The uptake rates were calculated as follows (Dugdale and Wilkerson 1986; Kanda et al. 2003):

$$\text{Uptake rate}\left(\mu\text{mol N l}^{-1}\text{h}^{-1}\right) = P \times \Delta I_{\text{p}} / [T \times [(I_0 S_{\text{a}} + I_{\text{r}} S_{\text{t}})/(S_{\text{a}} + S_{\text{t}}) - I_0]]$$

where P is the amount of particulate N in post-incubation sample, ΔI_p is the increase in ^{15}N atom% in particulate N during incubation, S_a and S_t are ambient and added concentration, respectively, I_r and I_0 are ^{15}N atom% of added tracer and natural ^{15}N atom%, and T is the incubation time. This equation assumes no formation of nutrient during the incubation, and therefore, rates presented here are representative of potential rates.

The relative uptake of NO_3^- and NH_4^+ was also calculated using the relative preference index (RPI; McCarthy et al. 1977):

$$\text{RPI}_x = \left[U_x/\left(U_x + U_y\right)\right]/[X/(X + Y)],$$

where X and Y are concentrations of NO_3^- and NH_4^+, respectively; U_x and U_y represent the corresponding uptake rates.

2 N_2 Fixation

Experiments to measure N_2 fixation rates were conducted using a $^{15}N_2$ bubble method (Montoya et al. 1996) using $^{15}N_2$ (98+ atom%) tracer gas procured from Cambridge Isotope Laboratories (CIL). These experiments were performed at all stations along the Cochin and Mangalore transects at surface, 10, and 20 m depths, except at nearshore stations, where it was performed only in surface waters. Experiments were also performed in a *Trichodesmium* bloom patch observed around MR2-0 off Mangalore. Similar experiments were conducted in the surface waters of estuarine stations. However, samples from two estuarine stations (5 and 6) were lost during mass spectrometric analysis.

Water samples collected with a clean bucket (for estuary) and Niskin sampler (for coastal waters) were transferred to four 250-ml borosilicate bottles (three lights and one dark). These bottles were filled to the brim and sealed tightly with septum caps to ensure the absence of air bubbles. Five tenths milliliter of ^{15}N-enriched N_2 gas was injected into the sample bottles using a chromatographic syringe followed by gentle mixing and 4 h of incubation. Post-incubated samples were filtered onto pre-combusted 25-mm Whatman GF/F filters and dried at 50 °C overnight. Ambient concentrations of dissolved N_2 gas were estimated using the gas solubility table of Weiss (1970), and the N_2 fixation rates were calculated using Montoya et al. (1996).

The $^{15}N_2$ bubble method (Montoya et al. 1996) used during the present study has some drawbacks. A main drawback is the incomplete dissolution of $^{15}N_2$ gas in the sample within the incubation time, which may underestimate the N_2 fixation rates (Mohr et al. 2010; Großkopf et al. 2012). The release of fixed N, during the incubation, as regenerated N forms (Capone et al. 1994; Bronk et al. 1994; Mulholland et al. 2004) may also contribute to an underestimation. A recent paper by Dabundo et al. (2014) reported other N forms (NH_4^+, NO_3^-, and N_2O) as impurities in the N_2 tracer gas supplied by different distributers, which can cause misinterpretation of the N_2 fixation rates. However, N_2 gas supplied by the CIL (used during the present study) had only very low concentrations of NH_4^+ (0.052 μmol/1 mol of N_2 gas) and non-detectable NO_3^- and N_2O compared to the gas supplied by Sigma-Aldrich (Dabundo et al. 2014).

Results

Environmental and Hydrographic Parameters

Estuary

In general, the estuary was partially cloudy and rainy during the sampling period due to pre-monsoon swells. The surface salinity ranged from 1.58 to 15.57 with a maximum at station 2 (Table 2). Station 3 also exhibited relatively high salinity due to tidal influence. Due to closure of the dam, station 6 showed no signature of saline water invasion as indicated by near zero salinity. The pH in the estuary ranged from 6.03 to 7.33 (Table 2). In general, the Cochin estuary is characterized as a nutrient replete system throughout the year with temporal and spatial fluctuations (Jyothibabu et al. 2006; Martin et al. 2008). During the present study, pre-monsoon rainfall influenced the nutrient concentrations in the estuary, as it brought terrestrial washouts along with the surface runoff. Nitrate and NH_4^+ concentrations varied from 1.25 to 26.6 and 2.11–75.2 μM, respectively (Table 2). The highest concentrations for NO_3^- (26.6 μM) and NH_4^+ (75.2 μM) were observed at station 1, which receives bioavailable N loading from nearby industries and the Periyar River. Station 6 had higher NO_3^- (25.3 μM) and relatively low NH_4^+ (5.02 μM) concentrations due to discharge from agricultural fields and tourist house boats. DON and DOP concentrations in the estuary ranged from 0.56 to 14.3 and 0.05–0.28 μM, respectively. TN and TP in the estuary ranged from 11 to 118 and 0.38 to 3.70 μM, respectively. Inorganic forms dominated the TN (78 %) and TP (86 %) pools at estuarine locations; hence TN:TP is considered as a measure of nutrient stoichiometry in the discussion. Although Si concentrations were high throughout the Cochin estuary, it was relatively low and varied narrowly at stations 1–4 (44.8±3.8 μM) compared to the southernmost stations 5 and 6 (70 and 112 μM, respectively). Chl *a* showed large variation (8.0±5.9 μg l^{-1}) in the estuary with a maximum value at station 2 (17.2 μg l^{-1}) and a minimum at station 1 (1.77 μg l^{-1}; Table 2).

