Microscale distribution of oxygen and nitrate in sediment inhabited by *Nereis diversicolor*: spatial patterns and estimated reaction rates

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ABSTRACT: Profiles of oxygen and nitrate were measured with microsensors in sediment surrounding burrow structures of the polychaete *Nereis diversicolor* in a shallow mudflat in Limfjorden, Denmark. Rates and spatial distribution of reactions involving oxygen and nitrate (oxygen consumption, nitrification and nitrate reduction) in the vicinity of burrow structures and surface sediment were calculated and identified from concentration profiles. The burrow walls of the natural *N. diversicolor* population increased the surface area of the sediment–water interface several-fold and introduced oxic microniches with high metabolic activity into otherwise anoxic sediment. Nitrification and nitrate reduction were spatially separated processes, with nitrification restricted to the 0.9 to 1.5 mm thick oxic zone and nitrate-reduction occurring as a strictly anaerobic process in the nitrate-containing layers next to the oxic zone. At a density of 2560 individuals m^{-2}, burrow walls of *N. diversicolor* account for 12 to 40% of bulk sediment oxygen uptake and 50 to 77% and 58 to 82% of bulk nitrification and nitrate reduction, respectively. Thus, burrow structures of macro-infauna are potentially a major sediment compartment involved in nitrogen cycling and may promote loss of bioavailable nitrogen from the sediment.

KEY WORDS: NO_3^-/NO_2^- biosensor · Nitrogen cycling · Sediments · Bioturbation · *Nereis diversicolor* · Burrow walls

INTRODUCTION

Nitrogen cycling in sediments has been studied intensively during recent decades, and several approaches have been used to quantify nitrification and denitrification/nitrate reduction. These include mass balance (Lomstein et al. 1998), inhibition techniques (Ormeland & Capone 1988), diagenetic modeling (Aller 1988), isotope techniques (Koike & Hattori 1978, Nielsen 1992) and microsensors (Binnerup et al. 1992, Jensen et al. 1993, Lorenzen et al. 1998). It is now widely recognized that ammonium oxidation by nitrifying bacteria is crucial in generating nitrate for anaerobic denitrification, leading to loss of nitrogen to the

atmosphere. It was realized early that the activity of burrowing macrofauna significantly enhanced nitrogen cycling in sediments by extending the oxic sediment–water interface into otherwise anoxic sediment (Henriksen et al. 1980). Irrespective of approach, animal species, location, and sediment type, most studies have revealed that burrow structures increase ammonium efflux from the sediment and increase nitrification as well as denitrification and nitrate uptake by the sediment (Henriksen et al. 1980, Kristensen et al. 1985, Pelegrí et al. 1994, Mayer et al. 1995, Pelegrí & Blackburn 1995, Svensson & Leonardson 1996, Gilbert et al. 1998). Animal density, size, and irrigation rates determine the degree to which the processes are stim-

ulated, and species with high irrigation rates (e.g. *Nereis diversicolor* and *Corophium volutator*) have the most pronounced impact. Introduction of the isotope pairing technique (Nielsen 1992) further revealed that coupled nitrification/denitrification processes in burrows are stimulated in excess of what is predicted by assuming a simple extension of the sediment–water interface, probably due to temporally and spatially decreased diffusion distances in the burrow wall (Pelegri & Blackburn 1995, Svensson & Leonhardson 1996).

Burrow structures are often considered a physical and chemical extension of the sediment surface with compressed solute and reaction contours (Aller 1988). Although they are known as biogeochemical hot-spots with high organic loading, bacterial numbers, and activity (Kristensen 1988, Reichardt 1988, Hansen et al. 1996, Phillips & Lovell 1999), relatively few studies have quantified and located biogeochemical reactions in the vicinity of the infaunal burrows. Direct measurements in the sediment immediately surrounding burrow structures have shown that this unique environment increases oxygen consumption (Binnerup et al. 1992), hampers sulfate reduction rates (Gribsholt et al. 2003, Nielsen et al. 2003) and increases potential nitrification and denitrification (Kristensen 1985, Mayer et al. 1995) compared to the surrounding sediment. In the past, nitrification denitrification processes in sediment surrounding burrow structures were measured from coarsely dissected burrows (low resolution) followed by slurry incubation (Kristensen 1985, Mayer et al. 1995) or by modeling similar coarse profiles of nitrate (Aller 1988). The development and introduction of NO_3^-/NO_2^- biosensors (Larsen et al. 1997) has made it possible to obtain high-resolution (µm-scale) profiles in sediments so that nitrification/nitrate reduction rates can be determined via diffusion–reaction modeling without the limitations and disadvantages associated with slurry experiments. These biosensors have proved excellent for measurements in marine surface sediments and freshwater microalgal biofilms (Larsen et al. 1997, Lorenzen et al. 1998), and are potentially a very powerful tool for studying nitrification and denitrification in stratified microbial communities.

The aim of the present study was to resolve the radial O_2 and NO_3^- distribution around burrows of *Nereis diversicolor* in a shallow Danish Fjord by the use of microsensors and to quantify nitrification/nitrate reduction processes associated with burrow walls. Oxygen consumption, net nitrification, and nitrate reduction were calculated from the measured O_2 and NO_3^- profiles using a diffusion–reaction model. The contribution of nitrification/nitrate reduction around burrow walls to total sediment nitrification/nitrate reduction was evaluated and related to total sediment oxygen consumption.

