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Bioturbation effects of *Chironomus riparius* on the benthic N-cycle as measured using microsensors and microbiological assays

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ABSTRACT: *Chironomus riparius* (Diptera) larvae were added to laboratory microcosms containing defaunated sediments sampled at 2 NO_3-polluted field sites. Following a 3 wk incubation, the larval influence on the sedimentary nitrogen conversions was studied using microsensors (O_2, NO_3^-, NH_4^+) and microbial bulk parameters (microbial biomass, community respiration). At the sediment surface the chironomid larvae fed on particles (deposit-feeding layer), while in the subsurface zone the larvae moved through the sediment and ventilated transient or permanent burrows (ventilation layer). In the deposit-feeding layer of the chironomids, NO_3^- production and NH_4^+ consumption were lower and microbial biomass decreased. In the ventilation layer of the chironomids, NO_3^- and NH_4^+ conversion maxima were shifted downwards, and both microbial biomass and community respiration were increased. The observed changes in the vertical stratigraphy of the benthic microbial community were ascribed to the depth-specific larval behaviour as: (1) particle ingestion and removal of adhering microorganisms in the deposit-feeding layer; and (2) stimulation of subsurface microorganisms due to an increased supply of O_2 and NO_3^- along with larval ventilation.

KEY WORDS: Freshwater sediment · Nitrogen cycle · *Chironomus riparius* · Bioturbation · Microsensor · Microbial biomass · Community respiration

INTRODUCTION

Against the background of the continual NO_3^- pollution of surface waters in Central Europe (Hellmann 1992), sediments of aquatic ecosystems have received considerable attention as possible NO_3^- sources and sinks (Bowden 1986, Howard-Williams & Downes 1993). The benthic macrofauna stimulates both nitrification, i.e. the microbial production of NO_3^-, (Mayer et al. 1995) and denitrification, i.e. the microbial consumption of NO_3^- (Kristensen et al. 1985, Pelegri & Blackburn 1994, 1995, Svensson 1997, 1998, Gilbert et al. 1998, Tuominen et al. 1999). The stimulatory effect on nitrification has been ascribed to the increased supply of O_2 from the water column to the sediment and to the excretion of NH_4^+ by animals (Mayer et al. 1995). The stimulatory effect on denitrification has been ascribed to the increased sediment/water interface area (Pelegri & Blackburn 1995), to the additional NO_3^- supply from the water column (Svensson 1997, Gilbert et al. 1998) and to a tighter coupling of nitrification and denitrification (Pelegri & Blackburn 1994, Svensson 1998, Tuominen et al. 1999).

In a previous study, the enhancement of nitrification in the presence of bioturbating animals was assessed from slurry incubations and specific inhibition of NO_2^- oxidation (Mayer et al. 1995). The animal-induced stimulation of denitrification has been quantified by determining the fate of $^{15}NO_3^-$ enrichments in the overlying water of bioturbated sediments (Pelegri et al. 1994, Pelegri & Blackburn 1995, Svensson 1997, 1998).

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The vertical distribution of nitrogenous solutes has been recorded in the pore-water on the scale of a few millimetres or centimetres (Pelegri & Blackburn 1994, Tuominen et al. 1999). The low spatial resolution of these concentration profiles prevents calculation of local NO_3^- consumption rates in the microenvironment of burrowing animals. The destructive nature of pore-water extraction did not allow a single sample to be analysed with respect to both its content of nitrogenous solutes and to its microbial characteristics. These limitations can be overcome by using ion selective microsensors in combination with small scale applications of microbial bulk parameters.

In this study of bioturbation, we hypothesised that the presence of *Chironomus riparius* larvae can either suppress or stimulate the benthic microbial community depending on the sediment depth of consideration: At the sediment surface, the larvae deposit feed and thereby reduce the microbial biomass associated with the sediment and detritus particles (Johnson et al. 1989, van de Bund et al. 1994). Larval grazing at the sediment surface might thus lower the abundance and activity of microorganisms involved in the nitrogen cycle. In subsurface sediment layers, however, the larvae construct and ventilate transient or permanent burrows creating additional surfaces for microbial colonisation which are supplied with O_2 and NO_3^- from the overlying water (Kristensen et al. 1985, Mayer et al. 1995, Pelegri & Blackburn 1995, Svensson & Leonardson 1996, Svensson 1997, 1998). Larval ventilation of subsurface burrow structures might thus increase the abundance and activity of microorganisms involved in the nitrogen cycle.

The stimulatory or suppressive effects of chironomid bioturbation on the distribution and activity of the benthic microbial community were studied in replicate sediment microcosms, which were either devoid of or inhabited by *Chironomus riparius* larvae. Microbial biomass and community respiration were quantified in sediment slices of 2 mm thickness. Pore-water concentrations and conversion rates of O_2, NO_3^- and NH_4^+ were measured using microsensors. Our hypothesis was tested for organic-poor sand versus organic-rich silt, taken from 2 NO_3-polluted habitats of chironomids.

MATERIALS AND METHODS

Origin of sediments and chironomids. Surface sediment (0 to 15 cm) was collected from 2 contrasting field sites that contained chironomids. Sandy deposits were sampled in the littoral zone of the Grietherorter Altrhein (GAR), a disconnected oxbow of the Lower River Rhine, Germany, and silty sediment material was sampled from the Liblarer Mühlengraben (LMG), a man-made brook near Cologne, Germany. Both field sites are NO_3-polluted, either due to groundwater seepage through the sediments (GAR) or drainage water from farmland and effluents from a municipal wastewater treatment plant (LMG).

