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# Distribution and activity of nitrifying bacteria in natural stream sediment versus laboratory sediment microcosms

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ABSTRACT: Nitrification was studied with microsensors and fluorescence *in situ* hybridization (FISH) in sandy sediment of a small lowland stream. Comparative measurements were performed in both intact field sediment ('natural sediment') and sediment from the same site after processing for a laboratory incubation experiment ('manipulated sediment'). In natural sediment, the nitrification activity and abundance of nitrifiers were markedly low. In contrast, nitrification activity in manipulated sediment (sieved, homogenized, and incubated for 5 wk in the laboratory with $NH_4^+$-enriched stream water) was significantly higher. Similarly, abundances of $NH_4^+$-oxidizing β-proteobacteria (AOB) and $NO_2^-$-oxidizing *Nitrospira* spp. directly at the sediment surface were markedly higher than in natural sediment. AOB mixed into deep sediment layers by homogenization disappeared more quickly than *Nitrospira* spp., suggesting that the latter were more persistent under anoxic conditions. Higher activity and abundance of nitrifiers near the sediment–water interface of manipulated sediment were explained by (1) the additional $NH_4^+$ supply via the overlying water and (2) the adverse conditions for nitrification in the field. In conclusion, the snapshot measurement in natural sediment revealed the spatial heterogeneity created by stream dynamics, whereas the sediment manipulation provided semi-natural microcosms with reduced heterogeneity suitable for factorial experiments.

KEY WORDS: Freshwater sediment · N-cycle · Nitrification · Microcosm · Fluorescence *in situ* hybridization · Microsensors



## INTRODUCTION

Metabolic activities of nitrifying bacteria, which mediate the microbial oxidation of $NH_4^+$ to $NO_3^-$, have been measured with microsensors in intact field sediments (Lorenzen et al. 1998, Meyer et al. 2001) as well as in model, i.e. sieved and homogenized, sediments (Jensen et al. 1993, Stief et al. 2002). Only fine scale measurements permit the precise localization of the narrow surface layer of active nitrification that may be overlooked by conventional pore water analysis (Rysgaard et al. 1995) or mass balance studies (Christensen et al. 2000). Using microsensors, the influence of other sedimentary processes like $O_2$ production by photosynthesis on nitrification and the coupling of nitrification and denitrification has been demonstrated (Meyer et al. 2001, Risgaard-Petersen 2003). However, measuring rates of nitrification does not provide information about the identity of particular microorganisms involved in nitrification or about their spatial organization. So far, the identification and quantification of nitrifying bacteria in natural sediments has been addressed by cultivation-dependent techniques (Smorczewski & Schmidt 1991, Pauer & Auer 2000). Thereby, however, the actual size and structure of microbial communities in general (Amann et al. 1995) and of nitrifying populations in particular (Hall et al. 1996) can be severely misjudged. To date this bias has been circumvented by using cultivation-independent techniques such as FISH (fluorescence *in situ* hybridization; Stahl 1995,

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Amann & Kühl 1998). Ideally, structural and functional *in situ* analysis are combined to adequately characterize microbial populations (Schramm et al. 1996). For instance, microsensors and FISH have been combined to study nitrification in wastewater biofilms and aggregates (Schramm et al. 1996, Okabe et al. 1999).

While measurements in naturally heterogeneous field sediments help depict the spatial and temporal dynamics of a particular habitat, working with sieved and homogenized sediments is a commonly used approach for factorial experiments under defined conditions. Sieving removes physical obstacles that may disturb measurements, and homogenization produces a high degree of similarity between replicate sediment cores (Svensson & Leonardson 1996, Hansen & Jensen 1998). On the other hand, the pre-treatment of sediments has been questioned because it destroys the vertical microbial stratification and has a negative effect on the persistence of slow growing members of the microbial community, e.g. nitrifiers mixed into anoxic subsurface layers (Findlay et al. 1990, Svensson et al. 2001). Recovery of these microorganisms and the establishment of a close-to-natural microbial stratification may take several weeks under laboratory conditions (Tuominen et al. 1999). For this reason pre-incubation of the manipulated sediments is usually scheduled prior to the actual experimental treatments (Svensson et al. 2001, Stief et al. 2003).

The present study aimed primarily to investigate the activity and distribution of nitrifying bacteria *in situ*, i.e. in natural stream sediment with microsensors and FISH. Secondly, we studied the rates of nitrification after the same natural sediment was sieved, homogenized and incubated in natural stream water and at ambient temperature. The same set of methods was used to follow the reorganization of nitrification in this manipulated sediment during a 5 wk incubation in the laboratory.

