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Water–Sediment Exchanges Control Microbial Processes Associated with Leaf Litter Degradation in the Hyporheic Zone: a Microcosm Study

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Abstract The present study aimed to experimentally quantify the influence of a reduction of surface sediment permeability on microbial characteristics and ecological processes (respiration and leaf litter decomposition) occurring in the hyporheic zone (i.e. the sedimentary interface between surface water and groundwater). The physical structure of the water–sediment interface was manipulated by adding a 2-cm layer of coarse sand (unclogged systems) or fine sand (clogged systems) at the sediment surface of slow filtration columns filled with a heterogeneous gravel/sand sedimentary matrix. The influence of clogging was quantified through measurements of hydraulic conductivity, water chemistry, microbial abundances and activities and associated processes (decomposition of alder leaf litter inserted at a depth of 9 cm in sediments, oxygen and nitrate consumption by microorganisms). Fine

sand deposits drastically reduced hydraulic conductivity (by around 8-fold in comparison with unclogged systems topped by coarse sand) and associated water flow, leading to a sharp decrease in oxygen (reaching less than 1 mg L^{-1} at 3 cm depth) and nitrate concentrations with depth in sediments. The shift from aerobic to anaerobic conditions in clogged systems favoured the establishment of denitrifying bacteria living on sediments. Analyses performed on buried leaf litter showed a reduction by 30% of organic matter decomposition in clogged systems in comparison with unclogged systems. This reduction was linked to a negative influence of clogging on the activities and abundances of leaf-associated microorganisms. Finally, our study clearly demonstrated that microbial processes involved in organic matter decomposition were dependent on hydraulic conductivity and oxygen availability in the hyporheic zone.

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Introduction

In streams, the interface zone between surface and interstitial water (i.e. the hyporheic zone [27, 50]) is recognized for its structural and functional significance on the whole-stream ecosystem functioning [13, 45]. This significance, often illustrated by the relative contribution of subsurface activities on the whole-stream metabolism, depends not only on the proportion and retention time of stream discharge routing through the subsurface sedimentary matrix but also on process rates mediated by metabolically active microbial communities [13, 23]. The microbial biofilm developed on hyporheic sediments is very efficient in the uptake, immobilization and transformation of both dissolved and particulate organic substances [37–39, 52]. These substances are used as preferential electron donors in redox reactions, which generate free energy for microbial growth and maintenance. According to predictable thermodynamic sequences (based on free energy yields), dissolved oxygen (DO) is the preferential electron acceptor used for organic matter (OM) mineralization. The sediment characteristics greatly influence the biomass of the biofilm [2, 7] by controlling the availability of both resources (DO, nutrients and OM) supplied from the surface through regulation of hydrologic flow paths between the stream and the hyporheic zone [35] and habitat (total surface of sediment particles) for microbial assemblages. They also control the nature and rates of microbially mediated processes occurring in the hyporheic zone [33, 53, 57].

The reduction in hydraulic conductivity often results from the modifications of riverbed physical characteristics by the clogging of top sediment layers through excessive deposits of fine sedimentary particles originating from different terrestrial sources [63]. By reducing permeability, clogging may greatly modify DO supply and influence the overall succession of biogeochemical processes within the hyporheic zone. In clogged systems, aerobic microbial processes such as aerobic respiration and nitrification may be reduced in favour of anoxic processes such as denitrification, fermentation and methanogenesis [11, 32]. These processes are often, but not always, less efficient than aerobic processes for the degradation of dissolved and particulate OM. In marine sediments, labile OM was shown to be degraded at comparable rates in the presence or absence of oxygen whereas refractory OM degrades more slowly under anoxic than oxic conditions [28]. In hyporheic sediments, several field experiments [4, 10, 61] were performed to analyse OM degradation under different environmental conditions using buried leaf litter. Although these studies suggested that OM degradation rates were positively linked with DO supply from surface water, other confounding factors such as temperature, nutrient supply, density of detritivore invertebrates or microbial diversity

could also partially explain these results. Therefore, experimental works are greatly needed in order to quantify the influence of environmental factors (sediment characteristics, temperature, nature and concentration of pollutants) on water chemistry, the microbial compartment (abundance and activities) and associated ecological processes such as OM processing.

Here, we developed an experimental procedure in the laboratory to evaluate the influence of clogging by fine sediment deposition on microbial activities and OM processing in hyporheic sediments. We manipulated the structure of the sedimentary matrix by adding a fine-grained sediment layer at the top of a sand–gravel-based sediment in slow filtration columns. Leaf litter was incorporated into the sediment in order to assess coarse particulate OM (CPOM) degradation in the hyporheic system. The influence of fine sediment deposition was studied through determinations of hydraulic conductivity (clogging), characteristics of microbial communities (abundances, activities) and rates of associated ecological processes (leaf litter breakdown, aerobic respiration and denitrification). We expected fine sediment deposits to reduce hydraulic conductivity and associated supply of DO from the surface, impacting the whole electron acceptor sequence. This change may affect the microbial communities both quantitatively by reduction of microbial abundances and qualitatively by modification of microbial activities. Consequently, we hypothesized that clogging-induced modification of the hyporheic microbial compartment would reduce the breakdown rate of buried OM, a process that has been used as a functional indicator of stream ecosystem integrity [5, 21, 64]. Here, while it is specifically applied to the hyporheic microbial compartment, we expect a similarly sensitive response to clogging as a major factor of stream ecosystem impairment.

Methods

Experimental Design

Experiments were carried out in slow filtration cylindrical columns (Fig. 1; height=35 cm and inside diameter=10 cm [42]) at constant temperature ($15\pm 0.5^\circ\text{C}$) under a 12:12-h light/dark cycle. Each column ($n=8$) was filled by eight successive additions of gravels (2–4 mm diameter, 300 g) and sand (0.1–1 mm, 170 g) collected from the Rhône River and previously elutriated, cleaned with deionised water to eliminate CPOM. To test the influence of clogging, the sedimentary matrix was topped by an additive layer of fine sand (90% of particles <0.16 mm diameter; specific area $1,465.00\pm 33.17$ cm^2 g^{-1} dry sediment, mean \pm SD on $n=3$ samples) or coarse sand (90% >0.29 mm; specific area 59.37 ± 0.34 cm^2 g^{-1} dry sediment; $n=3$ samples).