MATERIALS AND METHODS

Sampling location and sediment handling. Sediment cores were collected in May and September 2000 from a shallow mud flat at Aggersund (Limfjorden, Denmark). The sampling site was located next to a small freshwater outlet and was permanently water-covered, with a salinity of about 20 ppt during sampling periods. No rooted plants were observed at the location, and bottom-dwellers such as the polychaete *Nereis diversicolor* and the crustacean *Corophium volutator* dominated the infaunal community.

Undisturbed sediment cores were sampled with transparent acrylic core tubes (8 cm inner diameter) on both sampling occasions. After return to the laboratory, sediment cores were acclimatized for at least 24 h at 15°C before further handling. Profile measurements were conducted on selected individual burrows (O_2 and NO_3^-/NO_2^-) from cores on both sampling occasions, whereas whole-core flux measurements were conducted only on sediment cores sampled in September 2000.

Radial O_2 and NO_3^- profiles. Profiles of O_2 and NO_3^-/NO_2^- were obtained vertically at the sediment surface (September only) and radially in sediment surrounding *Nereis diversicolor* (on both occasions) (Fig. 1A). To obtain radial profiles, sediment cores containing *N. diversicolor* were gently split to expose the burrow surface (Fig. 1B). Individual burrows walls and the surrounding sediment were positioned in plastic trays and placed in seawater from the sampling location prior to measurement. Vertical profiles were obtained from intact cores.

In May 2000, burrow walls received 1 of 3 treatments: (1) NH_4^+-enriched seawater to a concentration of 100 µM, (2) NH_4^+-enriched seawater (100 µM) saturated with acetylene (20 Pa), and (3) unamended seawater. Radial burrow profiles of O_2 and NO_3^- were measured from 0 cm to approximately 10 cm along the burrow shaft. The low concentration of acetylene in Treatment 2 was intended to inhibit nitrification activity without affecting denitrification (Klemedtsson et al. 1988). In September 2000, only 100 µM NH_4^+-amended seawater was used, as the nitrification rates under natural ammonium concentrations were too slow to measure. Radial burrow profiles of O_2 and NO_3^- were measured at 3 vertical depth intervals (0–3, 3–6, and 6–9 cm) along the burrow shaft to determine whether depth in the sediment was important. In addition, vertical profiles were obtained from the sediment surface to compare rates at the surface with rates in the burrow wall.

Radial O_2 profiles were measured by gently introducing a Clark type microelectrode (10 µm tip, lower detection limit 0.5 µM) (Revsbech 1989) vertically into the exposed burrow wall in steps of 100 µm. The elec-

trode was held by a micromanipulator and connected to a picoammeter and strip-chart recorder. The electrode was calibrated by a 2-point calibration prior to measurements (i.e. in fully O_2-saturated seawater of known temperature and salinity and by introducing the electrode into anoxic sediment).

Radial NO_3^- profiles were measured on the same burrows, and in the vicinity of the spot at which O_2 profiles were obtained by a NO_3^-/NO_2^- biosensor (tip width of 35 µm, lower detection limit 0.5 µM) that had been pre-incubated for 1 d (Larsen et al. 1997) and calibrated by a 4-point calibration in water of known NO_3^- concentration prior to measurements. The sensor was positioned just above the exposed burrow wall surface and was introduced vertically into the sediment in 100 to 250 µm steps using a micromanipulator. The measured signals were amplified by a picoammeter and recorded on a strip-chart recorder. The profiles were obtained within approximately 30 min after burrow wall exposure. Vertical NO_3^- profiles were obtained using the same biosensor for subsamples of surface sediment from undisturbed cores. Because NO_2^- usually accounts for only a minor fraction of total NO_3^-/NO_2^- in coastal environments, in the following text, all NO_3^-/NO_2^- profiles obtained will be referred to as NO_3^- profiles.

All O_2 and NO_3^- profiles were modeled using the program 'PROFILE' (Berg et al. 1998) to obtain estimates of net substrate consumption or production at all measured depths. Fick's second law of diffusion, including a production and consumption term, was used as the basis of the calculations. Porosity in the burrow wall was assumed to be equal to bulk sediment porosity. Steady-state conditions were assumed, as equilibrium between diffusion transport and reactions is attained within a few minutes at distances <2 mm.

Flux measurements. Total sediment metabolism was determined in September 2000 as CO_2 and O_2 flux across an inundated sediment–water interface using the closed-core technique (Kristensen et al. 1988), with 5 cores equipped with magnetic stirrers and driven by a central magnet. The cores were sealed by rubber stoppers and incubated for 2 to 4 h in darkness at 15°C. Water samples for total CO_2 (TCO_2) and O_2 measurement were taken before and after sealing the cores. The stirring rate was constantly below the resuspension level, and the O_2 concentration of the headwater never dropped below 70 % of air saturation. Samples for TCO_2 were immediately preserved by addition of 10 µl saturated $HgCl_2$ solution ml^{-1} water sample and were later analyzed with the flow injection/diffusion cell technique (Hall & Aller 1992). Samples for O_2 determination were measured immediately after sampling with a Clark-type microelectrode (Revsbech 1989).

