

Effects of Tubificid Worm Bioturbation on Freshwater Sediment Biogeochemistry

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Abstract The effects of freshwater infaunal invertebrates on sediment geochemical properties were studied through an experimental approach using indoor microcosms during a 56-day experiment. The bioturbating organisms were tubificid worms, which consume sediment at depth and deposit undigested material at the sediment–water interface. Bioturbation intensity was determined using fluorescent tracers, and the distribution of redox-sensitive compounds was studied from replicate experimental units handled 7, 14, 21, 28 and 56 days after tubificid colonization. Worm activity transferred reduced particles and pore water at the sediment surface at a rate of 0.14 cm day^{-1} . Compared to control experimental units, this recycled material represented at the end a several centimetre-thick layer enriched in water content, dissolved nitrate and sulphate, and depleted in oxygen, ammonium and dissolved Mn(II). Tubificids consumed O_2 in bottom water, so that the sediment was anoxic, allowing a direct flux of dissolved reduced species into overlying water. Lower ammonium and Mn(II) concentrations and fluxes in anoxic sediment possibly resulted from a decrease in anaerobic microbial metabolism due to competition for labile organic carbon with tubificids. Higher sulphate concentration resulted from burial of surface waters with particle at the sediment surface, but not from bio-irrigation of burrows. Nitrate was produced in anoxic condition, as observed in almost every mixed modern sediments.

Keywords Bioturbation · Sediment · Tubificids · Early diagenesis

1 Introduction

Early diagenetic processes in modern sediments are often assumed to be in steady state. The work of Bjørn Sundby shows that in reality, transient state diagenesis is the rule,

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because of changing organic carbon input, oxygen content of bottom water, sedimentation rate or biological activity (Sundby 2006). The mixing activity of infaunal macrobenthos is called bioturbation (Richter 1952; Rhoads 1974; Aller 1982). Bioturbation can be defined as the result of burrowing, feeding, irrigating and defecating activities of animal species living at the surface and/or within the sediment superficial layers (Rhoads 1974). This activity causes sediment mixing and solute transport across the sediment–water interface (Boudreau 1997, 1999). The physical and chemical properties of the sediment can be altered. Bioturbation significantly affects the distribution of biogeochemical species inside the sediment and exerts an important control on the biogeochemical fluxes at the sediment–water interface (e.g. Yingst and Rhoads 1980; Aller 1982; Blackburn 1988; Meysman et al. 2006). Because of the bioturbation process, organic material and electron acceptors available for bacteria are redistributed in the sediment column (Aller 1982), strongly influencing the pathways, rates and extent of organic matter mineralization during early diagenesis (e.g. Kristensen 1985; Aller 1994; Gilbert et al. 2003). The role of organisms in sedimentary particle redistribution depends on which of five functional groups they belong to (for review see Gerino et al. 2003).

Laboratory experiments are a useful tools to demonstrate the role played by benthic faunal activity on biogeochemical properties of sediments (Aller 1994; Petersen et al. 1998; Loreau et al. 2001; Raffaelli et al. 2003; Giller et al. 2004; Ciutat et al. 2005; Mermillod-Blondin et al. 2005; Michaud et al. 2005; Norling et al. 2007). Aller (1994) showed that particle reworking and irrigation activities of benthic fauna promoted remineralization of organic matter in oscillating redox conditions. Michaud et al. (2005) have detailed the effects of two contrasted bioturbators (biodiffuser and gallery-diffuser) on sediment oxygen uptake rates. Experimental studies were also performed to investigate the influence of both the individual behaviour and the biodiversity of benthic fauna on organic mineralization and transport of solute in marine or continental sediments (Emmerson et al. 2001; Raffaelli et al. 2003; Mermillod-Blondin et al. 2005; Norling et al. 2007; Michaud et al. 2009). Macrofaunal diversity can significantly decrease (Mermillod-Blondin et al. 2005) or increase (Michaud et al. 2009) biogeochemical fluxes at the sediment–water interface, beyond the simple addition of individual species effects. Decreases in biodiversity can alter the role of biogeochemical engineers played by benthic macrofauna (Braeckman et al. 2010). Experimental work was also undertaken to investigate the interference between bioturbation and the chemistry of contaminants such as trace metals (e.g. Vale and Sundby 1998; Petersen et al. 1998; Ciutat et al. 2005, 2007).

The aim of this study was to investigate the effects of freshwater infaunal invertebrates on the geochemical properties of the sediment. An experimental approach was developed using indoor microcosms containing natural sediment and overlying water. The bioturbating organisms were tubificid worms; these worms are one of the major bioturbation agents in freshwater environments (Fisher et al. 1980). They feed head down in muddy sediments, consume sediments at depth and deposit undigested material at the sediment–water interface, causing downward sediment migration. Such sediment could be recycled through the feeding zone many times before ultimate burial below the deepest feeding zone (Matisoff et al. 1999). Tubificids are conveyor-belt deposit feeders. This bioturbation mode, called bioadvection, leads to a direct link between two sediment layers that are very different geochemically, that is, the oxidized superficial layer and the reduced subsurface layer (Fisher et al. 1980). This study aims to better understand how bioadvection modifies redox chemistry of the sediments. Dense tubificid populations can rapidly rework bottom deposits through selective ingestion of silt and clay (Ciutat et al. 2006). The rate at which these organisms modify the first diagenetic steps following colonization is the focus of this

research. Bioturbation intensity and the distribution of redox-sensitive compounds were studied during a 56-day experiment, initially designed to study the role of bioturbation on Cd fluxes across the sediment–water interface (Ciutat et al. 2005; Delmotte et al. 2007); here, the focus is on the behaviour of major redox species.

2 Materials and Methods

2.1 Microcosm Structure and Experimental Design

An experimental unit consisted of a sediment and water column, enclosed in a glass container (Fig. 1). The sediment was sampled from the Garonne river banks, upstream from Bordeaux (southwest France). The median grain size was 15 μm . Particulate organic carbon content was 1.55 % dry weight and the initial porosity was 74 %. The sediment was sieved through a 1-mm mesh to remove macrofauna and then frozen at -20°C for 1 week to kill any animals. Sediment was introduced into each unit and then dechlorinated tap water was carefully added to the upper part of the units to avoid disturbance at the sediment surface. The tubificid worms consisted of a mixture of three species that are closely related taxonomically and are similar in size (1.5–2.2 mg fresh wt): *Tubifex tubifex*, *Limnodrilus hoffmeisteri* and *Limnodrilus claparedeianus*. They were collected in a natural environment (GREBIL, Arry, France) and were acclimated for 15 days in the laboratory at 20°C in large tanks with Garonne river sediment. Batches of 250 ± 0.5 mg fresh wt. of worms were prepared, without prior sorting in order to respect the heterogeneous size distribution of natural communities. Each batch corresponded to 133 ± 5 worms/unit, or about 60,000 worms m^{-2} , which was close to the maximal densities found in the natural environment (McCall and Fisher 1980).