## MATERIALS AND METHODS

**Sampling site.** Sandy sediment for measurements in natural and manipulated sediment was sampled in May 2002 from a small lowland stream (the Rittrumer Mühlenbach, N. Germany). The stream crosses an area of glacial sand accumulation in the Wildeshausener Geest. The average flow velocity of 20 cm $s^{-1}$ caused ripple formation on the sediment surface, with detritus mainly accumulating in the troughs of this ripple system. Concentrations of $NH_4^+$ and $NO_3^-$ in the overlying water on the day of sampling were 10 and 500 µmol $l^{-1}$, respectively. On various sampling occasions during 2000 and 2003, concentrations ranged from 6 to 24 and 325 to 557 µmol $l^{-1}$ $NH_4^+$ and $NO_3^-$, respectively. The organic content of the sediment ranged between 0.9 and 2.4%, and the sediment was only sparsely inhabited by macrofauna.

**Experimental design.** For the analysis of natural sediment, intact sediment cores of 15 cm length and 7.5 cm diameter were taken in replicates with Plexiglas® cylinders from a water depth of 50 cm. To avoid disturbing the sediment surface the enclosed water phase was tightly sealed and the cylinders were carefully transported to the lab within 45 min. The microsensor measurements were performed immediately in natural stream water and at ambient temperature (9.6°C). During the measurements the overlying water was mixed to ensure the formation of a diffusive boundary layer. For the analysis of manipulated sediment, in the stretch of water, surface sediment (0 to 5 cm) was collected with a flat shovel and transferred into buckets. In the laboratory the sediment was immediately sieved to remove macrofauna, large detritus and pebbles, then poured into beakers (diameter = 9 cm) up to a height of 13 cm, and allowed to settle overnight. Six beakers were submersed in 3 opaque basins containing 15 l of unfiltered stream water, and were incubated in the dark at ambient temperature (9.6°C). $O_2$ concentration in the overlying water was adjusted to air saturation. In order to avoid depletion of $NH_4^+$ in the overlying water, aliquots from a $NH_4Cl$ stock solution were repeatedly added to the basins to give a final concentration of 50 µmol $l^{-1}$ $NH_4^+$. Concentrations of $NH_4^+$, $NO_3^-$ and $NO_2^-$ in the basins were checked regularly using photometric test kits (Merck). After 3 and 5 wk of incubation, microsensor measurements and sediment samples for FISH were taken.

**Microsensor measurements.** Microsensors for $O_2$ (Revsbech 1989), $NO_3^-$, and $NH_4^+$ (de Beer et al. 1997) were prepared, calibrated, and operated in a measuring set-up as described previously (Stief et al. 2002). Replicate profiles were recorded at randomly chosen positions of the sediment surface down to a depth of 10 mm. High resolution profiles of local solute conversion rates were calculated as the 2nd derivative of the concentration profiles (de Beer & Stoodley 2000; available at: www.springerlink.com, publication no. 430). Local $NO_3^-$ production in the oxic sediment layer was considered to be the best measure of nitrification activity, as in freshwater sediments no other process that produces significant amounts of $NO_3^-$ is known, and dissimilatory consumption of $NO_3^-$ is insignificant. Net conversion rates of $O_2$, $NH_4^+$, and $NO_3^-$ within the oxic sediment layer were calculated by integrating the local conversion rates across the depth of $O_2$ penetration. Diffusion coefficients in water needed for these calculations were taken from the literature (Schramm et al. 1999b) and corrected for the measuring temperature (9.6°C) by applying the Stokes-Einstein relation. The sediment diffusion coefficients of $O_2$, $NH_4^+$, and $NO_3^-$

were calculated from profiles measured with a diffusivity sensor (Unisense A/S) (Revsbech et al. 1998, Stief et al. 2003).

**FISH and total cell counts.** After the microsensor profiles had been recorded, 1 sediment core (diameter = 2.5 cm) was taken from each sediment cylinder (or beaker) and sectioned horizontally into 2 mm thick slices down to a depth of 10 mm. Sediment slices were prepared for FISH on gelatine-coated microscope slides as described by Altmann et al. (2003). A set of oligonucleotide probes specific for (1) *Eubacteria* (probe mix EUB 338, Amann et al. 1990; EUB II and EUB III, Daims et al. 1999), (2) $NH_4^+$-oxidizing β-proteobacteria (NSO 1225, Mobarry et al. 1996), and (3) the $NO_2^-$-oxidizing genera *Nitrobacter* (NIT 3, Wagner et al. 1996) and *Nitrospira* (NTSPA 662, Daims et al. 2000) was used. Probes NIT 3 and NTSPA 662 were used with an equimolar amount of a competitor oligonucleotide as indicated in the references. To check for unspecific binding, samples were hybridized with control probe NON 338 (Manz et al. 1992). All probes were purchased labelled with the fluorescent dye CY3 (Hybaid Interactiva). FISH and counter staining of all cells with 4′,6-diamino-2-phenylindole (DAPI; 0.5 µg $ml^{-1}$) were performed according to published protocols (Pernthaler et al. 1998). Counting was adapted to the low numbers of FISH-positive cells and their uneven distribution in the aliquots as described in (Altmann et al. 2003), with at least 20 (NTSPA 662) and 200 to 400 microscopic fields (NSO 1225) being analysed. Due to the low abundance of $NH_4^+$-oxidizing β-proteobacteria (as detected with the group-specific probe NSO 1225) FISH at a higher taxonomic level was not attempted. Samples with exceptionally poor dispersal of cells were excluded from counting and were replaced by newly prepared samples. To correct for cell losses during hybridization on microscope slides, total bacterial cell numbers were determined separately by DAPI-staining of sonicated and diluted sediment samples on black membrane polycarbonate filters (pore size, 0.2 µm; Osmonics). The absolute numbers of FISH-positive cells were calculated for each probe using the relative FISH-positive counts (as percentage of DAPI-stained cells) and the total cell counts.

