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Microbial activities in the burrow environment of the potamal mayfly *Ephoron virgo*

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SUMMARY

1. The impact of burrowing larvae of *Ephoron virgo* (Ephemeroptera, Polymitarcidae) on sediment microbiology has not been previously investigated because of difficulties in sampling the sediment of large rivers under *in situ* conditions. Therefore, we conducted experiments in the on-ship Ecological Rhine Station of the University of Cologne (Germany), in which ambient conditions of the River Rhine can be closely mimicked.
2. In two consecutive seasons, experimental flow channels were stocked with *Ephoron* larvae and continuously supplied with water taken directly from the River Rhine. Sediment from the immediate vicinity of *Ephoron* burrows (i.e. U-shaped cavities reaching 10–80 mm deep into the sediment) and bulk sediment samples were analysed for (i) particulate organic matter content, (ii) microscale *in situ* distribution of O_2, NO_3^-, and NH_4^+, and (iii) potential activities of exoenzymes.
3. Sediment surrounding the *Ephoron* burrows had markedly higher organic matter contents and exoenzyme activities compared with the bulk sediment. Microsensor measurements demonstrated that local O_2 and NO_3^- penetration into the sediment were greatly enhanced by larval ventilation behaviour. Volumetric O_2 and NO_3^- turnover rates that were calculated from steady state concentration profiles measured directly in the burrow lining were considerably higher than at the sediment surface.
4. In the sediment of the fast flowing River Rhine *Ephoron* burrows are preferential sites of organic matter accumulation and dissolved oxidant penetration. Our data suggest that the burrows are surrounded by a highly active microbial community that responds to the inputs from the water column with elevated O_2 and NO_3^- turnover, and release of exoenzymes into the sediment pore water. Especially during periods of mass occurrence, the larvae of *E. virgo* may thus significantly contribute (i) to the ecological connection between the water column and the sediment and (ii) to biogeochemical processing of organic matter in the riverbed.

Keywords: animal-microbe interactions, bioirrigation, bioturbation, Ephemeroptera, microbial activity, microsensor, Rhine, stream sediment

Introduction

Around 1990 the burrowing mayfly *Ephoron virgo* (Olivier 1791) returned to the River Rhine. The mass flight of the large mayflies near street lamps on bridges attracted great public and scientific interest, since the species had been absent from the polluted river for decades (Schöll, 1993; Kureck & Fontes, 1996; Kureck, 1996). In contrast, the larval life within the riverbed is poorly known; only by using a diving bell could population densities of 500–1000 larvae m^{-2} be determined during years of mass occurrence (Schöll, 1993). These occasionally high larval abundances

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suggest a measurable impact of larval sediment bioturbation and bioirrigation on benthic processes. Solute exchange between the water column and sediment pore water can be greatly enhanced because of active ventilation of animal burrows (Matisoff & Wang, 1998). Additionally, biodeposition of fine particles from the water column into animal-inhabited sediment has been reported (Vaughn & Hakenkamp, 2001). The latter may be of particular relevance in streams where current velocities are often too high to allow substantial sedimentation of seston particles.

The sediment burrows of various macroinvertebrates have been recognised as sites of high microbial activity in aquatic ecosystems. The benthic N-cycle and the mineralisation of organic matter are among the most widely studied microbial processes that are stimulated by bioturbation and bioirrigation (N-cycle: e.g. Svensson, Enrich-Prast & Leonardson (2001) and references therein; mineralisation: e.g. Kristensen (2000) and references therein). For instance, nitrification can be stimulated in burrow walls that are periodically aerated because of ventilation and exposed to NH_4^+ excreted by the inhabiting animal (Mayer, Schaffner & Kemp, 1995). In contrast, denitrification is promoted by the facilitated NO_3^- penetration into deep sediment layers that become anoxic periodically (Svensson & Leonardson, 1996). Oscillating O_2 and redox conditions, however, have been shown to stimulate organic matter degradation in periodically ventilated layers or when organic matter is frequently buried and reburied (Aller, 1994; Kristensen, 2001).

Surprisingly little attention has been paid to bioturbation effects on the microbiology of stream sediments (Vaughn & Hakenkamp, 2001). Nevertheless, even large rivers are colonised by macrofaunal species that may alter sediment properties (Zanetell & Peckarsky, 1996). We investigated the immediate vicinity of *Ephoron* burrows that reach 10–80 mm deep into the sediment for existing changes in sediment properties that might in turn alter sediment microbiology. Experiments were designed to test the hypothesis that sediment intimately surrounding the *Ephoron* burrows and the bulk sediment differ with respect to organic matter content, O_2 and inorganic nitrogen distribution, and potential exoenzyme activities. Fine-particulate organic matter may preferably accumulate in the sediment cavities created by the larvae and serve as a dietary basis for microbial growth. Oxygen and NO_3^- may be introduced to deep sediment layers by larval ventilation and serve as terminal electron acceptors for the metabolic oxidation of organic matter. Exoenzymes may preferably be released by microbes into the pore water of sediment surrounding *Ephoron* burrows and may thus indicate the greater extent of local organic matter degradation. Experiments were performed under semi-natural habitat conditions of *E. virgo* in flow channels, which continuously received water from the River Rhine.

Methods

Study site

Experiments were conducted in the on-ship Ecological Rhine Station of the University of Cologne (Germany). The ship is permanently located on the Lower River Rhine near Köln-Bayenthal (km 684.5). Experimental flow channels in the laboratory were continuously supplied with water collected by pumps submersed below the water table of the river. Before entering the flow channels, the water was filtered through a mesh to remove predators and suspended particles larger than 300 μm. Water discharge was high enough to avoid significant changes in temperature and chemistry of the river water. All experimental incubations were exposed to the natural photoperiod.