The *Chironomus riparius* (Meigen) larvae were taken from a laboratory bred population originating from the silty deposits of LMG, where they occurred in densities of up to several 10s of 1000s of individuals per m^2 during the summer of 1995. The larvae were reared in aquaria filled with a thin layer of ground dolomite and an aerated synthetic freshwater medium (Stief & Neumann 1998). Larvae were regularly fed with a suspension of shredded leaves of *Urtica* sp. and exposed to 15°C and a light:dark cycle of 12:12 h.

Microcosm preparation and incubation. The sediments were passed through a 1 mm mesh to remove pebbles, large detritus particles and indigenous macrofauna. After thorough mixing they were apportioned into cylindrical Perspex sediment containers of 2 sizes (9.5 and 19 cm in diameter, both 13 cm high) to give a final sediment height of 10 cm. The compacting sediments were covered by aerated synthetic freshwater (Stief & Neumann 1998). Sediment microcosms were allowed to stabilise for 2 wk at 15°C and a light:dark cycle of 12:12 h without further manipulations.

For experiments, a known amount of 4th instar larvae was introduced into the sediment microcosms (0 or 1 ind. cm^{-2}). During the experimental incubation no *Urtica* sp. suspension was added, meaning that the sediment and its microbial community served as the only food source for the larvae. The water column above the sediments was supplied with 15 ml h^{-1} of synthetic freshwater containing 475 µmol l^{-1} NO_3^-. This corresponded to dilution rates of 0.08 and 0.02 h^{-1} in the small and large sediment containers, respectively. The microcosms were inspected daily for dead larvae or emerged midges that were replaced by new larvae. After a 3 wk incubation, all analytical procedures were completed within 6 d. Subsequently, the sediment cores were sieved in order to retrieve and quantify the larvae still alive.

Experimental design. Six sediment microcosms were run each with organic-poor sediment (site GAR, large sediment containers) and organic-rich sediment (site LMG, small sediment containers). Three microcosms of each sediment type served as control treatments to which no chironomid larvae were added, while the other 3 microcosms served as experimental treatments with 1 *Chironomus riparius* larva cm^{-2} each.

Microsensor measurements (microbial nitrogen conversions) were made between Days 18 and 24 of the incubation at randomly chosen spots of the micro-

cosms. Extractable ATP content (microbial biomass estimate) and INT reduction capacity (respiratory activity estimate) were analysed in sliced sediment subcores between Days 21 and 25 of incubation after the microsensor measurements had been completed in a microcosm.

Physico-chemical sediment characteristics were either determined using subsamples of the homogenised source material (for organic content, protein content, and grain-size distribution) or observed through the container wall at the end of the sediment incubation (thickness of oxidised layer).

Microsensor measurements and data interpretation. LIX-type microsensors, selective for NO_3^- and NH_4^+, and Clark-type O_2 microsensors were constructed as have been described (de Beer & van den Heuvel 1988, Revsbech 1989, Sweerts & de Beer 1989). Individual microsensors were calibrated in synthetic freshwater (Stief & Neumann 1998) with known amounts of the respective ions. Diffusion coefficients of NO_3^-, NH_4^+ and O_2 at 15°C were taken as 1.44×10^{-5}, 1.50×10^{-5} and 1.83×10^{-5} $cm^2\ s^{-1}$, respectively. The microsensors were driven into the sediments by a computer controlled micromanipulator at step sizes of between 100 and 1000 µm. Vertical pore-water microprofiling was repeated 1 to 3× in each sediment microcosm. During these measurements the water column was supplied with new synthetic freshwater at a known dilution rate. The mixing of the water column and the maintainance of a diffusive boundary layer above the sediment surface was accomplished by sparging the overlying water with air.

The concentration profiles were converted into a vertical sequence of local conversion rates applying an extension of Fick's law of diffusion:

$$J = \varphi D(\delta C/\delta x) \quad (1)$$

where J is the flux, φ is the sediment porosity, D is the diffusion coefficient, and δC is the concentration gradient along the distance δx. Local fluxes at the exemplary chosen depths 1 and 2 were approximated as:

$$J_1 = \varphi_1 D(C_0 - C_2)/(x_0 - x_2) \quad (2)$$

$$J_2 = \varphi_2 D(C_1 - C_3)/(x_1 - x_3) \quad (3)$$

The volumetric conversion rate within the layer defined by the depths 1 and 2 was then calculated as:

$$R_{1,2} = (J_1 - J_2)/(x_1 - x_2) \quad (4)$$

Two consecutive layers were lumped together according to $(R_1 + R_2)/2 = R_{1'}$, $(R_3 + R_4)/2 = R_{2'}$, etc. and afterwards the weighed running average of 3 consecutive layers was calculated according to $(0.5 \times R_{0'} + R_{1'} + 0.5 \times R_{2'})/2 = R_{1''}$, $(0.5 \times R_{1'} + R_{2'} + 0.5 \times R_{3'})/2 = R_{2''}$, etc. These 2 smoothing procedures helped highlight the important features of the profiles, but led to a slight smearing of production-consumption zones.

The depth-integrated flux J_{di} of a solute was calculated as the sum of all R values multiplied by the thickness of a single conversion zone:

$$J_{di} = (\Sigma R_i)(x_{i+1} - x_i) \quad (5)$$

(from $i = 1$ to $i =$ the maximum number of conversion zones). In the case of NH_4^+, the depth-integrated flux was determined for the anoxic zone of the sediment column rather than across the sediment/water interface. For this purpose the linear part of the concentration profile below the oxic/anoxic interface was used together with Eq. (1) to calculate the NH_4^+ release into the oxic zone (de Beer et al. 1991).

