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Probing the microenvironment of freshwater sediment macrofauna: Implications of deposit-feeding and bioirrigation for nitrogen cycling

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Abstract

The effect of sediment-dwelling macrofauna on nitrifying bacteria was investigated by direct probing in their overlapping habitat, i.e., the upper few millimeters of freshwater sediments. Microsensors (O_2, NH_4^+, NO_3^-, and diffusivity) were used at the sediment surface and inside animal burrows to record steady-state and dynamic distributions of reactants, respectively. Short-term changes of metabolic activity (actual and potential nitrification rates) and long-term changes of abundance (fluorescence in situ hybridization) of nitrifying bacteria were determined. The presence of insect larvae (*Chironomus riparius*) increased the availability of O_2 and NO_3^- in the sediment pore water and inside animal burrows, suggesting promotion of nitrification and dissimilatory NO_3^- reduction, particularly in the burrowing layer of *C. riparius*. At the sediment surface (i.e., in the feeding layer of *C. riparius*), however, nitrification was inhibited by low NH_4^+ availability and high macrofaunal grazing pressure. Consequently, both actual and potential nitrification rates decreased in the feeding layer. Inside burrows, no net nitrification was detected, despite high NH_4^+ availability and frequent O_2 injections by larval ventilation activity. Conversely, burrows were sites of NH_4^+ production and NO_3^- consumption. Nevertheless, the abundance of nitrifying bacteria increased measurably in the burrowing layer after prolonged incubation, but only in sediments in which the larvae were able to construct and ventilate stable burrows.

Benthic aquatic macrofauna thoroughly alter the distribution of microbial populations in aquatic sediments. The animals excavate sediment for burrow construction and continuously redistribute particles because of locomotion and feeding (Krantzberg 1985). Thereby, particle-associated microbial populations become spatially arranged in a three-dimensional (3-D) mosaic rather than in a 1-D vertical sequence (Fenchel 1996*b*; Kristensen 2000). New associations arise that allow metabolic pathways to interact in a complex way. In addition, subsurface microbial populations become directly exposed to water and solutes from the water column because animals ventilate their burrows for oxygen acquisition (Riisgaard and Larsen 2005). Ventilation also enhances solute exchange between the water column and subsurface microbial populations through advective water transport through burrows (Aller and Aller 1998). Aside from these indirect effects, animals directly interact with microbes by feeding on them, which has a profound impact on microbial metabolic activity and biomass (Johnson et al. 1989, Van De Bund et al. 1994). Ingestion and egestion of bacteria often takes place at different sites in the sediment and thereby further promotes the spatial redistribution of microbes (Matisoff et al. 1999).

Studies on bioturbation and bioirrigation in freshwater ecosystems are severely underrepresented relative to studies in coastal marine environments (Fenchel et al. 1998). Freshwater macrofauna have a smaller average body size and burrow depth than marine macrofauna. Consequently, immediate interactions of freshwater macrofauna and microbes are limited to the sediment surface and the relatively small burrows (Stief and de Beer 2002; Stief et al. 2004). Their habitat spatially coincides with the layer of the highest metabolic activities of bacteria in sediments (Aller and Aller 1986; Haglund et al. 2003). Inevitably, microbial populations close to the sediment surface are influenced by macrofaunal bioturbation, bioirrigation, and feeding. These animal activities affect the spatial distribution, the physical supply of reactants for microbially driven oxidation–reduction reactions, and the ultimate survival of microorganisms, respectively. The microbial responses range from short-term change of metabolic activity to long-term change of abundance and distribution. An appropriate investigation of animal–microbe interactions in freshwater sediments must therefore reconcile approaches on small spatial scales and broad temporal scales.

We carried out laboratory experiments on the effects of *Chironomus riparius* larvae on the microbial nitrogen cycle (nitrification in particular) in freshwater sediments. *C. riparius* is a typical and often highly abundant inhabitant of soft sediments in lowland streams, ditches, and lake shores (Armitage et al. 1995). Because of its high tolerance to organic and inorganic pollution and O_2 depletion, *C. riparius* is also widespread in saprobic streams and eutrophic lakes with intense nutrient turnover in the sediment. The larva constructs a U-shaped burrow that reaches approximately 10 mm into the sediment and is intermittently ventilated with oxygenated surface water (Leuchs 1986). *C. riparius* leaves the burrow only to feed on particulate organic matter that has settled onto the

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sediment surface (Hölker and Stief 2005). Microbial nitrogen turnover is rather intense in the typical habitats of *C. riparius*, because the precursors of nitrification (NH_4^+ from organic matter mineralization) and dissimilatory NO_3^- reduction (NO_3^- from surface or groundwater pollution) are highly abundant. The layers of nitrification and dissimilatory NO_3^- reduction are intersected by the burrows of *C. riparius*. Additionally, the layer of nitrification is subject to the feeding activities of *C. riparius* larvae.

Our hypotheses were that *C. riparius* larvae (1) increase the availability of reactants for nitrification (and dissimilatory NO_3^- reduction), in particular in the burrowing layer; (2) increase the metabolic activity and abundance of nitrifying bacteria in the burrowing layer; and (3) decrease the metabolic activity and abundance of nitrifying bacteria in the feeding layer (Fig. 1). The availability of reactants was measured with O_2, NH_4^+, and NO_3^- microsensors at the sediment surface and inside ventilated burrows. Loosening of the sediment packing by animals was revealed with diffusivity microsensors. Actual nitrification rates at the sediment surface were derived from the curvature of steady-state NO_3^- concentration profiles. Potential nitrification rates were measured in the feeding and the burrowing layer using specific inhibitors for nitrification in slurry incubations. The abundance of nitrifying bacteria was quantified with fluorescence in situ hybridization (FISH) in two of four tested sediments after a prolonged incubation time. We compared the effects of animal presence on nitrifying bacteria in four sediments that varied in grain size composition and organic matter content, but commonly contain *C. riparius*, as these features may influence animal behavior and the response of nitrifying bacteria.

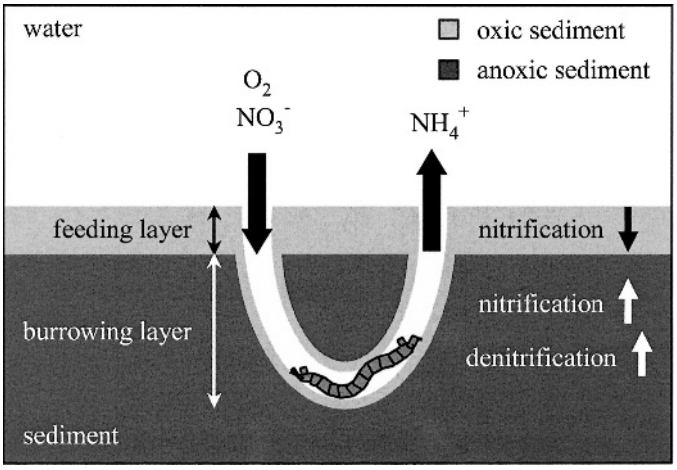

Fig. 1. Conceptual model of the interaction of macrofaunal behavior (feeding, bioirrigation), availability of reactants in the sediment (O_2, NH_4^+, and NO_3^-), and metabolic activity and abundance of nitrifying bacteria (and of NO_3^- reducing bacteria). It is hypothesized that deposit feeding decreases the metabolic activity and abundance of nitrifying bacteria in the feeding layer and that bioirrigation increases metabolic activity and abundance of nitrifying bacteria (and of NO_3^- reducing bacteria) in the burrowing layer.