Coastal

Nearshore surface waters of the Cochin transect were less saline (CH1-0; 33.90) compared to those of Mangalore (MR1-0; 35.2), whereas all other stations showed higher salinity (34.6–35.8). The depth variation in salinity was insignificant in both transects. The sea surface temperature (SST) varied from 30.0 to 31.4 °C with a maximum at MR1-0 (data not shown). Nearshore stations of both transects, sampled

Table 2 Estuarine environmental parameters during the sampling period

Station	Salinity	Chl *a*	NO_3^-	NH_4^+	DON	TN	DIP	DOP	TP	TN/TP	Si
1	1.58	1.77	26.6	75.2	14.3	117	3.64	0.05	3.69	31.9	47.6
2	15.6	17.2	1.25	5.22	13.3	20.4	2.95	0.20	3.15	6.50	42.1
3	13.0	8.06	4.97	13.7	1.62	23.1	2.68	0.13	2.81	8.24	41
4	6.27	6.44	8.65	2.11	3.03	14.2	0.51	0.28	0.79	18.1	48.5
5	5.31	2.53	5.20	4.36	0.56	10.7	0.30	0.09	0.39	27.9	70.9
6	0.26	12.2	25.3	5.02	6.30	37.3	0.54	0.27	0.81	46.4	112

Unit for Chl *a* is in microgram per liter; NH_4^+, NO_3^-, DON, DIP, TP, and Si are in micromole per liter

TN sum total of NO_3^-, NH_4^+, NO_2^-, and DON during low tide, showed high surface concentrations of NO_3^- and NH_4^+ compared to other coastal stations and were relatively higher off Cochin (CH1-0; 0.89 and 1.94 μM, respectively) compared to off Mangalore (MR1-0; 0.24 and 0.92 μM, respectively). However, the coastal nutrient concentrations were less than in the estuary. In general, NO_3^- concentrations were less than NH_4^+ in surface waters and both increased with depth (Fig. 2). DON and DOP concentrations off Cochin (0.17–9.51 and 0.01–0.40 μM, respectively) were lower than at off Mangalore (2.7–19.6 and 0.12–0.76 μM, respectively). TN and TP varied from 3.11 to 22.6 and 0.23 to 1.38 μM, respectively, in the Cochin transect and from 4.4 to 21.3 and 0.23 to 0.85 μM, respectively, in the Mangalore transect. Chl *a* remained high at nearshore stations (2.60 and 2.31 μg l^{-1} for CH1-0 and MR1-0, respectively) and low (0.05–0.44 μg l^{-1}) at other locations, except at CH3-40 (2.04 μg l^{-1}; Fig. 3).

Nitrogen Uptake Rates

Estuary

NH_4^+ uptake rates in the Cochin estuary ranged from 0.32 to 0.91 μmol N l^{-1} h^{-1} with an average of 0.59±0.22 μmol N l^{-1} h^{-1}, whereas NO_3^- uptake rates ranged from 0.01 to 0.38 μmol N l^{-1} h^{-1} with an average of 0.20±0.14 μmol N l^{-1} h^{-1} (Fig. 4). The highest NH_4^+ uptake rate among the five estuarine stations was observed at station 2 (0.91 μmol N l^{-1} h^{-1}), whereas NO_3^- uptake rate was the highest at station 4 (0.38 μmol N l^{-1} h^{-1}). NO_3^- and NH_4^+ uptake rates at freshwater Vembanad Lake (station 6) were 0.24 and 0.95 μmol N l^{-1} h^{-1}, respectively. For estuarine stations (1–5), NH_4^+ uptake rates showed a significantly high positive correlation with salinity (R^2=0.88), whereas NO_3^- uptake rates showed a relatively low correlation (R^2=0.15; Fig. 5a). Due to the major contribution of NH_4^+ uptake, total uptake (NO_3^- + NH_4^+) also showed a significant positive correlation with salinity. Similarly, NH_4^+ and total uptake rates also showed significant positive correlation with Chl *a* (R^2=0.94; Fig. 5b) and pH (R^2=0.69; Fig. 5c). A significant negative correlation was observed between TN:TP and salinity (Fig. 6a). Importantly, NH_4^+ uptake and N_2 fixation rates showed a significant negative correlation with TN:TP in the estuary (Fig. 6b).