The microsensor-based fluxes (henceforth referred to as depth-integrated fluxes, J_{di}) were cross-checked using the solute concentration in the continuously diluted overlying water. These overlying water-based fluxes (henceforth referred to as overlying water fluxes, J_{ow}) can be calculated as:

$$J_{ow} = (C_{ow} - C_{dil})\, V/A \quad (6)$$

where C_{ow} is the solute concentration in the overlying water, C_{dil} is the solute concentration in the supplied synthetic freshwater, V is the flow rate, and A is the cross-sectional area of the sediment.

Microbial biomass and community respiration. ***Microbial biomass:*** The extractable amount of ATP was determined for the layers 0 to 2, 2 to 6 and 6 to 10 mm using a modified version of the protocols of Karl & LaRock (1975) and Karl & Craven (1980). A sediment volume of 1 cm^3 (devoid of larvae) was suspended in 5 ml of the ice cold extractant (48 mmol l^{-1} EDTA-Na_2 in 1 mol l^{-1} phosphoric acid) and stored on ice for 30 min. After shaking once again, the suspension was centrifuged and the supernatant diluted 1:50 with sterile deionised water and its pH adjusted to 7.8 with NaOH. A luciferase assay kit (Sigma Chemical) and the luminometer TD 20 (Turner Designs) were used to perform the firefly bioluminescence reaction. After addition of known amounts of cellular-free ATP to sediment samples, an ATP loss of 15% was noted. All measured results were thus corrected for this apparent loss.

Community respiration: The capacity of the electron transport system (ETS) of the benthic microbial community was determined through the reduction of INT to INT-formazan (Blenkinsopp & Lock 1990). A sediment volume of 1 cm^3 from the layers 0 to 2, 2 to 6 and 6 to 10 mm (devoid of larvae) was incubated with 5 ml of 0.02% INT for 2 h at 15°C in centrifuge tubes. Non-converted INT was removed by centrifuging, discarding the supernatant and washing the pellet with filter-sterilised synthetic freshwater (repeated 3×).

INT-formazan was extracted from the remaining pellet with 98% methanol for 1 h at 4°C and sonication for 5 min. The extract was centrifuged again and the extinction of the supernatant was determined at 480 nm. The extinction values of formaline-killed replicate samples were subtracted from those of the living samples. INT-formazan formation rates were converted to volumetric O_2 consumption rates (Relexans 1996).

Physico-chemical sediment characteristics. ***Organic and protein content:*** Approximately 20 ml of each of the homogenised sediments were poured into precombusted glass beakers and dried to constant dry weight. The dried samples were combusted at 550°C for 3 h. Organic content was estimated as weight loss during this process. The extractable protein content was determined according to Rausch (1981) using 150 to 1000 mg of dried sediment of the homogenised source material. Both analytical procedures were replicated 4 to 10 times.

Thickness of oxidised layer: Redox-dependent colour change in the sediment column was observed through the transparent container wall at the end of the incubations. The light-brown top layer of the sediments was defined as the oxidised layer.

Mean grain diameter: Approximately 250 ml of wet sediment was sieved through a set of analytical sieves with the mesh sizes 63, 160, 200, 250, 400 and 630 µm. The mean grain diameter d_{50} was determined from the grain-size distribution curve.

RESULTS

Sediments and larvae

Sediments

Details on organic content, protein content and mean grain size are given in Table 1. The sediment collected from the GAR was a light-brown, organic-poor sand with a low content of visible detritus. Protein could be extracted only in trace amounts from this sediment relative to the dark-brown, organic-rich silt taken from the LMG. In both sediment types the redox-dependent change of sediment colour moved deeper down into the sediment in the presence of chironomid larvae (Table 2). In the non-bioturbated sediments the light-brown oxidised layer had an invariable thickness, while in the bioturbated sediments a few oxidised halos were observed around chironomid burrows.

Table 1. Initial physico-chemical sediment characteristics. Means (±SD, number of repeated analyses) are given

	Homogenised source material	
	Organic-poor sediment	Organic-rich sediment
Organic content (mg g^{-1})	12.9 (±1.6, 8)	43.8 (±1.9, 10)
Protein content (mg g^{-1})	1.1 (±0.1, 4)	18.2 (±0.7, 4)
Mean grain diameter (µm)	306 (±12, 4)	108 (±5, 4)

Table 2. Depth of redox discontinuity. Depth range of the colour change of the sediment from light-brown to dark-brown (organic-poor) or black (organic-rich) is given

Sediment type	Depth of redox discontinuity (mm)	
	Without *Chironomus*	With *Chironomus*
Organic-poor	9–10	10–13
Organic-rich	5	5–7

Larvae

Larval burrowing activity in the 2 sediment types was identical in that fewer than 10% of the added chironomids constructed a permanent, U-shaped burrow reaching down to 10 mm. Other individuals moved within the subsurface zone of the sediments at depths of between 2 and 6 mm. Ventilation activity of the larvae in the subsurface zone was sometimes detected as rhythmic up and down movements of the sediment surface. The larvae collected and ingested sediment or detritus particles at the sediment surface. A small amount of shredded *Urtica* sp. leaves placed around a burrow outlet was picked up by the larvae within a few minutes. Chironomid locomotive and feeding activity made the sediment surfaces look fluffier than occurred in the non-bioturbated control microcosms. More adult midges emerged during the experimental incubation in the organic-rich than in the organic-poor sediments (40 and 25%, respectively). Up to 35 and 50% of the added larvae were found alive at the end of the incubation in the organic-rich and the organic-poor sediments, respectively. The larvae that neither emerged nor were found alive were assumed to have died. Larval mortality accounted for up to 25% of the total additions in both sediment types. Only a few dead individuals were retrieved; the remaining larvae had decomposed.