Materials and methods

Experimental design—Sediment from four freshwater sites was used for microcosm experiments with and without *C. riparius* larvae. Grain size distribution and organic matter content varied widely between the four sediments and larvae were expected to adapt their burrowing and feeding behavior to these sediment characteristics. Sediment from the 0–10 cm layer was defaunated by sieving (1-mm mesh aperture) and freezing (−20°C for 48 h). For each of the four experiments (Fig. 2), sediment was homogenized and poured into 18 replicate glass beakers (800 mL) that were distributed among six aquaria (12 liters). The aquaria were filled with aerated tap water up to at least 3 cm above the rim of the sediment beakers. Thereby, three sediment beakers shared one water body. Tap water and natural stream water had a similar chemical composition, hardness, and pH. However, the low NO_3^- and NH_4^+ contents of the tap water (<30 and <1 μmol L^{-1}, respectively) were advantageous for control treatments at low NO_3^- and NH_4^+ concentrations, which could not be prepared with natural stream water. One sediment beaker each was sacrificed for microprofiling, measurements of potential nitrification, and FISH analysis during the incubation period (Fig. 2).

Sediment microcosms were preincubated at 15°C for 2 weeks to allow equilibration of the sediments (Stief et al. 2003). After that period, 60 *C. riparius* larvae from our own laboratory culture (Hölker and Stief 2005) were added to each of nine sediment beakers, giving a density of 10,000 individuals m^{-2}, which is typical of eutrophic freshwater ecosystems. The remaining nine beakers served as controls. Sediment beakers with and without *C. riparius* larvae were never together in the same aquarium. Incubation of the equilibrated sediments was continued under a 16 : 8 light : dark regime for 4 weeks to avoid induction of diapause in *C. riparius* (Goddeeris et al. 2001). Microsensor measurements were carried out during weeks 2 and 3. Potential nitrification rates were measured during week 3. Samples for FISH of nitrifying bacteria were taken at the end of week 4. Sediment characterization was conducted with surplus homogenized sediment (Fig. 2).

Sediment sampling sites were: Rittrumer Mühlenbach (RMB), a lowland stream crossing an area of glacial sand accumulation in the north of Germany; Liblarer Mühlengraben, an artificial ditch running through the lowlands in the west of Germany, upper reach (LMG-1); Liblarer Mühlengraben, lower reach (LMG-2); and Millinger Landwehr (MLW), an artificial ditch in the floodplain of the lower river Rhine in the northwest of Germany. Site LMG-1 was characterized by a higher organic content of the sediment and a markedly lower abundance of chironomids compared to site LMG-2. Sampling at each site was undertaken between 2001 and 2003. FISH was carried out in two additional but identically performed experiments in 2000 for which sediment was sampled only at sites RMB and MLW, which showed the largest differences in organic matter content and median grain size.

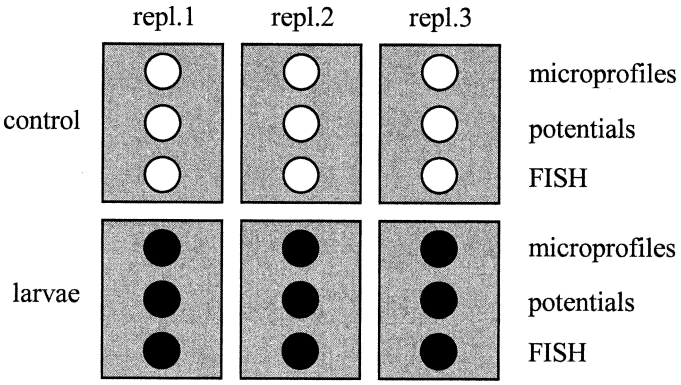

Fig. 2. Experimental design. Sediment-filled beakers (circles) were placed in water-filled aquaria (squares). Open circles symbolize sediment without *C. riparius* larvae (control), closed circles symbolize sediment with *C. riparius* larvae (larvae). Each treatment was replicated three times (Repl. 1–3). Beakers were retrieved for microsensor measurements during weeks 2 and 3, for potential nitrification assays during week 3, and for FISH analysis after week 4. The experiment was repeated with four different sediment types.

Sediment characterization—The content of organic matter of the noncalciferous sediments was estimated as the weight loss on ignition. Sediment subsamples of 5 g in dry mass were combusted at 550°C for 3 h. The grain size distribution was obtained by sieve analysis of sediment subsamples (250 mL wet volume).

Microsensor measurements—Microsensors for O_2 (Revsbech 1989), NH_4^+, and NO_3^- (de Beer et al. 1997) were constructed in our laboratory, whereas diffusivity probes equipped with an H_2 transducer (Revsbech et al. 1998) were purchased from Unisense A/S. The 90% response times of the microsensors were as follows: O_2, 10 s; NO_3^-, 25 s; NH_4^+, 25 s; and diffusivity, 180 s. Oxygen, NO_3^-, and NH_4^+ microsensors were calibrated and operated in a measuring setup as previously described (Stief and de Beer 2002). Diffusivity probes were calibrated in a layer of glass beads (40–60 μm) of known diffusivity (Revsbech et al. 1998). Since microbial H_2 consumption in the sediment leads to an overestimation of diffusivity, the sediment with the highest organic content, MLW, was additionally profiled before and after inhibition of microbial activity with 4% paraformaldehyde. All microsensor calibrations and measurements were conducted at 15°C. At least 12 h before the microsensor measurements, the sediment beakers to be analyzed were transferred to an aquarium filled with aerated tap water containing 50 μmol L^{-1} NH_4^+ and 300 or 500 μmol L^{-1} NO_3^-. Two types of measurements were done: (1) vertical concentration profiles were recorded across the sediment surface and (2) concentration time series were recorded inside the burrows at fixed positions in the ventilation current created by the larvae. Vertical profiles were recorded at increments of 200 μm from 2 mm above to 10 mm below the sediment surface, thereby covering the average burrow depth of *C. riparius* (fourth larval stage) observed in our experimental incubations. Local volumetric NO_3^- production rates were calculated using a 1-D diffusion–reaction model (de Beer and Stoodley 2000) for steady-state concentration profiles that prevail in the space between the *C. riparius* burrows where particle and pore water displacement is not significantly affected by animal activities. The diffusion coefficient of NO_3^- in water (15°C) was taken as 1.54×10^{-5} cm^2 s^{-1} (Li and Gregory 1974). Local diffusion coefficients throughout the upper 10 mm of the sediments were obtained with diffusivity probes, which is a superior approach compared to the more common use of porosity-based diffusivity estimates. A luminophore tracer experiment in the RMB sediment revealed a particle diffusion coefficient of 0.8–2.5 $\times$ 10^{-6} cm^2 s^{-1}, which the 1-D diffusion–reaction model was not corrected for.

