Testing the functional redundancy of *Limnodrilus* and *Tubifex* (Oligochaeta, Tubificidae) in hyporheic sediments: an experimental study in microcosms

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Abstract: Most tubificid worms are classified in the same functional group by their similar bioturbation and feeding activities in fine sediments of lakes. The objective of this study was to test the functional redundancy of two genera of tubificids (*Limnodrilus* and *Tubifex*) at two densities in coarse sediments using slow filtration columns. We measured the effects of the worms on particle redistribution, organic matter processing, nutrient fluxes, and microbial characteristics. The results showed that *Limnodrilus* and *Tubifex* created the same stimulation of aerobic and anaerobic microbial activities in the sediment. However, 50 *Tubifex* had a greater effect than 50 *Limnodrilus* on microbial processes. Furthermore, at a density of 100 oligochaetes per column, only *Tubifex* significantly increased particle redistribution and oxygen consumption in the first centimetre of the sediment. In contrast, *Limnodrilus* more often modified microbial activity in the deeper layers of the columns. The functional redundancy of the two genera of tubificid worms was validated by most measured processes. However, the variability within functional groups cannot be neglected because each genus had its functional peculiarity in the ecosystem.

Résumé : En raison de leur mode de déplacement et d'alimentation dans les sédiments fins des lacs, la plupart des oligochètes tubificidés sont classés dans le même groupe fonctionnel. L'objectif de cette étude est de tester la redondance fonctionnelle entre deux genres de tubificidés (*Limnodrilus* et *Tubifex*) pour deux densités en organismes dans des sédiments grossiers en utilisant des colonnes infiltrantes. L'effet des vers est mesuré sur la redistribution des particules, la décomposition de la matière organique, les flux de nutriments et plusieurs paramètres microbiens. Les résultats indiquent que *Limnodrilus* et *Tubifex* produisent une stimulation des activités microbiennes aérobie et anaérobie dans le sédiment. Cependant, 50 *Tubifex* ont un effet plus important sur l'activité microbienne que 50 *Limnodrilus*. A une densité de 100 individus par colonne, seul *Tubifex* augmente significativement la redistribution des particules et la consommation en oxygène dans les premiers cm de sédiment. Au contraire, *Limnodrilus* semble être plus actif que *Tubifex* en profondeur. La redondance des deux genres de vers est validée par la plupart des processus mesurés. Cependant, la variabilité à l'intérieur des groupes fonctionnels ne peut être négligée car chaque genre a sa spécificité dans le fonctionnement de l'écosystème.

Introduction

In recent years, several investigators tried to link species diversity with ecosystem processes (Lawton 1994; Palmer et al. 1997). Studies dealing with the functional aspect of diversity have described the role of species in ecosystems by using the functional group concept. A functional group is a set of species having a similar role in ecosystem processes (Chapin et al. 1992).

In fine sediments of lakes (mud and fine sand), tubificid worms such as *Tubifex tubifex* and *Limnodrilus hoffmeisteri* have been classified in the functional group of conveyor-belt deposit feeders (McCall and Tevesz 1982). The activities of these worms in fine sediments (feeding and bioturbation)

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play a significant role in the processing of organic matter and nutrient cycling in both aerobic and anaerobic conditions (Torreiter et al. 1994; van de Bund et al. 1994). However, in hyporheic habitats characterized by coarse sediments (gravel and sand) and convection flow of water into the sediment, the functional classification of worms has not been established yet because the effect of oligochaetes on system function remains poorly known (Boulton 2000). In a previous study performed in slow filtration columns filled with coarse sediments (gravel and sand), we showed a large effect of *Limnodrilus* tubificid worms on aerobic and anaerobic microbial activities (Mermillod-Blondin et al. 2000*a*).

The objective of the present study was to determine if the classification of tubificid worms established for lake sediments was also valid in hyporheic habitats. According to the redundancy model (Walker 1992), if two taxa of tubificids belong to the same functional group, they should create similar effects on ecosystem processes with a possible difference in effect intensity (François et al. 1999) or in effect location. In the present study, the redundancy within the tubificid group was investigated in hyporheic sediments using representatives of two genera (*Limnodrilus* and *Tubifex*) that are widely distributed and commonly co-occur in freshwater sediments (Brinkhurst 1966; Lindegaard and Dall 1988). However, *Tubifex* was found less often than *Limnodrilus* in coarse sediments in natural systems (Lindegaard and Dall 1988). Most studies (Juget 1979; Sauter and Güde 1996) also indicated that *Tubifex* was less adapted than *Limnodrilus* to ingest coarse sediments despite the fact that *Tubifex tubifex* did not exhibit a higher preference for silt–clay (<63 μm) than for coarse sand (500–850 μm) in experimental conditions (Lazim and Learner 1987). Therefore, in comparison with fine sediments, these two genera could act differently in hyporheic habitats because they differ in their ecological requirements. To test the redundancy within the tubificid group, the effects of two worm densities (50 and 100 oligochaetes per column) of *Limnodrilus* and *Tubifex* on ecosystem processes were measured and compared. Four sets of parameters were used to estimate the effect of oligochaetes on system functioning: particle redistribution by the worms (luminophore method), biofilm characteristics (numbers of ETS-active bacteria (with an active electron transport system) and total bacteria), microbial processes (denitrification potential and hydrolytic activity), and water chemistry resulting from microbial processes (NH_4^+, NO_2^-, NO_3^-, dissolved organic carbon (DOC), and oxygen concentrations).

Materials and methods

Sediment preparation

In an 8-L plastic flask, 5.5 kg of calcinated (550°C) sand (grain size 60–630 μm) were combined with 72.5 g of cellulose powder (20 μm) as a source of organic carbon commonly found in natural sediment. The final concentration of particulate organic matter in sand (1.3% of dry sand) corresponded to contents measured in secondary channels of the River Rhône (Mermillod-Blondin et al. 2000*b*). Dechlorinated tap water (2 L) containing 2 $mg \cdot L^{-1}$ DOC and 20 $mg \cdot L^{-1}$ NO_3^- was added to the sand. This sediment was inoculated with an extract of natural bacteria obtained from a pond sediment as in Mermillod-Blondin et al. (2000*a*). A natural extract of bacteria was used to reproduce most bacterial processes occurring in natural systems.

Experimental device

According to the experimental system developed by Danielopol and Niederreiter (in Torreiter et al. 1994 and Griebler 1996), the experiments took place in gravel–sand filtration columns (50-cm height and 10-cm diameter). Each column was filled to a height of 40 cm with calcinated (550°C) fine gravel (4–5 mm) and the sand previously incubated with bacteria during 4 days as explained above. Constant masses of gravel (590 g) and incubated sand (210 g) were alternately added to obtain a heterogeneous interface with presence of zones where sand was trapped and zones with macropores. A water column (6–10 cm) was left at the surface of the sediment to simulate the overlying water of the river. Five centimetres above the sediment surface and 1, 5, 15, and 35 cm below the sediment surface, openings were present in the columns to collect water and fine sediment with a syringe (see details in Mermillod-Blondin et al. 2000*a*).

The experiments were performed in a constant temperature room at 15 ± 0.5°C, and the light was controlled on a 12 h light – 12 h dark cycle in the overlying water. In contrast, the sediment of the column was kept in the dark to suppress photoautotrophic growth. Dechlorinated tap water permanently aerated to keep high oxygen content was continuously fed into the upper part of the four columns with the same peristaltic pump. Before entering the columns, this water was enriched with nitrate (potassium nitrate to obtain a final concentration of 20 $mg \cdot L^{-1}$ NO_3^-) and DOC (2 $mg \cdot L^{-1}$ potassium acetate) with another peristaltic pump. These concentrations of nutrients and DOC were chosen according to values measured in the braided channels of the River Rhône (Bornette and Amoros 1991; Mermillod-Blondin et al. 2000*b*). The resulting infiltration rate in columns was 2.0 ± 0.1 $mL \cdot min^{-1}$ (1.53 $cm \cdot h^{-1}$). The first measurements were run 3 days after the beginning of the water flow so that the initial hydraulic properties were stabilized.

Experimental design

One experiment consisted of four columns: three columns with the same oligochaete assemblages (oligochaete columns) and one column without oligochaetes used as a control. A previous experiment performed with four control columns showed similar spatial and temporal patterns of solutes and microbes in the four columns during 20 days. No significant differences were measured among the four columns, whereas in all of them, the same significant differences appeared in solute and microbial distributions among depths and dates (Table 1). This low variability between control columns of the same experiment indicated that the use of one control per experiment was acceptable to assess the effects of worms on system functioning.