Sediment parameters. Separate cores for determination of sediment parameters were sliced into sections representing the 3 depths at which burrow profiles were obtained (0–3, 3–6 and 6–9 cm). Sediment density was determined as the weight of a known sediment volume, while sediment water content was determined as weight loss after drying sediment for 12 h at 105°C.
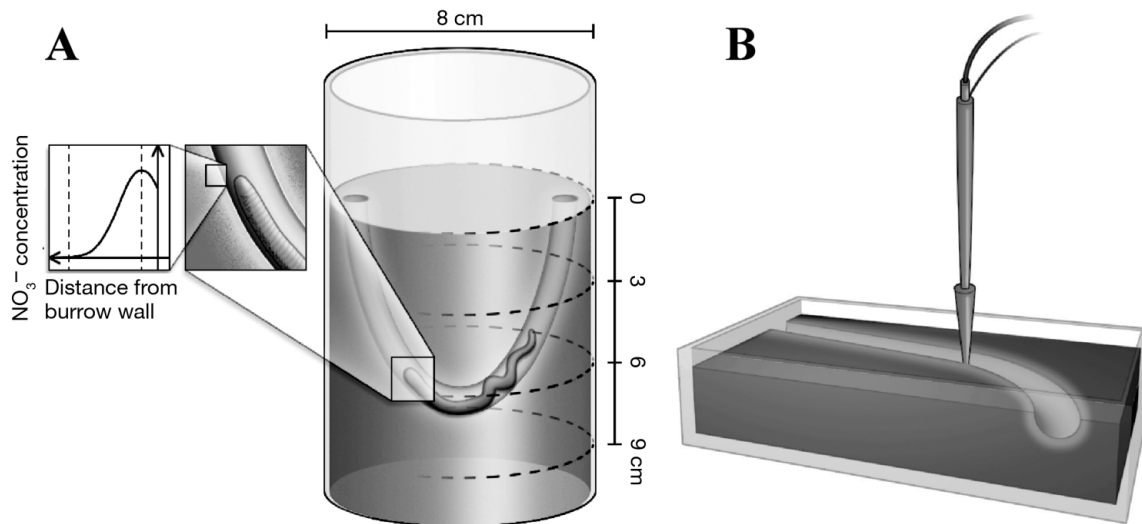

Fig. 1. *Nereis diversicolor.* Diagrams of burrows and sampling procedure. (A) Plexiglas core containing worm in its burrow and close-up of the burrow wall; sampling depths shown as intervals; diagram on left shows typical distribution of nitrate radial from burrow wall outward into the sediment obtained by micro-scale NO_3^-/NO_2^- biosensor; dashed lines represent outer limits of nitrification and denitrification zones. (B) Exposed burrow in plastic tray with micro-scale NO_x biosensor inserted perpendicular to burrow wall

Sediment from 10 sediment cores of known diameter was sieved through 1 mm mesh to obtain an estimate of *Nereis diversicolor* abundance. All worms were removed, counted, preserved in 4% formaldehyde, and later used for determination of individual dry weight (DW) as weight loss after drying for 12 h at 60°C. Burrow length and diameter for the *N. diversicolor* population were calculated by applying individual (ind.) body DW/body wet weight (WW), body WW/burrow wall surface, and body WW/burrow volume correlations, as suggested by Kristensen (1984) and Vedel & Riisgaard (1993). Since the relationships were originally made for *N. virens*, it was assumed that the closely related *N. diversicolor* and *N. virens* have identical body weight/burrow proportions.

RESULTS

General observations

Sediment from the sampling locality was muddy in appearance with an almost constant porosity with depth (0.44 to 0.50 ml pore water cm^{-3}). Nitrate concentrations in the water overlying the sediment surface were 29.8 µM in May 2000 and 4.5 µM in September 2000. The sampling site was heavily bioturbated by *Nereis diversicolor*, which were present at a density of 2560 ± 207 ind. m^{-2} and a biomass of 27.6 ± 5.2 g Dw m^{-2}. The size distribution was dominated by individuals of <5 mg Dw (>50%). Average burrow diameter, burrow lengths, and total burrow wall surface were calculated as 1.96 mm, 14.0 cm, and 2.3 m^2 wall surface m^{-2} sediment surface, respectively.

Sediment metabolism

Sediment metabolism in September 2000, measured as O_2 uptake, was 156 ± 10.4 mmol m^{-2} d^{-1} (n = 5). The corresponding TCO_2 release rate was 218 ± 19.0 mmol m^{-2} d^{-1} (n = 5), providing a respiratory quotient of 1.4.

