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Do tubificid worms influence organic matter processing and fate of pollutants in stormwater sediments deposited at the surface of infiltration systems?

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Abstract

The purpose of this study was to quantify the influences of tubificid worms on the biogeochemical functioning of an infiltration system impacted by a stormwater sediment deposit. Effects of worms with stormwater sediment deposit were compared with effects of worms with two other natural sediment deposits (one low and one rich-particulate organic matter deposits). We measured the effects of invertebrates on sediment reworking, organic matter processing, solute fluxes, microbial characteristics, and pollutant release from stormwater deposit to water. Our results showed that tubificid worms had slight effects on microbial activities in presence of the stormwater deposit whereas they significantly stimulated microbial activities in columns impacted by the other two deposits. High contents of labile organic matter contained in stormwater sediments probably led to very strong microbial activities that could not be easily stimulated by worm activities. Moreover, tubificid worms did not influence the fate of pollutants (heavy metals and PAHs) contained in the stormwater deposit. In conclusion, our study demonstrated that the organic matter characteristics of the stormwater sediments limited the efficiency of tubificid worms to stimulate organic matter mineralization in infiltration systems.

Keywords: Polluted sediments; Bioturbation; Microbial activities; Biogeochemical processes; Slow infiltration columns

38 # 1. Introduction

39 Management of urban stormwater consists in collecting and pouring stormwater into
40 rivers, ponds or infiltration basins (Marsalek and Marsalek, 1997; Barraud et al., 2002). Such
41 discharges of urban stormwater may cause numerous adverse effects including the import of
42 heavy metals, organic compounds and pathogens to receiving waters (Pitt et al., 1999). Most
43 pollutants transported by stormwater are associated with suspended sediments (Pitt et al.,
44 1999), these sediments being retained into the beds of rivers and infiltration basins. For
45 example, Datry et al. (2003b) estimated that a total of 4588 kg of particulate organic carbon,
46 284 kg of particulate nitrogen, 284 kg of particulate phosphorus, 128 kg of hydrocarbons, and
47 153 kg of heavy metals (Zn, Pb, Cu, Cr, Ni, and Cd) were retained in a small infiltration basin
48 draining an urban catchment of 2.5 ha. Such characteristics of stormwater sediments may
49 strongly affect biogeochemical processes occurring at the water-sediment interface (Datry et
50 al., 2003a, b; Nogaro et al., in press).

51 Despite high pollutant contents, stormwater deposits may be colonised by invertebrate taxa
52 adapted to life in suboxic and contamined environments such as tubificids worms (Datry et
53 al., 2003a). In stormwater sediments, these worms can create dense networks of burrows and
54 galleries (Mermillod-Blondin et al., 2005). Most bioturbation activities of worms could
55 stimulate the solute exchanges (oxygen and metabolites) across the water-sediment interface
56 and then the microbial processes such as nitrification and denitrification (Pelegri and
57 Blackburn, 1995; Svensson et al., 2001). In stormwater deposits, Mermillod-Blondin et al.
58 (2005) showed that tubificids could stimulate the organic matter mineralization and the
59 release of nutrients and pollutants in stagnant systems. However, the influence of worms on
60 the mineralization of stormwater deposits in infiltration systems (such as infiltration basins or
61 hyporheic zone of rivers) has never been studied.

62 The aim of this study was to quantify the worm effects on mineralization rates of
63 stormwater deposits (collected in urban area). These effects were compared with those

measured in two natural sediment deposits characterized by different particulate organic matter (POM) contents in order to determine if the urban deposits specifically influenced worm effects in the infiltration system. With these aims, our experiment were done using infiltration columns filled with gravel and sand with inputs of sediment deposits with different characteristics (POM) in surface and supplied by water under a constant flow rate. We measured the effects of invertebrates on sediment reworking, organic matter processing (O_2 uptake), solute fluxes (fluxes of NO_3^-, NO_2^-, NH_4^+, and COD), microbial characteristics (biomass, functional diversity and activities), and pollutant release rates from stormwater deposits to water. Our first hypothesis was that the effects of tubificid worms in stormwater deposits would be significantly different than the effects measured in other deposits due to the physico-chemical characteristics of the three sediment deposits (quantity of POM, quality of POM-C/N and C/P ratios-, and occurrence of pollutants). We also hypothesized that tubificid worms could modify the fate of pollutants (hydrocarbons and heavy metals) in the system impacted by stormwater sediment deposits.

2. Materials and methods

2.1. Sediment columns

Experiments were carried out in slow infiltration columns (Mermillod-Blondin et al., 2000). Each column (height = 45 cm and inside diameter = 10 cm) was constituted by association of four experimental modules (10 cm high) topped by a fifth module 5 cm high. We used 18 columns which were filled with a mixture of gravel and sand in order to constitute a deep layer of heterogeneous sediment (28 cm depth) with a 2 cm sediment layer in surface. Three different types of sediment deposit were added at the surface: (1) a stormwater (STORM) deposit, (2) a particulate organic matter-rich (POM-rich) deposit, and (3) a particulate organic matter-low (POM-low) deposit.

90 ***2.2. Preparation of heterogeneous sediments and sediment deposits***

91 *2.2.1. Heterogeneous sediment (gravel and sand)*

92 Gravel and sand were collected from the Rhône River. Gravel was sieved manually to
93 select particle sizes ranging from 5 to 8 mm and then was cleaned with deionized water before
94 being dried at 60°C. Before filling the columns, 18 kg of dry sand were manually mixed with
95 90 g of fibrous cellulose powder (0.5% of the sediment weight) to stimulate the microbial
96 growth. A volume of 10 L of synthetic water (96 mg L^{-1} $NaHCO_3$, 39.4 mg L^{-1} $CaSO_4,2H_2O$,
97 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}
98 $(CH_3CO_2)_2CaH_2O$) was added to the sand which was inoculated with an extract of natural
99 bacteria as described by Mermillod-Blondin et al. (2000).
100
101 *2.2.2. Different types of fine sediment deposits*

102 The STORM deposit was collected on a stormwater infiltration basin located on the
103 campus of the University Claude Bernard (Lyon, France). The POM-rich and the POM-low
104 deposits were collected on braided channels of the Rhône River at about 80 km east of Lyon.
105 These three types of fine sediment deposit were sieved (<1000 µm) and homogenized in the
106 laboratory before use. The particle size distributions of different fine deposits (STORM,
107 POM-low, and POM-rich deposits) were determined by a laser diffraction granulometer
108 (Mastersizer 2000, Malvern Instrument, UK). The STORM, the POM-rich, and the POM-low
109 deposits were characterized by a high proportion of fine sediment particles with respectively
110 42-48, 45-52, and 47-54 % of volume of particles lower than 100 µm.
111
112 ***2.3. Experimental design***

113 Each column was filled with the sand previously incubated and gravels to a height of 28
114 cm on day -11 (11 days before fauna deposit). Constant masses of gravels (600 g) and
115 incubated sand (215 g) were alternately added (five times) and finally 150 g of sand were

116 added at the sediment surface in order to obtain a heterogeneous interface corresponding as
117 much as possible to river natural sediments. On day -9, the columns were supplied with
118 synthetic water with a peristaltic pump controlling a constant infiltration flow rate of 1.5 ml
119 min^{-1}. On day -6, 100 g of incubated sand were added in each column before that of surface
120 deposit to prevent a too important penetration of the surface deposit in the heterogeneous
121 matrix. Then, we added 250 g of each sediment deposit (STORM, POM-low and POM-rich
122 deposits) in each deposit treatment (6 columns per deposit treatment). The total height of
123 sediment in each column was 30 cm (28 cm of heterogeneous sediment and 2 cm of fine
124 deposit). The use of a 2 cm layer of fine deposit was in accordance with the thickness of fine
125 sediments reported on the bed of infiltration basins (Bedell et al., 2004) and rivers (Wood and
126 Armitage, 1997). About 10 cm of water was left above the sediment surface.