## RESULTS

### Natural sediment

Concentration profiles of $O_2$, $NH_4^+$, and $NO_3^-$ are presented in Fig. 1. To highlight the similarity between replicate cores, profiles are shown for each individual core. In all 3 cores $O_2$ became depleted within the uppermost 2 to 3 mm of the sediment. $NO_3^-$ was continuously depleted over a depth of approximately 6 mm in Cores 1 and 2, but in Core 3 it did not reach 0 µmol $l^{-1}$ within the sampled sediment depth. $NH_4^+$ concentration in Cores 2 and 3 decreased slightly within the upper 2 mm and increased again in the deeper layers. In contrast, Core 3 was characterized by high $NH_4^+$ concentration in deeper layers and a steep concentration gradient at the oxic-anoxic interface. $NO_3^-$ production in the oxic layer, i.e. nitrification activity, was detected down to a depth of 1 mm and averaged 0.152 µmol $cm^{-3}$ $h^{-1}$ (SD = 0.091, n = 9). $NO_3^-$ consumption in the anoxic layer down to 10 mm averaged –0.036 µmol $cm^{-3}$ $h^{-1}$ (SD = 0.024, n = 9).

Total cell numbers in the natural sediment were 8 to 13.9 × $10^9$ cells $cm^{-3}$ and were evenly distributed throughout the sampled sediment depth (data not shown). Overall detection rate with FISH, as determined with combined oligonucleotide probes EUB 338, EUB II, and EUB III, was 40 to 50 % of total bacterial cells. Unspecific signals (i.e. binding of probe NON 338 and autofluorescent cells) were not detected. Absolute abundances and the vertical distribution of

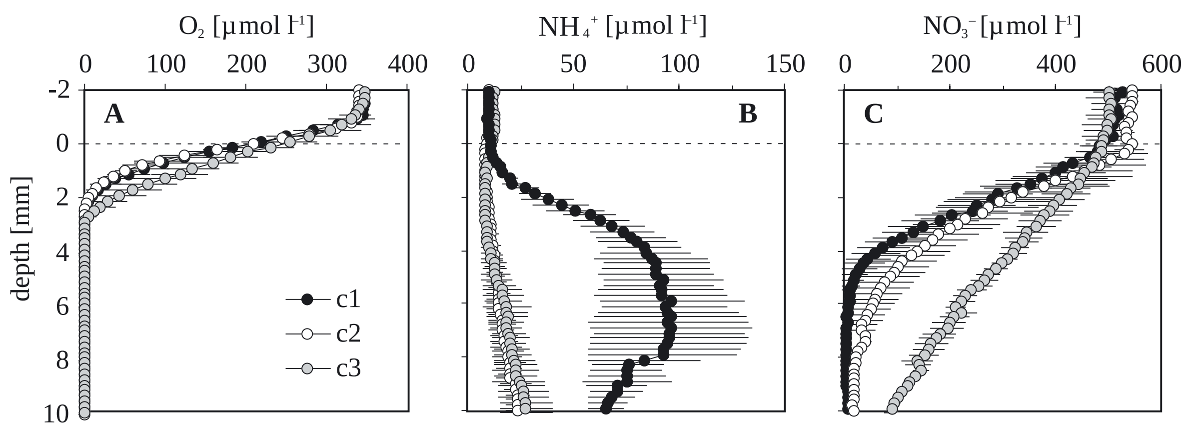


Fig. 1. Vertical concentration profiles of (A) $O_2$, (B) $NH_4^+$, and (C) $NO_3^-$ measured in natural sediment of a lowland stream. Average profiles (±1 SD, n = 3) within each of the 3 replicate sediment cores (c1–3) are shown. Dotted line indicates sediment surface

