

Nitrogen cycling in a deep ocean margin sediment (Sagami Bay, Japan)

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Abstract

On the basis of in situ NO_3^- microprofiles and chamber incubations complemented by laboratory-based assessments of anammox and denitrification we evaluate the nitrogen turnover of an ocean margin sediment at 1450-m water depth. In situ NO_3^- profiles horizontally separated by 12 mm reflected highly variable NO_3^- penetration depths, NO_3^- consumption rates, and nitrification. On average the turnover time of the pore-water NO_3^- pool was ~ 0.2 d. Net release of NH_4^+ during mineralization ($0.95 \text{ mmol m}^{-2} \text{ d}^{-1}$) sustained a net efflux of ammonia (53%), nitrification (24%), and anammox activity (23%). The sediment had a relatively high in situ net influx of NO_3^- ($1.44 \text{ mmol m}^{-2} \text{ d}^{-1}$) that balanced the N_2 production as assessed by onboard tracer experiments. N_2 production was attributed to prokaryotic denitrification (59%), anammox (37%), and foraminifera-based denitrification (4%). Anammox thereby represented an important nutrient sink, but the N_2 production was dominated by denitrification. Despite the fact that NO_3^- stored inside foraminifera represented $\sim 80\%$ of the total benthic NO_3^- pool, the slow intracellular NO_3^- turnover that, on average, sustained foraminifera metabolism for 12–52 d, contributed only to a minor extent to the overall N_2 production. The microbial activity in the surface sediment is a net nutrient sink of $\sim 1.1 \text{ mmol N m}^{-2} \text{ d}^{-1}$, which aligns with many studies performed in coastal and shelf environments. Continental margin areas can act as significant N sinks and play an important role in regional N budgets.

The importance of shelf and coastal sediments for regional and global nitrogen cycling has been emphasized by a number of studies (Devol and Christensen 1993; Galloway et al. 2004; Purvaja et al. 2008), but in the context of benthic nitrogen turnover the deeper continental margin sediments remain an understudied region of the ocean. This is despite the fact that sediments along the ocean margins early on were realized to represent sites of intensified mineralization and carbon retention with a large model-predicted contribution to the marine nitrogen cycle (Jahnke et al. 1990; Walsh 1991; Middelburg et al. 1996). One major limitation has been the difficulties of performing in situ studies and the recognition that fluxes and pore-water profiles measured in cores sampled from greater depths appear to be affected by recovery artefacts (Aller et al. 1998; Glud et al. 1999; Hall et al. 2007). Typically, pore-water profiles measured onboard reflect large subsurface peaks in dissolved organic nitrogen, ammonia, and nitrate that most likely are related to cell lysis, which leads to elevated and unrealistic N-fluxes during laboratory incubations (Aller et al. 1998; Hall et al. 2007). The numbers of in situ quantifications of benthic nutrient and N_2 exchange rates (Berelson et al. 1990; Devol 1991; Brunnegård et al. 2004) or of in situ nutrient distribution (Aller et al. 1998) beyond the coastal ocean are still very limited.

In recent years the conceptual complexity of the benthic nitrogen cycling has increased by the discoveries of

anammox activity (Thamdrup and Dalsgaard 2002), NO_3^- -storing bacteria (Fossing et al. 1995; McHatton et al. 1996), and benthic foraminifera capable of storing and respiring NO_3^- (Risgaard-Petersen et al. 2006). These discoveries challenge and in some cases compromise previous studies, and they have renewed the interest in understanding and quantifying the benthic nitrogen turnover. The number of studies assessing the relative importance of anammox for benthic nitrogen turnover is steadily increasing (Rysgaard et al. 2004; Dalsgaard et al. 2005) while only few studies at present have evaluated the importance of benthic foraminifera (Risgaard-Petersen et al. 2006; Høglund et al. 2008). However, to our knowledge all published experimental studies on benthic nitrogen turnover that incorporate and account for anammox and foraminiferal denitrification have been restricted to coastal sediments.

In the present study we combine in situ measurements of NO_3^- microprofiles and benthic nutrient exchange rates with laboratory-based assessments of anammox, prokaryotic-, and foraminifera-based denitrification rates to obtain a complete budget for nitrogen turnover in a 1450-m-deep ocean margin sediment. This is, to our knowledge, the first time that NO_3^- microprofiles have been measured at any significant water depth and that a coherent direct assessment of anammox and foraminifera-based denitrification has been carried out beyond the coastal zone. The implications and importance of the findings are discussed in the context of the recent advances within benthic nitrogen cycling.

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Methods

Study site—Sagami Bay is a 3000-km² large embayment at Honshu (Japan) facing the Pacific Ocean (Fig. 1). The bathymetry is dominated by a central canyon extending from the sill of the shallow, eutrophic Tokyo Bay to the central basin of Sagami Bay with a maximum water depth around 1500 m. The ‘basin’ opens up and continues down to the abyssal plains of the Pacific (Fig. 1). During 1996 to 1999 the bay was studied by a large-scale interdisciplinary research program that primarily focused on pelagic–benthic coupling (Kitazato et al. 2003). The study showed that the depth-integrated chlorophyll *a* concentration for the upper 50 m varies between 20 mg m⁻² and 80 mg m⁻² with peak values during early spring (Kanda et al. 2003). The estimated annual primary production roughly equals 12 mol C m⁻² yr⁻¹ while the vertical annual sedimentation of organic material as quantified by sediment traps amounts to 2 mol C m⁻² yr⁻¹ (Nakatsuka et al. 2003). The vertical transport of organic matter is, however, complemented by downslope transport of relatively refractory erosion material from the basin sides (Soh 2003). Intense mineralization in the water column results in a relatively stable O₂ minimum zone reaching minimum values ~55 μmol L⁻¹ to 60 μmol L⁻¹ (~15% air-saturation) at 1200–1400-m water depth. The bottom-water temperature remains stable at 2.5°C.

The present study was conducted at a site at 1450-m water depth in central Sagami Bay (35°00.86′N 139°21.59′E [in previous literature referred to as OBBII]) from the R/V *Natsushima*. This vessel acts as the mother-ship for the Remotely Operated Vehicle (ROV) *Hyperdolphin*, which was used for positioning our profiling instrument, deploying our benthic chamber and for recovering sediment cores for onboard investigations. Data were collected on three successive cruises conducted during the periods of 22–27 March 2006, 08–16 December 2006, and 17–23 January 2008 (NT06-05, NT06-23, and NT08-02, respectively).

In situ microprofile measurements and chamber incubations—Microprofiles were measured by a slightly updated and modified version of a previously described profiling instrument (Gundersen and Jørgensen 1990; Glud et al. 1994). The profiler carried an array of nine microsensors for measuring microprofiles of O₂, NO₃⁻, H₂S, and resistivity across the sediment–water interface (for sensor description see below). Single deployments hosted different combinations of these sensors. During two cruises (Mar 2006 and Jan 2008) the central measuring cylinder and the elevator was placed on a horizontally moving sledge capable of making a 90-cm transect in predefined increments (Glud et al. 2009). The microprofiling unit was mounted within a benthic lander tripod that was released from the ship and sank towards the seafloor at a speed of 25 m min⁻¹. Before the measuring routine was initiated the ROV moved the tripod to a site that remained undisturbed from any potential bow-wave effect from the landing of the tripod (Glud et al. 2005). Once the lander was positioned the ROV activated the measuring routine of