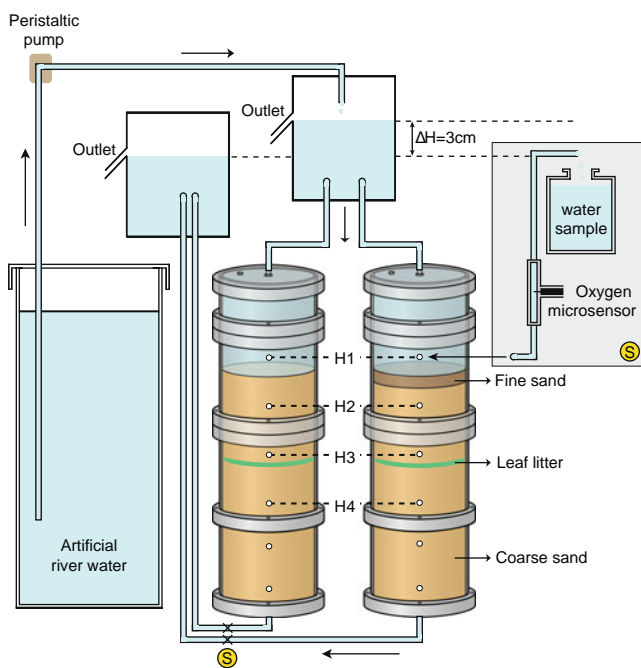


Figure 1 Schematic representation of the experimental device: example with two slow filtration columns impacted or not by fine sediment deposits (“fine sand” and “coarse sand” treatment, respectively). Water sampling procedure is indicated using circled “S”

Analyses performed before the start of the experiment indicated that the amounts of total organic C, total N and P per dry mass of sediment were significantly higher in the fine sand ($16.6 \pm 1.6 \text{ g kg}^{-1}$ of total organic C, $1.35 \pm 0.06 \text{ g kg}^{-1}$ of total N and $6.62 \pm 1.36 \text{ mg kg}^{-1}$ of total P) than in the coarse sand ($0.97 \pm 0.08 \text{ g kg}^{-1}$ of total organic C, $0.17 \pm 0.03 \text{ g kg}^{-1}$ of total N and $3.76 \pm 3.47 \text{ mg kg}^{-1}$ of total P).

While the top of the column was exposed to dark–light cycle, the whole-sediment layer was kept in the dark (using three layers of black adhesive tape) to suppress photoautotrophic processes. During sediment installation, a set of 35 discs (diameter 12 mm) of fresh leaves of alder (*Alnus glutinosa* (L.) Gaertn.), a common riparian tree species characterized by fast leaf degradation [1, 51], was inserted between two circular sieves (3 mm mesh) at a depth of 9 cm below the sediment surface. Discs were cut avoiding central veins, from leaves collected in the riparian zone of the Rhône River during abscission (October 2008) and conditioned in small-mesh bags immersed in a nearby river (located on the campus of the University Claude Bernard, Lyon, France) for 10 days, i.e. a time sufficient to allow microbial colonization [60]. After installation of sediment and leaf litter, all columns were provided by the top with artificially river water ($96 \text{ mg L}^{-1} \text{ NaHCO}_3$, $39.4 \text{ mg L}^{-1} \text{ CaSO}_4 \cdot 2\text{H}_2\text{O}$, $60 \text{ mg L}^{-1} \text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$, $4 \text{ mg L}^{-1} \text{ KCl}$,

$19 \text{ mg L}^{-1} \text{ Ca(NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $1.6 \text{ mg L}^{-1} (\text{CH}_3\text{CO}_2)_2 \cdot \text{CaH}_2\text{O}$; $\text{pH}=7.5$ [62]) saturated with DO by an air pump, at a constant hydraulic head ($\Delta H=3 \text{ cm}$). About 10 cm of water was left above the sediment surface. Openings at different depths on each column allowed sampling water at centimetric scale and different times during the experiment.

During the experiment, the hydraulic discharge rate was measured and water was sampled at four depths for chemical analyses, every 10 days for all columns. At the end of the experiment, several microbial variables were measured on leaf discs and in sediment collected at three layers. We also quantified the dry mass loss of buried leaf litter during the course of the experiment.

Physicochemical Analyses

Every 10 days from T0 to T5 (50 days), the outlet of each column was closed and water was shunted and sampled at +2 cm: overlying water (H1), –3 cm: below the top sediment layer (H2), –8 cm: above leaf litter (H3), and –13 cm: below leaf litter (H4) in similar hydraulic pressure conditions (Fig. 1). An oxygen microsensor probe fitted in a glass tube (OX 500, Unisense, Aarhus, Denmark) was used to determine O_2 concentration without contact with atmospheric oxygen during water sampling. Ammonium, nitrate, nitrite, orthophosphate and sulphate concentrations were measured following standard colorimetric methods [22] after filtration through Whatman GF/F filters (pore size $0.7 \mu\text{m}$; Millipore, Billerica, MA, USA) by using an automatic analyser (Easychem Plus, Systea, Anagni, Italy). For DOC measurements, water samples were filtered through Whatman HAWP filters (pore size $0.45 \mu\text{m}$; Millipore, Billerica, MA, USA) and acidified with three drops of HCl (35%). The DOC concentration in water samples was measured with a total carbon analyzer (multi-N/C 3100, Analytik Jena, Jena, Germany) based on combustion at 900°C after removal of dissolved inorganic C with HCl and CO_2 stripping under O_2 flow.

Average O_2 and NO_3 uptake rates (UR; expressed in milligrams per litre per hour) between sampling points (i.e. H1, H2, H3 and H4) were calculated by using the following formula: $\text{UR} = \Delta C \times Q/V$, where ΔC was the difference in O_2 or NO_3^- concentration (milligrams per litre) between sampling points, Q was the hydraulic discharge rate in the column (litres per hour) and V was the volume of water (litres) contained in the column between two sampling points.

After water collection, openings used for sampling were closed and outlet of each column was opened. Hydraulic discharge rate (litres per hour) was obtained for each column by weighting the volume of water released at the

outlet (with constant hydraulic pressure) during 3 h and by assuming that water density was 1 kg L^{-1} . Hydraulic conductivity (K , expressed in centimetres per hour) was calculated by using a form of Darcy's law: $K = Q/(A \times \Delta H/l)$, where Q was the hydraulic discharge rate in the column (litres per hour), A was the surface of the sedimentary matrix (78.54 cm^2 for all columns), ΔH was the difference in hydraulic head (3 cm for all columns) and l was the height of the sedimentary matrix (25 cm for all columns).