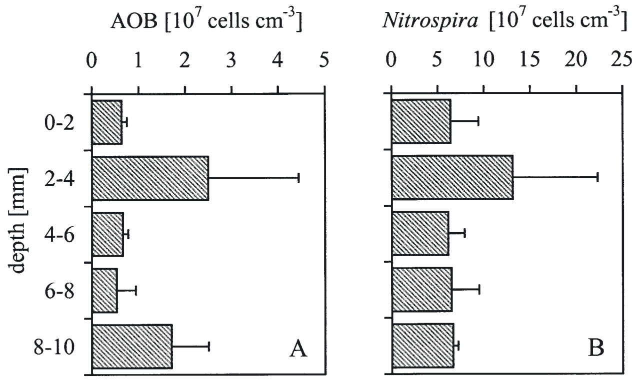


Fig. 2. Vertical distributions of (A) $NH_4^+$-oxidizing β-proteobacteria (AOB) and (B) $NO_2^-$-oxidizing *Nitrospira* spp. as revealed with FISH in natural sediment. Average abundances (+1 SD) of 3 replicate sediment cores are shown

AOB and NOB are shown in Fig. 2. AOB abundance was highest in the 2 to 4 mm layer, but peaked again at a depth of 8 to 10 mm, accounting for 0.26 and 0.16% of total cells, respectively. FISH indicated that NOB were represented by the genus *Nitrospira*, whereas the commonly isolated genus *Nitrobacter* spp. was not detected. Cells of *Nitrospira* spp. were 5 to 9 times more abundant than AOB and here the maximum numbers were also found in the 2 to 4 mm layer, where they made up 1.2% of all bacterial cells. Cells of *Nitrospira* spp. were also detected in remarkably high abundances in the anoxic layer of the sediment.

## Manipulated sediment

In contrast to the natural sediment, the high similarity between replicate cores of the manipulated sediment allowed averaging of all microprofiles measured in the 3 replicate cores within one sampling date. Averaged concentration profiles of $O_2$, $NH_4^+$, and $NO_3^-$ recorded after 3 and 5 wk of incubation in the laboratory are shown in Fig. 3. $O_2$ penetration depth after 3 wk was 7 mm. $NH_4^+$ concentration, which was held constant at 50 µmol $l^{-1}$ in the overlying water during the measurements, decreased within the upper 1 mm of the sediment and increased substantially below a depth of 7 mm. The $NO_3^-$ concentration peak in the upper 1 mm of sediment was followed by an extended zone of slightly decreasing $NO_3^-$ concentration, but $NO_3^-$ was not completely consumed within the sampled sediment depth. After 5 wk of incubation the concentrations of $O_2$, $NH_4^+$, and $NO_3^-$ showed the same vertical patterns as 2 wk before. $NO_3^-$ production in the oxic layer, i.e. nitrification activity, was highest in the 0 to 1 mm layer and only marginal down to a depth of 7 mm. $NO_3^-$ production near the sediment surface averaged 0.118 (SD = 0.059, n = 9) and 0.178 µmol $cm^{-3}$ $h^{-1}$ (SD = 0.063, n = 9) after 3 and 5 wk, respectively. In the 1 to 7 mm layer, rates were 0.019 (SD = 0.025, n = 9) and 0.012 µmol $cm^{-3}$ $h^{-1}$ (SD = 0.008, n = 9) after 3 and 5 wk, respectively. $NO_3^-$ consumption in the anoxic layer (7 to 10 mm) averaged –0.004 (SD = 0.050, n = 9) and –0.003 µmol $cm^{-3}$ $h^{-1}$ (SD = 0.034, n = 9) after 3 and 5 wk, respectively.

Total cell numbers after the 3 wk incubation were 9.5 to 11.1 × $10^9$ $cm^{-3}$ showing a homogeneous distribution throughout the sampled sediment depth (data not shown). Numbers in the 0 to 2 mm layer were 9.9 × $10^9$ $cm^{-3}$ after another 2 wk of sediment incubation, but were conspicuously lower in deeper layers, i.e. 4.1 × $10^9$ $cm^{-3}$. Overall detection rate with FISH, as revealed by hybridization with the EUB probe mix, was 70 to 80% in the oxic sediment and 50 to 60% in deeper layers. Unspecific signals due to binding of control probe NON 338 or autofluorescence were not detected. Absolute abundances of AOB and NOB varied spatially and temporally (Fig. 4): after the 3 wk incubation, the maximum abundance of AOB was found in the 0 to