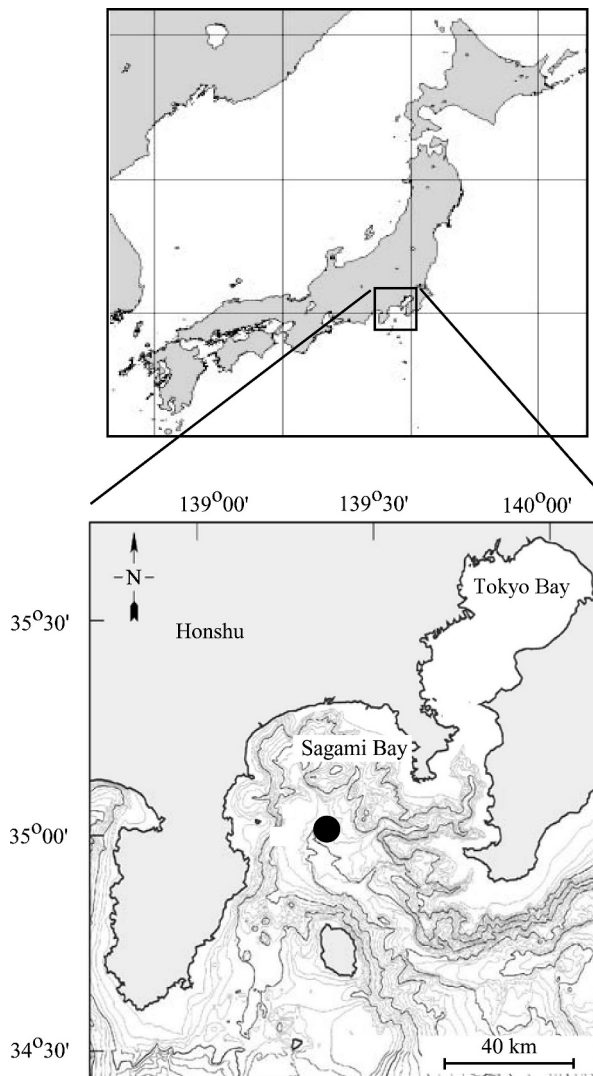


Fig. 1. Sagami Bay with the investigated site (OBBII) indicated by a black dot (modified from Glud et al. 2005). The thick isolines indicate 500-, 1000-, and 1500-m water depth.

the microprofiler by pushing a mechanical switch, and after a small delay the electronic cylinder carrying the micro-sensor array moved continuously towards the sediment surface. As the resistivity sensor recorded an abrupt 10% drop in the signal, which indicated the relative position of the sediment surface, the vertical movement stopped and the electronic cylinder receded 3.0 cm. Subsequently, sensors were moved towards the sediment surface in increments of 100 μm or 200 μm for a total distance of 7.0 cm, before moving back to the initial vertical start position. The profiling unit then moved sideward by the predefined distance (i.e., 12 mm) and the vertical measuring routine was repeated. At the end of the measuring procedure the ROV recovered the lander by grabbing a rope on top of the frame and lifting the whole lander tripod to the sea surface.

During some deployments the ROV also carried and positioned our chamber module at 50–80-m distance from

the profiling lander. The module consisted of a small carbon-fiber frame, holding a circular, benthic Plexiglas chamber (inside diam. 19 cm, ht 33 cm). The lid sealing the chamber carried two calibrated O₂ mini-electrodes (Glud et al. 2000), a rotating stirrer, and a one-way valve allowing water to escape as the chamber was pushed into the sediment by the ROV. The water height within the transparent chamber was determined from the ROV camera focusing on measuring sticks glued to the chamber wall. The O₂ sensor signals were continuously recorded and at a regular time interval five subsamples of 55 mL were taken from the enclosed water volume. The water was replaced by ambient water via a valve in the chamber lid and the dilution was later accounted for in the flux calculations (see below). Niskin bottles placed on the ROV were used to take samples of the bottom water. The bottom water was used for sensor calibrations and to determine the initial solute concentration during the chamber deployments. At the end of the incubations the ROV grabbed the chamber frame and brought it back to the research vessel. Upon instrument recovery water samples collected from the chamber module and the ROV were immediately frozen (at -18°C). The samples were later analyzed for (NO₃⁻ + NO₂⁻) and NH₄⁺ concentration. NO₃⁻ (including NO₂⁻) was determined by chemiluminescence after reduction to NO (Braman and Hendrix 1989), while NH₄⁺ was measured spectrophotometrically by the salicylate-hypochlorite method (Bower and Holm-Hansen 1980). The applied analysis measures the sum of NO₃⁻ + NO₂⁻, but due to the generally low concentrations of NO₂⁻ the results are in the following referred to as NO₃⁻ concentrations. Oxygen concentrations of the bottom-water samples recovered by the ROV were determined onboard by Winkler titration. The total exchange rates of O₂, NO₃⁻, and NH₄⁺ were calculated from the linear concentration changes over time accounting for the enclosed water volume, sample dilution, and the sediment area.

Microsensors and microprofile calculations—In essence, the microsensors and microprofile calculations applied in situ and in the laboratory were the same. Resistivity, R_s, was in both instances measured by a two-wire probe having an overall tip diameter of 1.0 mm. The distance between the two emerging electrode wires was, however, only 0.4 mm and tests in homogenized, fine-grained sediment indicated that a signal shift at the surface was resolved at a resolution of ~0.5 mm. Profiles of formation resistivity factor, F, were calculated by dividing the sensor signal at each sediment depth, R_s with the constant signal of the overlying water, R_w, (i.e., $F = R_s : R_w$), (Ullman and Aller 1982). Subsequently the effective sediment diffusive transport coefficient, D_e, was calculated as $D_e = D_0 / (\phi F)$ where, D₀, is the temperature-, pressure-, and salinity-corrected molecular diffusivity in water, and ϕ the sediment porosity (Ullman and Aller 1982). The latter was determined at a depth resolution of 0.5 cm from sediment slices as the weight loss after 24 h at 100°C multiplied with the specific density. The samples were subsequently used for determination of the organic content as the weight loss after ignition at 450°C for 24 h.

The applied Clark-type O₂ microelectrodes had tip diameters of ~5 μm, t₉₀ response times <2 s and stirring sensitivities <2% (Revsbech 1989; Glud et al. 2000). The linear response of each microelectrode was calibrated using the signals in water overlying the sediment of a known O₂ concentration and in sediment layers presumed anoxic as indicated by low constant sensor signals. The relative position of the sediment surface was estimated from each individual profile by a shift in the linear concentration gradient of the diffusive boundary layer (DBL). The O₂ penetration depth was quantified as the distance between the estimated sediment surface and the depth at which the sensor signal reached a constant low value (anoxic signal).

The NO₃⁻ microsensors were essentially biosensors consisting of a culture of denitrifiers encapsulated in a microcapillary in front of an amperometric N₂O micro-sensor (Larsen et al. 1997). As such, the sensor is sensitive to NO₃⁻, NO₂⁻, and N₂O but for convenience we in the following refer to the results as NO₃⁻ concentrations. The presently applied sensors were in several aspects improved as compared to the original description: (1) most importantly, the selected N₂O reductase-deficient bacteria strain was able to metabolize efficiently at low temperature; (2) the membrane materials were improved; and (3) an electrophoretic sensitivity-control polarization across the outer membrane was imposed to enhance supply of anions to the entrapped bacteria (Kjær et al. 1999). The improved NO₃⁻ microbiosensor will be described in detail elsewhere (Revsbech and Glud unpubl.). The sensors applied during the cruise had tip diameters of 70–100 μm, t₉₀ response times ~60 s, and negligible stirring sensitivity. The linear sensor response was calibrated against signals in water overlying the sediment of a known NO₃⁻ concentration and in sediment layers presumed nitrate-free as indicated by low constant sensor signals. The relative position of the sediment surface was estimated from each individual profile as for the O₂ microprofiles. The applied amperometric H₂S microsensors (Kuhl et al. 1998) detected no free H₂S above the detection limit of 1 μmol L⁻¹ on any of the cruises neither in situ nor in recovered sediment cores.

