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Relative influence of bioturbation and predation on organic matter processing in river sediments: a microcosm experiment

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SUMMARY

1. Our objective was to measure the effects of bioturbation and predation on the physical characteristics and biogeochemical processes in river sediments.
2. We investigated the impacts of tubificid worms tested separately and together with an omnivore (*Gammarus pulex*), which does feed on tubificids, on sediment distribution, water flux, sediment organic carbon, biofilm biomass and microbial activities, and the concentrations of dissolved oxygen, dissolved organic carbon, PO_4^{3-}, NO_3^-, NO_2^- and NH_4^+ in slow filtration sand–gravel columns. We hypothesised that gammarids, which exploit the top 2–3 cm of the sediment, would modify the impact of worms at the sediment surface.
3. In experiments both with and without gammarids, bioturbation by the tubificids modified both the distribution of surface particles in the sediment column and water flux. In addition, microbial aerobic (oxygen consumption) and anaerobic (denitrification and fermentative decomposition of organic matter) processes in the sediment were stimulated in the presence of tubificid worms. However, *G. pulex* did not affect either the density or bioturbation activity of the tubificid worms.
4. Bioturbation by the benthos can be a major process in river habitats, contributing to the retention of organic matter in sediment dynamics. The presence of at least one predator had no effect on bioturbation in sediments. In such systems, physical heterogeneity may be sufficient for tubificids to escape from generalist predators, though more specialised ones might have more effect.

Keywords: ecosystem engineers, functional diversity, interstitial invertebrates, microbial activity, river sediments

Introduction

In lotic freshwater systems, the role of benthic invertebrates has mainly been attributed to their feeding behaviour (functional feeding groups; Cummins, 1974; Cummins & Klug, 1979; Jonsson &

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Malmqvist, 2003). In recent studies, the importance of 'ecological engineering' by fauna at the water–sediment interface of rivers has been investigated (Pringle *et al.*, 1993; Flecker, 1996; Zanetell & Peckarsky, 1996; Wotton *et al.*, 1998; Statzner *et al.*, 2000), and shows that invertebrates can also affect ecosystems through bioturbation (displacement, burrowing, structure building).

While the influence of bioturbation by the benthos on the physical and chemical characteristics of the sediment, and on the microbiota, has been widely examined in soft marine and lacustrine sediments (Aller, 1988; van de Bund, Goedkoop & Johnson, 1994; Hansen & Kristensen, 1997; Stief & de Beer, 2002), less is known of the coarse sediments and greater interstitial flows of rivers. To study the effect of invertebrates in such hyporheic habitats, that play a key role in the metabolism of rivers (Boulton *et al.*, 1998), Mermillod-Blondin *et al.* (2000a) developed an experimental approach for controlling the high physical heterogeneity of the system. Mermillod-Blondin *et al.* (2002) demonstrated that three detritivorous invertebrates often associated with river sediments (asellids, chironomids and tubificid worms) produced various effects on microbial activity via bioturbation. For example, the galleries of tubificid worms and their egestion of faecal pellets stimulated denitrification and organic matter mineralisation, whereas chironomids reduced organic matter processing in the upper 5 cm of the sediment.

Several studies have shown that predation can exert a major impact on aquatic communities and, indirectly, on ecosystem processes (e.g. Menge *et al.*, 1994; Dahl & Greenberg, 1998; Hulot *et al.*, 2000). Although the significance of biotic interactions is well known in several terrestrial and aquatic habitats, the effects of the interactions between invertebrates on ecosystem processes has not been studied at the sediment–water interface of rivers. Our purpose here was to measure experimentally bioturbation by tubificid worms and to assess the impact on this bioturbation of an omnivorous amphipod (*Gammarus pulex*, Linné, 1758), an active predator on several invertebrate species in laboratory experiments (Dick, Montgomery & Elwood, 1999; Kelly, Dick & Montgomery, 2002a,b).

Predators often have their main impact by modifying the behaviour of their prey rather than by direct consumption (Douglas, Forrester & Cooper, 1994; Rosenberg & Selander, 2000; Usio, 2000). Therefore, we hypothesised that *G. pulex*, which use only the top layer of the sediment, would cause tubificids to use different habitats, change their activity, or alter their foraging behaviour. Previous studies showed that tubificid worms rework the surface sediment and egest faecal pellets (Mermillod-Blondin *et al.*, 2001, 2002). Therefore, the amphipod might limit the occurrence and activity of tubificids at the sediment surface, thus indirectly reducing microbial activity and organic matter degradation. To measure the impact of different combinations of species, we measured: (i) the physical impact of invertebrates in the system (sediment reworking, hydrodynamics), (ii) the impact of invertebrates on biogeochemical processes (dissolved oxygen, NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, and dissolved and particulate organic carbon) and (iii) microbial variables (microbial protein content, respiratory activity, hydrolytic activity).

Methods

Experimental design

We used gravel–sand filtration columns (40 cm in height, 10 cm in diameter) modified from those used by Danielopol & Niederreiter (1990); Torreiter, Pitaksintorn-Watanamahart & Danielopol (1994) and Griebler (1996). Each column ($n = 12$) was filled to a height of 30 cm with ashed (550 °C) fine gravel (4–5 mm) and with sand (60–630 μm) that had previously been incubated for 4 days with bacteria and cellulose powder (0.5% of the sediment volume) as a source of particulate organic matter. This produced a final concentration of organic carbon equivalent to that naturally measured in secondary channels of the River Rhône (Mermillod-Blondin *et al.*, 2000b). We alternated gravel (560 g) and incubated sand (220 g) in columns to obtain a 30-cm deep heterogeneous sediment layer with interstitial pores. This system was characterised by a high variability in solute transport as a result of the simultaneous rapid circulation through macropores and slow circulation in the sandy matrix (Mermillod-Blondin *et al.*, 2000a). About 9 cm of water was left above the sediment surface. At different depths, ports in the column permitted the collection of water and fine sediments with a syringe. Further details of the columns were given by Mermillod-Blondin *et al.* (2000a).

The experiment was performed at a constant temperature of 15 ± 0.5 °C with a 12 : 12 light : dark cycle (applied to the overlying water). The sedimentary part of the column was kept in the dark to suppress photo-autotrophic growth. Artificial water (96 mg L^{-1} of $NaHCO_3$, 60 mg L^{-1} of $CaSO_4 + 2H_2O$, 60 mg L^{-1} of $MgSO_4 + 7H_2O$, and 4 mg L^{-1} of KCl), aerated to maintain high oxygen concentrations, was continuously fed from the top to the bottom of the columns with a peristaltic pump. Before entering the columns, the water was enriched with a nutrient solution (35 mg L^{-1} KNO_3, 0.232 mg L^{-1} H_3BO_3, 0.174 mg L^{-1} $ZnSO_4 + 7H_2O$, 0.116 mg L^{-1} $Fe(NH_4)_2(SO_4)_2 + 6H_2O$, 0.114 mg L^{-1} $CoCl_2$, 0.022 mg L^{-1} $(NH_4)_6Mo_7O_{24} + 4H_2O$, 0.008 mg L^{-1} $CuSO_4 + 5H_2O$, and 0.008 mg L^{-1} of $MnSO_4 + 4H_2O$) with another peristaltic pump. Potassium acetate was used as the predominant source of assimilable organic carbon and was added with a peristaltic pump to supply 2 mg L^{-1} of carbon to columns throughout the experiment. The resulting infiltration rate was 2.0 ± 0.1 mL min^{-1} (volumetric flux density or darcian velocity: 1.53 cm h^{-1}), which generated an interstitial water velocity of 5.9 cm h^{-1}. The first measurements were made three days after the beginning of water flow to allow the initial hydraulic properties to stabilise.

Three replicate columns of each of the following treatments were used: (i) control without fauna, (ii) four gammarids (*G. pulex*), (iii) 100 tubificid worms (*Tubifex tubifex*, Müller, 1774), and (iv) four gammarids and 100 tubificid worms. These densities were equivalent to 520 and 13 000 individuals m^{-2} for gammarids and tubificid worms, respectively, similar to mean densities observed in natural gravel–sand sediments of braided channels of the River Rhône (Fruget, 1989; Martinet, 1993). To acclimatise them to the experimental conditions (granulometry and food), animals were maintained in the laboratory for more than 30 days before being placed in the microcosms.

Before the experiment, the ability of *G. pulex* to feed on *T. tubifex* was tested in the laboratory. Kelly *et al.* (2002a,b) showed that *G. pulex* can be an active predator in the laboratory. Preliminary trials in our laboratory showed that indeed *Gammarus* was a voracious predator of *T. tubifex* and was capable of reducing the number of its prey and/or changing their activity.

The initial measurements in the columns allowed us to measure the variability in the conditions among the four experimental treatments before the introduction of the invertebrates. After this control period, tubificids were placed in the overlying water of the columns. *Gammarus pulex* were then introduced after all tubificid worms had burrowed into the sediment.

Water flux using bromide as tracer was measured 1 day before (−1) the introduction of invertebrates and 20 days after their introduction (+20). Sediment reworking was estimated on day 21. Dissolved oxygen and the concentration of dissolved organic carbon were measured at five depths (5 cm above the sediment surface and 1, 5, 10 and 25 cm below the sediment surface) on days 0, 4, 8, 12, 16 and 20. On the same days, NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-} were measured at three depths (5 cm above the sediment surface and 5 and 25 cm below the sediment surface). Bacterial parameters (content of microbial protein, hydrolytic activity and respiratory activity) were measured at the sediment surface on day 0 and in six layers in the sediment (0–2, 4–6, 8–10, 13–15, 18–20 and 25–27 cm) at the end of the experiment. These sampling depths were chosen according to Mermillod-Blondin *et al.* (2000a) in order to analyse both aerobic and anaerobic processes in columns.