For the time series measurements inside burrows, short-term nitrogen addition experiments were carried out. Two hours before the microsensor measurements were started, the overlying water concentrations of NH_4^+ and NO_3^- were adjusted to (1) 3 μmol L^{-1} NH_4^+ and 30 μmol L^{-1} NO_3^- (no N addition), (2) 45 μmol L^{-1} NH_4^+ and 42 μmol L^{-1} NO_3^- (NH_4^+ addition), and (3) 6 μmol L^{-1} NH_4^+ and 290 μmol L^{-1} NO_3^- (NO_3^- addition). One microsensor at a time was inserted into a burrow that was visible through the transparent beaker wall. Time series were then recorded at a depth of 6 mm for 30 min and a temporal resolution of 3 s. Long-term recordings of O_2 and NO_3^- concentration were made at different depths of the same burrow for 1–10 h. Only those time series are presented for which it was visually confirmed that the microsensor tip remained in the lumen of the burrow throughout the recording time.

Potential nitrification rates—One sediment core (25 mm diameter) from each of three replicate beakers was taken (i.e., three larvae and three control cores). The cores were sliced into layers 0–2, 2–4, 4–6, 6–8, and 8–10 mm using a core extruder. Slices (1 cm^3 wet volume) were immediately transferred to sterile flasks containing 100 mL of filtered (0.2 μm) tap water and a magnetic stirring bar. For measuring the potential NH_4^+ oxidation rate, tap water was enriched with 20 mmol L^{-1} $NaClO_3$, a specific inhibitor of NO_2^- oxidation (Belser and Mays 1980). For measuring the potential NO_2^- oxidation rate, tap water was enriched with 10 mg L^{-1} allylthiourea, a specific inhibitor of NH_4^+ oxidation (Hall 1984). Initial NH_4^+ and NO_2^- concentrations were 50 and 25 μmol L^{-1}, respectively. Preliminary experiments indicated that at these concentrations substrate limitation did not occur over an incubation time of up to 8 h. The flasks were closed with sterile rolls of cellulose tissue through which a hypodermic needle was inserted for aeration. The sediment–water mixtures were slurried on a multiple magnetic stirrer (Multipoint HP15, Variomag). Subsamples of the slurry (5 mL) were taken after 0, 2, and 4 h of incubation at 15°C. Subsamples were centrifuged for 10 min at 4,000 $\times$ g and the NO_2^- concentration of the supernatant was measured colorimetrically according to Strickland and Parsons (1968). In the NH_4^+ and NO_2^- oxidation assays, linear increases and decreases of NO_2^- concentration with time were recorded, respectively. Rates were expressed as amount of NO_2^- produced (NH_4^+ oxidation) or consumed (NO_2^- oxidation)

per unit sediment volume and time (μmol cm^{-3} h^{-1}). Linearity of the NO_2^- concentration change was exemplarily verified for an 8-h incubation with five samplings.

Quantification of (nitrifying) bacteria—One sediment core (25 mm diameter) from each of three replicate beakers was taken (i.e., three larvae and three control cores). Sediment slices (see above) were prepared for FISH on gelatin-coated microscope slides as described by Altmann et al. (2003). The FISH procedure, the set of CY3-labeled oligonucleotide probes, the total cell counts, and the quantification of FISH-positive cells followed the protocols in Altmann et al. (2004*a*). Nitrifying bacteria could be detected with probes NSO 1225 (NH_4^+-oxidizing β-proteobacteria, Mobarry et al. 1996) and NTSPA 662 (NO_2^- -oxidizing *Nitrospira* spp., Daims et al. 2000), but not with NIT 3 (NO_2^- -oxidizing *Nitrobacter* spp., Wagner et al. 1996). The combined counts of NH_4^+- and NO_2^--oxidizing bacteria were expressed as cell number per unit sediment volume (cells cm^{-3}). Total bacterial cell numbers were determined separately by DAPI staining of sonicated and diluted sediment samples on black membrane polycarbonate filters (0.2 μm; Osmonics Inc.). All cell counting was done using an epifluorescence microscope (Zeiss, Axiophot II, Carl Zeiss).

Statistics—Pearson's correlation coefficients were calculated for bivariate comparisons of the sedimentary organic matter content with O_2 and NO_3^- penetration depth, maximum pore water concentration of NH_4^+, and actual NO_3^- production rate. Significant effects of animal presence on actual and potential nitrification rate were identified with Student's *t*-test, assuming Gauss distribution of triplicate data. For testing actual rates, we calculated one average rate for the complete NO_3^--producing layer, the thickness of which varied between sediments and treatments. As potential rates were determined in experimentally separated and thus independent sediment layers, comparisons between treatments were made for single sediment layers. Significant effects of animal presence on the abundance of total bacteria and nitrifying bacteria were identified with two-way analysis of variance. Fixed factors were animal presence and sediment depth. All analyses were carried out using SPSS®, version 11.5.1 (SPSS Inc.).

Results

Sediment characterization—Sediment characteristics are summarized in Table 1. According to grain size distribution and organic matter content, the four sediments can be put into the following order: (1) RMB sediment (coarse-grained, low organic content), (2) LMG-1 and LMG-2 sediments (fine-grained, intermediate organic content), and (3) MLW sediment (fine-grained, high organic content). Animal behavior divided the sediment column into two functional layers: (1) *C. riparius* larvae were feeding in the 0–2 mm layer of the sediments (feeding layer) and (2) they built stable burrows in the 2–10 mm layer of the sediments (burrowing layer) (compare Fig. 1). However, stable burrows were almost never observed in RMB sediment and only rarely in LMG-1 sediment. In those two sediments the larvae moved just underneath the surface with the burrows collapsing behind them, probably because the larvae were unable to glue together the particles of these sediments. This is consistent with the ability of chironomid larvae to colonize the sites RMB and LMG-1 only at low abundance and the sites LMG-2 and MLW at high abundance.

Table 1. Sediment characteristics.

Field site	Grain size (μm)*			Loss on ignition (%)
	D_{25}	D_{50}	D_{75}	
RMB	190	270	380	0.9±0.1
LMG-1	120	160	230	2.7±0.1
LMG-2	110	160	280	2.0±0.1
MLW	110	180	260	5.1±0.1

* D_{25}, D_{50}, and D_{75}: grain size at 25%, 50%, and 75% of the cumulative grain size distribution curve. Loss on ignition: Means±SD are given (n=4–8).

Microsensor measurements in sediments—Oxygen penetration depth was similar in all control sediments and did not correlate with organic matter content ($r = 0.09$, $p > 0.05$, $n = 21$). Animal presence increased O_2 penetration depth in all but one sediment type (LMG-1, Fig. 3D). The animal-related increase of O_2 penetration depth was markedly high in the organic-poor RMB sediment (Fig. 3A). Ammonium added to the overlying water was almost completely consumed (or immobilized) within the oxic layer of all sediment types. In the anoxic layer, NH_4^+ concentration consistently increased without reaching a plateau within the depth of record. Animal presence decreased the slope of this concentration increase without exception. Maximum NH_4^+ concentration at 10 mm depth was positively correlated with organic matter content ($r = 0.89$, $p < 0.01$, $n = 27$). Animal presence neutralized this correlation to a large degree. Nitrate concentration peaked in the oxic layer of all sediments and decreased in the anoxic layer. Nitrate penetration depth was negatively correlated with organic matter content ($r = -0.72$, $p < 0.01$, $n = 22$). In the organic-poor RMB sediment, NO_3^- was not depleted within the depth of record (Fig. 3C). Animal presence flattened out the NO_3^- concentration peaks and generally increased NO_3^- penetration. Occasionally during profiling the microsensor passed a ventilated animal burrow, as was noticed from sudden increases of O_2 and NO_3^- concentrations in the burrowing layer of *C. riparius*. In the averaged concentration profiles, these incidences produced irregularities of otherwise smooth profiles and great standard errors of the mean (Fig. 3D,F).