The bioturbation activity of tubificids was analysed using fluorescent particulate luminophores tracers. Luminophore tracers are natural sand particles coated with fluorescent paint. Two different grain sizes were used: $\phi = 63\text{--}100$ and $100\text{--}315$ μm (Geologisch-paleontologisches Institut and Museum of Kiel University, Germany). In each unit, a mixture of 10 g (wet wt.) of Garonne sediment with 0.45 and 0.6 g of the two types of luminophores was deposited at the sediment surface in the form of a 3-mm-thick frozen

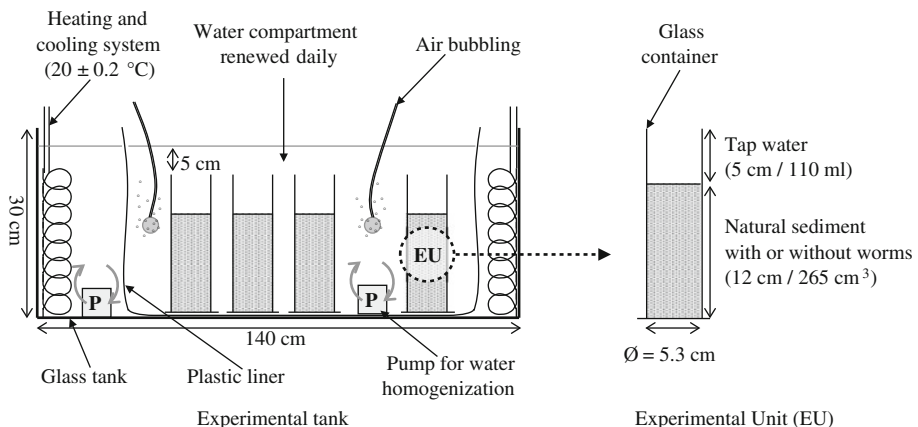


Fig. 1 Experimental system used in the present study

mud cake 24 h after introduction of the worms. The units corresponding to the two experimental treatments (control and with tubificids, noted –Tub and +Tub) were distributed into two different experimental tanks (Fig. 1). The experiment lasted 56 days, with five sampling times: 7, 14, 21, 28 and 56 days. For +Tub treatments and each exposure duration, seven units were set up: two replicates for the bioturbation study with fluorescent tracers and five for sediment analyses. For the control, two or three units were used for sediment analyses. Five units were kept for the microelectrode monitoring during the whole experiment. In total, 50 units were prepared. Water was renewed in each tank daily. Temperature, turbidity, pH and dissolved oxygen concentration in the water column were measured daily, using probes (EverSafe Thermometer; HI 93703 turbidity meter; Hanna Instruments; MP120 pH-meter; Mettler-Toledo; MO128 dissolved oxygen meter; Mettler-Toledo).

2.2 Sampling

Concentrations of O_2 , Mn^{2+} , Fe^{2+} and H_2S in the pore waters and in the overlying waters were measured by voltammetry. This technique has a relatively low detection limit for Mn^{2+} and Fe^{2+} compared with conventional techniques such as furnace atomic absorption spectrometry, but it provides simultaneous profiles of the redox species with a vertical resolution of 1 mm with very limited disturbance of the sediment structure. We used voltammetric gold amalgam microelectrodes made by sealing a 100- μm gold wire in a glass tube and plating mercury onto the polished exposed gold surface, as described by Brendel and Luther (1995) and Luther et al. (1998). Measurements were made with an Au/Hg microelectrode as working electrode, a 0.5 mm diameter platinum wire as counter electrode, and a saturated Ag/AgCl electrode as reference electrode. The counter and the references electrodes were introduced at the top of the experimental unit, a few centimetres from the working electrode. An Analytical Instrument Systems, Inc. model DLK-100 electrochemical analyser was used for all measurements. Oxygen was determined using linear sweep voltammetry, scanning from -0.1 to -1.7 V at a rate of 200 mV s^{-1} without a pre-concentration step. Mn^{2+} and Fe^{2+} measurements were performed using cathodic stripping voltammetry (CSV) in the square-wave mode, scanning from -0.1 to -1.7 V at a rate of 200 mV s^{-1} with an equilibration step at -0.1 V for 30 s. Calibration of dissolved Mn^{2+} was performed with standard solutions. We obtained a linear slope of $1\text{ nA} = 2.9 \pm 0.2\text{ }\mu\text{M}$. Data are presented in NA units, because it is the high vertical resolution shape of the profiles that is of interest. We used a micromanipulator to obtain millimetre scale depth resolution intervals with minimum sediment disturbance. Oxygen profiles were obtained for the whole experiment, whereas CSV profiles (Mn^{2+} , Fe^{2+} and H_2S) were performed in +Tub units only.

The vertical distribution of redox compounds in sediment pore water and particulate fractions was analysed for the two experimental treatments, after the five exposure durations. The water column was carefully removed from the units, and the sediment was sliced into six layers: 0–0.5, 0.5–1, 1–2, 2–3, 3–5 and 5–12 cm. Each layer was weighed (wet wt.) and centrifuged under an atmosphere of nitrogen for 20 min at 5,000 rpm. The supernatant was collected and filtered through 0.2- μm acetate cellulose membranes purged with N_2 . The pellets were lyophilized and weighed to determine the wet wt./dry wt. ratio for each sediment layer. One aliquot of pore water was frozen at $-25\text{ }^\circ\text{C}$ for nutrient analysis and another was filtered and acidified to pH 1.6 with trace metal grade HNO_3 for dissolved Mn and Fe analysis. Porosity was determined by comparison of the weight of wet and freeze-dried sediment. Porosity was calculated using a mean solid fraction density of 2.65 g cm^{-3} .

(Berner 1980). Particulate Mn was determined on homogenized freeze-dried sediments using a nitric acid solution. This reagent was able to extract all Mn(III,IV)-oxides and Mn(II)-carbonates in sediment. Total nitrate ($\text{NO}_3^- + \text{NO}_2^-$) was analysed by the flow injection method described by Anderson (1979). Ammonium (NH_4^+) was analysed by flow-through conductivity method (Hall and Aller 1992). The precision of the N-species analytical procedures was better than 5 %. Dissolved Fe concentrations were determined by the ferrozine procedure (Stookey 1970). The precision was better than 10 %. Pore water and particulate Mn were measured by flame atomic absorption spectrometry. Sulphate was measured with a nephelometric method based on barium-sulphate precipitate formation, with a precision better than 2 %. The volume of pore waters extracted from the sediment was less than 2 ml for some samples. Therefore, it was not possible to measure dissolved Mn, Fe or sulphate in all the samples or in all sediment units.

2.3 Bioturbation

To analyse the vertical distribution of luminophores in the sediment fractions, the water column was carefully removed with a syringe and the sediment was cut into six layers: 0–0.5, 0.5–1, 1–2, 2–3, 3–5 and 5–12 cm. Each layer was mechanically mixed, and 5 mL was collected and lyophilized. Three 100 mg samples of each lyophilized sediment layer were analysed using an epifluorescence UV microscope at 360 nm (Olympus Optical Co., BH2-RFC Reflected Light Fluorescence Attachment, Hamburg, Germany). All the luminophores of both sizes were counted at fourfold magnification. Luminophore concentrations in the sediment are expressed in grams per cubic centimetre. Tracer concentrations were standardized for homogenizing graph scales.

2.4 Diffusive Flux Calculation

Molecular diffusion is the main transport mechanism of solutes in muddy sediment (Berner 1980). In bioturbated sediment, total solute fluxes are a combination of molecular diffusive fluxes, plus an advective fraction. The vertical diffusive flux of dissolved species is directly proportional to the concentration gradient ($\Delta C/\Delta Z$). In the sediment, it is calculated using Fick's first law as:

$$J = -\phi D_s (\Delta C / \Delta Z)$$

where J is the flux, ϕ is the porosity, and D_s is the bulk sediment diffusion coefficient corrected by tortuosity, that is, $D_s = D_0/\theta^2$, where θ is tortuosity and D_0 the molecular diffusion coefficient in water (Berner 1980). The D_0 values obtained from the literature were corrected for in situ temperature at the time of sampling; tortuosity (θ^2) was assumed to be equal to $1 - \ln(\phi^2)$ (Boudreau 1996).

3 Results

The temperature, pH and dissolved oxygen concentration of tank water were constant throughout the experiments and were equal to 19.8 ± 0.18 °C, 8.4 ± 0.2 and 100 ± 0.4 % saturation, respectively. Oxygen saturation was at 284 μM . Significant differences were observed, however, in turbidity measured daily. The mean turbidity values during the experiments were 10.3 ± 1.3 formazin turbidity units (FTU) for the +Tub condition and 0.2 ± 0.1 FTU for the –Tub condition.

The concentration of pore water dissolved oxygen decreased with depth, from values close to saturation in tank water to zero at a maximum depth of 5 mm below the sediment–water interface (Fig. 2). The penetration depth of O_2 and the profile gradient remained similar for the condition +Tub throughout the experiment. In the presence of worms, the consumption of O_2 occurred in the first millimetres above the sediment–water interface in a layer of water containing a dense aggregation of posterior end of tubificid worm bodies, so that the sediment was anoxic below 1 mm depth in almost all profiles taken during the experiment. A deeper peak of dissolved oxygen, as would be expected if bioirrigation was taking place, was not observed.

