Applied and Environmental Microbiology, Oct. 2005, p. 6142–6149 Vol. 71, No. 10
0099-2240/05/$08.00+0 doi:10.1128/AEM.71.10.6142–6149.2005

Correlation between Anammox Activity and Microscale Distribution of Nitrite in a Subtropical Mangrove Sediment

Rikke Louise Meyer,[1]* Nils Risgaard-Petersen,[2] and Diane Elizabeth Allen[3]

Advanced Wastewater Management Centre[1] and Department of Botany,[3] The University of Queensland, St. Lucia, Queensland 4072, Australia, and National Environmental Research Institute, Department of Marine Ecology, Vejlsøvej 25, DK-8600 Silkeborg, Denmark[2]

Received 22 January 2005/Accepted 10 May 2005

The distribution of anaerobic ammonium oxidation (anammox) in nature has been addressed by only a few environmental studies, and our understanding of how anammox bacteria compete for substrates in natural environments is therefore limited. In this study, we measure the potential anammox rates in sediment from four locations in a subtropical tidal river system. Porewater profiles of NO_x^- (NO_2^- plus NO_3^-) and NO_2^- were measured with microscale biosensors, and the availability of NO_2^- was compared with the potential for anammox activity. The potential rate of anammox increased with increasing distance from the mouth of the river and correlated strongly with the production of nitrite in the sediment and with the average concentration or total pool of nitrite in the suboxic sediment layer. Nitrite accumulated both from nitrification and from NO_x^- reduction, though NO_x^- reduction was shown to have the greatest impact on the availability of nitrite in the suboxic sediment layer. This finding suggests that denitrification, though using NO_2^- as a substrate, also provides a substrate for the anammox process, which has been suggested in previous studies where microscale NO_2^- profiles were not measured.

Anammox (anaerobic ammonium oxidation) is the anaerobic conversion of NO_2^- and NH_4^+ to N_2. Although its existence was suggested as early as 1965 (24, 25), the first direct evidence for this process came from studies of activated sludge from a wastewater treatment plant in The Netherlands only a decade ago (21). The process has since been studied extensively in enrichment cultures with a view to using it to treat nitrogen-rich wastewaters (34, 37, 41). A number of recent studies have also reported the presence of anammox in the natural environment, namely, in estuarine and offshore sediments (27, 31, 38, 39), in permanently anoxic bodies of water (7, 17), and in multiyear sea ice (30). Anammox may be an important pathway in global N cycling, since it can account for as much as 67% of benthic N_2 production (8, 27, 31, 38, 39), the remainder being produced by denitrification. However, the characterization of the biogeography of anammox, its significance compared to denitrification, and its regulation in nature is still incomplete, since the methods used to detect the presence and activity of anammox bacteria have become available only recently.

According to present knowledge, anammox is carried out by a few anaerobic, autotrophic bacteria belonging to the order *Planctomycetales* (32, 33). These bacteria have not been isolated in pure culture, and the current information about their physiology has been obtained from enrichment culture studies (11, 36). While enrichment culture studies have provided substantial insight into the physiology of the enriched species of anammox bacteria, these data may not be directly applicable to understanding of anammox in natural systems. The anammox organisms so far identified in marine systems, though still *Planctomycetales*, are only distantly related to the anammox bacteria studied in enrichment cultures (17, 27). Therefore, knowledge about anammox in nature must still come from a continuous effort to measure the distribution of the process in various environments and to relate biogeographical data to the physiochemical parameters that may influence the process. However, due to the limited information about factors controlling the process in natural systems, selection of those parameters must be based on hypotheses.

Stable-isotope (^{15}N) experiments in sediments have established that NO_2^- and not NO_3^- is the oxidized substrate for the anammox process (8, 27, 38, 39), although recent research suggests that some cultivated anammox bacteria can reduce NO_3^- to NO_2^- with propionate as the electron donor (15). Furthermore, marine anammox bacteria, in contrast to their competitors, the denitrifiers, appear to require a continuous supply of NO_x^- (NO_2^- plus NO_3^-) to maintain an active enzyme apparatus (26). Given that NH_4^+ is rarely limiting in sediments (see, e.g., reference 28), the availability of NO_2^- most likely controls the abundance of active anammox bacteria.

A consequence of this hypothesis is that the activity and presumably the abundance of these bacteria are strongly linked to the activity of other bacterial groups, since nitrite is produced as an intermediate in both nitrification and denitrification. The abundance of free NO_2^- in the sediment is, therefore, a result of the complex balance between diffusive transport, aerobic NH_4^+ and NO_2^- oxidation, and anaerobic NO_3^- and NO_2^- reduction. The availability of NO_2^-, as well as the identification of the principal source for net NO_2^- production in sediments, is still not well studied, due to the scarcity of techniques that allow measurements of NO_2^- with sufficiently high spatial resolution. However, with the advent of ion selective microsensors

* Corresponding author. Mailing address: Advanced Wastewater Management Centre, The University of Queensland, St. Lucia, QLD 4072, Australia. Phone: 61 7 3346 9021. Fax: 61 7 3365 4726. E-mail: rikke.meyer@awmc.uq.edu.au.

(10) and novel biosensors for NO_2^- (22), it is now possible to address this issue.

In the present study, we investigate the occurrence and significance of the anammox process relative to denitrification, and we correlate the process to the availability of NO_2^- in sediment from four locations in an Australian subtropical tidal river fringed by mangrove vegetation. We thereby add to the still limited database on the biogeography of the anammox process in sediments, which currently covers only temperate and Arctic regions. Nutrient cycling in subtropical rivers and estuaries differs from that in temperate estuaries, primarily due to the different timing and magnitude of freshwater input, since the rainfall in subtropical regions is highly variable, with long dry periods during the winter months and intermittent but intense rain events during the summer months. The nutrient loading in subtropical rivers and estuaries therefore varies greatly over the year (12, 13). Biosensors for NO_x^- (18) and a novel sensor for NO_2^- (22) were used to measure the concentration and spatial distribution of NO_x^- and NO_2^- in the sediment on a micrometer scale. This detailed information about the availability of oxidized nitrogen species enabled us to investigate whether there is a link between the distribution of the anammox process, its potential activity, and the availability of NO_2^- in the suboxic sediment layers harboring the process.

It is possible to quantify production and consumption processes and to determine their vertical distribution in sediments from concentration profiles of given chemical species by using Fick's second law of diffusion (1, 2, 16). In the present study we use the method of Berg et al. (3) to determine the distribution and size of NO_3^- and NO_2^- production/consumption processes. From the physical location of these processes in relation to the oxygen profile, we seek to establish whether aerobic NO_2^- production (i.e., aerobic ammonia oxidation) or anaerobic NO_2^- production (i.e., NO_3^- reduction) is the principal NO_2^- source for the anammox process.