The four oligochaete treatments were (*i*) 50 individuals of *Limnodrilus* per column, (*ii*) 50 individuals of *Tubifex* per column, (*iii*) 100 individuals of *Limnodrilus* per column, and (*iv*) 100 individuals of *Tubifex* per column. The densities tested were equivalent to 6500 $individuals \cdot m^{-2}$ and 13 000 $individuals \cdot m^{-2}$ and approached tubificid densities of natural coarse sediments observed in Lake Esrom (Lindegaard and Dall 1988) or tubificid densities used in the columns filled with coarse-textured sediments by Chatarpaul et al. (1980). Oligochaetes were obtained from two sites: a dead arm of the Rhône about 20 km upstream of Lyon and the River Semène. All worms without capillary chaeta collected in the dead arm of the Rhône were represented by two species of the *Limnodrilus* genus (80% *Limnodrilus hoffmeisteri* and 20% *Limnodrilus claparedeanus*), whereas all worms collected in the River Semène belonged to the species *Tubifex tubifex*. Therefore, worms collected at these two sites were separated in two groups (*Limnodrilus* and *Tubifex*) without excessive manipulations that can damage the organisms. For acclimatization, the two genera of worms were kept separately under similar experimental conditions (granulometry and food) for more than 15 days before introduction into the columns. The first measurements (day 0) confirmed constency

Table 1. Test of the variability between four control columns of the same experiment (20 days) on chemical and microbial parameters using two-way ANOVA (p values).

Parameters	Tests	Factors	p value
O_2 concentrations	ANOVA column × depth	Column	0.47 (ns)
		Depth	0.0001 (***)
		Interaction	0.58 (ns)
	ANOVA column × date	Column	0.996 (ns)
		Date	0.94 (ns)
		Interaction	0.99 (ns)
Dissolved organic carbon concentrations	ANOVA column × depth	Column	0.63 (ns)
		Depth	0.003 (**)
		Interaction	0.12 (ns)
	ANOVA column × date	Column	0.87 (ns)
		Date	0.043 (*)
		Interaction	0.58 (ns)
NO_3^- concentrations at 35-cm depth	ANOVA column × date	Column	0.95 (ns)
		Date	0.02 (*)
		Interaction	0.996 (ns)
Total number of bacteria	ANOVA column × depth	Column	0.468 (ns)
		Depth	0.0001 (***)
		Interaction	0.335 (ns)
	ANOVA column × date	Column	0.635 (ns)
		Date	0.082 (ns)
		Interaction	0.792 (ns)
Number of ETS-active bacteria	ANOVA column × depth	Column	0.331 (ns)
		Depth	0.01 (*)
		Interaction	0.819 (ns)
	ANOVA column × date	Column	0.223 (ns)
		Date	0.0005 (***)
		Interaction	0.341 (ns)

Note: Significance levels at which the null hypothesis was rejected: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant; $n = 4$ for depths (5 cm above and 5 cm, 15 cm, and 35 cm below the sediment surface), and $n = 5$ for dates (days 0, 5, 10, 15, and 20). ETS-active bacteria were bacteria with an active electron transport system.

in conditions among the four columns before the introduction of the tubificid worms. After this control, tubificid worms were introduced into the overlying water of each oligochaete column. To eliminate the variability in worm population from one experiment to another, all tubicid worms used had a biovolume of 4 mm^3 (15- to 20-mm length and 5-mm diameter).

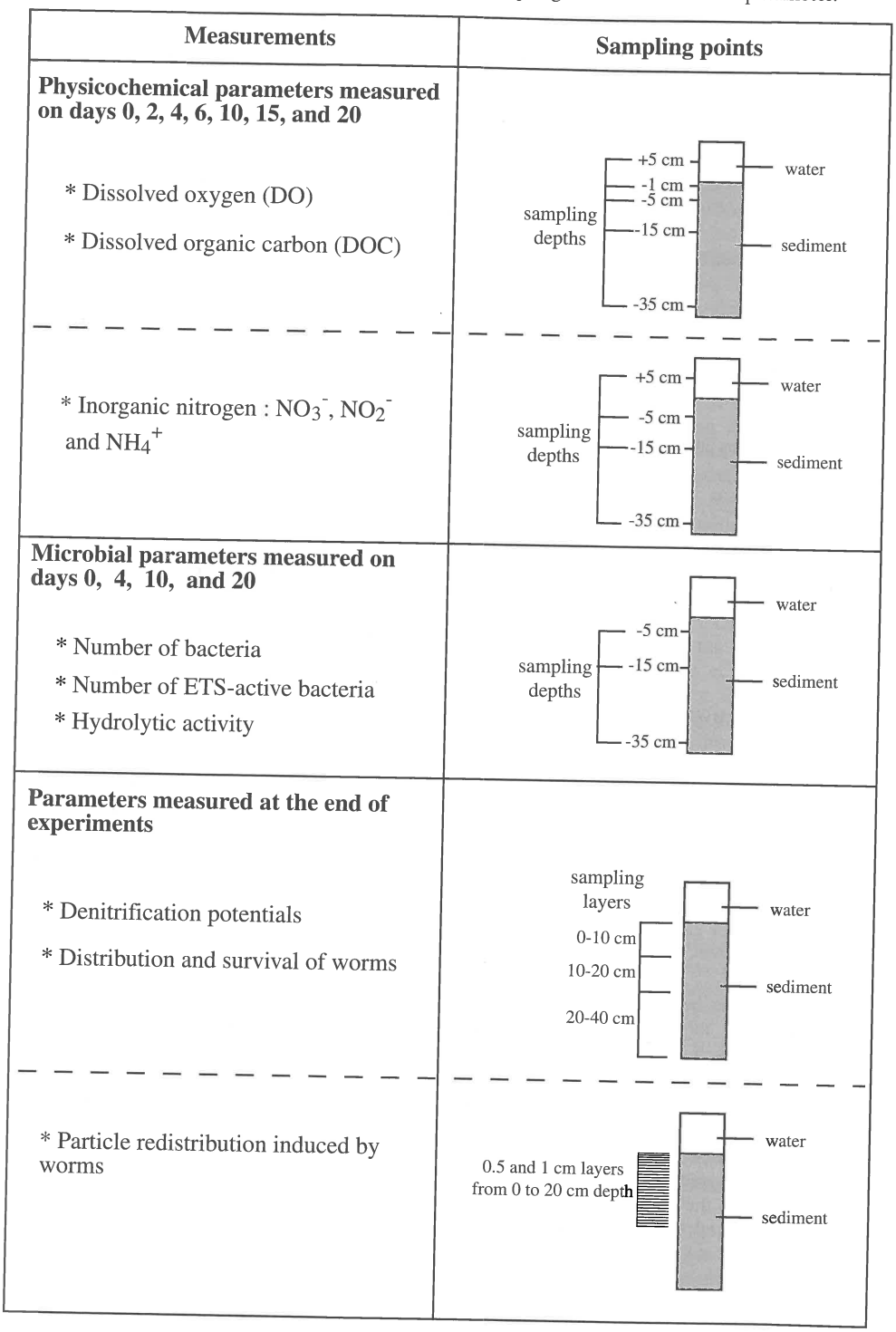
During the 20 days of the experiment, physical, chemical, and bacteriological parameters were measured at different depths and dates in the four columns to evaluate the role of the oligochaetes (Fig. 1). Particle redistribution induced by worms and denitrification potentials of the microorganisms developed on sediment were evaluated at the end of the experiments. Dissolved oxygen (O_2) and DOC concentrations were measured on days 0, 2, 4, 6, 10, 15, and 20 at five depths (5 cm above the sediment surface and 1, 5, 15, and 35 cm below the sediment surface). On the same days, nitrate, nitrite, and ammonium concentrations were measured at four depths (5 cm above the sediment surface and 5, 15, and 35 cm below the sediment surface). Microbial parameters (bacterial abundance, number of ETS-active bacteria, and hydrolytic activity) were measured in the sediment sampled with a syringe at three depths (5, 15, and 35 cm below the sediment surface) on days 0, 4, 10, and 20 of the experiment. These microbial parameters were not measured at a depth of 1 cm because there was not enough sand for analysis at this depth.

According to a previous study (Mermillod-Blondin et al. 2000*a*), NO_3^- concentrations were only presented at the 35-cm depth where they were used to indicate anaerobic processes (denitrification) in the columns. In the same way, DOC results were considered at depths of 1 cm and 35 cm as indicators of aerobic mineralization in surface sediment and anaerobic processes in deeper layers, respectively.