Coastal Arabian Sea

NO_3^- and NH_4^+ uptake in the coastal Arabian Sea varied from 0.003 to 0.18 and 0.02 to 0.34 μmol N l^{-1} h^{-1}, respectively. Surface NO_3^- and NH_4^+ uptake rates were significantly higher off Cochin than that off Mangalore, with an exception of NH_4^+ uptake rate at the outer shelf station (CH4-0; 0.10 μmol N l^{-1} h^{-1}; Fig. 7). Depth profiles of uptake rates suggest a subsurface maxima between 10 and 20 m depth ranges for Cochin transect, whereas subsurface minima in uptake rates were observed at 20 m for Mangalore transect (Fig. 8). Depth integrated column uptake was calculated using a trapezoidal method (Table 3). In general, column uptake was significantly lower for NO_3^- than for NH_4^+.

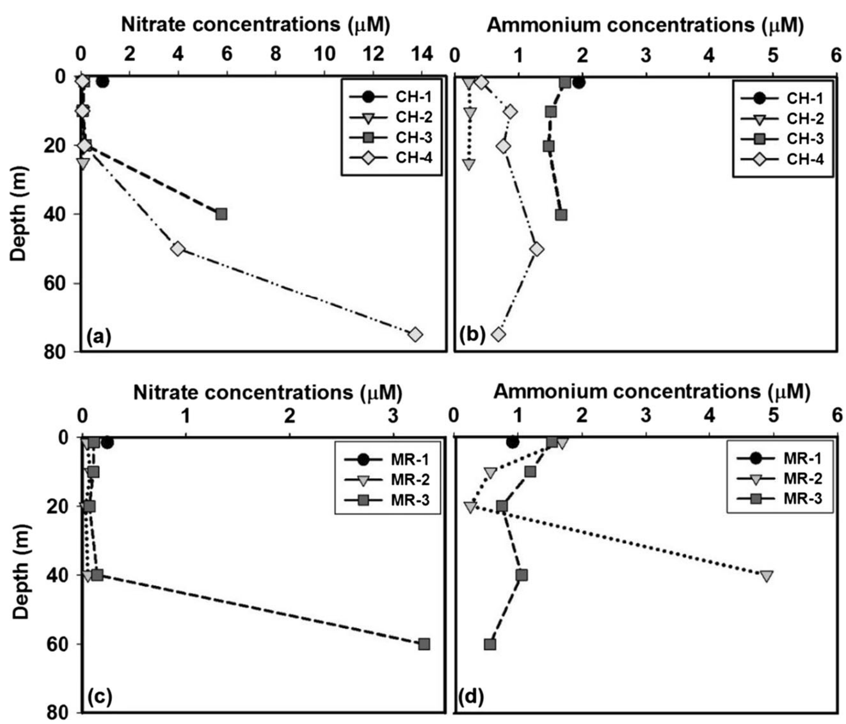


Fig. 2 Depth profiles of nutrient concentrations in the coastal Arabian Sea: **a** NO_3^- (Off Cochin—CH), **b** NH_4^+ (Off Cochin—CH), **c** NO_3^- (Off Mangalore—MR), and **d** NH_4^+ (Off Mangalore—MR)

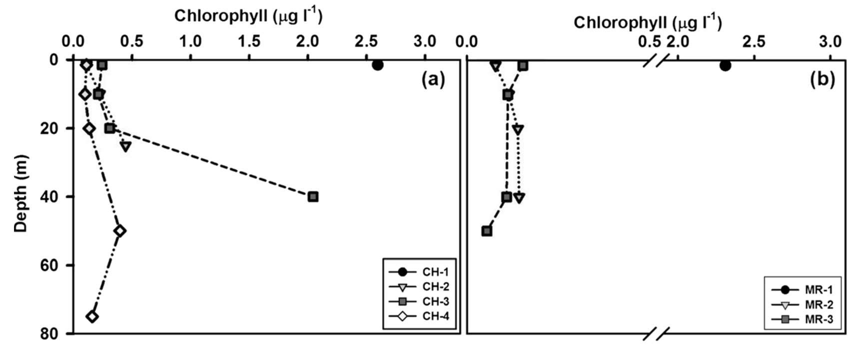


Fig. 3 Depth profiles of chlorophyll *a* in the coastal Arabian Sea: **a** Off Cochin (CH) and **b** Off Mangalore (MR) stations

N_2 Fixation

Despite high DIN concentrations in the estuary, considerable N_2 fixation rates were observed (0.59–1.3 nmol N l^{-1} h^{-1}). The highest rate was observed at station 2 and the lowest at station 1 (Fig. 4). Even though these rates are significantly lower than the DIN uptake rates, they provide evidence of diazotrophic activity in this eutrophic estuary. In general, N_2 fixation in the coastal ocean at our regular stations ranged from 0.33 to 0.55 nmol N l^{-1} h^{-1}. The nearshore surface waters off Cochin (CH1-0 : 0.55 nmol N l^{-1} h^{-1}) and Mangalore (MR1-0 : 0.46 nmol N l^{-1} h^{-1}) showed higher N_2 fixation rates among the coastal non-bloom stations. The N_2 fixation rate at *Trichodesmium* bloom patch (MR2-0 : 3.6 nmol N l^{-1} h^{-1}) was the highest observed in our study (Fig. 7).