Experimental set-up

Experiments were run in 2000 and 2001. For the experiment in 2000, river sediment was collected and sieved through a 1-mm mesh in order to remove pebbles and mussel shells. The sieved sediment (median grain size: 200 μm) was filled into a Plexiglas® flow channel of 600 × 20 × 10 cm in length × height × width, respectively. After settling, the flow channel was continuously supplied with river water. The resulting water level was 3–5 cm and the mean current velocity was 19 cm s^{-1}. Water passed over the sediment only once and left the flow channel via the outlet. Observations in the 2000 study suggested that *Ephoron* larvae promoted the introduction of organic matter into the sediment. Therefore, in 2001, quartz sand was used as an inorganic model substrate in order to set off the larval introduction of organics into

deep sediment layers. In February 2001, two Plexiglas® flow channels (300 × 20 × 8 cm in length × height × width, respectively) were filled with quartz sand (median grain size: 215 μm) and supplied with river water as described above. In both experiments, animals were introduced into the sediments during early spring: in March 2000, young larvae of *E. virgo* bred from field-collected eggs of the previous year were added to the experimental flow channel. In March 2001, however, only one flow channel was stocked with *Ephoron* larvae, while the second channel served as a control. Settling larvae constructed vertical U-shaped burrows in the sediment, some of which were visible from the side through the transparent flow channels. (The transparent walls had a dark cover that was removed only before the measurements or for visual inspections.) During spring and summer the larvae gained body mass until they reached a body length of about 15–17 mm. Their burrows penetrated the sediments as deeply as 10–80 mm (July/August 2000 and 2001). At that time, microsensor measurements were performed and exoenzyme activities and organic matter distribution were determined in the sediments.

Microsensor measurements

Microsensors for O_2 (Revsbech, 1989), NH_4^+, and NO_3^- (De Beer *et al.*, 1997) were prepared, calibrated, and operated in a measuring set-up as previously described (Stief & De Beer, 2002). As can be seen in Fig. 1, the burrow lining can be considered as an extension of the sediment surface that reaches into the sediment, whereas the burrow lumen can be considered as an extension of the water column that reaches into the sediment. Therefore, three measuring approaches were chosen (Fig. 1): (i) vertical concentration profiles were recorded across the sediment surface (horizontal distance of sensor tip from any *Ephoron* burrow outlet: 10 mm), (ii) microsensor profiles were measured across the lining of animal burrows that could be observed through the transparent channel wall (vertical distance of sensor tip from sediment surface: 20 mm), and (iii) concentration time series were recorded inside the burrows at fixed positions in the ventilation current created by the abdominal gills of the larva (vertical distances of sensor tip from sediment surface: 10–25 mm, duration: 15–60 min, temporal resolution: 3 s).

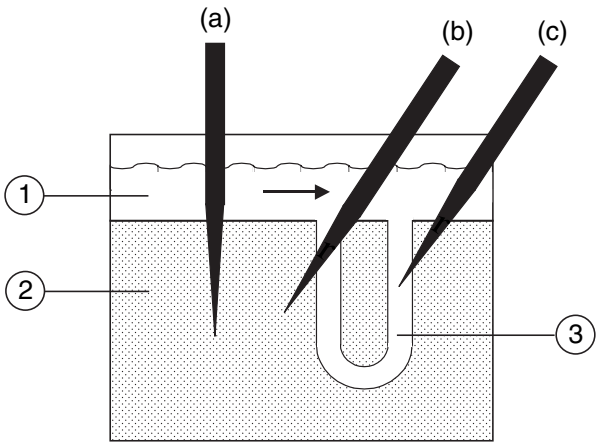

Fig. 1 Microsensor measurements in experimental flow channels (side view). 1: water column, 2: bulk sediment, 3: *Ephoron* burrow. Arrow indicates flow direction. (a) measurement across sediment surface (sensor tip was >10 mm away from any burrow outlet), (b) measurement across burrow lining (sensor tip was 20 mm below sediment surface), (c) measurement in burrow lumen (sensor tip was 10–25 mm below sediment surface).

Local conversion rates of O_2, NH_4^+, and NO_3^- were calculated by applying (i) a one-dimensional diffusion-reaction model to the profiles across the sediment surface (De Beer & Stoodley, 2000) and (ii) the corresponding equation for radial diffusion to the profiles across the burrow lining (Brune, Frenzel & Cypionka, 2000). Diffusion coefficients of O_2, NH_4^+, and NO_3^- at 23 °C in the water phase were taken as 2.12×10^{-5}, 1.81×10^{-5}, and 1.67×10^{-5} cm^2 s^{-1}, respectively (Stief, De Beer & Neumann, 2002). Solute diffusivity in the sediment was determined using a diffusivity probe (Unisense A/S, Denmark, Revsbech, Nielsen & Ramsing, 1998) at several positions in the channel with the water flow switched off. In the 2001 study, the same probe type was also used to detect horizontal pore water movement that may be imposed by the overlying water current (i.e. Brinkman flow). This was carried out by comparing vertical diffusivity profiles with and without water flow in the channel. Horizontal pore water movement was at best detected in the upper 400 μm of the sediment, but the actual velocity could not be determined. Therefore, the layer 0–400 μm had to be omitted from the calculation of solute conversion rates that was based on a diffusion-reaction model rather than an advection model. Because of technical problems, NH_4^+ profiles could not be measured in 2000.