Methods of analysis

Particle redistribution in the columns was quantified by the luminophore tracer technique (Gérino et al. 1994). To reproduce the grain-size heterogeneity of the sand present in the columns, two size fractions of luminophores (natural sediment particles dyed with fluorescent paint) were used: 63- to 100-μm particles (pink) and 100- to 350-μm particles (yellow). These two fractions were cumulated in the results analysis. On day 0 of each experiment, a mixture of the two fractions (0.5 g per fraction) was deposited at the surface of the sediment of the four columns before oligochaete introduction. After a 20-day period, water was evacuated from all columns, and the columns were opened to sample sediment and luminophores. The top 2 cm of each column were collected in 0.5-cm layers, and the next 18 cm were collected in 1-cm layers. Each layer was homogenized and a 1-g subsample was dried at 50°C for luminophore counting. The number of luminophores was estimated under an ultraviolet (UV) light microscope and converted into grams of tracers per gram of dry sediment. The effect of oligochaetes on

Fig. 1. Schematic representation indicating the dates and positions of sampling for each measured parameter.



particulate matter transport was estimated with a diffusive–advective type transport model (Gérino et al. 1994) adapted to simulate vertical distributions of luminophores. Biological diffusive transport (D_b, $cm^2 \cdot year^{-1}$) was defined as omnidirectional transport in the sediment column, by analogy with molecular diffusion in water. The biodiffusion generates a spreading of tracers in the sediment column (Guinasso and Schink 1975) by exchanges of small amounts of material with adjacent sediment. In control columns with continuous water flowing through the sediment column, tracer migration is due to hydrodynamic effects and gives an estimation of physical diffusion D. In fauna columns, the diffusive term is called total diffusion (D_t) and corresponds to the addition of both diffusive transport (D) and biodiffusion (D_b). Vertical bioadvective transport (V_b, $cm \cdot year^{-1}$) is created by head-down deposit feeders such as tubificid worms that feed in the sediment and defecate pellets at the sediment surface (conveyor-belt organisms). This mode of feeding (called bioadvection) accelerates the rate of sediment and pore water burial within the feeding zone. The global

bioadvective coefficient V_t takes into account hydrodynamic effects (V) and bioadvective transport (V_b). The fundamental model equation for tracer distribution in experimental columns is

$$(1) \qquad \frac{\partial C}{\partial t} = D_t \frac{\partial^2 C}{\partial z^2} - V_t \frac{\partial C}{\partial z}$$

where t = time, z = depth, and C = tracer concentration. Luminophores are typically conservative tracers so that the model does not include a reactive term.

Luminophores were added as pulse input at the surface of the sediment at the beginning of each experiment so that the model was applied under non-steady-state conditions. Thus model eq. 1 was used with the upper boundary condition of an instantaneous source of unit strength (maximal C of tracer) at $z = 0$ at $t = 0$, a lower boundary $C \rightarrow 0$ at $z \rightarrow \infty$, and initial condition $C = 0$ at $z > 0$. The general solution was given by Officer and Lynch (1982) as

$$(2) \qquad c(z,t) = \frac{1}{\sqrt{\pi D t}} \exp\left(-\frac{(z - Vt)^2}{4Dt}\right) - \frac{V}{2D} \exp\left(\frac{Vz}{2D}\right) \operatorname{erfc} \frac{z + Vt}{\sqrt{4Dt}}$$

where

$$\operatorname{erfc}(x) = 1 - \frac{2}{\sqrt{(\pi)}} \int_0^x e^{-t^2} dt$$

and c is a normalized concentration relative to unit input. Transport parameters for luminophores were estimated for each column using a least squares fit of the theoretical profile obtained with eq. 2 to the measured concentration pattern.

Oxygen concentration was measured using a circulating system provided with a Clarke-type electrode connected to an Orbisphere oxymeter (model 3600). This procedure allowed suppression of the contact between the water samples and atmospheric oxygen. Nitrate, nitrite, and ammonium contents were measured using colorimetric Hach methods (Hach Company, Loveland, Co.) after filtration through Whatman glass fiber filters (GF/F type, mesh 0.7 µm).

DOC was measured with a Dohrman DC80 total carbon analyser based on UV-promoted potassium persulfate oxidation (precision ±1%) after removing inorganic carbon with orthophosphoric acid (1 µL·mL^{-1}) and CO_2 stripping under 10 min of oxygen flow. In case an increase in DOC versus depth was noted in a column, additional analyses by Capillary Ion Analyzer were performed to detect the possible occurrence of volatile fatty acids in the interstitial water outlet of the column (as observed in Mermillod-Blondin et al. 2000*a*).

Total bacteria were estimated by epifluorescence after DAPI staining (4′,6-diamino-2-phenylindole, Porter and Feig 1980). Wet sediment (0.3–0.5 g) was dispersed in 40 mL of filtered (on a 0.2-µm acetate membrane) distilled water and 10 mL of a solution of pyrophosphate (0.002 M, final concentration) by sonication (Bioblock Vibracell 72041, for 180 s, power 2, duty cycle 50%). One millilitre of the suspension was filtered through a Whatman glass fiber filter (GF/D type, mesh 2.7 µm) to eliminate the coarsest particles. Staining was performed by addition of 0.1 mL DAPI solution (100 µg·L^{-1}) to 1 mL of the filtrate. After 10 min of incubation, the suspension was filtered on a GTBP Millipore membrane (mesh 0.2 µm), which was then washed, air-dried, and mounted in low-fluorescence immersion oil on a microscope glass slide. Bacterial counts were performed using an epifluorescence microscope (Zeiss microscope fitted with a 200-W mercury burner and a Zeiss 365-nm excitation filter). At least 30 randomly selected fields were counted per slide. Results were expressed as numbers of bacteria per gram of dry sediment.

The number of ETS-active bacteria was measured using CTC staining (5-cyano-2,3-ditolyltetrazolium chloride, Rodriguez et al. 1992). Wet sediment (0.3–0.5 g) was incubated with a 1.38 mg·L^{-1} CTC solution (final concentration) for 3 h at 20°C and then sonicated (same sonication as for DAPI with a dilution in 20 mL of pyrophosphate solution). Five millilitres of the suspension was filtered through a Whatman glass fiber filter (GF/D type, mesh 2.7 µm). The filtrate was filtered through a GTBP Millipore membrane (mesh 0.2 µm), which was then washed, air-dried, and mounted in low-fluorescence immersion oil on a microscope glass slide. Bacteria were counted with an epifluorescence microscope fitted with a 200-W mercury burner, a blue (420-nm) excitation filter (Zeiss model BP 390-420), and a 540-nm barrier filter (Zeiss model LP 540). At least 30 randomly selected fields were counted per slide. Results were expressed as numbers of bacteria per gram of dry sediment.

Hydrolytic activity was measured using fluorescein diacetate (FDA) as substrate for hydrolases (Fontvieille et al. 1992). Wet sediment (0.5–1 g) was incubated in 3 mL of phosphate buffer (pH 7.6) with 0.1 mL FDA solution (2 mg·mL^{-1}) at 20°C in the dark until the green colour of fluorescein became visible (1 to 3 h). The reaction was stopped by freezing the sediment after addition of 3 mL of mercuric chloride solution (200 mg·L^{-1}). The absorbance of this solution at 490 nm was measured after filtration through a acetate membrane (HAWP type, mesh 0.45 µm). Results were expressed as micromoles of hydrolysed FDA·h^{-1}·g dry weight^{-1}.

At the end of each experiment, wet sediments in each column were collected from three depths: 0-10 cm, 10–20 cm, and 20–40 cm h. Sediments were then dried at 30°C for 1 week, and sand was separated from gravel by filtration on a 1-mm mesh net. Denitrification potentials were measured according to the method described by Lensi et al. (1985). Dry sand (10 g) was placed in 150 mL flasks and 3 mL of sterilized distilled water containing KNO_3 (0.1 mg N·g^{-1} dry sand), glucose (1 mg C·g^{-1} dry sand), and glutamic acid (1 mg C·g^{-1} dry sand) were added. The mixture was gently homogenized. The flasks were then sealed with rubber stoppers. The air of each flask was evacuated and replaced by a 90:10 He–C_2H_2 mixture to provide anaerobic conditions and inhibition of N_2O reductase activity. After incubation at 24°C for 24 h, gas samples were analyzed for N_2O with an electron capture gas-detector chromatograph Varian 3400 Cx. Denitrification potentials were expressed as milligrams of N_2O-N produced per gram of sediment. As a complementary analysis, denitrification potentials were also measured on three particle sizes of the sediment (gravel, coarse sand (>315 µm), and fine sand (<315 µm)) to explain the importance of sediment size on denitrification potentials. This analysis was performed using sediments collected in the control column of the experiment with 100 *Tubifex*. Six replicate samples of each sediment grain size were analysed according to the method previously described (Lensi et al. 1985).

At the end of each experiment, the living worms were counted at three depths (0–10 cm, 10–20 cm, and 20–40 cm) of each fauna column. The number of collected worms was used to estimate worm mortality and also to give information about the vertical distribution of worms in columns.