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 with the ergosterol quantification method [19] in which saponified products were obtained using methanol refluxing prior to saponification reaction (KOH/methanol), following the protocol detailed in [46]. Ergosterol was isolated from saponified products by using Oasis HLB 3 cc extracting columns (Waters Corporation, Milford, MA, USA) and elution with isopropanol. Mass of ergosterol in the sample was then calculated by using HPLC system (HPLC 360/442, Kontron, Eching, Germany). Fungal biomass was estimated from ergosterol amounts using a 182 conversion factor determined for aquatic hyphomycetes, which are known to dominate fungal assemblages on decomposing litter [20]. Results were expressed in milligrams of fungi per gram dry mass of leaf litter.

Bacterial Abundances

During column dismantling (day 29), two leaf discs and three sediment samples (10 g on SED1 0–2 cm, SED2 5–7 cm and SED3 10–12 cm depth) were immediately collected and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS; 0.13 M NaCl, 7 mM NaHPO₄, 3 mM NaH₂PO₄, pH=7.2) for 10 h. Fixed samples were subsequently washed twice in PBS and stored in ethanol and PBS (50:50) at 20°C . After storage (2 weeks), leaf discs or 0.5 g of fixed sediment was homogenized in 20 mL of 0.1% pyrophosphate in PBS using a sonicator with a 2-mm-diameter probe at 50 W for two periods of 60 s. All homogenized samples were finally supplemented with the detergent NP-40 (Flucka, Buchs, Switzerland) 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} ; Sigma, Buchs, Switzerland) according

to [46] and [43]. Total abundance of bacteria (TOTB; DAPI-bacteria) and abundance of active bacteria (ACTB; Cy3-bacteria) were expressed as numbers of bacteria and numbers of active eubacteria (hybridized with EUB 338 [30]) per gram dry leaf litter or dry sediment.

Microbial Activities

All microbial activities were measured within the 24 h following column dismantling, leaf discs and sediment being 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 discs (two times) by fluorimetry using constant volume of substrate analogues: 4-methylumbelliferyl- β -D-glucoside (MUF-glu; 750 μM , 2 mL), 4-methylumbelliferyl- β -D-xyloside (MUF-xyl; 1,000 μM , 2 mL) and L-leucine-4-methyl coumarinyl-7-amide HCl (MAC-leu; 1,000 μM , 2 mL), respectively. Incubation at 20°C (40 min) was stopped by transferring into boiling water before centrifugation (5,000 G; 4,851 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 (SAFIRE, TECAN Group Ltd, Switzerland) 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. Litter dry mass was determined at the end of analyses to express results as nanomoles of hydrolysed compound per hour per gram dry leaf litter. For each sample, values were corrected by the fluorimetric signal obtained with a formaldehyde-killed control (measurements realised in similar conditions on two discs previously treated for 30 min with a 39% formaldehyde solution).

Potential Aerobic Respiration and Anaerobic Denitrification Activities They were determined on leaf discs and on sediment samples obtained at the end of the experiment from three layers (SED1, SED2 and SED3) following the slurry technique [17]. Leaf discs ($n=4$ for respiration and $n=6$ for denitrification) or sediment samples (10 and 20 g wet sediment for aerobic respiration and anaerobic denitrification, respectively) were placed in 150-mL flasks supplemented with feeding solutions to optimize microbial activity. For the measurements of CO₂ production (respiration), the incubation was conducted 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 N₂O production (denitrification), the incubation was conducted under anaerobic conditions with a N₂ atmosphere. The feeding solution was a mixture of 5 mL of a KNO₃

(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 N₂ saturated atmosphere to stop N₂O reductase activity. CO₂ and N₂O productions were calculated from measurements of concentrations at 2 and 6 h incubations by using gas chromatography on a microcatharometer (M200 microgas chromatograph, MTI Analytical Instruments, Richmond, CA, USA). Leaf discs and sediment were then dried (70°C for 48 h). Results were expressed in micrograms of C or N per hour per gram dry mass. Results were also related to the total abundance of bacteria to estimate specific potential respiration and denitrification activities, expressed in nanograms of C or N per hour per bacterial cell.

Leaf Litter Breakdown

For each column, the total dry mass of leaf litter after 59 days was calculated as the sum of the dry mass of discs used in microbial analyses and the dry mass obtained for discs that were not used for microbial analyses (common drying method 70°C for 48 h), with correction for the set of five discs that were freeze-dried for fungal biomass assessment. Attention was made to select discs at similar average decomposition stage within each subset of leaf discs, so that the determination of total mass was reliable. The total dry mass remaining was compared to the initial dry mass determined on five additional sets of 35 alder discs (228.80±6.25 mg, mean ± SD) at the start of the experiment.

Data Treatment

Repeated measures of hydraulic conductivity were analysed using one-way repeated measures analysis of variance (RM-ANOVA1) with “clogging” (“coarse sand” vs. “fine sand”) as main factor whereas profiles in DO, DOC, NH₄⁺, NO₃⁻, NO₂⁻, SO₄²⁻ and PO₄³⁻ were analysed using RM-ANOVA2 with “clogging” and “depth” as main factors. Effects within experimental units (“Time” and interactions with “Time”) were tested by using Greenhouse–Geisser univariate test because of violation of the sphericity assumption (Mauchly’s *W*) [65].

Uptakes rates and microbial characteristics measured on sediment (TOTB, ACTB, ratio active/total bacteria, potential aerobic respiration, potential denitrification and specific potential aerobic respiratory and denitrification activities) were analysed using ANOVA2 with “clogging” and “depth” as main factors. When significant differences were detected among treatments, we used the contrasts method to determine which treatments differed [9].

Influence of clogging on data obtained on buried leaf litter (fungal biomass, total abundance of bacteria, abundance of active bacteria, percentage of active bacteria, enzymatic

activities, potential aerobic respiration, potential denitrification and specific potential respiratory and denitrification activities) was examined using *t* test. Hydraulic conductivity, enzymatic activities, potential respiratory activity and denitrification were log-transformed before statistical analysis in order to fit the assumption of homogeneity of variance. Statistical analyses were performed using JMP 7.0 (SAS Institute, Cary, NC, USA), version 2.6.0. Significance for all statistical tests was accepted at $\alpha < 0.05$.

Results

Physicochemical Analyses

Hydraulic Conductivity

Mean (± SD) hydraulic conductivity measured for the “coarse sand” treatment was 15.95±4.81 cm h⁻¹. Hydraulic conductivity was about 8-fold lower for the “fine sand” treatment with a mean value of 2.02±0.67 cm h⁻¹ (Fig. 2, RM-ANOVA1: $F_{(1, 6)}=497.94$, $P < 10^{-4}$). A significant decrease in hydraulic conductivity was measured during the course of the experiment (Greenhouse–Geisser test: $F_{(1.4, 8.4)}=9.88$, $P < 10^{-3}$), particularly between T3 and T5 for “coarse sand” treatment (Fig. 2). Table 1 indicates the correspondence between hydraulic conductivities and flow rates measured in columns.