Methods of analysis

Water flux. Bromide (Br^-) movement was used to trace the pattern of infiltration through the sediment (Taniguchi & Sharma, 1990) on days −1 and 20. On both dates, a solution of $CaBr_2$ was added to 200 mL of the overlying water to obtain a Br^- concentration of 5 mg L^{-1} in all columns. This solution was applied as a pulse (over 1.7 h) and tracer outflow was measured in samples collected at the column outlets over 18 h (each half hour for the first 8 h and every hour thereafter). The bromide content was evaluated with a capillary ion analyser (Waters Quanta 4000, Milford, MA, U.S.A.). The effluent tracer concentration was analysed in order to measure mass balance and mean residence time of the tracer in each column (Jury & Roth, 1990). The mean residence time obtained was compared with the theoretical residence time of the tracer calculated from the experimental conditions (dimension of the columns: 40 cm in height, 10 cm in diameter; sediment height: 31 cm; flow rate: 2 mL min^{-1}; porosity: 26%; time of tracer application: 1.7 h). According to a previous study (Mermillod-Blondin *et al.*, 2003a), a two-region (mobile–immobile water) model with a first-order exchange of solutes

(MIM model, Gaudet *et al*., 1977; Schoen, Gaudet & Elrick, 1999) was used to predict column water flux. This model simulates heterogeneity in the fluxes by partitioning the water into a mobile region (solute transport by advection) and an immobile region (solute transport by diffusion). The MIM equations are (Gaudet *et al*., 1977):

$$\frac{\partial C_m}{\partial t} + \frac{\theta_{im}}{\theta_m}\frac{\partial C_{im}}{\partial t} = D_m \frac{\partial^2 C_m}{\partial z^2} - \frac{q}{\theta_m}\frac{\partial C_m}{\partial z}$$

and

$$\frac{\partial C_{im}}{\partial t} = \frac{\alpha}{\theta_{im}}(C_m - C_{im})$$

Initial conditions:

$$C_m(z,0) = C_{im}(z,0) = 0$$

Lower boundary conditions:

$$C_m(\infty,t) = C_{im}(\infty,t) = 0$$

Third-type upper boundary conditions:

$$-D_m\theta_m\frac{\partial C_m}{\partial x} + qC_m\big|_{z=0} = qC_0 \quad (0 < t \le t_0)$$
$$= 0 \qquad (t_0 < t)$$

where C_m is the concentration in the mobile region (g cm^{-3}), C_{im} the concentration in the immobile region (g cm^{-3}), C_0 the input (pulse) concentration (g cm^{-3}), t the time (h), D_m the dispersion coefficient (cm^2 h^{-1}), z the depth (cm), q the darcian flux (cm h^{-1}), θ_m the volumetric water content in the mobile region (cm^3 cm^{-3}), θ_{im} the volumetric water content in the immobile region (cm^3 cm^{-3}), $\theta = \theta_m + \theta_{im}$ and α the solute exchange rate (h^{-1}) between the two regions.

We used the analytical solution for a pulsed input of solute, with a third type (flux-type) boundary condition, found in Toride, Feike & Van Genuchten (1993) (p. 2170 in Table 2). There are three parameters to fit: D_m, α, and θ_m or θ_{im}, as they are related. Choice for the 'best' fits was carried out by eye, as automatic procedures failed to take into account the tail of the breakthrough curves (Schoen *et al*., 1999).

Sediment reworking. Sediment reworking in the columns was quantified by the luminophore tracer technique (Gerino, 1990; Gerino, Stora & Durbec, 1994). On day 0 of each experiment, 1 g of luminophores (natural sediment particles of 100–350 μm in size, dyed with yellow fluorescent paint) was deposited at the sediment surface of the four columns after introduction of the invertebrates. After 21 days, the water layer was removed and columns were opened to sample sediment and luminophores. The top 2 cm were sampled in slices 0.5 cm in thickness while the lower 18 cm were sampled in 1 cm slices. Each layer was homogenised and a 1 g sub-sample was dried at 50 °C for luminophore counting. The number of luminophores was estimated with a UV light microscope and converted into g tracer g^{-1} dry sediment. Sediment transport at the interface as a result of the different invertebrate assemblages was estimated by comparing the vertical profiles of luminophores obtained in the columns.

Chemical analyses. Oxygen concentration was measured with the technique developed by Mermillod-Blondin *et al*. (2000a). At each depth, 30 mL of water were sampled in a bottle full of nitrogen. This water was then pumped at a rate of 20 mL min^{-1} and circulated through an Orbisphere-type respirometer chamber provided with a Clarke-type electrode connected to an Orbisphere oxymeter (model 3600; Orbisphere Laboratories, Neuchâtel, Switzerland). This procedure allowed the measurement of dissolved oxygen concentrations in the samples without contact with atmospheric oxygen. NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-} concentrations were measured using colorimetric HACH methods (HACH Company, Loveland, CO, U.S.A.) after filtration through GF/F filters.

The N-NO_3^- was measured from 25 mL water samples using the Nitraver 5 reagent (HACH). Cadmium metal contained in the reagent reduced the nitrate present in the sample to nitrite. The nitrite ion reacted in an acidic medium with sulphanilic acid to form an intermediate diazonium salt, which coupled to gentisic acid to form an amber-coloured product, directly proportional to the amount of nitrate present in the sample. The concentration of nitrate was then estimated from the absorbance of the sample measured at 400 nm using a DR2000 spectrophotometer (HACH). With this method, we obtained a standard deviation of ±0.10 mg L^{-1} N-NO_3^-.

The N-NO_2^- was measured from 25 mL water samples using the Nitriver 3 reagent (HACH). Nitrite present in the sample reacted with sulphanilic acid to form an intermediate diazonium salt. This coupled with chromotropic acid to produce a coloured complex directly proportional to the amount of nitrite present in the sample. The concentration of nitrite was

then estimated from the absorbance of the sample measured at 507 nm using a DR2000 spectrophotometer. The standard deviation associated with this method was ± 0.005 mg L^{-1} $N\text{-}NO_2^-$.

The $N\text{-}NH_4^+$ was measured from 25 mL water samples using the Nessler method. We added 0.5 mL of rochelle salt and 0.5 mL of Nessler reagent (HACH) to the sample. Rochelle salt aided the colour formation in the reaction of Nessler reagent with ammonium ions. A yellow colour was formed proportional to the ammonia concentration. The concentration of ammonium was then estimated from the absorbance of the sample measured at 425 nm using a DR2000 spectrophotometer. This method had a standard deviation of ±0.02 mg L^{-1} $N\text{-}NH_4^+$.

The PO_4^{3-} was directly measured from 25 mL water samples using the Phosver 3 reagent (HACH). Orthophosphate reacted with molybdate in an acid medium to produce a phosphomolybdate complex. Ascorbic acid then reduced the complex, giving an intense molybdenum blue colour proportional to the concentration of orthophosphate in the sample. The concentration of orthophosphate was then estimated from the absorbance of the sample measured at 890 nm using a DR2000 spectrophotometer. We obtained a standard deviation of ±0.002 mg L^{-1} PO_4^{3-} with this method.

Dissolved organic carbon was measured with a Dohrman DC80 total carbon analyser (Dohrman Division, Xertex Corporation, Santa Clara, CA, U.S.A.) based on UV-promoted potassium persulphate oxidation (precision ± 1%) after removing inorganic carbon with orthophosphoric acid (1 μL mL^{-1}) and carbon dioxide stripping under 10 min of oxygen flow. When a DOC increase was noted in the columns, additional analyses with a capillary ion analyser were performed to detect the possible occurrence of volatile fatty acids in the interstitial water outlet of the columns. The sediment organic carbon was measured in the dry fine sediment collected on day 0 (before packing the columns) and at the end of the experiment (day 21) from three layers (0–10, 10–20 and 20–40 cm below the sediment surface). From this sediment, 0.3 g of fine sediment (<125 μm) were added to 50 mL of deionised water and 0.5 mL of orthophosphoric acid and then homogenised for 5 min using a vortex mixer. An oxygen flow was applied to samples for 15 min to purge inorganic carbon. The sample was then homogenised and a sub-sample of 40 μL was injected into a furnace (800 °C). The carbon dioxide produced by burning the sub-sample was determined by infrared detection. The quantity of carbon was expressed as mg g^{-1} of dry sediment (‰).

Microbial analyses. Total protein was directly measured from the wet sediment (1 g) according to the micro-Lowry method modified by Peterson (1977) using the Sigma Protein Assay Kit (P 5656 Sigma Diagnostics, St Louis, MO, U.S.A.).

Hydrolytic activity of biofilms was estimated using the fluorescein diacetate (FDA) hydrolysis method (Jørgensen, Eriksen & Jensen, 1992), where 1 g of wet sediment was placed in 4.5 mL of a pH 7.6 phosphate buffer with 0.15 mL of 4.8 mM FDA solution. The incubation was maintained until the green colour of fluorescein had appeared (1–3 h) and so that the FDA concentration was never limiting for enzymatic processes. The reaction was stopped by freezing the sample after the addition of 5 mL of an $HgCl_2$ solution (400 mg L^{-1}). The fluorescein concentration was estimated from the absorbance of the filtered supernatant (0.45 μm; Millipore, Billerica, MA, U.S.A.) measured at 490 nm.

Electron transport system (ETS) activity was measured with 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) using a protocol modified from Houri Davignon, Relexans & Etcheher (1989). One gram of wet sediment was incubated in a 0.02% INT solution (final solution) for 2 h at 15 °C and then filtered on a nylon membrane (0.22 μm, MSI). Controls were prepared by adding formaldehyde (2% final) to the INT solution. Extraction of INT-formazan was made in vials containing 5 mL of methanol. Each vial was sonicated for 30 s at power 4 and 80% active cycle using a Vibracell sonicator (Type 72401; 300 W, 20 KHz; Bioblock, Illkirch, France) fitted with a 3 mm microtip to increase the solvent extraction yield (Maurines-Carboneill *et al.*, 1998). The INT-formazan extract was measured by a spectrophotometer adjusted to 480 nm against blank control. The quantity of INT-formazan was computed by using the molar extinction coefficient of 18 000 M^{-1} cm^{-1} at 480 nm.

