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Sedimentary context controls the influence of ecosystem engineering by bioturbators on microbial processes in river sediments

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By modifying the physical environment, ecosystem engineers can have inordinately large effects on surrounding communities and ecosystem functioning. However, the significance of engineering in ecosystems greatly depends on the physical characteristics of the engineered habitats. Mechanisms underlying such context-dependent impact of engineers remain poorly understood even though they are crucial to establish general predictions concerning the contribution of engineers to ecosystem structure and function.

The present study aimed to decrypt such mechanisms by determining how the environmental context modulates the effects of ecosystem engineers (bioturbators) on microorganisms in river sediments. To test the effects of environmental context on the role of bioturbators in sediments, we used mesocosms and recreated two sedimentary contexts in the laboratory by adding a layer of either fine or coarse sand at the top of a gravel-sand matrix. For each sediment context, we examined how the sediment reworking activity of a bioturbating tubificid worm (*Tubifex tubifex*) generated changes in the physical (sediment structure and permeability) and abiotic environments (hydraulic discharge, water chemistry) of microorganisms. Microbial characteristics (abundances, activities) and leaf litter decomposition – a major microbially-mediated ecological process – were measured to evaluate the impact of bioturbation on biotic compartment.

Our results showed that the permeability, the availability of oxygen and the activities of microorganisms were reduced in sediments covered with fine sand, in comparison with sediments covered with coarse sand. *Tubifex tubifex* significantly increased permeability (by about six-fold), restored aerobic conditions and ultimately stimulated microbial communities (resulting in a 30% increase in leaf litter breakdown rate) in sediments covered with fine sand. In contrast *T. tubifex* had low effects in sediments topped by coarse sand, where O_2 was already available for hyporheic microorganisms. Our study supports the idea that context dependency mainly modulates the effects of engineering by controlling the ability of engineers to create changes on abiotic (O_2 in the present study) factors that are limiting for surrounding communities.

Habitat modification by engineer organisms has been recognized as a major ecological process with important consequences for biodiversity and ecosystem functions in a wide range of ecosystems (Lavelle et al. 1997, Crooks 2002, Badano et al. 2006, Mermillod-Blondin and Rosenberg 2006, Wright and Jones 2006, Wright et al. 2006, Daleo et al. 2007, Badano and Marquet 2008, Gutiérrez et al. 2011). Ecosystem engineers can have disproportionate effects on communities by modifying their surrounding physical environment (Jones et al. 1994, see Fig. 1 for detailed engineering sequence). The beaver *Castor canadensis* is a classical example of ecosystem engineer. Its activities (essentially dam-building) can increase 1) the proportion of flooded soils (water and wetlands) in the landscape (Johnston and Naiman 1990) and 2) the retention of sediment, organic material (Naiman et al. 1986) and nutrients (Naiman and Melillo 1984) by decreasing water velocity, ultimately affecting the structure of animal and plant communities in the landscape (Naiman et al. 1988, Hägglund and Sjöberg 1999, Wright et al. 2002, Anderson and Rosemond 2007). Beside this emblematic example of beavers, there is a broad diversity of engineer organisms and of engineering mechanisms (Berke 2010) that significantly impact structure and functions of ecosystems. For example, the sediment reworking activities of bioturbators can have marked effects on microbial communities developing on sediments by affecting hydrological fluxes and biogeochemistry at the water–sediment interface (Aller 1994, Mermillod-Blondin and Rosenberg 2006, Nogaro et al. 2009). These bioturbating organisms are recognized to have significant influences on surrounding species

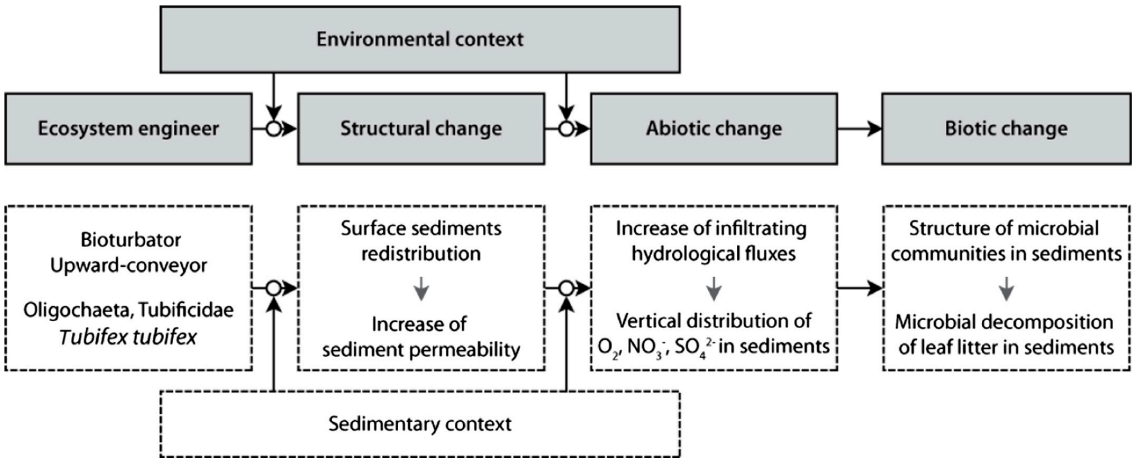

Figure 1. Successive steps for 1) a general case of ecosystem engineering sequence (in grey) and 2) the effects of *T. tubifex* on microorganisms in sedimentary habitats (study case, in white). Note that feedbacks to the engineer and other relationships in the ecosystem engineering are not represented here but can exist (Gutiérrez and Jones 2008, Jones et al. 2008).

and communities (e.g. microorganisms, zooplankton, vegetation, benthic invertebrates) in a broad range of marine and freshwater ecosystems (Meysman et al. 2006, Gyllström et al. 2008, Mermillod-Blondin and Lemoine 2010, Creed et al. 2010). Whatever the engineering mechanisms involved (e.g. through bioturbation, physical engineering …), a change in resource availability relative to the unengineered state may suffice to observe positive or adverse engineering effects on communities (Bertness 1984a, b, Jones et al. 1997, Menge 2000). The framework of ecosystem engineers also suggests that the strongest engineering-induced changes on resources resulted in the strongest effects in communities (Jones et al. 1994, Gutiérrez et al. 2003). Therefore, the importance of an ecosystem engineering activity also depends on the environmental context in which it happens (Crain and Bertness 2006, Wright and Jones 2006). Because ecosystem engineers affect communities through environmentally mediated interactions, a given engineering process can have contrasted effects on biological communities across environmental gradients and thus appear as idiosyncratic (Moore 2006, Wright et al. 2006). However, most studies dealing with ecosystem engineering only examined the relationships between the engineer (e.g. number of individuals) and 1) the physical characteristics of the environment or 2) the other species, in a unique environmental context. To establish general principles and predictions, there is a need to fully understand mechanisms underlying context dependency and thus to examine the whole 'cause–effect relationships' sequence (Fig. 1) in contrasted environments.