Water Chemistry

Concentrations of O₂ in the interstitial water significantly decreased with depth in the sedimentary matrix (Fig. 3; RM-ANOVA2: $F_{(3, 24)}=1,779.21$ and 127.01 for O₂ and NO₃⁻, respectively, $P < 10^{-4}$ for both). The decreases in O₂ and NO₃⁻ in the sedimentary matrix were significantly higher in “fine sand” than in “coarse sand” treatment (RM-ANOVA2: interaction $F_{(3, 24)}=491.28$ and 134.53, for O₂ and NO₃⁻, respectively, $P < 10^{-4}$), in particular in the top sediment layer, i.e. between H1 and H2. For instance, O₂

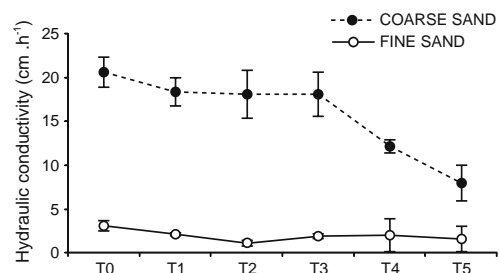


Figure 2 Hydraulic conductivity measured every 10 days (from T0 to T5) in sediment columns impacted or not by fine sediment deposits (“fine sand” and “coarse sand” treatment, respectively)

Table 1 Hydraulic conductivity and hydraulic discharge rates measured in sediment columns impacted or not by fine sediment deposits (“fine sand” and “coarse sand” treatment, respectively) at six dates during the course of the experiment (mean \pm SD, $n=4$)

	T0	T1	T2	T3	T4	T5
Hydraulic conductivity (cm h^{-1})						
Coarse sand	20.7 \pm 1.71	18.42 \pm 1.57	18.18 \pm 2.73	18.18 \pm 2.52	12.21 \pm 0.79	8.02 \pm 2.08
Fine sand	3.13 \pm 0.56	2.17 \pm 0.13	1.13 \pm 0.31	1.95 \pm 0.25	2.09 \pm 1.87	1.63 \pm 1.41
Hydraulic discharge rate (mL min^{-1})						
Coarse sand	3.25 \pm 0.27	2.89 \pm 0.25	2.86 \pm 0.43	2.86 \pm 0.40	1.92 \pm 0.12	1.26 \pm 0.33
Fine sand	0.49 \pm 0.09	0.34 \pm 0.02	0.18 \pm 0.05	0.31 \pm 0.04	0.33 \pm 0.29	0.26 \pm 0.22

concentrations were dramatically reduced by about 87% to reach $0.86 \pm 0.49 \text{ mg O}_2 \text{ L}^{-1}$ (mean \pm SD) between H1 and H2 in “fine sand” treatment whereas this reduction was limited to less than 13% in “coarse sand” treatment. Consequently, concentrations of O_2 and NO_3^- in interstitial water were significantly lower in “fine sand” than in “coarse sand” treatment (RM-ANOVA2: $F_{(1, 24)}=8,186.24$ and $1,216.71$, respectively, $P < 10^{-4}$ for both). From the measurements of O_2 and NO_3^- concentrations, our calculations showed that clogging by fine sand significantly influenced the vertical distribution of O_2 and NO_3^- uptake

rates in the sedimentary column (Fig. 4; ANOVA2 interaction: $F_{(2, 18)}=19.85$ and 116.91 , respectively, $P < 10^{-4}$ for both). Although average O_2 uptake rates were similar for the two treatments in the top sediment layer (Fig. 4a; contrast: $|t|_{18}=0.98$, $P=0.85$), clogging significantly decreased O_2 uptake rates in the deeper sediment layers (contrast: $|t|_{18}=3.20$ and $|t|_{18}=7.93$ for layer 2 and layer 3, respectively, $P < 10^{-4}$ for both). In contrast, clogging significantly increased NO_3^- uptake rates in the sedimentary column (Fig. 4b; ANOVA2: $F_{(1, 24)}=204.83$, $P < 10^{-4}$) and especially in the top sediment layer (contrast: $|t|_{18}=20.72$, $P < 10^{-4}$).

