Transformation and transport of inorganic nitrogen in sediments of a southeast Asian mangrove forest

Erik Kristensen1,*, Mikael H. Jensen1,2, Gary T. Banta3, Kim Hansen1, Marianne Holmer1, Gary M. King1

1Institute of Biology, Odense University, DK-5230 Odense M, Denmark
2Nature Management and Water Quality Division, County of Fyn, Ørbækvej 100, DK-5220 Odense SØ, Denmark
3Department of Life Sciences and Chemistry, Roskilde University, Box 260, DK-4000 Roskilde, Denmark
4Darling Marine Center, University of Maine, Walpole, Maine 04573, USA

ABSTRACT: The most important inorganic nitrogen transformations (nitrification, denitrification and N fixation) and DIN fluxes in darkened and inundated sediments were studied during the dry season in the Ao Nam Bor mangrove forest at the island of Phuket, Thailand. Dark fluxes of NH4+ were low (not significantly different from zero) and tended to be directed into the sediment in the area vegetated by the tree Rhizophora apiculata (204 pmol m-2 d-1) and out of the sediment in an adjacent unvegetated area (80 pmol m-2 d-1). NO3- appeared to be taken up by the sediment in both areas, although at rates not significantly different from zero (134 and 85 pmol m-2 d-1, respectively). The trend for higher uptake of DIN in the vegetated area may be related to microbial assimilation during degradation of nitrogen-poor tree litter. Nitrification rates, estimated from potential assays using oxygen penetration depth or measured as coupled nitrification-denitrification using a 15N isotope-pairing technique, were also very low (12 to 74 pmol m-2 d-1). Nitrification rates appeared higher and penetrated deeper in the vegetated than the unvegetated sediments as substantiated by higher concentrations of porewater NO3-. Denitrification rates were 3.5 times higher in the vegetated (46 pmol m-2 d-1) than the unvegetated sediments (13 pmol m-2 d-1). Since more than 90% of the NO3- needed by denitrifiers originated from nitrification (coupled nitrification-denitrification), only 1 to 2% of the measured NO3- influx from the overlying water was consumed by denitrification. N fixation (284 to 390 pmol m-2 d-1) in the present mangrove sediments was estimated to account for about 10% of the net demand by primary producers. About half of the measured N fixation was due to fixation by sulfate reducing bacteria. The Ao Nam Bor mangrove forest was characterized by low concentrations, fluxes and rates of microbial transformations of DIN, suggesting a tight coupling between mineralization and assimilation processes. These nitrogen-poor sediments acted as sinks for nitrogen, due to microbial assimilation, and the presence of trees in the vegetated sediment was evident as a 50% higher net retention of DIN.

KEY WORDS: Nitrogen cycling · Nitrification · Denitrification · N fixation · Mangrove sediments

INTRODUCTION

Most intertidal coasts in the tropics are lined with mangrove forests (Por 1984). This widespread and productive ecosystem supports a unique assembly of animals and plants, and provides both an important linkage and buffer zone between land and ocean (Boto & Wellington 1988, Morell & Corredor 1993). However, since mangrove forests have been threatened by massive destruction during the last decades caused by charcoal logging and shrimp farming (Chansang et al. 1982, Hatcher et al. 1989, Macintosh 1996), there is an urgent need for detailed knowledge on the functional role of mangroves with respect to transport and transformations of nutrients.

The role of mangroves in the exchange of nutrients with coastal waters has been examined in an increasing number of studies in recent years (Boto & Robertson 1990, Morell & Corredor 1993). Although the general knowledge on mangrove nutrient dynamics has improved, the results are in a number of cases equivocal and sometimes conflicting, emphasizing the need

*E-mail: ebk@biology.ou.dk

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for further studies in this important environment. A characteristic feature of these forests is very low levels of dissolved and particulate nutrients, and the forests generally appear to be N or P limited (Boto & Wellington 1983, Alongi 1996). As a consequence, they possess a high capacity for retaining and recycling nutrients by several mechanisms that reduce export (Twilley et al. 1986). Wiebe (1989) also suggested that mangroves are a sink for nutrients, but warned that such generalization may be tentative. The extent to which mangroves exchange nutrients with adjacent waters depends upon a variety of factors, like geomorphology, tidal regime, climate and groundwater inputs (Alongi 1989, Alongi et al. 1992).

Studies on nitrogen cycling have mostly been concerned with the transport of dissolved and particulate forms between mangrove forest and the ocean, whereas the role of sediment processes has been addressed to a lesser extent (Boto & Wellington 1988, Morell & Corredor 1993, Rivera-Monroy et al. 1995a, b, Alongi 1996). The general features of mangrove sediments with respect to inorganic nitrogen are low concentrations, low uptake rates from the tidal water, and low rates of transformation processes. Many of the sediment investigations have focused on porewater pools and the exchange of dissolved nitrogen between the sediment and tidal waters (Alongi et al. 1993, Alongi 1996), while others have dealt with specific microbial nitrogen transformations, like nitrogen fixation and denitrification (Morell & Corredor 1993, Nedwell et al. 1994, Rivera-Monroy et al. 1995b). However, there are to our knowledge only few mangrove studies in which an array of dissolved inorganic nitrogen transformations have been combined and examined simultaneously.