Solute diffusivity in the sediment decreased with depth, the decrease being strongest in the sandy RMB sediment and weakest in the silty MLW sediment (Fig. 4). Animal presence increased solute diffusivity in sediments LMG-2 and MLW in which stable larval burrows were observed, but not in sediments RMB and LMG-1 in which stable burrows were not observed. Additional measurements in

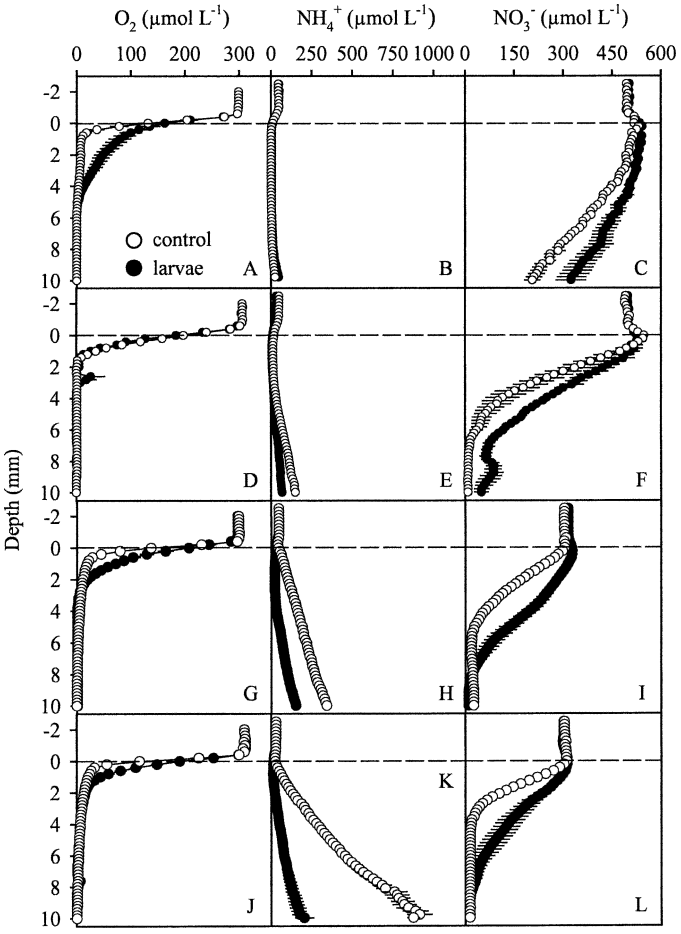

Fig. 3. Vertical O_2, NH_4^+, and NO_3^- concentration profiles in sediment microcosms with and without *C. riparius* larvae. Sediments were from sites (A–C) RMB, (D–F) LMG-1, (G–I) LMG-2, and (J–L) MLW. Incubation time was 2–3 weeks. Means±SE of 3–8 replicate microprofiles are given. Sediment surface is marked with dashed line.

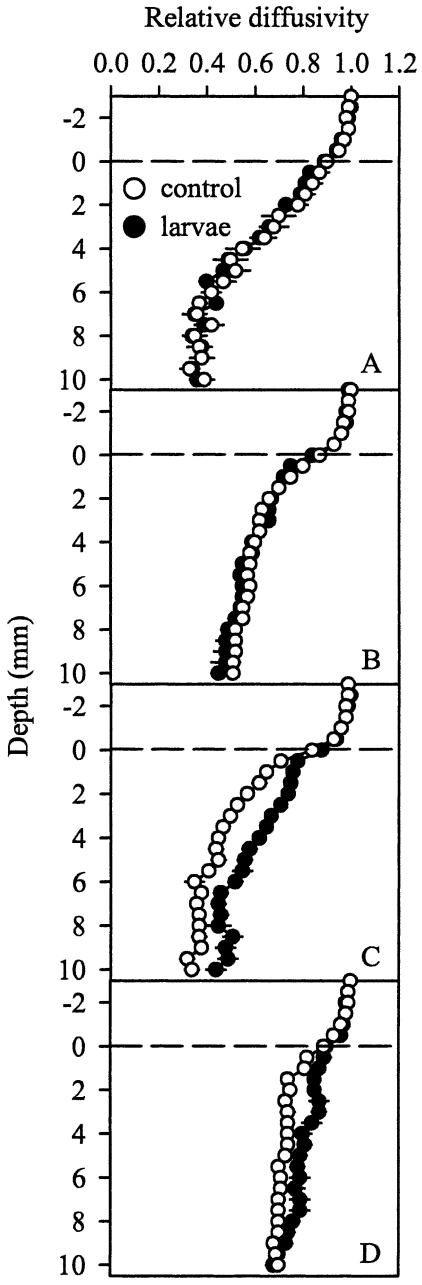

Fig. 4. Vertical profiles of relative diffusivity in sediment microcosms with and without *C. riparius* larvae. Relative diffusivity was calculated from measurements with a H_2-based diffusivity microsensor and is given as the fraction of diffusivity in the overlying water = 1. Sediments were from sites (A) RMB, (B) LMG-1, (C) LMG-2, and (D) MLW. All other information as given in Fig. 3.

sediment MLW before and after inhibition of microbial activity did not reveal significant differences in the diffusivity profiles (data not shown). Thus, sedimentary H_2 metabolism did not markedly influence the signal of the diffusivity sensor. In a few instances, we noticed unstable microsensor readings that were obviously related to a ventilation event in a burrow in vicinity of the microsensor tip. These profiles were not used for further analysis.

Actual NO_3^- production (as calculated from steady-state concentration profiles) was almost exclusively restricted to the upper 2 mm of the sediments (Fig. 5). Net production rates were highest in the upper 1 mm of control treatments and were positively correlated with organic matter content ($r = 0.80$, $p < 0.01$, $n = 22$). Animal presence significantly decreased NO_3^- production rates in all sediments (Student's *t*-test, LMG-1: $t = 3.1$, df = 9, $p < 0.05$; LMG-2: $t = 4.9$, df = 10, $p < 0.001$; MLW: $t = 6.7$, df = 10, $p < 0.001$), except for the organic-poor RMB sediment ($t = 1.0$, df = 14, $p > 0.05$).