The shape of dissolved component profiles obtained from pore water extraction was remarkably similar in replicate units that were processed at each time. Total dissolved Mn measured from extracted pore water samples (Fig. 3) was detected below the oxygen penetration depth in both conditions, indicating that the majority of the sediment was anoxic. The vertical profiles of dissolved Mn remained fairly constant in replicate units and during time from 7 to 56 days for all conditions. The dissolved Mn concentration ranged between 150 and 200 $\mu\text{mol L}^{-1}$ at the bottom of control units and decreased upward. Dissolved Mn concentrations were close to the detection limit of 1 $\mu\text{mol L}^{-1}$ in the oxic sediments. In the units with worms, dissolved Mn concentrations were between 120 and 170 $\mu\text{mol L}^{-1}$ at the bottom, the lowest values corresponding to the end of the experiment, and between 0 and 10 $\mu\text{mol L}^{-1}$ at the top. The concentration gradient just below the oxic

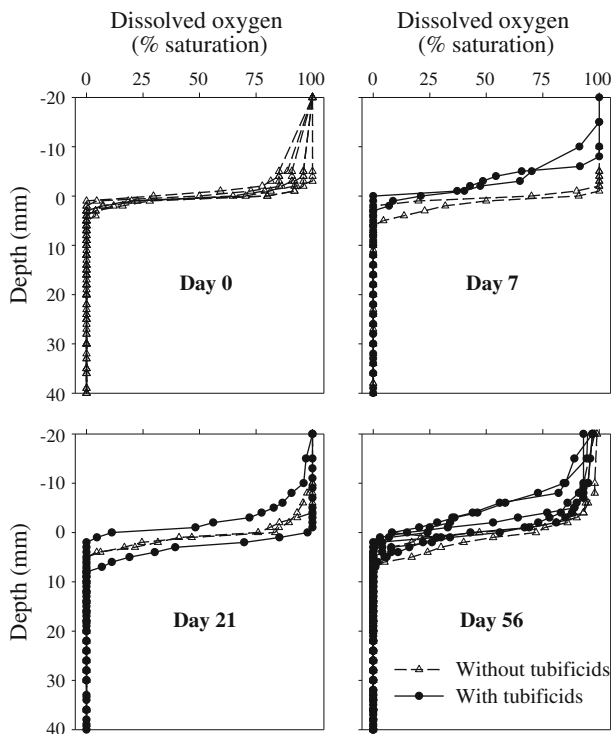
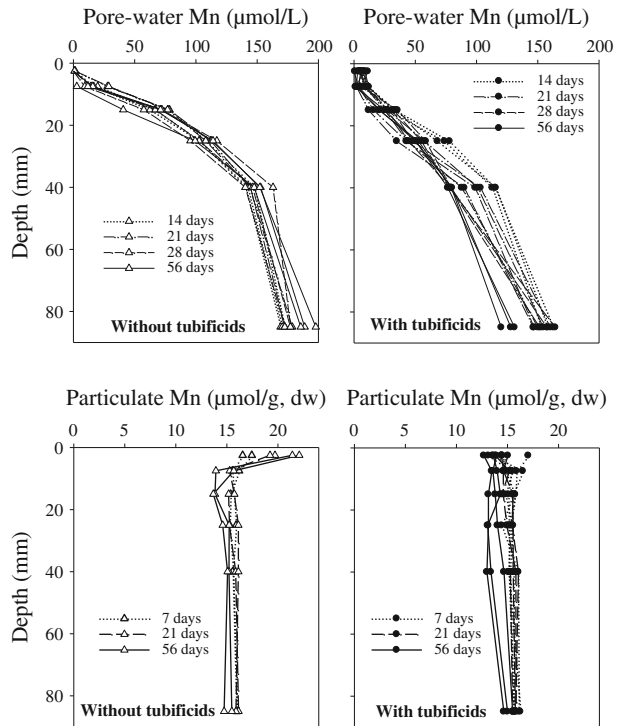


Fig. 2 Dissolved oxygen profiles measured just above the sediment–water interface and in the sediment pore water of control units and units with tubificids at time zero (just before worm introduction) and after 7, 21 and 56 days

Fig. 3 Evolution with time of vertical profiles of total dissolved Mn in pore water and particulate Mn concentrations in the sediment of control units and of units with tubificids, as determined by sediment slicing and pore water extraction. Each profile is from one experimental unit



layer was always significantly higher in the control units. The concentration gradient was, on average, 65 nmol cm^{-4} for the -Tub condition, and 25 nmol cm^{-4} for the +Tub condition. Dissolved Mn(II) from high vertical resolution profiles allowed to better characterize the distribution of Mn(II) in the upper part of the sediment (Fig. 4). Individual vertical profiles of Mn(II) measured with electrodes differed from dissolved Mn profiles from pore water extraction as is to be expected given the wide difference in spatial scale between the two approaches used. Replicate profiles from different EU (Fig. 4) or from the same EU (not shown) presented significant variability. The ≈ 100 dots represented in each panel of Fig. 4 would have represented only 4 or 5 data points of pore water extraction, suggesting that pore water extraction showed smoothed profiles that mask the real vertical and lateral variability. However, pore water smoothed profiles are useful to estimate mean fluxes. The depth of Mn(II) detection with electrodes was frequently located between 1 and 2 cm, that is, below the depth of Mn appearance measured from extracted pore water, but this was probably due to the differences in the lower detection limits of both approaches. A specific feature was observed on days 7 and 14 in each unit with tubificids: A peak of Mn(II) occurred in the upper 5 mm of the sediment column, followed by a 1- to 8-mm-thick layer where Mn(II) was below the detection limit. This surface Mn(II) peak was not detected at other times.

Profiles of particulate Mn showed maximum values in the top sediments (0–0.5 cm) of the worm-free units (Fig. 3). The maximum concentration increased with time, from $17 \pm 0.5 \text{ } \mu\text{mol g}^{-1}$ at 7 days to $21.5 \pm 1 \text{ } \mu\text{mol g}^{-1}$ at 56 days. The Mn concentrations decreased abruptly below the oxic layer to a constant background value of $15.5 \text{ } \mu\text{mol g}^{-1}$. The profiles also showed a minimum concentration, between 13 and $15 \text{ } \mu\text{mol g}^{-1}$, just