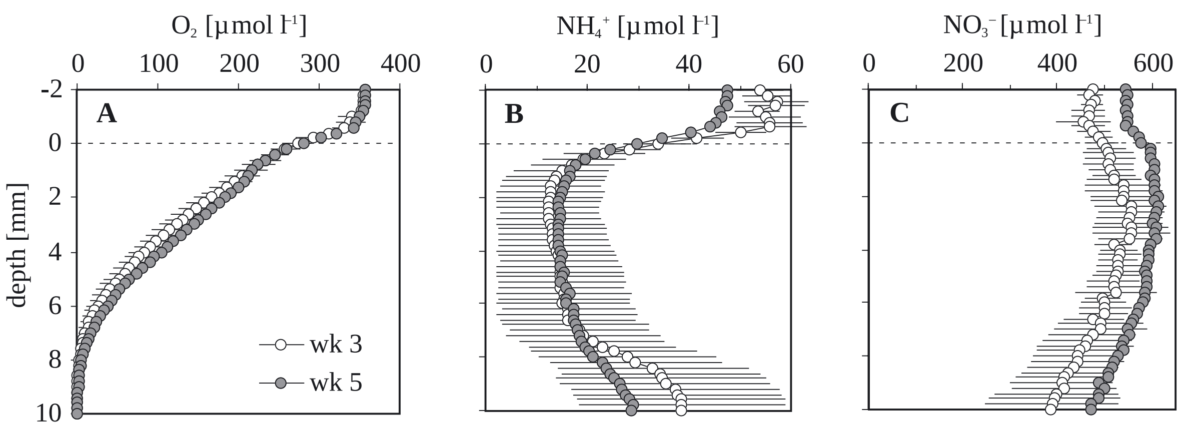


Fig. 3. Vertical concentration profiles of (A) $O_2$, (B) $NH_4^+$, and (C) $NO_3^-$ measured in manipulated sediment after 3 and 5 wk laboratory incubations. Average profiles (±1 SD, n = 9) of all 3 replicate sediment cores are shown. Dotted line indicates sediment surface

2 mm layer (Fig. 4A). In this layer, AOB made up 0.7 % of total bacterial cells. In deeper layers their proportion decreased to 0.04 % of total cells. After another 2 wk of incubation, the absolute abundances and the vertical distribution of the AOB remained unchanged (Fig. 4A). NOB were represented by the genus *Nitrospira*, whereas *Nitrobacter* spp. was not detected at any time during the incubation. Cells of *Nitrospira* spp. were evenly distributed over the whole sampled sediment depth after 3 wk of incubation and made up approximately 1.9 % of total bacterial cells (Fig. 4B). After 5 wk, however, a distinct abundance maximum had developed at the sediment surface and cell numbers there had increased to 3.6 % of total cells (Fig. 4B).

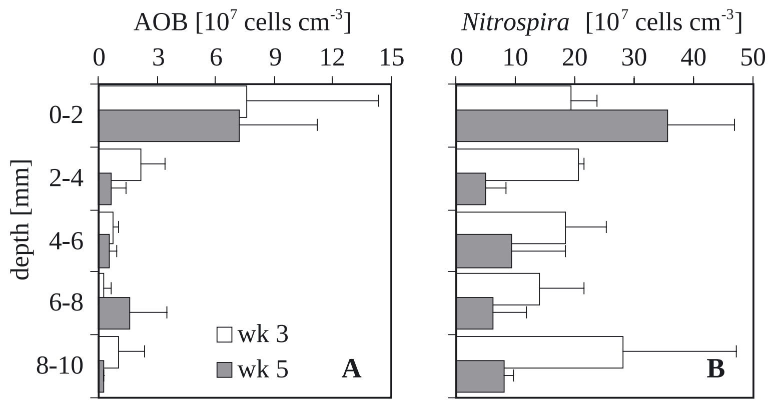


Fig. 4. Vertical distribution of (A) $NH_4^+$-oxidizing β-proteobacteria (AOB) and (B) $NO_2^-$-oxidizing *Nitrospira* spp. as revealed with FISH in manipulated sediment after 3 and 5 wk laboratory incubation. Average abundances (+1 SD) of 3 replicate sediment cores each are shown

**Comparison of natural and manipulated sediment**

The net conversion rates of $O_2$, $NH_4^+$, and $NO_3^-$ within the oxic layer of both natural and manipulated sediments are given in Fig. 5. Neither rate changed significantly in the manipulated sediment between Weeks 3 and 5 of the laboratory incubation (paired *t*-test, p > 0.05). In contrast, both $NH_4^+$ consumption and $NO_3^-$ production rates were significantly higher in manipulated than in natural sediment (Student's *t*-test, p < 0.001 and p < 0.01 for $NH_4^+$ and $NO_3^-$, respectively). Due to the high variation of the $NO_3^-$ production after 3 wk of incubation, no significant difference was found in this case (Student's *t*-test, p > 0.05). The increase in $NO_3^-$ production was not due to higher rates in the 0 to 1 mm layer (see above), but rather due to an extension of the $NO_3^-$-producing zone down to a depth of 7 mm. $O_2$ consumption was significantly lower in manipulated than in natural sediment (Student's *t*-test, p < 0.01). Variability of rates in manipulated sediment was lower than in natural sediment. This was indicated by lower coefficients of variation when considering the total number of 9 microprofiles per treatment that were measured in 3 replicate sediment cores (Table 1).