Organic matter distribution

Sediment cores (25 mm diameter) were taken from the flow channels at sites with and without *Ephoron* burrows. Cores that were not supposed to contain burrows were at least 10 mm away from any burrow outlet. The sediment layers 0–2, 10–12, 20–22, and 30–32 mm were obtained by slicing the sediments with a core extruder. Care was taken to remove any *Ephoron* larva from the sediment to be analysed. Slices were dried at 60 °C for 72 h and then combusted at 550 °C for 3 h. Weight loss on combustion was taken as an estimate of the organic matter content of the sediment.

Exoenzyme activity

Potential activities of α-glucosidase (indicative of microbial degradation of labile polysaccharides, e.g. storage compounds), β-glucosidase (→ refractory polysaccharides, e.g. structural polymers), chitobiase (→ chitinous compounds, e.g. skeletal elements of crustacean zooplankton), and aminopeptidase (→ proteins, humic complexes) in the sediment pore water were determined using fluorescently labelled substrate analogues (Hoppe, 1983; Boetius, 1995). 4-methylumbelliferyl-α-D-glucoside, 4-methylumbelliferyl-β-D-glucoside, 4-methylumbelliferyl-N-Acetyl-β-D-glucosamide (Sigma Chemicals), and L-Leucin-4-methyl-7-coumarinylamide-hydrochloride (Fluka) were dissolved in ethylenglycolmonomethylether at concentrations of 20, 20, 6.7, and 20 mmol L^{-1}, respectively, and stored at −20 °C (Boschker & Cappenberg, 1998). Sediment cores (25 mm diameter) were taken and sliced in the same way as for the organic matter analysis. Each sediment slice was divided into four equal parts (0.25 mL each), diluted with 2.15 mL (except for chitobiase assay: 1.95 mL) sterile and particle-free river water, and mixed with 0.10 mL (except for chitobiase assay: 0.30 mL) of substrate analogue. Final substrate concentration in the assay was thus 800 μmol L^{-1}. Incubation took place in the dark and at ambient water temperature (21–23 °C). After 20, 40, and 60 min, 0.20 mL subsamples were taken from each suspension and diluted with 3.80 mL of sterile carbonate buffer (pH 10) that stopped the enzymatic reaction in the subsample (Boschker & Cappenberg, 1998). After centrifugation, the light emission of the liberated fluorescent label in the supernatant was measured in a fluorimeter (Perkin Elmer, LS 50 B). Excitation/emission wavelengths were 364/445 nm and 380/440 nm for 4-methylumbelliferyl (MUF) and 4-methylcoumarinyl-7-amide (MCA), respectively. The linear increase of MUF (or MCA) concentration during the incubation was used to calculate the potential volumetric cleavage rate of the respective substrate. Calibration was done with known concentrations of MUF (or MCA) dissolved and stored in ethylenglycolmonomethylether and readily diluted with carbonate buffer.

Statistics

Samples of organic matter content and exoenzyme activity were checked for Gauss distribution (Kolmogorov–Smirnov test) and homogeneity of variances (Levene test). Data meeting these criteria were used in two-factorial ANOVA statistics with *Ephoron* presence and sediment depth as the fixed factors. As *post hoc* tests were not possible for the 2-step factor *Ephoron* presence, multiple one-sided *t*-tests with α adjustment according to Hommel (1988) were carried out. The application of one-sided *t*-tests was motivated by the well-founded assumption that *Ephoron* larvae increase both the organic matter content and the exoenzyme activities in the immediate neighbourhood of their burrows. All statistical analyses were performed using the software package SPSS 12.0 (SPSS Inc.).

Results

Microsensor measurements

O_2 concentration in the surface sediment, i.e. at distances of >10 mm from any *Ephoron* burrow outlet, decreased sharply in the top few millimeters (Fig. 2a,c). In 2000 and 2001 the sediment was anoxic below a depth of 4.1 ± 0.1 and 2.6 ± 0.1 mm, respectively (means ± S.E., n = 16 and 8). Maximum O_2 consumption rates were located at a depth of 1 mm (Fig. 2a,c). NO_3^- penetration depths were 7.3 ± 0.5 and 5.0 ± 0.2 mm (means ± SE, n = 7 and 9) in 2000 and 2001, respectively (Fig. 2b,d). The vertical shape of the profiles translated into NO_3^- production near the sediment surface and NO_3^- consumption in the layers below. In 2001, NH_4^+ concentration in the water column was as low as 1 μmol L^{-1} and increased significantly below a sediment depth of about 1 mm