In the present study, we examine context dependency in river sediments. The functioning of lotic ecosystems partly depends on microbially-mediated biogeochemical processes (nutrient cycling, organic matter (hereafter OM) processing) occuring in the hyporheic zone (hereafter HZ, sedimentary interface between surface water and groundwater) (Grimm and Fisher 1984, Pusch and Schwoerbel 1994, Findlay 1995, Naegeli and Uehlinger 1997, Boulton et al. 1998, Fellows et al. 2001). By controlling hydrological exchanges at the water-sediment interface, and hence chemical conditions (e.g. availability of dissolved oxygen DO) in the HZ, sediment permeability is a crucial factor controlling the structure and activities of hyporheic microbial communities (Valett et al. 1990, Brunke and Gonser 1997, Mermillod-Blondin and Rosenberg 2006). In this context, bioturbators can have a major influence on the ecological functioning of the HZ through modification of sediment structure and permeability (Nogaro et al. 2009, Nogaro and Mermillod-Blondin 2009). However, the magnitude of bioturbation influences on permeability and then hyporheic microbial processes is expected to depend on physical characteristics of sediments (sedimentary context; Hakenkamp and Palmer 2000, Mermillod-Blondin 2011). By applying the conceptual framework of ecosystem engineering in rivers, bioturbators would have major influence on hyporheic ecological processes in sedimentary contexts where: 1) they are able to drastically modify habitat physical characteristics by actively reworking sediment, 2) these physical changes result in alteration of the permeability in the sedimentary habitat and finally 3) the changes in permeability modify resource availability for interstitial microorganisms involved in ecological processes (Fig. 1). In riverbeds covered by excessive deposition of fine sedimentary particles, permeability, hydrological exchanges and the associated input of resources (e.g. O_2) from the surface are reported to be low (Beschta and Jackson 1979, Schälchli 1992, Wood and Armitage 1997) and are supposed to constrain hyporheic microbial communities. We expected bioturbation to have higher effects on hyporheic microbial communities in such constraining sedimentary context with fine sand than in coarse sand systems with high permeability and large O_2 availability.

The aim of our study was therefore to examine how the environmental context (sediment characteristics) modulates the effects of an active bioturbator (as ecosystem engineer) in river sediments. We tested the effects of the bioturbator *Tubifex tubifex* (Oligochaeta, Tubificidae) on the characteristics and activities of microbial communities in two sedimentary habitats with contrasted textures (topped by coarse sand vs topped by fine sand) by using mesocosms (slow filtration columns). For each habitat, we broke down and examined the ecosystem-engineering process into detailed intermediate

steps (Fig. 1) including sediment reworking, and physical (permeability) and chemical abiotic (availability of nutrients and electron acceptors used for OM mineralization) changes on the sedimentary habitats. The ecosystem-engineering effects (biotic changes) were measured on the characteristics (bacterial and fungal abundances) and activities (potential aerobic respiration, potential denitrification, hydrolytic exoenzymatic activities) of the microbial community developing on leaves buried in sediments. Decomposition of leaf litter (measurement of breakdown rates) – a crucial microbially-mediated ecological process in river sediment – was considered as the final step in the engineering process.

Methods

Experimental design

To address how sedimentary context modulates the effects of a bioturbator at the water-sediment interface of rivers, we employed a factorial experimental approach in which the occurrence of *Tubifex tubifex* and the texture of surface sediments were manipulated. Experiments were carried out in mesocosms (n = 16 slow filtration columns, height = 35 cm and inside diameter = 10 cm; Mermillod-Blondin et al. 2005, see Fig. 1 in Navel et al. 2011) filled with sediment, at constant temperature (15 ± 0.5°C) under a 12 h light/12 h dark cycle.

Mesocosms were filled by successively adding gravel (2–4 mm diameter, 300 g) and then sand (100–1000 µm, 170 g), eight times. We manipulated the the texture of surface sediments by adding a 2 cm thick layer of either fine sand (90% of particles < 150 µm diameter, 'fine sand treatment', n = 8 columns) or coarse sand (90% of particles >300 µm diameter, 'coarse sand treatment', n = 8 columns). The thickness of this top sediment layer was in accordance with observations reported from riverbeds impacted by fine sediment deposits (Wood and Armitage 1997). Analyses performed before the start of the experiment indicated that the sediment surface colonizable by microorganisms (specific area) and the amounts of total organic carbon (TOC), nitrogen (TN) and phosphorus (TP) in the sediment were higher in the fine than in the coarse sand (Table 1). All the sedimentary material was collected from the Rhône River, elutriated and cleaned with deionised water to eliminate fauna and coarse particulate organic matter (CPOM). The whole-sediment layer was kept in the dark to suppress possible photoautotrophic processes.