MATERIALS AND METHODS

Study site and sampling. Sediment was collected from the Logan/Albert River system in subtropical southeast Queensland, Australia (Fig. 1). The catchment of the rivers covers 3,740 m^2 (12). Both rivers arise in forested national parks, and the catchment overall is dominated by grazing land and natural forest. The upper reaches have been cleared for grazing and agriculture (some sugar cane), and the lower reaches flow through rural residential and urban areas. The two rivers join 11.2 km from the river mouth, and the tidal limit occurs about 16 km upstream of this junction. While the upper reaches are relatively pristine, the Logan estuary is characterized by poor water quality, with high turbidity and high nitrogen and phosphorus levels. Primary sources of nutrients are two wastewater treatment plants located in Logan River (19.6 km upstream) and Albert River (11.5 km upstream). Nutrient levels increase with distance from the mouth and reach a maximum approximately 23 km upstream (12). Four study sites were chosen in a transect up the Logan and Albert rivers, with station 1 located just outside the mouth of the river and station 4 located furthest upstream in Logan River (Fig. 1). Monthly water-phase nutrient monitoring at stations 2, 3, and 4 by the state Environmental Protection Agency shows that annual ranges of concentrations of oxidized nitrogen species are 0.8 to 9.9 μM at station 2, 22 to 44.7 μM at station 3, and 27 to 93.2 μM at station 4 (12). The annual range of ammonium concentrations is 0.2 to 2.3 μM at station 1, 0.3 to 27 μM at station 3, and 1.6 to 52.5 μM at station 4 (12).

Four replicate sediment cores were sampled from stations 1 to 4 (Fig. 1) using 29-mm-inner-diameter plastic cores. Cores used for microsensor measurements were transported to the laboratory within 4 h of sampling. The sediment was pushed up so that the sediment surface was flush with the edge of the core. The cores from each site were incubated in a 10-liter aquarium containing in situ water sparged with air at room temperature (24°C). The water in the aquarium was changed every 2 days. Cores were incubated in the aquarium for at least 24 h before commencement of measurements. At the end of the microsensor measurements, these cores were used for determination of the sediment porosity by measuring the wet weight and dry weight (after 24 h of drying at 105°C) of the upper 5 mm of the sediment cores.

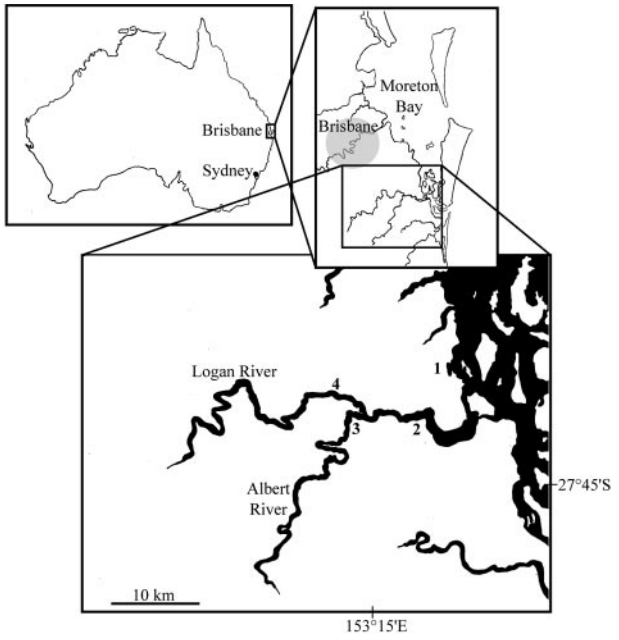

FIG. 1. Map of the Logan and Albert River system and its location in Australia. Stations 1 to 4 indicate where sediment was sampled. Adapted from Australian Environmental Protection agency maps, with permission.

Sediment used for potential anammox and denitrification measurements in anoxic slurry incubations was collected with 29-mm-inner-diameter cores, and the top 10 mm of sediment was pushed out of the core and transferred to a 50-ml plastic container. Sediments from the top 10 mm of approximately 20 cores from each site were mixed in the plastic container, transported to the laboratory, and stored at room temperature (24°C) overnight before preincubations for the anammox measurements were started. The in situ temperature of Logan and Albert rivers ranges from 18 to 28°C annually (12).

Microsensors and measurements of porewater profiles. Porewater profiles of NO_x^-, NO_2^-, and O_2 were measured in all sediments by using microsensors for O_2 (23) and biosensors for NO_x^- (18) and for NO_2^-. The NO_2^- sensor was constructed like the NO_x^- biosensor except that the culture of bacteria used (*Stenotrophomonas nitritireducens*) was deficient in NO_3^- reductase (22). Concentrations of NO_x^-, NO_2^-, and O_2 in porewater were measured as three or more replicate profiles from at least two different cores, as described by Meyer et al. (20). Profiles of NO_3^- were calculated by subtracting the averaged NO_2^- profile from the averaged NO_x^- profile. Water-phase samples for NO_x^- and NO_2^- were collected during profile measurements and used for calibration of the sensors. Concentrations of nitrate plus NO_2^- (NO_x^-) were determined using the vanadium chloride reduction method (5) on a NO_x^- analyzer (model 42c; Thermo Environmental Instruments). Nitrite concentrations were determined by a standard colorimetric method described by Grasshoff et al. (14).

Profiles of NO_x^-, NO_2^-, NO_3^-, and O_2 production were calculated from the respective individual concentration profiles and from the average profile by using the numerical method of Berg et al. (3). For a direct comparison of each process in the different sediment layers, calculations of production profiles were performed using the same increments on the depth scale, resulting in the same spatial resolution. Depth-integrated consumption or production rates (per m^2) of NO_2^- and NO_x^- were calculated from each replicate production profile, and the diffusion coefficients used in these calculations were calculated as the free diffusion coefficient of the respective species multiplied by the sediment porosity (40). The free diffusion coefficients for O_2, NO_3^-, and NO_2^- at 24°C were 2.22×10^{-5} cm^2 s^{-1}, 1.81×10^{-5} cm^2 s^{-1}, and 1.82×10^{-5} cm^2 s^{-1}, respectively, according to Broecker and Peng (6) and Li and Gregory (19). The diffusion coefficient for NO_3^- was used in the calculation of NO_x^- production.

TABLE 1. Production of $^{29}N_2$ and $^{30}N_2$ in anoxic slurries from stations 1 to 4 after incubation with $^{15}NH_4^+$ either alone or with $^{14}NO_3^-$

Station	Concn (μM) of the indicated radiolabeled nitrogen species[a] after:			
	Incubation i[b]		Incubation ii[c]	
	$^{29}N_2$	$^{30}N_2$	$^{29}N_2$	$^{30}N_2$
1	−0.0003 ± 0.0015	−0.00002 ± 0.0003	−0.0038 ± 0.0013	−0.0003 ± 0.0011
2	−0.0123 ± 0.0018	0.0007 ± 0.0002	0.3430 ± 0.0107	0.0052 ± 0.0013
3	−0.0245 ± 0.0032	0.0090 ± 0.0012	0.1432 ± 0.0110	0.0070 ± 0.0015
4	−0.0139 ± 0.0025	0.0066 ± 0.0011	0.2213 ± 0.0038	0.0033 ± 0.0007

[a] Values are averages ± standard errors (n = 3 to 4) and are calculated as the end point concentration minus the background.
[b] Incubation with $^{15}NH_4^+$ alone.
[c] Incubation with $^{15}NH_4^+$ and $^{14}NO_3^-$.