The purpose of this study was to determine the most important inorganic nitrogen transformations in the Ao Nam Bor mangrove forest in Thailand. Simultaneous measurements of NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-} fluxes, nitrogen fixation and denitrification (Morell & Corredor 1993, Nedwell et al. 1994, Rivera-Monroy et al. 1995b) have been combined and examined simultaneously.

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**MATERIALS AND METHODS**

**Study site.** Samples were collected in the dry season during January 1992 in the 300 m wide mangrove forest Ao Nam Bor in Makham Bay, ca 4 km south of Phuket Town on the east coast of Phuket Island, Thailand. A detailed description of the study site is given by Kristensen et al. (1995). Two sampling stations in the low- and mid-intertidal zone, respectively, were chosen for this study.

The mid-intertidal Stn 2 was located within the mangrove forest between 'prop' roots of Rhizophora apiculata ca 50 m from the seaward fringe. The sediment was heterogeneous and composed of grey-brown silt (90% of particles < 63 \(\mu m\) with an organic content of about 3% C and 0.15% N (molar C:N ratio of about 24). Only the upper 4 to 6 cm was devoid of roots (silt zone); below was a deep root zone with peat-like appearance. The silt zone was never black and sulfidic, whereas scattered spots were apparent around dead roots in the root zone. This location was moderately affected by burrowing fauna (sesarmid crabs and sipunculids; Kristensen et al. 1994). The average diurnal water cover for January 1992 was 7.3 h.

Stn 3 was located in the low-intertidal zone on the tidal flat ca 100 m outside the mangrove forest. The sediment appeared more sandy in the upper centimetres (40% of particles < 63 \(\mu m\) than at the other station. The sediment below was homogeneous and consisted of silty, non-sulfidic sand (70% of particles < 63 \(\mu m\) down to ca 30 cm depth followed by a zone of coarse coral sand. The organic content increased from about 0.5% C and 0.02% N in the upper 5 cm to 1.0% C and 0.04% N below 10 cm depth (molar C:N ratio of about 30). The dominating burrowing animals at this station were ocyopodid crabs, mudskippers and small (1 to 3 cm long) polychaete worms of unknown species (Kristensen et al. 1994). The average diurnal water cover for January 1992 was 9.6 h. Salinity and water temperature during sampling were 33 to 35% and 28 to 33\(^\circ\)C at both stations.

**Fluxes and porewater profiles.** For determination of dissolved inorganic nitrogen (DIN = NH\textsubscript{4}\textsuperscript{+}, NO\textsubscript{2}\textsuperscript{-} and NO\textsubscript{3}\textsuperscript{-}) flux across the sediment-water interface during inundation, 4 sediment cores (8 cm in diameter and 13 cm deep) were sampled per station during low tide. In the laboratory, the still air-exposed, but darkened, cores were pre-incubated at in situ temperature (29\(^\circ\)C). After 1 to 4 h, seawater was carefully added, resulting in a water column height of 9 cm. Flux rates of DIN were obtained by standard closed-core incubations of 2 to 7 h duration in darkness according to Kristensen et al. (1991). Water samples for DIN analysis were taken at the start and end of incubation, assuming linear concentration change with time. This was occasionally verified by sequential samplings (data not shown). The samples were GF/C filtered and stored frozen in polyethylene vials until analysis using standard autoanalyzer methods of Solorzano (1969) for NH\textsubscript{4}\textsuperscript{+} and Armstrong et al. (1967) for NO\textsubscript{2}\textsuperscript{-} and NO\textsubscript{3}\textsuperscript{-}. As NO\textsubscript{2}\textsuperscript{-} concentrations were very low and constant, this compound is included here in the presented NO\textsubscript{3}\textsuperscript{-} data.
Three sediment cores (5 cm in diameter and 30 cm deep) for porewater extraction were sampled at each of the 2 stations. Within 2 h of sampling, the cores were sectioned into 1 and 2 cm segments, and porewater was extracted by centrifugation for 5 to 10 min at 2000 rpm (250 × g). The supernatant porewater was GF/C filtered and stored frozen for later DIN analysis as mentioned above.

**Potential nitrification.** Potential nitrification was determined in aerobic sediment slurries as the accumulation of NO$_3^-$ after addition of chlorate. Immediately after sampling during low tide, 2 sediment cores (5 cm diameter, 20 cm deep) were subcored to 15 mm depth by 5 ml cut-off syringes. The subcores were extruded and sectioned into 0–2, 2–4, 4–7, 7–10 and 10–15 mm depth intervals. Duplicate samples of about 0.3 g wet weight from each section were transferred to 30 ml serum bottles containing 10 ml of seawater enriched with NH$_4$Cl, KH$_2$PO$_4$ and NaClO$_3$ to concentrations of 500 μM, 50 μM and 10 mM, respectively. The addition of chlorate (NaClO$_3$) inhibits the oxidation of NO$_3^-$, which will accumulate in the slurry and can easily be analyzed (Belser & Mays 1980). The serum bottles were incubated aerobically in the dark at in situ temperature (29°C) on a shaking table. After 0, 3, 6 and 10 h, subsamples of 1.5 ml were taken and centrifuged at 3000 rpm (375 × g) for 5 min. The supernatant was stored frozen until spectrophotometric analysis according to Strickland & Parsons (1972). Potential nitrification was determined from the slope of NO$_3^-$ increase with time and related to sediment volume.