O_2 and NO_3^- profiles

Radial profiles of oxygen in May and September 2000 were all concave in shape due to active removal of oxygen by heterotrophic activity and reoxidation of reduced metabolites (Fig. 2). O_2 disappeared rapidly and was below detection limit 0.90 to 1.30 mm into the burrow wall irrespective of depth, treatment or time (Table 1). Radial nitrate profiles from unamended and NH_4^+-enriched burrows showed a production zone close to the burrow wall with fairly pronounced sub-

surface peaks, followed by a consumption zone in which nitrate rapidly declined to below the detection limit (Fig. 3A,B). This pattern was similar to the vertical nitrate profile obtained from the sediment surface layer (Fig. 3C). Addition of NH_4^+ to the overlying water in May 2000 increased nitrate production rates in burrows, as indicated by a larger subsurface nitrate peak, but did not change the general shape of the profile (Fig. 3A). Although the NO_3^- level was substantially lower in September than May, the pattern of the profiles remained similar at all depths, with an NO_3^- production zone adjacent to the burrow wall followed by an outer consumption zone (Fig. 3B). The radial NO_3^- penetration depth did not change significantly with season or treatment (p > 0.05), and was not significantly different from vertical NO_3^- penetration into the sediment surface layer in September 2000 (p > 0.05). The zone of net nitrate production (indicated by the subsurface peaks of NO_3^-) closely followed oxygen penetration depth and was apparent to a depth of 0.94 to 1.48 mm zone into the burrow wall (Table 1). NO_3^- was consumed by the sediment in a zone extending from the oxygen penetration boundary to the NO_3^- detection boundary 2.01 to 2.58 mm into the sediment (Table 1), a zone 0.94 to 1.28 mm wide. The presence of acetylene blocked the nitrification reaction in the oxic zone, inducing subsurface NO_3^- production. The resulting profile indicates an almost linear diffusion-controlled decrease in NO_3^- concentration with increasing depth. Nitrate-reducing bacteria were not inhibited by the relatively low acetylene concentration in the pore water (20 Pa acetylene), and active nitrate uptake forced a concave curvature to the profile below the oxygen penetration depth.

Volume-specific rates of oxygen consumption, nitrification and nitrate reduction varied with depth in an irregular pattern within the respective production and consumption zones. For comparison, rates were depth-integrated and are presented as mean volume-specific rates for each production and consumption zone in the following text.

The mean volume-specific O_2 consumption rates increased slightly from 10 to 14 µmol cm^{-3} d^{-1} after NH_4^+ addition in May (Table 2). Mean rates of oxygen consumption in NH_4^+-amended sediment were of comparable magnitude in May and September. However, rates appeared to vary down (i.e. along) the burrow, with the mean oxygen consumption rate at 3 to 6 cm depth significantly different from the rates at 0 to 3 cm and 6 to 9 cm depths (p < 0.05). This difference is somewhat unexpected, as there were no down-burrow differences in NO_3^- production or NO_3^- consumption. The difference was most probably due to small-scale variability, but further investigation is needed to confirm this.
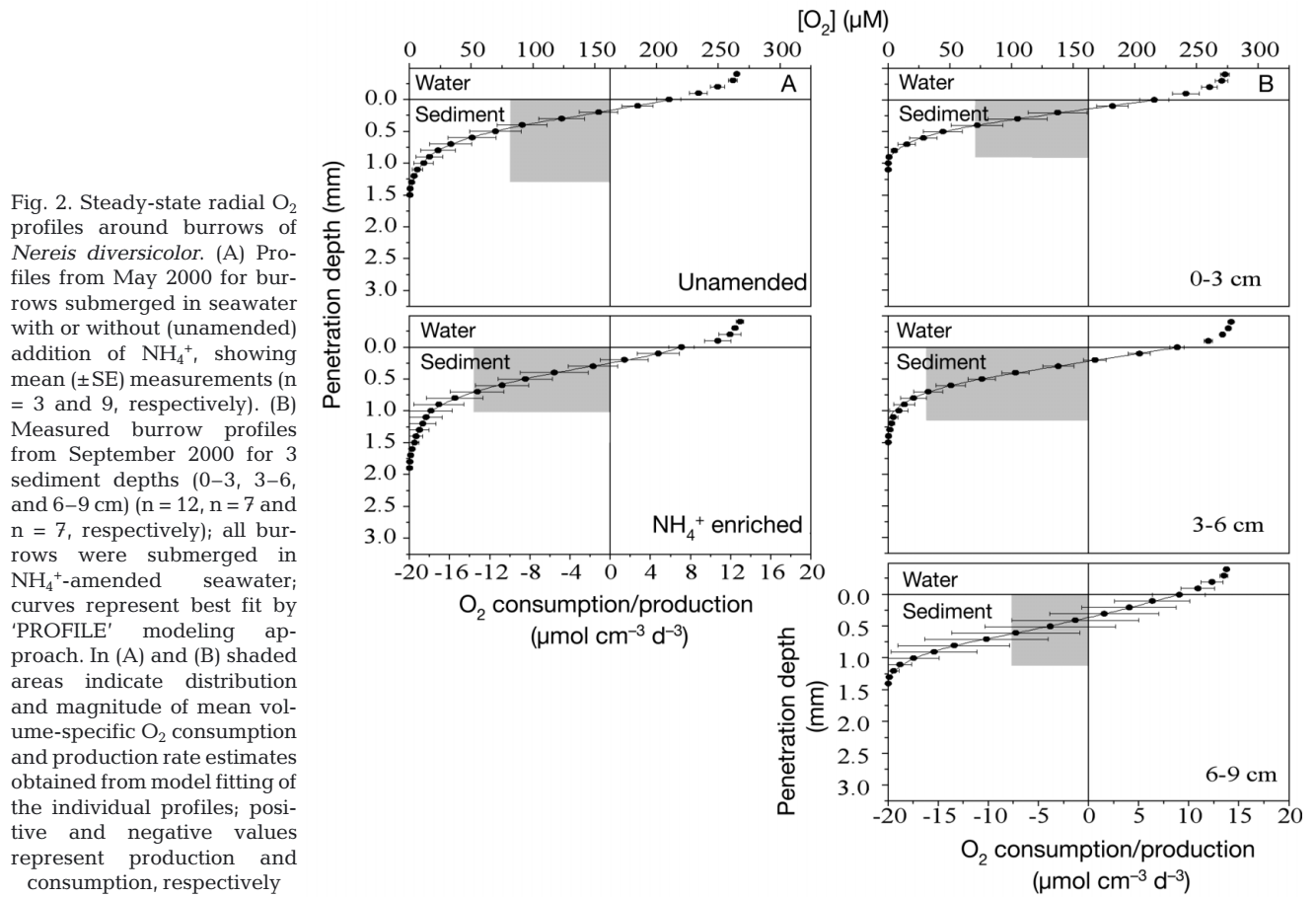
Fig. 2. Steady-state radial O_2 profiles around burrows of *Nereis diversicolor*. (A) Profiles from May 2000 for burrows submerged in seawater with or without (unamended) addition of NH_4^+, showing mean (±SE) measurements (n = 3 and 9, respectively). (B) Measured burrow profiles from September 2000 for 3 sediment depths (0–3, 3–6, and 6–9 cm) (n = 12, n = 7 and n = 7, respectively); all burrows were submerged in NH_4^+-amended seawater; curves represent best fit by 'PROFILE' modeling approach. In (A) and (B) shaded areas indicate distribution and magnitude of mean volume-specific O_2 consumption and production rate estimates obtained from model fitting of the individual profiles; positive and negative values represent production and consumption, respectively