127 On day 0, tubificids were introduced in the columns (in free water). For each surface
128 deposit treatment (STORM, POM-rich, and POM-low deposits), two fauna treatments were
129 performed with three replicates per treatment: (1) without invertebrate (controls) and (2) with
130 160 tubificid worms per experimental unit. The invertebrate densities used in our experiment
131 (20 400 individuals. m^{-2}) were typical for lakes and streams (McCall and Fisher, 1980).
132 Tubificids were collected from the Rhône River and were about 60% *Tubifex sp.* and 40%
133 *Limnodrilus sp.* For acclimation to experimental conditions (particle size and temperature),
134 animals were kept in the laboratory for more than 10 days before use in infiltration columns.
135 At the end of the experiment, we recovered 68.8 ± 21.7 % (mean ± SD, n = 9) of the animals
136 added initially. Experiments were performed at constant temperature (15 ± 0.5 °C) and the
137 light was controlled on a 12 h light, 12 h dark cycle in the overlying water. In contrast, the
138 sediment of the column was kept in the dark to suppress photoautotrophic growth.
139

140 ***2.4. Methods of analysis***

141 *2.4.1. Chemical composition of the sediment deposits and the heterogeneous sediment*

142 Concentrations of particulate organic matter (POM), particulate organic carbon (POC),
143 particulate nitrogen (PN), particulate phosphorus (PP), heavy metals, and polycyclic aromatic
144 hydrocarbons (PAHs) were determined in the three sediment deposits (STORM, POM-rich,
145 and POM-low deposits) and in the sand of the heterogeneous sediment. The measurements
146 were performed in fresh material before use in experimental columns to characterize the
147 chemical properties of the different deposits and the bed sediment layer.
148 POM content was determined as loss upon ignition at 550°C for 5h. POC, PN, PP, and
149 heavy metal analyses were performed by the Central Service of Analysis of the French
150 National Centre for Scientific Research (CNRS) in Lyon (France) following standard methods
151 (Buchanan, 1984; Hedges and Stern, 1984). PAH analyses were performed by the Health and
152 Environmental Laboratory of Lyon using a HPLC with fluorescence detectors (Agilent 1100).
153
154 *2.4.2. Sediment reworking analyses*
155 Luminophores (natural sediment particles covered with a luminescent paint) were used to
156 estimate sedimentary reworking by invertebrates in the columns. The day after introduction of
157 the invertebrates, 1g of yellow luminophores (160–315 μm) was deposited at the sediment
158 surface of each column. At the end of the experiment, the water layer was carefully pumped
159 out and the sediment of each column was cut into slices. The top 3 cm were sliced at 0.5 cm
160 thickness while the next 7 cm were sliced in 1 cm layers. Each slice was sieved to remove
161 gravel (with a sieve of 2 mm) and recover living organisms (with a sieve of 500 μm). Five g
162 of sieved sediment were taken from each slice and dried at 40°C for 48h before being mixed
163 delicately to homogenize the sediment without breaking the luminophores. Luminophores
164 were counted with a U.V. light microscope and expressed as number of luminophore per gram
165 of dry sediment. Sediment transport at the interface due to the invertebrate activities was
166 estimated by comparing the vertical profiles of luminophores in each treatment (i.e. control

and tubificid). Vertical profiles of luminophores in the sediment were obtained from percentages of luminophore found in each slice for each column.

2.4.3. Physico-chemical analyses

Physico-chemical analyses were performed in water samples of slow infiltration columns to study the biogeochemical processes in sedimentary columns. We measured dissolved oxygen (O_2), nitrate, nitrite, ammonium, dissolved organic carbon (DOC) in water samples taken from different depths (+5 cm above and -1, -5, -15, and -25 cm below the water-sediment interface) of slow filtration columns and at days 0 (before the fauna introduction), 2 (= 2 days after the fauna introduction), 6, 10, 14, and 20. Measurements of nitrates, ammonium, and DOC were also performed in the tank containing the supplied synthetic water and at -25 cm below the water-sediment interface of each column every second day during the course of the experiment (from days 1 to 20). The average release rate of each solute for each column and then, the average release rate per fauna treatment (control vs. tubificid) for each deposit treatment were calculated.

Dissolved O_2 measurements were done according to the method of Mermillod-Blondin et al. (2000) using a 3600 Orbisphere model oxygen meter. Nitrate, nitrite and ammonium contents were measured using colorimetric HACH methods according to Mermillod-Blondin et al. (2000). For DOC measurements, water samples were measured according to the method of Mermillod-Blondin et al. (2000) using a Dohrman DC 80 “Total carbon analyser”.

2.4.4. Microbial analyses

Microbial analyses were performed in sediment at different depth to study the influence of worms on microbial communities, activities and functional diversity in presence of different sediment deposits. At the end of experiments (on day 22), the water layer was carefully pumped and the sediment of each column was collected from four depth layers (0-3, 3-6, 13-

16, and 23-26 cm). Each layer was sieved to remove gravel (with a sieve of 2 mm) and recover living organisms (with a sieve of 500 µm).

The DNA intercalating dye (DAPI) and a Cy3-probe (EUB 338, eubacteria) were used on sediment samples to determine the total numbers of bacteria stained with DAPI and the percentages of active eubacteria (% EUB/DAPI). Two g of wet sediment were taken in each layer and were prepared according to Mermillod-Blondin et al. (2005). Numbers of DAPI and Cy3-bacteria were counted separately from the same field in order to calculate the percentages of active bacteria (EUB/DAPI) and the total numbers of bacteria stained with DAPI from each analyzed field. Results were expressed as numbers of bacteria g^{-1} of sediment dry weight (DW).

Activity (global Average Well Colour Development) and functional diversity (number of substrates used) were measured with Biolog ECO microplates. Details concerning the methods of analysis were given in Nogaro et al. (in press).

Aerobic respiration and denitrification were performed following the slurry technique (Furutani et al., 1984). About 10 g of wet sieved sediment of each sediment layer were prepared according to Nogaro et al. (in press). Results were expressed as µg of C or N h^{-1} g^{-1} sediment DW. Hydrolytic activity was measured using fluorescein diacetate (FDA) as substrate for hydrolases (Fontvieille et al., 1992). Three wet sieved sediment samples (0.95–1.05 g) of each sediment layer were prepared following Nogaro et al. (in press). Results were expressed as micromoles of hydrolyzed FDA h^{-1} g^{-1} of sediment DW.

2.4.5. Hydrocarbon and heavy metal release from stormwater sediment deposit to water

Concentrations of 4 heavy metals and 15 hydrocarbons widely found in stormwater sediments (Datry et al., 2003b) were measured in water at the outlet of the columns on days 1, 8 and 15 of the experiment in the columns with STORM deposit in surface (Table 2).