Anammox and denitrification measurements. Potential rates of anammox and denitrification were estimated by the method of Thamdrup and Dalsgaard (38), modified according to the work of Risgaard-Petersen et al. (27). Briefly, 1 ml of the sediment collected for anammox measurements was transferred to 10-ml Exetainer vials (Labco, High Wycombe, United Kingdom) containing a 5-mm glass bead for mixing. The vials were filled with N_2-sparged in situ water. Exetainers were preincubated on a rotating disk mixer for approximately 48 h to eliminate any oxygen or NO_x^-. Three parallel incubations were performed in triplicate: incubation i, with 100 μM $^{15}NH_4^+$ (^{15}N atom%, 99.3%); incubation ii, with 100 μM $^{15}NH_4^+$ plus 100 μM $^{14}NO_3^-$; and incubation iii, with 100 μM $^{15}NO_3^-$ (^{15}N atom%, 98%). Incubation iii for station 1 sediment was also amended with 100 μM $^{14}NH_4^+$ to ensure that ammonium was not limiting the process in this nutrient-poor sediment. This resulted in sets of nine vials, which were prepared in five replicates for each station, and the reaction in each set was stopped after 0, 1, 2, 3, or 4 h, respectively. Incubation vials were handled in an anaerobic chamber for injection of the substrates under strictly anoxic conditions. The reaction was stopped by injecting 0.25 ml 7 M $ZnCl_2$. Incubation i was a negative control, and formation of $^{14}N^{15}N$ ($^{29}N_2$) in incubation ii was taken as evidence of the presence of anammox (38). Anammox and denitrification rates were calculated from the production rates of $^{29}N_2$ and $^{15}N^{15}N$ ($^{30}N_2$) in incubation iii according to the method of Thamdrup and Dalsgaard (38). ^{15}N-labeled N_2 was measured by gas chromatography/mass spectrometry (RoboPrep-G+ in line with Tracermass; Europa Scientific) as described by Risgaard-Petersen and Rysgaard (29).

Previous studies have shown that identical anammox rates are obtained in incubations with $^{15}NO_2^-$ and $^{15}NO_3^-$ (8, 27, 39), but as a check for the availability of NO_2^- in the sediment slurries, a parallel set of slurries (in triplicate) was set up in which 100 μM NO_3^- was added and NO_2^- was measured after 1, 2, 3, and 4 h of incubation. This test showed that NO_2^- was present in all samples (at 0.8 to 10.5 μM), and there was no significant correlation with time or stations.

RESULTS

Anammox and denitrification potential. There was no indication of ^{15}N-labeled N_2 accumulation in samples incubated with $^{15}NH_4^+$ only (Table 1). Incubation with $^{15}NH_4^+$ plus $^{14}NO_3^-$ resulted in significantly higher $^{29}N_2$ concentrations (Table 1) in samples from stations 2, 3, and 4 than in the corresponding samples incubated with $^{15}NH_4^+$ alone ($P < 0.003$ by the Student t test), while $^{30}N_2$ concentrations were unaffected ($P > 0.15$ by the Student t test). At station 1, incubation with $^{15}NH_4^+$ plus $^{14}NO_3$ had no effect on the presence of either $^{29}N_2$ or $^{30}N_2$ at the end of the incubation. These results demonstrated the presence of the anammox process at all stations except station 1. In agreement with these results, incubations with $^{15}NO_3^-$ suggested the presence of anammox activity at stations 2, 3, and 4, located in the Logan/Albert river system, whereas the process was below the detection limit at station 1, located outside the river mouth (Fig. 1 and 2). The potential anammox rates differed significantly among the four stations ($P < 0.001$ by analysis of variance [ANOVA]). The highest activity was found upstream, at station 4, and the process rate decreased gradually as one approached the mouth of the river. The potential rates of denitrification followed a similar, significant trend ($P < 0.001$ by ANOVA), with the highest rates at station 4 and the lowest at station 1 (Fig. 2). Despite these parallel trends, anammox contributed less and less to N_2 production as one approached the mouth of the system. Anammox contributed 9, 5, 2, and 0% to N_2 production at stations 4, 3, 2, and 1, respectively.

Porewater concentration and production profiles of NO_x^- and NO_2^-. Microscale porewater profiles of NO_x^- and NO_2^- were very different at the different stations investigated (Fig. 3). The general trend was a successive downstream decrease in the overall content of NO_x^- and NO_2^- in the sediment. The average NO_2^- concentration, as well as the total pool of NO_2^- in the suboxic zone, was highest at station 4 (Table 2), and both of these parameters decreased significantly ($P < 0.001$ by ANOVA) at stations located closer to the mouth of the river. At station 1, NO_x^- and NO_2^- in the suboxic zone approached values close to the detection limit of the microsensors (0.2 μM). These overall differences in porewater NO_x^- and NO_2^- concentrations among the four stations investigated were due to differences in water-phase concentrations of the respective ion species and differences in NO_x^- and NO_2^- production/consumption rates within the sediment, as summarized in Table 2 and described in further detail below.

At station 1, the NO_x^- concentration in the overlying water was only 2 μM, and with no NO_x^- production within the

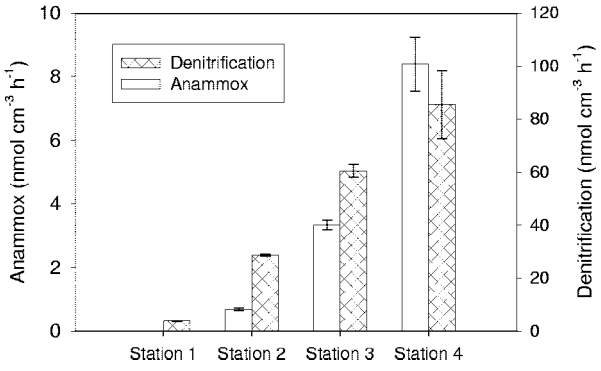

FIG. 2. Potential anammox rates ($NH_4^+ + NO_2^- \rightarrow N_2$) (hatched bars) and denitrification rates (organic e^- donor + $2NO_x^- \rightarrow N_2$) (white bars) at stations 1 to 4. Rates are given in nmol N cm^{-3} h^{-1}. Error bars, standard errors. Note different scales for anammox and denitrification.