Survival of animals and tubificid distribution at the end of the experiment. At the end of the experiment, living worms were counted in six layers (0–2, 2–6, 6–10, 10–15, 15–20, and 20–30 cm) in each column with tubificids. The number of worms collected was used to

estimate the survival and to provide information about the vertical distribution of animals in columns. The number of living gammarids was counted every day.

Data treatment

For chemical variables measured on day 0 of the experiment, we tested treatment and depth effect by a two-way ANOVA with treatment (control, gammarids only, tubificids only and gammarids plus tubificids) and depth as the main effects (Statistica 5^{TM}, Statsoft, Tulsa, OK, U.S.A.). For microbial parameters measured at the sediment surface on day 0, the treatment effect was tested with a one-way ANOVA. These tests allowed us to measure the variability in conditions among the columns for each treatment before the introduction of the invertebrates.

For chemical variables measured at different times, we tested treatment and depth effects by repeated measurements (days 4, 8, 12, 16 and 20) with a two-way ANOVA with treatment (control, gammarids, tubificids and gammarids plus tubificids) and depth as the main effects. If significant differences were detected among treatments, Scheffé *post hoc* tests were performed to determine which treatment differed. For variables measured at the end of the experiment, the effects of treatments and depths were compared using two-way ANOVA (taxon × depth effects for microbial variables and coefficients of sediment reworking). Data on the number of living worms found at the end of the experiments were expressed as percentages. We compared the percentage of living tubificids between treatments with a one-way ANOVA. The number of worms found at each of the six sampling layers was also expressed as a percentage. We tested the treatment and depth effects on worm distribution using a two-way ANOVA with treatment and depth as the main effects. When necessary, data were log or square-root transformed to fit the assumption of homoscedasticity; variables expressed as percentages (microbial variables) were arcsine transformed.

Results

Patterns in columns before the introduction of invertebrates

Before the introduction of animals, the results of the tracer experiment showed that similar bromide evolutions were observed in the four experimental treatments (example in Fig. 1a) although the mass balance varied from 0.9 to 1.17 (Table 1). The ratio of tracer applied (C/C_0, where C is the bromide concentration of the interstitial water drawn from the columns and C_0 is the bromide concentration of the applied solute pulse) tended to show a similar distribution with time among columns (with values ranging from 0 to 0.35, Fig. 1a,b). Peak concentration was observed within 4.0 to 5.0 h after tracer application. After a rapid transport of tracer during the first 4 h the concentration of bromide decreased slowly, becoming undetectable in the interstitial water 15 h after introduction. The MIM model gave a good approximation of measured values (Table 1; Fig. 1a) with the following parameters: 50% of mobile region (θ_m), 0.1 h^{-1} as the solute exchange rate (α) between mobile and immobile water, and a dispersion coefficient (D_m) of 8 cm^2 h^{-1}.

On day 0, oxygen and DOC concentrations decreased with depth in all columns (Fig. 2a,b; two-way ANOVA, $P < 0.001$, depth effect for these two solutes) without a significant difference among treatments (two-way ANOVA, $P > 0.2$, treatment effect). The concentrations of NH_4^+, NO_2^-, NO_3^- and PO_4^{3-} did not vary significantly among treatments before the introduction of animals (two-way ANOVA, $P > 0.4$ for treatment effect). In comparison with oxygen and DOC, the concentrations of these four solutes varied only slightly among depths (Figs 3a,b & 4a,b). However, a significant decrease in NO_3^- and a significant increase in NO_2^- were measured with depth (two-way ANOVA, $P < 0.05$, depth effect for NO_3^- and NO_2). Like the physico-chemical parameters, the microbial measurements at the sediment surface of the twelve columns on day 0 showed similar values in the four treatments (one-way ANOVA, $P > 0.8$ for the three microbial parameters).

Effects of treatments

Particle redistribution. In the control columns, most luminophores (93 ± 4%) were found at the sediment surface at the end of the experiment (Fig. 5) whereas more than 20% of the luminophores had migrated down into the sediment in animal treatments (mean values: 21% with gammarids, 26% with tubificids, 33% with gammarids plus tubificids). The percentages of luminophores remaining at the sediment

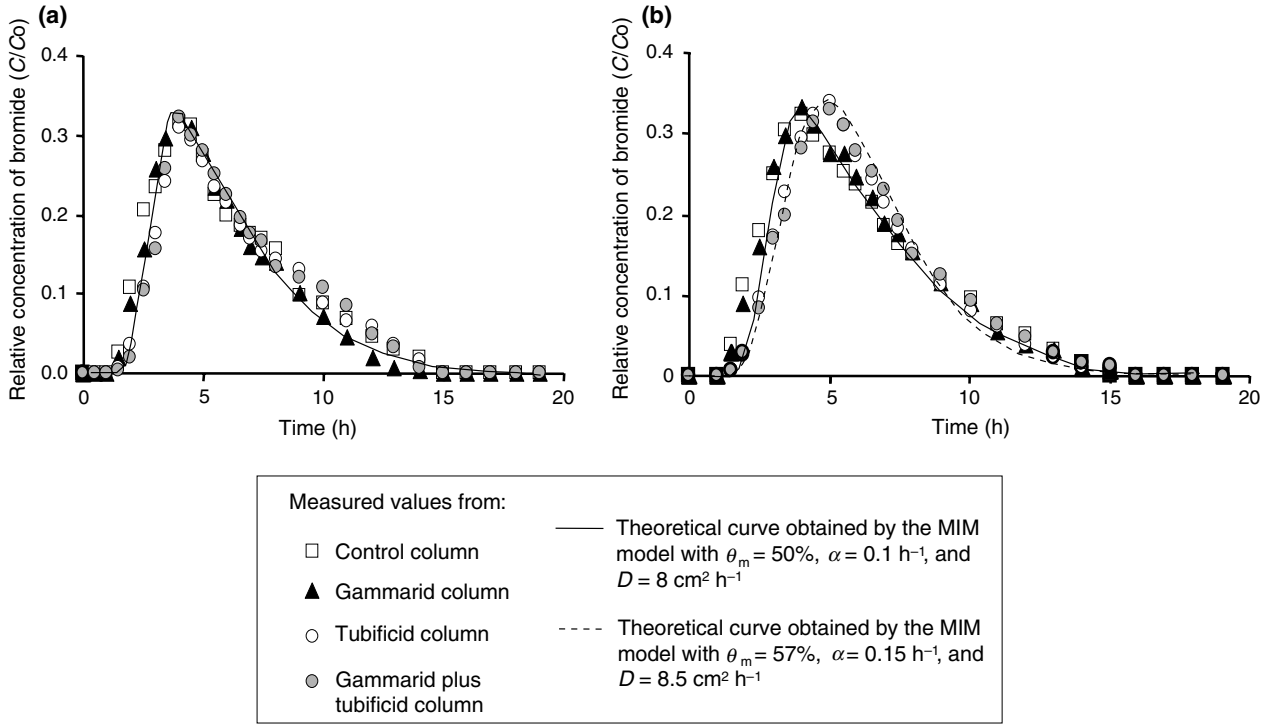

Fig. 1 Changes in relative concentration (C/C_0) of Br^- with time at 31 cm in depth (outlet) (a) in four columns (one column per treatment) before introduction of the invertebrates, (b) in the same four columns (one column per treatment) on day 20. Theoretical curves obtained by the MIM model are represented on graphs.

Table 1 Mass balances (quantity of tracer measured at column outlets/quantity of injected tracer) and retardation factors (mean time of tracer transport measured/mean time of tracer transport expected with the experimental conditions) measured during the tracer experiments as well as parameters used in the MIM model to fit with measured values obtained during tracer experiments. θ_m is the volumetric water content in the mobile region, α is the solute exchange rate between mobile and immobile region and D_m is the water dispersion coefficient

Date	Column	Mass balance	Retardation factor	θ_m	α	D_m
Day-1	C1	1.1409	1.0025	0.5	0.1	8
	C2	1.089	0.9727	0.5	0.1	8
	C3	1.0009	0.9389	0.5	0.1	8
	G1	1.0906	0.9143	0.5	0.1	8
	G2	1.0456	0.9233	0.5	0.1	8
	G3	0.9688	0.9149	0.5	0.1	8
	T1	1.1724	0.999	0.5	0.1	8
	T2	1.0961	1.095	0.5	0.1	8
	T3	0.8998	0.9375	0.5	0.1	8
	(G + T)1	1.1568	1.0184	0.5	0.1	8
	(G + T)2	1.0974	1.0322	0.5	0.1	8
	(G + T)3	0.9496	1.0021	0.5	0.1	8
Day 20	C1	0.9756	1.0108	0.5	0.1	8
	C2	1.0766	0.9404	0.5	0.1	8
	C3	1.1172	0.9978	0.5	0.1	8
	G1	1.0454	0.9669	0.5	0.1	8
	G2	0.9946	0.9198	0.5	0.1	8
	G3	1.1868	1.0498	0.5	0.1	8
	T1	1.0353	0.9584	0.58	0.15	8
	T2	1.0273	1.0607	0.55	0.14	8
	T3	1.0599	1.0623	0.55	0.13	9
	(G + T)1	0.9917	1.0456	0.6	0.16	8
	(G + T)2	1.0587	1.0604	0.55	0.15	8
	(G + T)3	1.115	1.1022	0.55	0.13	9

C1, C2, C3: control columns; G1, G2, G3: gammarid columns; T1, T2, T3: tubificid columns; (G + T)1, (G + T)2, (G + T)3: gammarid plus tubificid columns.