218 Analyses of hydrocarbons and metals in water were performed by the Health and
219 Environmental Laboratory of Lyon following standard methods (Clesceri et al., 1998).
220
221 ***2.5. Data treatment***
222 We tested the effects of fauna treatment (control and tubificid) and sediment deposit
223 treatment (STORM, POM-low, and POM-rich deposits) on the percentage of luminophore
224 found at each depth using a two-way analysis of variance (ANOVA). If significant differences
225 were detected, Scheffé post hoc tests were performed to determine if the effect of tubificids
226 were observed for each deposit treatment.
227 For physico-chemical variables (O_2, NO_3^-, NH_4^+ and COD), we tested the homogeneity
228 among the columns on day 0 (before the fauna deposition) using a two-way ANOVA for each
229 type of surface deposit with fauna treatment (i.e. control and tubificid) and depth (+ 5 cm
230 above and -1, -5, -15, -25 cm below the water-sediment interface) as main effects. After fauna
231 addition, a two-way repeated measures ANOVA (RM-ANOVA) was used on physico-
232 chemical variables to detect differences among fauna treatments and depths using time as
233 repeated factor (days 2, 6, 10, 14, and 20). If significant differences were detected among
234 fauna treatments, Scheffé post hoc tests were performed to determine the differences among
235 fauna treatments for each depth and time. For the average release rates of solutes (NH_4^+ and
236 DOC) from days 1 to 20, we tested the effects of fauna treatment and surface deposit
237 treatment using a two way ANOVA and Scheffé post hoc tests.
238 For bacterial measurements, we tested the fauna treatment and depth effects using a two-
239 way ANOVA and Scheffé post hoc tests for each type of surface deposit. We tested the fauna
240 treatment and time (measurements performed at days 1, 8 and 15) on metal and PAH release
241 rates using two-way ANOVA with fauna treatment and time as main effects.
242 When necessary, data were log-transformed, and data expressed as percentages (% of
243 luminophore and active bacteria) were arcsine-transformed before statistical analysis, to fit

244 the assumption of homoscedasticity. Statistical analyses were performed using Statistica 6 TM
245 *(Statsoft, Tulsa, OK, USA).*
246

247 # 3. Results

248 ### *3.1. Composition of the surface sediment deposits and the heterogeneous sediment*

249 The STORM and the POM-rich deposits had high POM and POC contents compared to
250 the POM-low deposit (Table 1). The concentrations of PN and PP were higher in the STORM
251 deposit than in the two other deposits. The STORM and the POM-low deposits had
252 comparable atomic ratio of C/N and C/P which were higher than those of the POM-rich
253 deposit. Moreover, STORM deposit was characterized by high concentrations of heavy metals
254 and hydrocarbons whereas no pollutants were detected in the other sediment deposits.

255 Before use in experimental column, the sand of the heterogeneous sediment layer was
256 characterized by 17.3 ± 1.2, 7.7 ± 0.6, 0.3 ± 0.06, and 0.16 ± 0.01 $g.kg^{-1}$ sed. DW of POM,
257 POC, PN, and PP, respectively (mean ± SD, n = 3). No pollutants (heavy metals and
258 hydrocarbons) were detected in the sand in all experimental columns at the beginning and at
259 the end of the experiment.
260

261 ### *3.2. Sediment reworking analyses*

262 In the control columns, more than 97 % of the luminophores were in the upper layer (0.5
263 cm depth) of sediments at the end of the experiment (Fig. 1). In the animal treatments, the
264 percentages of luminophores in this upper layer were 60.5 ± 11.1, 84.7 ± 4.9, and 63.7 ± 15.8
265 % in presence of STORM, POM-low, and POM-rich deposits, respectively. These
266 percentages were significantly different among control and tubificid treatments depending on
267 the type of deposit (Fig. 1, two-way ANOVA, interaction "fauna x deposit" effect, $p<0.05$).
268 The tubificids significantly buried luminophores at depth (Scheffé post hoc tests, $p<0.001$)
269 and with a same intensity in presence of STORM and POM-rich deposits treatments (Scheffé

post hoc test, $p>0.05$). In contrast, the percentages of luminophores measured at depth were not significantly different among control and tubificid treatments in the POM-low deposit treatment (Scheffé post hoc test, $p>0.05$).

3.3. Physico-chemical analyses

On day 0 (before the fauna addition), no differences in concentrations of dissolved O_2, NO_3^-, NO_2^-, NH_4^+, and DOC were measured between the groups of columns assigned to each treatment (control vs. tubificid) whatever the type of surface deposit (two-way ANOVAs, fauna effect, $p>0.05$). During the experiment, oxygen and nitrate concentrations decreased significantly with depth in all columns (two-way RM-ANOVAs, depth effect, $p<0.05$, see Fig. 2 for day 14). After animal addition, occurrence of worms significantly reduced O_2 concentrations with all sediment deposits (two-way RM-ANOVAs, fauna effect, $p<0.001$). This reduction of O_2 concentrations by worms was however slight with the STORM deposit because Scheffé post hoc tests did not detect any effect of worms for each depth with STORM deposit ($p>0.05$, see Fig. 2a for day 14). In contrast, the influence of worms on O_2 concentrations was higher in the two other deposit treatments (POM-rich and POM-low deposits) where occurrence of worms led to significant reduction of O_2 concentrations in interstitial water for different depths (Scheffé post hoc tests, $p<0.05$, see Figs. 2b and 2c for day 14).

NO_3^- concentrations were not significantly affected by tubificid worms during the experiment for the three sediment deposits (two-way RM-ANOVAs, fauna effect, $p>0.05$, see Fig. 2 for day 14).

Nitrite concentrations were very low (<0.2 mg L^{-1}) in all columns throughout the experiment and no significant differences were detected between control and tubificid treatments in all deposit treatments (two-way RM-ANOVAs, fauna effect, $p>0.05$).

295 During the experiment, NH_4^+ and DOC concentrations significantly increased with depth
296 in the STORM and the POM-rich deposit treatments (two-way RM-ANOVAs, depth effect,
297 *p<0.001*). In the STORM deposit treatment, tubificid worms significantly increased the
298 concentrations of NH_4^+ in interstitial water (two-way RM-ANOVA, fauna effect, *p<0.001*)
299 despite no significant influence on concentrations for each depth (Scheffé post hoc tests,
300 *p>0.05*, see Fig. 2a for day 14). Such increased production of NH_4^+ due to worms was also
301 measured in the POM-rich deposit columns (two-way RM-ANOVA, fauna effect, *p<0.001*)
302 with significant effects of worms observed at different depths (Scheffé post hoc tests, *p<0.05*,
303 see Fig. 2b for day 14). In presence of POM-low deposit, ammonium concentrations remained
304 lower than 0.3 mg L^{-1} during the experiment with no significant difference between the fauna
305 treatments (two-way RM-ANOVA, animal effect, *p>0.05,* see Fig. 2c for day 14). Tubificid
306 worms did not significantly influence DOC concentrations in the STORM deposit treatment,
307 (two-way RM-ANOVA, fauna effect, *p>0.05*). In contrast, tubificid worms increased the
308 DOC concentrations in interstitial water with the two other deposits (two-way RM-ANOVAs,
309 fauna effect, *p<0.05*), this increase being the highest with the POM-rich deposit (see data of
310 day 14 on Fig. 2b).