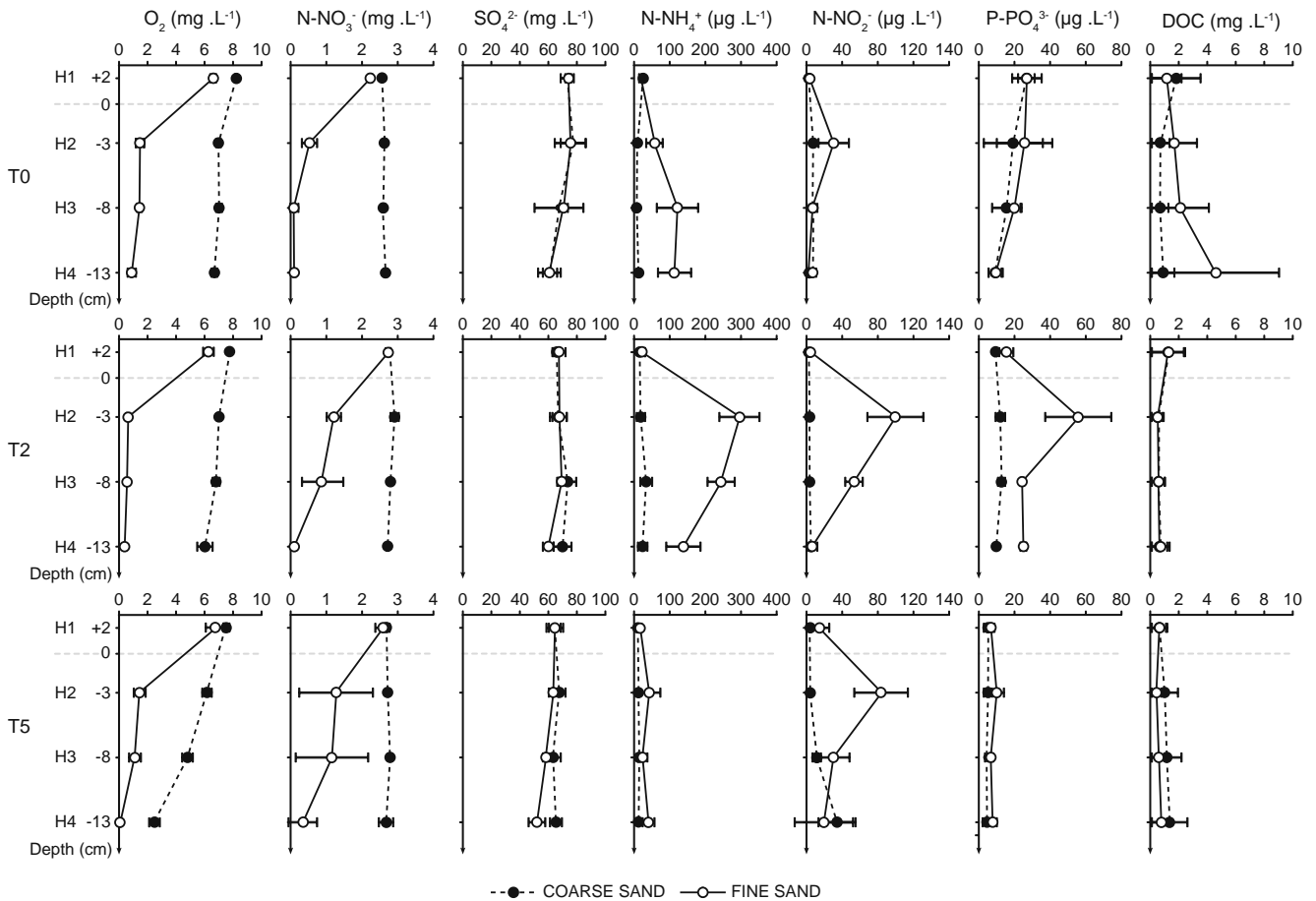


Figure 3 Depth profiles for O_2 , N-NO_3^- , N-NO_2^- , N-NH_4^+ , DOC, SO_4^{2-} and PO_4^{3-} concentrations determined at four depths (from H1—2 cm above sediment interface to H4—11 cm below sediment interface) in

sediment columns clogged or not by fine sediment deposits (“fine sand” and “coarse sand” treatment, respectively) on dates T0, T2 (20 days) and T5 (50 days)

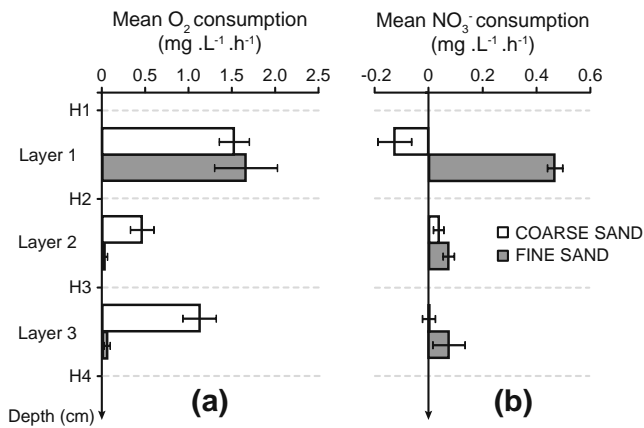


Figure 4 Average uptake rates for **a** dissolved oxygen and **b** nitrate, calculated between four depths (from H1—2 cm above sediment interface to H4—11 cm below sediment interface) in sediment columns impacted or not by fine sediment deposits (“fine sand” and “coarse sand” treatment, respectively)

As observed for O_2 and NO_3^- , SO_4^{2-} concentration in the sedimentary matrix was influenced by clogging with fine sand (RM-ANOVA2: $F_{(1, 24)}=15.67$, $P=6.10^{-4}$) and depth (RM-ANOVA2: $F_{(3, 24)}=9.26$, $P=3.10^{-4}$). However, no significant difference in decrease of SO_4^{2-} concentrations was measured between “fine sand” and “coarse sand” treatments (Fig. 3; RM-ANOVA2 interactions $F_{(3, 24)}=2.77$, $P=0.06$).

The concentrations of NH_4^+ , NO_2^- , PO_4^{3-} and DOC in the overall sedimentary matrix were significantly higher in the “fine sand” treatment than in the “coarse sand” treatment (RM-ANOVA2: $F_{(1, 24)}=93.02$, $F_{(1, 24)}=665.57$, $F_{(1, 24)}=193.27$ and $F_{(3, 24)}=16.13$, for DOC, NH_4^+ , NO_2^- and PO_4^{3-} , respectively, $P<6.10^{-4}$ for all). Peaks of DOC, NH_4^+ , NO_2^- and PO_4^{3-} concentrations were recorded in the sedimentary matrix at several dates for the “fine sand” treatment (especially at the H2 layer and at the T2 sampling date) whereas such peaks were not observed for the “coarse sand” treatment (Fig. 3).

Therefore, the effect of depth on nutrient concentrations was significantly influenced by clogging (ANOVA2: interaction $F_{(3, 24)}=29.01$, $F_{(3, 24)}=96.77$, $F_{(3, 24)}=71.14$ and $F_{(3, 24)}=6.03$, respectively, $P<4.10^{-3}$ for all).

Microbial Analyses on Sediment

TOTB and ACTB significantly decreased with depth in sediment (Fig. 5a, b; ANOVA2: $F_{(2, 18)}=416.92$ and 351.24 for total abundance and abundance of active bacteria, respectively, $P<10^{-4}$ for both variables). These decreases were significantly higher in “fine sand” treatment (ANOVA2: interaction $F_{(2, 18)}=136.39$ and $F_{(2, 18)}=129.14$ for TOTB and ACTB, respectively, $P<10^{-4}$ for the two variables) due to higher densities of bacteria in the top 2 cm of sediment (2.20- and 2.38-fold higher, contrast: $|t|_{18}=21.12$ and $|t|_{18}=19.32$, for TOTB and ACTB, respectively, $P<10^{-4}$ for both). The percentage of active bacteria also decreased with depth in sediment (Fig. 5c; ANOVA2: $F_{(2, 18)}=48.18$, $P<10^{-4}$). Clogging by fine sand significantly decreased the percentage of active bacteria (ANOVA2: $F_{(1, 18)}=8.10$, $P=0.01$) in the sediment, in particular at 10–12 cm (contrast: $|t|_{18}=5.82$, $P<10^{-4}$).