Potential nitrification rates—While actual NO_3^- production was limited to the sediment surface (Fig. 5), potential NH_4^+ and NO_2^- oxidation was measurable down to 10 mm (Fig. 6). Rates were highest at the sediment surface and decreased with depth. Ammonium and NO_2^- oxidation rates were positively correlated with each other ($r = 0.93$, $p < 0.01$, $n = 120$). Potential NO_2^- oxidation rates (equivalent to potential NO_3^- production rates) were 6–13

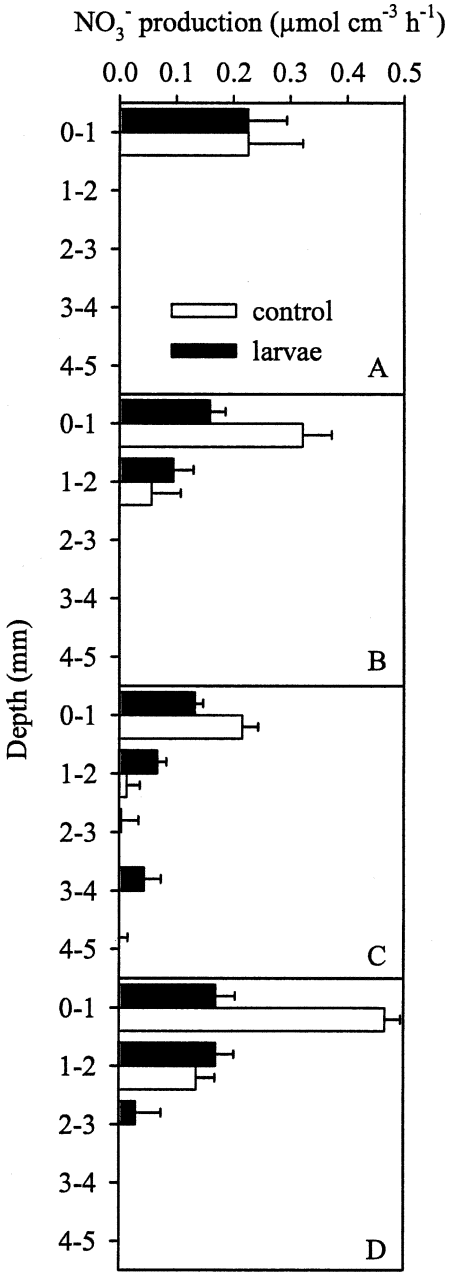

Fig. 5. Actual NO_3^- production rates in sediment microcosms with and without *C. riparius* larvae. Rates were calculated from the NO_3^- concentration profiles in Fig. 3 C, F, I, and L. Nitrate consumption rates are not shown for clarity. Sediments were from sites (A) RMB, (B) LMG-1, (C) LMG-2, and (D) MLW. See text for statistics.

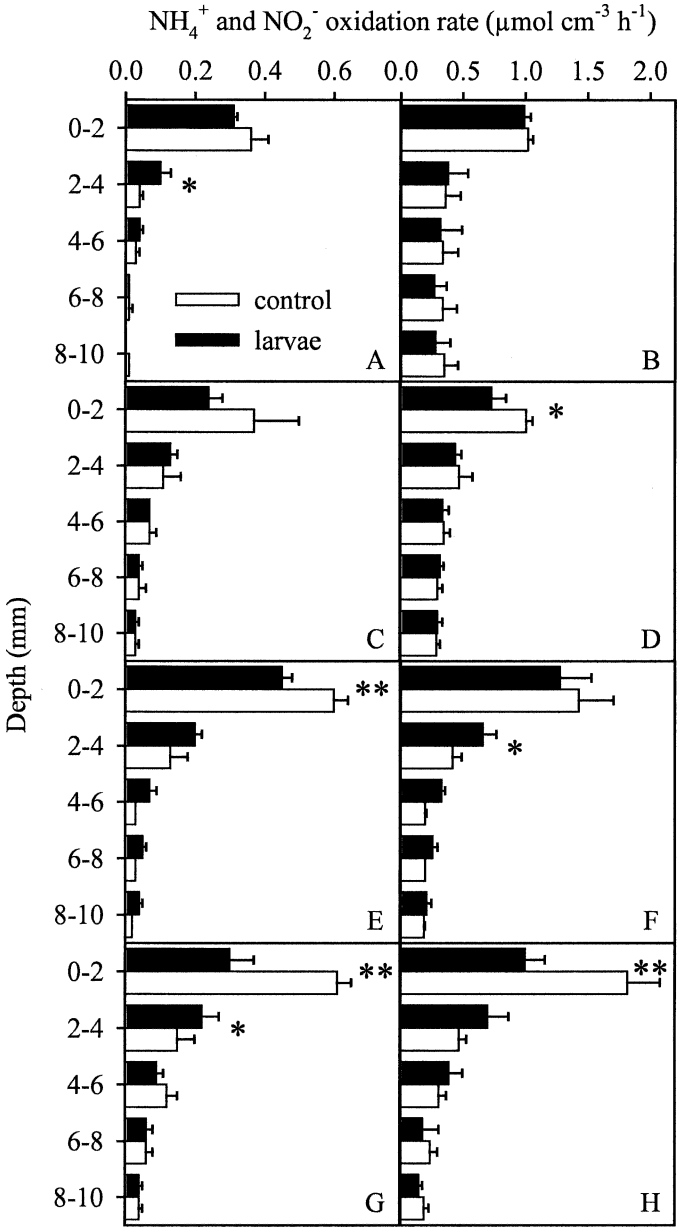

Fig. 6. Potential nitrification rates (i.e., NH_4^+ and NO_2^- oxidation rates) in sediment slices sampled from microcosms with and without *C. riparius* larvae. Sediments were from sites (A, B) RMB, (C, D) LMG-1, (E, F) LMG-2, and (G, H) MLW. Means+SE of three replicate microcosms are given. Asterisks mark significant differences between control and larvae treatments within a sediment layer (Student's *t*-test: *, $p < 0.05$; **, $p < 0.01$).

times higher than actual NO_3^- production rates (averaged for the feeding layer and for control and larvae treatments). Animal presence decreased the NH_4^+ or NO_2^- oxidation rates in the feeding layer of all sediments, except for the RMB sediment (Student's *t*-test, as marked in Fig. 6). Animal presence increased NH_4^+ and NO_2^- oxidation rates in the 2–4 mm layer in some cases (Student's *t*-test, as marked in Fig. 6). In any sediment layer deeper than 4 mm, animal-related effects on NH_4^+ and NO_2^- oxidation rates were not observed.