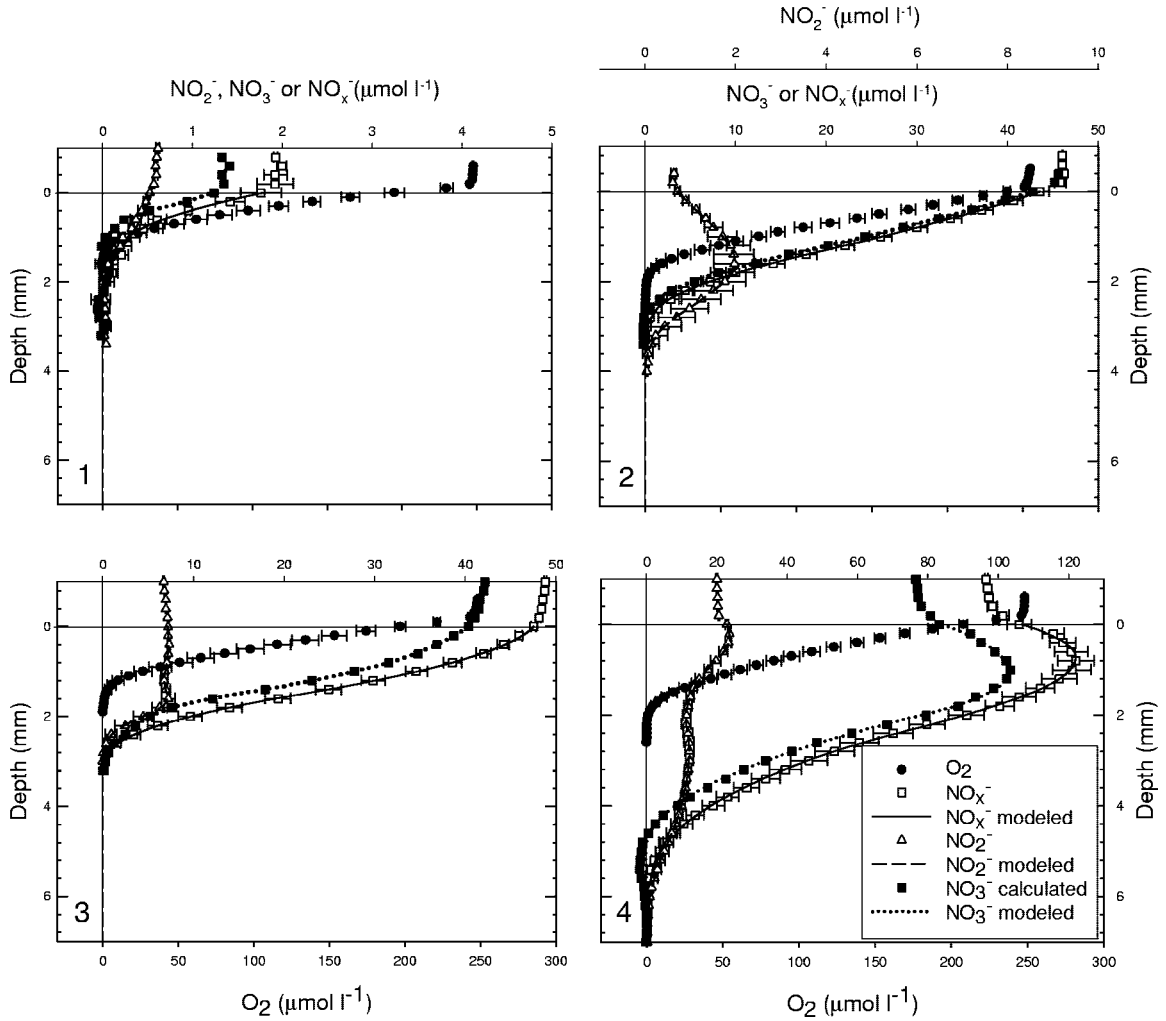

FIG. 3. Measured porewater profiles of NO_x^-, NO_2^-, and O_2, and calculated profiles of NO_3^-. Profiles shown are averages; error bars, standard errors (n = 3 to 6). NO_3^- profiles are calculated as the average NO_2^- profile subtracted from the average NO_x^- profile from each station. Station numbers are given in lower left corners.

sediment (Fig. 4), NO_x^- was depleted at a depth of 2.2 mm. This site had the poorest availability of oxidized nitrogen species. Station 2 also had very little NO_2^- (<1 μM) in the water phase but had a substantial amount of NO_x^- (46 μM). There was a small net production of NO_x^- in the oxic part of this sediment. A net production of NO_2^- led to a small accumulation of NO_2^-, with a peak concentration of approximately 2 μM near the oxic-suboxic interface. Oxygen penetrated 2 mm into this sediment, but NO_x^- penetrated somewhat deeper, forming a suboxic zone extending 2 mm further.

Station 3 had a similar level of NO_3^- in the water phase (42 μM) but had somewhat more NO_2^- (7 μM). Production of NO_x^- was evident throughout the oxic sediment layer (Fig. 4), whereas two separate zones of net NO_2^- production were measured: an aerobic production zone near the sediment surface, associated with net NO_x^- production, and an anaerobic

TABLE 2. Rates of NO_x^- and NO_2^- production and consumption,[a] and average NO_2^- concentration and integrated NO_2^- pool in the suboxic layer

Station	NO_x^- production	NO_x^- consumption	NO_2^- production	NO_2^- consumption	Avg suboxic NO_2^- concn (μM)[b]	Total suboxic pool of NO_2^- (nmol cm^{-2})[b]
1	0.3 ± 0.3	13.7 ± 4.2	0.4 ± 0.4	2.2 ± 0.6	0.03 ± 0.02	0.006 ± 0.005
2	36.5 ± 11.1	107.3 ± 6.4	17.5 ± 4.1	12.2 ± 3.7	0.99 ± 0.18	0.20 ± 0.06
3	104.0 ± 7.4	130.8 ± 6.6	74.1 ± 11.8	73.5 ± 10.2	3.00 ± 0.36	0.46 ± 0.07
4	467.7 ± 26.8	264.9 ± 20.4	153.9 ± 11.6	120.2 ± 7.3	6.84 ± 0.56	3.27 ± 0.35

[a] Expressed in micromoles per square meter per hour. Values are averages ± standard errors (n = 3 to 6) and are calculated by integration of replicate production profiles.
[b] Calculated from individual concentration profiles (n = 3 to 6).