Microbial oxygen and nitrogen conversions

General observations

The concentration profiles in Fig. 1A–F represent the steady state distribution of solutes after 3 wk incubations. Their smooth curvature indicates continuous

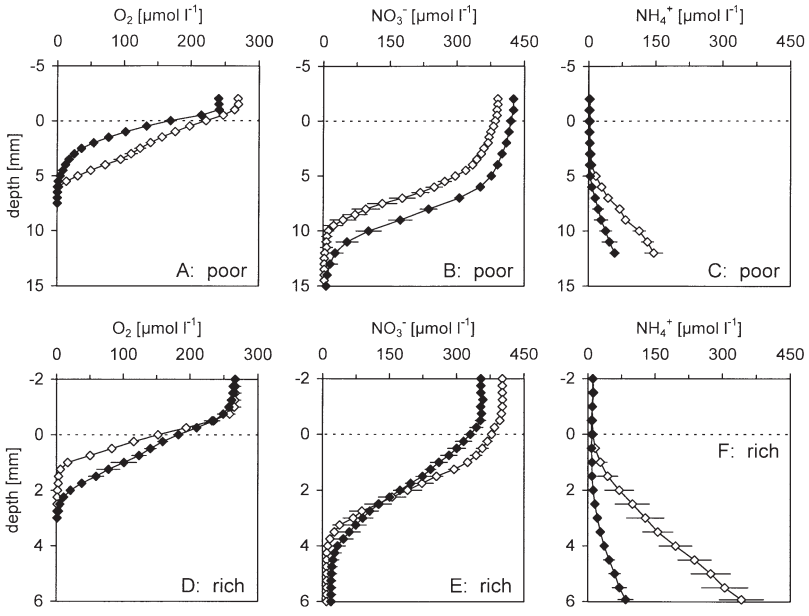

Fig. 1. Vertical concentration profiles in sediment microcosms without *Chironomus riparius* (0 larvae cm^{-2}, ◇) or with *C. riparius* (1 larva cm^{-2}, ◆). Means (±SD) of 3 microcosm replicates with 1 to 3 repeated profiles each are shown. 'poor' = organic-poor sediment, 'rich' = organic-rich sediment

profiles, uninterrupted by the unique microenvironment of animal burrows. In Fig. 2A,B,C it can be seen, however, that pore-water NO_3^- and NH_4^+ concentrations approximated bulk-water values at a relatively great sediment depth. Irregular curvatures of this type occurred in 3 out of 81 profile recordings and will be referred to as burrow crossings.

O_2 conversions

In the organic-poor sediment, the presence of larvae increased local O_2 consumption rates near the sediment surface (Fig. 3A), while in the organic-rich sediment these rates were lower near the sediment surface and higher in the subsurface layer (Fig. 3D). In a depth-integrated budget of the organic-poor sediment, an animal-related increase of sedimentary O_2 consumption was seen (Fig. 4A). The organic-rich sediment, in contrast, consumed less O_2 in the presence of chironomids (Fig. 4D).

NO_3^- conversions

Bioturbation generally caused a greater NO_3^- penetration depth (Fig. 1B,E) and a downward relocation of both the NO_3-producing and the NO_3-consuming zones (Fig. 3B,E). In the organic-poor sediment, the maximum local conversion rates remained unchanged after the 3 wk colonisation with chironomids. In the organic-rich sediment, however, the local NO_3^- production and consumption rates were lower when animals were present. Integrated over depth, the presence of chironomid larvae decreased (in organic-poor sediment) or increased (in organic-rich sediment) the sedimentary NO_3^- consumption (Fig. 4B,E), but these differences were not statistically significant when based on microsensor measurements (i.e. depth-integrated fluxes, J_{di}). In contrast to this result, the overlying water fluxes, J_{ow}, indicated significant differences in the sedimentary NO_3^- between the bioturbated and the non-bioturbated sediment (Fig. 4C,F).

NH_4^+ conversions

The 3 wk bioturbation activity by *Chironomus riparius* erased the NH_4^+ concentration gradients (Fig. 1C,F), resulting in lower local NH_4^+ consumption rates near the sediment surface and lower local NH_4^+ production rates deep in the sediment (Fig. 3C,F). Based on the microprofiles presented here, an NH_4^+ flux across the sediment/water interface was not detected in either direction. Conversely, an NH_4^+ flux from the anoxic zone up into the oxic zone of the sediment could be calculated from the steepness of the concentration gradients in the anoxic zone (Fig. 4C,F). The 3 wk presence of *C. riparius* larvae led to lower NH_4^+ fluxes up into the oxic layer, but this trend was only significant in the organic-rich sediment.

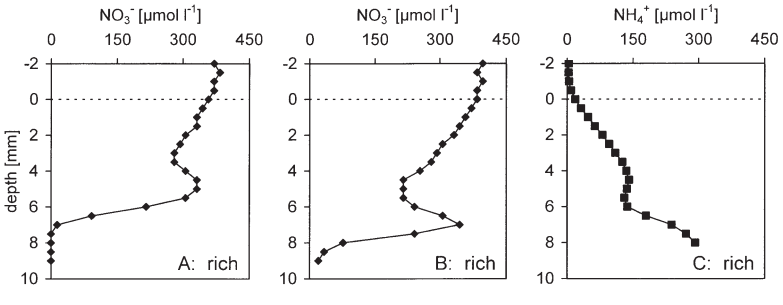

Fig. 2. Vertical concentration profiles in bioturbated microcosms (1 larva cm^{-2}), as measured with microsensors at randomly chosen spots of the sediment cores. Profiles exhibiting conspicuous irregularities of their curvature are shown