Nitrification appeared to be NH_4^+-limited in unamended burrow walls, as indicated by an increase in net nitrification from 674 to 2173 nmol cm^{-3} d^{-1} after NH_4^+ addition in May (Table 2). Addition of acetylene effectively inhibited nitrification in the burrow wall, indicating that acetylene-inhibited bacteria mediated nitrate production in the oxic zone (Klemedtsson et al.

1988). The potential activity of nitrifying bacteria (when NH_4^+ was added) generally decreased from May to September. Nitrification was so low in September that it could only be detected after addition of NH_4^+ (data not shown), and even then still exhibited relatively low rates of 182 to 721 nmol cm^{-3} d^{-1}. Burrow wall nitrification did not change significantly with

Table 1. *Nereis diversicolor* burrows. Radial penetration depths of oxygen, nitrate production, and nitrate from walls of burrows submerged in pure seawater, in seawater containing 100 µM NH_4^+, or NH_4^+-enriched seawater with added acetylene (May), and for burrows submerged in seawater containing 100 µM NH_4^+ (September). Depths are means (±SE) for all measured profiles. nd: no data

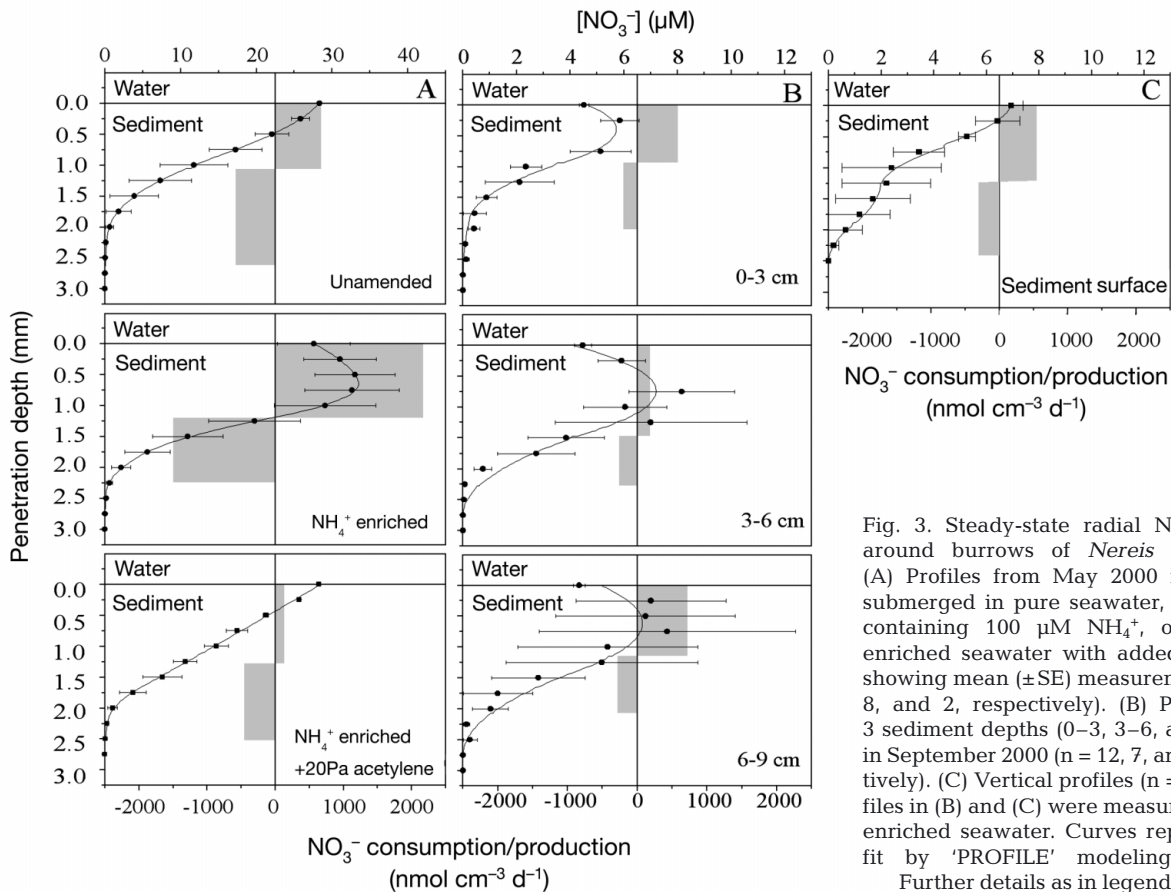|	O_2 penetration (mm)	NO_3^- production depth (mm)	NO_3^- penetration (mm)
May			
+ 0 µM NH_4^+	1.30 ± 0.30 (n = 3)	1.05 ± 0.25 (n = 3)	2.58 ± 0.22 (n = 3)
+ 100 µM NH_4^+	1.01 ± 0.10 (n = 9)	1.20 ± 0.11 (n = 8)	2.24 ± 0.17 (n = 8)
+ 100 µM NH_4^+ + acetylene	nd	nd	2.50 ± 0.25 (n = 2)
September			
Surface	nd	1.23 ± 0.65 (n = 2)	2.13 ± 0.38 (n = 2)
0–3 cm	0.90 ± 0.07 (n = 12)	0.94 ± 0.17 (n = 12)	2.01 ± 0.18 (n = 12)
3–6 cm	1.15 ± 0.08 (n = 7)	1.48 ± 0.21 (n = 7)	2.28 ± 0.16 (n = 7)
6–9 cm	1.13 ± 0.13 (n = 7)	1.14 ± 0.25 (n = 7)	2.07 ± 0.19 (n = 7)

Fig. 3. Steady-state radial NO_3^- profiles around burrows of *Nereis diversicolor*. (A) Profiles from May 2000 for burrows submerged in pure seawater, in seawater containing 100 µM NH_4^+, or in NH_4^+-enriched seawater with added acetylene; showing mean (±SE) measurements (n = 3, 8, and 2, respectively). (B) Profiles from 3 sediment depths (0–3, 3–6, and 6–9 cm) in September 2000 (n = 12, 7, and 7, respectively). (C) Vertical profiles (n = 2). All profiles in (B) and (C) were measured in NH_4^+-enriched seawater. Curves represent best fit by 'PROFILE' modeling approach. Further details as in legend to Fig. 2