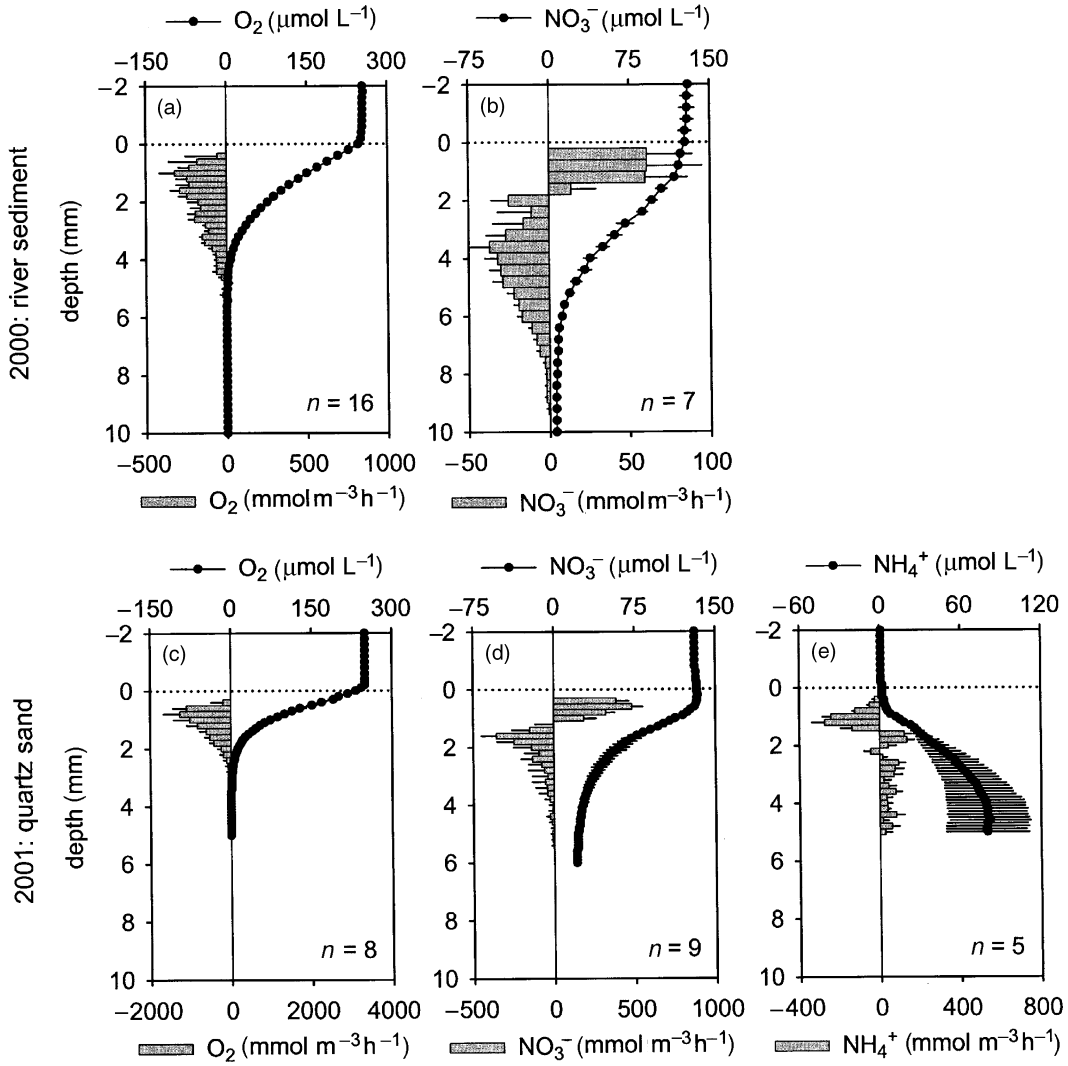

Fig. 2 Microsensor measurements across the sediment surface. Vertical profiles of O_2, NO_3^-, and NH_4^+ concentrations across the sediment surface (dotted line) and volumetric conversion rates calculated thereof (a and b: 2000, river sediment; c, d and e: 2001, quartz sand). Profiles were recorded at least 10 mm from any *Ephoron* burrow outlet. Mean ± SE are shown.

(Fig. 2e). A plateau was reached at 4–5 mm in the sediment with a mean concentration of 80 µmol L^{-1} NH_4^+. The shape of the profile translated into a surface layer of NH_4^+ consumption and a subsurface layer of NH_4^+ production.

In 2000, one NO_3^- and three O_2 concentration profiles across the lining of three different *Ephoron* burrows (50–55 mm deep) could be measured (Fig. 3a,b). O_2 penetrated 2 mm into the sediment surrounding the larval burrows, which indicated O_2 consumption rates in the burrow lining considerably higher than in the surface sediment (compare Figs 2a & 3a). NO_3^- concentration peaked at 1.5 mm from the burrow and then decreased to approximately the overlying water concentration (Fig. 3b). The first 2.5 mm of the burrow lining were thus characterised by NO_3^- production, while in the subsequent 1.5 mm, NO_3^- was consumed. Local rates of NO_3^- production and consumption were 1–2 orders of magnitude higher than near the surface sediment (compare Figs 2b & 3b). In 2001, six NO_3^- and three O_2 profiles in the lining of six different *Ephoron* burrows (35–45 mm deep) could be measured (Fig. 3c–e). O_2 penetrated 1.5 mm into the burrow lining and local O_2 consumption rates were in the order of those measured in the surface sediment (compare Figs 2c and 3c). NO_3^- profiles into the burrow vicinity varied considerably in