311 The average release rates of NH_4^+ and DOC measured from day 1 to day 20 (Table 2) were
312 significantly different among fauna treatments (control vs. tubificid) and surface deposit
313 treatments (two-way ANOVAs, fauna effect and deposit effect, *p<0.05*). The mean releases
314 of ammonium and DOC were not significantly different between the control and the tubificid
315 treatments in presence of STORM and POM-low deposits (Scheffé post hoc tests, *p>0.05*). In
316 contrast, in presence of POM-rich deposit, tubificid worms increased by 40 and 30 % the
317 release rates of NH_4^+ and DOC, respectively (Scheffé post hoc tests, *p<0.05*).

318

319 ***3.4. Microbial analyses***

320 At the end of the experiment, the total numbers of bacteria (stained with DAPI) decreased
321 significantly with depth with no significant difference among the fauna treatments for the 3

deposits (Fig. 3, two-way ANOVAs, fauna effect, $p>0.05$, depth effect, $p<0.001$). The percentages of active bacteria (% EUB/DAPI) also decreased significantly with depth for all deposit treatments (Fig. 3, two-way ANOVAs, depth effect, $p<0.001$). The percentages of active bacteria were not significantly different among fauna treatments (control vs. tubificid) in the STORM deposit treatment (Scheffé post-hoc tests, $p>0.05$). In contrast, in presence of POM-low deposits, the percentages of active bacteria were increased by more than 30 % in tubificid treatment in the first two layers of sediment (0 – 3 and 3 – 6 cm) compared to the controls (Scheffé post-hoc tests, $p<0.01$). In the POM-rich deposit columns, these percentages of active bacteria were also higher in presence of fauna for the first three sediment layers (0–3, 3–6 and 13–16 cm; Scheffé post-hoc tests, $p<0.05$).

The microbial activities measured with biolog (global AWCD) at the end of the experiment varied significantly with depth for the 3 sediment deposits but no significant differences were detected among control and tubificid treatments (Fig. 3, two-way ANOVAs, depth effect, $p<0.001$; fauna effect, $p>0.05$). The functional diversity measured with Biolog (numbers of substrates used), varied also significantly with depth for the 3 sediment deposits (Fig. 3, two-way ANOVAs, depth effect, $p<0.05$). Moreover, significant differences among control and tubificid treatments were only detected in the STORM deposit treatment (two-way ANOVA, fauna effect, $p<0.05$) where the functional diversities in the two first sediment layers (0–3 and 3-6 cm) were increased by more than 40 % in tubificid treatment compared to the controls (Scheffé post–hoc tests, $p<0.05$).

At the end of the experiment, respiratory, denitrification, and hydrolytic activities in presence of the STORM deposit decreased significantly with depth with no significant difference among fauna treatments (Fig. 4, two-way ANOVAs, fauna effect, $p>0.05$, depth effect, $p<0.001$). In the POM-low deposit treatment, respiration, denitrification and hydrolytic activities were very low in all sediment layers (i.e. for hydrolytic activity: <0.02 $\mu mol.h^{-1}.g^{-1}$ sed. DW) and did not show significant differences among fauna treatments and depths (Fig. 4,

two-way ANOVAs, fauna effect, $p>0.05$, depth effect, $p>0.05$). In contrast, respiration and denitrification potentials decreased significantly with depth in the POM-rich deposit treatment (Fig. 4, two-way ANOVAs, depth effect, $p<0.001$). No significant effect of fauna was detected in the POM-rich deposit treatment for the respiration potential (two-way ANOVA, fauna effect, $p>0.05$), whereas denitrification and hydrolytic activities were increased by 94% and 100% respectively in the first sediment layer by the presence of tubificid worms (two-way ANOVAs, fauna effect, $p<0.001$, Scheffé post–hoc tests, $p<0.01$).

3.5. Hydrocarbon and heavy metal release from stormwater deposit to water

Only 3 PAHs (acenaphthene, naphtalene and phenanthrene) and one heavy metal (lead) were detected in water at the outlet of all the columns with STORM deposit in surface during all the experiment (Table 3). Concerning the releases of these pollutants (naphtalene, acenaphthene, phenanthrene and lead) from STORM deposit to water, no significant differences were detected between control and fauna treatments during the experiment (two-way ANOVAs, fauna effect, $p>0.05$).

4. Discussion

4.1. Effects of worms

Our results showed that several processes (O_2 uptake, DOC and NH_4^+ productions, hydrolytic activity …) could be stimulated by worm activities in sediments. For instance, all the columns showed a sharp decrease of oxygen and nitrate with depth (due to aerobic microbial degradation of OM and denitrification) and, this oxygen decrease was enhanced by the presence of worms. The direct effects of tubificid worms on O_2 uptake (own respiration of worms, McCall and Fisher, 1980; Mermillod-Blondin et al., 2005) could explain less than 0.03 mg L^{-1} of differences in oxygen concentrations measured between control and fauna columns. Because reduction of O_2 concentration due to worms were higher than 0.3 mg L^{-1} in

374 the 5 top cm of sediments with POM-rich and POM-low deposits (Fig. 2), the influence of
375 worms was predominantly due to a stimulation of microbial activity in these two deposits (as
376 demonstrated by the percentage of active eubacteria in POM-low and POM-rich deposit
377 treatments). According to several studies (e.g. Chatarpaul et al., 1980; Mermillod-Blondin et
378 al., 2000; Svensson et al., 2001), the worm effects on biogeochemical processes were due to
379 their particular mode of feeding and reworking activities. Burrowing, construction of
380 galleries, feeding and production of faecal pellets by tubificid worms increased the exchanges
381 of solutes and particles across the water-sediment interface, enhanced the accessibility of O_2
382 and nutrients to anaerobic environments, and, as a consequence, induced a stimulation of
383 microbial rates and pathways (Mermillod-Blondin et al., 2000; Svensson et al., 2001). During
384 the experiment, faecal pellets were observed at the sediment surface of tubificid columns for
385 all surface deposit treatments. Such pelletized layer at the sediment surface could be a
386 favourable area for the development of microbes and could act as a high biogeochemical
387 reactive zone. Moreover, the grazing of fine particles and attached bacteria by worms could
388 stimulate the bacterial growth rates as shown with nematodes by Traunspurger et al (1997).
389 All these feeding and reworking activities probably stimulated the aerobic and anaerobic
390 bacterial communities and the microbial growth in sediment columns. However, the tubificid
391 worms did not affect similarly microbial processes in the three sediment deposits. According
392 to our hypothesis, the effects of worms depended on the physico-chemical characteristics of
393 the sediments (POM quantity and quality-C/N and C/P ratios-).
394
395 ***4.2. Interactions between tubificid effects and physico-chemical characteristics of the***
396 ***stormwater deposit***
397 *4.2.1. Tubificid effects in STORM and POM-low deposits = influence of the POM quantity*
398 The quantity of POM measured in the STORM deposit was more than 3-fold higher than
399 those measured in the POM-low deposit whereas the qualities of POM (expressed as C/N and