Data treatment

The effects of each oligochaete treatment were evaluated by comparing values obtained with and without oligochaetes for each experimental set, date, and variable. To describe the effect of oligochaete on processes (sediment fluxes and microbial activities), the use of percentage or ratio gave a direct estimation of stimulation compared to the processes in the control column. For number of bacteria, values were expressed as percentage of the control because a mean difference of 10^3 bacteria could be significant although it only represents 0.01% of bacteria present in control

Fig. 2. Vertical distributions of luminophores obtained in the oligochaete (solid) and control columns (open) of the experiment with 100 *Tubifex*. Values in oligochaete columns are means ± confidence intervals (n = 3).

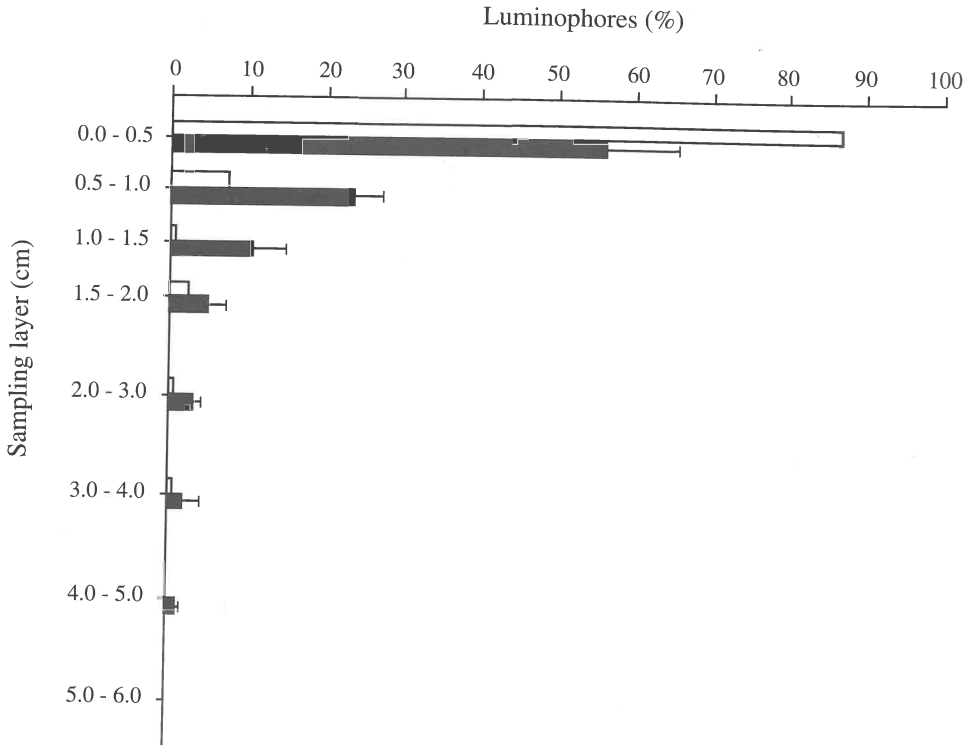


Table 2. Comparison of diffusive mixing rates of sediment estimated in control (D) and oligochaete (D_b) columns with the four oligochaete treatments.

Oligochaete treatment	D_b/D	p value
50 *Limnodrilus*	0.35	0.08 (ns)
50 *Tubifex*	0.71	0.13 (ns)
100 *Limnodrilus*	0.80	0.07 (ns)
100 *Tubifex*	4.0	0.008 (**)

Note: Oligochaete impact on transport intensity is expressed as a ratio of average biodiffusion on simple diffusion measured in the control of the same experiment. ns, not significant; *, $p < 0.05$; **, $p < 0.01$.

columns (10^7 bacteria). In contrast, we used differences rather than percentages for chemical variables because the change expressed as a percentage was highly dependent on the control value. For example, a difference of 20% in O_2 concentration did not have the same significance with 2.0 mg·L^{-1} O_2 in control (difference of 0.4 mg·L^{-1}) as with 5 mg·L^{-1} O_2 in the control (difference of 1 mg·L^{-1}). The impact of oligochaete was clearly higher (250%) in the second case.

Therefore, oligochaete effects on particle distribution were estimated at the end of each experiment by the ratio between mean particle flux in oligochaete columns and the flux measured in the control column (n = 3, differences between three oligochaete columns and the control column of the same experiment). Microbial parameters were rapidly modified by tubificid worms, and significant effects were measured on day 4 of the experiment (as observed in Mermillod-Blondin et al. 2000*a*). Therefore, worm effects on microbial characteristics were estimated by calculating the average difference between oligochaete and control columns (expressed as a percentage of the control values) measured on days 4, 10, and 20 (n = 9, differences between three oligochaete columns and the control column of the same experiment at three dates). This analysis indicated the mean effect of each worm treatment on microbial parameters at each depth during the experiment. Despite a rapid modification of microbial parameters by the worms, the impact of oligochaetes on O_2, DOC, and NO_3^- concentrations was observed only after 10 days of the experiment (as observed in Mermillod-Blondin et al. 2000*a*). Therefore, the effect of oligochaetes on these solutes was estimated on the last two sampling dates when both aerobic and anaerobic processes were modified by the worms. For these variables, we calculated the average differences between oligochaete and control columns measured on days 15 and 20 at each depth (n = 6, differences between three oligochaete columns and the control column of the same experiment at two dates).

For each experimental set, parametric t tests (in comparison to the theoretical value of 0, Sokal and Rohlf 1969) were performed on the differences calculated between the oligochaete column data and the control column data for each depth and each variable. This analysis made it possible to test the null hypothesis that oligochaete treatment did not create changes in sediment redistribution, nutrient concentrations, and microbial distributions. When $p <$ 0.05, differences were considered significant and were indicated in the figures with an asterisk (*). Parameters expressed as percentages (sediment fluxes and microbial parameters) were arcsine transformed (Sokal and Rohlf 1969). To test the difference between the effects of *Limnodrilus* and those of *Tubifex*, parameters expressed as ratios (particle fluxes), differences (chemical parameters), and percentages of the difference (microbial parameters) between the fauna and control columns were compared using the parametric t test for each density (comparison of the effects of *Limnodrilus* and those of *Tubifex* at both densities). Denitrification potentials obtained from three sediment grain sizes were compared using a one-way analysis of variance (ANOVA). Subsequently, Fisher's PLSD (protected least significant difference) comparisons were used to evaluate differences in denitrification potentials between each sediment grain size. Data on number of living worms found at the end of experiments were expressed as percentages and

Table 3. Average values of O_2 concentrations ($mg \cdot L^{-1}$) measured during days 15 and 20 at the five depths in control and oligochaete columns for the four experiments.

	Oligochaete treatment							
	50 *Limnodrilus*		50 *Tubifex*		100 *Limnodrilus*		100 *Tubifex*	
Sampling depth	Control	With fauna	Control	With fauna	Control	With fauna	Control	With fauna
+5 cm	5.7 (0.39)	5.37 (0.26)	5.15 (0.49)	4.97 (0.17)	5.55 (0.49)	5.72 (0.16)	5.95 (0.10)	5.77 (0.07)
–1 cm	3.7 (0.05)	3.15 (0.42)	3.4 (0.20)	2.7 (0.26)	3.95 (0.49)	3.48 (0.18)	3.3 (0.20)	2.93 (0.11)
–5 cm	2.5 (0.20)	2.32 (0.14)	2.85 (0.29)	2.12 (0.06)	2.6 (0.20)	2.0 (0.16)	2.7 (0.20)	2.02 (0.12)
–15 cm	2.0 (0.05)	1.9 (0.07)	2.1 (0.05)	1.95 (0.08)	1.95 (0.10)	1.38 (0.15)	2.05 (0.10)	1.72 (0.09)
–35 cm	1.85 (0.10)	1.78 (0.06)	1.85 (0.10)	1.67 (0.11)	1.67 (0.11)	1.17 (0.07)	1.95 (0.10)	1.55 (0.08)

Note: Values in parentheses are confidence intervals.

were arcsine transformed. The percentage of living worms was compared between oligochaete treatments using a one-way ANOVA. The numbers of worms found at the three sampling layers were also expressed as percentages and were arcsine transformed. The effects of depth and oligochaete treatment on worm distributions were tested using a two-way ANOVA (depth × experiment). Statistical analyses were performed using Statview F-5.0 (SAS Institute Inc., Cary, N.C.).

Results

Effect of worms on particle redistribution

Tracer distributions in control and all fauna columns exhibited an exponential decrease with sediment depth typical of diffusion-like transport without any occurrence of advection processes (as in the experiment with 100 *Tubifex*, Fig. 2). A small quantity of the tracer was found below 1.5 cm in the control while the tracer occasionally migrated 4 cm down into the sediment when oligochaetes were present (Fig. 2). Biological change expressed as the ratio between the average diffusion coefficients ($n = 3$) estimated with oligochaetes and the diffusion coefficient estimated in the control column of the same experiment are indicated in Table 2. Fifty *Limnodrilus* or 50 *Tubifex* per column did not significantly modify surface particle distribution. However, 100 *Tubifex* per column significantly increased the diffusive particle transport, whereas 100 *Limnodrilus* had no effect on particle distribution.