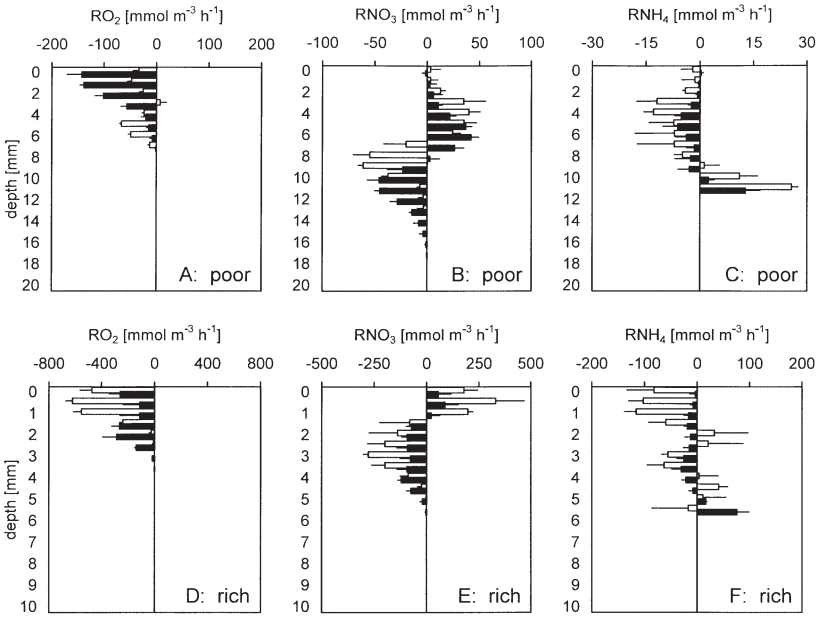

Fig. 3. Local production and consumption rates of O_2, NO_3^- and NH_4^+ as derived from concentration profiles. Non-bioturbated cores (open bars), bioturbated cores (closed bars). Positive values correspond to production and negative values to consumption. Means (±SD) of 3 microcosm replicates with 1 to 3 repeated profiles each are shown

Microbial bulk parameters

Microbial biomass

In all incubated sediments, microbial biomass was greatest at the sediment surface and then decreased with depth (Fig. 5A,B). The 3 wk presence of chironomid larvae levelled off these vertical microbial biomass gradients. At the end of the 3 wk incubation with *Chironomus riparius* larvae, the microbial biomass of the 0 to 2 mm layer was reduced in both sediment types tested. Within the 2 to 6 and 6 to 10 mm layers, however, the presence of chironomid larvae caused an amplified microbial biomass.

Community respiration

In the absence of chironomid larvae the respiratory activity of the microbial community decreased at greater sediment depths (Fig. 5C). However, after colonisation of the sediments with

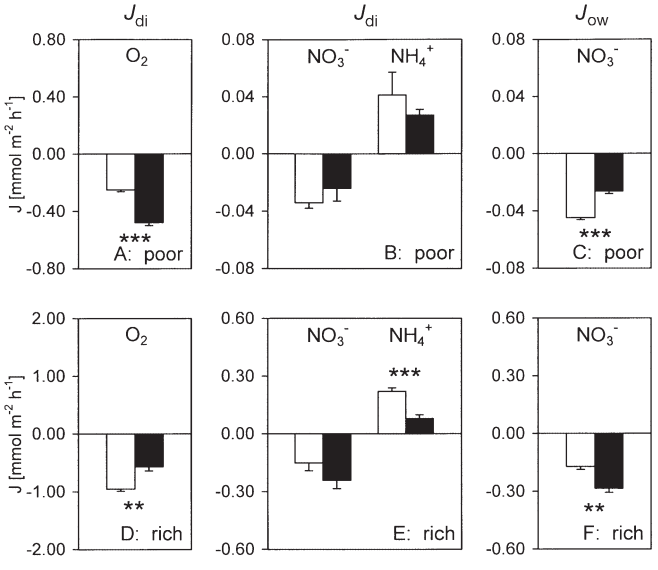

Fig. 4. Fluxes of O_2 (oxic zone) NO_3^- (oxic and anoxic zone), and NH_4^+ (anoxic zone), as derived from punctiform concentration profiles (= depth-integrated fluxes, J_{di}: A,B,D,E) or from overlying water concentrations (= overlying water fluxes, J_{ow}: C,F). Non-bioturbated cores (open columns), bioturbated cores (closed columns). Positive values correspond to an efflux and negative values to an influx. Means (±SD) of 3 microcosm replicates with 1 to 3 repeated profiles each are shown. Student's *t*-tests between experimental and control treatments revealed significant differences at $p < 0.01$ (**) and $p < 0.001$ (***)