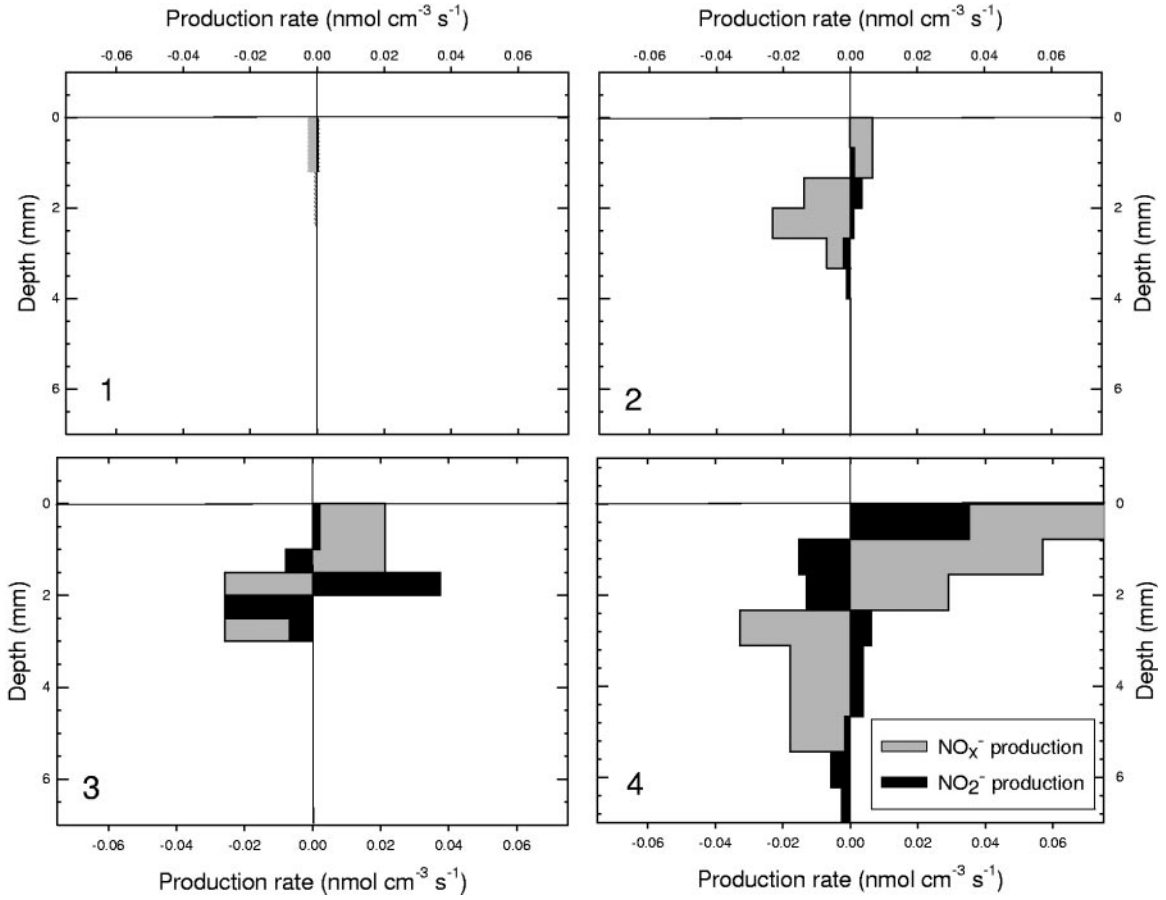

FIG. 4. Profiles of net NO_x^- and NO_2^- production calculated from the average NO_x^- and NO_2^- concentration profiles. It should be noted that the division between aerobic and anaerobic processes at station 2 does not occur exactly at the oxic-suboxic interface. This is due to the somewhat coarse increments in the zonation of the production profile obtained by the modeling procedure used. Station numbers are given in lower left corners.

production zone just below the oxic-suboxic interface, associated with net NO_x^- consumption. These zones were separated by a net NO_2^- consumption zone at a 1- to 1.5-mm depth. The total penetration depth of NO_x^- was approximately 3 mm, leaving a suboxic zone of 1.5 mm.

The porewater concentration, penetration depth, and transformation rates of NO_2^- and NO_x^- were highest at station 4. The water-phase NO_x^- concentration was 96 μM, and a high aerobic NO_x^- production led to a 120 μM peak 1 mm below the sediment surface. NO_x^- penetrated 6 to 7 mm into the sediment, and with an oxygen penetration of 2 mm, this led to a deep suboxic zone spanning as much as 5 mm. Nitrite concentrations in the water phase were as high as 20 μM, and NO_2^- also penetrated up to 7 mm into the sediment. The distribution of net NO_2^- production in the sediment followed a pattern similar to that found at station 3. An aerobic NO_2^- production zone associated with net NO_x^- production was found near the surface, and below this zone, net NO_2^- consumption was observed. A second NO_2^- production zone associated with anaerobic net NO_x^- consumption was situated at a depth of 2.4 to 4.7 mm, below which NO_2^- was consumed and depleted.

DISCUSSION

Until recently, anammox was a completely unnoticed process in the global N cycle, yet with the increasing attention paid to it, its widespread distribution has been demonstrated in several marine ecosystems of temperate and Arctic regions (7, 17, 27, 30, 38, 39). The present study demonstrates the presence of anammox in a subtropical mangrove system and thereby adds to the diversity of environments where anammox has been detected, underlining the apparently ubiquitous nature of the process.

Potential rates of anammox in the Logan River sediments (0.5 to 8 nmol N cm^{-3} h^{-1} [Fig. 2]) were within the range reported for other sediments investigated for anammox thus far, such as temperate estuaries and offshore marine sediments (9). Interestingly, such a similarity between sites is not found for potential denitrification rates, which can differ by several orders of magnitude (9, 27, 38, 39).

The contribution of anammox to sediment N_2 production in the Logan River system (0 to 9%) was relatively low and within the range reported previously for temperate estuaries such as the Thames estuary in the United Kingdom (39) and the Danish

estuary Randers Fjord (27). Thus, both in temperate and in subtropical estuaries, denitrification appears to govern benthic N_2 production, in contrast to offshore sediments, where anammox has been found to dominate (38).

Anammox and NO_2^- in the sediment. The anammox distribution found in the Logan River was shown to decrease, both in activity and in its contribution to N_2 production, at downstream locations in the river system (Fig. 2). This distribution is similar to the pattern reported by Trimmer et al. for the Thames estuary in the United Kingdom (39). These authors observed a significant correlation between the contribution of anammox to N_2 production and the organic carbon content of the sediment. Based on this observation, they proposed that the decrease in anammox activity and in its contribution to N_2 production along the Thames estuary was caused by a decrease in sediment reactivity. They argued that the anammox population was reliant on a NO_2^- supply that was proportional to the rate of NO_3^- reduction, which, in turn, was correlated with sediment reactivity. Thus, the authors suggested, though they did not directly show, that NO_2^- was the substrate limiting the abundance of anammox bacteria.

The direct measurements of NO_2^- in the Logan River sediment show a strong correlation between the potential anammox rate and the in situ availability of NO_2^- measured as (i) NO_2^- production ($r^2 = 0.9933$) (Fig. 2; Table 2), (ii) the average suboxic NO_2^- concentration ($r^2 = 0.9971$) (Fig. 2; Table 2), or (iii) the integrated pool (mol cm^{-2}) of nitrite in the suboxic sediment layer ($r^2 = 0.9273$) (Fig. 2; Table 2). Thus, at the present concentration level, NO_2^- appears to control the sediment capacity for anammox. Assuming that the anammox potential is proportional to the density of active anammox bacteria, these correlations support the hypothesis that the abundance of active anammox bacteria is determined by the NO_2^- supply to the suboxic sediment layer, as proposed by Trimmer et al. (39). We note that the statistics are based on only four sets of independent observations and that, theoretically, the correlations could therefore reflect a random interdependency rather than a mechanistic dependency. However, the strong correlation between the anammox potential and the in situ availability of NO_2^- highly supports the hypothesis of NO_2^- being a key regulator of anammox in this sediment.