Effects of worms on O_2 contents

In all columns, DOC showed a sharp drop with depth, but O_2 concentration at 35 cm was always higher than 1 $mg \cdot L^{-1}$ (Table 3). All oligochaete treatments significantly decreased O_2 concentrations at all depths in the sediment (Fig. 3). The differences in O_2 concentrations were greatest in the first 5 cm of the sediment. At 15 cm and 35 cm, these differences were lower except with 100 *Limnodrilus*. With a density of 50 worms per column, no significant differences were measured between the effects of *Limnodrilus* and those of *Tubifex* on O_2 concentration at depths of 1, 15, and 35 cm. However, a significantly higher O_2 depletion was measured with *Tubifex* than with *Limnodrilus* at 5 cm ($p < 0.001$, t test). With a density of 100 worms per column, the effects of *Tubifex* and *Limnodrilus* on O_2 concentration were not significantly different at 1 and 5 cm in the sediment. However, at depths of 15 and 35 cm, *Limnodrilus* produced a higher O_2 depletion than *Tubifex* ($p < 0.05$, t test).

Effect of worms on DOC and NO_3^- contents

At 1 cm, 50 *Tubifex* had a significantly different effect on DOC concentration, whereas 50 *Limnodrilus* did not(Fig. 4*a*, $p < 0.05$, t test). The presence of *Tubifex* produced lower DOC concentration than in the control, whereas the presence of *Limnodrilus* had no effect on DOC concentration. In contrast, both oligochaete treatments with 100 tubificid worms significantly decreased DOC concentration at 1 cm. At 35 cm, despite no significant effect resulting from the presence of 50 and 100 *Limnodrilus* per column, DOC concentrations tended to increase in all experiments with the same intensity (Fig. 4*b*). Complementary analyses indicated that this DOC production was due to volatile fatty acids (malic, acetic, and propionic acids). At the two densities, *Tubifex* and *Limnodrilus* did not produce any significantly different effect ($p > 0.5$, t tests).

In contrast with DOC concentrations, the concentration of NO_3^- at 35 cm decreased with most oligochaete treatments (Fig. 4*c*). No significant differences in decrease of NO_3^- concentration were measured between the treatments with 50 *Limnodrilus* and 50 *Tubifex* per column ($p > 0.2$, t test), and the same result occurred with 100 individuals of each genus ($p > 0.5$, t test).

Effect of worms on microbial parameters

With 50 worms per column, *Tubifex* significantly increased the total number of bacteria at 5 and 35 cm, whereas *Limnodrilus* had no significant effect on this parameter (Fig. 5*a*). However, there were no significant differences between the effects of the two genera on total numbers of bacteria at all depths ($p > 0.1$, t tests). At a density of 100 worms, *Limnodrilus* and *Tubifex* had similar effects on bacterial abundance at the three depths ($p > 0.5$, t tests) despite the fact that the worm effects were not always significant in comparison with the control (*Tubifex* at 5 and 15 cm).

With 50 worms per column, *Limnodrilus* and *Tubifex* produced significantly different changes on number of ETS-active bacteria at depths of 5 ($p < 0.03$, t test) and 15 cm ($p < 0.01$, t test). *Tubifex* sp. significantly increased the number of ETS-active bacteria at 5 cm, whereas *Limnodrilus* had no effect (Fig. 5*b*). In contrast, at 15 cm, *Tubifex* had a negative effect on the number of ETS-active bacteria, whereas *Limnodrilus* increased, but not significantly, this parameter. At 35 cm, *Limnodrilus* and *Tubifex* did not significantly modify the number of ETS-active bacteria. At a density of 100 worms, the two genera created a similar and significant increase in numbers of ETS-active bacteria at depths of 5 and 15 cm ($p > 0.05$, t tests). However, at 35 cm, the two

Fig. 3. Differences in O_2 concentrations ($mg \cdot L^{-1}$) between fauna and control columns for each oligochaete treatment (open, 50 *Limnodrilus*; solid, 50 *Tubifex*; shaded, 100 *Limnodrilus*; cross-hatched, 100 *Tubifex*) at (*a*) 1 cm, (*b*) 5 cm, (*c*) 15 cm, and (*d*) 35 cm below the sediment surface. Values are means ± confidence intervals ($n = 6$). * indicates a significant difference between control and oligochaete columns.

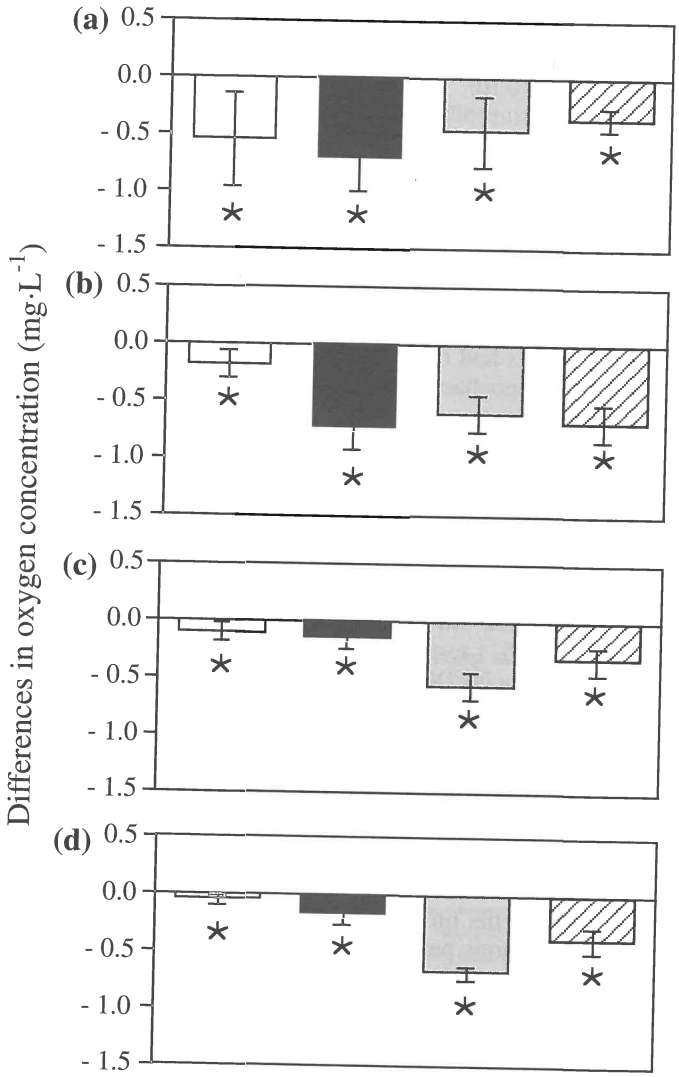
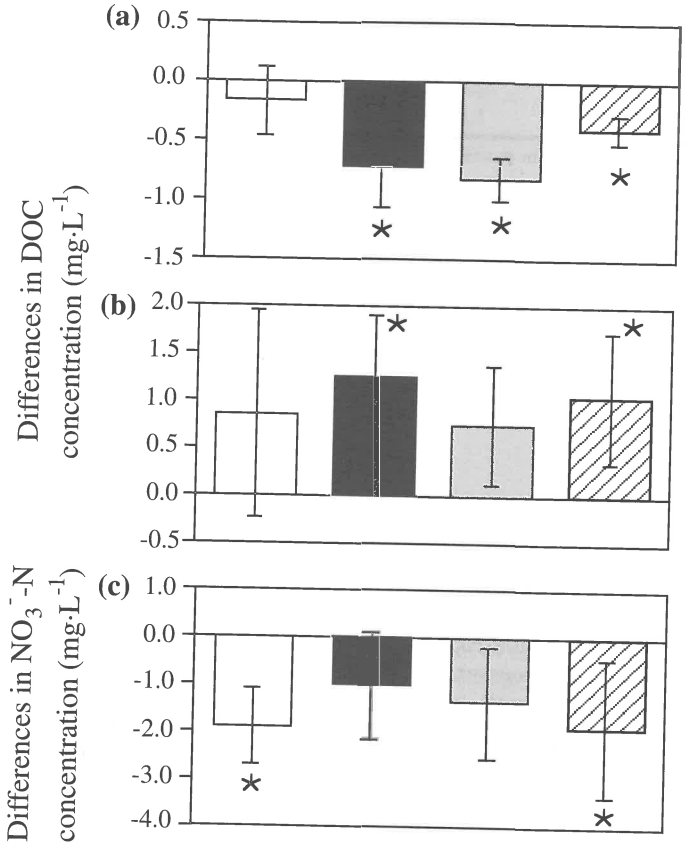


Fig. 4. Differences in DOC at (*a*) 1 cm and (*b*) 35 cm and in (*c*) NO_3^--N concentrations at 35 cm between fauna and control columns for each oligochaete treatment (open, 50 *Limnodrilus*; solid, 50 *Tubifex*; shaded, 100 *Limnodrilus*; cross-hatched, 100 *Tubifex*). Values are means ± confidence intervals ($n = 6$). * indicates a significant difference between control and oligochaete columns.



genera had significantly different effects on active bacteria ($p < 0.05$, *t* test). *Limnodrilus* significantly increased the number of active bacteria, whereas the 100 *Tubifex* had no effect on this parameter.