Leaves of alder *Alnus glutinosa* – a common species along rivers characterized by fast leaf degradation (Abelho 2001) – were collected from the riparian zone of the Rhône River during abscission (October 2008). Leaves were conditioned in small-mesh bags immersed in a nearby river (located on the campus of the Univ. Claude Bernard Lyon 1, Lyon, France) for 10 days, (i.e. a time sufficient to allow microbial colonization, Suberkropp and Chauvet 1995) and then cut into discs (diameter: 12 mm) avoiding central veins. During sediment installation, a set of 35 leaf discs was inserted between two circular sieves (3 mm mesh) at a depth of 9 cm below the sediment surface in each mesocosm. After installation of sediment and leaf litter, aerated artificial river water (96 mg l^{-1} $NaHCO_3$, 39.4 mg l^{-1} $CaSO_4$ $2H_2O$, 60 mg l^{-1} $MgSO_4$ $7H_2O$, 4 mg l^{-1} KCl, 19 mg l^{-1} $Ca(NO_3)_2$ $4H_2O$ and 1.6 mg l^{-1} $(CH_3CO_2)_2CaH_2O$; pH = 7.5; US EPA 1991) was supplied at a the top of mesocosms by applying a constant hydraulic head (ΔH = 3 cm) to generate a vertical infiltration of water in sediments. About 10 cm of water was left above the sediment surface. Openings in each mesocosm allowed water sampling during the experiment.

Seven days after sediment installation (T7) (time necessary to obtain physico-chemical stabilization of the system), we added a set of 100 individuals of *T. tubifex* to half of the experimental units (n = 4 per treatment). The density of tubificid worms in the experimental units (around 12 800 individuals m^{-2}) was in accordance with densities reported in field studies (Fruget 1989, Martinet 1993). *Tubifex tubifex* is a common deposit feeder that inhabits sandy and muddy habitats, which can actively rework sedimentary particles and produce biogenic structures, affecting O_2 and nutrients concentrations in sediments (McCall and Fisher 1980, Nogaro and Mermillod-Blondin 2009). The potential impact of *T. tubifex* on leaf litter degradation was expected to result from the influence of *T. tubifex* as ecosystem engineers rather than from direct feeding on leaf litter. To verify that *T. tubifex* did not feed on leaves, we conducted a preliminary experiment using aerated aquatic mesocosms in which 35 alder leaf discs were deposited at the surface of a fine layer of sediment for 59 days. We measured that the occurrence of 100 individuals *T. tubifex* did not significantly influence leaf litter breakdown rate, microbial abundances and activities associated with leaf litter (Navel et al. unpubl.).

During the main experiment, hydraulic discharge rate was measured and water was sampled every 10 days at four depths to determine O_2, NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}, SO_4^{2-} and dissolved organic carbon (DOC) concentrations, for all mesocosms. At the end of the experiment, mesocosms were dismantled and sediment was cut into slices to quantify sediment reworking and vertical distribution of invertebrates. Fungal biomass, total bacterial abundance, abundance of active eubacteria, potential aerobic and anaerobic activities and enzymatic activities involved in C and N cycles were determined on leaf discs, as described below. Leaf discs were then dried and weighed to quantify mass loss during the experiment.

Physico-chemical analyses

Every 10 days starting with day 6, a day before fauna addition (T6, T16, T26, T36, T46 and T56), the outlet of each

Table 1. Specific area, total organic carbon (TOC), total nitrogen (TN) and total phosphorus (TP) of the sediment used as top-sediment layer (mean ± SD, n = 4 for specific area, n = 5 for TOC, TN and TP).

	Specific area (cm^2 g^{-1})	TOC (g kg^{-1})	TN (g kg^{-1})	TP (mg kg^{-1})
Coarse sand	59.37 ± 0.34	0.97 ± 0.08	0.17 ± 0.03	3.76 ± 3.47
Fine sand	1465.00 ± 33.17	16.6 ± 1.6	1.35 ± 0.06	6.62 ± 1.36

column was closed and water was shunted and sampled at +2 cm above (H1) and −3 cm (H2), −8 cm (H3) and −13 cm (H4) below water-sediment interface under similar hydraulic pressure conditions. An oxygen micro-sensor probe fitted in a glass tube was used to determine O_2 concentration without contact with atmospheric oxygen during sampling. NH_4^+, NO_3^-, NO_2^-, PO_4^{3-} and SO_4^{2-} concentrations were determined following standard colorimetric methods (Grashoff et al. 1983) after filtration through Whatman GF/F filters (pore size: 0.7 µm) using an automatic analyzer. For DOC measurements, water samples were filtered through Whatman HAWP filters (pore size: 0.45 µm) and acidified with three drops of HCl (35%). The DOC concentration in water samples was measured with a total carbon analyzer based on combustion at 900°C after removal of DOC with HCl and CO_2 stripping under O_2 flow.

Sediment reworking analyses

Particle redistribution induced by worms in the sedimentary matrix was estimated by the luminophore tracer technique (Gérino 1990). In each column, natural sediment particles (150–300 µm) dyed with yellow luminescent paint were deposited uniformly at the top of the sedimentary matrix a few hours after the introduction of *T. tubifex* (at T7). During column dismantling (T59), the top 4 cm of sediment were cut into 0.5 cm thick slices, dried at 40°C (48 h) and homogenized before counting luminophores on 500 mg subsamples under UV light (three replicates per sampled slice). Vertical distribution of luminophores in the sediment was obtained by expressing the density of particles (number g^{-1} dry sediment) obtained for each slice as percentage of the total amount of luminophores obtained for the whole top 4 cm sediment layer.

Vertical distribution of tubificid worms

After collecting subsamples on the top sediment for luminophore counting, sediment was pooled into 5 cm thick sediment slices that were sieved (using a 500 µm diameter sieve) to collect living tubificids. Individuals recovered in each slice were preserved in 96% ethanol and counted under a dissecting microscope. For each column, the vertical distribution of tubificid worms in the sediment was determined by reporting the abundance of worms in each slice to the total amount of worms retrieved in the overall sedimentary column (results for each slice were expressed as percentage).

Microbial analyses

Fungal biomass

For each column, five leaf discs collected at the end of the experiment were stored at −80°C and freeze-dried for 12 h before analysis. Fungal biomass was estimated using the ergosterol quantification method with methanol refluxing prior to saponification reaction using KOH/methanol (Gessner et al. 2003), following the protocol detailed in Navel et al. (2010). Ergosterol was isolated from saponified products with extracting columns and elution with isopropanol. Ergosterol mass in the sample was then determined using HPLC. Fungal biomass was estimated from ergosterol mass using a 182 conversion factor determined for aquatic hyphomycetes, which are known to dominate fungal assemblages of decomposing litter (Gessner and Chauvet 1993). Results were expressed in mg fungi g^{-1} dry mass of leaf litter.