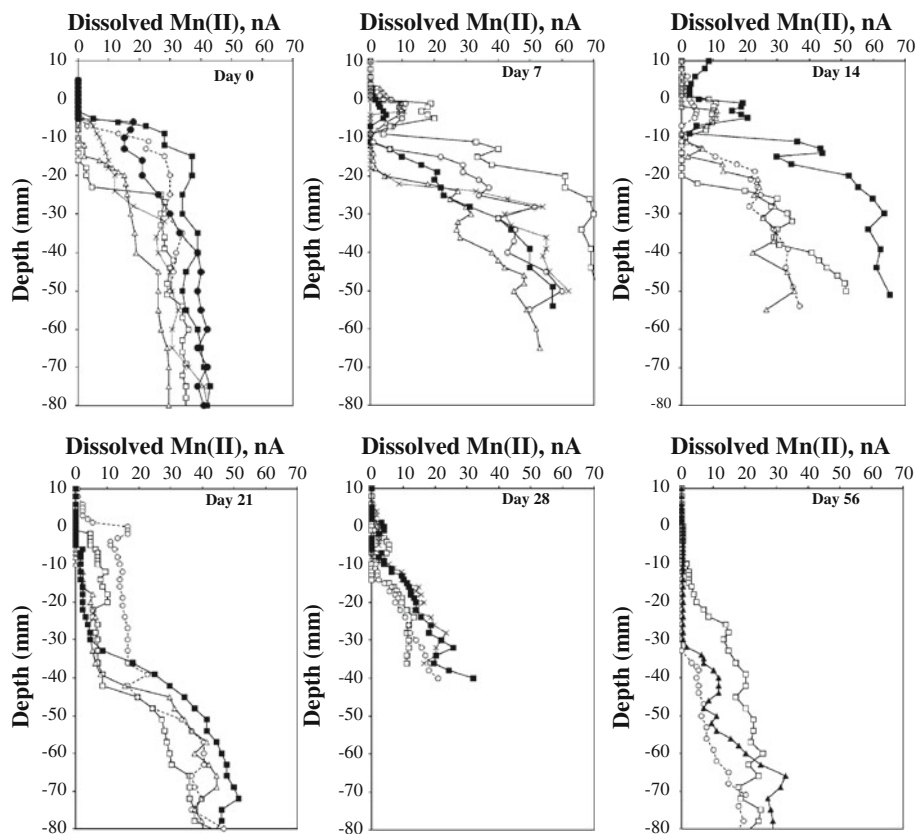


Fig. 4 Vertical profiles of pore water dissolved Mn(II) from polarographic electrode measurements before tubificid introduction (day 0) and 7, 14, 21, 28 and 56 days after colonization. The different profiles correspond to measurements in different experimental units (EU). Electrochemical response of Mn(II) on Au/Hg mini-electrodes is given in nA. Electrode calibration gave $1\text{ nA} = 2.9 \pm 0.2 \mu\text{M}$

below the oxic layer at 56 days. Particulate Mn concentrations in the +Tub condition remained fairly constant with depth and time. The average concentrations were $15 \mu\text{mol g}^{-1}$ at 7 and 21 days, and $13\text{--}14 \mu\text{mol g}^{-1}$ at 56 days.

Profiles of dissolved Fe(II) were difficult to extract from microelectrode measurements, probably since values were beneath lower detection limits ($20 \mu\text{M}$). Data from extracted pore water were patchy because several samples were depleted after analyses of other parameters. Data are presented in Table 1. Dissolved Fe(II) was detected in anoxic parts of units. Concentrations remained generally below $20 \mu\text{mol L}^{-1}$ in +Tub units, except at the bottom of units after 21 days, where most of the samples were between 40 and $100 \mu\text{mol L}^{-1}$. The accumulation of dissolved Fe(II) was greater in control units. Concentrations reached values between 100 and $200 \mu\text{mol L}^{-1}$ in the bottom part of control units at 56 days.

Dissolved nitrate was always below $1 \mu\text{mol L}^{-1}$ in tank water. Units without tubificids showed maximum nitrate concentrations in the 0–0.5 cm sample, where dissolved oxygen was present. Maximum values were below $50 \mu\text{mol L}^{-1}$ during the first 2 weeks and around $100 \mu\text{mol L}^{-1}$ after 3 weeks (Fig. 5). Lower nitrate concentrations were detected

Table 1 Concentration of dissolved Fe(II) in pore waters

Depth (cm)	Pore water Fe(II) ($\mu\text{mol L}^{-1}$)							
	Without tubificids			With tubificids				
7 days								
0–0.5				5	3			
0.5–1								
1–2	15		12		10			
2–3							37	
3–5	28		29	38	2	32	13	
>5	23		43		25			
14 days								
0–0.5	1				>	>	>	>
0.5–1								
1–2			2			>		>
2–3	2	3	2	>		1		
3–5	43	9	8	2	1	3	5	5
>5		7	9	8	5	10	6	6
21 days								
0–0.5					2			>
0.5–1								
1–2		13	13		3	1	>	>
2–3	11	38	52		3	>	>	>
3–5	51	65	69	7	9	1	3	7
>5	85	418	60	93	35	8	13	12
28 days								
0–0.5								
0.5–1								
1–2		16		4	35	3	3	4
2–3		36			5	3	3	3
3–5	71	72	76	7	9	7	3	4
>5	45	51	52	53	42	47	83	60
56 days								
0–0.5					3	3		
0.5–1					3		3	
1–2	17	11	21	4	6	2	3	3
2–3	58	25	74	3	14	4	16	4
3–5	125	112	109	23	15	146	51	12
>5	120	181	161	46	28	57	40	41

in the 0.5–1 cm depth samples, and values close to zero were measured below, except in some subsurface samples in the first 2 weeks. In the presence of tubificids, the dissolved nitrate also increased with time. The nitrate maximum was not always located at the sediment surface, but at depths of 0.5–1 cm or 1–2 cm, that is, in the anoxic part of the sedimentary column.

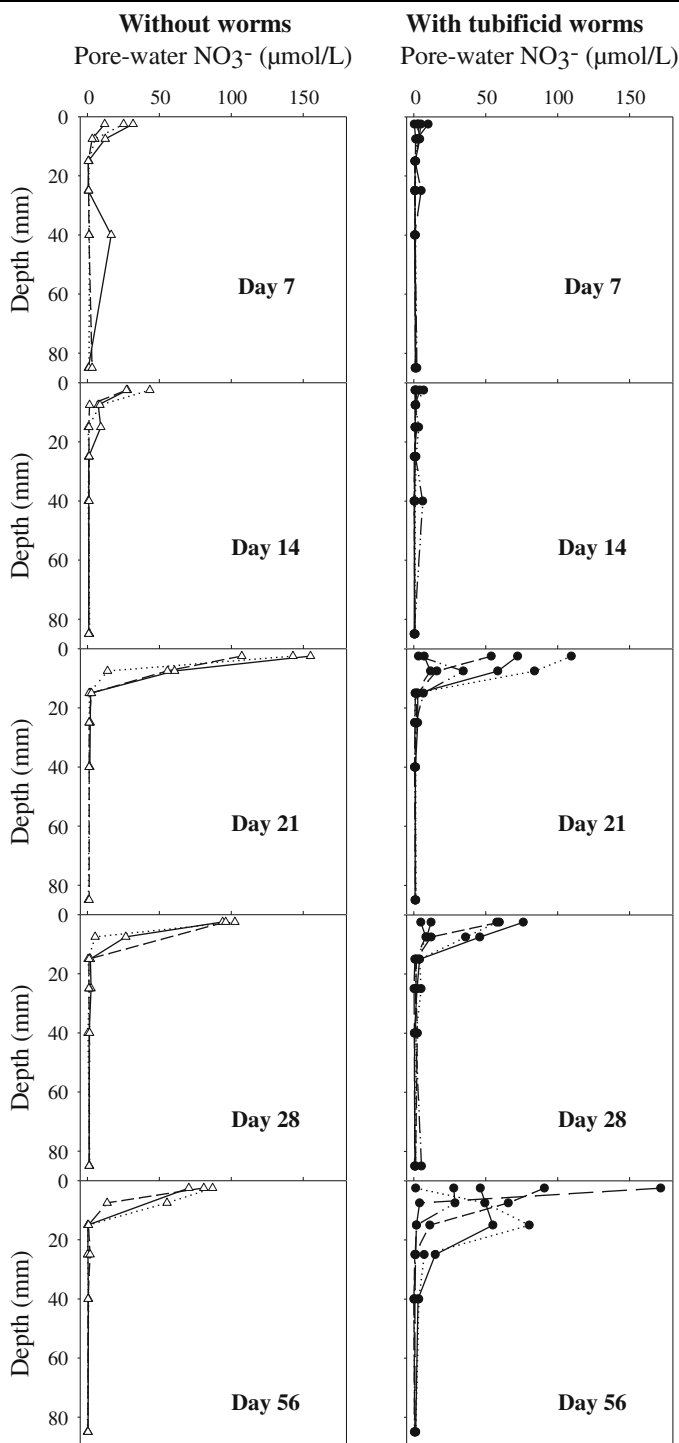
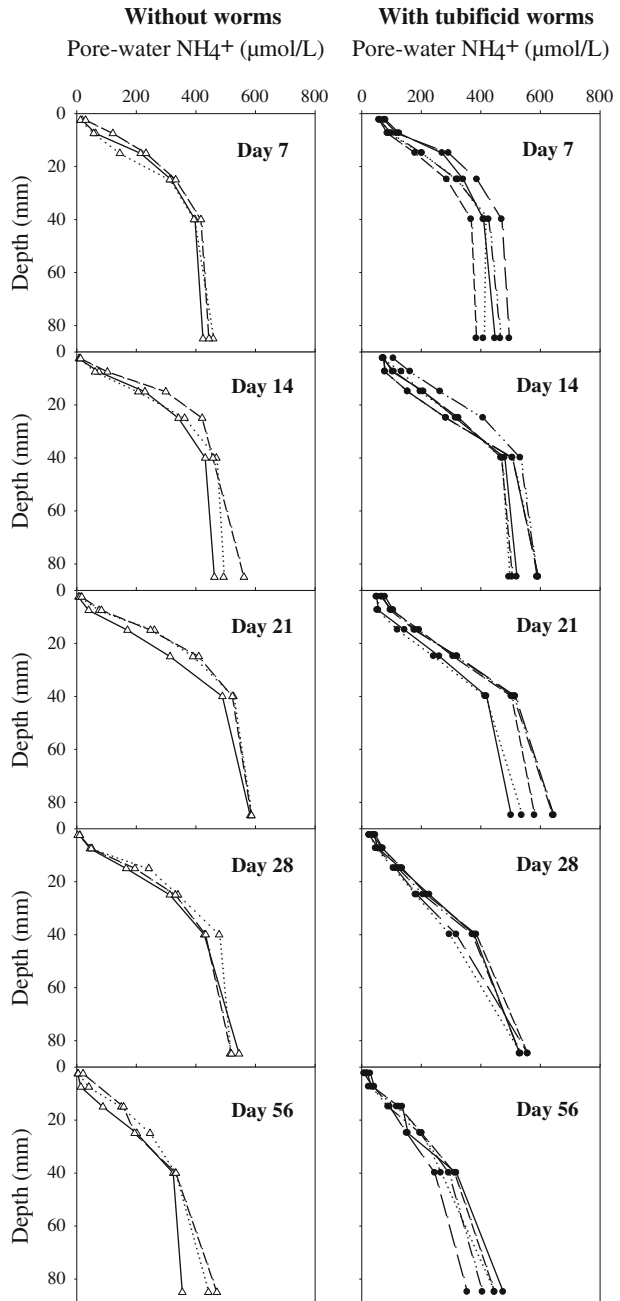