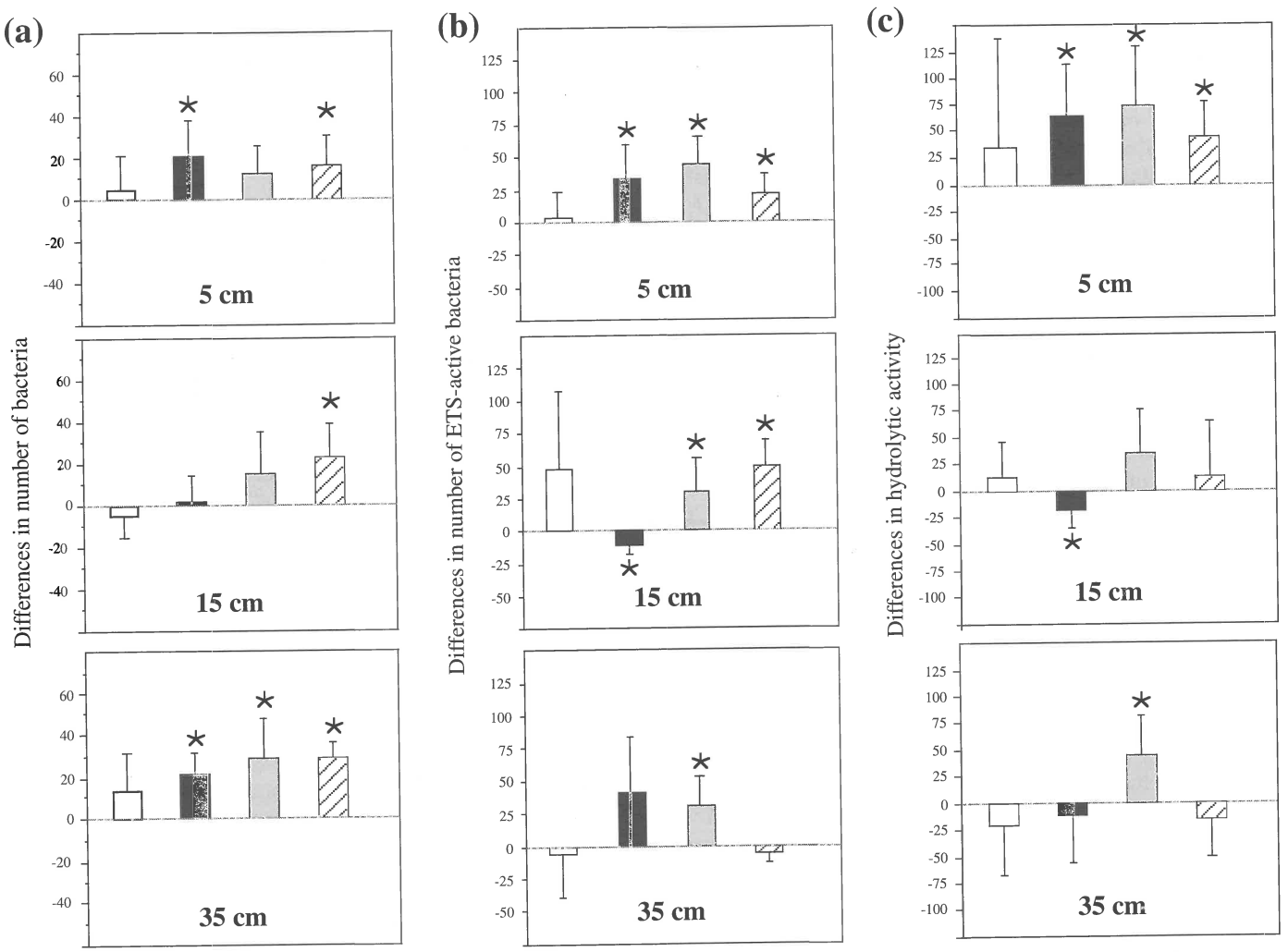
All oligochaete treatments stimulated hydrolytic activity at 5 cm below the sediment surface except the 50 individuals of *Limnodrilus* (Fig. 5*c*). With 50 oligochaetes per column, despite no significant differences between the two genera at the three depths ($p > 0.15$, *t* tests), *Limnodrilus* did not significantly modify the hydrolytic activity, whereas *Tubifex* had a positive effect at 5 cm and a negative effect at 15 cm. With a density of 100 oligochaetes, *Limnodrilus* and *Tubifex* did not produce significantly different changes in hydrolytic activity except at 35 cm ($p < 0.02$, *t* test), where only *Limnodrilus* had a positive and significant effect.

The differences in denitrification potentials resulting from oligochaete treatments are shown in Table 4. Although the presence of worms significantly modified the denitrification potentials in three cases, no clear effect of all oligochaete treatments was observed owing to a high heterogeneity. The denitrification potentials measured on three particle sizes (gravel, coarse sand (>315 µm), and fine sand (<315 µm)) were clearly higher in fine sand (22.9 ± 3.6 mg N_2O-N·g^{-1} sediment) than in the other two fractions ($p < 0.001$, one-way ANOVA), which showed no significantly different values (3.6 ± 0.4 mg N_2O-N·g^{-1} sediment in the coarse sand and 4.1 ± 1.2 mg N_2O-N·g^{-1} sediment in the gravel ($p = 0.72$, Fisher's PLSD)).

Estimated mortality and distribution of worms at the end of the experiments

Whatever the experiment, the percentage of living worms varied from 79% to 90% (Table 5) and was not significantly different among experiments ($p = 0.86$, one-way ANOVA). The number of worms found at each layer of sediment showed that most worms (60–86%) were observed in the first 10 cm of sediment ($p < 0.001$, depth effect, two-way ANOVA). Furthermore, only individuals of *Limnodrilus* were found in the deeper layer of sediment at the end of the experiment using 100 *Limnodrilus* (Table 5). However, for

Fig. 5. Differences between fauna and control columns (expressed as percentages of the controls) in numbers of (*a*) bacteria and (*b*) bacteria with an active electron transport system (ETS-active bacteria) and (*c*) hydrolytic activity at three depths for each oligochaete treatment (open, 50 *Limnodrilus*; solid, 50 *Tubifex*; shaded, 100 *Limnodrilus*; cross-hatched, 100 *Tubifex*). Values are means ± confidence intervals (n = 9). * indicates a significant difference between control and oligochaete columns.



the two tested densities of worms, no significant differences were measured between the vertical distribution of *Limnodrilus* and that of *Tubifex* ($p > 0.98$, experiment effect, two-way ANOVA).

Discussion

Our results show that oligochaete effects on sediment redistribution, aerobic respiration, denitrification, and organic matter mineralization depend on the worm populations. However, before interpreting worm effects, the physical characteristics of the system must be briefly discussed and linked to the biogeochemical functioning.

Physical heterogeneity of the system

As observed in a previous study (Mermillod-Blondin et al. 2000*a*), the occurrence of denitrification (NO_3^- decrease) and fermentative processes (production of volatile fatty acids resulting from the anaerobic degradation of the cellulose powder) at O_2 concentrations (mean values measured at each sampling depth) always above 1.0 mg·L^{-1} indicated that anaerobic processes occurred locally in microenvironment. The high physical and chemical (solution and solid phase) heterogeneity of the porous medium controlled the local availability of nutrients, which led to a heterogeneous distribution of microbial activities (as in Murphy et al. 1997). For example, the strong differences in denitrification potentials measured between three sediments with different particle sizes was in accordance with other studies (Dodds et al. 1996; Griebler 1996) and indicated a preponderant effect of particle size and particle distribution on microbial colonization and the occurrence of aerobic and anaerobic microenvironments. Thus, the experimental system presented a physical heterogeneity similar to that observed in studies on the hyporheic zone (Danielopol 1989; Triska et al. 1993; Mermillod-Blondin et al. 2000*b*).

Functional redundancy of *Limnodrilus* and *Tubifex*

The hypothesis that the representatives of the two genera were redundant in coarse sediments was validated with respect to most of the measures used to assess their impact on the system. *Limnodrilus* and *Tubifex* clearly produced the same kind of change on chemical parameters resulting from

Table 4. Differences in denitrification potential between oligochaete treatment and control (expressed as percentages) for each experiment at three layers of sediment.