Bacterial abundances

During column dismantling (at T59), two leaf discs were immediately collected and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS; 0.13 M NaCl, 7 mM $NaHPO_4$, 3 mM NaH_2PO_4; pH = 7.2) for 10 h. Fixed samples were washed twice in PBS and were stored in ethanol and PBS (50:50) at 20°C. After storage (two weeks), leaf discs were homogenized in 20 ml of 0.1% pyrophosphate in PBS using a sonicator with a 2 mm-diameter probe set at a power of 50 W for two periods of 60 s. All homogenized samples were finally supplemented with detergent to a final concentration of 0.01%. Aliquots (10 µl) of homogenized samples were spotted onto gelatine-coated slides and were hybridized with Cy3-labelled oligonucleotide probe (mix of EUB 338, EUB 338 II and EUB 338 III, eubacteria) and concomitantly stained with the DNA intercalating dye DAPI (200 ng μl^{-1}) according to Navel et al. (2010). Abundances of DAPI- and Cy3-bacteria were expressed as numbers of bacteria and active eubacteria (hybridized with EUB 338, Karner and Fuhrman 1997) reported per g of dry leaf.

Microbial activities

All microbial activities were measured within the 24 h following columns dismantling, with leaf discs stored at 4°C before analysis.

Enzymatic activities

β–glucosidase (EC: 3.2.1.21), β–xylosidase (EC: 3.2.1.37) and leucine aminopeptidase (EC: 3.4.11.1) activities were measured on two replicates of two discs (for each activity and each mesocosm) by fluorimetry using constant volume of substrate analogs: 4-methylumbelliferyl-ß-D-glucoside (MUF-glu; 750 µM, 2 ml), 4-methylumbelliferyl-xylosidase (MUF-xyl; 1000 µM, 2 ml) and L-leucine-4-methyl coumarinyl-7-amideHCl (MCA-leu; 1000 µM, 2 ml), respectively. Incubation at 20°C (40 min) was stopped by transferring into boiling water before centrifugation (5000 g; 4851 rpm, 3 min). Fluorimetry measurements were realised on a mix of supernatant (300 µl) and buffer (30 µl, pH 10.4) using a microplate reader with excitation wavelength of 363 nm and emission wavelengths of 441 nm for MUF-glu and MUF-xyl. Wavelengths were set at 343 nm (excitation) and 436 nm (emission) for MCA-leu. At the end of analyses, all the two-discs samples were dried (drying at 70°C for 48 h) and weighted to express results as nmol of hydrolysed compound h^{-1} g^{-1} dry leaf litter. For each mesocosm, each enzymatic activity calculated as the mean value obtained for the two replicates of two discs, corrected by the fluorimetric signal obtained with a formaldehyde-killed control (measurements realized in similar conditions on two discs previously treated 30 min with a 39% formaldehyde solution).

Aerobic respiration and anaerobic denitrification

Potential aerobic respiration and anaerobic denitrification activities were measured on leaf discs following the slurry

technique (Furutani et al. 1984). Leaf discs (n = 4 for respiration and n = 6 for denitrification) were placed in 150 ml flasks supplemented with feeding solutions to optimize microbial activity. For the measurements of CO_2 production (respiration), leaf discs were incubated under aerobic conditions with 5 ml of a feeding solution of glucose (7.5 g l^{-1}) and glutamic acid (7.3 g l^{-1}). For the measurements of N_2O production (denitrification), the incubation was under anaerobic conditions with a N_2 atmosphere. The feeding solution was a mixture of 5 ml of a KNO_3 (2.2 g l^{-1}), glucose (7.5 g l^{-1}) and glutamic acid (7.3 g l^{-1}) solution. Acetylene (10% v/v) was introduced in N_2 saturated atmosphere to stop N_2O-reductase activity. For each sample, CO_2 and N_2O productions were calculated from differences of concentrations measured after 2 h and 6 h of incubation by using gas chromatography on a microcatharometer. After the drying of leaf discs (70°C for 48 h), results were expressed in μg of C or N h^{-1} g^{-1} dry leaf litter.

Leaf litter degradation

For each column, the total dry mass of leaf litter after 59 days was calculated as the sum of the dry masses of samples used in microbial analyses and that measured for the remaining leaf material (common drying method: 70°C for 48 h), with correction for the set of five discs that were freeze-dried for fungal biomass assessment. Results were compared to the initial dry mass determined on five additional sets of 35 alder discs (228.8 ± 6.25 mg) at the start of the experiment.

Data analysis

Repeated measures of permeability were analyzed using mixed model analysis of variance with 'sediment' ('coarse sand' vs 'fine sand'), 'worms' ('with' vs 'without') and 'time' as fixed factors, and 'mesocosm' as random factor. Repeated measures of vertical profiles in O_2, DOC, NH_4^+, NO_3^-, NO_2^-, SO_4^{2-} and PO_4^{3-} were analysed similarly, with 'depth' as additive fixed factor. Vertical distribution of tubificid worms was studied by using two-way analysis of variance (ANOVA) with 'sediment', and 'depth' as main factor. Vertical distribution of luminophores was studied by using similar procedure with 'sediment', 'depth' and 'worms' as main factors. Data obtained on buried leaf litter (daily dry mass loss, fungal biomass, total abundance of bacteria, abundance of active bacteria, % active bacteria, enzymatic activities, potential aerobic respiration and potential denitrification) were examined using two-way ANOVAs with 'sediment' and 'worms' as main factors. The method of contrasts was used to determine significant differences between treatments (Crawley 2002).

Permeability and microbial activities on leaves (glucosidase, leucine aminopeptidase activity and potential denitrification activities) were log-transformed before statistical analysis in order to fit the assumption of homoscedasticity. Abundances of luminophores and worms retrieved at the end of experiment for each layer within a same column were expressed as percentages of the total abundance for the whole column, and were arcsine-transformed before analyses. Statistical analyses were performed using JMP ver. 8.0.1 (SAS Inst., Cary, NC, USA). Significance for all statistical tests was accepted at $\alpha < 0.05$.