Processes responsible for delivery of NO_2^- to anammox bacteria. The supply of NO_2^- to the suboxic zone in the sediment is the result of interactions between a series of physical and microbial processes including diffusion, aerobic NH_4^+ and NO_2^- oxidation, and anaerobic NO_3^- and NO_2^- reduction. From the porewater profiles of NO_x^- and NO_3^-, it is possible to infer these processes, by calculating the NO_x^- and NO_3^- production profiles and relating the physical location of production and consumption to the distribution of oxygen in the sediment.

Production profiles were again calculated by the method of Berg et al. (3). Production of NO_x^- in the oxic zone (calculated from the NO_x^- profiles) represents the production of NO_2^- plus NO_3^- and is therefore equivalent to the rate of aerobic NH_4^+ oxidation. Production of NO_3^- in the oxic zone (calculated from the NO_3^- profile) is equivalent to the rate of aerobic NO_2^- oxidation. Consumption of NO_3^- in the suboxic zone represents anaerobic NO_3^- reduction, while consumption of NO_x^- represents anaerobic NO_2^- reduction—the latter being denitrification, anammox, and/or nitrite ammonification. The process rates and their distribution in the sediment at stations 2 to 4 are shown in Fig. 5. Because nitrite did not accumulate from sediment processes at station 1, this site was omitted from the analysis.

Aerobic NH_4^+ oxidation was constant through the oxic sediment layer at stations 2 and 3 but decreased with depth at station 4. The gradient in NH_4^+ oxidation found at station 4 may reflect the availability of NH_4^+ in the sediment. The concentration of NH_4^+ in Logan River at station 4 was as high as 52 μM around the time of sampling (P. Maxwell, Environmental Protection Agency, Queensland, Australia, personal

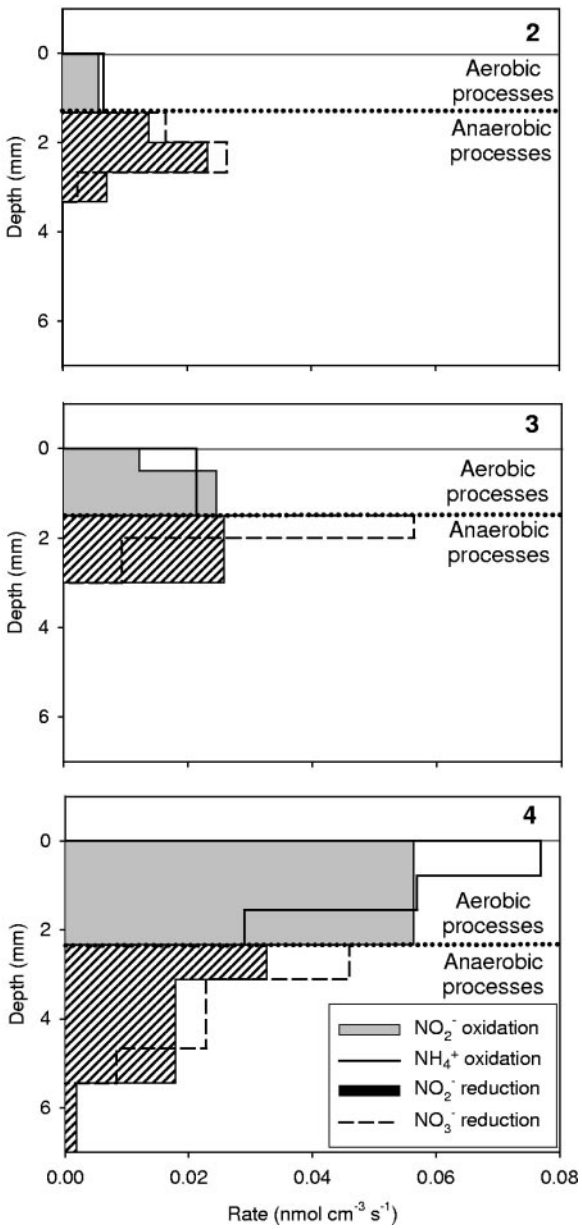

FIG. 5. Aerobic NH_4^+ and NO_2^- oxidation profiles, and anaerobic NO_3^- and NO_2^- reduction profiles, at stations 2 to 4. The dotted line indicates the separation of aerobic and anaerobic processes in the sediment.

communication), and NH_4^+ from the water phase as opposed to the sediment is therefore likely to be the primary substrate source for nitrification. Altmann et al. (1) also found the highest NH_4^+ oxidation rate in the part of the oxic zone closest to the sediment surface in a study of sediment microcosms overlaid with 50 μM NH_4^+. This positioning of the NH_4^+ oxidation coincided with the abundance of ammonia-oxiding bacteria and was ascribed to the flux of NH_4^+ into the sediment from the overlying water column.

At stations 3 and 4, the difference between NH_4^+ and NO_2^- oxidation was highest close to the sediment surface, causing the highest nitrification-associated NO_2^- accumulation here. At station 2, aerobic NH_4^+ oxidation was relatively low compared to that at the other stations and almost matched the rate of NO_2^- oxidation, leading to very low net NO_2^- production in the oxic zone.

Nitrate reduction rates exceeded NO_2^- reduction rates in the upper part of the suboxic sediment layer at all stations, while the opposite was observed deeper in the sediment. This imbalance suggests that NO_2^- accumulated as an intermediate in denitrification and/or nitrate ammonification. Nitrite may be lost from the cell at high NO_3^- concentrations due to differences in the kinetics of the NO_3^- and NO_2^- reductases (4), which is consistent with our observation of NO_2^- accumulation in the suboxic sediment layers having the highest NO_3^- concentration and the highest NO_3^- reduction rate (Fig. 3, 4, and 5). Stief et al. (35) found a similar distribution of NO_2^- production from NO_x^- reduction in freshwater microcosms.

Although NO_2^- accumulated from both aerobic NH_4^+ oxidation and anaerobic NO_3^- reduction, the detailed analysis of the size and distribution of NO_2^--producing processes in the sediment suggests that NO_3^- reduction rather than NH_4^+ oxidation was the major direct source of NO_2^- in the suboxic sediment layer, where anammox occurs. Figure 6 shows a more conceptual presentation of the process rates presented in Fig. 5, as well as the net diffusive fluxes between the water, the oxic sediment layer, and the suboxic sediment layer.

Not all nitrite accumulating from nitrification was accessible to anaerobic processes; a significant fraction was either lost by diffusion to the water column or further oxidized to NO_3^- before reaching the suboxic sediment layer. Most of the NO_2^- was oxidized to NO_3^-, and there was an efflux of NO_2^- to the overlying water at all stations (Fig. 6). The net diffusion of NO_2^- from the suboxic to the oxic sediment layer demonstrates that aerobic NO_2^- production was not sufficient to drive a net flux of NO_2^- to the sediment below.