C/P ratios) measured in these two deposits were similar. This difference in POM quantity could explain the lower microbial metabolism measured in the POM-low deposit treatment than those measured in the STORM deposit treatment (as detailed in Nogaro et al., in press). For instance, in control columns higher mean releases of ammonium (× 3.8) and DOC (× 4.3) at depth were measured in presence of the STORM deposit than with the POM-low deposit. The bacterial biomass (numbers of total bacteria) and activities (global AWCD, respiration, denitrification and hydrolytic activities) were also relatively low in the POM-low deposit treatment in comparison with the STORM deposit (Nogaro et al., in press). Less substrate could lead to a lower stimulation of the microbial metabolism and / or a lower activity of invertebrates in the sediment (in particular the feeding activity, Gremare et al., 2004). The depth profiles of luminophores were in accordance with this assumption because a lower bioturbation activity of worms was measured in the POM-low deposit treatment in comparison with the STORM deposit treatment (with a high POM content). However, if worms had a lower sediment reworking activity in the POM-low deposit treatment, the worm effects on oxygen uptake and number of active eubacteria were more effective in presence of POM-low deposit than with STORM deposits. Kristensen et al. (1992) stated that the influence of benthic organisms in POM mineralization was positively related to the quantity of the POM. In our system, the higher quantity of organic particles in the STORM deposit probably led to an increase of the feeding and the burrowing activities of worms but also to lower effects of fauna on sediment metabolism than in presence of a sediment with a lower OM content. The high rates of POM mineralization measured in control columns with STORM deposit probably limited the ability of worms to increase this mineralization rates. For instance, oxygen concentrations measured on day 10 decreased sharply from the surface (7.3 ± 0.1 mg L^{-1}) to 5 cm depth (1.1 ± 0.05 mg L^{-1}) in control columns with STORM deposit whereas this decrease was less marked in controls with POM-low deposit (from 7.7 ± 0.1 mg L^{-1} at the surface to 3.4 ± 0.3 mg L^{-1} at 5 cm depth). In these conditions, the aerobic microbial

426 activity (O_2 consumption) in STORM deposit could not be easily stimulated by worms
427 whereas a stimulation of O_2 consumption could occur in the POM-low deposit treatment
428 because the respiration process was less saturated. Therefore, the relationship between worm
429 activities in the sediment and their effects on microbial metabolism depends strongly on the
430 POM resources of the sedimentary habitat.
431
432 *4.2.2. Tubificid effects on STORM and POM-rich deposits = influence of the POM quality*
433 Despite similar bioturbation activities in the STORM and the POM-rich deposits, the
434 worms did not affect similarly the biogeochemical processes in these two types of sediment
435 deposits characterized by high and comparable contents of POM. This difference may be
436 linked to differences in quality of POM between STORM and the POM-rich deposits: the
437 STORM deposit was characterized by a higher quality (lability) of its POM (indicated by
438 lower C/N and C/P ratios) than the POM-rich deposit (Table 1).
439 In the POM-rich deposit, several processes (O_2 uptake, DOC and NH_4^+ productions,
440 hydrolytic activity …) were stimulated by worms whereas such stimulations did not occur in
441 the STORM deposit at the exception of the functional diversity of micro-organisms measured
442 with Biolog. This increase of the functional diversity of microbial communities (Fig. 3) by
443 more than 40 % in the two first sediment layers of the STORM deposit treatment was due to
444 an increase of the diversity of carbon substrates due to worm activities. This specific effect of
445 tubificid worms could be due to the chemical composition of the STORM sediment and, in
446 particular, its high ability to produce a high diversity of carbon substrates. However, despite
447 this worm effect on the microbial diversity, worms did not influence the microbial activities
448 in presence of the STORM deposit. The higher effects of worms in the POM-rich treatment in
449 comparison with the STORM deposit treatment may be linked to the differences in microbial
450 activities measured without fauna in the two deposits. For instance, in control columns
451 denitrification and hydrolytic activities were respectively 2.7-, and 3.9-fold higher in the first

452 sediment layer (0–3 cm) in the STORM deposit treatment compared to the same layer in the
453 POM-rich deposit treatment. It was probable that the worms did not influence the
454 biogeochemical processes and microbial metabolism in STORM deposit treatment because of
455 the high mineralization rates occurring in the stormwater deposit. As discussed in the section
456 4.2.1., the microbial metabolism was probably saturated by the high content of labile POM in
457 the STORM deposit reducing the ability of worms to stimulate biogeochemical processes. In
458 marine sediments, several studies (Andersen and Kristensen, 1992; Kristensen et al., 1992;
459 Hansen and Kristensen, 1998) showed that the benthic organisms had a greater influence on
460 the mineralization of refractory POM than on that of the labile POM in marine sediments.
461 According to these studies, we also observed a higher effect of worms on mineralization in
462 the POM-rich deposit characterized by a relatively old and refractory POM (with the highest
463 C/N) in comparison with the effects measured in the STORM deposit. Therefore, our results
464 highlight that the role of the fauna in sediments depends on the lability of the POM. More
465 precisely, the organisms which reworked the refractory POM could increase its availability to
466 an aerobic and anaerobic decomposition by micro-organisms whereas organisms could not
467 increase strongly the availability of labile POM to micro-organisms.
468
469 ***4.3. Interactions between tubificid worms and pollutant content in stormwater deposits***
470 Our second hypothesis of an effect of tubificid worms on the fate of pollutants
471 (hydrocarbons and heavy metals) in the STORM deposit treatment was not validated. We
472 expected that worm activities in the sediment (in particular the reworking and feeding
473 activities of sediment particles) would lead to a stimulation of the pollutant release from the
474 stormwater deposit to water. Invertebrate bioturbation was commonly recognized to have a
475 significant effect on pollutant transports in the sediment (Caradec et al., 2004). It has also
476 been shown that the particular mode of feeding of tubificid worms (conveyor-belt species)
477 could influence the pollutant release (hydrocarbons) from sediment to water by increasing the

478 resuspension of particles and the exchanges between sediment and water (Reible et al., 1996).
479 In our study, only some of the most soluble PAHs (naphthalene, acenaphthene and
480 phenanthrene) and one heavy metal (lead) were detected in water at the outlet of the columns
481 during the experiment. The concentrations of pollutants released in water were relatively low
482 (< 90 $ng.L^{-1}$ for PAHs and < 25 $\mu g.L^{-1}$ for the lead) and were not enhanced in presence of
483 tubificid worms. Such results were in accordance with Mermillod-Blondin et al. (2005) who
484 did not detect any effect of fauna on pollutant release from stormwater deposit to the
485 overlying water of stagnant systems. As shown by Datry et al. (2003b) in the field, the
486 stormwater deposits present in infiltration basins act as a sink for hydrocarbons and heavy
487 metals. It has also been demonstrated in marine sediments that the physico-chemical
488 characteristics (hydrophobicity and particle reactivity) of sediments could control the
489 influence of animal bioturbation on pollutant dynamic (Banta and Andersen, 2003). Our
490 experiments therefore highlighted that the characteristics of the stormwater deposit lead to a
491 high immobilisation of the pollutants in the system. From a management point of view, it is
492 however necessary to analyse the long-term chemical interactions between sediment matrix
493 and pollutants in order to quantify the water contamination potential due to accumulation of
494 pollutants in infiltration systems.
495

496 ## 5. Conclusion

497 ***Are activities of tubificid worms useful in management of stormwater sediments?***

498 Our study showed that the influence of invertebrates on biogeochemical processes and
499 microbial communities was greatly affected by the characteristics of the surface deposit
500 (quantity and quality of the POM) which control the activities of micro-organisms and
501 invertebrates in aquatic ecosystems. The high lability of POM of the STORM deposit induced
502 a high microbial activity which can not be easily stimulated by worms in infiltration
503 conditions.