Results

Influence of sediment physical characteristics on sediment reworking activity

The physical structure of the habitat influenced the vertical distribution of tubificid worms in sediments (Fig. 2; 'sediment-by-depth interaction effect': $F_{3,24} = 83.44$, $p < 10^{-4}$). While the major part of individuals were retrieved in the top sediment layer when covered by fine sand (93% in the first 5 cm), most worms were retrieved deeper in the sediment of columns covered with coarse sand (around 65% were found below 10 cm depth). The presence of worms in systems increased the transport of luminophores from the sediment surface to the sedimentary column (Fig. 2; 'worms-by-depth interaction effect': $F_{7,96} = 29.78$, $p < 10^{-4}$). This effect of worms on luminophore profiles was strongly influenced by the physical characteristics of the sedimentary habitat ($F_{7,96} = 9.61$, $p < 10^{-4}$). The percentage of luminophores buried below the '0–0.5 cm' sediment layer was around 4% in the 'coarse sand' treatment whereas it was around 24% in the 'fine sand' treatment (Fig. 2).

Influence of sediment characteristics on hydraulic exchanges and microbial processes in controls without worms

Mean (±SD) permeability measured for the 'fine sand' treatment was about 8-fold lower than for the 'coarse sand' treatment (Fig. 3; 2.02 ± 0.67 and 15.95 ± 4.81 cm h^{-1}, respectively). The decreases with depth of O_2 and NO_3^- concentrations (Fig. 4; $F_{3,276} = 933.73$ and 134.90, respectively, $p < 10^{-4}$ for both) in the interstitial water were stronger in 'fine sand' than in 'coarse sand' treatment ('sediment-by-depth interaction effect': $F_{3,276} = 200.85$ and 159.26 for O_2 and NO_3^- respectively, $p < 10^{-4}$ for both). This difference was particularly marked in the top sediment layer (O_2 concentrations reduced by about 87% and 13% in the 'fine sand' and the 'coarse sand' treatments, respectively), and led to lower O_2 and NO_3^- concentrations in the treatment covered by fine sand ($F_{1,12} = 631.70$ and 409.18 for O_2 and NO_3^- respectively, $p < 10^{-4}$ for both). Peaks of DOC, NH_4^+, NO_2^- and PO_4^{3-} concentrations were only recorded in the 'fine sand' treatment, leading to higher concentrations of these solutes in 'fine sand' than in 'coarse sand' treatment (Fig. 4; $F_{1,12} = 93.02$, 665.57, 193.27, 16.13, for DOC, NH_4^+, NO_2^- and PO_4^{3-} respectively, $p < 10^{-4}$ for all).

Determinations of the dry mass of leaf litter retrieved at the end of the experiment (Fig. 5A) showed that the daily mass loss rate was 31% lower in the 'fine sand' treatment than in the 'coarse sand' treatment ($F_{1,12} = 14.39$, $p = 0.043$). In parallel, the total abundance of bacteria (Fig. 5B), the abundance of active bacteria (Fig. 5C), the fungal biomass (Fig. 5D), the glucosidase activity (Fig. 5H) and the leucine aminopeptidase activity (Fig. 5I) were significantly lower in 'fine sand' than in 'coarse sand' treatment (contrasts: comparisons without *T. tubifex*: $|t|_{12} = 2.57$, 2.87, 2.50, 5.18 and 7.14, respectively, $p < 0.028$ for all). Potential aerobic respiration (Fig. 5E), potential denitrification (Fig. 5F) and xylosidase activities measured on leaves were not significantly

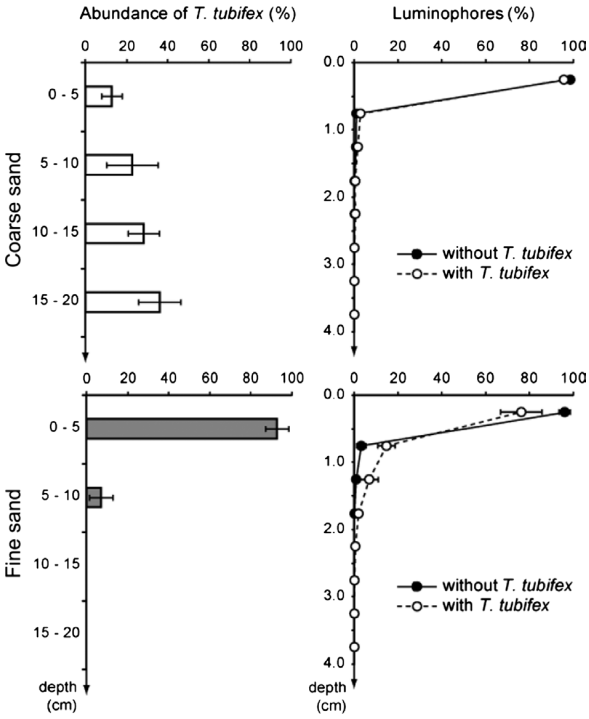

Figure 2. Vertical distribution of *T. tubifex* in sediment and their associated effect on vertical profiles of luminophores in sediment columns covered by coarse sand (upper panels) and fine sand (lower panels).

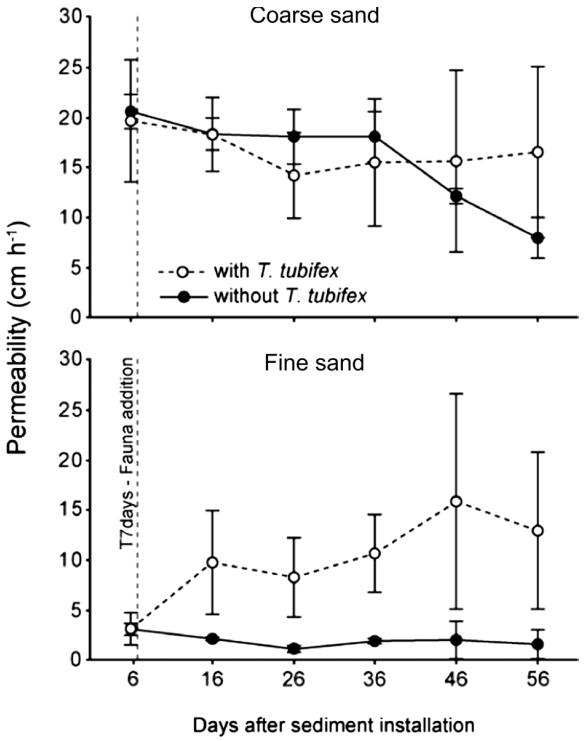

Figure 3. Effect of *T. tubifex* on hydraulic conductivity measured every 10 days in sediment columns covered by coarse sand or fine sand.

influenced by sedimentary conditions (contrasts: $|t|_{12} = 1.43$, 0.99 and 0.76, respectively, $p > 0.176$ for all).