depth in the sediment (p > 0.05), with mean rates similar to those at the sediment surface.

Nitrate reduction increased from 579 to 1496 nmol cm^{-3} d^{-1} in May after NH_4^+ enrichment in response to the increased NO_3^- production (Table 2). The acetylene-amended burrows displayed nitrate reduction below the oxygen penetration depth at a rate of only

453 nmol cm^{-3} d^{-1}, fed from the overlying water. In September, nitrate reduction was low at 194 to 284 nmol cm^{-3} d^{-1}, consistent with the low nitrification rates. Nitrate reduction in the surface sediment was slightly higher (307 nmol cm^{-3} d^{-1}) than in the burrow wall, although the difference was not significant (p > 0.05).

Table 2. Volume-specific rates of O_2 consumption, nitrification, and nitrate reduction in sediment surrounding burrow walls of *Nereis diversicolor* for unamended, 100 µM NH_4^+-amended, and acetylene-amended treatments in May 2000, and for surface and burrow walls, both 100 µM NH_4^+-amended, in September 2000. Rates obtained from best-curve fits to measured oxygen and nitrate profiles. Value are mean (±SE) for all measured profiles. nd: no data

	O_2 consumption (µmol cm^{-3} d^{-1})	Nitrification (nmol cm^{-3} d^{-1})	Nitrate reduction (nmol cm^{-3} d^{-1})
May			
+0 µM NH_4^+	10.0 ± 0.3 (n = 3)	674 ± 246 (n = 3)	579 ± 43 (n = 3)
+100 µM NH_4^+	13.6 ± 0.5 (n = 9)	2173 ± 354 (n = 8)	1496 ± 122 (n = 8)
+100 µM NH_4^+ + acetylene	nd		453 ± 129 (n = 2)
September			
Surface (+100 µM NH_4^+)	nd	545 ± 454 (n = 2)	307 ± 52 (n = 2)
0–3 cm (+100 µM NH_4^+)	11.3 ± 3.1 (n = 4)	588 ± 267 (n = 12)	194 ± 60 (n = 13)
3–6 cm (+100 µM NH_4^+)	16.2 ± 2.6 (n = 6)	182 ± 43 (n = 7)	260 ± 79 (n = 7)
6–9 cm (+100 µM NH_4^+)	7.7 ± 2.4 (n = 4)	721 ± 290 (n = 7)	284 ± 89 (n = 8)