The vertical diffusive exchange (J) of O₂ or NO₃⁻ was calculated from Fick's first law of diffusion $J = D_0 (dC/dz)$, where, D₀, is the molecular diffusion coefficient of O₂ or NO₃⁻ at the in situ temperature, salinity, and hydrostatic pressure, and C is the O₂ (or NO₃⁻) concentration at depth z within the DBL (Rasmussen and Jørgensen 1992). From the measured NO₃⁻ microsensor profiles the consumption or production of NO₃⁻ (R_x) was calculated assuming steady-state solute distribution and by using Fick's second law of diffusion: $R_x = D_e d^2C/d^2z$, where C is the solute concentration at depth z (Crank 1975). The approach was facilitated by using the freely available software package for concentration profile interpretation PROFILE (Berg et al. 1998).

Onboard sediment-core investigations—On each cruise the ROV recovered 15–24 sediment cores in Plexiglas liners with an inner diameter of 8.2 cm. Upon recovery the cores were uncapped and subsampled into smaller liners with a

5.2-cm inner diameter. These cores were submerged in previously sampled bottom water kept at in situ temperature and O_2 tension. Rotating small magnets, receiving momentum from a centrally placed large magnet, were attached inside all core liners to ensure a well-mixed overlying water phase. After 12 h preincubation 2–4 cores were used for onboard O_2 , NO_3^- , and H_2S microprofile measurements at a vertical resolution of 100 μm or 200 μm , the procedure essentially followed the description of Rasmussen and Jørgensen (1992). A total of six sediment cores were used to measure the total exchange of O_2 , NO_3^- , and NH_4^+ . The cores were capped but O_2 microelectrodes perforating the lid were used to monitor the O_2 concentrations during the 4–8-h-long incubations. The O_2 concentration was never allowed to decline by >15% of the initial value during the incubations. At the end of the respective incubations water samples for NO_3^- and NH_4^+ were taken, stored, and analyzed as described above. The total solute exchange rates were calculated from concentration changes during the incubations as described above.

After incubation the decapped cores were left submerged in the bottom water for 12 h before they were used to measure denitrification by the isotope pairing technique (IPT; Nielsen 1992; Risgaard-Petersen and Rysgaard 1995). ^{15}N -labelled NO_3^- was added to a final concentration of 30–35 $\mu mol L^{-1}$ in the incubation bath, and as the bottom-water NO_3^- concentration ranged from 40 $\mu mol L^{-1}$ to 42 $\mu mol L^{-1}$ this resulted in a specific labeling of ~45%. Before and 2 h after tracer addition, five replicate samples for the determination of the total NO_3^- in the incubation bath were taken. After 5 h of preincubation, which allowed the tracer to diffuse into the denitrifying zone, all cores were recapped. The individual cores were processed at predefined time intervals during the overall 18-h-long incubation period. From each core samples for ^{15}N labeling of N_2 were taken from the enclosed water volume and the slurried surface sediment (0–5 cm) as described by Nielsen (1992). Samples for ^{15}N - N_2 measurements were preserved in 12.6-mL glass vials (Exetainer, Labco) containing 250 μL $ZnCl_2$ solution (50% w:w).

The relative abundance and concentrations of $^{14}N^{15}N$, and $^{15}N^{15}N$ were determined using a gas chromatograph coupled to a triple-collector isotopic ratio mass spectrometer (RoboPrep-G⁺ in line with TracerMass, Europa Scientific for March 2006, Thermo DeltaPlus for later cruises) as described by Risgaard-Petersen and Rysgaard (1995). The denitrification rates were calculated according to Risgaard-Petersen et al. (2003) for sediments hosting both denitrification and anammox activity, using the relative importance of anammox as determined independently in batch incubations (*see below*).

The relative contribution of anammox and denitrification to the overall N_2 production was determined by previously described approaches using homogenized sediment amended with ^{15}N -labelled compounds (Thamdrup and Dalsgaard 2002; Rysgaard et al. 2004). In March 2006, homogenized sediment was incubated in gas-tight plastic bags (Thamdrup and Dalsgaard 2002), while on later

cruises, sediment was slurried and incubated in glass vials (Rysgaard et al. 2004). In short, for bag incubations five cores were sectioned under N_2 and the depth intervals 0–2, 2–4, and 4–6 cm were pooled separately, homogenized, and split into three equal portions each, which were then amended with $^{15}NH_4^+$, $^{15}NH_4^+ + ^{14}NO_3^-$, and $^{15}NO_3^-$, respectively, at ~50 $\mu mol L^{-1}$ each, and, still in an N_2 atmosphere, loaded into gas-tight plastic bags (Hansen et al. 2000). The sediment batches were subsampled five times during a 3-d anoxic incubation at in situ temperature. Pore water was extracted by centrifugation and analyzed for N_2 , NO_3^- , and NH_4^+ as previously described (Thamdrup and Dalsgaard 2002).

For the slurry approach applied on later cruises, the oxic zone (as determined by the microprofile measurements above) of four sediment cores was eliminated and the underlying 3 cm of sediment of all cores was pooled and homogenized under a N_2 atmosphere in a glove bag. A 2.5-mL subsample of homogenized sediment was added to a series of gas-tight glass vials (12.6 mL; Exetainer, Labco), which were subsequently filled with artificial NO_3^- - and NH_4^+ -free, anoxic seawater (or seawater of known NO_3^- and NH_4^+ concentration). The slurries were left for 8–18 h under N_2 atmosphere to ensure elimination of trace amounts of O_2 and reduce the inherent NO_3^- concentration to a minimum. Four series of treatments, each consisting of five vials were established: (1) addition of 80 $\mu mol L^{-1}$ $^{15}NH_4^+$; (2) addition of 80 $\mu mol L^{-1}$ $^{15}NH_4^+$ and $^{14}NO_3^-$; (3) addition of 80 $\mu mol L^{-1}$ $^{14}NH_4^+$ and $^{15}NO_3^-$; and (4) addition of 80 $\mu mol L^{-1}$ $^{15}NO_3^-$. The capped vials were incubated at in situ temperature and in darkness for 3–30 h. At each sampling time one vial from each series was stopped by injecting 400 μL $ZnCl_2$ solution (50% w:w) for preservation until analysis (*see above*).

The isotope composition of N_2 for all experiments, and the concentrations of NO_3^- and NH_4^+ from the bag incubation were analyzed as described for the whole-core incubations. The respective N_2 production rates via denitrification and anammox were calculated according to Thamdrup and Dalsgaard (2002).

Benthic foraminifera—On two cruises, one sediment core was sectioned in six depth intervals down to a sediment depth of 7.0 cm. Each sediment slice was sieved through a 125- μm mesh and all shell-bearing benthic foraminifera filled with active cytoplasm in the respective slices were sampled and identified to the species level under a dissection microscope. All foraminifera were photographed together with 400- μm -diameter glass beads for later size determination. Subsequently groups and, in some cases, individual foraminifera were frozen in 1.5-mL Eppendorf™ centrifuge tubes at $-80^\circ C$, which lysed the cells. Later 0.5 mL of NO_3^- -free distilled water was added to the respective tubes hosting the lysed cells and after 20-s sonication the concentration of NO_3^- was measured as described above. The measured integrated intracellular NO_3^- pool was recalculated into a pore-water concentration as if it had been evenly distributed in the pore water of the respective sediment layers.