**Denitrification.** A modification of the $^{15}$N isotope-pairing technique of Nielsen (1992) was used for measurement of in situ denitrification rates. A total of 7 to 11 sediment cores (3.6 cm in diameter and 5 cm deep) from each station were taken at low tide and transported to the laboratory for further processing within an hour. In the laboratory, cores were carefully supplied with air-saturated seawater (from the sampling site) to a depth of 5 cm, and submerged in a darkened tank containing about 30 l of the same water. Cores were equipped with magnetic stirring bars receiving momentum from a rotating external magnet (60 rpm). The cores were then allowed to equilibrate uncapped for 3 to 4 h at in situ temperature (29°C).

The denitrification assay was initiated by adding $^{15}$NO$_3^-$ (99%) to the tank water at a final concentration of 30 μM. The ambient $^{15}$N level was assumed negligible. The uncapped cores were pre-incubated for 12 to 14 h with $^{15}$NO$_3^-$ in order to obtain a near steady state (>90%) efflux of $^{15}$N$_2$ and $^{15}$N$_2$ (Nielsen 1992). The incubation was initiated by capping the cores with inverted glass petri dishes. Preliminary experiments had shown that this was sufficient to prevent exchange of dissolved gases. After incubation for 1 to 4 h (tests showed that O$_2$ never decreased below 75% of air saturation), cores were removed from the incubation tank, uncapped and 45 ml of water (final sample) was taken immediately from each core using a 50 ml plastic syringe. Care was taken not to introduce air bubbles into the syringe. Triplicate water samples were taken from the tank water for determination of initial (start sample) DIN concentrations and isotopic composition as mentioned above.

After placing a hypodermic needle on the tip of each syringe, 5 ml of the sample was discarded and 5 ml of pure O$_2$ was slowly sucked into the syringe, which was stoppered by a rubber stopper. Then the syringe was shaken vigorously for 2 min to equilibrate dissolved N$_2$ with the headspace, and a 3 ml gas sample was withdrawn into a pre-evacuated Venoject tube (Terumo Corp., Belgium) immediately after removing the rubber stopper. The remaining water sample was frozen for later DIN analysis as previously described.

Nitrogen gas isotopic composition was analyzed by injecting 0.2 ml of the Venoject subsamples into a gas chromatograph in line with a Sira Series II isotope ratio mass spectrometer. Denitrification rates were calculated from $^{2}$N$_2$ and $^{15}$N$_2$ production rates during incubations as described by Nielsen (1992).

**Nitrogen fixation.** Sediment nitrogen fixation was determined by a modified version of the classic acetylene reduction technique (Stewart et al. 1967, Capone 1988). Sediment cores similar to those used for porewater extraction (5 cm i.d.) were sectioned into 1 cm slices and the depth intervals 0–1, 5–6 and 14–15 cm (Stn 2) or 19–20 cm (Stn 3) were used for the N-fixation assay. Triplicate subsamples of about 3 g sediment from each depth interval were transferred to 30 ml serum bottles together with 2 ml of deoxygenated seawater (N$_2$ purged). Although all visible Rhizophora apiculata roots were removed from Stn 2 subsamples, small roots may still have been present. Subsequently, the bottles were flushed with N$_2$ for a few minutes before being sealed with butyl stoppers and placed on a shaking table in the dark at in situ temperature (29°C). After a few minutes of equilibration, 3 ml of the gas phase was evacuated and replaced with 3 ml of C$_2$H$_2$. Sampling was initiated about 20 min later and repeated 5 times at 10 to 15 min intervals. During sampling, 0.5 ml gas was withdrawn using a 1 ml syringe (dispo glass) and transferred in triplicates to Venoject tubes. The Venojects had been previously opened, cleaned and sealed with stoppers that did not release hydrocarbons. A vacuum was created in the Venojects immediately prior to injecting samples. Samples were assayed after bringing the Venojects to atmospheric pressure with air and adding 0.5 ml of an ammoniacal silver nitrate solution to precipitate
acetylene as silver acetylide (David et al. 1980). Subsamples of 0.5 ml from the Venloject headspace were analyzed on a Shimadzu GC-14A equipped with a Porapak Q column in series with a capillary column containing DB-1 and detected by FID. N fixation was estimated based on the theoretical 3:1 ratio between ethylene produced and N₂ fixed (e.g. Capone 1988), since no calibration was done here against a direct (e.g. ¹⁵N) method.

In order to determine the impact of a surplus inorganic nitrogen source and the role of sulfate reducing bacteria on N fixation in the sediment, 2 parallel series from the 5 to 6 cm depth interval were added either NH₄⁺ to a concentration of 2 mM or the specific inhibitor of bacterial sulfate reduction, Na₂MoO₄, to a concentration of 20 mM. The sampling and analysis were done as mentioned above for unamended sediment.

The association of N-fixing bacteria with roots of mangrove trees was determined by adding 50 to 100 μg live roots to the incubation bottles instead of sediment. Fine and live roots from the tree Rhizophora apiculata were carefully removed from the sediment and rinsed in seawater to remove adhered particles before being transferred to the incubation bottles. The sampling and analysis were done as mentioned above for sediment incubations.