In comparison with these infiltration conditions, Mermillod-Blondin et al. (2005) showed that tubificid worms increased the O_2 uptake (+35%) and the releases of NH_4^+ and DOC by 2- and 3-fold respectively, in stormwater deposits under stagnant conditions. These effects were due to the stimulation of microbial communities by enhancing the solute exchanges at the water-sediment interface. In our infiltration columns, the physically-induced flow rates within sediments probably played a significant role in the high metabolism of the STORM deposit treatment. For instance, Mermillod-Blondin et al. (2005) measured values of hydrolytic activity in the upper sediment layers (0-1 and 1-3 cm) of control columns (without fauna) were between 0.1 and 0.15 $\mu mol\ h^{-1}\ g^{-1}$ sed. DW whereas our values in infiltration conditions were 2-fold higher in the first sediment layer (0-3 cm) of control columns. Oxygen and metabolites (NO_3^-) which were supplied at constant flow rate in the sediment columns during all the experiment enhanced probably the sediment metabolism in comparison with stagnant conditions. Under infiltration conditions, bioturbation (bioirrigation) only modulates slightly the supply of O_2 and nutriments for bacteria in infiltration systems whereas the same organism activity can produce fluxes of solutes at the water-sediment interface of stagnant systems and then can strongly influence microbial activities in sediments (Gerino et al., 2003; Mermillod-Blondin and Rosenberg, in press).

Therefore, our experiments suggest that efficiency of tubificid worms to stimulate organic matter processing in stormwater sediments would be optimized in stagnant systems such as retention ponds rather than in infiltration systems. Our conclusions should be however extended to other stormwater sediments. Considering the links between chemical characteristics of the stormwater deposit (complex composition with high contents of POM and contaminants) and the potentiality of tubificid worms to stimulate organic matter mineralization, testing the effect of tubificid worms in a set of stormwater deposits originating from different drainage area (urban, industrial, agricultural zones) is greatly needed to make generalizations.

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617

618 ## Figure Captions

619 Fig. 1. Depth profiles of luminophore for (a) the STORM, (b) the POM-rich, and (c) the
620 POM-low deposits at the end of the experiment (mean ± SD, n = 3). Asterisks (*) indicate a
621 significant difference between control and tubificid columns.
622

623 Fig. 2. Depth profiles of O_2, $N\text{-}NO_3^-$, $N\text{-}NH_4^+$, and DOC concentrations on day 14 for (a) the
624 STORM, (b) the POM-rich, and (c) the POM-low deposits (mean ± SD, n = 3). Asterisks (*)
625 indicate a significant difference between control and tubificid columns.
626

627 Fig. 3. Depth profiles of total numbers of bacteria (DAPI), % of active bacteria (%
628 EUB/DAPI), activity measured with biolog (global AWCD), and functional diversity
629 measured with Biolog (number of substrates) for (a) the STORM, (b) the POM-rich, and (c)
630 the POM-low deposits at the end of the experiment (mean ± SD, n = 3). Asterisks (*) indicate
631 a significant difference between control and tubificid columns.

632 Fig. 4. Depth profiles of respiratory, denitrification, and hydrolytic activities for (a) the
633 STORM, (b) the POM-rich, and (c) the POM-low deposits at the end of the experiment (mean
634 ± SD, n = 3). Asterisks (*) indicate a significant difference between control and tubificid
635 columns.

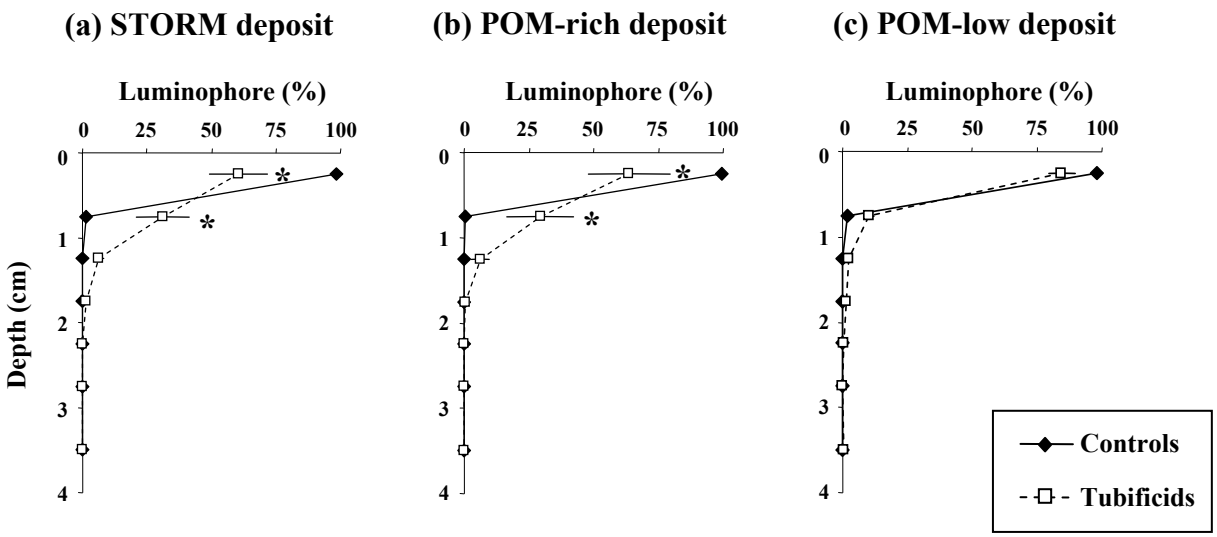
(a) STORM deposit
(b) POM-rich deposit
(c) POM-low deposit
Luminophore (%)
0
25
50
75
100
Depth (cm)
0
1
2
3
4
*
Controls
Tubificids

Fig. 1.