Discussion

Coastal and estuarine ecosystems receiving high anthropogenic inputs are susceptible to ecological changes. The understanding of the aftermaths of such human-induced ecological changes on the rates of biogeochemical processes in large tropical estuaries and coastal areas is incomplete. The Cochin estuary and the adjacent coastal region are among the most vulnerable ecosystems, vis-à-vis eutrophication, in India due to increased anthropogenic activities. This estuary was highly autotrophic five decades ago (Qasim et al. 1969) but has evolved into a heterotrophic system due to vast urbanization causing organic enrichment (Shoji et al. 2008; Gupta et al. 2009). The high N uptake rates observed during the present study corroborate the above statement and are comparable with the values reported for estuaries elsewhere affected by increased N loading (Mulholland et al. 2003; Middelburg and Nieuwenhuize 2000b). This study suggests that anthropogenic influences can propagate to coastal regions via estuaries and regulate N and C cycling intensity. The imbalance in TN/TP along with hydrographic and environmental parameters also influenced DIN uptake, particularly NH_4^+ uptake rates, under the nutrient replete condition of the estuary. Evidence of N_2 fixation in the estuary despite high DIN concentrations disproved one of our central hypotheses presented above.

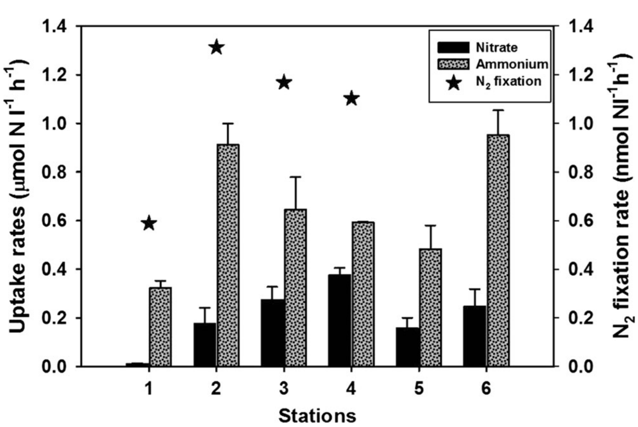


Fig. 4 N uptake and N_2 fixation rates in the Cochin estuary (*1–5*) and Vembanad Lake (*6*). *Error bars* represent 1σ

Nitrogen Sources to the Cochin Estuary

Our results indicate that the Cochin estuary sustains high N uptake rates. One of the prime reasons could be the maintenance of nutrient replete conditions due to inorganic and organic N loadings into the estuary from several sources, including industrial effluents, domestic sewage, agricultural wastes, aquaculture discharges, port activities, coir wastes, and boat tourism. The shallow photic depth (~1 m, equivalent to 10–30 % of entire water column) of the estuary during the study period further suggests that mineralization of organic matter in the dark bottom waters could be another source of DIN. The leaching/remineralization of resuspended sediments due to navigational activities in the lower estuary are important sources of N (Gopalan et al. 1983; Martin et al. 2010). The mesohaline regions in the estuary receive 7–11 times high organic matter as lateral input as compared to the inputs through rivers (Shoji et al. 2008; Gupta et al. 2009). Another possible source of N to the estuarine and coastal waters is atmospheric deposition (Paerl 1993, 1995). A recent study reported atmospheric NO_3^- and NH_4^+ inputs to the Arabian Sea to be around 6.5 and 0.4 nmol m^{-3}, respectively (Srinivas and Sarin 2013), which is too low to make a significant contribution to the DIN pool of the estuary or the coastal waters.

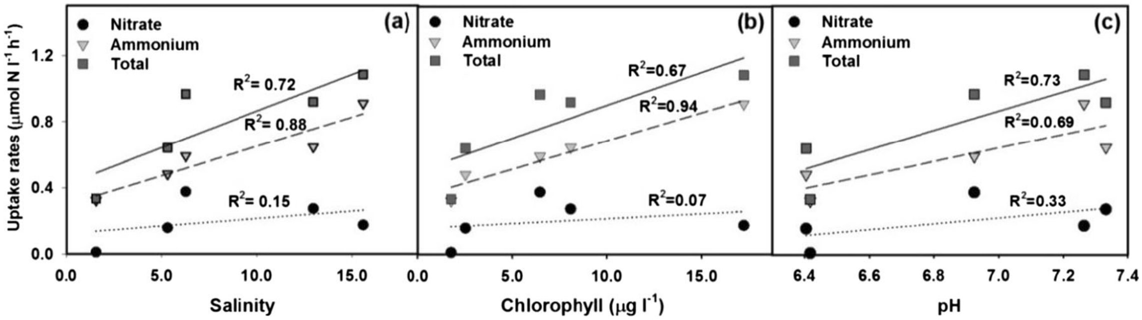


Fig. 5 Relationships between N uptake rates and **a** salinity, **b** chlorophyll *a*, and **c** pH in the Cochin estuary

Effect of N Uptake in the Cochin Estuary

The turnover times of NO_3^- and NH_4^+ pools in the estuary, calculated by dividing the nutrient concentrations with the respective uptake rates, varied from 7.13 h (station 2) to 2419 h (station 1) for NO_3^-, whereas it varied from 3.56 h (station 4) to 232 h (station 1) for NH_4^+ (Fig. 9a). Overall, the turnover of DIN pool in the estuary was faster except at station 1 (relatively longer turn over time due to higher ambient concentrations and lower uptake rates), which indicates that the continuous supply of nutrients through rivers and anthropogenic activities is assimilated and recycled fast in the estuary, underscoring its dynamic nature.