Influence of tubificid worms on hydrologic exchanges, biogeochemical processes and CPOM processing in the two sedimentary contexts

The influence of tubificid worms on permeability (Fig. 3) and water chemistry (Fig. 4) was dependant on the physical characteristics of the top sediment ('sediment-by-worms interaction effect': $F_{1,12} = 24.67$, 48.41, 147.73, 13.76, 96.24 and 4.04 for hydraulic conductivity, O_2, NO_3^-, SO_4^{2-}, NH_4^+ and PO_4^{3-} concentrations, respectively, $p < 0.044$ for all). While worms had weak influence on permeability and concentrations of solutes in 'coarse sand' treatment, they increased permeability by more than six-fold in 'fine sand' treatment. Th e presence of tubificid worms increased O_2 and NO_3^- concentrations and strongly reduced the peaks of solutes (DOC, NH_4^+, NO_2^- and PO_4^{3-}) released in the sedimentary columns with 'fine sand' treatment.

Similarly, the influence of *T. tubifex* on microbial characteristics and associated processing of buried leaf litter was dependant on the physical structure of the sedimentary habitat (Fig. 4). We did not observe any influence of *T. tubifex* on microbial characteristics measured on leaves buried in 'coarse sand' systems (Fig. 5; contrasts: $|t|_{12} = 0.24$, 0.83, 0.02, 1.32, 0.97 and 1.05 for total abundance of bacteria, abundance of active bacteria, fungal biomass, xylosidase, glucosidase and leucine aminopeptidase, respectively, $p > 0.207$ for all) except for potential aerobic respiratory activity ($|t|_{12} = 2.77$, $p < 0.018$). In contrast, *T. tubifex* had a positive influence on most microbial variables in 'fine sand' treatment (contrasts: $|t|_{12} = 4.63$, 4.42, 3.05, 8.04 and 4.46 for total abundance of bacteria, abundance of active bacteria, xylosidase and leucine aminopeptidase respectively, $p < 0.009$ for all; and $|t|_{12} = 1.98$, $p = 0.071$ for glucosidase activity) with the exception of potential denitrification ($|t|_{12} = 0.21$, $p < 0.835$).

In parallel, *T. tubifex* increased the daily loss rate of leaf litter mass in the 'fine sand' treatment by about 30% (Fig. 4A; $|t|_{12} = 3.40$, $p < 0.006$) where they counteracted the negative influence of fine sediment deposition on leaf litter degradation ($F_{1,12} = 5.61$, $p = 0.036$). This effect was not observed in the 'coarse sand' treatment (Fig. 5A, $|t|_{12} = 0.17$, $p = 0.867$).

Discussion

Contrasted biogeochemical processes induced by sediment characteristics

Our study confirmed the expectation that the biogeochemical functioning of the hyporheic zone is strongly influenced by the sedimentary context. The high permeability and high hydraulic discharge rates in systems topped by a 2-cm thick layer of coarse sand generated aerobic conditions (O_2 concentration > 2 mg l^{-1}) throughout the sedimentary matrix (to a depth of 13 cm). As a consequence of the O_2 availability, NO_3^- and SO_4^{2-} – as less energetically favourable electron acceptors for OM mineralization (Hedin et al.

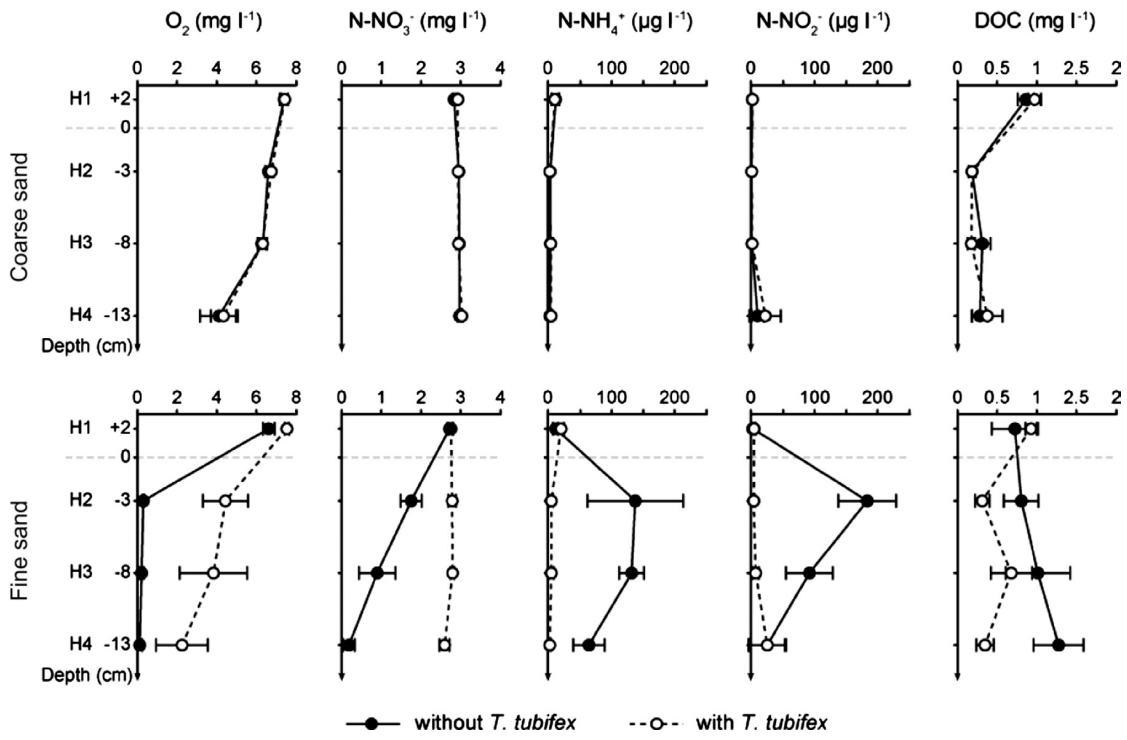

Figure 4. Effect of *T. tubifex* on depth profiles for O_2, $N\text{-}NO_3^-$, $N\text{-}NH_4^+$, $N\text{-}NO_2^-$ and DOC concentrations determined at four depths (from H1: 2 cm above sediment interface, to H4: 13 cm below sediment interface) after 36 days in sediment columns covered by coarse sand (upper panels) or fine sand (lower panels).