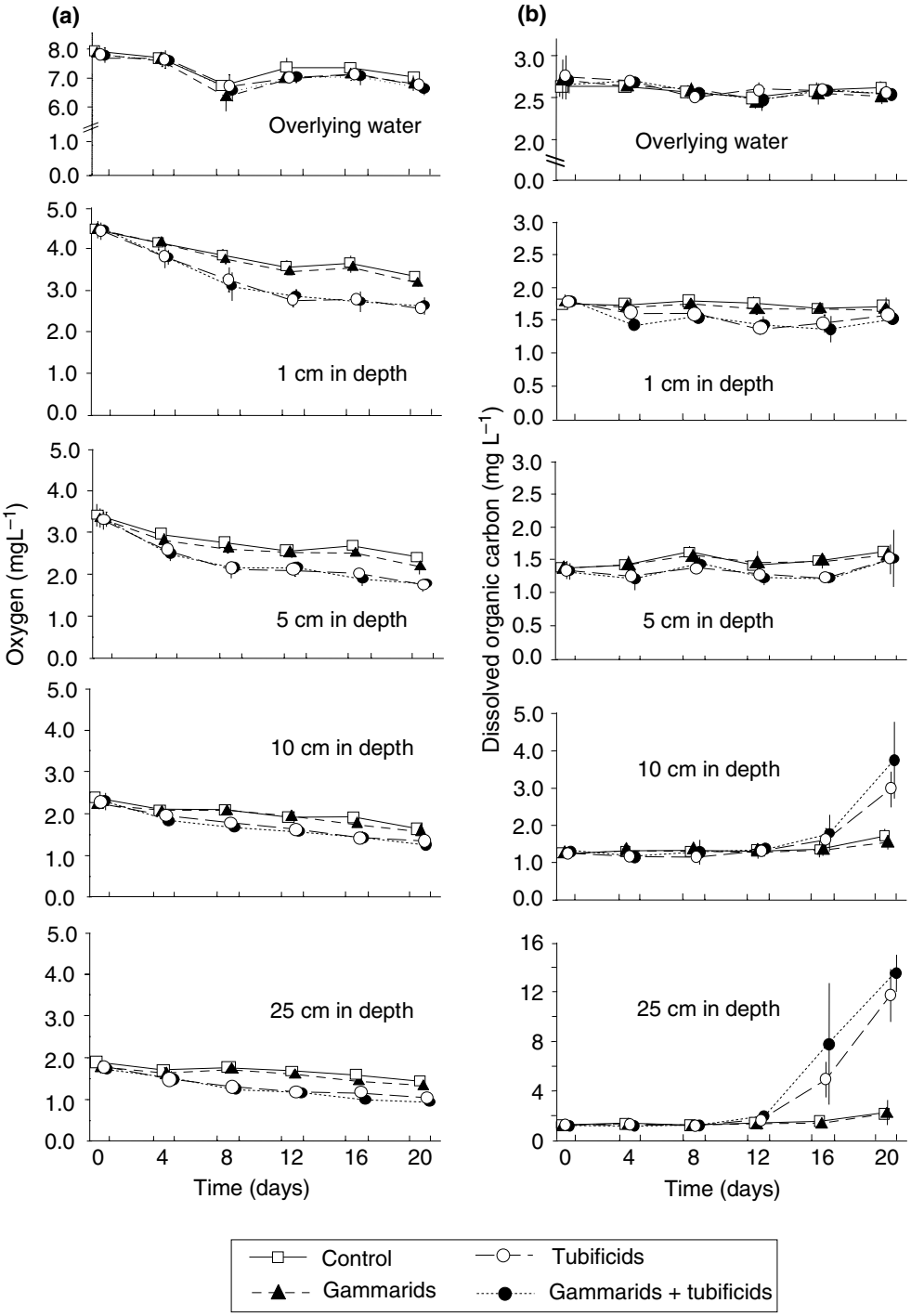

Fig. 2 Concentration of (a) oxygen and (b) DOC at 5 cm above and 1, 5, 10 and 25 cm below the water–sediment interface for the four treatments (means ± confidence intervals, n = three columns per treatment) during the experiments.

surface were significantly different among treatments (one-way ANOVA, $P < 0.01$). The two treatments with tubificids decreased the quantity of particle tracer at the sediment surface significantly compared with the control (Scheffé *post hoc* tests, $P < 0.05$) whereas the gammarid treatment did not produce a significantly higher transport of tracer than the control (Scheffé *post hoc* test, $P > 0.05$).

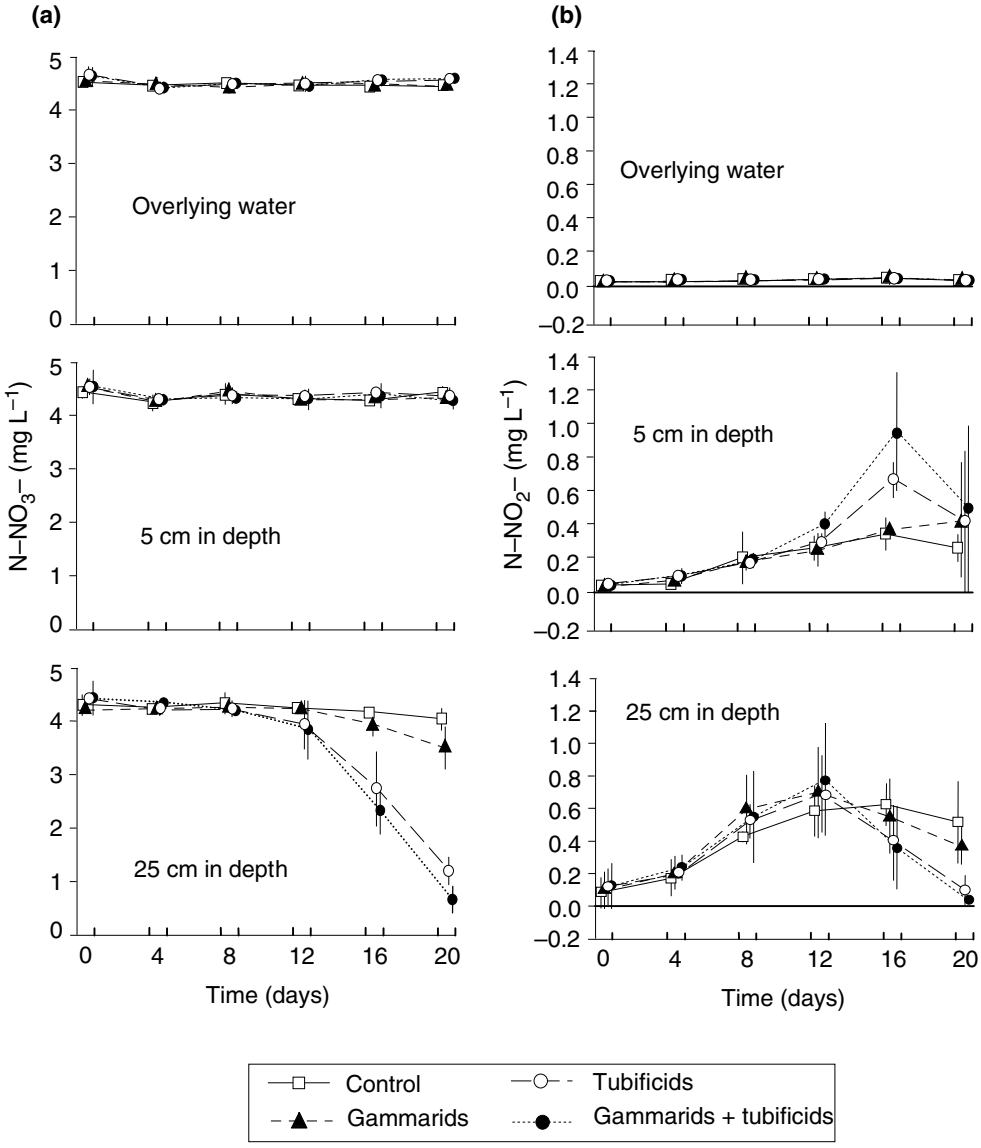

Fig. 3 Concentration of (a) NO_3^- and (b) NO_2^- at 5 cm above and 5 and 25 cm below the water–sediment interface for the four treatments (means ± confidence intervals, n = three columns per treatment) during the experiments.

Water fluxes. As obtained by the conservative-solute transport model, measurements made on day −1 and day 20 in the control and gammarid columns were not significantly different (Fig. 1b; Table 1, $P > 0.5$; comparisons of θ_m and α between day −1 and day 21 for the control and gammarid treatments, t-tests). In contrast, the columns with tubificids and gammarids plus tubificids exhibited different infiltration patterns on day 20 than on day −1 (Fig. 1b). The partition between mobile and immobile region was significantly modified with these two treatments (Table 1). A shift in the peak of bromide concentration was observed in presence of tubificids and gammarids plus tubificids (Fig. 1b). The model gave a better fit between theoretical and measured values after the change of the proportion of mobile water and the solute exchange rate between mobile and immobile region (Table 1; as exemplified on Fig. 1b). The results of this hydrodynamic modelling showed that the presence of tubificids and gammarids plus tubificids significantly increased the proportion of mobile region (θ_m) and, as a consequence, the solute exchange rate (α) between mobile and immobile region ($P < 0.05$; comparisons of θ_m and α between day −1