A balance between potential nitrification (800 nmol N l^{-1} h^{-1}) and conservative ammonification (~1 μmol l^{-1} h^{-1}) rates were observed for the Cochin estuary (Miranda et al. 2008). Any further removal of NH_4^+ (in turn DIN) through microbial assimilation, as observed during the present study, and subsequent nitrification and denitrification in the system should lower or mitigate the NH_4^+ pollution in the estuary. However, the sustained higher NH_4^+ concentration in the estuary points to the contrary. It is obvious that high rates of biological assimilation and nitrification rates in this estuary are only moderately effective in mitigating NH_4^+ pollution. Therefore, external sources of nutrients need to be monitored and controlled to alleviate NH_4^+ pollution in the estuary. Preliminarily, the fast assimilation of NH_4^+ (present study) and its mineralization (Miranda et al. 2008) in the Cochin estuary are indicators of estuarine responses towards increased anthropogenic activities (Shoji et al. 2008; Gupta et al. 2009).

Influence of TN:TP and Environmental Conditions on N Assimilation

Although estuaries, in general, receive high nutrient loadings, phytoplankton growth may eventually be limited by the nutrient which is proportionately low relative to the others. Phosphorus is often the nutrient limiting primary production in freshwater and estuarine systems (Schindler 1977; Hecky and Kilham 1988; Yin and Shan 2001) where N:P plays an important role in regulating N metabolism. In the Cochin estuary, TN:TP varied from 6.5:1 to 32:1 and showed strong negative correlation with salinity (Fig. 6a). This observation implies that the freshwater supplied to the estuary by the rivers contains a relatively higher proportion of N than P. The highest TN (118 μM) and TP (3.6 μM) during the present study were observed at station 1, a location where the river Periyar meets the estuary, but still showed comparative limitation of P (TN:TP ~31.9:1).

Importantly, the negative relationship observed during the present study between TN:TP and NH_4^+ uptake rates (as well as N_2 fixation; Fig. 6b) suggests that the N assimilation in the Cochin estuary, in general, is co-limited by P. Higher DIN uptake and N_2 fixation rates were observed at locations where TN:TP was low. Station 1, despite having very high TN and

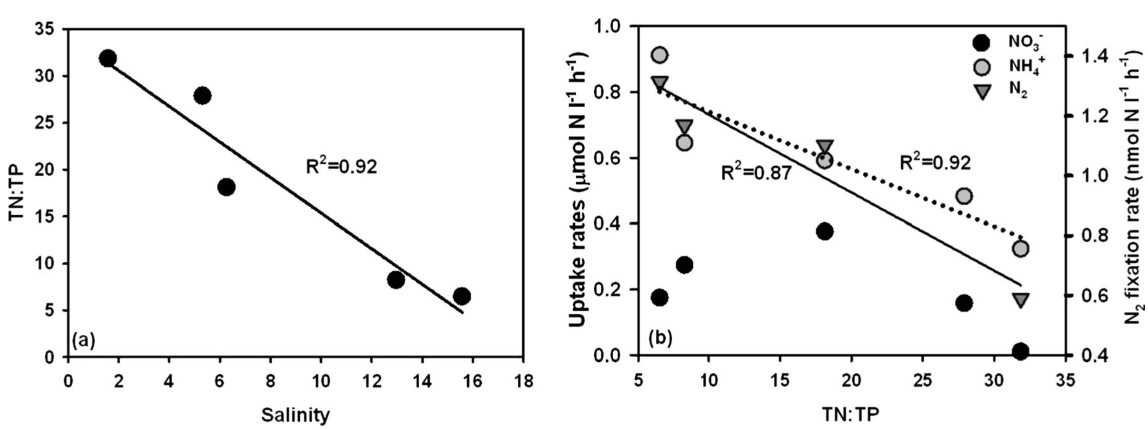


Fig. 6 **a** TN/TP along the salinity gradient. **b** Relationship between DIN uptake and N_2 fixation rates with TN/TP in the Cochin estuary