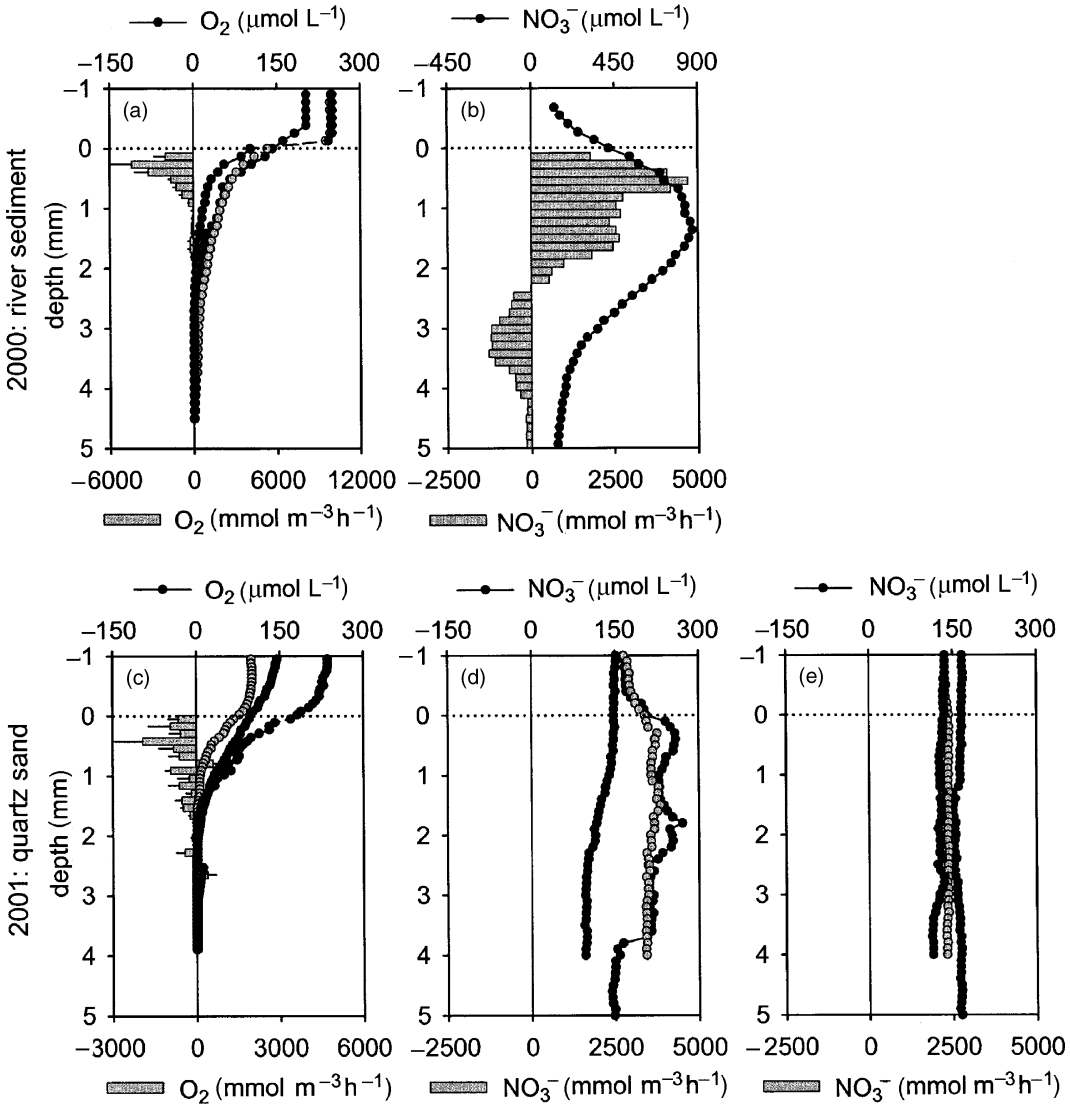

Fig. 3 Microsensor measurements across the burrow lining. O_2 and NO_3^- concentration profiles across the *Ephoron* burrow lining (dotted line) and conversion rates calculated thereof (a and b: 2000, river sediment; c, d and e: 2001, quartz sand). Because of heterogenous profile curvatures, NO_3^- conversion rates could not be calculated in 2001 (d and e). Sensor tip was positioned 20 mm from the sediment surface. Mean ± SE are shown where applicable. Profiles recorded in different burrows have different shades.

shape: three profiles exhibited concave curvatures near the burrow lining (Fig. 3d), whereas the remaining three profiles showed inconclusive curvatures (Fig. 3e). Because of the heterogenous character of these profiles it was not attempted to calculate volumetric conversion rates.

O_2 and NO_3^- concentrations inside the *Ephoron* burrows were remarkably stable and close to those in the water column. Only a few conspicuous deviations from this rule were observed. Fig. 4 shows four representative time series that extended over 15 min each. A total of 15 time series was recorded ($9 \times O_2$ and $6 \times NO_3^-$) in different burrows. Concentration fluctuations were mostly moderate and sudden changes were apparently related to larval locomotion (e.g. Fig. 4a,b and d). In only a few cases larval gill beating caused periodic concentration changes (e.g. Fig. 4c). O_2 concentration in the ventilation current was on mean 32 µmol L^{-1} lower than in the water column, which corresponded to a mean decrease in air saturation of 13% during the passage of the burrow. In four out of six burrows, NO_3^- concentration was lower (7%), while in the remaining two burrows NO_3^- concentration was higher than in the water column

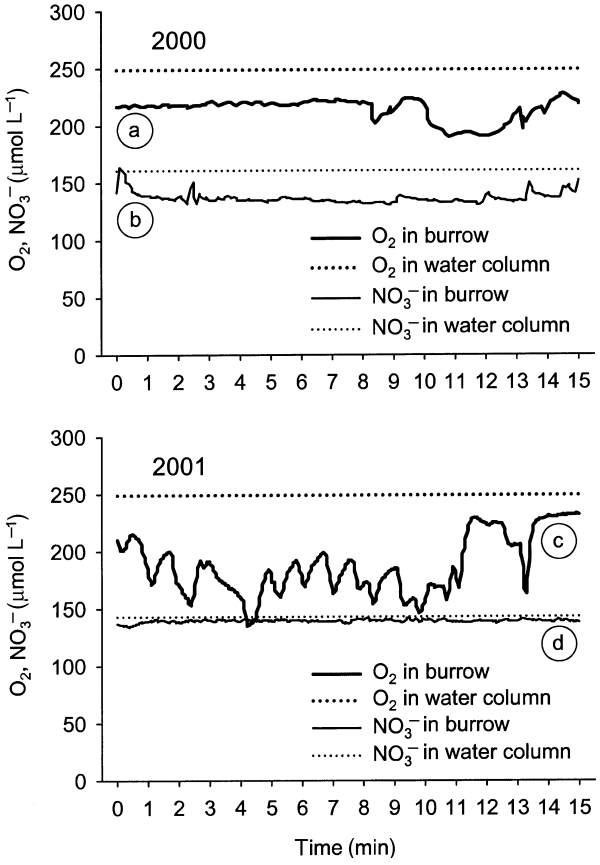

Fig. 4 Microsensor measurements within burrows. Four representative concentration time series within *Ephoron* burrows and the water column are shown. Microsensors were positioned at distances of 25 (a), 10 (b), 15 (c), and 10 mm (d) from the sediment surface. Depths of burrows were 50 (a), 10 (b), 37 (c), and 25 mm (d).