**RESULTS**

**Sediment-water fluxes**

The dark flux of dissolved inorganic nitrogen (NH₄⁺ and NO₃⁻) across the sediment-water interface was low and mostly directed into the sediment (Table 1). At Stn 2, within the mangrove forest, both NH₄⁺ and NO₃⁻ tended to be taken up by the sediment from low concentrations in the overlying water. The situation at Stn 3 was similar for NO₃⁻, but NH₄⁺ appeared to be released here despite a 4 times higher concentration in the overlying water. It should be noted, however, that none of the average fluxes presented in Table 1 were significantly different from 0 (t-test, \(p > 0.05\)) due to high inter-core variability.

**Porewater profiles**

The 2 examined stations exhibited different depth profiles of dissolved NH₄⁺ and NO₃⁻ in the sediment (Fig. 1). Profiles of NH₄⁺ showed much higher concentrations at the non-vegetated Stn 3 than at the forested Stn 2. At the former, the concentration of NH₄⁺ increased to a level of 200 to 300 μM at 5 cm depth, remained almost constant down to about 12 cm, and increased again below this depth to 400-500 μM at 27 cm. NH₄⁺ remained at a low level between 10 and 50 μM at all depths down to 27 cm at Stn 2, with a small peak around 5 to 7 cm. The porewater concentration of NO₃⁻ showed the opposite pattern, with the highest level at Stn 2. However, the concentrations were generally very low and with high inter-core variability. NO₃⁻ remained at a level around 2 μM in the entire depth interval at Stn 2, whereas this level was approached only within the upper 3 cm at Stn 3. NO₃⁻ was hardly detectable below 5 cm depth at the latter station.

**Potential nitrification**

Although rates of potential nitrification appeared higher at Stn 2 than Stn 3 (Fig. 2), the difference was not significant at any depth (t-test, \(p > 0.05\)). Rates varied between 20 and 60 nmol cm⁻³ d⁻¹ within the upper

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**Table 1. Dark flux (mean ± SD, \(n = 4\)) of NH₄⁺ (\(J_{NH4}^-\)) and NO₃⁻ (\(J_{NO3}^-\)) across the sediment-water interface at Stns 2 and 3 in the Ao Nam Bor mangrove forest. Negative values indicate fluxes directed into the sediment. The overlying water concentration is shown for comparison.**

<table>
<thead>
<tr>
<th></th>
<th>(J_{NH4}^-) (μmol m⁻² d⁻¹)</th>
<th>[NH₄⁺] (μM)</th>
<th>(J_{NO3}^-) (μmol m⁻² d⁻¹)</th>
<th>[NO₃⁻] (μM)</th>
</tr>
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<tbody>
<tr>
<td>Stn 2</td>
<td>-204 ± 429</td>
<td>1.2</td>
<td>-134 ± 136</td>
<td>0.69</td>
</tr>
<tr>
<td>Stn 3</td>
<td>80 ± 371</td>
<td>4.7</td>
<td>-85 ± 292</td>
<td>0.63</td>
</tr>
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Table 2. Area-specific nitrogen transformations (μmol N m⁻² d⁻¹, mean ± SD of n measurements) at Stns 2 and 3 in sediment of the Ao Nam Bor mangrove forest. (1) Estimated nitrification based on potential nitrification measurements and the oxygen penetration depth; (2) estimated nitrification based on the degree of coupling between nitrification and denitrification found by the isotope-pairing technique; (3) measured denitrification based on the isotope-pairing technique; and (4) depth integrated (0 to 15 cm) N fixation based on slurry incubations.

<table>
<thead>
<tr>
<th></th>
<th>Stn 2</th>
<th>Stn 3</th>
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<tr>
<td>(1) Nitrification (pot)</td>
<td>59 ± 51</td>
<td>2 ± 10</td>
</tr>
<tr>
<td>(2) Nitrification (I⁵N)</td>
<td>43 ± 9</td>
<td>4 ± 12</td>
</tr>
<tr>
<td>(3) Denitrification</td>
<td>46 ± 9</td>
<td>4 ± 13</td>
</tr>
<tr>
<td>(4) N fixation (0 to 15 cm)</td>
<td>284 ± 10</td>
<td>3 ± 390 ± 21</td>
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</table>

13 mm of the sediment column at the former station. However, none of the variations with depth were significantly different due to high inter-core variability. Stn 3 was different, showing gradually, but significantly (p < 0.05), decreasing rates (from 40 to 16 nmol cm⁻³ d⁻¹) with depth from the surface down to 13 mm. The low variability in potential nitrification substantiates the relatively homogeneous composition of the upper sediment layers at this station.

Since nitrification is an aerobic process, the depth of oxygen penetration at the sediment surface is an indicator of the depth of the nitrification zone, and can be used to provide a maximum estimate of the surficial nitrification rate (Hansen et al. 1981). Oxygen penetration depths in darkness of 1.65 mm at Stn 2 and 1.9 mm at Stn 3 have previously been recorded in the Ao Nam Bor mangrove during the dry season (Kristensen et al. 1994). Based on these oxygen data and the potential nitrification results presented here, the maximum area-specific nitrification rates are estimated to be 59 and 74 mmol m⁻² d⁻¹ at Stns 2 and 3, respectively (Table 2).