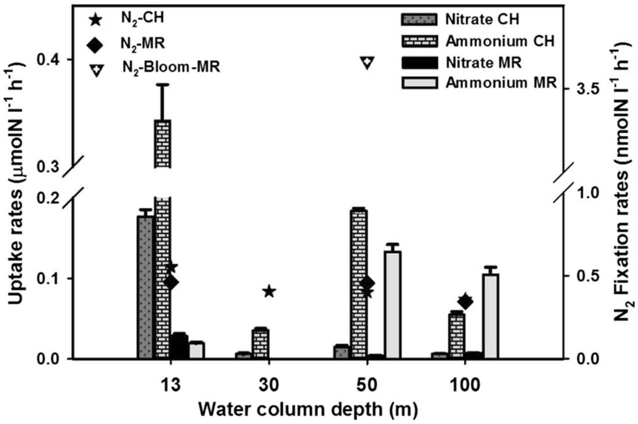


Fig. 7 Surface NO_3^- and NH_4^+ uptake along with N_2 fixation rates at the Off Cochin and Off Mangalore stations. *Error bars* represent 1σ

TP (also the highest DIN; Fig. 6b), showed the lowest DIN uptake and N_2 fixation rates, whereas station 2, with lower DIN concentrations, showed the highest uptake rates. The above mentioned negative relationship holds for inorganic fractions (DIN:DIP) as well (R^2=0.74 with NH_4^+ uptake and R^2=0.76 with N_2 fixation) in the estuary.

The DIN uptake rates in the estuary did not show significant relationships with NO_3^- and NH_4^+ concentrations, possibly due to their dependence on P and other environmental parameters. A significant positive correlation of NH_4^+ uptake rates with salinity (R^2=0.88), pH (R^2=0.69), and Chl *a* (R^2= 0.94; Fig. 5) indicates that these parameters might have influenced the N uptake as well. In general, NO_3^- uptake did not depend on any of these parameters in the estuary. The turbidity, cloudiness, and preference for NH_4^+ may have caused fluctuations in NO_3^- uptake rates and poor correlations with the environmental parameters (Kanda et al. 1989).

Table 3 Column integrated uptake rates (mmol N m^{-2} h^{-1}) in the coastal waters

Stations	Depth (m)	NH_4^+	NO_3^-
Off Cochin	30	1.05	0.93
	50	7.41	0.41
	100	8.08	0.97
Off Mangalore	50	6.25	0.28
	100	5.71	0.38

Nitrogen Uptake in the Coastal Waters

The maximum uptake rates for NO_3^- (0.18 µmol N l^{-1} h^{-1}) and NH_4^+ (0.34 µmol N l^{-1} h^{-1}; Fig. 8) along the coastal transects were observed in the nearshore surface waters of Cochin (CH1-0) with the highest ambient concentrations of NO_3^- (0.89 µM) and NH_4^+ (1.94 µM; Fig. 2). These rates are near the lower limit of the uptake rates observed in the Cochin estuary. Interestingly, a similar station off Mangalore (MR1-0) had low uptake rates (0.03 and 0.02 µmol N l^{-1} h^{-1} for NO_3^- and NH_4^+, respectively) and ambient concentrations (0.24 and 0.97 µM, respectively; Fig. 2). The corresponding surface

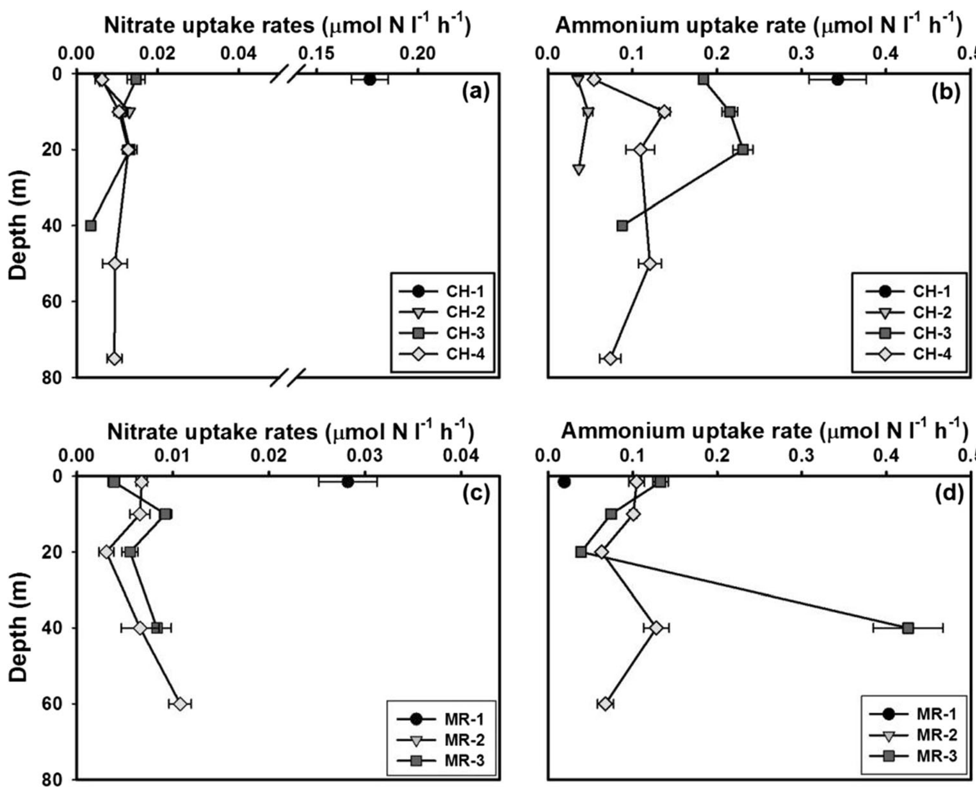
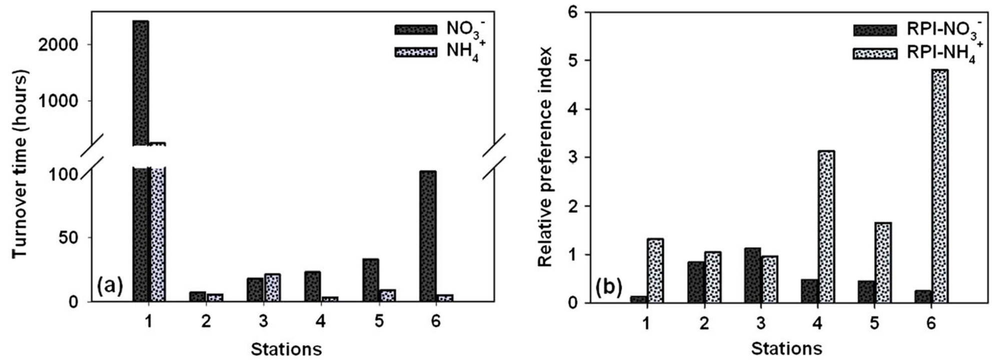