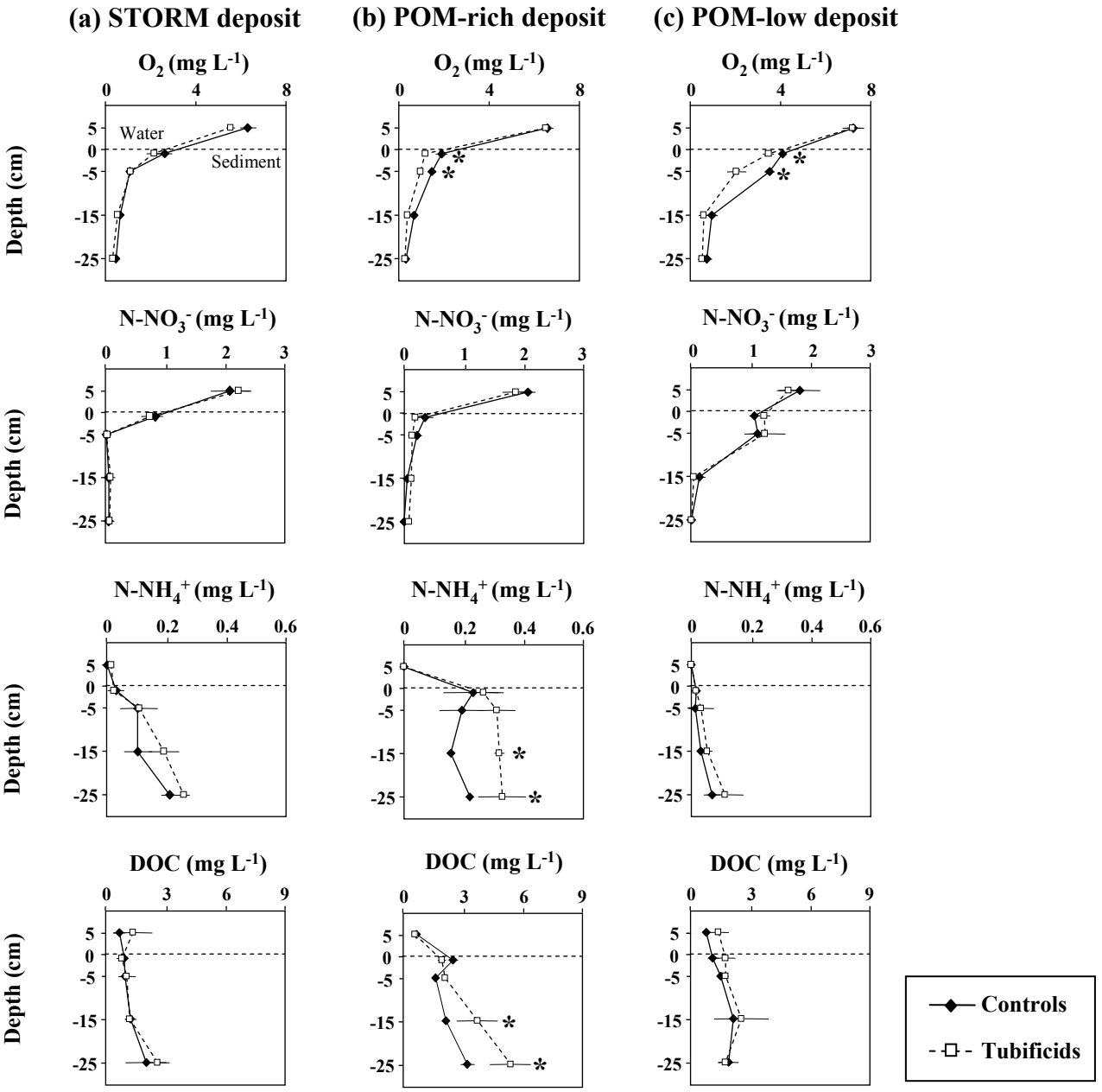
(a) STORM deposit
(b) POM-rich deposit
(c) POM-low deposit
O2 (mg L-1)
N-NO3- (mg L-1)
N-NH4+ (mg L-1)
DOC (mg L-1)
Depth (cm)
Water
Sediment
Controls
Tubificids

Fig. 2.

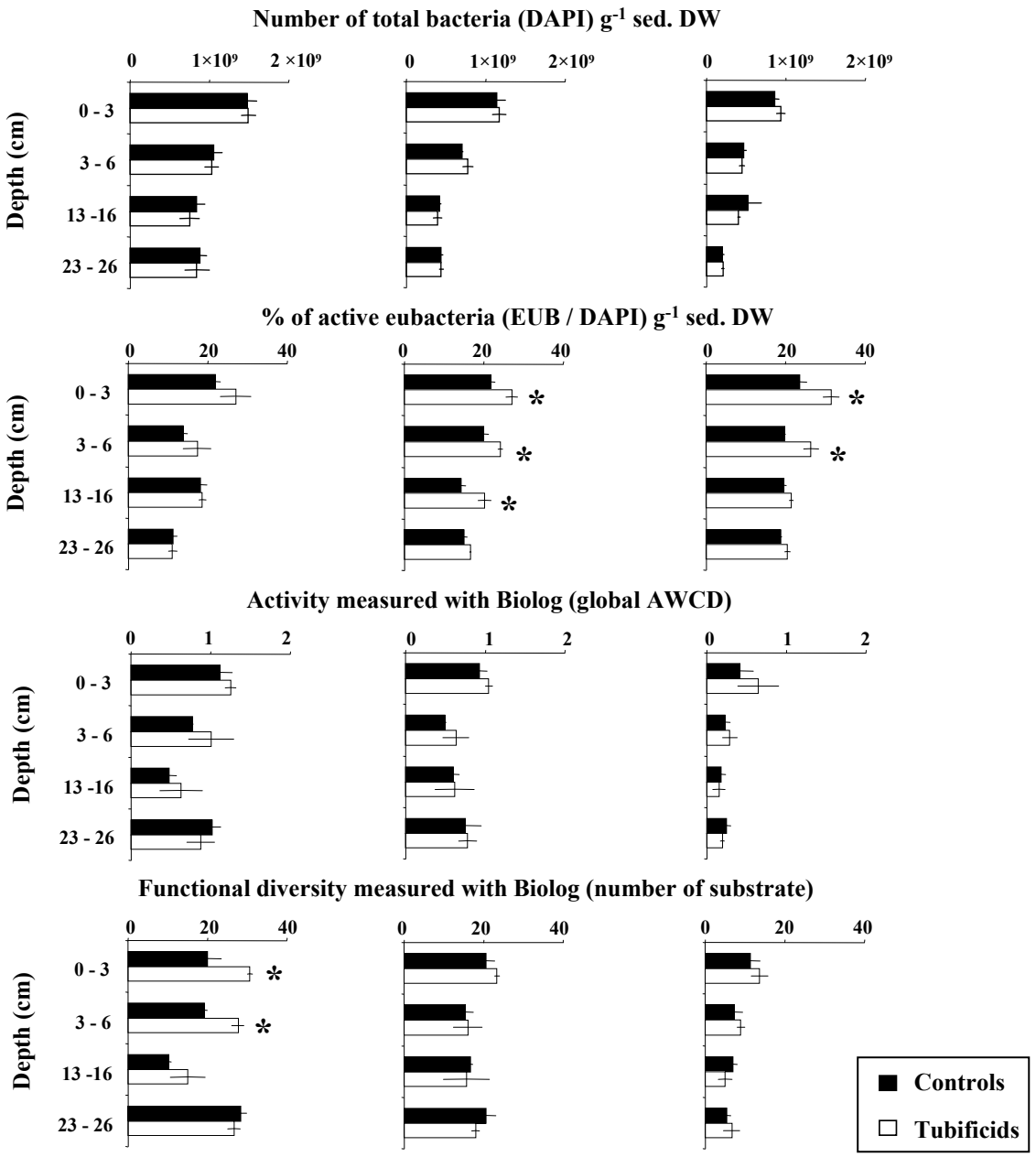
(a) STORM deposit
(b) POM-rich deposit
(c) POM-low deposit
Number of total bacteria (DAPI) g-1 sed. DW
0
1×10⁹
2 ×10⁹
2×10⁹
Depth (cm)
0 - 3
3 - 6
13 -16
23 - 26
% of active eubacteria (EUB / DAPI) g-1 sed. DW
0
20
40
*
Activity measured with Biolog (global AWCD)
0
1
2
Functional diversity measured with Biolog (number of substrate)
Controls
Tubificids

Fig. 3.