Potential aerobic respiratory (Fig. 5d) and denitrification (Fig. 5e) activities determined in the three sediment layers significantly decreased with depth (ANOVA2: $F_{(2, 18)}=5.51$, $P<0.02$ and $F_{(2, 18)}=100.33$, $P<10^{-4}$, respectively). These potential activities were higher in “fine sand” than in “coarse sand” treatment (ANOVA2: $F_{(1, 18)}=9.52$, $P<5.10^{-3}$ and $F_{(1, 18)}=362.24$, $P<10^{-4}$, respectively). The influence of clogging by fine sand on potential denitrification was observed at all sediment layers (contrast: $|t|_{18}=16.36$, $|t|_{18}=6.62$ and $|t|_{18}=9.99$ for SED1, SED2 and SED3, respectively, $P<10^{-4}$ for all) whereas clogging only stimulated aerobic respiration in the top sediment layer (contrast: $|t|_{18}=2.55$, $P=0.02$ for SED1; $|t|_{18}=1.24$, $P=0.23$

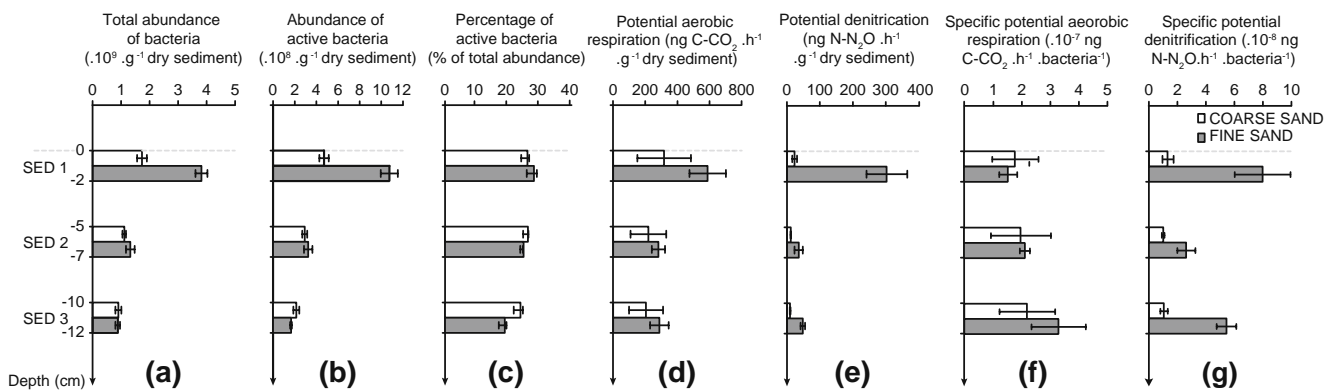


Figure 5 Microbial characteristics: total abundance of bacteria (a), abundance (b) and ratio (c) of active bacteria, potential aerobic respiration (d), potential denitrification (e), specific aerobic respiration (f) and specific denitrification (g) determined at three sediment layers:

SED1 (0–2 cm), SED2 (5–7 cm) and SED3 (10–12 cm) in sediment columns impacted or not by fine sediment deposits (“fine sand” and “coarse sand” treatment, respectively)

for SED2 and $|t|_{18}=1.56$, $P=0.14$ for SED3). The significant increase with depth (ANOVA2: $F_{(2, 18)}=3.88$, $P=0.04$) observed for specific potential aerobic respiration (aerobic respiratory activity reported per bacterial cell) was not influenced by clogging (Fig. 5f; ANOVA2: interaction: $F_{(2, 18)}=1.53$, $P=0.24$). In contrast, specific potential denitrification was greatly stimulated by clogging with fine sand (Fig. 5g; ANOVA2: $F_{(1, 18)}=129.49$, $P<10^{-4}$), at all depths in the sedimentary matrix (contrast: $|t|_{18}=10.39$, $P<10^{-4}$ for SED1; $|t|_{18}=2.48$, $P=0.02$ for SED2 and $|t|_{18}=6.84$, $P<10^{-4}$ for SED3).

Determinations on Buried POM

Determinations of the dry mass of leaf litter retrieved at the end of the experiment (Fig. 6a) showed that the daily mass loss rate was 30% higher in “coarse sand” than in “fine sand” treatment (t test: $|t|_6=4.28$, $P<6.10^{-3}$). In parallel, the total abundance of bacteria (Fig. 6b), the abundance of

active bacteria (Fig. 6c) and the percentage of active bacteria (Fig. 6d) were higher in “coarse sand” than in “fine sand” treatment (t test: $|t|_6=2.94$, $|t|_6=3.70$ and $|t|_6=6.69$, respectively, $P<0.03$ for all). Although no significant difference in fungal biomass on leaf litter was detected between “fine sand” and “coarse sand” treatments (t test: $|t|_6=1.92$, $P=0.10$), mean value of fungal biomass was 40% higher in “coarse sand” treatment (Fig. 6e). The potential aerobic respiration (Fig. 6f) and denitrification (Fig. 6g) activities of microorganisms developed on leaves were not significantly influenced by clogging (t test: $|t|_6=1.40$, $P=0.21$ and $|t|_6=1.01$, $P=0.35$, respectively). In parallel, specific potential denitrification rate was not influenced by clogging with fine sand (Fig. 6i; t test: $|t|_6=1.76$, $P=0.13$) whereas specific aerobic respiration was higher in “coarse sand” than in “fine sand” treatment (Fig. 6h; t test: $|t|_6=2.49$, $P<0.05$).

While xylosidase activity (Fig. 6j) was not influenced by clogging (t test: $|t|_6=0.94$, $P=0.38$), glucosidase and leucine