In sediment layers of maximum abundance (i.e. 2 to 4 mm in natural and 0 to 2 mm in manipulated sediment) the cell numbers of AOB and *Nitrospira* spp. were not significantly different in the 2 sediment types (Student's *t*-test, p > 0.05). However, in the 0 to 2 mm layer AOB and *Nitrospira* spp. abundances were markedly higher in manipulated than in natural sediment. AOB and *Nitrospira* spp. abundances in the 0 to 2 mm layer remained unchanged between Weeks 3 and 5 of incubation (paired *t*-test, p > 0.05). Between-core variability of *Nitrospira* spp., but not AOB, abundance was lower in manipulated than in natural sediment (Table 2).

## DISCUSSION

### Natural sediment

The investigated stream sediment was characterized by a remarkably low nitrification activity, as indicated by the absence of pronounced $NO_3^-$ peaks in the majority of the microsensor profiles. $NO_3^-$ production

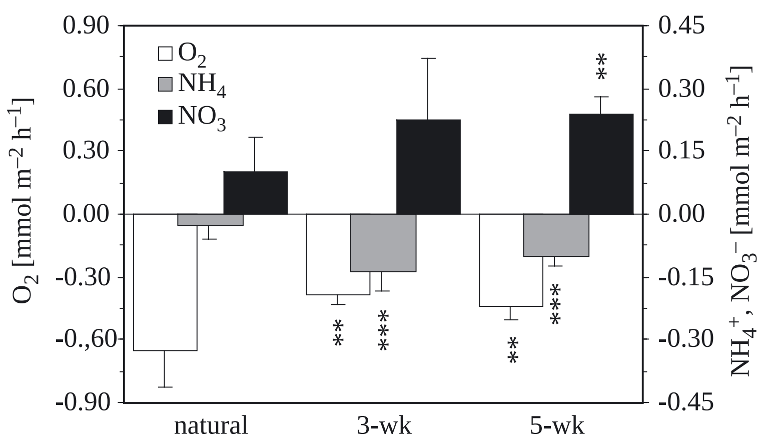


Fig. 5. Net conversion rates of $O_2$, $NH_4^+$, and $NO_3^-$ in the oxic layer of natural and manipulated sediment (3 and 5 wk) as calculated from the concentration profiles in Figs. 1 & 3. Average rates across 3 replicate sediment cores with 3 profiles each (+1 SD, n = 9) are given. ** and *** indicate significant differences between natural and manipulated sediment at p < 0.01 and 0.001 (Student's *t*-test)

was only observed in the 0 to 1 mm layer. The sediment as a whole consumed $NO_3^-$, which was indicated by the concentration decrease in the diffusive boundary layer above the sediment. Dissimilatory $NO_3^-$ reduction in the anoxic sediment layers was probably the principal pathway of $NO_3^-$ consumption in this sediment. Additional measurements performed in illuminated sediments (light intensity: 500 µE $m^{-2}$ $s^{-1}$, data not shown) did not reveal measurable photosynthetic activity, hence in our sediments, photosynthesis can be ruled out as a significant sink for $NO_3^-$. The local volumetric rates of $NO_3^-$ consumption in the anoxic sediment layer were at the lower end of the rates reported in the literature (Lorenzen et al. 1998, Meyer et al. 2001). These low rates of N cycling in our sediment probably resulted, firstly, from the low concentration of labile organic matter (2.4% in the 0 to 10 mm layer), which limited the supply of $NH_4^+$ and $CO_2$ for nitrification and electron donors for dissimilatory $NO_3^-$ reduction. Secondly, the low rates may be indicative of the physical dynamics in the natural stream sediment, as water currents permanently reshape the sediment surface, e.g. cut off formerly oxic layers from $O_2$ (Wulff et al. 1997). Such physical disturbances might inhibit nitrification in particular because of its dependence on $O_2$ (Sloth et al. 1995). Moreover, nitrifying bacteria are characterized by low growth kinetics, meaning that recovery from disturbances may be particularly slow (Findlay et al. 1990, Svensson et al. 2001). Supportive of the dynamic character of the natural stream sediment was the high heterogeneity of solute conversions found within and between the replicate sediment cores.

Table 1. Between-core variability of net conversion rates of $O_2$, $NH_4^+$, and $NO_3^-$ in the oxic layer of sediments as calculated from microsensor profiles

| Sediment type | Core no. | Time | No. of cores | No. of profiles | Coefficient of variation (%)[a] $O_2$ | $NH_4^+$ | $NO_3^-$ |
|---|---|---|---|---|---|---|---|
| Natural | 1–3 | Week 0 | 3 | 9 | 26 | 110 | 87 |
| Manipulated | 4–6 | Week 3 | 3 | 9 | 12 | 35 | 65 |
| Manipulated | 7–9 | Week 5 | 3 | 9 | 15 | 24 | 17 |

[a]Coefficients of variation were calculated for n = 9 profiles measured in 3 replicate cores. Net conversion rates are plotted in Fig. 5