Oligochaete treatment	Sampling layers (cm)	Mean differences	95% CI	Oligochaete treatment vs. control p value
50 *Limnodrilus*	0–10	–34.31	13.11	0.036 (*)
	10–20	–10.31	46.62	0.707 (ns)
	20–40	–45.72	68.56	0.321 (ns)
50 *Tubifex*	0–10	934.56	187.09	0.001 (**)
	10–20	138.39	289.04	0.447 (ns)
	20–40	–10.67	68.21	0.788 (ns)
100 *Limnodrilus*	0–10	88.33	84.87	0.177 (ns)
	10–20	28.33	54.69	0.447 (ns)
	20–40	152.86	134.18	0.157 (ns)
100 *Tubifex*	0–10	15.70	23.02	0.313 (ns)
	10–20	–32.30	46.84	0.309 (ns)
	20–40	616.63	58.8	0.002 (**)

Note: Mean differences and confidence intervals (CI) of differences (n = 3). Results of Student's t tests performed to compare oligochaete treatment and control are indicated in the last column. ns, not significant; *, $p < 0.05$; **, $p < 0.01$.

Table 5. Numbers of living worms found at the end of the four experiments in oligochaete columns at three sediment layers.

	Oligochaete treatment			
	50 *Limnodrilus*	50 *Tubifex*	100 *Limnodrulus*	100 *Tubifex*
Oligochaete column 1				
0–10 cm	41	39	61	74
10–20 cm	3	2	16	8
20–40 cm	0	0	2	0
% of living worms	88	82	79	82
Oligochaete column 2				
0–10 cm	35	38	77	76
10–20 cm	5	4	8	9
20–40 cm	0	0	0	0
% of living worms	80	84	85	85
Oligochaete column 3				
0–10 cm	40	43	82	77
10–20 cm	0	1	6	4
20–40 cm	0	0	2	0
% of living worms	80	88	90	81

Note: Estimated percentages of living worms were indicated for each fauna column.

microbial processes. As observed by many authors in other aquatic systems (Chatarpaul et al. 1980; Torreiter et al. 1994; van de Bund et al. 1994), tubificid worms played a significant role in the processing of organic matter both in aerobic and anaerobic conditions. Most measurements (oxygen concentrations, number of bacteria, and hydrolytic activity) indicated that 100 *Limnodrilus* and 100 *Tubifex* did not produce any significantly different increase in microbial activity in the first 5 cm of the sediment. At the 35-cm depth, the results of NO_3^- and DOC measurements showed that *Limnodrilus* and *Tubifex* also had no significantly different effects on anaerobic processes.

As observed in a previous study (Mermillod-Blondin et al. 2000*a*), O_2 concentrations rapidly declined with sediment depth, presumably owing to microbial respiration. Columns with oligochaetes showed significantly higher uptakes of O_2 in the sediment than did control columns. As the respiration of the worms can only explain less than 10% of the maximum effect of each oligochaete treatment on O_2 concentration (McCall and Fisher 1980), higher O_2 uptakes in oligochaete columns undoubtedly resulted from interactions between oligochaetes and bacteria. The greater effect of most worm treatments on O_2 concentrations at 1-cm and 5-cm depths in comparison with 15-cm and 35-cm depths (except in the experiment with 100 *Limnodrilus*) suggests that the two genera of worms modified predominantly the functioning of the first 5 cm of the sediment. By stimulating microbial respiration, tubificid worms created a difference in O_2 concentration between control and oligochaete columns in the upper layer. This difference was smaller deeper in the sediment because respiration was lower in oligochaete columns than in control columns. In the upper layer of the control columns, O_2 and DOC concentrations were greater than in the oligochaete columns, thus allowing a higher microbial respiration in deeper

layers. The higher impact of tubificid worms in the upper layer of the sediment was confirmed by the high percentage of worms found in the 0- to 10-cm layer at the end of the experiments (more than 75%), indicating that worm activities were concentrated in the first 10 cm of the sediment.

The functional redundancy of the representatives of the two genera was certainly due to the fact that *Limnodrilus* and *Tubifex* have similar feeding and bioturbation activities in the coarse sediments of the hyporheic system. As indicated by luminophore profiles, there was no advective transport of luminophores in the sediment owing to oligochaete activity or downward water flow. In contrast, tubificid worms produce a bioadvection (i.e., sediment mixing resulting from particle ingestion within the sediment and egestion at the surface) of surface particles in the fine sediments of lakes (McCall and Tevesz 1982). This biologically induced sediment transport was certainly not measured in our experimental system because most sediment grains were too coarse to be ingested (>200 µm, Juget 1979) by the tested worms. In these conditions, we can suppose that tubificid worms fed selectively on fine particles (cellulose powder) in experimental columns, according to the findings of Rodriguez et al. (2000) for *Tubifex tubifex*. Although biologically mediated sediment fluxes were not intense owing to the low fine sediment fraction in the column, 100 *Tubifex* significantly increased the diffusive transport of luminophores in columns. This sediment transport might be due to the presence of tubes and burrows observed in sandy zones presents on inner wall of the columns in all oligochaete experiments. These structures may act as supplementary pathways that facilitated surface tracer propagation in the sediment column (diffusive transport of luminophores). However, if the two genera produced tubes and burrows, that 100 *Tubifex* had a significant effect on luminophore transport whereas 100 *Limnodrilus* did not suggested differences in depth burrowing between the two groups of worms (discussed below).

By feeding on fine particles and associated microflora, the oligochaetes kept the biofilm in an active physiological state, stimulating the microbial activity. Tubificid worms are well known to be head-down deposit feeders that defecate pellets with lower numbers of bacteria at the sediment surface (Wavre and Brinkhurst 1971). Despite low content of fine sediment in the experimental conditions, faecal pellets were observed at the sediment surface, indicating that worms could increase the colonizable area for bacteria by modifying sediment structure at the water–sediment interface. Furthermore, the continuous creation of new burrows and pathways also modified the dispersion of the water flux in the sediment. By increasing the flux dispersion, oligochaetes enhanced the nutrient availability in all microenvironments of the columns, stimulating both aerobic and anaerobic microbial activities. Therefore, the stimulation of anaerobic processes in the deeper layers was not only linked to the low oxygen concentrations induced by oligochaetes but it could also be due to a better water flow dispersion through the burrows. Burrowing by oligochaetes favoured the introduction of NO_3^- into new and deeper reactive niches, as those described by Murphy et al. (1997), where denitrification occurred. This effect was clearly exemplified by the presence of 50 *Limnodrilus*, which decreased NO_3^- concentration at the 35-cm depth but had a low impact on oxygen concentration at the same depth (O_2 depletion owing to 50 *Limnodrilus* was 0.07 $mg \cdot L^{-1}$). Furthermore, if potential denitrification was not significantly modified by oligochaetes, these worms clearly modified the expression of this potentiality in the columns. Thus, oligochaete activities such as biofilm feeding, faecal pellet production, and new pathway creation should all produce a stimulation of microbial processes. The particle redistribution may also increase microbial activity in the deeper strata of the sediment by increasing the quantity of particulate organic matter.

Death of worms in the columns was estimated at the end of the experiments as 10–21% of the fauna introduced. Decomposition of worms could serve as carbon for bacteria and might increase microbial processes. However, no difference in DOC production was observed at the 35-cm depth between oligochaete treatments, whereas twofold higher numbers of worms did not survive in experiments with 100 individuals (mean = 16 dead worms per column) in comparison with experiments with 50 individuals (mean = 8.2 dead worms per column). This result indicates that DOC was not linked to the number of dead worms, suggesting that the decay of few individuals had no significant effect on microbial processes.

Despite a functional redundancy observed for most consequences of their biological activities, some dissimilarity was observed between the effects of the two genera of tubificid worms. With a density of 50 oligochaetes per column, *Tubifex* produced a higher increase in aerobic microbial activity than did *Limnodrilus* in the first 5 cm of the sediment columns. This difference between worm activities could be linked to the water temperature of 15°C used in the experiments. Several studies (see review in McCall and Tevesz 1982) showed that the feeding rates of three species of worms were highly dependant on temperature. The feeding rate of *Tubifex tubifex* was found to be four times higher than that of *Limnodrilus hoffmeisteri* at 15°C, our experimental temperature. Therefore, that the effect of 50 *Tubifex* was higher than that of 50 *Limnodrilus* in our hyporheic system could be due to different optimum temperatures of the two genera. However, this result is not contrary to a redundancy of the two genera because two members of a same functional group produce effects of the same nature on ecosystem processes but with a possible difference in effect intensity (François et al. 1999).