Denitrification

The addition of 30 μM I⁵NO₃⁻ to the overlying water in cores used for the denitrification assay increased the total NO₃⁻ uptake by a factor of 7 to 10 (750 ± 300 μmol m⁻² d⁻¹ at Stn 2 and 1000 ± 390 μmol m⁻² d⁻¹ at Stn 3) compared to unamended cores (Table 1). Anyway, based on the isotope-pairing theory of Nielsen (1992), the formation of single-labelled (I⁴N₁⁵N) and double-labelled (I⁵N₁⁵N) dinitrogen can be used to estimate total denitrification of the naturally occurring I⁴NO₃⁻. By this method it is also possible to differentiate between denitrification of NO₃⁻ diffusing from the overlying water (uncoupled denitrification) and NO₃⁻ from nitrification within the sediment (coupled denitrification).

Total area-specific denitrification at the 2 stations in the Ao Nam Bor mangrove was low (Table 2). The rates measured at Stn 2 (46 μmol m⁻² d⁻¹) were almost 4 times higher than at Stn 3 (13 μmol m⁻² d⁻¹). Coupled denitrification accounted for most of the total rates, 90 to 97% at Stn 2 and 87 to 94% at Stn 3, due to the low NO₃⁻ concentration in the overlying water. This indicates an area-specific coupled nitrification rate of 43 and 12 mmol m⁻² d⁻¹ at Stns 2 and 3, respectively.

Fixation of atmospheric dinitrogen was relatively low in the upper silt zone at Stn 2 (1 to 2 nmol N cm⁻³ d⁻¹), but attained rates of twice this level in the underlying root zone (3 nmol N cm⁻³ d⁻¹ at 14 to 15 cm depth, Fig. 3). The difference was probably related to a high N fixation associated with live roots of Rhizophora apiculata [262 ± 100 nmol (g ww)⁻¹ d⁻¹]. N fixation at Stn 3 was relatively constant with depth down to 20 cm at a level about 50 to 100% higher than in the silt zone at Stn 2. The depth-integrated rates were about 40% higher at Stn 3 than Stn 2 (Table 2).

There was no apparent inhibitory effect of NH₄⁺ in surplus on rates of N fixation in the depth interval 5 to 6 cm at either Stn 2 or Stn 3 (Fig. 3). However, additions of Na₂MoO₄ to the same depth interval reduced N fixation to 70% at Stn 2 and 40% at Stn 3 of the rates found in uninhibited slurries (Fig. 3), implying that sul-

![Potential nitrification (nmol cm⁻³ d⁻¹)](image)
Trophic), dissimilatory reduction to NH$_4^+$ (nitrate assimilation generally prefers NO$_3^-$ (Boto et al. 1985), a substrate available to R. hizophora apiculata (Fig. 1) to uptake by tree roots. However, as R. hizophora apiculata prefers NO$_3^-$ uptake by assimilation (autotrophic and heterotrophic), the assimilatory demand by benthic microalgae (Stanley et al. 1987, Welling et al. 1990) is a highly insufficient substrate. Accordingly, the cycling of inorganic nitrogen must be rapid, efficient (i.e., tight), but not sufficient to support bacterial growth in mangrove sediments. Unfortunately, no measurements of NH$_4^+$ turnover are available from the present study, but a rapid turnover of 1.5 to 9 d$^{-1}$ in the upper 4 to 8 cm of the sediment has been reported from other tropical mangrove forests (Blackburn et al. 1987, Nedwell et al. 1993). The microbial demand for nitrogen in the vegetated sediment must be particularly high at the sediment-water interface since the porewater profile of NH$_4^+$, in contrast to the measured fluxes, indicates that there should be a considerable release from the sediment during inundation. Several flux studies in mangrove sediments have suggested that dissolved inorganic nitrogen in tidal waters is actually trapped in the upper few centimeters of the sediment due to a tight coupling between microbial mineralization and assimilation (Stanley et al. 1987, Alongi 1998, 1996, Boto & Wellington 1988, Rivera-Monroy et al. 1995b). In the non-vegetated area at Ao Nam Bor, where no litter accumulation occurs and porewater NH$_4^+$ turnover is relatively high in the upper 0.5 cm of the sediment (Alongi 1996, Rivera-Monroy & Twilley 1996), the assimilatory demand by benthic microalgae may contribute to a lower NH$_4^+$ release than expected (Kristensen 1990), even when measured in darkness as here (Rysgaard et al. 1993). The measured fluxes and porewater concentrations of NH$_4^+$ and NO$_3^-$ in Ao Nam Bor sediments are similar to other tropical mangrove sediments (Rivera-Monroy et al. 1995b, Alongi 1996, Rivera-Monroy & Twilley 1996). The somewhat lower concentrations and higher import (although not significantly) of nutrients in darkened Rhizophora apiculata vegetated than in non-vegetated sediment at Ao Nam Bor suggest a mangrove tree related demand. Such a pattern was also noted by Alongi (1996) for Australian mangrove forests. It is tempting to relate the apparent reversal in NH$_4^+$ flux pattern between the 2 study areas (Table 1) together with an order of magnitude lower concentration of this solute in the porewater at the vegetated site (Fig. 1) to uptake by tree roots. However, as R. apiculata generally prefers NO$_3^-$ (Boto et al. 1985), a substantial part of the high NH$_4^+$ demand must instead be assigned to assimilation during microbial degradation of nutrient-poor tree tissues (litter, dead roots, twigs and trunks) in the sediment (Alongi 1996, Rivera-Monroy & Twilley 1996). Bacteria generally need substrates with an elemental C:N ratio below 10 for maintenance and growth (Fenchel & Blackburn 1979), so mangrove litter and other tree materials with C:N ratios of 100 or even more (Kristensen 1990) are a highly insufficient substrate. Accordingly, the cycling of inorganic nitrogen must be rapid, efficient (i.e., tight), but not sufficient to support bacterial growth in mangrove sediments. 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In the non-vegetated area at Ao Nam Bor, where no litter accumulation occurs and porewater NH$_4^+$ turnover is relatively high concentrations, the assimilatory demand by benthic microalgae may contribute to a lower NH$_4^+$ release than expected (Kristensen 1993), even when measured in darkness as here (Rysgaard et al. 1993). The measured fluxes and porewater concentrations of NH$_4^+$ and NO$_3^-$ in Ao Nam Bor sediments are similar to other tropical mangrove sediments (Rivera-Monroy et al. 1995b, Alongi 1996, Rivera-Monroy & Twilley 1996). The somewhat lower concentrations and higher import (although not significantly) of nutrients in darkened Rhizophora apiculata vegetated than in non-vegetated sediment at Ao Nam Bor suggest a mangrove tree related demand. Such a pattern was also noted by Alongi (1996) for Australian mangrove forests. It is tempting to relate the apparent reversal in NH$_4^+$ flux pattern between the 2 study areas (Table 1) together with an order of magnitude lower concentration of this solute in the porewater at the vegetated site (Fig. 1) to uptake by tree roots. However, as R. apiculata generally prefers NO$_3^-$ (Boto et al. 1985), a substantial part of the high NH$_4^+$ demand must instead be assigned to assimilation during microbial degradation of nutrient-poor tree tissues (litter, dead roots, twigs and trunks) in the sediment (Alongi 1996, Rivera-Monroy & Twilley 1996). Bacteria generally need substrates with an elemental C:N ratio below 10 for maintenance and growth (Fenchel & Blackburn 1979), so mangrove litter and other tree materials with C:N ratios of 100 or even more (Kristensen 1990) are a highly insufficient substrate. Accordingly, the cycling of inorganic nitrogen must be rapid, efficient (i.e., tight), but not sufficient to support bacterial growth in mangrove sediments. Unfortunately, no measurements of NH$_4^+$ turnover are available from the present study, but a rapid turnover of 1.5 to 9 d$^{-1}$ in the upper 4 to 8 cm of the sediment has been reported from other tropical mangrove forests (Blackburn et al. 1987, Nedwell et al. 1993). The microbial demand for nitrogen in the vegetated sediment must be particularly high at the sediment-water interface since the porewater profile of NH$_4^+$, in contrast to the measured fluxes, indicates that there should be a considerable release from the sediment during inundation. Several flux studies in mangrove sediments have suggested that dissolved inorganic nitrogen in tidal waters is actually trapped in the upper few centimeters of the sediment due to a tight coupling between microbial mineralization and assimilation (Stanley et al. 1987, Alongi 1998, 1996, Boto & Wellington 1988, Rivera-Monroy et al. 1995b). 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monification) and N₂ (denitrification) in the surface layers.