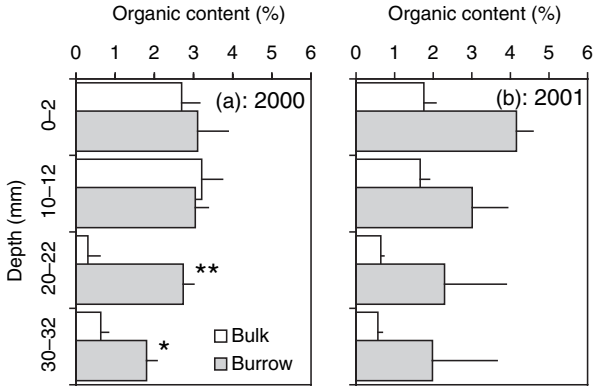

Fig. 5 Organic matter content of sediment. Vertical distribution in sediment samples without ('bulk') or with *Ephoron* burrows ('burrow') in 2000 (a, river sediment) and 2001 (b, quartz sand). Mean (+1 SE) of three replicate measurements are given. Asterisks indicate significantly higher organic contents.

(10%). Noticeably, the latter two burrows were also the by far largest burrows probed with NO_3^- microsensors (i.e. with the deepest point at 70 mm from the sediment surface).

Organic matter distribution

In the absence of *Ephoron* burrows, the fraction of combustible organic matter decreased from approximately 2–3% in the upper sediment layers to <1% in layers deeper than 20 mm (Fig. 5a,b). The presence of *Ephoron* burrows increased the mean organic matter content only in sediment layers deeper than 20 mm in the 2000 study. In the 2001 study, however, the presence of *Ephoron* burrows increased the organic matter content in all sampled sediment layers. Using ANOVA statistics, a significant effect of *Ephoron* presence on the vertical distribution of organic matter was revealed in both years of investigation (2000: $P < 0.01$; 2001: $P < 0.05$). Increases of organic matter were significant in sediment layers deeper than 20 mm (2000) and in the 0–2 mm layer (2001) (multiple one-sided *t*-test with α adjustment). The greatest relative increases occurred in the layers 20–22 and 30–32 mm (i.e. 2.9–9.0-fold increases) compared with the moderate changes in the layers 0–2 and 10–12 mm (i.e. 0.9–2.4-fold changes).

Exoenzyme activities

In the absence of *Ephoron* burrows, sedimentary exoenzyme activities typically declined with depth and were marginal below 30 and 20 mm in the years 2000 and 2001, respectively (Figs 6 & 7). Using ANOVA statistics, the significant effects of *Ephoron* presence on the vertical distribution of exoenzyme activities were revealed for β-glucosidase (2001: $P < 0.05$) and aminopeptidase (2000 & 2001: $P < 0.05$). When ANOVA statistics were restricted to the sediment layers 20–22 and 30–32 mm, the significant effects were more numerous, i.e. α-glucosidase (2000: $P < 0.01$; 2001: $P < 0.05$), β-glucosidase (2001: $P < 0.001$), and aminopeptidase (2000 & 2001: $P < 0.01$). More specifically, the presence of *Ephoron* burrows increased enzyme activities in some of the deep sediment layers (Figs 6 & 7). These increases were significant for all enzymes in layer 30–32 mm (2000) and for β-glucosidase in layer 20–22 mm (2001) (multiple one-sided *t*-test for

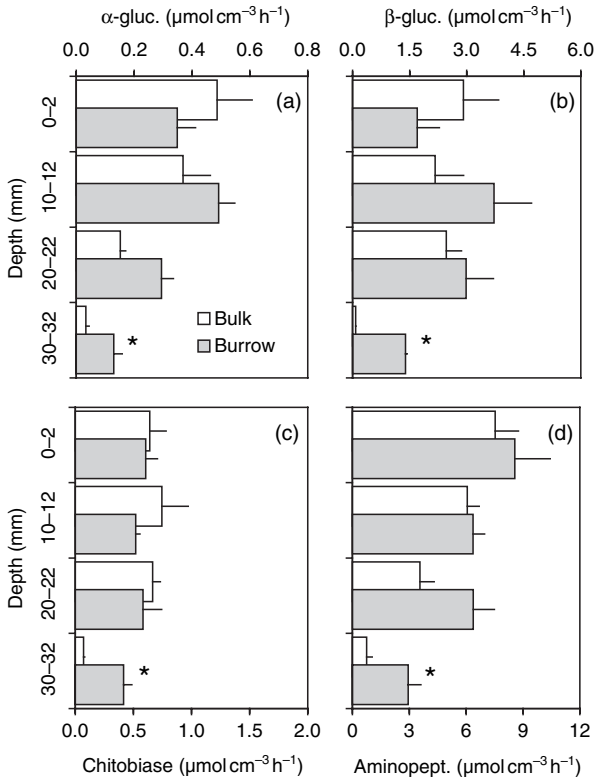

Fig. 6 Exoenzyme activity in sediment pore water. Vertical distribution in sediment samples without ('bulk') or with *Ephoron* burrows ('burrow') in 2000 (river sediment). (a) α-glucosidase; (b) β-glucosidase; (c) chitobiase; (d) aminopeptidase. Means (±1 SE) of three replicate measurements are given. Asterisks indicate significantly higher enzyme activity.