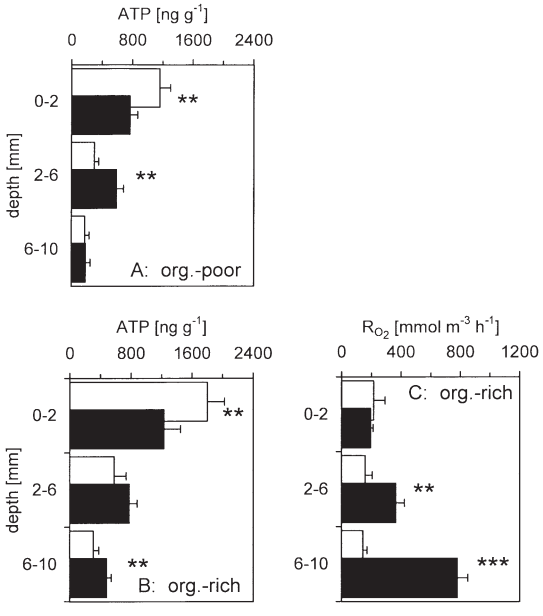

Fig. 5. Vertical distribution of microbial biomass (A,B) and microbial community respiration (C), as analysed in sliced subcores taken from sediment microcosms. Non-bioturbated cores (open columns), bioturbated cores (closed columns). Means (±SD) of 3 microcosm replicates are shown. Student's *t*-tests between experimental and control treatments revealed significant differences at $p < 0.01$ (**) and $p < 0.001$ (***)

Chironomus riparius larvae, conspicuous activity maxima appeared within the 2 to 6 and 6 to 10 mm layers. These elevations over background activities were statistically significant, but no change was observed within the 0 to 2 mm layer.

DISCUSSION

Microbial oxygen and nitrogen conversions

O_2 conversions

In the organic-poor sediment, the increase of the sedimentary O_2 consumption (0.23 mmol m^{-2} h^{-1}) was in the range of the presumable larval respiration rate as recalculated per unit area of sediment (0.19 mmol m^{-2} h^{-1}, Bairlein 1989). Since some of the larval O_2 consumption must have occurred during the time the larvae had spent on top of the sediments, the actual larval respiration rate per unit area of sediment would be lower than the expected 0.19 mmol m^{-2} h^{-1}. Thus, an unknown proportion of the increased sedimentary O_2 consumption could be attributed to an increased microbial community respiration. For high densities of *Tubifex tubifex* (Oligochaeta) a similar result was explained by the superficial accumulation of organic particles including the associated microorganisms (Pelegri & Blackburn 1995). Indeed, in the top layer of our sediments a higher content of combustible organic matter was found in the bioturbated than in the non-bioturbated treatments (7.7 ± 0.8% instead of 5.7 ± 0.6%, means ± SE, from a total of 8 samples from 3 replicate treatments each). The higher organic content could have supported the growth of heterotrophic microorganisms resulting in an increased sedimentary O_2 consumption. An animal-related increase of superficial nitrifying activity could explain the increased sedimentary O_2 consumption as well, but the NO_3^- and NH_4^+ microprofiles demonstrate that this was not the case (Fig. 3B,C).

In the organic-rich sediment, the sedimentary O_2 consumption decreased by the end of the 3 wk presence of *Chironomus riparius*. Similarly, bioturbation activity of the amphipod *Monoporeia affinis* caused a progressive aeration of an organic-rich sediment (Tuominen et al. 1999). Both observations could be due to the loss of microbial biomass close to the sediment surface as we observed using ATP extractions. The microbial community respiration in the same sediment layer, however, was not different in either the presence or absence of animals. This apparent contradiction can be resolved when one assumes that bioturbation effects on the distribution and the activity of the microbial community cancel each other out: Larval grazing of attached microorganisms could have reduced the microbial biomass (Johnson et al. 1989), but pore-water ventilation and larval secretion of organic or nitrogenous compounds might have stimulated the microbial respiratory activity (Svensson & Leonardson 1996).

NO_3^- conversions in the organic-poor sediment

NO_3^- production and consumption zones moved deeper down into organic-poor sediments when chironomids were present. This observation was true even though the diminished O_2 penetration depth may have caused the opposite result: the restriction of the NO_3^- production zone (the presumable nitrification zone) to the thinner layer of intense O_2 consumption and the relocation of the NO_3^- consumption zone (the presumable denitrification zone) in the direction of the oxic/anoxic interface. In spite of this prediction, the observed downward shift of the nitrification zone can be explained by the depletion of NH_4^+ following the long-term presence of *Chironomus riparius*. This depletion must have restricted the nitrification activity to a deeper layer in the sediment where NH_4^+ became available. Trace amounts of O_2 could be measured down to the depth of the observed maximum nitrification rate. The portion of nitrification activity below the O_2 penetration depth may be due to smearing from smoothing the local conversion rates. Denitrification might have become substrate limited from the continuous chironomid bioturbation as well: The animal-induced accumulation of organic matter in the top layer of the sediments occurred at the expense of deeper sediment layers (1.7 ± 0.2% instead of 2.4 ± 0.4%, means ± SE, from a total of 8 samples from 3 replicate treatments each) and this transfer might have created a lack of electron donors needed for denitrification. Following these ideas, O_2 was consumed in a thin top layer of accumulated organic matter, while NO_3^- was reduced less efficiently due to a lack of suitable organic compounds in deeper sediment layers. The chironomid bioturbation shifted both the nitrification and denitrification zone towards sediment layers in which the respective substrate demands were still met.

The sedimentary NO_3^- consumption in the presence or absence of *Chironomus riparius* showed identical trends, no matter if calculated from microprofiles or bulk-water samples. The latter method largely reduced the scatter of data because the lateral heterogeneities, as revealed by the microprofiles taken at separate spots of the sediment surface, were integrated to form a single value. Similar to earlier attempts to quantify the impact of burrowing animals on the benthic NO_3^- budget, the bulk-water samples

revealed significant differences between bioturbated and non-bioturbated sediment cores (Pelegri et al. 1994, Pelegri & Blackburn 1995, Svensson 1997, 1998). However, microsensors were needed to demonstrate the way these differences occurred (i.e. through vertical shifts of production and consumption zones).