Nitrification

The presence of NO₃⁻ in the porewater down to at least 30 cm depth in the vegetated sediment (Fig. 1) indicates active nitrification below the oxic surface layer. Active subsurface nitrification associated with roots of aquatic macrophytes has been observed in a number of cases (Reddy et al. 1989; Caffrey & Kemp 1992) and is due to downward translocation and release of O₂ by roots. The NO₃⁻ profile in the non-vegetated sediment, on the other hand, is typical for marine sediments where nitrification is restricted to the upper oxic surface layer (Henriksen & Kemp 1988). The depth pattern of potential nitrification parallels the observed NO₃⁻ profiles (Fig. 2). The generally higher rates with depth at the vegetated site, reflecting a higher population density or enzyme activity of nitrifying bacteria, suggests that nitrifiers are subject to superior (more oxic) conditions here compared with the non-vegetated sediment. However, numerous studies have confirmed that potential nitrification in sediments is only an indicator of nitrifiers being present, and not a measure of actual nitrification (e.g. Henriksen & Kemp 1988). The potential nitrification rates found here are in the low range of previously published rates from tropical mangrove sediments (0 to 300 nmol cm⁻³ d⁻¹; Iizumi 1986, Shaiful et al. 1986), and more than an order of magnitude lower than usually found in temperate coastal sediments (Henriksen & Kemp 1988).

When maximum area-specific nitrification rates at Ao Nam Bor were calculated from potential rates and oxygen penetration depths, almost similar rates were obtained in both sediment types (Table 2). These estimates are, however, uncertain due to a number of problems. First, nitrification in the oxic zone may be limited by ammonium availability compared to the potential assay where NH₄⁺ is provided in excess. The concentrations of NH₄⁺ in the surface sediment at Ao Nam Bor (20 and 60 µM in the upper 0.5 cm at Stns 2 and 3, respectively) are low compared with the half-saturation constant of ammonium oxidizers in culture (Kₘ = 50 to 70 µM), although natural samples have shown considerably lower kinetic constants (Kaplan 1983). Second, mixing and stirring of slurries often enhances microbial processes significantly compared to undisturbed sediment (Jorgensen 1978, Burdige 1989), due in part to the breakdown of diffusive barriers in the sediment matrix. Third, the use of oxygen penetration depths in darkened sediments for determination of diurnal rates may underestimate the nitrification rates during light exposure when microphytobenthic photosynthesis expands the oxic zone (Rysgaard et al. 1995).