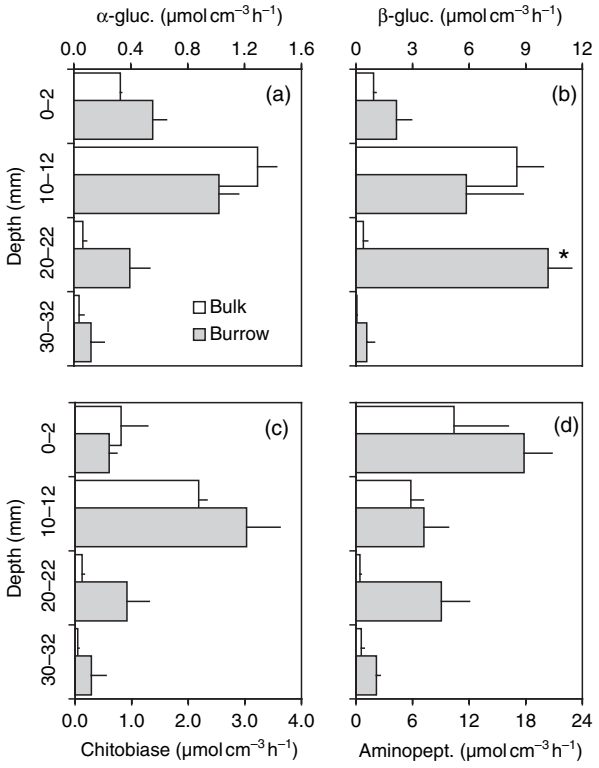

Fig. 7 Exoenzyme activity in sediment pore water. Vertical distribution in sediment samples without ('bulk') or with *Ephoron* burrows ('burrow') in 2001 (quartz sand). Means (±1 SE) of three replicate measurements are given. Asterisks indicate significantly higher enzyme activity.

each enzyme with α adjustment). In 2000, the greatest relative activity increases occurred in the 30–32 mm layer (i.e. 3.9–17.3-fold) compared with the moderate changes in the remaining layers (i.e. 0.6–1.9-fold). In 2001, the greatest relative activity increases occurred in the 20–22 mm layer (i.e. 6.2–25.8-fold) compared with the minor changes in the remaining layers (i.e. 0.7–10.9-fold).

Discussion

Organic matter accumulation

Benthic macrofauna can significantly enhance the sedimentation of fine particles from flowing water. Such biodeposition of seston may be because of macrofaunal filter feeding (Hakenkamp & Palmer, 1999; Vaughn & Hakenkamp, 2001) or the presence of sediment cavities with reduced current velocity (Graf & Rosenberg, 1997). Seston particles entering the *Ephoron* burrow are either filtered out by the larva, trapped in the burrow lining, or pass the burrow uninfluenced. Aside from the directly trapped particles, the filtered particles may eventually accumulate in the burrow environment, as *Ephoron* larvae attach their faeces to the burrow lining for stabilisation (Heinen, 1995). On the other hand, benthic macroinvertebrates can also enhance sediment resuspension rates and thereby reverse the vertical downward flux of particles (Graf & Rosenberg, 1997). Especially the mobile deposit-feeders increase the shear stress at the sediment/water interface and destabilise the sediment surface (Davis, 1993). Even if resuspension had occurred near the outlets of the *Ephoron* burrows, our data suggest that in subsurface sediment layers, *E. virgo* promoted biodeposition rather than bioresuspension of fine and organically rich particles. Suspended particles of the River Rhine have a high organic content, but a relatively low C/N-ratio and contain a great proportion of extracellular polymeric substances (Lartiges *et al.*, 2001). Like in other rivers they are focal

points of microbial colonisation within the water column (Zimmermann-Timm, 2002). Our results suggest that the immobilisation of organic particles and microbial aggregates in biogenic riverbed structures, e.g. sediment burrows, may promote microbial activities in an otherwise organically poor environment.

Oxygen and nitrogen re-distribution

Our microsensor measurements in the lumen of *Ephoron* burrows revealed O_2 and NO_3^- concentrations close to the water column values. This proved that larvae were able to oxygenate their immediate sediment environment by maintaining almost continuous ventilation current through their burrow. A similar bioirrigation pattern was also observed for the burrowing ephemerid *Hexagenia limbata* (Wang, Tessier & Hare, 2001). In contrast, pronounced fluctuations of burrow water O_2 concentrations were found for the marine polychaete *Nereis diversicolor* (Fenchel, 1996) and the freshwater alderfly *Sialis velata* (Wang *et al.*, 2001). As *E. virgo* and *H. limbata* are both filter feeders and sensitive to hypoxia (van der Geest *et al.*, 2002), they have to spend most of their time ventilating the burrows (Heinen, 1995; Wang *et al.*, 2001). Macroinvertebrates with a higher tolerance of O_2 depletion, however, start ventilating not until the burrow water falls short of O_2 (Fenchel, 1996; Wang *et al.*, 2001).