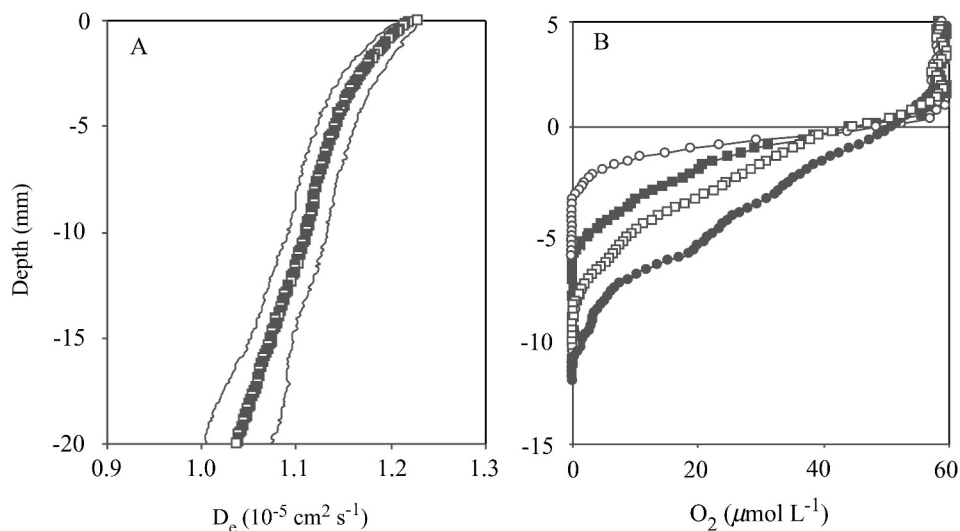


Fig. 2. (A) The average diffusive sediment transport coefficient (D_e) for O_2 as derived from resistivity profiles measured in situ ($n = 10$), profiles affected by infauna borrow structures were omitted. The profile can be converted to D_e for NO_3^- by multiplying with $D_{0,\text{O}_2} : D_{0,\text{NO}_3^-}$, equal to 0.83 at the in situ temperature (Boudreau 1997). The average profile plus or minus the standard deviation are included as solid lines. (B) Four selected in situ O_2 microprofiles measured in parallel with the resistivity profiles all within a sediment area of 130 cm^2 in central Sagami Bay. In both panels '0' at the ordinate indicate the estimated position of the sediment surface.

Results

Sediment characteristics and microprofile measurements—

The silty surface sediment of central Sagami Bay had an average porosity of 0.93 ± 0.03 that gradually declined to 0.86 ± 0.01 ($n = 6$) at 10-cm depth. The derived profiles of the diffusive sediment transport coefficient (D_e) for O_2 (and NO_3^-) exhibit an exponential decline with depth immediately below the sediment surface (Fig. 2A). From 4.0-mm depth the decline becomes linear down to 18.0–20.0-mm depth. The upper 2.0 cm of the surface sediment contained $3.0 \pm 0.4\%$ of organic matter (wet wt, $n = 6$). During the three cruises a total of 165 in situ O_2 microprofiles were measured (Glud et al. 2009). There was no statistical difference between either the O_2 penetration depth or the calculated diffusive O_2 uptake (DOU) measured during these three winter cruises (Dec, Jan, Mar). Altogether, 13% of the O_2 microprofiles were visually affected by bio-irrigation (indicated by distinct O_2 peaks in the otherwise anoxic sediment), and the average thickness of the oxic zone was $6.7 \pm 2.3 \text{ mm}$, while the average DOU amounted to $2.2 \pm 0.7 \text{ mmol m}^{-2} \text{ d}^{-1}$ (Glud et al. 2009). However, the measured microprofiles revealed an extensive small-scale variability. This is exemplified by the measurements performed during the January 2008 cruise (Fig. 2B), where the O_2 penetration depth as derived from 25 microprofiles obtained during two deployments ranged from 3.0 mm to 12.4 mm (average $7.1 \pm 2.1 \text{ mm}$), while the DOU ranged from $0.5 \text{ mmol m}^{-2} \text{ d}^{-1}$ to $3.5 \text{ mmol m}^{-2} \text{ d}^{-1}$ (average $1.42 \pm 0.74 \text{ mmol m}^{-2} \text{ d}^{-1}$). No free H_2S was measured at any time, neither in the laboratory nor in situ.

In total 13 in situ $\text{NO}_3^- + \text{NO}_2^-$ microprofiles were measured on two deployments during the cruise in

January 2008. The measurements were obtained along three mini-transects each consisting of 3–5 profiles measured at 12-mm horizontal intervals. The data exhibited an extensive small-scale variation as reflected by the highly variable nitrate distribution (Fig. 3). The $\text{NO}_3^- + \text{NO}_2^-$ penetration depth varied from 8.0 mm to 14.6 mm with an average of $10.4 \pm 1.7 \text{ mm}$ (ignoring one apparent irrigation event; Fig. 3C). Three profiles showed distinct nitrate peaks (Fig. 3B–D) as a result of surface associated nitrification, while the majority of profiles reflected a net-influx of NO_3^- to the sediment. Altogether the diffusive exchange of nitrate across the benthic interface, as derived from the concentration gradient at the sediment surface, ranged from $-0.67 \text{ mmol m}^{-2} \text{ d}^{-1}$ to $1.15 \text{ mmol m}^{-2} \text{ d}^{-1}$ and the average value amounted to $0.53 \pm 0.61 \text{ mmol m}^{-2} \text{ d}^{-1}$. The net production–consumption of $\text{NO}_3^- + \text{NO}_2^-$ for the individual microprofiles, as quantified from the described curve-fitting procedure, mostly reflected a typical pattern of a presumed oxic surface zone with nitrification or no $\text{NO}_3^- + \text{NO}_2^-$ turnover followed by a zone of net consumption of $\text{NO}_3^- + \text{NO}_2^-$ by denitrification or anammox activity (Fig. 3).

In situ exchange data and laboratory-based measurements—

The bottom-water concentrations of NO_3^- and NH_4^+ during all three cruises were similar and, on average, amounted to $40.2 \pm 0.8 \mu\text{mol L}^{-1}$ and $1.1 \pm 0.5 \mu\text{mol L}^{-1}$, respectively. A total of four in situ chamber incubations were performed during the three cruises, providing an average NO_3^- uptake rate of $1.44 \pm 0.70 \text{ mmol m}^{-2} \text{ d}^{-1}$ and an ammonium exchange rate of $-0.49 \pm 0.25 \text{ mmol m}^{-2} \text{ d}^{-1}$ (Table 1; Fig. 4). The corresponding values for