The similarity between nitrification rates coupled to denitrification by the non-destructive ¹⁵N technique and those estimated from potential rates in the vegetated sediment (Table 2) suggests that the various problems associated with the latter technique may in our case have balanced out and probably by chance resulted in similar areal rates. Coupled nitrification determined by ¹⁵N should represent all nitrification in the sediment when no loss of NO₃⁻ occurs as indicated by the measured influx from the overlying water (Nielsen 1992). However, if a high coupled nitrification-assimilation occurred in the surface layers as suggested from profiles and fluxes of NO₃⁻ (as mentioned previously), then nitrification determined from ¹⁵N is underestimated concurrently. The slurry-estimated areal rate of nitrification at the non-vegetated sediment was 5 to 6 times higher than rates determined from ¹⁵N, probably due to the uncertainties mentioned above. Based on the coupled nitrification data from the isotope-pairing technique, the vegetated area supported a 3.5 times higher area-specific nitrification rate than the non-vegetated area. Since both techniques for determination of areal nitrification rates only include the oxic few mm thick surface layer of the sediment, the present results from vegetated sediment are probably underestimates due to translocation and release of O₂ to subsurface layers where nitrification may occur.

Denitrification

In accordance with the low NO₃⁻ levels, denitrification rates in the Ao Nam Bor mangrove forest were very low (Table 2). There are few other studies available on denitrification in mangrove sediments, and those of Rivera-Monroy et al. (1995b) and Rivera-Monroy & Twilley (1996), using a ¹⁵N enrichment technique, confirm the low rates. Actually, denitrification was barely detectable at in situ NO₃⁻ concentrations in these studies, and only ranged between 40 and 200 µmol m⁻² d⁻¹ in sediments amended with up to 200 µM NO₃⁻. The 3.5 times higher area-specific denitrification rate in the vegetated compared to the non-vegetated sediment at Ao Nam Bor was caused by the higher nitrification rate and thus higher porewater NO₃⁻ concentrations. Mangrove forests receiving large NO₃⁻ discharges from sewage treatments plants show relatively high denitrification rates (using mass balance or C₂H₂ blockage techniques) at a level 1 to 2 orders of magnitude higher than in the present study (Nedwell 1975, Corredor & Morell 1994). The denitrifi-
cation rates in these polluted mangrove forest are, however, comparable to rates (using the \(^{15}\)N isotope-pairing technique) obtained from unpolluted and relatively \(\text{NO}_3^-\) poor coastal sediments in temperate areas (e.g. Rysgaard et al. 1995), substantiating that the capacity for denitrification in mangrove sediments is generally low and not only related to \(\text{NO}_3^-\) availability.

Since more than 90% of the \(\text{NO}_3^-\) needed by denitrifiers in the present study originated from nitrification, only 1 to 2% of the \(\text{NO}_3^-\) influx from the overlying water was consumed by denitrification. This is in contrast to the results of Rivera-Monroy & Twilley (1996), where no coupled nitrification-denitrification was detectable in a mangrove forest from Mexico. Anyway, the present data largely support their final conclusion that mangrove sediments are sinks for \(\text{NO}_3^-\) from tidal waters due to assimilation by sediment bacteria and tree roots, but not by denitrification. The possibility of \(\text{NO}_3^-\) removal by reduction to \(\text{NH}_4^+\), as frequently observed in other coastal sediments (Koike & Sørensen 1988), was not addressed in the present study, but Rivera-Monroy & Twilley (1996) found that generally less than 2% of added \(^{15}\)NO\(_3^-\) (25 to 200 \(\mu\)M) was recovered as \(^{15}\)NH\(_4^+\) in a Mexican mangrove sediment.

### N fixation

As in the case of the potential nitrification assay, the slurry estimated rates of N fixation (Table 2) are subject to uncertainties. First, the slurry technique as used here may overestimate rates measured by non-destructive whole-core techniques by up to a factor of 40 (Welsh et al. 1996). Slurrying may release labile substrates from both roots and the sediment matrix as well as alleviating the natural diffusion limitation in sediments. Second, the theoretical 3:1 ratio used to transform acetylene reduction rates into N fixation is probably too low. While the theoretical ratio of 3:1 appears reasonable for pure cultures of bacteria (Stewart et al. 1967), measured ratios in marine sediments generally range between 2:1 and 10:1, with extreme values as high as 100:1 (Seitzinger & Garber 1987, Howarth et al. 1988). Third, simple depth integration to obtain area-specific rates of sediment N fixation in vegetated areas may result in a considerable underestimate. A number of studies have demonstrated that N fixation in the rhizosphere of sediments vegetated by a variety of rooted plants can be very high due to supply of oxygen and labile carbon (Capone 1988, O'Donohue et al. 1991, Welsh et al. 1996). This is substantiated in the present study by root associated rates up to 300 times higher by weight than sediment rates. Furthermore, structures such as cyanobacterial mats, decomposing logs and algal covered prop roots may contribute considerably to N fixation in mangrove ecosystems (Gotto & Taylor 1975, Boto & Robertson 1990, Sheridan 1991).