Fig. 5 Evolution with time of vertical profiles of pore water nitrate concentrations in the sediment of control units and of units with tubificids. Each profile is from one experimental unit

Fig. 6 Evolution with time of vertical profiles of pore water ammonium concentrations in the sediment of control units and of units with tubificids. Each profile is from one experimental unit



Ammonium concentration in control units was close to zero in the top sample (0–5 mm) throughout the experiment (Fig. 6). Concentrations increased with depth below 5 mm depth and reached values between 400 and 600 $\mu\text{mol L}^{-1}$ in the bottom sample. The gradient of concentration below the oxic layer was linear between 0.5 and 3 cm depth. It was between 140 and 180 nmol cm^{-4} from 7 to 28 days. The gradient decreased below. At

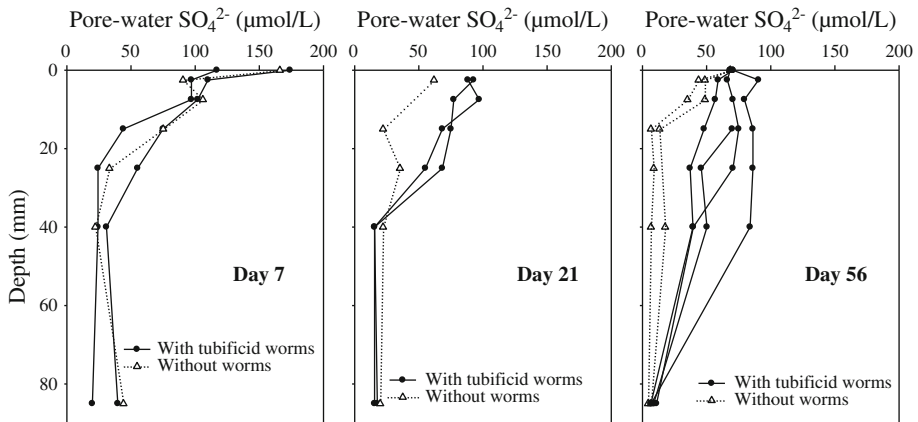


Fig. 7 Evolution with time of vertical profiles of pore water sulphate concentrations in the sediment of control units and of units with tubificids. Each profile is from one experimental unit

56 days, the gradient was slightly lower, with a mean value of 95 nmol cm^{-4} . In units containing tubificids, the shape of ammonium profiles was about the same as in control units, except that the concentrations in the top sample were above $50 \text{ } \mu\text{mol L}^{-1}$ from 7 to 21 days, and between 10 and $50 \text{ } \mu\text{mol L}^{-1}$ at 28 and 56 days. The profiles were remarkably linear between the surface and 4 cm depth. The concentration gradient was between 75 and 117 nmol cm^{-4} .

The concentration of dissolved sulphate in the water column changed during time, from $170 \text{ } \mu\text{mol L}^{-1}$ at 7 days to $70 \text{ } \mu\text{mol L}^{-1}$ at 56 days (see Fig. 7). These concentrations were higher than the concentration of the sediment pore water at the beginning of the experiments, which was close to $40 \text{ } \mu\text{mol L}^{-1}$. The dissolved sulphate concentrations at the top of the units decreased with time. For all conditions, the dissolved sulphate concentration at the bottom of the units also decreased with time, from about $40 \text{ } \mu\text{mol L}^{-1}$ at 7 days to less than $15 \text{ } \mu\text{mol L}^{-1}$ at 56 days. The dissolved sulphate evolution at intermediate depths, between 1 and 5 cm, depended on the conditions. In the absence of worms, the sulphate concentrations were lower than in the units with worms. Dissolved sulphide was not detected at any depth in the sediment column with the polarographic electrode, which had a detection limit of $0.1 \text{ } \mu\text{mol L}^{-1}$.

In the control condition, luminophores were confined to the upper sediment layers (Fig. 8). About 75 % were recovered in the top 0- to 0.5-cm layer and about 25 % were recovered just below, in the 0.5- to 1.0-cm layer. In the +Tub condition, the distribution of tracers changed with time. The luminophore content deepened progressively with time. Tracers were recovered in the 0.5- to 1-cm and the 1- to 2-cm layers after 7 days. After 21 days, 90 % of the luminophores were found in the 3- to 5-cm layer and the remaining was found below. At the end of the experiment, almost all the luminophore content was in the 5- to 12-cm layer.

The porosity profiles in experimental units decreased with depth (Fig. 9). In the control condition, the surface sample porosity was close to 80 %. Porosity decreased rapidly below to a value of 72 % at 7 days and 69 % at 56 days. In units containing tubificids, the porosity was higher at the surface, between 82 and 85 %. Below, the porosity in +Tub units was higher than that in -Tub units in samples that contained luminophores and