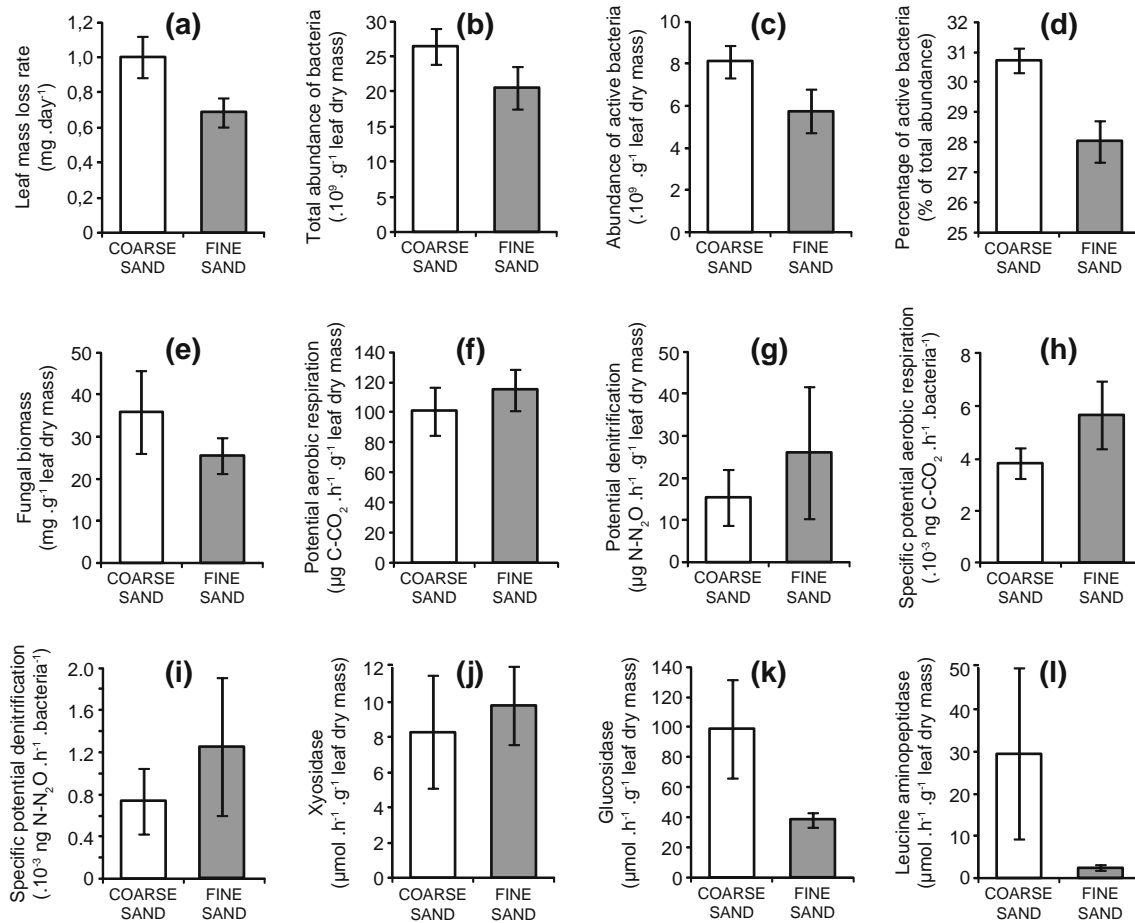


Figure 6 Leaf mass loss rate (a), total abundance of bacteria (b), abundance (c) and ratio (d) of active bacteria, fungal biomass (e), potential aerobic respiration (f) potential denitrification (g), specific aerobic respiration (h) and specific denitrification (i), xylosidase (j),

glucosidase (k) and leucine aminopeptidase activities (l), determined on leaves buried at 9 cm depth in sediment columns impacted or not by fine sediment deposits (“fine sand” and “coarse sand” treatment, respectively)

aminopeptidase activities associated with the decomposing leaves were significantly higher in “coarse sand” than in “fine sand” treatment (Fig. 6k, l; *t* test: $|t|_6=4.85$, $P<3.10^{-3}$ and $|t|_6=6.12$, $P=9.10^{-4}$, respectively).

Discussion

Clogging and Electron Acceptor Availability in Hyporheic Sediment

Our microcosm approach effectively reflected the influence of sediment clogging on hydraulic conductivity and associated biogeochemical processes occurring in the interstitial system. The deposition of fine-grained sediment particles that fulfilled interstices and generated a compact layer (2 cm height) at the surface of a porous (sand and gravel) sedimentary matrix resulted in a dramatic reduction of the hydraulic conductivity, i.e. from 16 to 2 cm h⁻¹. Such clogging process is comparable to observations from field studies showing that inputs of fine sediment into stream and river beds leading to physical clogging of the heterogeneous streambed interstices [3, 32, 53] can reduce both sediment permeability [57] and effective porosity [18]. In slow filtration columns (with a water–sediment interface of 78 cm²), the change in permeability due to fine sediment deposition reduced flow rates from 2.5 to 0.3 mL min⁻¹ (Table 1). This drastic reduction in water flow led to a low renewal of O₂ in sediments resulting in a sharp decrease in O₂ concentrations with depth. For instance, O₂ concentrations were lower than 0.6 mg L⁻¹ at 3 cm below the water–sediment interface of the systems clogged with fine sand whereas it remained higher than 2 mg L⁻¹ for the unclogged system in the whole-sediment column (up to 13 cm below the water–sediment interface). The shift from aerobic to anaerobic processes with the retention time of water in the sediment was in agreement with the classical suite of metabolic pathways commonly reported from natural systems: O₂ being consumed during OM mineralization in the oxic zone, followed by the consumption of NO₃⁻ (denitrification), manganese and iron oxides, SO₄²⁻ and carbon dioxide (from the most to the lowest energetically favourable mechanism by which microorganisms oxidize organic carbon material) [26, 31]. In systems clogged with fine sand, O₂ became limiting for microorganisms living in the sedimentary columns, which thus used nitrate as electron acceptor (denitrification) leading to a sharp decrease of NO₃⁻ with depth in the sediment. In the same way, the anoxic conditions observed in clogged systems induced a slight consumption of SO₄²⁻ at 13 cm depth in the sediment after 2 weeks of experiment. In contrast, we did not detect any significant reduction of NO₃⁻ and SO₄²⁻ concentrations with depth in the columns not impacted by fine sediment deposition. The rapid succession

of the electron acceptors (O₂, NO₃⁻ and SO₄²⁻) with decreasing redox potential, observed in our clogged systems was associated to high releases of NH₄⁺, PO₄³⁻ and DOC in the deepest sediment layers, mainly due to the anaerobic mineralization of OM. Indeed, as reported in previous studies using infiltration sediment columns [41, 49], the production of DOC in the sediment was probably linked to the production of volatile fatty acids resulting from the anaerobic degradation of the particulate OM [29]. Therefore, our experimental systems well reproduced the rapid shift from aerobic to anaerobic conditions like observed in field studies measuring the effects of sediment deposition on biogeochemistry [32].

Clogging and Bacterial Compartment Developed on Sediments

The influence of clogging on bacteria developed on sediments was predominantly observed on the top sediment layer (0–2 cm) due to differences in sediment characteristics (particle size and OM content). The difference in colonisable area between fine and coarse sands (1,465.00±33.17 and 59.37±0.34 cm² g⁻¹, respectively) may explain the higher bacterial abundances measured in the top layer of the clogged system in comparison with the unclogged one [34, 55, 56]. However, Nogaro et al. [47] did not detect any positive relationship between the proportion of fine sediments and the abundance of bacteria attached to sediments in French rivers (Usses, Drôme and Isère) impacted by fine sediment deposition. These authors suggested that fine sediment deposition did not affect bacterial abundances because it did not always increase the OM content of the sediment. Indeed, several studies [14, 15, 59] demonstrated that bacterial communities (biomass, production) developed on sediment depended on the quantity and lability of the OM. In the present study, bacterial abundances and potential aerobic respiration in the top layer of sediments (0–2 cm) were higher in the clogged than in unclogged systems because of a higher colonisable area for bacteria (×24.7) and a higher OM content (×17.1 for total organic carbon, ×7.8 for total nitrogen and ×1.9 for phosphorus) in fine than in coarse sand.