The rates found in the present study (Fig. 3) are comparable to previously published rates (100 to 200 \(\mu\)mol \(m^{-2} d^{-1}\)) from mangrove sediments using both slurry and whole-core \(\text{C}_2\text{H}_2\) techniques (Hicks & Silvester 1985, Iizumi 1986, Boto & Robertson 1990). The present data therefore confirm that mangrove sediments support relatively low N fixation compared with most intertidal sediments (e.g. seagrass beds and salt-marshes) from temperate climates (Capone 1988, Howarth et al. 1988, Welsh et al. 1996). However, in the present mangrove sediment, the microbial nitrogen supply (N fixation) seems to be up to an order of magnitude higher than the nitrogen loss (nitrification-denitrification) (Table 2).

The 30 to 60% reduction in N fixation after addition of molybdate to the slurries indicates that sulfate reducing bacteria contributed significantly to sediment N fixation (Fig. 3). For comparison, Welsh et al. (1996) found that addition of molybdate to slurries inhibited N fixation by more than 75% in sediments from seagrass beds. Sulfate reducers have previously been proposed to be the most important heterotrophic N-fixing group of organisms in marine sediments (Herbert 1975, Nедвед & Азиз 1980). Zuberer & Silver (1975) found that the most numerous N-fixing organism in mangrove sediments from Florida (USA) was the sulfate reducing bacteria of the genus Desulfovibrio.

\(\text{NH}_4^+\) can be quite important in regulating N fixation in aquatic environments due to repression of nitrogenase synthesis and a rapid and reversible inhibition of nitrogenase activity (Capone 1988). Since concentrations of \(\text{NH}_4^+\) are generally much higher in sediments than in water columns, \(\text{NH}_4^+\) inhibition of N fixation should be of more general significance in sediments. Studies of sedimentary N fixation have reported a threshold concentration for inhibition by \(\text{NH}_4^+\) of 100 to 200 \(\mu\)M (Carpenter et al. 1978, Teal et al. 1979). Nonetheless, in the present study no inhibition was observed when the \(\text{NH}_4^+\) concentration was increased from around 25 \(\mu\)M to about 2 mM \(\text{NH}_4^+\) (Fig. 3), suggesting a high degree of \(\text{NH}_4^+\) insensitivity by mangrove N-fixing organisms. Similarly, Welsh et al. (1997) found that although N fixation in seagrass sediments was inhibited by 60% when the \(\text{NH}_4^+\) concentration was increased from 0 to 50 \(\mu\)M, only little further inhibition occurred at concentrations up to 1 mM. This trend has been confirmed by culture studies, where a number of N-fixing organisms, including Desulfovibrio sp., appeared \(\text{NH}_4^+\) insensitive (Welsh et al. 1997). It is likely that factors other than \(\text{NH}_4^+\) inhibition are responsible for the low rates of N fixation in mangrove sediments.
Conclusions

Transformation rates and fluxes of dissolved inorganic nitrogen were low in sediments of the Ao Nam Bor mangrove. As indicated in the tentative N budgets in Fig. 4, darkened and inundated sediments imported more inorganic nitrogen by N fixation and uptake of NH$_4^+$ and NO$_3^-$ than was lost by denitrification. Accordingly, both the vegetated (Stn 2) and the non-vegetated (Stn 3) sediments acted as sinks for nitrogen due to autotrophic and heterotrophic microbial assimilation in these nitrogen-deprived environments. The presence of trees in the vegetated sediment was evident as a 50% higher net sediment retention of inorganic nitrogen, mostly due to assimilation during microbial degradation of nitrogen-poor litter and uptake by tree roots. However, it should be noted that the budgets in Fig. 4 do not represent 'true' diurnal values since (1) the blockage of NH$_4^+$ and NO$_3^-$ fluxes across the sediment surface during air exposure is ignored, and (2) DIN fluxes into the sediment as well as nitrification-denitrification may be enhanced in light due to microphytobenthic activity (Rysgaard et al. 1995).

Based on estimates of net primary production (microphytobenthos and Rhizophora apiculata) at Ao Nam Bor (Kristensen et al. 1995), dark NH$_4^+$ and NO$_3^-$ uptake by the sediment may account for 9% of the primary producers' nitrogen requirements in the vegetated area, while NH$_4^+$ efflux counteracted NO$_3^-$ uptake so that the sediment-water fluxes in the non-vegetated area contributed only 0.1% of the required nitrogen. The value found here for the vegetated area is about twice the estimated contribution of dissolved nitrogen fluxes in an Australian mangrove forest (Boto & Wellington 1988). Despite the same degree of uncertainty, the estimated heterotrophic N fixation in the sediment was equivalent to 7 and 10% of the net demand by primary producers in the vegetated and the non-vegetated areas, respectively. The former value is about 2 times higher than found by Boto & Robertson (1990). These authors also estimated that N fixation by all other structures in mangrove forests may be equal to the sediment contribution. Assuming the same here, N fixation in the vegetated area at Ao Nam Bor should account for up to 14% of the plant demand. Nitrification and denitrification, on the other hand, were insignificant in the mangrove sediments, as these processes only transformed inorganic nitrogen equivalent to less than 1% of net plant requirement. Consequently, as DIN fluxes and N fixation only provided up to 30% of the nitrogen requirement of primary producers, there must be other sources of nitrogen. Direct input of nitrogen as both precipitation and dry fall may contribute (e.g. Paerl 1993), but the low fluxes and process rates strongly indicate a major contribution by a tight coupling between mineralization and assimilation. Thus, nutrients in Ao Nam Bor sediments appear to be rapidly recycled with limited new supplies and losses.

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