NO_3^- conversions in the organic-rich sediment

The chironomid larvae helped create a smoother curvature of the NO_3^- profiles in the organic-rich sediments that resulted in lower local conversion rates of both NO_3^- production and consumption. Since vertical relocations of the respective conversion zones were of minor significance, the substrate demands of nitrification and denitrification were met at identical sediment depths regardless of whether *Chironomus riparius* larvae were present or not. From the lower local conversion rates, however, a relative depletion of NH_4^+ (as a substrate of nitrification) and organic compounds (as substrates of denitrification) could be inferred. The shortage of these compounds at the end of the sediment-animal incubation may be a result of the continuous pore-water flushing through larval ventilation (i.e. a loss of dissolved compounds to the overlying water through advective mass transfer). Animals may have also enhanced or stimulated the microbial conversion of these compounds at the beginning or during the whole incubation period. Neither one of these processes were actually quantified, but they could have created the substrate-depleted situation at the end of the incubation.

A significant increase of sedimentary NO_3^- consumption was seen in the presence of chironomid larvae in depth-integrated budgets. This result was obtained by both microprofiling and bulk-water sampling. While the latter method delivered statistically significant results (see above), only the microsensors helped elucidate the underlying mechanisms (alterations of the vertical sequence of local NO_3^- production and consumption rates).

NH_4^+ conversions

Irrespective of the sediment type, the NH_4^+ gradients were less steep after the 3 wk incubation with *Chironomus riparius*. Both the enhanced transport of NH_4^+ to the overlying water due to pore-water flushing and the enhanced microbial conversion of NH_4^+ within the sediments due to stimulation of nitrification could explain the relative NH_4^+ depletion at the end of the incubation. Experimental support for the latter hypothesis has been found in the form of elevated nitrification potentials within the burrow walls of various marine animal species (Mayer et al. 1995). The intermittent transport of O_2-rich water into the burrows together with the availability of NH_4^+ are believed to create favourable growth conditions for nitrifiers. In our case, however, the local NH_4^+ consumption rates close to the sediment surface were lower in the presence of *C. riparius*. This observation coincides with a lower extractable ATP content in the surface layer of our bioturbated sediments. Larval ingestion and digestion of particles and attached microorganisms is one possible way of lowering the abundance of, e.g. nitrifying bacteria. It has been shown for *Chironomus plumosus* that larval and bacterial abundances were negatively correlated to each other after a 10 d incubation in laboratory sediments (Johnson et al. 1989). Similarly, the 22 d bioturbation activity of *C. riparius* larvae at abundances of 5000 and 10 000 ind. m^{-2} led to a decrease of bacterial abundance in the oxidised surface layer of the sediments (van de Bund et al. 1994). In both cases, larval grazing of attached bacteria was considered to be responsible for the decrease in bacterial abundance close to the sediment surface.

The NH_4^+ concentration gradient below the oxic/anoxic interface within the sediment column was chosen to quantify the flux of NH_4^+ into the presumable nitrification zone (de Beer et al. 1991). In the presence of chironomid larvae, less NH_4^+ was supplied from the anoxic zone to the nitrification zone than in the non-bioturbated treatment. Similar observations have been made for *Chironomus plumosus* in that at the end of a laboratory incubation, interstitial NH_4^+ concentrations were lower when animals were present (Fukuhara & Sakamoto 1987). These authors concluded that mechanical bioturbation led to a gradual loss of NH_4^+ to the overlying water and that NH_4^+ excretion by the animals could not compensate for this loss.

Microbial bulk parameters

Biomass gradients

Animals with a burrowing behaviour similar to that of *Chironomus riparius* affect the spatial distribution of microorganisms by grazing (Johnson et al. 1989), particle translocation (Francois et al. 1997) or growth stimulation through ventilatory substrate supply (Svensson & Leonardson 1996). In our experiments with *C. riparius*, the significant decrease of the microbial biomass within the 0 to 2 mm layer could be ascribed to the deposit-feeding at the sediment surface (Johnson et al. 1989, van de Bund et al. 1994). In contrast, the greater microbial biomass in the main residence depth of the larvae (2 to 6 mm) could have been caused by the

stimulation of microbial growth through the supply of substrates from the overlying water (e.g. O_2 or NO_3^-). Particle translocation could have contributed to the flattening of the vertical biomass gradients as well.

The decreased microbial biomass at the sediment surface coincided with lower NH_4^+ and NO_3^- conversion rates, while in deeper sediment layers the increased microbial biomass coincided with downward relocated NH_4^+ and NO_3^- conversion rate maxima. Even though these observations suggest causal correlations, one should be careful to bring together a bulk parameter that integrates functional groups of microorganisms with the activities of a rather specific selection of microorganisms. Identification and quantification of functional groups of microorganisms are needed to correlate abundance and activities of microorganisms involved in the benthic nitrogen cycle.

Respiration gradients

Noticeable elevations of community respiration were only seen in the subsurface layers of the bioturbated sediment and not at the sediment surface. Chironomid larvae facilitate the transport of solutes from the overlying water down to subsurface microorganisms which are constrained by a diffusion-limited substrate supply. The additional availability of, e.g. O_2 and NO_3^-, probably increased the proportion of actively respiring microorganisms, i.e. microorganisms with an operating electron transport system (Relexans 1996). Nitrifying bacteria, for example, could have profited from O_2 in the ventilation currents, but because in our sediments NH_4^+ had been depleted at the sediment surface, an activation of nitrifiers was only possible in subsurface layers (e.g. in the 2 to 6 mm layer). This interpretation agrees with the findings of other authors on elevated nitrification potentials in animal burrows in the subsurface layers of the sediment (Kristensen et al. 1985, Mayer et al. 1995). Denitrifying bacteria could likewise have profited from NO_3^- having been introduced into deeper sediment layers (e.g. into the 6 to 10 mm layer). Gilbert et al. (1998) reported a similar stimulation pattern of the denitrification activity by a natural community of burrowing macrobenthos. The INT incubation technique constitutes another unspecific bulk parameter that integrates different functional groups of microorganisms (e.g. bacteria involved in the benthic cycling of nitrogen, sulphur, iron etc.). Thus, a direct correlation of the community respiration and more specific solute conversions (e.g. O_2, NO_3^- and NH_4^+) are not possible.