The biogeochemical implication of burrow ventilation is that oxidants (O_2, NO_3^-) are introduced to sediment layers in which otherwise reducing conditions prevail (Aller, 1994; Fenchel, 1996; Kristensen, 2000 & 2001). Our microsensor measurements prove that at least in our experimental setting, oxidants became available in layers ten times deeper than oxidant penetration into the sediment surface. A three-dimensional mosaic of oxic and anoxic sediment patches in deep sediment layers may allow aerobic and anaerobic pathways to proceed concurrently and thereby intensify their linkage. For instance, O_2-dependent processes, such as microbial nitrification, might establish in deep sediment layers that are otherwise not supplied with O_2 from the overlying water, but are closer to sites of elevated NH_4^+ production by sedimentary microbes or by the larvae themselves (Mayer *et al.*, 1995). Similarly, denitrification and NO_3^- ammonification might be stimulated both by the introduction of NO_3^- from the water column or by NO_3^- that is produced in adjacent oxic sediment patches (Kristensen, 2000 & 2001). When spatially and functionally interwoven, nitrification and denitrification/NO_3^- ammonification promote organic matter degradation by oxidising both NH_4^+ (nitrification) and organic compounds using NO_3^- as an electron acceptor (denitrification/NO_3^- ammonification). The nature of microbial NO_3^- conversions in the burrows, as inferred from the time series in the burrow lumen, were inconclusive although: in the two largest burrows NO_3^- concentrations were higher than in the water column, while in small burrows the opposite was true. Net production of NO_3^- in large (i.e. old) burrows may be because of the progressive establishment of nitrification in the burrow lining, which does not yet play a role in small (i.e. young) burrows.

Microprofiles recorded across the burrow lining are a more reliable source of quantitative information on microbial conversion rates. For instance, O_2 penetration into the burrow lining was less deep than into the sediment surface. This can to some degree be attributed to the different geometries of diffusion (radial diffusion versus diffusion across a plane, Wang *et al.*, 2001), but is principally the result of much higher O_2 consumption rates within the burrow lining than at the sediment surface. Similarly, some NO_3^- profiles across the burrow lining allowed us to calculate NO_3^- production and consumption rates several times higher than at the sediment surface. In 2001, however, many NO_3^- profiles were straight, suggesting the absence of NO_3^--consuming bacteria or organic substrates in the quartz sand surrounding the burrows. In summary, the direct comparison of O_2 and NO_3^- conversion rates calculated for the sediment surface and the burrow lining revealed much higher microbial activities inside the burrows, which has been previously suggested by other authors (Kristensen, Jensen & Aller, 1991; Pelegri & Blackburn, 1995; Svensson & Leonardson, 1996). However, future investigations should aim at mapping the microbial conversions along the entire burrow. Planar optodes selective for O_2 (Glud *et al.*, 1996) or NH_4^+ (O.S. Wolfbeis, University of Regensburg, personal communication) could be fixed to the transparent wall of the flow channel to get two-dimensional images of O_2 and NH_4^+ distribution in the burrow environment.

Organic matter degradation

Potential exoenzyme activities are quantitative measures of the available amount of polymeric organic

substrates to which sediment microorganisms respond by releasing enzymes into the pore water (Boschker & Cappenberg, 1998). The response is also qualitative, i.e. the release of a certain exoenzyme is coupled to the presence of its specific substrate. As the activities of some enzymes were significantly higher in the neighbourhood of *Ephoron* burrows, we conclude that this pattern reflects the local accumulation of the respective organic substrates. This finding is ecologically relevant, as the fine-particulate organic matter that is being trapped in the burrows constitutes the dietary basis for heterotrophic microorganisms. Significant differences were restricted to deep sediment strata interspersed with burrows and enriched in particulate organic matter.

Increased activities of hydrolytic enzymes have previously been reported for deep sea sediments inhabited by macrofauna with large burrows (Boetius, 1995). In that study the quick burial of freshly deposited organic matter has been made responsible for enhanced induction of microbial exoenzymes. High exoenzyme activities are also associated with suspended matter in the water column of the River Rhine (Admiraal & Tubbing, 1991). Thus, it seems likely that *Ephoron*-stimulated biodeposition of suspended matter imports both fine-particulate organic matter and exoenzyme-releasing microorganisms into the sediment. Moreover, bioirrigation by the animals continuously supplies O_2 and NO_3^- needed for the respiratory activity of heterotrophic microbes that are involved in the degradation of organic matter.

Validity of laboratory results

Given the chance of mass occurrence of *Ephoron* species in large rivers (Schäffer, 1757; Schöll, 1993; Kureck, 1996; Kureck & Fontes, 1996; Watanabe, Mori & Yoshitaka, 1999), the revealed small-scale bioturbation and bioirrigation effects may have physical, chemical and microbial implications on a larger scale. *E. virgo* may thereby contribute significantly to the connection of pelagic and benthic food webs in rivers and promote the microbial degradation of deposited seston particles. Despite this conceptual assessment of the *Ephoron* burrow, care must be taken before transferring our results to *in situ* conditions: (i) Our measurements were made on late-instar larvae and thus did not cover the full life span of *Ephoron* larvae. We assume, however, that late-instar larvae cause much more substantial alterations of the sediment microbiology than early-instar larvae, because the burrow lining of the latter may not be stable enough to support excessive microbial colonisation. (ii) Hydrodynamics near the sediment surface may have differed considerably between our experimental channels and the natural riverbed. For instance, flow velocity and pressure were probably lower than under *in situ* conditions, which may have increased particle deposition and decreased advective solute exchange between the sediment and water. Application of our laboratory results to field conditions may be even more difficult, because *Ephoron* burrows have often been found underneath stones (by A. Kureck, unpublished observations), which has unpredictable consequences for the hydrodynamics. It would thus be desirable either to perform experiments similar to ours directly in the riverbed or to mimick a wider range of environmental conditions as closely as possible in the laboratory.

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