Fig. 8 Depth profiles of the NO_3^- and NH_4^+ uptake rates in coastal waters: **a** NO_3^- (CH), **b** NH_4^+ (CH), **c** NO_3^- (MR), and **d** NH_4^+ (MR). CH and MR indicate Cochin and Mangalore transects, respectively. *Error bars* represent 1σ

Fig. 9 **a** Turnover time of NO_3^- and NH_4^+ and **b** relative preference index of NH_4^+ and NO_3^- at the estuarine stations (*1*–*5*) and Vembanad Lake (*6*)



salinity off Cochin was low (CH1-0:33.9) compared to that of Mangalore (MR1-0:35.2), possibly indicating a greater influence of tidal-driven estuarine inputs on the uptake rates at the Cochin coast than at Mangalore.

In general, the uptake rates for NO_3^- (0.006–0.18 μmol N l^{-1} h^{-1}) and NH_4^+ (0.04–0.34 μmol N l^{-1} h^{-1}) were higher in the off Cochin surface waters than along the Mangalore transect (0.003–0.028 and 0.02–0.42 μmol N l^{-1} h^{-1} for NO_3^- and NH_4^+, respectively). This pattern is due to the nutrient-rich estuarine water discharge to the nearshore region of the Cochin transect. The total DIN uptake rates observed in estuarine and coastal waters are significantly higher than the corresponding rates observed under bloom conditions in the Arabian Sea (Kumar et al. 2010; Gandhi et al. 2010). Unlike the Cochin estuary, DIN uptake and N_2 fixation rates in the coastal waters did not relate to nutrient stoichiometry or any other environmental variables. This trend suggests the major role of varying light conditions and phytoplankton composition with depth (Kanda et al. 1989).

Preferred Substrate and Size Fractionation

Our data showed a consistently higher NH_4^+ than NO_3^- uptake rates. An $RPI_{(nitrate)}$ <1 indicates preference for NH_4^+ (McCarthy et al. 1977). During the present study, $RPI_{(nitrate)}$ was less than 1 at most of the stations and rarely exceeded 2, except at the Mangalore nearshore surface station (MR1-0; RPI=2.88). Overall, $RPI_{(nitrate)}$ ranged from 0.05 to 2.88 in the coastal waters and 0.13 to 1.12 in the estuarine waters (Fig. 9b). The $RPI_{(nitrate)}$ did not correlate with NO_3^- or NH_4^+ concentrations in the estuarine or coastal waters.

The results from the present study suggest unequivocally that NH_4^+ was assimilated in preference to NO_3^-, and primary production was supported mainly by the regenerated N. These observations are consistent with N uptake studies from the open Arabian Sea (Sambrotto 2001; Kumar et al. 2010). The preference for reduced or recycled forms of N such as NH_4^+ is well known in the aquatic environments (e.g., Dugdale and Goering 1967; Glibert et al. 1982; Rees et al. 1995) due to higher energy requirement for the assimilation of NO_3^- (McCarthy and Carpenter 1983). Inhibition of NO_3^- uptake by NH_4^+ could be one of the reasons for lower NO_3^- uptake, a commonly observed feature in aquatic ecosystems (e.g., Glibert et al. 1982; Harrison et al. 1996; Rees et al. 1995). Although the extent of inhibition is variable, the inhibition appears to be greater under NH_4^+-rich conditions and the least when phytoplankton are N-starved (Conway 1977; Dortch 1990). During the present study, ambient conditions were nutrient replete, particularly in the estuarine region, which may have contributed to relatively lower NO_3^- uptake. Another reason may be higher than required addition of $^{15}NH_4^+$ tracer during the experiment, which could trigger artificial stimulation of NH_4^+ uptake (Watts and Owens 1999); however, the addition of NH_4^+ was within the recommended limits (i.e., less than 10 % of ambient). Iron and silicon limitation can also cause NO_3^- underutilization (Landry et al. 1997), but the high concentrations of dissolved iron (up to 459 μg l^{-1} (Balachandran 2001) and 663 μg l^{-1} (Anju et al. 2011)) and Si (present study) indicate that Fe and Si are not limiting in the Cochin estuary.