Microsensor measurements in burrows—Periodical changes of O_2, NH_4^+, and NO_3^- concentrations were observed in *C. riparius* burrows in the MLW sediment (Fig. 7; exception: Fig. 7B, NO_3^-). The intermittent burrow ventilation due to larval undulation behavior generated this periodicity. Fast and slow concentration changes alternated and they corresponded to the onset and cessation of larval undulation, respectively. Larval undulation behavior started when O_2 was entirely depleted inside the burrow, except in the NO_3^- addition experiment. Period length was

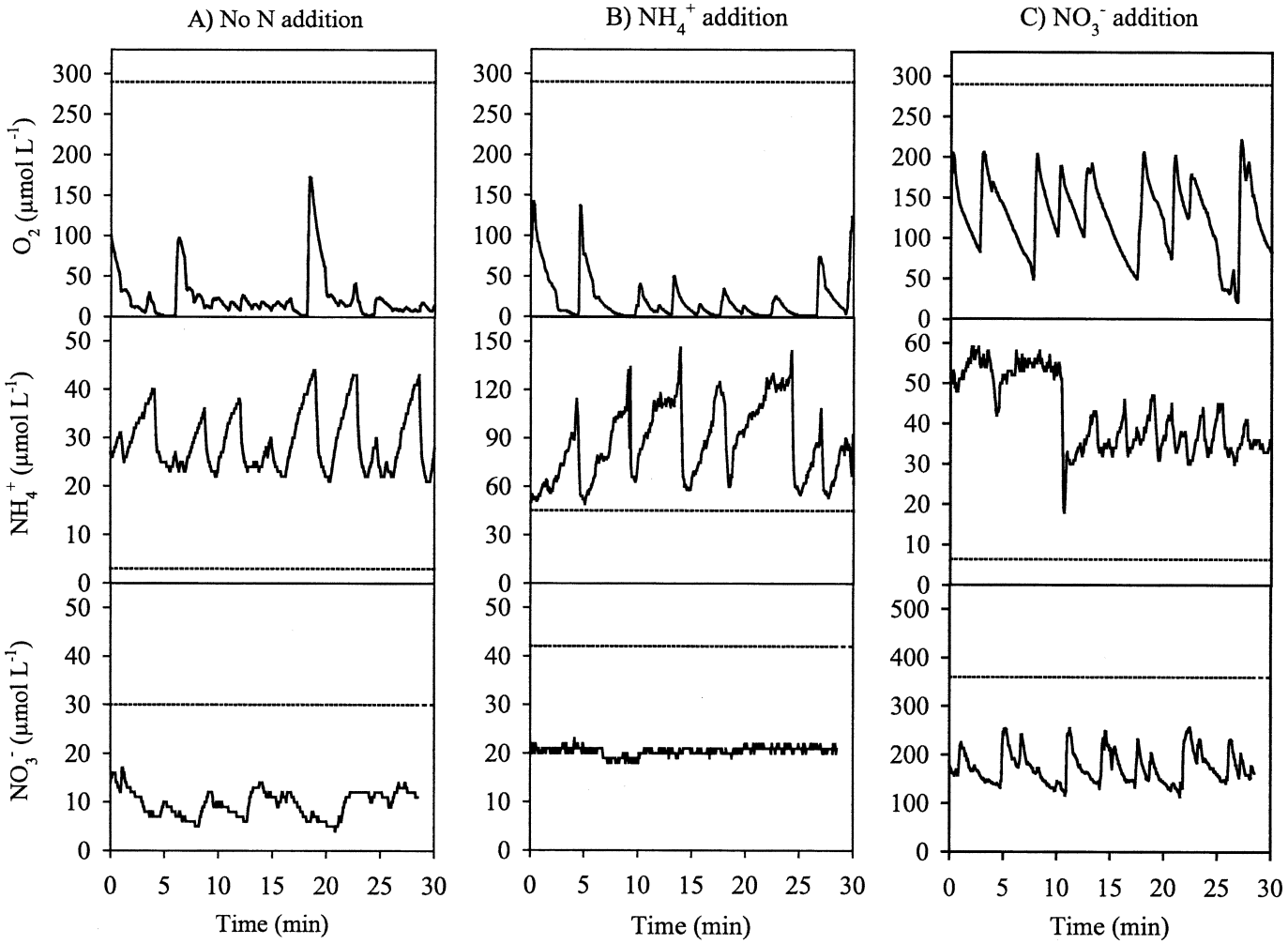

Fig. 7. Microsensor measurements inside burrows of *C. riparius* larvae over time (A) without addition of inorganic nitrogen to the overlying water, (B) with addition of NH_4^+ (final concentration 45 μmol L^{-1}), (C) with addition of NO_3^- (final concentration 290 μmol L^{-1}). Sediment was from site MLW. The microsensor tips were placed at fixed positions in the center of the burrow lumen and at a distance of 6 mm from the sediment surface. Concentrations in the overlying water are given by dotted lines.

mostly 3–6 min. Occasionally, double spikes (e.g., Fig. 7A, NH_4^+ and 7C, NO_3^-) or temporary interruptions (e.g., Fig. 7C, NH_4^+) compromised the overall rhythm. Oxygen and NO_3^- concentrations were lower and NH_4^+ concentrations were higher inside the burrows than in the water column. Therefore, burrow ventilation transported O_2 and NO_3^- into the burrows, whereas it transported NH_4^+ out of the burrows. Raising the water column concentrations of NH_4^+ and NO_3^- above their background values led to higher NH_4^+ and NO_3^- concentrations inside the burrows. The fluctuation of NH_4^+ and NO_3^- concentrations, however, was the same as without NH_4^+ and NO_3^- additions. Overall levels of O_2 and NO_3^- concentrations inside the same burrow were lower at a distance from the sediment surface of 8 mm compared to 4 mm (Fig. 8). Moreover, the periodical change of NO_3^- concentration was more pronounced at the deeper than at the shallower position. Long-term recordings of O_2 concentration demonstrated the steadiness of larval undulation behavior (Fig. 8A,B). Persistent animal burrows were also detected in the LMG-2 sediment, but microsensor measurements were not successful. In the RMB and LMG-1 sediments, animal burrows were absent and very rare, respectively.

The 90% response time of the microsensors (see Materials and methods) caused a temporal shift of a few seconds between the observed beginning or ending of larval ventilation and the recorded concentration changes. The period length, however, was depicted correctly. Since the ventilation periods lasted on average half a minute and were thus in the range of the 90% response times, the observed concentration maxima (O_2, NO_3^-) and minima (NH_4^+) were probably under- and overestimates, respectively. Conversely, the rest periods were considerably longer than the 90% response times and thus the recorded concentrations probably corresponded much better to the true values inside the burrows.

Quantification of (nitrifying) bacteria—Total bacterial counts revealed an almost even cell distribution across the depth of record (Fig. 9A,B). Cell abundance was 3–4 ×

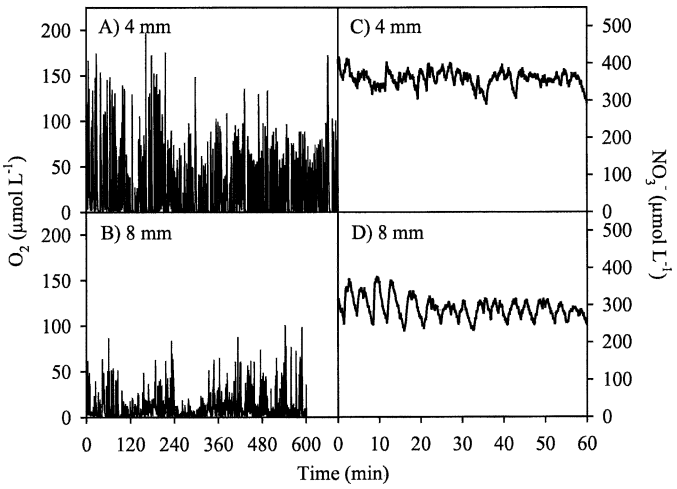

Fig. 8. (A, B) Oxygen and (C, D) NO_3^- amplitudes inside burrows of *C. riparius* larvae over time. The microsensor tips were placed at fixed positions in the center of the burrow lumen and at distances of either 4 or 8 mm from the sediment surface. Sediment was from site MLW. Nitrate concentration in the overlying water was 500 µmol L^{-1}.