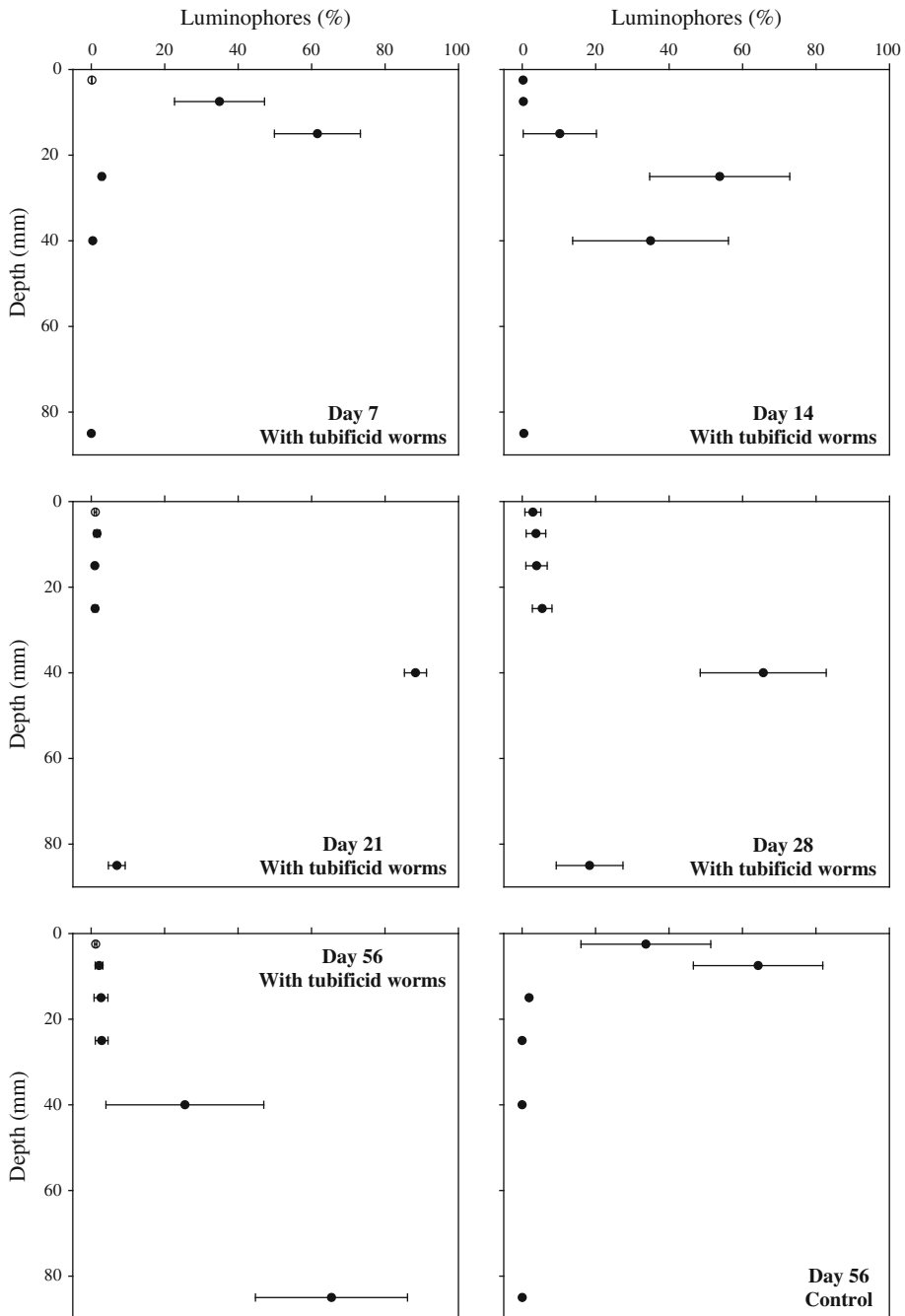


Fig. 8 Vertical profiles of the proportion of luminophores measured after 56 days for the control and after 7, 14, 21, 28 and 56 days for the tubificid-colonized sediment. Values are means of three replicate tracers counts (\pm standard deviation). Values represent the percentage of luminophores counted in a layer versus the total counted in each unit

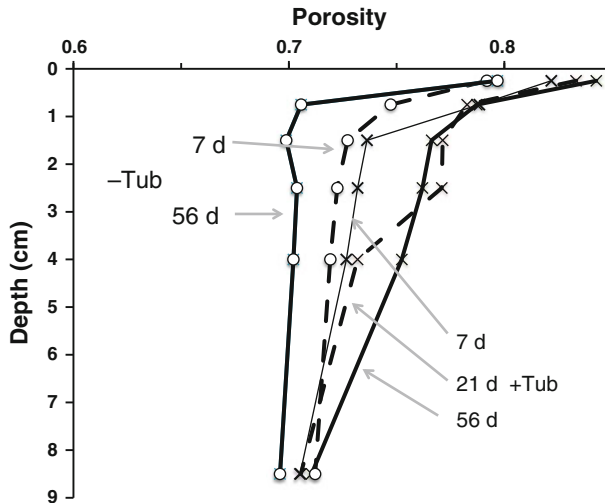


Fig. 9 Vertical profiles and evolution with time of sediment porosity measured after 7 and 56 days for the control units (–Tub) and after 7, 21 and 56 days for the tubificid-colonized sediment (+Tub). Each profile is an average of three replicates (standard deviation <0.012)

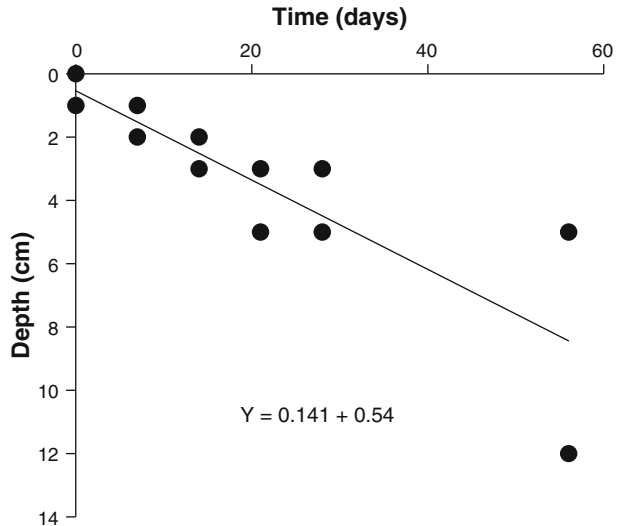
samples situated above. Hence, the positive anomaly of porosity propagated downward with the luminophores.

4 Discussion

4.1 Particle Mixing

In the absence of bioturbation, luminophores were recovered at the top of the sediment column showing that the particle mixing did not occur. Some luminophores were recovered in the 0.5- to 1.0-cm layer, probably because the sediment layer containing the particulate tracers was almost as thick as the first sampled sediment layer. Tracer distribution in +Tub units illustrates the effect of bioadvection. Tubificids are known to ingest anoxic sediment at depth and reject it at the sediment surface. This process generates the rapid burial of surface sediment by the accumulation of faecal pellets at the sediment surface. An increase in interstitial water content of re-worked surface sediment is shown by the porosity measurements. A consequence of this may be an increase in diffusive fluxes at the sediment–water interface, as these fluxes depend on porosity (e.g., Boudreau 1997). The solid volume fraction becomes lower in surface sediment. Therefore, tubificid bioturbation fluidizes the sediment, which may become more sensitive to resuspension and erosion (McCall and Tevesz 1982; Davis 1993), as confirmed here by turbidity measurements in tank water. Luminophores, which are too coarse to be ingested by tubificids (Juget 1978; Rodriguez et al. 2001), migrate vertically in the sediment as far as the ingestion zone is reached, where they accumulate. The occurrence of luminophores in the 5- to 12-cm layer at 28 days indicates that the maximal ingestion depth is located in this layer. In the present experiment, the average advection velocity of luminophores, as deduced from the migration of the luminophore maximum, is 0.14 cm day^{-1} (Fig. 10). This rate is similar to

Fig. 10 Vertical migration with time of luminophores, as represented by the sediment sampling depth containing the greatest concentration of luminophores at each sampling date



previous bioadvection rates estimated experimentally from similar tubificid densities (Fisher et al. 1980; McCall and Fisher 1980; Davis 1974).

Bioturbation creates two layers: a top layer, the thickness of which increases with time, corresponding to accumulation of faecal pellets of ingested anoxic sediment, and a bottom layer of initially “fresh” sediment inhabited with worms. Profiles of dissolved compounds that present smooth profiles versus depth in pore waters, such as ammonium or total dissolved Mn do not show a break in the concentration gradient specifically located at the boundary between both layers. This suggests that the transport of solutes is more efficient than the transport of particles. The advective transport of particles can be modelled as a biodiffusive transport (Boudreau 1997). The biodiffusive coefficient deduced from the present experiment has a mean value of $3.0 \text{ cm}^2 \text{ year}^{-1}$ (Ciutat et al. 2005; Delmotte et al. 2007). This value is at least one order of magnitude lower than that of molecular diffusion of dissolved ions in pore waters. For example, the sediment diffusion coefficients of dissolved Mn and NH_4^+ are 108 and $30 \text{ cm}^2 \text{ year}^{-1}$, respectively, as deduced from tracer values corrected from porosity. Therefore, the propagation of a solute front by molecular diffusion in pore waters is much faster than pellet accumulation at the surface. The presence of worms could also induce bioirrigation of water in burrows. Several authors have argued, however, that tubificid bioirrigation is negligible (McCall and Fisher 1980; Kresoski and Robbins 1981).