1998) – were not consumed for OM mineralization. In these conditions, we did not observe any significant production of solutes linked to anaerobic OM degradation in sediments (i.e. NH_4^+, PO_4^{3-} and DOC, Nogaro et al. 2007). Permeability and hydraulic discharge rates were 85% lower in sediment covered by a 2-cm thick layer of fine sand, in comparison with systems topped by coarse sand. As a consequence of the reduced hydraulic discharge rates, we observed sharp decreases in O_2 and NO_3^- concentrations along depth in these systems. The rapid along-depth succession of metabolic pathways was in accordance with predictable thermodynamic sequences (based on free energy yields): O_2 being consumed first during OM mineralization in the oxic zone, followed by the consumption of NO_3^- (denitrification), manganese and iron oxides, SO_4^{2-} and carbon dioxide (Hedin et al. 1998, Baker et al. 2000, Kristensen 2000). Since O_2 was limiting in the first centimeters of sediments, most of the sedimentary matrix (and thus buried leaf litter) was under anaerobic conditions, leading to the release of NH_4^+, PO_4^{3-} and DOC. In such O_2-limited system, microbial abundances (total abundance of bacteria, abundance of active bacteria and fungal biomass) and activities (glucosidase and leucine aminopeptidase activities) were altered, and rates for microbially-mediated ecological processes occurring in the HZ were reduced (leaf litter decomposition was 30% lower than in not-stressed coarse-sand treatment).

The contrasts in biogeochemical conditions between the two sedimentary contexts were consistent with field studies showing that reduced hydrologic exchanges due to clogging favoured the occurrence of anaerobic processes such as denitrification, sulphato-reduction and methanogenesis (Dahm et al. 1987, Brunke and Gonser 1997, Boulton et al. 1998, Lefebvre et al. 2004). Our results are also in accordance with other studies showing that biogeochemical conditions, in particular the availability of electron acceptors (mainly O_2 and NO_3^-), strongly affect 1) the fungal colonization of leaves (Medeiros et al. 2009) and microbial enzymatic activities such as cellulase and peptidase activities (Montuelle and Volat 1997) and 2) OM degradation rates (Chauvet 1988, Claret et al. 1998, Dahm et al. 1998, Lefebvre et al. 2005). It is therefore clear that sedimentary contexts that lead to low hydrological exchanges and O_2 concentration in sediments limit the growth and activity of microbial communities and ultimately the rates of microbially-mediated ecological process occurring in the HZ. Finally, we efficiently recreated the hydrological and biogeochemical functioning of two contrasted sedimentary contexts and highlighted the key role played by O_2 as resource for microorganisms in sedimentary habitats.

Modulation of bioturbator effects on biogeochemical processes by sedimentary context

The present study confirmed our hypothesis that the effects of bioturbators on permeability and microbially-mediated processes depend on the sedimentary context.

As upward conveyors (feeding on sediment at depth and ejecting faecal pellets at the sediment–water interface, Fisher et al. 1980, McCall and Fisher 1980), tubificid worms can