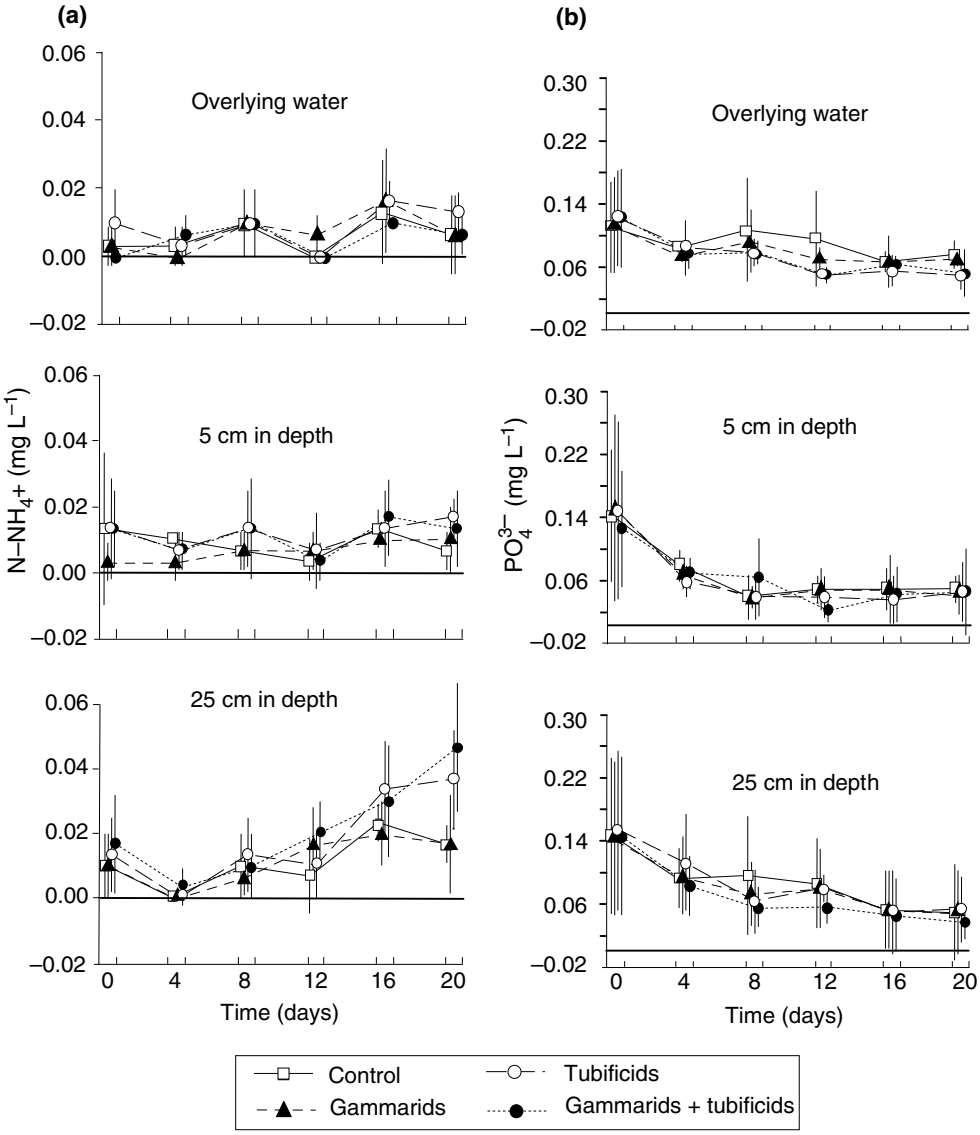

Fig. 4 Concentration of (a) NH_4^+ and (b) PO_4^{3-} at 5 cm above and 5 and 25 cm below the water–sediment interface for the four treatments (means ± confidence intervals, n = three columns per treatment) during the experiments.

and day 20 for the tubificid and gammarid plus tubificid treatments, *t*-tests).

Physico-chemical and microbial parameters. Oxygen concentration always exhibited a sharp decrease with depth in all columns (Fig. 2a; two-way ANOVA, $P < 0.001$). For instance, on day 20 in the control columns, average values decreased from 7.1 mg L^{-1} in the surface water to 3.3 mg L^{-1} at 1 cm in depth, 2.4 mg L^{-1} at 5 cm in depth, 1.6 mg L^{-1} at 10 cm in depth, and about 1.4 mg L^{-1} at 25 cm in depth. From day 4 to 20, dissolved oxygen concentration was significantly different among treatments (two-way ANOVA, $P < 0.001$). The three invertebrate treatments significantly reduced the oxygen concentrations compared with the control (Scheffé *post hoc* tests, $P < 0.005$, comparison with oxygen concentrations in the control). However, the gammarid treatment produced a lower oxygen decrease than the two treatments with tubificids (Fig. 2a; Scheffé *post hoc* tests, $P < 0.001$), which created a similar effect on oxygen gradients in columns (Scheffé *post hoc* test, $P > 0.42$).

During the experiment, DOC concentration showed different patterns depending on day and depth (Fig. 2b; two-way ANOVA, $P < 0.001$). From day 4 to

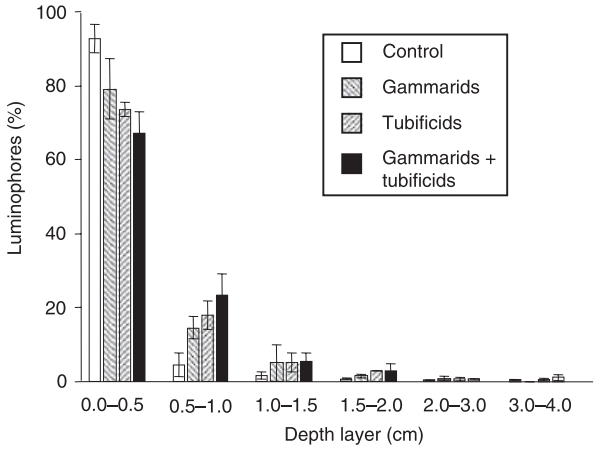

Fig. 5 Luminophore depth profiles for the four treatments (means ± confidence intervals, n = three columns per treatment).

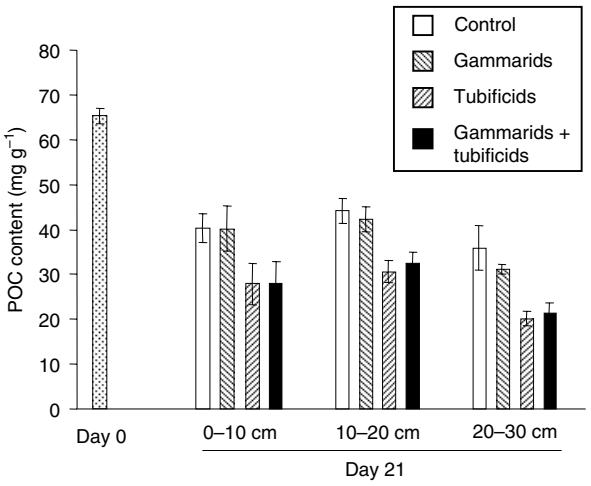

Fig. 6 Proportion of particulate organic carbon per gram of dry sediment on day 0 (in fine sediment introduced in columns) and in the three sediment layers at the end of the experiment in the four treatments (means ± confidence intervals, n = three columns per treatment).

8, DOC concentration decreased with depth in the sediment, as we observed with the oxygen concentration profiles. From day 12 to 20, DOC concentration increased sharply at 10 and 25 cm in depth in columns with tubificids and gammarids plus tubificids, whereas smaller increases were observed in other treatments (Fig. 2b). Significant differences in DOC concentration were measured among the four treatments (two-way ANOVA, $P < 0.001$). The DOC concentration in the two treatments with tubificids was significantly different than that measured in the gammarid and control treatments (Scheffé *post hoc* test, $P < 0.001$). However, the effects of invertebrates depended on depth (T × D effect, two-way ANOVA, $P < 0.001$): the tubificid and gammarid plus tubificid treatments led to a lower DOC concentration than in the control in the first layers of the sediment (1 and 5 cm in depth) whereas DOC concentration increased deeper from day 12 to 20 (Fig. 2b). Additional analyses with a capillary ion analyser detected four kinds of volatile fatty acids (acetic, propionic, malic and formic acids) in water sampled from column outlets on days when an increase in DOC concentration was measured at 25 cm in depth. Concentrations were high enough to be responsible for the DOC production recorded.

The sediment organic carbon measured in three layers of the sediment showed that more than 35% of the particulate organic matter in all columns was lost from the sediment after 20 days (Fig. 6). This POC decrease depended on the treatments (two-way ANOVA, $P < 0.001$). In the presence of gammarids, the amount of particulate organic carbon measured in the sediment at the end of the experiment was similar to the control (Scheffé *post hoc* test, $P > 0.49$). In contrast, the two treatments with tubificids produced a similar loss of particulate organic carbon that was higher than those measured in the control at all sediment layers (Scheffé *post hoc* test, $P < 0.001$).

From day 4 to 8, N-NO_3^- concentration (Fig. 3a) was relatively stable at all depths and in all columns, with values ranging from 4.1 to 4.5 mg L^{-1}. From day 12 to 20, a strong decrease in NO_3^- concentration was observed at 25 cm depth in treatments with tubificids and gammarids plus tubificids whereas no such decrease was measured in the control and the gammarid columns (two-way ANOVA, $P < 0.001$, significant differences between treatments). Scheffé *post hoc* tests showed that the two treatments with tubificids produced changes in NO_3^- concentrations ($P < 0.001$) whereas the gammarid treatment did not have a significant effect on this parameter ($P > 0.52$, comparison between gammarid and control treatments).

In all columns, NO_2^- concentration (Fig. 3b) varied significantly with depth and time during the experiment (two-way ANOVA, $P < 0.001$). The concentration increased with depth during the first 12 days in all columns (Fig. 3b). On day 12, a NO_2^- peak was observed at 25 cm depth in the tubificid and gammarid plus tubificid treatments. Another peak was observed for the same treatments at 5 cm depth on day 16. Despite the observation of such peaks in

the two treatments with tubificids only, no significant differences were measured among treatments (two-way ANOVA, $P > 0.6$). Throughout the experiment, NH_4^+ concentration was low and ranged from 0.00 to 0.06 mg L^{-1} (Fig. 4a). Ammonium concentration varied slightly with depth and date without any clear link to the presence of invertebrates. Similarly, no pattern with invertebrate activities was observed for PO_4^{3-} concentrations during the experiments (Fig. 4b; two-way ANOVA, $P > 0.6$).