Although small phytoplankton and heterotrophic bacteria play a significant role in N uptake (Laws et al. 1985; Kirchman 1994; Middelburg and Nieuwenhuize 2000b), we did not focus on plankton size effects. The nanoplankton (2–20 μm) contributes about 80 % of primary production in the Cochin estuary and coastal waters followed by microplankton (>20 μm) and picoplankton (0.2–2.0 μm) during summer monsoon (Martin et al. 2010). Similarly, >75 % of productivity in the north-western Arabian Sea was supported by nano and pico fractions (Watts and Owens 1999), whereas contribution of heterotrophic bacteria can be as high as 70 % of total NH_4^+ uptake in some aquatic ecosystems (Gardner et al. 2004). The present study could not estimate the contribution of bacteria on DIN uptake rates because the bacteria lost during filtration through GF/F filters (Watts and Owens 1999) were not quantified. Therefore, the uptake of DIN by bacteria and the role of DON as an alternative source of N should be addressed in future studies.

N_2 Fixation in the Estuary and Coastal Waters

N_2 fixation rates were measured to clarify the relevance of diazotrophic activity in the eutrophic Cochin estuary and the coastal Arabian Sea. *Trichodesmium*, a major diazotroph, fixes atmospheric N and contributes to the bioavailable N pool in aquatic systems (Capone et al. 1997; Mulholland and Capone 2009; Subramanian and Carpenter 1994). A gradual decline in *Trichodesmium* abundance from 1970 to 2005 along with an increase in nutrient loading was observed in the Cochin estuary (Qasim et al. 1969; Thomson 2002; Martin et al. 2013). Our recent observations indicate the presence of diazotrophs, particularly *Synechocystis* and *Synechococcus*, in the Cochin estuary (Jabir et al., personal communication).

N_2 fixation rates in DIN-enriched waters are generally expected to be low because high NH_4^+ concentrations (>1 μM) can suppress the nitrogenase activity (Mulholland and Capone 2001). A significant negative correlation (R^2=0.89) of N_2 fixation rates with NH_4^+ concentrations was observed in the Cochin estuary as well (figure not shown). Interestingly, in contrast to results in coastal waters, positive correlations of estuarine N_2 fixation rates with NH_4^+ uptake (R^2=0.89), NO_3^- uptake (R^2=0.45), and total DIN uptake (R^2=0.97) rates were also observed (figure not shown). The occurrence of diazotrophic activity in nutrient replete waters of the estuary and its relationship with DIN rates is intriguing but it may stem from the fact, as discussed earlier, that the N assimilation rates in the estuary depend more on N:P ratios than on DIN concentrations alone. DIN uptake as well as N_2 fixation rates increase with the relative availability of P.

The air was warm with intense solar radiation (SST: 30.02 to 31.44 °C) during the time of our coastal observations. Warm, calm, and oxic conditions facilitate proliferation of *Trichodesmium erythraeum* (Jabir et al. 2013). High concentrations of dissolved iron and P in inner shelf waters of Cochin (Balachandran 2001; Anju et al. 2011) enhance N_2 fixation in this region.

Based on the estimations from Mulholland et al. (2006), N_2 fixation was examined as a new N source here. Assuming that a maximum of 50 % of the fixed N is released in the form NH_4^+, the contribution of N_2 fixation to the NH_4^+ pool in the Cochin estuary was less than 0.05 % (0.04±0.042 %). This result was mainly due to higher ambient NH_4^+ concentration in the estuary. Similar calculations for coastal stations revealed a contribution of less than 0.5 % (average ~0.17±0.14 %) with the highest at the *Trichodesmium* bloom patch (0.42 %). N_2 fixation was not an apparent significant source of new N to the Cochin estuary and the coastal Arabian Sea. Previous studies in the open Arabian Sea, however, indicate that *Trichodesmium* bloom patches are a significant source of new N in the surface waters (Bange et al. 2000; Gandhi et al. 2011).

Conclusions

Significantly higher DIN uptake rates in the Cochin estuary and at a nearshore location in the adjacent Arabian Sea compared to other coastal locations provide a clear evidence of anthropogenic effects on N uptake dynamics in the region. These results suggest that nutrients delivered via rivers from anthropogenic activities upstream have the potential ability to modulate the estuarine-coastal biogeochemistry. Measurable N_2 fixation rates despite very high DIN concentrations were observed in the estuary, which disproved our central hypothesis. Significant negative correlations of NH_4^+ uptake and N_2 fixation rates with TN:TP suggest that the N assimilation in the estuary depends more on the relative balance of N and P than on just N or P alone.

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