At a density of 100 individuals per column, *Limnodrilus* and *Tubifex* modified the same environmental parameters but at different locations and with different intensities. *Limnodrilus* induced a greater O_2 depletion and a greater increase of biofilm parameters (bacterial activities) at the 35-cm depth. In contrast, the luminophore results showed that *Tubifex* induced greater changes in the top 5 cm than did *Limnodrilus* by producing significant particle redistribution. As observed by Juget (1984) in the alluvial plain of the French Upper Rhône, these results seem to indicate that *Limnodrilus* burrow deeper than *Tubifex* into the sediment in the experimental system. Despite no significant differences between *Limnodrilus* and *Tubifex*, only individuals of *Limnodrilus* were found in the deeper layer of the sediment at the end of experiments, suggesting a deeper penetration of

this genus in coarse sediments. In our experimental conditions, the low fraction of grains smaller than 63 μm could explain the better use of the sediment by *Limnodrilus* because this genus is more tolerant than *Tubifex* of low contents of clay and silt in the sediments (Sauter and Güde 1996). Juget (1979) reported that *Limnodrilus hoffmeisteri* could ingest a larger range of grain sizes than *Tubifex tubifex*. However, the differences in colonized depths between the two genera could also be due to other factors, such as differences in musculature. Juget (University of Lyon, Lyon, France, personal communication) found that *Limnodrilus hoffmeisteri* had a greater musculature (ratio between the longitudinal muscle diameter and animal diameter of 7.8 ± 1.5%) than *Tubifex tubifex* (4.3 ± 1.2%). Therefore, *Limnodrilus* might present a better adaptation to live deeper in the sediment than *Tubifex*. Further investigations would be useful to determine the relative importance of each factor on the differences in worm activity distributions.

In a laboratory experiment, François et al. (1999) measured differences in the intensity of sediment reworking between two bivalves of the same functional group. They pointed out that the specific variability within functional groups is crucial to link diversity and ecosystem function. The differences observed between *Limnodrilus* and *Tubifex* in the present study also indicate that the functional diversity within the functional group of tubificid worms cannot be neglected. For example, in natural systems, the differences in particle redistribution intensities between the two genera can have great consequences on system function because the hyporheic zone depends on the supply of organic matter from the surface (Danielopol 1989). Therefore, the two genera of tubificids function differently at the interface by modifying the transfer of organic matter into the hyporheic system (Gilbert et al. 1997). The present study also indicates that the functional redundancy of two species (or genera) cannot be observed in all ecosystem processes. In a sense, each species (or genus) has its functional peculiarity in an ecosystem.

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References

Bornette, G., and Amoros, C. 1991. Aquatic vegetation and hydrology of a braided river floodplain. J. Veg. Sci. **2**: 497–512.

Boulton, A.J. 2000. The functional role of the hyporheos. Verh. Int. Verein. Limnol. **27**: 51–63.

Brinkhurst, R.O. 1966. The Tubificidae (Oligochaeta) of polluted waters. Verh. Int. Verein. Limnol. **16**: 54–859.

Chapin, F.S., Schulze, E.-D., and Mooney, H.A. 1992. Biodiversity and ecosystem processes. Trends Ecol. Evol. **7**: 107–108.

Chatarpaul, L., Robinson, J.B., and Kaushik, N.K. 1980. Effects of tubificid worms on denitrification and nitrification in stream sediment. Can. J. Fish. Aquat. Sci. **37**: 656–663.

Danielopol, D.L. 1989. Groundwater fauna associated with riverine aquifers. J. N. Am. Benthol. Soc. **8**: 18–35.

Dodds, W.K., Randel, C.A., and Edler, C.C. 1996. Microcosms for aquifer research: application to colonization of various sized particles by ground-water microorganisms. Ground Water, **34**: 756–759.

Fontvieille, D., Outaguereouine, A., and Thevenot, D.R. 1992. Fluorescein diacetate hydrolysis as a measure of microbial activity in aquatic systems. Application to activated sludge. Environ. Technol. **13**: 531–540.

François, F., Dalègre, K., Gilbert, F., and Stora, G. 1999. Variabilité spécifique à l'intérieur des groupes fonctionnels. Etude du remaniement sédimentaire de deux bivalves Veneridae, *Ruditapes decussatus* et *Venerupis aurea*. C. R. Acad. Sci. Paris, **322**: 339–345.

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

Gilbert, F., Stora, G., Bonin, P., LeDréau, Y., Mille, G., and Bertrand, J.-C. 1997. Hydrocarbon influence on denitrification in bioturbated Mediterranean coastal sediments. Hydrobiologia, **345**: 67–77.

Griebler, C. 1996. Some applications for the DMSO-reduction method as a new tool to determine the microbial activity in water-saturated sediments. Arch. Hydrobiol. Suppl. **113**: 405–410.

Guinasso, N.L., Jr, and Schink, D.R. 1975. Quantitative estimates of biological mixing rates in abyssal sediments. Geophys. Res. **80**: 3032–3043.

Juget, J. 1979. La texture granulométrique des sédiments et le régime alimentaire des oligochètes limnicoles. Hydrobiologia, **65**: 145–154.

Juget, J. 1984. Oligochaeta of the epigean and underground fauna of the alluvial plain of the French Upper Rhône (biotypological trial). Hydrobiologia, **115**: 175–182.

Lawton, J.H. 1994. What do species do in ecosystems ? Oikos, **71**: 367–374.

Lazim, M.N., and Learner, M.A. 1987. The influence of sediment composition and leaf litter on the distribution of tubificid worms (Oligochaeta). Oecologia, **72**: 131–136.

Lensi, R., Gourbière, F., and Josserand, A. 1985. Measurement of small amounts of nitrate in an acid soil by N_2O production. Soil Biol. Biochem. **17**: 733–734.

Lindegaard, C., and Dall, P.C. 1988. Abundance and distribution of Oligochaeta in the exposed littoral zone of lake Esrom, Denmark. Arch. Hydrobiol. Suppl. **81**: 533–562.

McCall, P.L., and Fisher, J.B. 1980. Effects of tubificid oligochaetes on physical and chemical properties of Lake Erie sediments. *In* Aquatic oligochaete biology. *Edited by* R.O. Brinkhurst and D.G. Cook. Plenum Press, New York. pp. 253–317.

McCall, P.L., and Tevesz, M.J.S. 1982. The effects of benthos on physical properties of freshwater sediments. *In* Animal–sediment relations. The biogenic alteration of sediments. *Edited by* P.L. McCall and M.J.S. Tevesz. Plenum Press, New York. pp. 105–176.

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

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

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

Officer, C.B., and Lynch, D.R. 1982. Interpretation procedures for the determination of sediment parameters from time-dependent flux inputs. Earth Planet. Sci. Lett. **61**: 55–62.

Palmer, M.A., Covich, A.P., Finlay, B.J., Gibert, J., Hyde, K.D., Johnson, R.K., Kairesalo, T., Lake, P.S., Lovell, C.R., Naiman, R.J., Ricci, C., Sabater, F.F., and Strayer, D.L. 1997. Biodiversity and ecosystem processes in freshwater sediments. Ambio, **26**: 571–577.

Porter, K.G., and Feig, Y.S. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25**: 943–948.

Rodriguez, G.G., Phipps, D., Ishiguro, K., and Ridgway, H.F. 1992. Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. Appl. Environ. Microbiol. **58**: 1801–1808.

Rodriguez, P., Martinez-Madrid, M., Navarro, E., and Arrate, J.A. 2000. Selective feeding by *Tubifex tubifex* (Oligochaeta: Annelida). *In* VIII International Symposium on Aquatic Oligochaeta, 18–22 July, Bilbao. University of the Basque Country, Spain.

Sauter, G., and Güde, H. 1996. Influence of grain size on the distribution of tubificid oligochaete species. Hydrobiologia, **334**: 97–101.

Sokal, R.R., and Rohlf, J. 1969. Biometry: the principles and practice of statistics in biological research. W.H. Freeman and Co., Kent, England.

Torreiter, P., Pitaksintorn-Watanamahart, P., and Danielopol, D.L. 1994. The activity of oligochaetes in relation to their ecological role in slow filtration columns. *In* Second International Conference on Ground Water Ecology, 27–30 March, Atlanta. U.S. EPA, AWRA, Herndon, Va. pp. 85–94.

Triska, F.J., Duff, J.H., and 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.

van de Bund, W.J., Goedkoop, W., and Johnson, R.K. 1994. Effects of deposit-feeder activity on bacterial production and abundance in profundal lake sediment. J. N. Am. Benthol. Soc. **13**: 532–539.

Walker, B.H. 1992. Biodiversity and ecological redundancy. Conserv. Biol. **6**: 18–23.

Wavre, M., and Brinkhurst, R.O. 1971. Interaction between some tubificid oligochaetes and bacteria found in the sediments of Toronto Harbour, Ontario. J. Fish. Res. Board Can. **28**: 335–341.