The three microbial parameters varied significantly with depth and treatment (Fig. 7a–c, two-way ANOVA, $P < 0.001$). Protein content was highest in the shallowest and deepest layers of the sediment, whereas hydrolytic and respiratory activities were the highest in the upper layer of the sediment. For the three microbial parameters, the gammarid treatment had values similar to the control (Scheffé *post hoc* tests, $P > 0.75$). In contrast, the two treatments with tubificids (tubificid and gammarid plus tubificid treatments) had a significantly higher protein content and microbial activity in comparison with the control (Fig. 7a–c, Scheffé *post hoc* tests, $P < 0.05$). Moreover, there was no significant difference between microbial characteristics measured in microcosms with tubificids and those measured with gammarids plus tubificids (Scheffé *post hoc* tests, $P > 0.05$).

Estimated survival of animals and distribution of tubificids at the end of the experiments. The percentage of living tubificids varied from 91 to 97% and was unrelated to the presence of *G. pulex* (Student's *t*-test, $P > 0.85$). The percentages of worms found in each sediment layer showed that the number of tubificids decreased with depth (Fig. 8, two-way ANOVA, $P < 0.01$). Most living tubificids (>75%) were observed in the first 10 cm of sediment. No difference in vertical distribution of worms was observed between columns with or without gammarids (two-way ANOVA, $P > 0.95$). No mortality of gammarids was observed during the experiment.

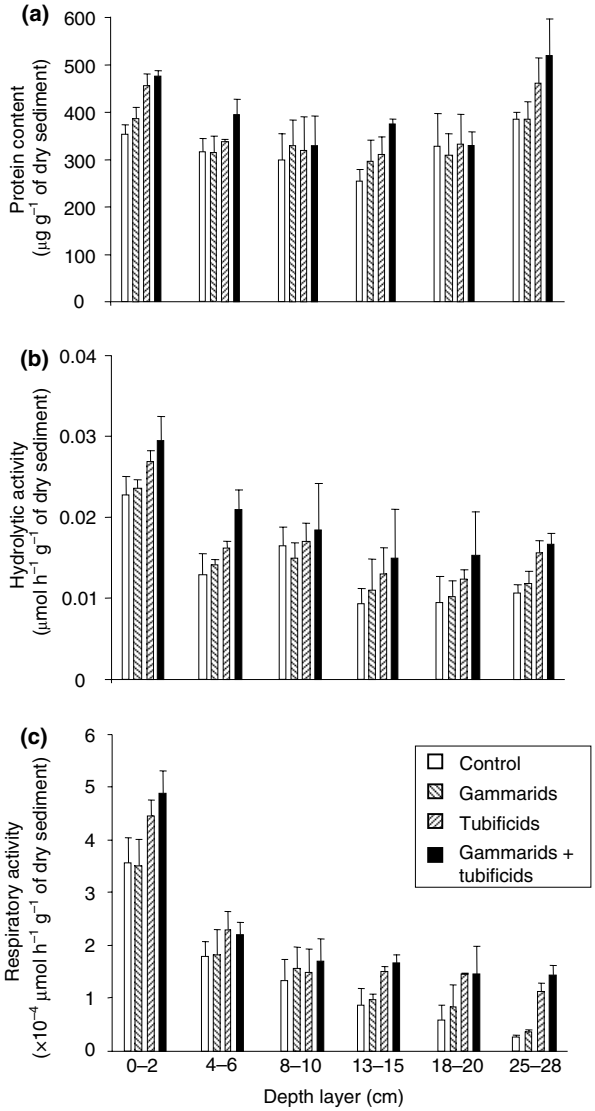

Fig. 7 Depth profiles of (a) protein content, (b) hydrolytic activity and (c) respiratory activity at six sediment layers for the four treatments (means ± confidence intervals, n = three columns per treatment) on day 20.

Discussion

Assessment of the variability among columns before the addition of invertebrates

The lack of significant differences in hydrodynamic, chemical and microbial parameters among the four treatments before the addition of invertebrates indicates that the heterogeneity of the sediment (gravel and sand) did not cause a difference in functioning among columns. The bromide experiment clearly demonstrated the low variability in hydrodynamics among columns. However, the tracer experiment showed a wide range of solute transport velocities in each column. For each column, the tracer transport was modelled by a 50% mobile water phase (where solute transport took place by advection) and a 50% immobile water phase (where solute transport took

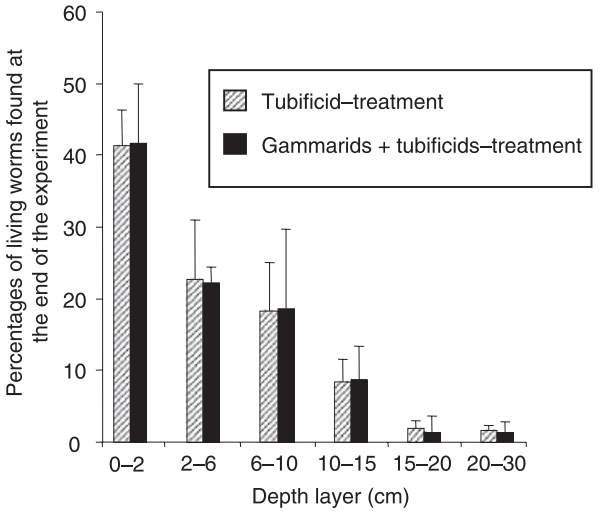

Fig. 8 Vertical distribution of the tubificid worms at the end of the experiment for the tubificid and the gammarid plus tubificid treatments.

place by diffusion). As observed in previous studies (Mermillod-Blondin *et al.*, 2000a, 2003a), the high physical and chemical (solution and solid phase) heterogeneity of the porous medium controlled the local availability of nutrients, leading to a heterogeneous distribution of microbial activity (Bott & Kaplan, 1985; Murphy *et al.*, 1997; Thullner *et al.*, 2002). The experimental system included aerobic and anaerobic micro-environments and exhibited a heterogeneity similar to that observed in natural hyporheic zones (Triska, Duff & Avanzino, 1993; Storey, Fulthorpe & Williams, 1999).

Effect of the invertebrate treatments on the physical habitat

The luminophore results indicated that the occurrence of tubificids (in either the presence or the absence of gammarids) clearly modified sediment transport in the porous media. Tubificids are known to ingest particles within the sediment and egest faecal pellets at the surface (Fisher *et al.*, 1980). They can also produce galleries in a wide range of sediments (Rogaar, 1980). The activity of worms (gallery building, faecal pellet formation) led to a higher penetration of luminophores into the sediment in comparison with control and gammarid only treatments. These results agree with a previous study (Mermillod-Blondin *et al.*, 2001) showing that tubificids modified the physical habitat and increased the penetration of surface particles into the sediment column (infiltration through galleries and macropores).

The modification of the sediment structure was accompanied by significant differences in the hydrodynamics in microcosms with worms. The bromide experiments showed that the creation of structures by tubificids modified the partitioning of mobile–immobile regions of water and increased the solute exchange rate between mobile and immobile region in micro-environments of the sediment columns. The use of columns with 31 cm depth of sediment in the present study allowed us to observe such changes in water partitioning whereas, in a former study (Mermillod-Blondin *et al.*, 2003a), we did not measure such an impact with an assemblage of three invertebrates (tubificid worms, asellids and chironomids) using a greater sediment depth (41 cm sediment layer). The collection of worms at the end of the experiment showed that tubificids predominantly used the upper 10 cm of the columns. Because of this vertical distribution, we presume that the use of a 41 cm sediment layer did not enable us in our previous experiments to measure any impact of invertebrates on water partitioning by collecting samples at the outlet of columns: the impact of invertebrates may have been masked by the water transport of the tracer from the bottom of the zone bioturbated by tubificids (approximately 10 cm) to the column outlets. Thus, the present study using shorter columns gave us a more precise description of the effect of tubificids on the hydrodynamics in river sediments characterised by advective fluxes of water and a wide range of particle sizes.

Effect of the invertebrate treatments on the microbial processes

As discussed in previous studies (Mermillod-Blondin *et al.*, 2000a, 2001, 2003a), chemical parameters reflected the microbial processes in columns: (i) the decrease of oxygen concentration with depth in columns was because of microbial respiration, (ii) the decrease of NO_3^- concentration without strong production of NO_2^- and/or NH_4^+ resulted from denitrification processes, and (iii) the appearance of volatile fatty acids (DOC) in the columns was because of an anaerobic microbial process (degradation of particulate organic matter in anoxic micro-environments). The lower oxygen concentration in the sediment and the lower DOC concentration at 1 cm in

depth with tubificids indicated a stimulation of aerobic microbial processes by worms. This interpretation is supported by the microbial measurements, which indicated a stimulation of hydrolytic and respiratory activities and an increase in the protein content with tubificids. Because of the water flow from the top to the bottom of columns, the reduction of oxygen concentration in the upper layer of sediment with tubificids led to a reduction of oxygen supply in deeper layers. As a consequence, anaerobic microbial activities were stimulated in the deepest layers of the sediment in the presence of tubificids. For instance, the decrease in NO_3^- concentration and the increase of DOC at 25 cm in depth were more than 10 times higher in the presence of *T. tubifex*.

The higher anaerobic consumption of particulate organic matter (leading to the production of volatile acids) in columns with tubificids resulted in a significantly higher loss of sediment organic carbon in the presence of worms. An approximately 30% greater decrease in particulate organic matter was observed with worms in comparison with the control at the three sampling layers. Therefore, the activities of tubificids (with or without gammarids) enhanced organic matter and nutrient processing by microorganisms in the sediments. In contrast, organic matter processing was not affected by gammarids. In our study system, characterised by coarse sediment and no leaf litter, omnivorous gammarids probably fed on biofilms that developed on sediment surfaces and on cellulose powder. However, the feeding activity of this invertebrate, which did not significantly affect the physical habitat (sediment reworking and hydrodynamics), did not affect microbial activities in the top 2 cm of the sediment (the layer in which gammarids were observed during the experiment).