4.2 Transient Redox Processes

The present experiment allows us to directly compare early diagenesis redox reactions in bioturbated and non-bioturbated freshwater sediment. Despite the smoothing effect due to molecular diffusion, profiles of bioturbated sediments were slightly different from undisturbed sediments. Oxygen penetration depth was shallower in bioturbated sediments. In both conditions, nitrate accumulated in the sediment. However, nitrate accumulated only in the oxic layer in the absence of tubificids, whereas it also accumulated in an anoxic subsurface layer in bioturbated sediment on day 56. Dissolved ammonium gradients were of the same order of magnitude in both conditions, suggesting that the diffusive flux was

almost the same. However, the gradient reached the sediment surface in +Tub condition, implying that ammonium escaped the sediment, while the gradient did not cross the subsurface oxic layer in the –Tub condition. Dissolved Mn increased with depth in the anoxic sediment, but a minimum was observed at about 1 cm depth with the presence of tubificids. Particulate Mn accumulated at the surface of the units without worms, whereas particulate Mn profiles remained constant with depth in the presence of worms. Dissolved iron was much higher in control units after 56 days, whereas sulphate concentrations became lower in these units.

All these observations indicate that redox processes were of different intensity or nature, although the general scheme was the same for both conditions. The decrease in oxidizing compounds below the surface and the accumulation of reduced species reflect a well-established depth zonation of redox reactions in which oxygen is reduced near the sediment–water interface, followed by the reduction in nitrate, manganese oxides, iron oxides and sulphate. This succession is driven by the preferential use of the electron acceptor that yields the highest amount of free energy in the terminal step of the bacterially mediated oxidation of organic matter (Froelich et al. 1979). In general, bioturbation interferes significantly with this sequence; it modifies the partitioning between solid and dissolved phases (Vale and Sundby 1998), and the transfer between sediments and overlying water. Tubificid bioturbation appears to interfere only slightly.

The gradient of O₂ at the sediment water interface was often higher in control units than in tubificid-containing units (Fig. 2). However, the calculated diffusive O₂ flux was almost equal in both conditions (Table 2), because the lower gradient of +Tub units was compensated for a higher porosity of the surface sediment. The rapid consumption of O₂ was due to oxic respiration by microorganisms and oxidation of reduced products that diffused from below. In the +Tub experiments, worm respiration also contributed to the oxygen consumption. Tubificids do not irrigate their burrows (McCall and Fisher 1980). Their respiratory exchanges are achieved by cutaneous diffusion across the surface of the posterior end of the body, which often remains in the water column. This may explain the position of the gradient, which occurs above the sediment–water interface. Therefore, although the diffusive flux of O₂ was the same in +Tub condition as in –Tub condition (Table 2), total O₂ consumption was probably much higher in the +Tub condition due to an additional O₂ uptake in the water column because of worm metabolism.

Table 2 Averaged vertical fluxes of dissolved species in mmol m^{−2} day^{−1} calculated from the maximum concentration gradients (negative and positive values correspond to downward and upward fluxes, respectively)

Days	O ₂ –Tub	O ₂ +Tub	Mn ²⁺ –Tub	Mn ²⁺ +Tub	NH ₄ ⁺ –Tub	NH ₄ ⁺ +Tub
7	−12.0	−10.0	–	–	0.100	0.085
14			0.130	0.087	0.109	0.077
21	−9.1	−10.0	0.135	0.080	0.117	0.090
28			0.139	0.045	0.126	0.065
56	−5.6	−6.9	0.125	0.065	0.070	0.085

The mean standard deviation was 20 %

$$D_0(\text{O}_2 \text{ at } 20^\circ\text{C}) = 1.91 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$$

$$D_0(\text{Mn}^{2+} \text{ at } 20^\circ\text{C}) = 5.44 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$$

$$D_0(\text{NH}_4^+ \text{ at } 20^\circ\text{C}) = 1.66 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$$

The contribution of dissolved reduced species oxidation to O_2 uptake was probably low, because the upward flux of dissolved reduced species was two orders of magnitude lower than the downward flux of O_2 (Table 2). In bioturbated sediments, some of the particles advected to the surface probably also reacted with oxygen.

Dissolved Mn concentrations were high and relatively steady in anoxic sediments, suggesting that the upward diffusive flux was balanced by dissolved Mn production. Dissolved Mn production probably resulted from the reduction in Mn oxide present in sediments. The rapid establishment of high dissolved Mn concentrations was also observed in mixed turbiditic or flood sediments (Anschutz et al. 2002; Deflandre et al. 2002). Since the sediment put in the experimental units consisted of homogenized muds from the Garonne river, the whole sediment column contained particulate reducible Mn(III, IV) oxides initially present at the sediment surface only. Recent studies showed that dissolved Mn(III) was an intermediate and transient species during Mn oxide reduction (Trouwborst et al. 2006; Madison et al. 2011), which may explain differences in profile shape obtained from pore water extraction (total dissolved Mn) and microelectrodes (Mn(II)). Dissolved Mn concentrations were close to zero just below the sediment–water interface, probably because soluble Mn precipitated back as insoluble Mn(III) or Mn(IV) in the oxic layer. Precipitation of upward diffusing Mn is a dominant phenomenon that enriches oxidized surface sediment in manganese (Sundby et al. 1981). Particulate Mn concentrations increased with time in the oxic layer only in the worm-free experiments. The presence of a particulate Mn maximum in the oxic layer is commonly observed in modern sediments (Burdige 1993). Particulate Mn content in the top sediment layer can be estimated from the concentration of Mn and the solid fraction volume ($1 - \phi$) (Mouret et al. 2009). One centimetre square of the 0.5-cm-thick upper interval contained 0.278 g of particles at a mean Mn concentration of $19.2 \mu\text{mol g}^{-1}$ at 21 days, that is, $5.34 \mu\text{mol}$ of solid Mn. The Mn inventory was 5.86 at 56 days. Mn enrichment was, therefore, $14.8 \text{ nmol cm}^{-2} \text{ day}^{-1}$. This value is close to the mean Mn(II) diffusive flux of this time interval ($13.3 \text{ nmol cm}^{-2} \text{ day}^{-1}$; Table 2), suggesting that dissolved Mn precipitated quantitatively in the oxic layer. Consequently dissolved Mn did not escape the sediment in the absence of bioturbation.

In the presence of worms, the shape of the dissolved Mn profile suggests that Mn precipitated in the oxic layer, but Mn oxides did not accumulate in this layer. The bioadvection velocity (0.14 cm day^{-1}) deduced from the distribution of the luminophores suggests that oxidized Mn would have been rapidly transported back to the anoxic layers below, preventing establishment of a solid Mn peak. Nevertheless, a fraction of the upward flux of dissolved Mn may have escaped the sediment to satisfy the material balance: particulate Mn concentrations were lower at 56 days than before. The difference is accounted for by Mn export to tank water. Similarly, mass balance considerations have shown that the burial flux of manganese in bioturbated coastal environments can be lower than the sedimentation flux. (Sundby et al. 1981; Trefrey and Presley 1982). Bjorn Sundby, in his review paper on transient benthic processes (Sundby 2006), presented a synthesis of processes that affect manganese when benthic organisms bring about a net flux of oxidized manganese. The factors that favour release of dissolved Mn include the slow oxidation kinetics of Mn(II) by oxygen (Stumm and Morgan 1981), excursions of the Mn(II)/Mn(IV) redox boundary to, or close to, the sediment surface (Aller 1994; Thamdrup et al. 1994; Gobeil et al. 1997), and desorption of Mn(II) from particulate matter moved to the sediment–water interface by bioturbation (Slomp et al. 1997). Polarographic electrode profiles clearly show that dissolved Mn(II) reached the sediment surface after the introduction of tubificids, whereas Mn(II) was detected below 5 mm depth before worm addition (Fig. 4).