As the clogging by fine sand greatly modified the aerobic conditions and the interstitial water chemistry (NO₃⁻, NH₄⁺, PO₄³⁻ and DOC) of the whole sedimentary column, microorganisms were expected to be affected not only on the top sediment layer (0–2 cm) but also at depth of the sedimentary column. According to the differences in aerobic conditions and NO₃⁻ consumption between clogged and unclogged systems, the microbial communities on sediments exhibited a higher denitrification potential per bacterial cell in depth

in clogged conditions. Such a result indicates that the shift from aerobic to anaerobic conditions (with changes in availability of electron acceptors) induced by clogging with fine sand favoured the establishment of denitrifying bacteria within bacterial assemblage living on sediments. Despite this change, the enrichment of interstitial water with NH_4^+ , PO_4^{3-} and DOC due to clogging did not stimulate bacterial development in deep sediment. In slow filtration columns, Nogaro et al. [48] showed that the deposition of OM-rich sediments on a sandy matrix could stimulate the bacterial abundances and activities at depth in a sandy matrix. These stimulations were mainly related to the quantity and quality of DOC released by the OM-rich sediments and transported deep down by the infiltrating flow. In our study, average release rates of DOC between superficial water and the bottom of the sedimentary matrix in treatment impacted by fine sediment deposits was $1.4 \text{ mg DOC day}^{-1}$, i.e. more than 5-fold lower than in the study of Nogaro et al. [48]. In our conditions, the quantity of dissolved OM transferred from the top sediment layer to the deepest layer was not large enough to significantly stimulate the bacterial compartment.

Clogging and Processing of OM in Hyporheic Sediments: Biogeochemical Processes and Leaf Litter Breakdown

The main influence of clogging on microorganisms developed on sediments was related to changes in microbial functionality, like the development of denitrifying bacteria. However, such bacterial changes did not inform us about the capacity of the sedimentary system to process OM. Based on O_2 and NO_3^- uptake rates measured between 3 and 13 cm depth in our columns, we assessed the influence of clogging with fine sand on the potential capacity for the microbial assemblages of the sediment column to consume organic carbon. SO_4^{2-} consumption (reduction) was not integrated into our calculations because vertical gradients of SO_4^{2-} concentrations were not significantly different between the clogged and unclogged columns. Assuming a consumption of 1 mol of carbon for 1 mol of O_2 consumed during the aerobic degradation of the OM and a consumption of 1 mol of carbon for 4/5 mol of NO_3^- consumed during the denitrification process [26], we estimated that $35.5 \pm 18.7 \text{ mg C-CH}_2\text{O}$ (83% by using NO_3^-) and $138.7 \pm 17.7 \text{ mg C-CH}_2\text{O}$ (95% by using O_2) were respectively consumed in the clogged and unclogged systems during the experiment. These results suggest that clogging by fine sediment deposition greatly reduces the processing of organic carbon in sediments. However, such assessment was potentially biased by the quantity of OM in the sedimentary columns which modulated microbial abundances and activities. For instance, the release of DOC from the top sediment layer could both favour microbial development and reduce the availability of electron acceptor necessary for OM

degradation in depth. The assessment of OM processing using biogeochemical process is also dependent on the ratio between C consumed and O_2 (or NO_3^-) uptake rate which mainly depends on the quality of the OM consumed. Therefore, the use of buried leaf litter is expected to be more pertinent to assess the influence of environmental conditions on OM processing in hyporheic sediments. Our results showed that clogging with fine sand reduced by 30% the breakdown of buried leaf litter. This substantial influence of clogging on OM processing was, however, less marked than what it would be expected from aerobic respiration and denitrification rates (reduction by 75%), highlighting limitations when relying only on biogeochemical process rates to estimate particulate OM degradation (see above). The decrease in leaf litter breakdown rates due to clogging was clearly linked to reductions in several microbial parameters measured on leaves (abundances of bacteria, percentage of active bacteria, glucosidase activity and leucine aminopeptidase activity). This adverse influence of clogging on microbes involved in OM degradation resulted from the low availability of electron acceptors (mainly dissolved O_2 and NO_3^-) necessary for OM degradation [6, 33, 40]. The lower (-40%) mean fungal biomass determined in the clogged system could also be related to changes in O_2 concentrations because low O_2 availability could dramatically reduce fungal colonization of leaves [40]. Thus, the reduction of exoenzymatic activities under clogged conditions were probably linked to the adverse influence of fine sediment clogging and associated anaerobic conditions on microbial abundances and biomasses [58]. We can also hypothesize that a shift from aerobiosis to anaerobiosis could have partially influenced exoenzymatic activities [44], but such biochemical control is still under debate [38, 44]. Therefore and in accordance with field studies [16, 32], the present work highlights the predominant role played by the sediment structure and water-sediment exchanges (O_2 availability) on the ability of hyporheic microorganisms to process OM.

Conclusions and Perspectives

While field experiments have demonstrated that leaf litter breakdown by microorganisms was controlled by the concentrations of nutrients in streams [25, 54, 60], other reported low leaf breakdown to be associated to low DO concentrations [61]. Our study clearly supports the idea that DO concentration is a major factor influencing the breakdown of buried leaf litter in hyporheic sediments.

In benthic habitats of streams, leaf litter breakdown, a process realized by the concerted action of invertebrates (especially shredders) and microorganisms (i.e. fungi and bacteria), is now used to assess stream ecological integrity [5, 21, 64] as it is significantly affected by stresses such as

eutrophication [24] and acidification [12]. Our study clearly indicates that the breakdown of buried leaf litter could also be used to assess the impact of a reduction of sediment permeability on hyporheic zone functioning. Although we did not consider the role played by hyporheic fauna on buried OM in the present study, hyporheic microorganisms are probably the key actors of OM processing in fine-textured sediments where the accessibility of buried OM is low for leaf-shredding invertebrates such as gammarid species [8, 46]. Therefore, the use of leaf litter breakdown in river sediments by relying on a relevant method [36] looks very promising to assess the influence of environmental conditions on functioning of the hyporheic zone.

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