Effect of ecosystem engineering on microbial processes

The stimulatory effect of invertebrates on microbial activity was certainly linked to ecosystem engineering by animals. In this system, the water flux that transports oxygen, NO_3^-, and DOC determined microbial activity and biomass in the porous medium (see Murphy *et al*., 1997; Thullner *et al*., 2002). Therefore, the impact of tubificids on hydrodynamics probably modified microbial activity by affecting the physical and chemical conditions in the sediment. The present study demonstrated that a higher solute exchange rate of water between mobile and immobile region occurred in the presence of tubificids, indicating that bioturbation activities increased the water exchanges between aerobic and anaerobic micro-zones of the columns. In such conditions, a higher supply of oxygen and nutrients occurred in anaerobic zones and created fluctuations in redox conditions. Aller (1994) demonstrated that redox oscillations stimulated the mineralisation rate of organic matter in anoxic marine sediments. He also demonstrated that particle reworking and burrow ventilation by benthic fauna can promote the re-mineralisation of organic matter by rapid switching from anaerobic to aerobic conditions in the sediment. In our microcosms, the improved transport of water from aerobic to anaerobic zones because of bioturbation certainly had the same consequence as in marine sediment. Tubificid worms enhanced nutrient availability in all microenvironments of the columns, stimulating both aerobic and anaerobic microbial activities.

Animals could also modify microbial activity by feeding on bacteria and thus keeping them in an active physiological state (Yingst & Rhoads, 1980). Nevertheless, Mermillod-Blondin *et al.* (2002) demonstrated that three detritivorous invertebrates (feeding on organic matter and associated bacteria), that have different bioturbation activities (biodiffusion of fine sediment, production of dense galleries, and production of U-shaped tubes), affected the microbial processes differently in the same sediments. These previous results demonstrated that microbial activity was more affected by the physical effects of invertebrates on structure and hydrodynamics of porous media than by direct feeding activities.

The present study not only supports previous findings obtained with an invertebrate assemblage made up of tubificid worms, asellids and chironomid larvae (Mermillod-Blondin *et al*., 2003a) but quantifies more precisely the role of tubificids as ecosystem engineers (Jones, Lawton & Shachak, 1994) in river sediments. Tubificids indirectly modulate the availability of resource flows to other species (microbes) by causing physical state changes in abiotic materials (modification of water fluxes). The impact of ecosystem engineers on microbial activity has often been demonstrated in muddy and sandy sediments in both marine (Aller, 1988; Pelegri, Nielsen & Blackburn, 1994; Hansen & Kristensen, 1997) and lacustrine (van de Bund *et al*., 1994; Svensson & Leonardson, 1996;

Stief & de Beer, 2002) habitats. In comparison with lake and marine systems, river sediments are characterised by coarse sediments and advective water fluxes that largely influence microbial processes (Boulton *et al*., 1998). Despite the use of an experimental system that is simpler than the natural environment, the present study demonstrated that ecosystem engineers such as tubificid worms can significantly alter the hydrological characteristics of such systems.

Relative impacts of engineering and predation pressure on ecosystem processes

The presence of gammarids in assemblages with tubificids did not alter either ecosystem functioning (oxygen and DOC consumptions, microbial activity) or the distribution of tubificids in the top 5 cm of the sediment. The lack of differences in tubificid density in columns with or without gammarids does not indicate that gammarids did not feed on worms in the experimental system. Tubificids often live in sediment with their posterior part protruding into the overlying water. As observed with other predators (reviewed by Giani, 1984), gammarids could have just consumed the posterior parts of several worms. As tubificid worms are known to regenerate their body (Wisniewski, 1978; Bouguenec & Giani, 1989), this loss of the posterior part may not have induced death or a significant decrease in tubificid density. In such predation conditions, we could imagine a deeper distribution of tubificids to escape gammarid activity, which was limited to the top 2 cm of the sediment, and/or a modification in worm behaviour as observed in the marine worm *Nereis virens* (Sars, 1835) (Miron *et al*., 1991). However, the fact that surface sediment reworking by worms was not significantly affected by the gammarids, which may act as surface predators in our experimental system, does not support this hypothesis. Furthermore, in columns with tubificid worms, we observed worm faecal pellets at the sediment surface both with and without *G. pulex*. All results demonstrate a low impact of the gammarids on worm activity at the water–sediment interface. The impact of the omnivore was negligible on system processes in comparison with the effect of the tubificid worms. We suppose that the composition of the system studied, with sandy–gravely zones and pore spaces of different sizes, are sufficiently heterogeneous for tubificid worms to escape from surface predators without strong behavioural modifications.

In surface sediments, organic matter mineralisation was explained mainly by the trophic structure of the benthic communities (Cummins, 1974; Cummins & Klug, 1979). In deeper sediments corresponding to the system studied here, several authors (Triska *et al*., 1989; Murphy *et al*., 1997; Claret, Marmonier & Bravard, 1998; Storey *et al*., 1999) demonstrated the importance of microbial processes on nutrient cycling and organic matter processing. This study is one of the first to demonstrate that non-trophic interactions (ecosystem engineering) significantly influenced the microbial processes occurring in such sediments. In a previous study, Mermillod-Blondin, Creuzé des Châtelliers & Gerino (2003b) demonstrated that the interaction between two tubificid worms (*Limnodrilus* and *Tubifex*) altered the sediment reworking of surface sediment. The present study showed that ecosystem engineering by a population of tubificid worms was not affected by an omnivorous species foraging at the water–sediment interface. Thus, there is no evidence for a substantial effect of trophic interactions such as predation on the functioning of river sediments (organic matter processing, nutrient cycling) whereas it has been previously shown in a similar system (Mermillod-Blondin *et al*., 2003b) that interactions within the same trophic group (tubificid worms) can affect system functioning. Furthermore, we also suggest that predation pressure in a natural environment is comparable with that observed in the present study because most hyporheic invertebrates function as browsers and deposit-feeders (Danielopol, 1989; Boulton, 2000) and true macro-invertebrate predators are scarce. In these conditions, interactions for space among bioturbators may have more influence on ecosystem functioning than predation. Further investigations with different kinds of predators [cyprinid fishes, the flatworm *Polycelis nigra* (Müller, 1774), or the amphipod *Dikerogammarus villosus* (Sowinsky, 1894)] are needed, however, to test the importance of non-trophic versus trophic interactions on the functioning of river sediments.

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References

Aller R.C. (1988) Benthic fauna and biogeochemical processes in marine sediments: the role of burrow structures. In: *Nitrogen Cycling in Coastal Marine Environments* (Eds T.H. Blackburn & J. Sørensen), pp. 301–338. John Wiley & Sons Ltd, New York.

Aller R.C. (1994) Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation. *Chemical Geology*, **114**, 331–345.

Bott T.L. & Kaplan L.A. (1985) Bacterial biomass, metabolic state, and activity in stream sediments: relation to environmental variables and multiple assay comparisons. *Applied and Environmental Microbiology*, **50**, 508–522.

Bouguenec V.G. & Giani N. (1989) Les oligochètes aquatiques en tant que proies des invertébrés et des vertébrés: une revue. *Acta Oecologica – Oecologia Applicata*, **10**, 177–196.

Boulton A.J. (2000) The functional role of the hyporheos. *Verhandlungen der Internationale Vereinigung für theoretische und angewandte Limnologie*, **27**, 51–63.

Boulton A.J., Findlay S., Marmonier P., Stanley E.H. & Valett H.M. (1998) The functional significance of the hyporheic zone in streams and rivers. *Annual Review of Ecology and Systematics*, **29**, 59–81.

van de Bund W.J., Goedkoop W. & Johnson R.K. (1994) Effects of deposit-feeder activity on bacterial production and abundance in profundal lake sediment. *Journal of the North American Benthological Society*, **13**, 532–539.

Claret C., Marmonier P. & Bravard J.P. (1998) Seasonal dynamics of nutrient and biofilm in interstitial habitats in two contrasting riffles in a regulated large river. *Aquatic Sciences*, **60**, 33–55.

Cummins K.W. (1974) Structure and function of stream ecosystems. *BioScience*, **24**, 631–641.

Cummins K.W. & Klug M.J. (1979) Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics*, **10**, 147–172.

Dahl J.G. & Greenberg L.A. (1998) Effects of fish predation and habitat type on stream benthic communities. *Hydrobiologia*, **361**, 67–76.

Danielopol D.L. (1989) Groundwater fauna associated with riverine aquifers. *Journal of the North American Benthological Society*, **8**, 18–35.

Danielopol D.L. & Niederreiter R. (1990) The measurement of dissolved oxygen in lacustrine sediments. Laboratory set-up and techniques. *Bulletin de l'Institut Géologique du Bassin d'Aquitaine (Bordeaux)*, **47–48**, 287–296.

Dick J.T.A., Montgomery W.I. & Elwood R.W. (1999) Intraguild predation may explain an amphipod replacement: evidence from laboratory populations. *Journal of Zoology*, **249**, 463–468.

Douglas P.L., Forrester G.E. & Cooper S.D. (1994) Effects of trout on the diel periodicity of drifting in baetid mayflies. *Oecologia*, **98**, 48–56.

Fisher J.B., Lick W.J., McCall P.L. & Robbins J.A. (1980) Vertical mixing of lake sediments by tubificid oligochaetes. *Journal of Geophysical Research*, **85**, 3997–4006.

Flecker A.S. (1996) Ecosystem engineering by a dominant detritivore in a diverse tropical stream. *Ecology*, **77**, 1845–1854.

Fruget J.-F. (1989) L'aménagement du bas-Rhône. Evolution du fleuve et influence sur les peuplements de macro-invertébrés benthiques. PhD thesis, University Lyon-I, France.

Gaudet J.-P., Jegat H., Vachaud G. & Wierenga P.J. (1977) Solute transfer with exchange between mobile and stagnant water, through unsaturated sand. *Soil Science Society of America Journal*, **41**, 665–671.

Gerino M. (1990) The effects of bioturbation on particle redistribution in Mediterranean coastal sediment – preliminary results. *Hydrobiologia*, **207**, 251–258.

Gerino M., Stora G. & Durbec J.-P. (1994) Quantitative estimation of biodiffusive and bioadvective sediment mixing: in situ experimental approach. *Oceanologica Acta*, **17**, 547–554.