DISCUSSION

Burrow structures extend the oxic sediment–water interface, and thus oxic microbial respiration and bacterial nitrification/nitrate reduction, deep into the otherwise anoxic sediment (Kristensen 1984, Fenchel 1996). Previous attempts to localize nitrification/nitrate reduction zones around burrows have been made on a coarse scale involving disruption of the sediment (Kristensen et al. 1985), but by the use of microsensors (biosensors), nitrate profiles can be obtained at high spatial resolution with only limited disturbance of the sediment matrix, thus eliminating flaws introduced by sediment disrupture. The profiles obtained in the present study confirmed on a microscale the previous assumptions (Aller 1988) that the nitrification zone is restricted to the narrow oxygen-containing zone in the burrow wall being spatially separated from an outer nitrate-reducing zone.

The bulk nitrification rates estimated in this study are at the lower end of those normally observed in Danish shallow estuaries and coastal waters (Binnerup et al. 1992, Jensen et al. 1996, Hansen & Kristensen 1998), but within the range of 0 to 1.8 mmol m^{-2} d^{-1} measured by NO$_3^-$/NO$_2^-$ sensors in another Danish fjord (Meyer et al. 2001). Only a few studies have measured nitrification rates in association with burrow structures (see Mayer et al. 1995 for review), and of these the majority report potential rates (slurry assays at high NH$_4^+$ concentrations) as opposed to measured rates. Only Aller (1988) has attempted to report actual rates. He estimated rates of 250 to 500 µM pore water d^{-1} at 2.5°C (~350 to 700 nmol cm^{-3} sediment d^{-1} at 15°C when corrected for temperature by Q_{10} ~2.5: Hansen et al. 1981) by modeling nitrogen profiles around burrows of *Nereis virens* reported in Kristensen et al. (1985). These rates are very similar to those obtained by our biosensor measurements, despite differences in locality, spatial resolution, and techniques. Since modeled profiles only represent net rates, the possibility cannot be excluded that in both studies nitrification was underestimated due to nitrate reduction in the oxic zone. However, the results of acetylene addition proved that nitrate enters the sediment and passes the oxic zone via diffusion and that nitrate reduction is confined to the anoxic layers. Thus, our nitrification estimates, as well as those of Aller (1988), represent true rates, validating the use of biosensors and models for such estimates.

The 3-fold increase in nitrification rates after NH$_4^+$-enrichment is consistent with the general observation that only a fraction of the nitrification potential is utilized under natural conditions. The magnitude of the rates was also consistent with the potential rates of 1950 to 2600 nmol cm^{-3} d^{-1} found for *Nereis virens* burrows by Kristensen et al. (1985), but at the lower end of the broad range of rates (1000 to 10 000 nmol cm^{-3} d^{-1}) reported for a variety of polychaete, bivalve and amphipod burrows (Mayer et al. 1995). Nereid burrows generally favor microbial activity such as nitrification through oxygen availability in a fine-grained environment enrichment with NH$_4^+$ from animal excreta and sedimentary metabolism (Kristensen 1985, Bartoli et al. 2000). However, in contrast to reports for most investigated macrofaunal species (Mayer et al. 1995), the nitrification potential of the burrow walls of *N. virens* did not exceed the activity at the sediment surface in our study. The cause for this discrepancy is not known, but as NH$_4^+$ availability appears to regulate nitrification activity (Mayer et al. 1995), O$_2$ and NH$_4^+$ may have been in short supply in the burrow walls in our study.

The nitrate profiles clearly show that nitrate reduction occurred in a narrow (0.9 to 1.5 mm thick) zone outside the oxic–anoxic interface around burrows. The rates obtained are typical for marine surface sediment (Herbert 1999) and close to the potential denitrification rates in *Nereis virens* burrow walls measured by Kristensen et al. (1985). The enhanced nitrate reduction rates when nitrification was stimulated after addition of NH$_4^+$ indicates that NO$_3^-$ availability is rate-limiting in the burrow wall. A general NO$_3^-$ limitation of nitrate-reduction explains the similarity of potential nitrate-reducing activity in the burrow walls and at the sediment surface despite the fact that the burrow walls are rich in labile organic carbon from the mucus lining and thus are potential 'hot-spots' for heterotrophic activity.