In contrast to NO_2^- produced aerobically, NO_2^- accumulating from NO_3^- reduction is directly available for anammox, because it is produced within the suboxic part of the sediment. Furthermore, nitrate reduction rates were higher than aerobic NH_4^+ oxidation rates at stations 2 and 3 but not at station 4 (Fig. 6). However, a high aerobic NO_2^- oxidation at station 4, exceeding the aerobic NH_4^+ oxidation, caused a substantial flux of NO_2^- from the suboxic sediment, which contributed to the aerobic consumption of NO_2^-. Hence, even though aerobic NH_4^+ oxidation was twice as high as anaerobic NO_3^- reduction in this sediment, nitrification was still driving a net transport of NO_2^- out of the suboxic sediment below.

It is clear that the direct contribution by nitrification to the NO_2^- pool in the suboxic sediment was negligible. Nitrification

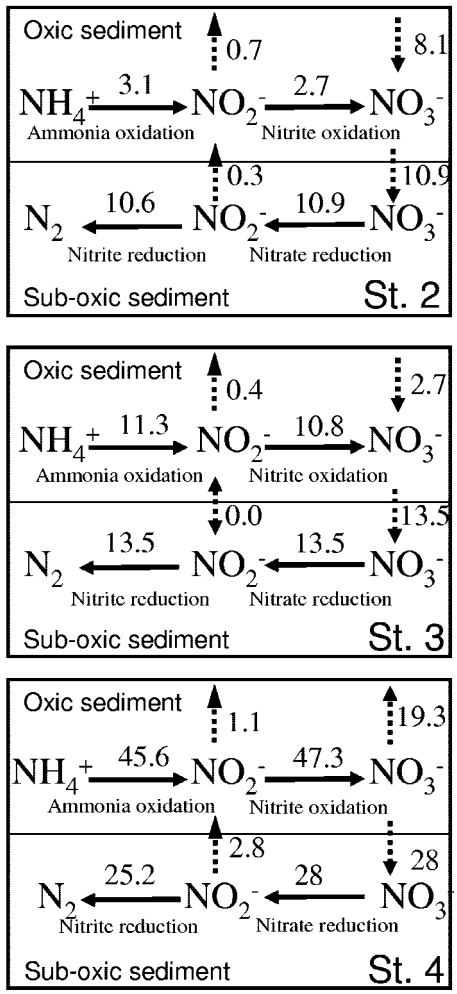

FIG. 6. Conceptual presentation of aerobic and anaerobic N transformation rates (solid arrows) and net diffusive fluxes (dotted arrows) between the water, the oxic sediment, and the suboxic sediment layer. All rates are in nmol cm^{-2} h^{-1}.

may, however, contribute indirectly as a source of NO_3^- for anaerobic NO_3^- reduction. This was not the case at station 2, where most NO_3^- was diffusing into the sediment from the overlying water (Fig. 6), but at station 3, NO_2^- oxidation could account for 80% of the NO_3^- found to be reduced anaerobically, and at station 4, the aerobic NO_2^- oxidation even drove a substantial flux of NO_3^- out of the sediment. In sediments where nitrification activity was high, this process could therefore be an important indirect source of NO_2^- for anammox.

Conclusions. This study verified the presence of anammox in the sediment of a subtropical mangrove system and thereby contributes to the still limited data about the biogeographical distribution of anammox. We demonstrated a strong correlation between the potential rates of anammox and the concentration and total pool of NO_2^- in the suboxic part of the sediment. Being able to link the potential anammox activity to the availability of free NO_2^- has brought us one step closer to making general statements about the distribution of anammox in marine sediments.

However, the conditions under which NO_2^- accumulates in natural systems are in general not well understood, and distribution of both NO_2^- and anammox should be studied at other locations, in both similar and different environments, to further validate the proposed link between anammox and NO_2^- availability. In this study, both nitrification and the intermediate steps in denitrification (i.e., NO_3^- reduction) caused accumulation of NO_2^- in the sediment; however, anaerobic NO_3^- reduction was the principal deliverer of NO_2^- to the zone where the anammox reaction would take place. Many organisms performing denitrification or nitrate ammonification are able to reduce both NO_3^- and NO_2^-. While these organisms compete with anammox bacteria for NO_2^-, they may also excrete NO_2^- as an intermediate in their metabolism. Paradoxically, anammox bacteria are in this way reliant on bacteria that are otherwise thought to be their competitors.

ACKNOWLEDGMENTS

We thank the Danish Natural Science Research Council and the Carlsberg Foundation, Denmark, for funding this work.