Table 2. Between-core variability of AOB and *Nitrospira* spp. abundance maxima near the sediment surface

| Sediment type | Core no. | Time | No. of cores | Coefficient of variation (%)[a] AOB | *Nitrospira* spp. |
|---|---|---|---|---|---|
| Natural | 1–3 | Week 0 | 3 | 78 | 70 |
| Manipulated | 4–6 | Week 3 | 3 | 88 | 23 |
| Manipulated | 7–9 | Week 5 | 3 | 56 | 32 |

[a]Coefficients of variation were calculated for n = 3 subcores taken from 3 replicate sediment cores. Nitrifier abundances are plotted in Figs. 2 & 4

Applying oligonucleotide probes specific for $NH_4^+$-oxidizing β-proteobacteria (AOB) and the $NO_2^-$-oxidizing genera *Nitrospira* and *Nitrobacter* we were able to localize and quantify members of the 2 physiological groups of nitrifying bacteria *in situ*, i.e. in a stream sediment sampled directly in the field. Most probable number counts of cultivable nitrifying bacteria in other natural freshwater sediments led to abundances 1 to 2 orders of magnitude lower than ours (Smorczewski & Schmidt 1991, Pauer & Auer 2000). This discrepancy is apparently due to the generally recognized bias of cultivation-dependent methods (Amann et al. 1995, Hall et al. 1996). In the investigated natural sediment the genus *Nitrobacter*, which is the most commonly isolated $NO_2^-$-oxidizer, was not detected with FISH. Instead, we identified and quantified the genus *Nitrospira* as a major representative of the $NO_2^-$-oxidizing bacteria. This genus has recently been detected in several habitats by means of comparative 16S rRNA analysis (Hovanec et al. 1998, Juretschko et al. 1998, Todorov et al. 2000). Our finding thus confirms the recently assumed dominance of *Nitrospira* spp. as opposed to *Nitrobacter* spp. not only in engineered systems and model sediments, but also in intact freshwater sediments.

Maximum abundance of both AOB and *Nitrospira* spp. in natural sediment occurred in the 2 to 4 mm layer, which was apparently anoxic during our microsensor measurements. Two main reasons for this discrepancy should be considered: (1) in the field, i.e. before the cores were taken and measured in the laboratory, $O_2$ may have also been present in the 2 to 4 mm layer. $O_2$ penetration depth may have decreased due to the lower current velocity during our measurements (Huettel & Rusch 2000). If this was the case, then under field conditions, the active fraction of the nitrifier population may indeed have settled deep in the oxic layer with a better $NH_4^+$ supply from below. (2) The layer of maximum abundance of AOB and *Nitrospira* spp. may represent the former surface of the sediment which had recently been sloped and cut off from $O_2$ supply by the horizontal shifting of ripple structures. Under the Rittrumer Mühlenbach conditions (flow velocity: 20 cm $s^{-1}$, median grain diameter: 300 µm, ripple height: 3 cm, ripple length: 20 cm) the down-stream migration of sand ripples by a full phase may only take a few hours (Fischer et al. 2003). Thus, the turnover time of sediment ripples might be too short for new abundance maxima of nitrifiers to be established at the sediment surface.

**Manipulated sediment**

In contrast to natural sediment, the sieved and homogenized sediment was characterized by a distinct nitrification zone in the uppermost layer. $NO_3^-$ production was highest in the 0 to 1 mm layer, but occurred at lower rates down to 7 mm. This was explained by the extended $O_2$ penetration depth compared to natural sediment. Nevertheless, nitrifiers obviously did not tap into the full potential of nitrification, as was indicated by the incomplete depletion of $NH_4^+$ within the oxic sediment layer. Since $O_2$ was present down to a depth of 7 mm, nitrification was limited neither by $NH_4^+$ and $O_2$, but more by other factors like a population size too small to consume the continuously supplied $NH_4^+$ to its limit, or a shortage of $CO_2$, which was internally produced by mineralization. However, nitrification activity as estimated from the $NO_3^-$ production rate within the oxic zone was comparable to other freshwater studies in which microsensors have been applied to experimentally manipulated sediments (Jensen et al. 1993, Stief et al. 2002). In contrast to the higher nitrification rates in the manipulated sediment, dissimilatory $NO_3^-$ consumption down to a depth of 10 mm was lower than in the natural sediment. This was most probably due to a lack of electron donors for $NO_3^-$ reduction as a consequence of the low sedimentary organic content. It seems likely that by sieving the sediment, the dissolved and fine particulate organic matter was partially washed out. Moreover, after the sediments had settled, a sharp separation into the sandy sediment at the bottom and a fine-particulate layer at the sediment surface of 2 to 3 mm thickness was observed. This phenomenon suggested that the sediment below the fine-particulate layer became impoverished in particulate organics. Indeed the organic content of manipulated sediment was lower compared to natural sediment (1.0 and 2.4 %, respectively, in the 0 to 10 mm layer). The activities of both nitrification and $NO_3^-$ consumption did not change during the 2 wk incubation, stressing the stability of the manipulated sediment during this period. Moreover, the sieving and homogenization procedure clearly reduced heterogeneity within and between the sediment cores, as was indicated by the low variability between replicate concentration profiles. Hence, the pre-treatment of sediment and a time course of 2 wk after initial restratification presents a suitable basis for the performance of factorial experiments in which a high degree of similarity is needed between replicate sediment cores.