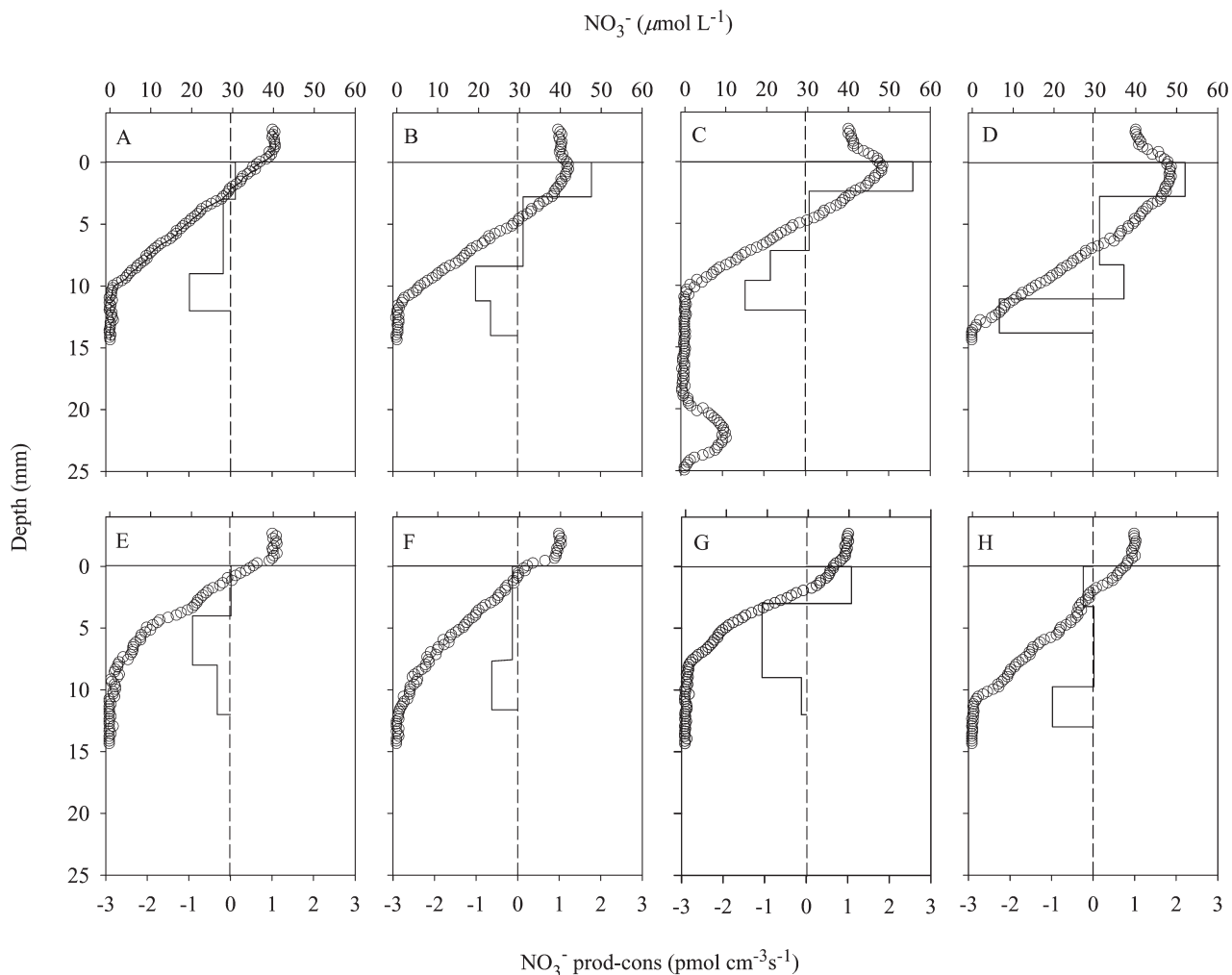


Fig. 3. Selected in situ NO_3^- microprofiles and derived volume-specific NO_3^- production and consumption (prod-cons). Profiles A–D are measured consecutively at an equidistant distance of 12 mm. Likewise, profiles E–F and G–H are separated by a distance of 12 mm.

the core incubations in the laboratory were $0.57 \pm 0.20 \text{ mmol m}^{-2} \text{ d}^{-1}$ and $-0.20 \pm 0.09 \text{ mmol m}^{-2} \text{ d}^{-1}$, respectively (Table 1).

Anammox made a substantial and stable contribution to N_2 production by batch incubations of homogenized or slurried sediment during all three cruises, with an average of $36 \pm 2\%$ (Table 1). In March 2006, incubations with

$^{15}\text{NO}_3^-$ yielded anammox contributions of 35%, 35%, and 39% at 0–2-, 2–4-, and 4–6-cm depth, respectively, and a similar mean contribution of 37% was determined for the parallel incubations with $^{15}\text{NH}_4^+ + ^{14}\text{NO}_3^-$, yielding a mean of 36% for all six incubations. This value was reproduced closely in slurries of surface sediment (0.5–3.5 cm) during the subsequent cruises. There was no

Table 1. Nutrient fluxes, denitrification ($\text{mmol m}^{-2} \text{ d}^{-1}$), and the relative importance of anammox for the N_2 production as measured in slurred surface sediment. The average values used in the budget calculations account for the different numbers of n .

	Mar 06	Dec 06	Jan 08	Mean
Lab				
NO_3^- flux	0.45 ± 0.13 ($n=6$)	0.96 ± 0.50 ($n=4$)	0.30 ± 0.20 ($n=6$)	0.57 ± 0.20
NH_4^+ flux	-0.18 ± 0.11 ($n=6$)	-0.28 ± 0.10 ($n=4$)	-0.15 ± 0.04 ($n=6$)	-0.20 ± 0.09
Denitrification	0.64 ± 0.31 ($n=6$)	0.79 ± 0.28 ($n=4$)	0.57 ± 0.12 ($n=6$)	0.67 ± 0.25
Anammox (%)	36 ± 3 ($n=6$)	36 ± 1 ($n=10$)	35 ± 2 ($n=10$)	36 ± 2
In situ				
NO_3^- flux	2.2 ($n=1$)	0.88 ± 0.60 ($n=2$)	1.80 ($n=1$)	1.44 ± 0.70
NH_4^+ flux	-0.41 ($n=1$)	-0.59 ± 0.28 ($n=2$)	-0.38 ($n=1$)	-0.49 ± 0.25

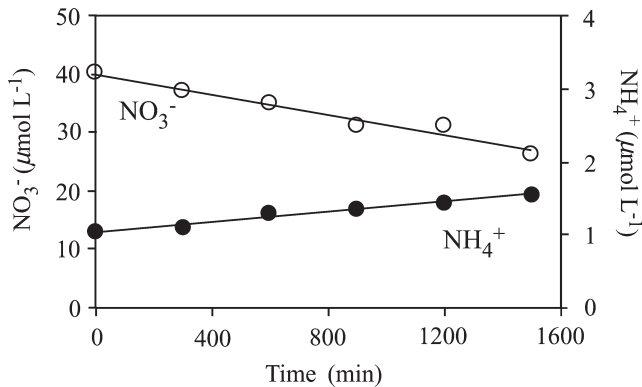


Fig. 4. NO_3^- and NH_4^+ concentrations in the overlying water during the benthic-chamber incubation of January 2008.

detectable production of ^{15}N -labeled N_2 in incubations with $^{15}\text{NH}_4^+$ without added NO_3^- in January 2008, while a slight sustained production of $^{14}\text{N}^{15}\text{N}$ was observed during the previous cruises, at a rate corresponding to <3% of the rate in the presence of $^{14}\text{NO}_3^-$. The denitrification rates as measured by IPT in the intact recovered sediment cores, with correction for the effect of anammox, were also not significantly different for the three respective cruises and the average amounted to $0.67 \pm 0.25 \text{ mmol m}^{-2} \text{ d}^{-1}$ (Table 1).

Benthic foraminifera—During the cruise in December 2006, 449 shell-bearing foraminifera of nine different species were encountered below a sediment area of 38 cm^2 . The community was strongly dominated by *Globobulimina affinis* and *G. pacifica*. Together the two

Globobulimina species accounted for >45% of all encountered specimens while *Chilostomella ovoidea* accounted for 22% and each of the remaining species for <10% (Fig. 5A). The distribution reflected an apparent succession with *Cyclammina cancellata*, *Uvigerina akitaensis*, and *Bolivina spissa* dominating in the surface sediment gradually replaced by *Textularia* sp., *Reophax*, *Chilostomella ovoidea*, and *Globobulimina* sp. in the deeper sediment strata. The size span of the individual foraminifera varied by a factor of >1000 from $0.8\text{-}\mu\text{L}$ large *Cyclammina cancellata* to small individuals of *Textularia kattgatensis* being $<0.0003 \mu\text{L}$. Given the complex 3-dimensional structure of the shells we were not able to reliably convert the sampled specimens into bio-volumes or biomass despite the scaled images obtained of each of the sampled specimens—this was especially true for the small species and size classes.