The contribution of *Nereis diversicolor* burrows to bulk oxygen consumption, nitrification, and nitrate reduction can be estimated from the size distribution of the *N. diversicolor* population and by applying the 2D cylindrical reaction diffusion model of Aller (1988) to the volume-specific oxygen, nitrification, and nitrate reduction rates. Based on this model, it was estimated that 40% of the total O$_2$ flux into the sediment occurred across the burrow wall surface (Table 3), showing that burrow walls are very important in bulk heterotrophic microbial activity of bioturbated sediments. The remaining 60% of the benthic oxygen consumption arose from oxygen flux across the sediment surface, animal uptake, and surface topography (Furukawa et al. 2000). Nitrification in the sediment surface and the burrow walls was a minor component in the total oxygen consumption, being responsible for about 4% of the total benthic oxygen consumption. Of the total nitrification, 77% occurred in the oxic burrow wall (Table 3); this is somewhat higher than previously reported contributions of 35 to

Table 3. Diffusive O_2 flux into sediment, nitrification and nitrate reduction rates in sediment inhabited by *Nereis diversicolor* population separated into burrow wall and surface contributions. Numbers in parentheses represent percentage contribution to bulk oxygen consumption, nitrification, and nitrate reduction by burrow walls. All estimates are for September 2000. nd: no data

Compartment	Diffusive O_2 flux (mmol m^{-2} d^{-1})	Nitrification (mmol m^{-2} d^{-1})	Nitrate reduction (mmol m^{-2} d^{-1})
Surface	nd	0.67	0.28
Burrow walls (100% ventilation)	61.1 (40%)	2.27 (77%)	1.28 (82%)
Burrow walls (30% ventilation)	18.3 (12%)	0.68 (50%)	0.38 (58%)

41% (Henriksen et al. 1980, Aller 1988, Kristensen et al. 1991). However, direct comparison of these values is not possible, as animal density, temperature, and oxygen penetration depths differed. Unfortunately, modeling of NO_3^- profiles does not provide an estimate of denitrification, since it represents net nitrate reduction, which does not discriminate denitrification from NO_3^- reduction to NH_4^+ via ammonification. Some studies have indicated that most of the reduced nitrate passes through the denitrification pathway (Binnerup et al. 1992, Pelegrí et al. 1994). However, other studies have shown that a large fraction of the NO_3^- may be reduced to ammonium in strongly reduced sediments with a surplus of electron donors (Christensen et al. 2000). The ratio of nitrate passing through the 2 pathways in sediment from Aggersund is not known. However, the sediment was heavily bioturbated and appeared relatively oxidized, indicating that denitrification was the main nitrate-reducing pathway driving the profiles. Thus, our estimates of NO_3^- reduction provide a maximum measure of denitrification. Under these assumptions, it was estimated that denitrification only contributed 0.9% of the total TCO_2 release (COH:NO_3^- stoichiometry of 1.25), and hence is of only minor importance in carbon oxidation. Nevertheless, this process is very important in nitrogen cycling and acts as a significant sink for combined nitrogen. The burrow walls of *N. diversicolor* increase the nitrate-reducing sediment volume more than 3-fold and account for 82% of bulk nitrate reduction (Table 3), indicating that *N. diversicolor* burrows are the most important structure for nitrogen cycling in the sediment. Our use of pore water profiles for biogeochemical budgets can only be crude estimates, as the calculations are based on measurements made under optimized conditions and assuming that the measured oxygen and nitrate penetrations depths represent the true *in situ* conditions, that rates are

uniform in all layers, and that nitrate concentrations in the overlying water are constant through time. However, nitrate and oxygen concentrations fluctuate in the burrow lumen as a consequence of the intermittent irrigation patterns of *N. diversicolor*, and may rapidly decrease during periods of rest (Kristensen et al. 1991). Due to these flaws, the calculated importance of burrow structures for total sediment oxygen uptake and nitrification/denitrification are probably overestimated. As *N. diversicolor* is known to ventilate its burrow approximately 30% of the time under natural conditions (Vedel et al. 1994), the estimated burrow wall contributions to bulk nitrification and nitrate reduction may not exceed 50, and 58%, respectively. These levels are similar to previous estimates of 35 to 41% for nitrification and 62 to 79% for denitrification for other bioturbated sediments (Henriksen et al. 1980, Aller 1988, Kristensen et al. 1991, Binnerup et al. 1992, Pelegrí et al. 1994). Animal and community densities also changes with season and sediment type under natural conditions. This also may affect the relative importance of nitrification and nitrate reduction pathways. Nevertheless bioturbation is undoubtedly very important for nitrogen cycling through the simultaneous stimulation of nitrification and nitrate reduction in sediments.

In conclusion, our data demonstrate that non-destructive micro-biosensors are a highly useful tool for determining fine-scale distributions of O_2 and NO_3^- in bioturbated sediments, and thus for locating zones involved in oxic respiration, nitrification, and nitrate reduction. The profiles obtained showed that bioturbation by *Nereis diversicolor* greatly affected sediment oxygen uptake, nitrification, and nitrate reduction by extending the sediment–water interface deep into otherwise anoxic sediment. Due to the intermittent irrigation patterns of *N. diversicolor*, nitrification and nitrate reduction contribute less to the overall nitrogen cycling than predicted from a simple extension of the surface area. However, it is clear that the presence of *N. diversicolor* has a large impact on sediment nitrogen cycling and accelerates the removal of bioavailable nitrogen through the simultaneous stimulation of nitrification and nitrate reduction.

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