Sediment manipulation most probably created a homogeneous vertical distribution of both AOB and *Nitrospira* spp. at the start of the experiment. During the laboratory incubation that followed, both populations showed a different pattern of vertical restratification: AOB were found in highest abundance in the 0 to 2 mm layer after only 3 wk and this was still the case after 5 wk of incubation. In contrast, cells of *Nitrospira* spp. were still homogeneously distributed after 3 wk of incubation, but eventually showed signs of vertical stratification after 5 wk of incubation. The AOB population quickly established an abundance maximum near the sediment-water interface at the expense of deeper layers, even though $O_2$ penetrated 7 mm deep into the sediment and was thus not limiting. This preferential growth in the 0 to 1 mm layer can be explained by the continuous supply of $NH_4^+$ via the overlying water, while in the deeper oxic layers AOB were probably $NH_4^+$-limited. For AOB that dominate in oligotrophic environments, a $K_m$ value of around 40 µmol $l^{-1}$ $NH_4^+$ was given (Schramm et al. 1999a), which is well above the minimum concentrations of 12 to 18 µmol $l^{-1}$ $NH_4^+$ measured in our sediments. The spatial 'plasticity' of nitrification activity in response to an $NH_4^+$ source was also demonstrated by Jensen et al. (1993). Given that $NO_2^-$ production by AOB was the only significant source of $NO_2^-$ in the sediment, cells of *Nitrospira* spp. were expected to thrive better at the sediment-water interface than in deeper layers, and this was indeed the case at the end of the experiment. Obviously, though, *Nitrospira* spp. in this experiment persisted longer under the unsuitable conditions (i.e. lack of $O_2$ and $NO_2^-$) in the deeper layers than the AOB. This superior persistence may have contributed to the delayed stratification of *Nitrospira* spp. as opposed to that of AOB. *Nitrospira* spp. might be adapted to survive or even thrive under anoxic conditions, or experience a particularly slow decay of ribosomes (Okabe et al. 1999).

**Comparison of natural and manipulated sediment**

Despite the destruction of the microbial stratification by sieving and homogenizing, the manipulated sediment was finally characterized by higher abundances of AOB and *Nitrospira* spp. and higher nitrification activities. This was probably due to the additional supply with $NH_4^+$, which can be a major factor limiting nitrification in sediments (de Beer et al. 1991, Jensen et al. 1993). On the other hand, sediment pre-treatment and laboratory incubation have obviously produced a loss of electron acceptors and $NH_4^+$ within the sediments, which resulted in lower consumption of $O_2$ and lower dissimilatory consumption of $NO_3^-$. Possible reasons for this loss are the washout of dissolved substances during sieving and the accumulation of fine-particulate organic matter at the sediment surface. This may constitute non-favorable conditions for the performance of both nitrification and denitrification because $NH_4^+$, $CO_2$, and electron donors may become limiting. In our case, however, limitation of $NH_4^+$ was avoided by supplying it via the overlying water.

As expected, natural sediment was more heterogeneous than manipulated sediment, which gives a rough idea of the spatial and temporal dynamics in the sampled stream habitat. Measurements conducted directly in the field are useful for investigations of the complexity of a particular habitat, for comparisons between different natural habitats and for investigations of the seasonality within certain habitats. The pre-treatment of the sediment, however, produced a high degree of similarity between replicate cores, which is useful for the performance of factorial experiments under defined conditions. In the present study the complementary use of both approaches also enabled us to address the adaptive capacities of sedimentary nitrifiers to disturbance.

## CONCLUSIONS

The combined application of microsensors and FISH for the investigation of nitrification in natural stream sediment was challenging because of the generally lower activities and cell abundances compared to waste water biofilms, for example. However, to date this is the only acceptable *in situ* approach to studying nitrification activity with the appropriate spatial resolution (microsensor approach) and to determining the abundance of nitrifiers without the bias of cultivation-dependent techniques (FISH approach). With the use of FISH, the 2 physiological groups of nitrifiers, AOB and NOB, could be separately studied with respect to both their prevalence in natural sediment and how they are affected by sediment manipulation and incubation. Microsensor data, on the other hand, helped to reveal which of the identified nitrifiers were actually active and what effect their activity had on the nutrient exchange between the sediment and the overlying water. In this sense, our field measurements represent a snapshot of the situation in a small stream at a given time of the year, whereas the laboratory incubation revealed the potential of the same sediment for nitrification and the ability of the inhabiting nitrifiers to adapt to changing conditions.

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