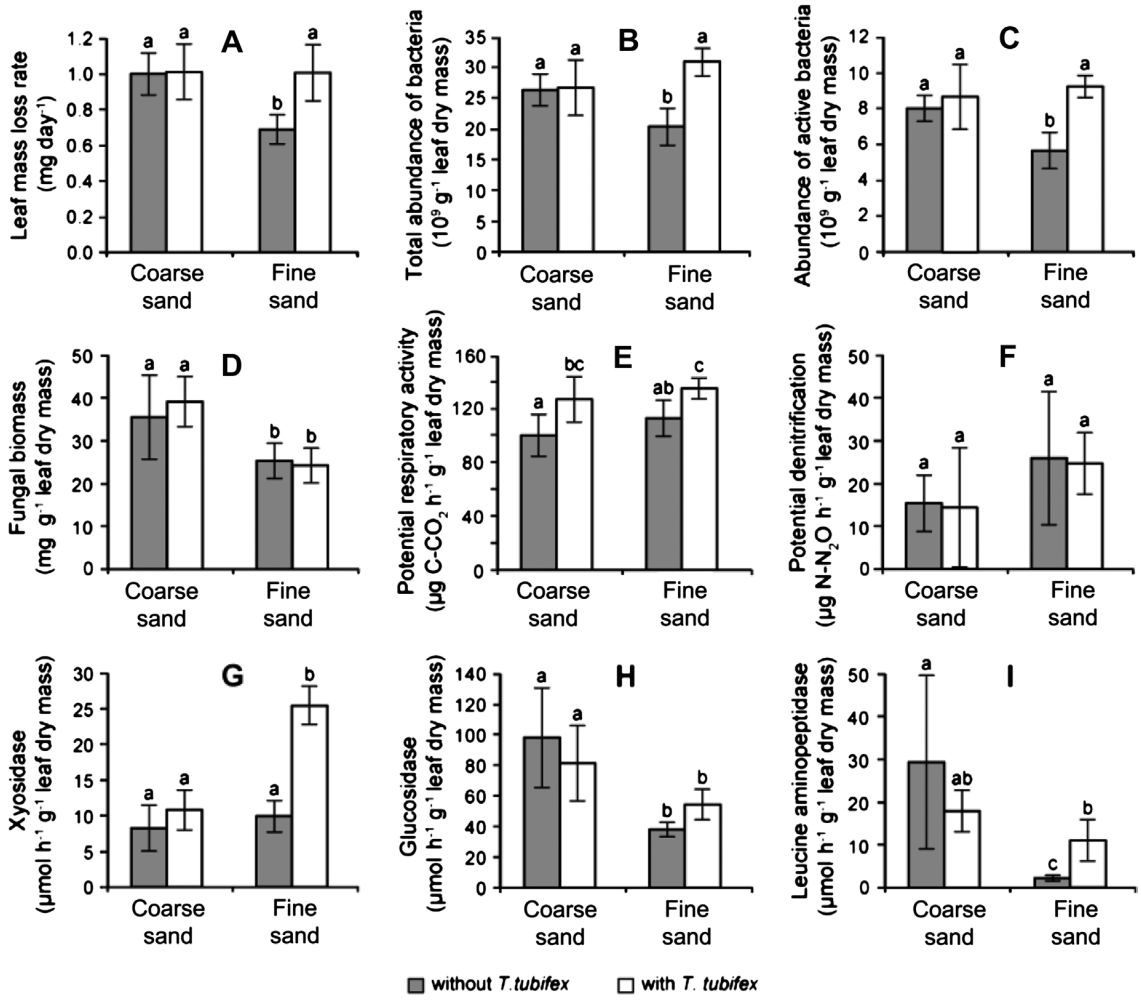

Figure 5. Effect of *T. tubifex* on leaf mass loss rate (A), and microbial characteristics measured at the end of the experiment on leaves buried at 9 cm depth in sediment columns covered by coarse sand or fine sand: total abundance of bacteria (B), abundance of active bacteria (C), fungal biomass (D), potential respiratory activity (E), potential denitrification (F), xylosidase (G), glucosidase (H), and leucine aminopeptidase activities (I).

build networks of tubes and burrows that may extend as deep as 20 cm in sediments. However, our results showed that both the bioturbation activity and the vertical distribution of tubificid worms were modulated by characteristics of the sediment. Using luminophore as particle tracers, we noted that tubificid worms significantly reworked the top of the sedimentary column with a fine sand layer whereas it was not the case with a coarse sand layer. This contrast in bioturbation activity was linked to the vertical distribution of worms. While worms used the whole sediment column in the systems topped with coarse sand, most tubificid worms were found in the top 0–5 cm in systems topped with fine sand. Fine sand probably acted as preferential feeding zone for *T. tubifex* (Rodriguez et al. 2001), which strongly influenced the vertical distribution of worms in the sedimentary column. The different bioturbation activities exhibited by *T. tubifex* in the two sedimentary contexts could explain their contrasting effects on permeability (see Fig. 1 for the successive effects of bioturbators). By producing galleries through the fine sand layer, *T. tubifex* create water pathways that counteracted the adverse effect of fine sediment deposition on water exchanges. The six-fold increase in permeabiliy due to *T. tubifex* in the fine-sand treatment stimulated the exchanges of water and O_2 from surface to deep sediment layers, restoring aerobic conditions in the sedimentary column (Fig. 1). Modification of aerobic-anaerobic conditions observed through the increase in O_2 and NO_3^- concentrations was also associated with a lack of NH_4^+, PO_4^{3-} and DOC accumulation in the sedimentary habitat bioturbated by tubificid worms. The increase in electron acceptors (O_2 and NO_3^-) availability for microorganisms with *T. tubifex* has stimulated microbial communities (abundances and activities) associated to the buried leaf litter, leading to an increase by 30% of leaf litter breakdown rate in sedimentary systems covered by fine sand (Fig. 1). In contrast, tubificid worms did not affect permeability and the subsequent chemical conditions (availability of electron acceptors) in sediments topped by coarse sand. Consequently, bioturbators did not influence microorganisms and microbially-mediated processes in sediments.

Our study clearly demonstrated that the contribution of bioturbating invertebrates on ecosystem processes was negatively correlated with the hydrologic exchanges occurring at the water-sediment interface of the studied system, supporting conclusions from other studies (Hakenkamp and Palmer 2000, Boulton et al. 2002, Mermillod-Blondin 2011). Bioturbators are able to strongly influence water fluxes (through biological decolmation) in sedimentary habitats characterized by low hydrologic exchanges (affected by the deposition of fine sediment particles in the present study), whereas they only slightly modulate existing water fluxes in habitats with high hydrologic exchanges. While the weak influence of bioturbators on hydraulic conductivity in systems covered with coarse sand could be linked to their low sediment reworking activity (Fig. 2), it could also have resulted from the reduced ability of bioturbation to increase hydrologic exchanges in a system that is already highly permeable.

Contribution of the present study to the theoretical framework of ecosystem engineers

Most studies dealing with ecosystem engineering have quantified the effects of engineers on communities in a given habitat without taking into account the modulation of organism engineering by environmental conditions (Crain and Bertness 2006). Our study clearly demonstrates that the influences of bioturbators as engineers may vary across environmental contexts. Few studies have already reported similar observations for various types of ecosystems and various types of ecosystem engineers (Spooner and Vaughn 2006, Nogaro et al. 2009, Quierós et al. 2011) but they did not finely decrypt the complete mechanisms by which ecosystem engineers and habitat characteristics interacted to shape biological communities and/or ecosystem functions. More precisely, the environmental context can influence the impacts of engineers on communities by modulating 1) the degree with which engineering activities (or structures) generate physical changes in the environment and/or 2) the degree with which physical changes can generate abiotic changes in the environment (Fig. 1, see also Jones et al. 2010). By examining the whole engineering process as a detailed sequence of successive cause–effect relationships, our study concluded that the impact of bioturbators on hyporheic microbial processes was mainly linked with their ability to generate changes on abiotic factors that are limiting for microorganisms (i.e. O_2). This conclusion supports the general idea that the magnitude of engineering impacts on communities depends on the degree to which engineers modulate the availability of limiting abiotic factor(s) in comparison with the unengineered habitat (Gutiérrez et al. 2003, Gutiérrez and Jones 2006, Jones et al. 2010). Finally, quantifying the importance of ecosystem engineering in ecosystems needs to determine which factors are limiting for communities and which engineering activities are able to change these limiting factors.

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