Giani N. (1984) Contribution à l'étude de la faune d'eau douce et plus particulièrement des Oligochètes. II- Les Oligochètes aquatiques: taxonomie, répartition et écologie. PhD thesis, Université Paul Sabatier, Toulouse, France.

Griebler C. (1996) Some applications for the DMSO-reduction method as a new tool to determine the microbial activity in water-saturated sediments. *Archiv für Hydrobiologie Supplement*, **113**, 405–410.

Hansen K. & Kristensen E. (1997) Impact of macrofaunal recolonization on benthic metabolism and nutrient fluxes in a shallow marine sediment previously overgrown with macroalgal mats. *Estuarine Coastal and Shelf Science*, **45**, 613–628.

Houri Davignon C., Relexans J.C. & Etcheher H. (1989) Measurements of actual electron transport system (ETS) activity in marine sediments by incubation with INT. *Environmental Technology*, **10**, 91–100.

Hulot F.D., Lacroix G., Lescher-Moutoué F. & Loreau M. (2000) Functional diversity governs ecosystem response to nutrient enrichment. *Nature*, **405**, 340–344.

Jørgensen P.E., Eriksen T. & Jensen B.K. (1992) Estimation of viable biomass in wastewater and activated sludge by determination of ATP, oxygen utilisation rate and FDA hydrolysis. *Water Research*, **26**, 1495–1501.

Jones C.G., Lawton J.H. & Shachak M. (1994) Organisms as ecosystem engineers. *Oikos*, **69**, 373–386.

Jonsson M. & Malmqvist B. (2003) Mechanisms behind positive diversity effects on ecosystem functioning: testing the facilitation and interference hypotheses. *Oecologia*, **134**, 554–559.

Jury W.A. & Roth K. (1990) *Transfer Functions and Solute Movement Through Soils – Theory and Applications*. Birkhäuser Verlag, Basel.

Kelly D.W., Dick J.T.A. & Montgomery W.I. (2002a) The functional role of *Gammarus* (Crustacea, Amphipoda): shredders, predators, or both? *Hydrobiologia*, **485**, 199–203.

Kelly D.W., Dick J.T.A. & Montgomery W.I. (2002b) Predation on mayfly nymph, *Baetis rhodani*, by native and introduced *Gammarus*: direct effects and the facilitation of predation by salmonids. *Freshwater Biology*, **47**, 1257–1268.

Martinet F. (1993) Le macrobenthos limnivore, descripteur des flux organiques liés aux sédiments: exemples dans diverses annexes fluviales du Rhône. PhD thesis, University Lyon-I, France.

Maurines-Carboneill C., Pernelle J.J., Morin L., Sachon G. & Leblon G. (1998) Relevance of the INT test response as an indicator of the ETS activity in monitoring heterotrophic aerobic bacterial populations in activated sludges. *Water Research*, **32**, 1213–1221.

Menge B.A., Berlow E.L., Blanchette C.A., Navarrete S.A. & Yamada S.B. (1994) The keystone species concept: variation in interaction strength in a rocky intertidal habitat. *Ecological Monographs*, **64**, 249–286.

Mermillod-Blondin F., Creuzé des Châtelliers M., Gerino M. & Gaudet J.-P. (2000a) Testing the effect of *Limnodrilus* sp. (Oligochaeta, Tubificidae) on organic matter and nutrient processing in the hyporheic zone: a microcosm method. *Archiv für Hydrobiologie*, **149**, 467–487.

Mermillod-Blondin F., Creuzé des Châtelliers M., Marmonier P. & Dole-Olivier M.-J. (2000b) Distribution of solutes, microbes and invertebrates in river sediments along a riffle-pool-riffle sequence. *Freshwater Biology*, **44**, 255–269.

Mermillod-Blondin F., Gerino M., Degrange V., Lensi R., Chassé J.-L., Rard M. & Creuzé des Châtelliers M. (2001) Testing the functional redundancy of *Limnodrilus* spp. and *Tubifex* sp. (Oligochaeta, Tubificidae) in hyporheic sediments: an experimental study in microcosms. *Canadian Journal of Fisheries and Aquatic Sciences*, **58**, 1747–1759.

Mermillod-Blondin F., Gerino M., Degrange V. & Creuzé des Châtelliers M. (2002) Functional diversity of bioturbation by 3 detritivorous hyporheic invertebrates: an experimental study in microcosms. *Journal of the North American Benthological Society*, **21**, 132–149.

Mermillod-Blondin F., Gaudet J.-P., Gerino M., Desrosiers G. & Creuzé des Châtelliers M. (2003a) Influence of macroinvertebrates on physico-chemical and microbial processes in hyporheic sediments. *Hydrological Processes*, **17**, 779–794.

Mermillod-Blondin F., Creuzé des Châtelliers M. & Gerino M. (2003b) Effects of the interaction between tubificid worms on the functioning in hyporheic sediments: an experimental study in sediment columns. *Archiv für Hydrobiologie*, **156**, 203–223.

Miron G., Desrosiers G., Retière C. & Lambert R. (1991) Dispersion and prospecting behaviour of the polychaete *Nereis virens* (Sars) as a function of density. *Journal of Experimental Marine Biology and Ecology*, **145**, 65–77.

Murphy E.M., Ginn T.R., Chilakapati A., Resch C.T., Phillips J.L., Wietsma T.W. & Spadoni C.M. (1997) The influence of physical heterogeneity on microbial degradation and distribution in porous media. *Water Resources Research*, **33**, 1087–1103.

Pelegri S.P., Nielsen L.P. & Blackburn T.H. (1994) Denitrification in estuarine sediment stimulated by the irrigation activity of the amphipod *Corophium volutator*. *Marine Ecology Progress Series*, **105**, 285–290.

Peterson G.L. (1977) A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Analytical Biochemistry*, **83**, 346–356.

Pringle C.M., Blake G.A., Covich A.P., Buzby K.M. & Finley A. (1993) Effects of an omnivorous shrimp in a mountain tropical stream: sediment removal, disturbance of sessile invertebrates and enhancement of understory algal biomass. *Oecologia*, **93**, 1–11.

Rogaar H. (1980) The morphology of burrow structures made by tubificids. *Hydrobiologia*, **71**, 107–124.

Rosenberg R. & Selander E. (2000) Alarm signal response in the brittle star *Amphiura filiformis*. *Marine Biology*, **136**, 43–48.

Schoen R., Gaudet J.-P. & Elrick D.E. (1999) Modelling of solute transport in a large undisturbed lysimeter, during steady-state water flux. *Journal of Hydrology*, **215**, 82–93.

Statzner B., Fièvet E., Champagne J.-Y., Morel R. & Herouin E. (2000) Crayfish as geomorphic agents and ecosystem engineers: biological behavior affects sand and gravel erosion in experimental streams. *Limnology and Oceanography*, **45**, 1030–1040.

Stief P. & de Beer D. (2002) Bioturbation effects of *Chironomus riparius* on the benthic N-cycle as measured using microsensors and microbiological assays. *Aquatic Microbial Ecology*, **27**, 175–185.

Storey R.G., Fulthorpe R.R. & Williams D.D. (1999) Perspectives and predictions on the microbial ecology of the hyporheic zone. *Freshwater Biology*, **41**, 119–130.

Svensson J.M. & Leonardson L. (1996) Effect of bioturbation by tube-dwelling chironomid larvae on oxygen uptake and denitrification in eutrophic lake sediments. *Freshwater Biology*, **35**, 289–300.

Taniguchi M. & Sharma M.L. (1990) Solute and heat transport experiments for estimating recharge rate. *Journal of Hydrology*, **119**, 57–69.

Thullner M., Mauclaire L., Schroth M.H., Kinzelbach W. & Zeyer J. (2002) Interaction between water flow and spatial distribution of microbial growth in a two-dimensional flow field in saturated porous media. *Journal of Contaminant Hydrology*, **58**, 169–189.

Toride N., Feike J.L. & Van Genuchten M.T. (1993) A comprehensive set of analytical solutions for non-equilibrium solute transport with first-order decay and zero-order production. *Water Resources Research*, **29**, 2167–2182.

Torreiter P., Pitaksintorn-Watanamahart P. & Danielopol D.L. (1994) The activity of oligochaetes in relation to their ecological role in slow filtration columns. In: *Proceedings of the Second International Conference on Ground Water Ecology* (Eds J.A. Stanford & H.M. Valett), pp. 85–94. American Water Resources Association, Herndon.

Triska F.J., Duff J.H. & Avanzino R.J. (1993) The role of water exchange between a stream channel and its hyporheic zone in nitrogen cycling at the terrestrial-aquatic interface. *Hydrobiologia*, **251**, 167–184.

Triska F.J., Kennedy V.C., Avanzino R.J., Zellweger G.W. & Bencala K.E. (1989) Retention and transport of nutrients in a third order stream in north-western California: hyporheic processes. *Ecology*, **70**, 1893–1905.

Usio N. (2000) Effects of crayfish on leaf processing and invertebrate colonisation of leaves in a headwater stream: decoupling of a trophic cascade. *Oecologia*, **124**, 608–614.

Wisniewski R.J. (1978) Effect of predators on tubificidae groupings and their production in lakes. *Ekologia Polska*, **26**, 493–512.

Wotton R.S., Malmqvist B., Muotka T. & Larsson K. (1998) Fecal pellets from a dense aggregation of suspension-feeders in a stream: an example of ecosystem engineering. *Limnology and Oceanography*, **43**, 719–725.

Yingst J.Y. & Rhoads D.C. (1980) The role of bioturbation in the enhancement of microbial turnover rates in marine sediments. In: *Marine Benthic Dynamics* (Eds K.R. Tenore & B.C. Coull), pp. 407–422. University of South Carolina Press, Columbia.

Zanetell B.A. & Peckarsky B.L. (1996) Stoneflies as ecological engineers – hungry predators reduce fine sediments in stream beds. *Freshwater Biology*, **36**, 569–577.

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