The upward excursion of the Mn redox boundary is due to tubificid respiration. Worms probably reject at the surface sediment reduced Mn(II)-enriched pore water of Mn(II) adsorbed on particle with the deposition of faecal pellets. The minimum in Mn(II) signal of voltammetric measurements at around 1 cm depth cannot be related to Mn oxidation with O_2 , because O_2 was absent at this depth. Near the sediment–water interface, rapid and reversible adsorption on metals oxides can buffer the concentration of Mn(II) in the pore water (van der Zee et al. 2001, 2003). Adsorption of newly precipitated Mn oxides that are rapidly buried by faecal pellets may represent a trap for dissolved Mn(II) diffusing from below.

Nitrate accumulation in the oxic layer of sediment is the result of aerobic oxidation of upward diffusing ammonium. At the sediment–water interface, the downward flux of O_2 was large enough to oxidize the upward fluxes of NH_4^+ to produce nitrate (Table 2). The decrease in nitrate below the oxic layer was probably due to denitrification. Denitrification can also be supported by electron donors other than organic matter such as Fe^{2+} or NH_4^+ (Sørensen and Jørgensen 1987; Postma 1990; Mulder et al. 1995; Hyacinthe et al. 2001). The rate of denitrification can be estimated from the downward gradient of dissolved nitrate. This gradient was always lower than the upward gradient of dissolved ammonium, indicating that the nitrate produced from nitrification of ammonium was not totally converted to N_2 through denitrification. A significant part of nitrate probably escaped the sediment, since nitrate concentration in overlying water was always lower than that of the surface sediment. The vertical resolution of nitrate profiling is not satisfactory enough to estimate the diffusive flux of nitrate toward the water column.

At the beginning of the experiment, several samples located in the anoxic part of the sediment showed micromolar levels of dissolved nitrate. In bioturbated experimental units, nitrate penetration depth was deeper than in control units. These observations suggest that nitrate production occurred in anaerobic conditions. Anaerobic nitrate production through ammonium oxidation with particulate Mn oxide is now recognized as a common process during non-steady-state diagenesis (e.g. Hulth et al. 1999; Anschutz et al. 2000; Mortimer et al. 2004; Chaillou et al. 2007). At the beginning of the experiment reactive Mn oxides were present in anoxic sediment because of initial sediment homogenization. In bioturbated sediment, a subsurface nitrate peak probably resulted from tubificid-induced burial of Mn oxide that precipitated in the oxic layer. The inventory of dissolved Fe(II) was also lower in +Tub units. The reduction in NO_3^- to N_2 by Fe^{2+} is thermodynamically favourable at all pH encountered in marine sediment (Luther et al. 1997). Because of the multiple pathways to reduce NO_3^- to N_2 under anoxic conditions (e.g. potential oxidants: organic carbon, Fe^{2+} , NH_4^+ , H_2S , AVS), nitrate did not accumulate in sediment pore waters.

In anoxic layers, the upward flux and the inventory of dissolved reduced species, such as Mn(II), ammonium (Table 2) or Fe(II) were always lower in +Tub condition than in –Tub condition. This suggests that anaerobic processes of organic matter mineralization are less intense when bioturbation occurs. After 56 days, the major part of the sediment (at least the upper 8 cm) was recycled by worms through ingestion and faecal pellet deposition at the sediment surface. The recycled sediment probably became depleted in labile organic matter and/or in anaerobic bacteria because of tubificid feeding. Therefore, the living biomass of worms accelerates organic mineralization, probably leaving less available organic carbon for anaerobic bacteria.

Dissolved sulphate concentrations in the pore water decreased from the top to the bottom, except in the recycled sediment layer (above luminophores). The gradient of sulphate was primarily due to the difference between initial pore water sulphate

concentration ($40 \mu\text{mol L}^{-1}$) and overlying water sulphate concentration ($170 \mu\text{mol L}^{-1}$ at 7 days, $70 \mu\text{mol L}^{-1}$ at 56 days). Nevertheless, the sulphate concentration became lower than $40 \mu\text{mol L}^{-1}$ at the bottom of the units, indicating that sulphate was consumed in the anoxic sediment, probably by bacterial sulphate reduction to sulphide (Jorgensen 1982). Dissolved sulphide was probably titrated by insoluble metal-sulphide precipitation because we did not detect any dissolved sulphide with the polarographic electrodes. The recycled sediment layer of bioturbated sediment became enriched in dissolved sulphate compared to control units. Apparently, sulphate reduction occurred only in non-recycled sediment. Porosity measurement indicates that the water volume of bioturbated sediments increased with time. This additional water content necessarily came from overlying sulphate-rich tank water. However, additional water represented only about 10 % of added water, which is not enough to explain an almost conservative sulphate behaviour in the mixed layer. The mixed sediment layer consisted of sediment parcels that spent time at the sediment–water interface. According to the sediment advection rate measured with luminophores, each sediment parcel spent 1 day between 0 and 0.14 cm depth. This is sufficient to permit diffusive equilibrium of sulphate between tank water and surface sediment pore water and may explain the steady sulphate concentration in recycled sediment. Recycling of sediment by tubificids prevents sulphate reduction, probably because of depletion of labile organic matter content due to worm metabolism.

5 Conclusion and Implication

Freshwater tubificids generate a water exchange of about $25 \text{ L m}^{-2} \text{ day}^{-1}$ (Woods 1975), which is important but not massive compared to *Chironomus riparius* or to *Nereis diversicolor*, which may induce $1,500 \text{ L m}^{-2} \text{ day}^{-1}$ (Leuchs 1986; Kristensen and Kostka 2005). Our results show that tubificid bioturbation does not have a drastic impact on the pore water geochemistry. However, the present study gives additional information on the first diagenetic steps following colonization of conveyor-belt deposit feeders within sediments. Although there are several studies on the evolution of sediment and pore water chemistry in bioturbated freshwater sediment, (e.g. Svensson 1997; Matisoff and Wang 1998; Gilbert et al. 2003; Mermillod-Blondin et al. 2005), the present kinetic experiment allows us to examine the onset of new conditions that evolve from a steady state to transient diagenetic situation due to sediment re-working (Sundby 2006). This experiment allows us to characterize redox reactions that occur when oxic particles are shifted toward anoxic sediment and when reduced material is advected to the sediment surface.

Tubificid metabolism prevents the establishment of an oxic layer at the top of the sediment. This has a major effect on exchange of redox-sensitive species across the sediment–water interface. We observed that the dissolved inorganic nitrogen species that left the sediment in colonized muds consisted of a mixture of ammonium and nitrate rather than nitrate alone, as observed in the control experiment. Anaerobic nitrate production has been observed here, as in recently deposited turbidites (Anschutz et al. 2002; Deflandre et al. 2002; Mucci et al. 2003; Chaillou et al. 2007). This process is probably the rule in non-steady-state diagenesis (Anschutz et al. 2000; Sundby 2006), where the $\text{NH}_4^+/\text{NO}_3^-$ couple is a major electron conveyor between Mn oxides and organic matter.

Colonized sediments progressively lost manganese because of the migration of the redox front at the sediment–water interface, and because of direct discharge at the surface of reduced pore waters by tubificids. In natural settings, this may have an influence on the transfer of minor or trace elements that can be adsorbed on metal-oxides. The out-flux of

material is probably partly balanced by rapid burial of sediment. A previous study showed that tubificids increased cadmium scavenging into the sediment because of renewal of adsorption site at the sediment surface (Ciutat et al. 2005). Our study showed that bioadvection was a pump of dissolved sulphate from the surface to the anoxic sediment.

Worm metabolism decreased the rate of anaerobic respiration processes, probably because tubificids were in competition with bacteria for labile organic carbon mineralization. Therefore, tubificids do not only influence early diagenesis processes through bioadvection, but they have a direct effect on sediment metabolism as well.

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