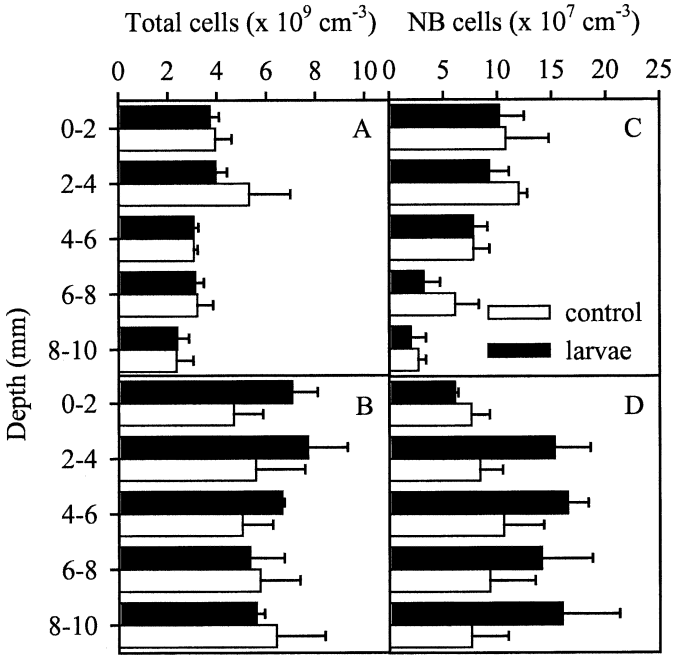

Fig. 9. (A, B) Total number of bacteria and (C, D) nitrifying bacteria (=NB cells) in sediment slices sampled from microcosms with and without *C. riparius* larvae. NB cells comprise NH_4^+ - and NO_2^- -oxidizing bacteria as identified with FISH. Incubation time was 4 weeks. Sediments were from sites RMB (panels A, C) and MLW (panels B, D). Means+SE of three replicate microcosms are given.

10^9 cm^{-3} and 5–7 × 10^9 cm^{-3} in the RMB and MLW sediment, respectively. Animal presence did not have a consistent and significant influence on cell abundance in either sediment type and in either functional sediment layer (two-way analysis of variance [ANOVA], RMB: $F_{1,20} = 0.521$, $p > 0.05$; MLW: $F_{1,20} = 1.244$, $p > 0.05$). FISH with the oligonucleotide probe mix EUB 338 (Amann et al. 1990), EUB II, and EUB III (Daims et al. 1999) revealed up to 75% of DAPI-stained cells as *Eubacteria* with a ribosome content sufficiently high for FISH detection (data not shown). In the MLW sediment, this percentage was constant down to a depth of 10 mm, whereas in the RMB sediment, it gradually decreased to 51% at a depth of 10 mm (data not shown). In the RMB sediment (Fig. 9C), the presence of animals had no significant effect on the abundance of nitrifying bacteria in any sediment layer (two-way ANOVA, $F_{1,20} = 1.250$, $p > 0.05$). In the MLW sediment (Fig. 9D), however, the presence of animals had a significant effect on the abundance of nitrifying bacteria in the burrowing layer of *C. riparius* (2–10 mm) (two-way ANOVA, $F_{1,16} = 5.977$, $p < 0.05$), but not in the feeding layer (0–2 mm) (Student's *t*-test, $p > 0.05$).

Discussion

Availability of reactants—C. riparius larvae increase the availability of O_2 but decrease the availability of NH_4^+ in the pore water of freshwater sediments (Stief and de Beer 2002, Altmann et al. 2004*b*), leaving open whether nitrification will be promoted or not. Two transport mechanisms for O_2 and NH_4^+ are conceivable. First, animal activities increase the overall sediment porosity by loosening the packing of particles and thereby increase diffusive transport. Second, animal burrows represent large pores that allow advective water and solute transport through them. With either mechanism, O_2 input from the water column and loss of pore water NH_4^+ to the water column will increase, though at different degrees of lateral homogeneity. Overall sediment porosity was indeed increased by *C. riparius* larvae in the two fine-grained sediments in which stable burrows were observed (LMG-2, MLW). Concomitantly, O_2 and NO_3^- penetrated deeper into the sediment, while pore water concentrations of NH_4^+ fell off. However, the same observations were also made in the two other sediments (RMB, LMG-1), even though the larvae did neither influence overall porosity nor construct stable burrows. In this case, the displacement of pore water as a consequence of frequent larval movements and intense particle reworking close to the sediment surface must be the cause. This stresses that burrow ventilation is not the only mechanism by which bioturbating organisms enhance pore water exchange with the water column.

Nevertheless, the burrows of *C. riparius* larvae were clearly sites of local O_2 and NO_3^- injection and NH_4^+ ejection due to larval ventilation behavior. Oxygen and NO_3^- availability inside the burrows was consequently higher than in the adjacent sediment, but lower than at the sediment surface. Ammonium availability was generally greater than at the sediment surface layer, but lower than in the pore water of identical sediment depth. The periodic changes of solute concentrations inside animal burrows are caused by a composite of advective transport between burrow and water column, diffusive transport between burrow and adjacent sediment, microbial production and consumption inside the burrow walls, and animal metab-

olism (Kristensen et al. 1991; Fenchel 1996*a*). *C. riparius* larvae show an intermittent ventilation behavior with rather frequent and short ventilation spurts (Leuchs 1986). Similar ventilation behavior was found for the alderfly *Sialis velata* (Wang et al. 2001). Other macrofauna species, however, have longer ventilation spurts, which makes burrow concentrations of O_2 and NO_3^- approach short-term (*Nereis virens*, Kristensen et al. 1991) or long-term plateaus (*Hexagenia limbata*, Wang et al. 2001; *Ephoron virgo*, Stief et al. 2004). These interspecific differences can be explained both by different O_2 demands and by different feeding habits: Unlike deposit-feeders (*C. riparius*) and predators (*S. velata*), filter-feeders (*N. virens*, *H. limbata*, *E. virgo*) have a second motivation for burrow ventilation, i.e., the acquisition of food, and therefore have very high ventilation rates (Riisgaard and Larsen 2005).

Control of metabolic activity—Availability of reactants for nitrification did not clearly predict the metabolic response of nitrifying bacteria to *C. riparius* larvae: At the surface of animal-inhabited sediments, O_2 availability increased, while NH_4^+ availability decreased. Actual and potential nitrification rates, however, both decreased at the surface of most animal-inhabited sediments (Stief and de Beer 2002; Altmann et al. 2004*b*; this study). What may tip the scales here are factors other than the bottom-up control via reactant supply to the microorganisms, namely top-down control by macrofauna. *C. riparius* larvae feed at the sediment surface on detritus particles and the associated microflora. It has been shown earlier, and is further supported by this study, that larval deposit-feeding does not measurably reduce the abundance of nitrifying bacteria (Altmann et al. 2004*b*). The gross top-down control of microorganisms, however, can be masked by compensatory regrowth (Plante and Wilde 2001). Additionally, the larvae may directly (i.e., not via reducing the abundance) control the metabolic activity of nitrifying bacteria. For instance, the passage through the anoxic gut (Stief and Eller 2006) may inactivate bacteria metabolically without killing them (Plante 2000, Altmann et al. 2004*b*).