As described above, the cells collected from the respective taxa were divided in up to three size classes and after freezing the cell-content was dissolved in 0.5 mL NO_3^- -free water. Only samples containing *Cyclammina cancellata* were not able to significantly raise the NO_3^- concentration above a background level of $0.5 \pm 0.2 \mu\text{mol L}^{-1}$ in tubes containing no cells. The background level is most likely related to air born N-contamination in the laboratory.

The NO_3^- concentration in tubes containing larger specimens of *G. affinis* increased up to $250 \mu\text{mol L}^{-1}$, which corresponded to an internal NO_3^- pool of $\sim 30 \text{ nmol cell}^{-1}$ or an average estimated internal nitrate concentration in the order of $\sim 300 \text{ mmol L}^{-1}$. On average small *Globobulimina* cells contained $2.3 \pm 1.8 \text{ nmol NO}_3^-$ ($n = 7$), while medium-sized and large cells on average contained

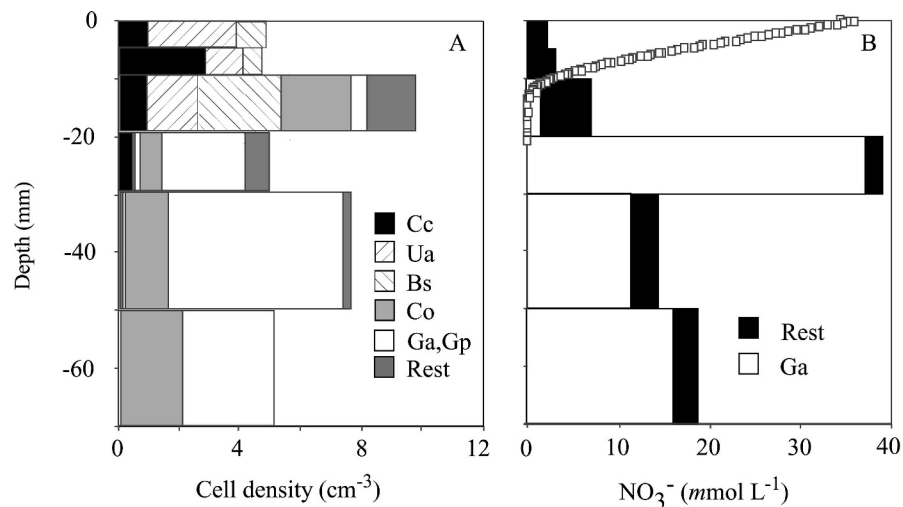


Fig. 5. (A) The depth distribution of shell-bearing benthic foraminifera as quantified in a sediment core (inner diam. 70 mm) recovered from central Sagami Bay in December 2006. The abbreviations represent the following species (Cc) *Cyclammina cancellata*, (Ua) *Uvigerina akitaensis*, (Bs) *Bolivina spissa*, (Co) *Chilostomella ovoidea*, (Ga) *Globobulimina affinis*, and (Gp) *G. pacifica*, while 'Rest' indicates different species encountered in low numbers. (B) Small open squares show the average in situ NO_3^- microprofile ($n = 13$), while the large boxes show the intracellular NO_3^- pools in the respective depth intervals expressed as average pore-water concentration for *G. affinis* and for the total foraminifera population.

7.9 ± 5.8 ($n = 7$) and 20.4 ± 9.3 ($n = 5$) nmol NO_3^- per cell, respectively (n indicates number of tubes containing the respective size classes that were analyzed). *Chilostomella ovoidea* cells on average contained 1.1 ± 0.5 nmol NO_3^- , while all remaining species contained <1 nmol NO_3^- cell $^{-1}$. Without correct assessment of the cell sizes it is difficult to estimate the internal nitrate concentration of especially the small species, but apparently several of the sampled species were able to internally accumulate NO_3^- above the ambient concentration. There was no apparent relation between sediment depth at which cells were sampled and their internal NO_3^- content.

The in situ microprofiles documented that free NO_3^- only was available in the upper 8–15 mm and in this zone the foraminifera-bound NO_3^- pool only accounted for a minor fraction of the total benthic NO_3^- pool (Fig. 5B). However, in the deeper sediment layers the NO_3^- pool stored by foraminifera corresponded to a total NO_3^- pore-water concentration of 14–39 $\mu\text{mol L}^{-1}$ (Fig. 5B). Integrated for the upper 70 mm of sediment, $\sim 80\%$ of the benthic NO_3^- pool was retained within the foraminifera and of this pool *Globobulimina* sp. accounted for $\sim 74\%$. A preliminary investigation on the foraminifera-bound NO_3^- pool, carried out already during the cruise in March 2006 and on basis of a smaller data set, essentially provided the same results. On that occasion *Globobulimina* sp. accounted for 30% of the sampled shell-bearing benthic foraminifera and that they contained up to 29 nmol NO_3^- cell $^{-1}$ (average 10 nmol NO_3^- cell $^{-1}$). It should be noted that on both occasions strongly agglutinated foraminifera cells were not collected and, thereby, they were not accounted for; we estimate that they accounted for ~ 10 – 20% of the total shell-bearing foraminifer biomass.

Discussion

During the last decade the marine nitrogen cycle has undergone major conceptual changes, and in many environments the quantitative importance of the various 'new' pathways is poorly constrained (Brandes et al. 2007). This is particularly true for the deep shelf and the abyssal plains. Taking advantage of the ongoing interdisciplinary research program in Sagami Bay, we have carried out a multifaceted approach to constrain the rates and pathways of benthic nitrogen turnover and constructed a nitrogen budget for this site.

Deposition and mineralization—The actual net-deposition of organic matter can be bracketed by measurements with sediment traps, which have caught fluxes of 66 $\text{mg C m}^{-2} \text{d}^{-1}$ and 241 $\text{mg C m}^{-2} \text{d}^{-1}$ at 350 m and 20 m above the seabed, respectively (Masuzawa et al. 2003). The difference may partly be explained by near-bottom, downslope transport of material bypassing the upper trap, and by local resuspension events that may add conversely to the deeper trap (Masuzawa et al. 2003). Accounting for the average C:N ratio (~ 8.2 w/w) of the organic matter caught in the traps (Nakatsuka et al. 2003) we estimate the average annual net deposition of particulate organic nitrogen in central Sagami Bay to between 0.6 mmol N

$\text{m}^{-2} \text{d}^{-1}$ and 2.1 $\text{mmol N m}^{-2} \text{d}^{-1}$. These values bracket the depth-integrated NH_4^+ mineralization rate of 0.95 $\text{mmol m}^{-2} \text{d}^{-1}$ as measured in a series of anoxic bag incubations of homogenized sediment from 0-cm to 10-cm depth (B. Thamdrup unpubl.). This procedure for estimating N mineralization is compromised by two opposing factors; sediment homogenization generally stimulates mineralization of organic material and, consequently, the ammonification rate (Hansen et al. 2000), but the net NH_4^+ accumulation does not account for any adsorption to sediment particles during the incubation of the newly homogenized sediment (Blackburn 1979, Hansen et al. 2000). The two factors scale quantitatively, and for the present budget calculations we assumed that they outbalance each other.