Aside from the enhanced nutrient supply, grazing on sedimentary bacteria may have caused increased microbial activity. Reichelt (1991) noted that bacterivorous feeding creates open surfaces that can be recolonised by microbial populations growing in log phase. Grazing pressure does not necessarily come from macrobenthic organisms; it may also originate from bacterivorous meiofauna (Reichardt 1988) or protozoa, for which the macrobenthic organisms were creating favourable growth conditions (Alongi 1985). Sieving our sediments through a 1 mm mesh should have preserved both groups of animals. Small nematodes were occasionally seen to move within the 0 to 2 mm layer, but no effort was made to find protozoa. When assuming that apart from the added macroinvertebrates (*Chironomus riparius*), bacterivorous meiofaunal species and protozoa were also present in our sediments, another trophic cascade seems possible: Chironomids could have grazed upon these small animals and thereby indirectly decreased the grazing pressure on microorganisms which in return should show higher abundances and activities. A similar scenario was indeed proposed by Lavrentyev et al. (2000) who showed that the bivalve *Dreissena polymorpha* removed a large proportion of protozoa (but not bacteria) and thereby indirectly stimulated microbial NH_4^+ conversions at the sediment/water interface. When applied to our experiments, the meiofaunal and protozoan abundances should have been diminished mainly in the deposit-feeding layer of *C. riparius*. The expected decrease in bacterivory should then have increased the bacterial abundances and activities, but this could not be confirmed: Microbial biomass was smaller, community respiration remained unchanged and activities of microbial conversions were mostly lower in the deposit-feeding layer of the chironomids.

Larval behaviour and experimental approach

Most chironomid larvae in our microcosms roamed and ventilated the subsurface zone of the sediments at random places. Only a few of them constructed permanent burrows. Accordingly, obvious burrow crossings by microsensors were quite rare (3 out of 81 profiles). Similar observations on the burrowing behaviour were made at the field site from which the larvae had been collected: At a larval abundance of several 10s of 1000s of individuals per m^2, individual burrows were not distinguishable. At a lower larval abundance (100 ind. m^{-2}), however, almost every single larva did construct its own U-shaped burrow. One explanation for the lack of spatially separated burrows could be competition for limited space in the top layer of sediments. Moreover, the experimental sediment homogenisation had buried fresh deposits of high nutritional value into deeper sediment layers, which could be exploited by subsurface grazing instead of deposit-feeding.

Larval burrows may also go undetected by microsensors because of their small inner diameter (ca. 2 mm), the relatively large theoretical distance between them (10 mm) and the microscale range of the microsensors. Archer & Devol (1992) postulated that O_2 microdistribution is governed by vertical diffusion (and not irrigation inside burrows or radial diffusion around burrows) as long as the horizontal distance between burrows is greater than the oxic zone surrounding them. This precondition was fulfilled in our case, because O_2 penetration into the sediment surface was 2 and 5 mm (in organic-rich and -poor sediment, respectively) and O_2 penetration into the burrow walls can be assumed to be only 40 to 70% of these values (Fenchel 1996). Thus, the presence of a few permanent larval burrows was of minor importance for the shape of most vertical concentration profiles that were interpreted using a 1D approach. The local uniqueness around individual burrows has been averaged out by taking microsensor measurements at random spots within 1 sediment core and by slicing subcores, which were as wide as 25 mm. Thus, the impact of *Chironomus riparius* on the distribution and activity of bacteria was described as laterally integrated alterations of the vertical gradients. This approach is similar to the extraction of pore-water from different sediment layers that also smoothes out some of the vertical and lateral heterogeneities around animal burrows (Tuominen et al. 1999, Mermillod-Blondin et al. 2000). The major advantage of microsensors is their superior spatial resolution in the vertical dimension which helps with interpreting possible interrelations between larval behaviour and the benthic microbial community. In the future ion-selective microsensors (NO_3^-, NO_2^-, NH_4^+) should also be used inside individual animal burrows to get some more insights into the microbial nitrogen conversions occurring in them. So far, this approach has been performed only with respect to O_2 distribution around burrows and O_2 fluctuations within burrows (Frenzel 1990, Fenchel 1996, Wang et al. 2001).

CONCLUSIONS

Our data suggest a correlation of the depth-specific behaviour of the chironomid larvae with changes in the distribution and activity of sediment microorganisms: (1) larval grazing in the deposit-feeding layer reduced the size of the microbial community as a whole (and maybe that of nitrifiers in particular); and (2) larval pore-water flushing in deeper sediment layers stimulated the growth and activity of subsurface microorganisms (e.g. nitrifiers and denitrifiers) via an increased supply of O_2 and NO_3^-. Chironomid bioturbation thus produced vertical shifts of microbial biomass, microbial community respiration and nitrogen conversion rates.

The examination of the benthic microbial community needs further refinements to address some of the microorganisms involved in the benthic nitrogen cycle with respect to abundance and activity: The sediments could be screened specifically for nitrifiers and denitrifiers using molecular techniques and inhibitors could be used to specifically quantify nitrification and denitrification.

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