Inside the burrows, O_2 availability was higher than in the adjacent sediment and NH_4^+ concentrations were not limiting. Nevertheless, net nitrification was not evident judging from the periodic changes of NH_4^+ and NO_3^- concentrations inside the burrows. But since gross nitrification goes undetected with this technique, the burrow walls might be sites of nitrification activity all the same. The latter was supported by elevated potential nitrification rates in the burrowing layer in the presence of *C. riparius* larvae and stable burrows. Enhanced potential nitrification rates have previously been measured with sediment sampled directly from macrofaunal burrows (Mayer et al. 1995). Rates were consistently higher than in the adjacent sediment and at the sediment surface, and they correlated well with pore water concentrations of NH_4^+. Our microsensor measurements provide evidence that inside the burrows of *C. riparius* the availability of NH_4^+ is higher than at the sediment surface, similar to what has been observed for the burrows of the marine polychaete *N. virens* (Kristensen 1984). Mineralization in the surrounding sediment and in the organic-rich burrow walls and also animal excretion contribute to the NH_4^+ enrichment of the burrow fluid. Potential nitrification rates correlate with the relative duration of the ventilation activity by different macrofauna species (Mayer et al. 1995). This duration determines the time-integrated O_2 concentration inside the burrow and thereby influences nitrification. In our experiments, time-integrated O_2 concentration inside burrows was higher near the openings and lower near the bend of the burrows, and so were the potential nitrification rates.

Hasty conclusions on strong stimulation of nitrification by species with high time-integrated O_2 concentrations (e.g., filter-feeders, see above) should be avoided, because persistent ventilation will deprive the burrows of NH_4^+. The price for higher O_2 availability is lower NH_4^+ availability, and vice versa. Instead of looking at the absolute reactant concentrations, one should focus on the periodicity of their availability inside burrows. Nitrifying bacteria might be more competitive than heterotrophic bacteria when exposed to changing O_2 conditions, because even during prolonged anoxia, nitrifying bacteria retain a high content of ribosomes (Morgenroth et al. 2000). Metabolic recovery is rapid, as could be convincingly demonstrated by measuring potential nitrification rates under nonlimiting reactant supply.

Control of abundance—Potential nitrification rates have been used as indices of metabolic activity and abundance of nitrifying bacteria (Mayer et al. 1995). However, they cannot be equated with either of them. Cell-specific metabolic activity varies largely with reactant availability and other environmental factors (Smorczewski and Schmidt 1991; Schramm et al. 1999) and therefore should not be used to estimate abundance. As an alternative, we quantified the actual abundance of nitrifying bacteria in the two most contrasting sediments (i.e., organic-poor sand vs. organic-rich silt). In the control treatments of both sediments, the vertical pattern of nitrifier abundance agreed reasonably well with that of the potential nitrification rate, i.e., at the oxic sediment surface both quantities were high, but also in the anoxic burrowing layer they were different from zero. The latter fact stresses once more the metabolic inducibility and the high content of ribosomes of nitrifying bacteria even after prolonged periods of anoxia (Mayer et al. 1995; Morgenroth et al. 2000). The presence of animals in the organic-poor sand affected neither nitrification rates nor nitrifier abundance. In the organic-rich silt, however, opposing effects of the larvae were observed for nitrifier abundance and nitrification rates: In the feeding layer, nitrifier abundance did not decrease, despite the markedly lower actual and potential nitrification rates, whereas in the burrowing layer, nitrifier abundance increased significantly, despite the almost identical potential nitrification rates. The major reason for these differential effects could be the different time points at which actual and potential nitrification rates (during week 3) the abundance of nitrifying bacteria (after week 4) were determined. First, the grazing effect by *C. riparius* larvae at the sediment surface may have been obscured by regrowth of nitrifiers in the meantime. Second, the growth of nitrifiers in the

burrowing layer may have been delayed by the only intermittent injection of O_2 into burrows, which probably induced only short-term metabolic activity of the nitrifiers.

Relevance for natural freshwater ecosystems—In pristine aquatic ecosystems, sedimentary nitrification oxidizes NH_4^+ from the degradation of deposited organic matter and thereby constitutes the major source of NO_3^-. Nitrate diffusing into anoxic sediment layers is reduced to either N_2 (denitrification) or NH_4^+ (dissimilatory NO_3^- reduction to NH_4^+, DNRA). The presence of burrowing macrofauna tightens the coupling of nitrification and dissimilatory NO_3^- reduction (Binnerup et al. 1992; Pelegri et al. 1994; Svensson 1998). Nitrate produced by nitrification is rapidly transported into animal burrows in which, due to hypoxic or anoxic conditions, dissimilatory NO_3^- reduction takes place. Our study indicates that in the presence of macrofauna the nitrification activity can be reduced at the sediment surface and emerge in the burrowing layer of the animals. Thereby, the spatial separation of nitrification and dissimilatory NO_3^- reduction in distinct sediment layers can be replaced by the temporal separation of these two processes due to the intermittent ventilation of burrows. It is noteworthy, however, that macrofaunal burrows act as sinks for NO_3^- irrespective of its origin. In most human-affected freshwater ecosystems, great amounts of NO_3^- are imported from terrestrial or groundwater environments (e.g., soil runoff from fertilized arable land or groundwater seepage). The imported inorganic nitrogen is then removed from or recycled within the freshwater ecosystems, depending on the share of denitrification and DNRA. To date it has not been investigated whether sediment-dwelling freshwater macrofauna have a significant influence on the share of denitrification and DNRA. Similarly, the macrofaunal effects on anammox, the anaerobic oxidation of NH_4^+ to N_2 (Mulder et al. 1995), remain to be explored.

The large-scale effect of sediment macrofauna on nitrogen dynamics depends on the animal species and benthic communities under consideration (Mayer et al. 1995). Feeding, burrowing, and ventilation behavior (and their effects on microbial populations) differ between animal species and are modified by sediment characteristics (Altmann et al. 2004*b*). In our experiments, *C. riparius* larvae were able construct and maintain stable burrows only in fine-grained sediments with at least a moderately high content of organic matter. It is obvious that nitrification in the burrowing layer can only be promoted by stable, deep-reaching burrows that are frequently ventilated. Also, the grazing effect on nitrification activity was most pronounced in fine-grained, organic-rich sediments, suggesting more intense or efficient deposit-feeding activity. A closer look reveals that the difference between control and larvae treatments was largely explained by the correlation between organic matter content and nitrification rates in the control treatments and that *C. riparius* larvae neutralized this correlation. Other macrofauna with different feeding and burrowing behaviors can affect the microbial nitrogen cycling in freshwater and marine sediments in different ways (Pelegri and Blackburn 1995; Svensson and Leonardson 1996; Kristensen 2000). What ultimately makes such macrobiological control of microbiological processes significant on the large scale is the often very high abundance of the typical inhabitants of both freshwater and marine ecosystems.

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