The benthic N burial can be estimated from a previous burial rate of organic carbon of ~ 16 $\text{mmol C m}^{-2} \text{d}^{-1}$ determined for a sediment depth of 10 cm (Glud et al. 2009) and the C:N ratio determined in the same depth (~ 8.7 w/w; T. Nakatsuka unpubl.). The retention of N below 10 cm of surface sediment as estimated by this procedure amounts to 1.5 $\text{mmol N m}^{-2} \text{d}^{-1}$. In this context it should, however, be noticed that downslope, near-bottom hyperpycnal transport of sediment material does contribute to the deposition in central Sagami Bay, which could circumvent the sediment traps (Soh 2003), and this probably explain the relatively high N retention calculated by this procedure.

Pore-water profiles of NH_4^+ show that N mineralization does occur in deeper sediment layers, but the vertical flux at 10-cm depth only amounts to 0.03 ± 0.01 $\text{mmol m}^{-2} \text{d}^{-1}$ ($n = 4$; B. Thamdrup unpubl.) and can in the present context be ignored.

Benthic NO_3^- distribution and exchange—The NO_3^- microsensor data revealed extensive small-scale variation where successive vertical profiles exhibited very different NO_3^- penetration depths and nitrogen turnover rates (Fig. 3). The sediment surface in some instances hosted a nitrifying community with a net NO_3^- production of >2 $\text{pmol cm}^{-3} \text{s}^{-1}$ while the surface in other profiles a few centimeters away showed almost no nitrifying activity. The volume-specific NO_3^- consumption in the deeper sediment layers showed a similar variation reaching maximum values >2 $\text{pmol cm}^{-3} \text{s}^{-1}$. The extensive variation aligns with previous (and parallel) O_2 microprofiles data that resolved a highly heterogeneous O_2 distribution in the same surface sediment (Fig. 2). This was ascribed to patchy distribution of the O_2 -consuming processes (i.e., the available electron donors) that exhibited a mosaic-like pattern of diagenetic hot spots (Glud et al. 2009). Clearly extrapolation from a few profiles in such sediments should be done with caution. However, the average depth-integrated nitrification activity of all 13 profiles amounted to 0.22 $\text{mmol m}^{-2} \text{d}^{-1}$ while the depth-integrated average nitrate consumption rate equaled 1.01 $\text{mmol m}^{-2} \text{d}^{-1}$ (Fig. 3). For profiles exhibiting a clear NO_3^- peak, on average 45% of the produced NO_3^- diffused upwards into the overlying water while the remainder helped sustain the underlying NO_3^- consumption. In other words, based on all the microprofile data,

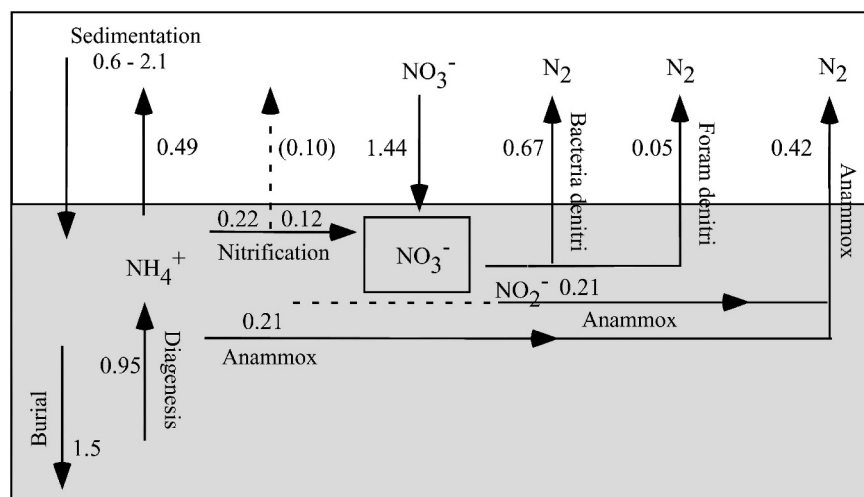


Fig. 6. A budget for benthic N turnover in the surface sediment (0–10 cm) of central Sagami Bay. All values ($\text{mmol N m}^{-2} \text{d}^{-1}$) are based on independent measurements, each with their own caveats and shortcomings and, as such, a complete mass balance is not expected (see text for details).

88% of the NO_3^- consumption in the sediment was supported by diffusive supply from the overlying water, while 12% was supported by sediment nitrification.

The average diffusive NO_3^- exchange as derived from the in situ microprofiles ($0.53 \pm 0.61 \text{ mmol m}^{-2} \text{d}^{-1}$) was somewhat lower than the average total NO_3^- uptake as measured by the benthic-chamber deployments ($1.44 \pm 0.82 \text{ mmol m}^{-2} \text{d}^{-1}$). The difference is not significant, but as the total exchange rate includes any contribution from bio-irrigation it is expected to exceed the diffusive mediated uptake. The macrofauna was dominated by a diverse polychaete infauna, and a single NO_3^- microprofile (Fig. 3C) indeed suggested that burrow irrigation may stimulate both the NO_3^- production and consumption as it has been observed for coastal sediments (Binnerup et al. 1992; Pelegri and Blackburn 1995; Webb and Eyre 2004). The fact that the laboratory-based whole-core incubations, which tend to underestimate natural infauna activity (Glud 2008), also underestimated the in situ nutrient exchange further suggest that fauna can have an importance for the benthic nitrogen exchange at this site (Table 1). As a measure for the benthic exchange of NO_3^- and NH_4^+ , the in situ chamber incubations remain the most reliable. The in situ efflux of NH_4^+ equaled $\sim 50\%$ of the net NH_4^+ production as measured from anoxic bag incubations (B. Thamdrup unpubl.), and the other $\sim 50\%$ balanced the nitrification activity inferred from the microprofiles and the anammox activity estimated from the onboard batch incubations (Fig. 6).

Anammox and denitrification—As techniques for in situ determination of anammox and denitrification have not yet been developed, we quantified these processes through a combination of batch and whole-core incubations onboard the ship. Apart from potential effects of sediment recovery, possible caveats associated with the batch incubations include changes in the labeling of NH_4^+ and NO_3^- during the incubations and differential effects of sediment

handling and amendments on denitrification and anammox (Thamdrup and Dalsgaard 2002). Changes in the labeling of NO_3^- are normally not expected during anoxic incubation, but in this sediment NO_3^- leaking from the large intracellular NO_3^- pool in foraminifera could dilute added $^{15}\text{NO}_3^-$, which would result in overestimation of anammox. The slight production of ^{15}N -labeled N_2 observed in some of the incubations with $^{15}\text{NH}_4^+$ and no added NO_3^- may be due to such leakage, but because this production was $<3\%$ of that observed with addition of $^{14}\text{NO}_3^-$, the leakage should not substantially affect our results. This was further supported by the fact that we calculated the same relative importance of anammox for sediment N_2 production in slurries containing $^{15}\text{NO}_3^-$ and $^{15}\text{NH}_4^+$.

A recent study suggests that sediment homogenization may lead to underestimation of the importance of anammox as compared to intact sediment cores (Trimmer et al. 2006). Thus, at a site where anammox, similar to our study, contributed 36% to N_2 production in a homogenized batch, the contribution was estimated to 48% in whole cores by means of an elegant method based on isotope analysis of both N_2 and N_2O . So far, however, the two approaches have only been compared at two sites with anammox, and it remains to be seen whether the difference is a general trend.