REFERENCES

1. **Altmann, D., P. Stief, R. Amann, and D. de Beer.** 2004. Distribution and activity of nitrifying bacteria in natural stream sediment versus laboratory sediment microcosms. Aquat. Microb. Ecol. **36:**73–81.
2. **Amann, R., and M. Kuhl.** 1998. In situ methods for assessment of microorganisms and their activities. Curr. Opin. Microbiol. **1:**352–358.
3. **Berg, P., N. Risgaard-Petersen, and S. Rysgaard.** 1998. Interpretation of measured concentration profiles in sediment pore water. Limnol. Oceanogr. **43:**1500–1510.
4. **Betlach, M. R., and J. M. Tiedje.** 1981. Kinetic explanation for accumulation of nitrite, nitric oxide, and nitrous oxide during bacterial denitrification. Appl. Environ. Microbiol. **42:**1074–1084.
5. **Braman, R. S., and S. A. Hendrix.** 1989. Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium(III) reduction with chemi-luminescence detection. Anal. Chem. **61:**2715–2718.
6. **Broecker, W. S., and T. H. Peng.** 1974. Gas exchange between air and sea. Tellus **26:**21–35.
7. **Dalsgaard, T., D. E. Canfield, J. Petersen, B. Thamdrup, and J. Acuna-Gonzalez.** 2003. N_2 production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. Nature **422:**606–608.
8. **Dalsgaard, T., and B. Thamdrup.** 2002. Factors controlling anaerobic ammonium oxidation with nitrite in marine sediments. Appl. Environ. Microbiol. **68:**3802–3808.
9. **Dalsgaard, T., B. Thamdrup, and D. E. Canfield.** 2005. Anaerobic ammonium oxidation (anammox) in the marine environment. Res. Microbiol. **156:**457–464.
10. **de Beer, D., A. Schramm, C. M. Santegoeds, and M. Kühl.** 1997. A nitrite microsensor for profiling environmental biofilms. Appl. Environ. Microbiol. **63:**973–977.
11. **Egli, K., U. Fanger, P. J. J. Alvarez, H. Siegrist, J. R. van der Meer, and A. J. B. Zehnder.** 2001. Enrichment and characterization of an anammox bacterium from a rotating biological contactor treating ammonium-rich leachate. Arch. Microbiol. **175:**198–207.
12. **EHMP.** 2004. Ecosystem Health Monitoring Program 2002–2003 annual technical report. Moreton Bay Waterways and Catchments Partnership, Brisbane, Australia.
13. **Eyre, B. D.** 2000. Regional evaluation of nutrient transformation and phytoplankton growth in nine river-dominated sub-tropical east Australian estuaries. Marine Ecol. Prog. Ser. **205:**61–83.
14. **Grasshoff, K., M. Erhardt, and K. Kremling.** 1983. Methods of seawater analysis, 2nd ed. Verlag Chemie, Weinheim, Germany.
15. **Guven, D., A. Dapena, B. Kartal, M. C. Schmid, B. Maas, K. van de Pas-Schoonen, S. Sozen, R. Mendez, H. J. M. Op den Camp, M. S. M. Jetten, M. Strous, and I. Schmidt.** 2005. Propionate oxidation by and methanol inhibition of anaerobic ammonium-oxidizing bacteria. Appl. Environ. Microbiol. **71:**1066–1071.
16. **Jorgensen, B. B., N. P. Revsbech, and Y. Cohen.** 1983. Photosynthesis and structure of benthic microbial mats: microelectrode and SEM studies of four cyanobacterial communities. Limnol. Oceanogr. **28:**1075–1093.
17. **Kuypers, M. M. M., A. O. Sliekers, G. Lavik, M. Schmid, B. B. Jorgensen, J. G. Kuenen, J. S. S. Damste, M. Strous, and M. S. M. Jetten.** 2003. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature **422:**608–611.
18. **Larsen, L. H., T. Kjær, and N. P. Revsbech.** 1997. A microscale NO_3^- biosensor for environmental applications. Anal. Chem. **69:**3527–3531.
19. **Li, Y.-H., and S. Gregory.** 1974. Diffusion of ions in sea water and in deep-sea sediments. Geochim. Cosmochim. Acta **38:**703–714.
20. **Meyer, R. L., T. Kjær, and N. P. Revsbech.** 2001. Use of NO_x^- microsensors to estimate the activity of sediment nitrification and NO_x^- consumption along an estuarine salinity, nitrate and light gradient. Aquat. Microb. Ecol. **26:**181–193.
21. **Mulder, A., A. A. Vandegraaf, L. A. Robertson, and J. G. Kuenen.** 1995. Anaerobic ammonium oxidation discovered in a denitrifying fluidized-bed reactor. FEMS Microbiol. Ecol. **16:**177–183.
22. **Nielsen, M., L. H. Larsen, M. S. M. Jetten, and N. P. Revsbech.** 2004. A bacterium-based NO_2^- biosensor for environmental applications. Appl. Environ. Microbiol. **70:**6551–6558.
23. **Revsbech, N. P.** 1989. An oxygen microsensor with a guard cathode. Limnol. Oceanogr. **34:**474–478.
24. **Richards, F. A.** 1965. Anoxic basins and fjords, p. 611–645. *In* J. P. Riley and G. Skirrow (ed.), Chemical oceanography, vol. 1. Academic Press, London, United Kingdom.
25. **Richards, F. A.** 1965. Chemical observations in some anoxic, sulfide-bearing basins and fjords, p. 215–232. *In* E. A. Pearson (ed.), Advances in water pollution research, vol. 3. Pergamon Press Inc., London, United Kingdom.
26. **Risgaard-Petersen, N., R. L. Meyer, and N. P. Revsbech.** 2005. Denitrification and anaerobic ammonium oxidation in sediments: effects of microphytobenthos and NO_3^-. Aquat. Microb. Ecol. **40:**67–76.
27. **Risgaard-Petersen, N., R. L. Meyer, M. Schmid, M. S. M. Jetten, A. Enrich-Prast, S. Rysgaard, and N. P. Revsbech.** 2004. Anaerobic ammonium oxidation in an estuarine sediment. Aquat. Microb. Ecol. **36:**293–304.
28. **Risgaard-Petersen, N., L. P. Nielsen, S. Rysgaard, T. Dalsgaard, and R. L. Meyer.** 2003. Application of the isotope pairing technique in sediments where anammox and denitrification coexist. Limnol. Oceanogr. Methods **1:**63–73.
29. **Risgaard-Petersen, N., and S. Rysgaard.** 1995. Nitrate reduction in sediments and waterlogged soils measured by ^{15}N techniques, p. 287–296. *In* K. Alef and P. Nannipieri (ed.), Methods in applied soil microbiology. Academic Press Inc., London, United Kingdom.
30. **Rysgaard, S., and R. N. Glud.** 2004. Anaerobic N_2 production in Arctic sea ice. Limnol. Oceanogr. **49:**86–94.
31. **Rysgaard, S., R. N. Glud, N. Risgaard-Petersen, and T. Dalsgaard.** 2004. Denitrification and anammox activity in Arctic marine sediments. Limnol. Oceanogr. **49:**1493–1502.
32. **Schmid, M., U. Twachtmann, M. Klein, M. Strous, S. Juretschko, M. Jetten, J. W. Metzger, K. H. Schleifer, and M. Wagner.** 2000. Molecular evidence for genus level diversity of bacteria capable of catalyzing anaerobic ammonium oxidation. Syst. Appl. Microbiol. **23:**93–106.
33. **Schmid, M., K. Walsh, R. Webb, W. I. C. Rijpstra, K. van de Pas-Schoonen, M. J. Verbruggen, T. Hill, B. Moffett, J. Fuerst, S. Schouten, J. S. S. Damste, J. Harris, P. Shaw, M. Jetten, and M. Strous.** 2003. *Candidatus* "Scalindua brodae," sp nov., *Candidatus* "Scalindua wagneri," sp nov., two new species of anaerobic ammonium oxidizing bacteria. Syst. Appl. Microbiol. **26:**529–538.
34. **Schmidt, I., O. Sliekers, M. Schmid, E. Bock, J. Fuerst, J. G. Kuenen, M. S. M. Jetten, and M. Strous.** 2003. New concepts of microbial treatment processes for nitrogen removal in wastewater. FEMS Microbiol. Rev. **27:**481–492.
35. **Stief, P., D. De Beer, and D. Neumann.** 2002. Small-scale distribution of interstitial nitrite in freshwater sediment microcosms: the role of nitrate and oxygen availability, and sediment permeability. Microb. Ecol. **43:**367–378.
36. **Strous, M., J. G. Kuenen, and M. S. M. Jetten.** 1999. Key physiology of anaerobic ammonium oxidation. Appl. Environ. Microbiol. **65:**3248–3250.
37. **Strous, M., E. VanGerven, P. Zheng, J. G. Kuenen, and M. S. M. Jetten.** 1997. Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (anammox) process in different reactor configurations. Water Res. **31:**1955–1962.
38. **Thamdrup, B., and T. Dalsgaard.** 2002. Production of N_2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. Appl. Environ. Microbiol. **68:**1312–1318.
39. **Trimmer, M., J. C. Nicholls, and B. Deflandre.** 2003. Anaerobic ammonium oxidation measured in sediments along the Thames estuary, United Kingdom. Appl. Environ. Microbiol. **69:**6447–6454.
40. **Ullman, W. J., and R. C. Aller.** 1982. Diffusion coefficients in nearshore marine sediments. Limnol. Oceanogr. **27:**552–556.
41. **van Dongen, U., M. S. M. Jetten, and M. C. M. van Loosdrecht.** 2001. The SHARON-Anammox process for treatment of ammonium rich wastewater. Water Sci. Technol. **44:**153–160.