The current database shows that the relative importance of anammox for benthic N_2 production ranges from below detection to a current maximum of $\sim 80\%$ at 700-m depth in central Skagerrak, Denmark (Dalsgaard et al. 2005). Generally, there appears to be a positive relation between the importance of anammox and increasing water depth, which relates to the overall decline in carbon mineralization rate (Dalsgaard et al. 2005; Engstrom et al. 2005). Given the water depth of central Sagami Bay (1450 m) and the fact that the overall benthic carbon mineralization rate is ~ 8 times lower than the activity in Skagerrak (Canfield et al. 1993; Glud et al. 2009) one might have expected a

relative importance of anammox that at least ranged with the ~80% found in Skagerrak. However, the high contribution in Skagerrak is likely related to competitive inhibition of denitrification by dissimilatory Mn reduction, which in turn is supported by an unusually high Mn oxide content (Dalsgaard and Thamdrup 2002). Moreover, one distinct difference between these two sites is the reduced O₂ availability in central Sagami Bay. The O₂ depletion could have limited the nitrification and the nitrite-fed anammox activity as compared to the more oxygenated environments of Skagerrak. Furthermore, a number of studies indicate that other environmental controls like NO₃⁻ and NO₂⁻ availability (Risgaard-Petersen et al. 2004; Rysgaard et al. 2004; Trimmer et al. 2005), temperature, and ecosystem stability (Rysgaard et al. 2004) also play a role for the relative importance of anammox in marine sediments. The present study shows that even though anammox is an important nitrogen sink, denitrification remains the dominant pathway for N₂ production at our study site (Fig. 6). If this can be extrapolated to ocean-margin sediments in general remains to be investigated.

Procedures for quantifying N₂ exchange rates and denitrification by IPT directly at the coastal seabed have been presented (Devol 1991; Nielsen and Glud 1996). However, contamination problems, shifts in hydrostatic pressure (i.e., gas solubility) and disturbances during chamber recovery make it a nontrivial task to transplant these approaches to greater depth. Therefore practically all direct assessments of N₂ production in sediments from deeper waters have been based on incubations of recovered sediment cores. To what extent recovery affects denitrification or anammox activity along the primary sediment-water interface in such studies is currently unknown. The NO₃⁻ consumption rates calculated from the in situ pore-water profiles and the chamber measurements do, however, scale with the N₂ production (i.e., prokaryotic denitrification and anammox) as quantified by tracer-additions experiments in the laboratory. The revised IPT procedure in combination with an independent assessment of the anammox activity may, for most sediments, provide relatively robust estimates of the prokaryotic denitrification (Risgaard-Petersen et al. 2003), but it does not account for the activity of organisms sustaining their denitrification by a large unlabelled intracellular NO₃⁻ pool.

The current sediments did not host a larger population of known NO₃⁻-accumulating bacteria, but NO₃⁻-accumulating foraminifera proved to hold ~1.3 mmol NO₃⁻ m⁻², which is ~5-fold more than the NO₃⁻ pool dissolved in the pore water. The average denitrification rate of *G. pseudospinesscens* cells has been estimated to ~560 pmol N individual⁻¹ d⁻¹ (range 226–902 pmol N individual⁻¹ d⁻¹; Risgaard-Petersen et al. 2006). Assuming that this value holds for the *Globobulimina* species of our study the average turnover time for this pool was 29 d (range 12–52 d). The *Globobulimina* cells can thereby on average move around in deeper NO₃⁻-free sediment layers for 29 d while living on their NO₃⁻ storage. Another recent study estimated the denitrification rate of the smaller foraminifera *Nonionella* cf. *stella* to be 84 ± 33 pmol N individual⁻¹ d⁻¹ (Høgslund et al. 2008). Applying this figure to the

remaining smaller nitrate-accumulating foraminifera of our study the average NO₃⁻ turnover time for this group amounted to 17 d. The turnover time of the intracellular NO₃⁻ pools is much slower than the turnover time of the pore-water NO₃⁻, which on the basis of the in situ microprofiles, is calculated to be ~0.2 d. Applying the cell-specific denitrification rates above to the foraminifera densities of Sagami Bay the total foraminifera denitrification equals 0.05 mmol N m⁻² d⁻¹, equivalent to just ~4% of the total N₂ production. Even though this value represents a minimum, because cells below 7-cm depth or smaller than 125 μm have not been accounted for and agglutinated cells were discarded, the value indicates that foraminifera, despite hosting a large intracellular NO₃⁻ pool, only play a minor role for NO₃⁻ turnover in the investigated sediment.

The benthic nitrogen turnover in central Sagami Bay—Based on the various measurements we have established a benthic nitrogen budget for central Sagami Bay (Fig. 6). Because the budget is based on different independent measurements and procedures that each have their caveats and limitations, the budget is not expected to balance, but nevertheless the budget appears surprisingly consistent. The NH₄⁺ that is released from the sediment or consumed by nitrification and anammox must be produced by ammonification as the settling organic material is mineralized. Microprofile measurements reflected an average nitrification activity of 0.22 mmol N m⁻² d⁻¹, while onboard tracer-addition experiments indicated that anammox consumed 0.21 mmol N m⁻² d⁻¹ in the form of NH₄⁺ (assuming a 1:1 stoichiometry between NO₂⁻ and NH₄⁺), and in situ chamber incubation showed an average efflux of -0.49 mmol N m⁻² d⁻¹ (Fig. 6). This sums-up to 0.92 mmol N m⁻² d⁻¹, which nearly matches the integrated NH₄⁺ release rate of 0.95 mmol N m⁻² d⁻¹ measured in anoxic bag incubations of the 0–10-cm depth interval (B. Thamdrup unpubl.). Based on the microprofiles, 0.10 mmol N m⁻² d⁻¹ of the NO₃⁻ produced during nitrification is released to the overlying water (an efflux that is included in the net NO₃⁻ uptake measured by the in situ chamber incubation) while the remainder directly supported the benthic N₂ production (Fig. 6). In total the benthic NO₃⁻ supply of 1.66 (1.44 + 0.12) mmol N m⁻² d⁻¹ exceeds the measured NO₃⁻ consumption of 0.93 (0.67 + 0.05 + 0.21) mmol N m⁻² d⁻¹ (Fig. 6). This may partly be ascribed to the large dynamic population of foraminifera that efficiently can accumulate NO₃⁻ at rates that exceeds their metabolic rate by a factor of 10 (Høgslund et al. 2008). Bacterial denitrification accounts for the major fraction of the N₂ production, followed by anammox and a minor contribution from foraminifera-mediated denitrification (Fig. 6). In addition to any sediment N burial, the integrated budget shows that the sediment of central Sagami Bay represents a regional N sink in the order of ~1.1 (0.67 + 0.05 + 0.42) mmol N m⁻² d⁻¹.

We are quite aware of the shortcomings and limitation of the respective techniques, which have been discussed in a number of studies (Groffman et al. 2006; Trimmer et al. 2006; Ferguson and Eyre 2007). The present study is based

on a combination of 'state of the art' in situ techniques and well-established, widely used, laboratory-based measuring routines. Caveats or shortcomings related to recovery artefacts cannot be excluded and potential nitrogen turnover associated with infauna burrows is not included. Further, all three measuring campaigns have been carried out during winter (Dec–Mar) and seasonal deposition events may change the outlined budget during summer. Nevertheless our study strongly suggests that denitrification and anammox are important N sinks in the investigated sediment. In fact the overall N_2 production of the present study ranges with many coast and shelf studies (Herbert 1999; Rysgaard et al. 2004; Purvaja et al. 2008). Central Sagami Bay is a high-deposition area and extrapolation of our findings to deep margin sediments in general should be done with caution. The bottom water is strongly depleted in O_2 (15% air-saturation), which presumably leads to relatively low nitrification rates (Middelburg et al. 1996) and could reduce the relative importance of anammox as compared to more oxygenated environments. The area does, however, bear resemblance to many ocean-margin and deep-fjord systems characterized by moderate upwelling and downslope material transport. The present study suggests that these areas indeed may prove to be important regional N sinks.

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