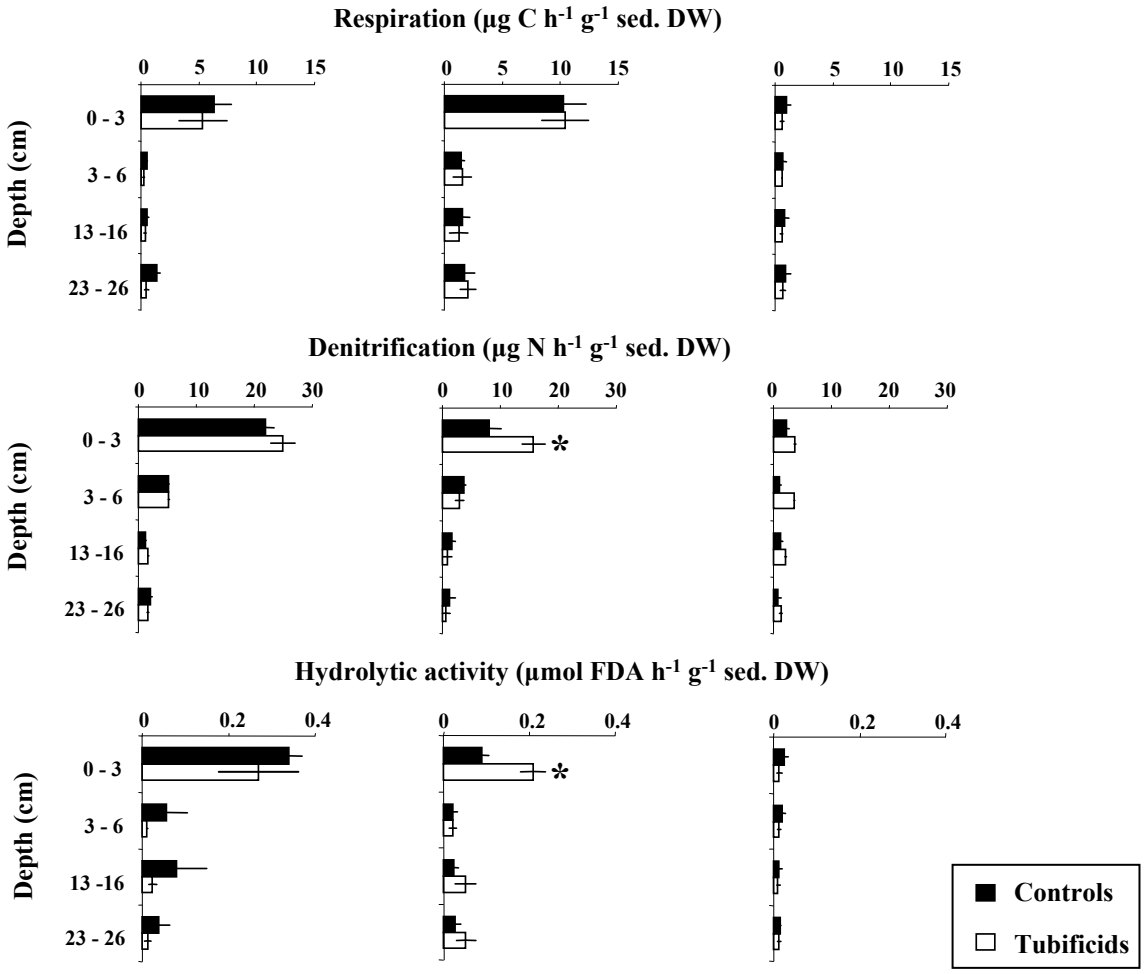
(a) STORM deposit
(b) POM-rich deposit
(c) POM-low deposit
Respiration (µg C h-1 g-1 sed. DW)
Denitrification (µg N h-1 g-1 sed. DW)
Hydrolytic activity (µmol FDA h-1 g-1 sed. DW)
Depth (cm)
0 - 3
3 - 6
13 -16
23 - 26
0 5 10 15
0 10 20 30
0 0.2 0.4
*
Controls
Tubificids

Fig. 4.

Table 1
Chemical composition of the fresh sediment deposits before their deposition in the columns (mean ± SD, n = 3). Modified from Nogaro et al. (in press)

Chemical composition	STORM deposit	POM-rich deposit	POM-low deposit
Nutrients (g kg^{-1} sed. DW)			
Particulate Organic Matter	70.0 ± 5.3	85.0 ± 2.6	20.3 ± 5.1
Particulate Organic Carbon	55.6 ± 1.6	37.8 ± 6.1	14.9 ± 6.1
Particulate Nitrogen	2.93 ± 0.47	1.10 ± 0.17	0.70 ± 0.20
Particulate Phosphorus	1.11 ± 0.15	0.41 ± 0.01	0.35 ± 0.02
Atomic C/N	22.4 ± 3.35	40.1 ± 7.55	24.9 ± 6.04
Atomic C/P	126.3 ± 14.8	230.5 ± 34.3	114.0 ± 37.5
Heavy metals (mg kg^{-1} sed. DW)			
Cadmium	2.8 ± 0.3	< 0.2	< 0.2
Copper	113.3 ± 14.2	< 10	< 10
Lead	265.3 ± 9.2	< 10	< 10
Zinc	77.0 ± 2.6	< 30	< 30
Polycyclic Aromatic Hydrocarbons (mg kg^{-1} sed. DW)			
Acenaphtene	0.26 ± 0.07	< 0.13	< 0.13
Anthracene	0.31 ± 0.09	< 0.13	< 0.13
Benzo (a) anthracene	1.09 ± 0.23	< 0.13	< 0.13
Benzo (a) pyrene	0.97 ± 0.19	< 0.13	< 0.13
Benzo (b) fluoranthene	0.82 ± 0.14	< 0.13	< 0.13
Benzo (k) fluoranthene	0.44 ± 0.09	< 0.13	< 0.13
Benzo (ghi) perylene	0.84 ± 0.12	< 0.13	< 0.13
Chrysene	1.22 ± 0.26	< 0.13	< 0.13
Dibenzo (a,b) anthracene	0.18 ± 0.03	< 0.13	< 0.13
Fluoranthene	2.54 ± 0.59	< 0.13	< 0.13
Fluorene	0.29 ± 0.06	< 0.13	< 0.13
Indeno (1,2,3 cd) pyrene	0.47 ± 0.08	< 0.13	< 0.13
Naphtalene	0.14 ± 0.03	< 0.13	< 0.13
Phenanthrene	1.78 ± 0.47	< 0.13	< 0.13
Pyrene	1.82 ± 0.39	< 0.13	< 0.13
2-méthyl naphtalene	< 0.13	< 0.13	< 0.13
2-méthyl fluoranthene	< 0.13	< 0.13	< 0.13

Table 2
Average release rates of solutes ($N\text{-}NH_4^+$ and DOC) in the control and the tubificid columns for the three deposit treatments from days 1 to 20 (mean ± SD, n = 3)

Release rates of solutes (mg d^{-1})	STORM deposit		POM-rich deposit		POM-low deposit	
	Controls	Tubificids	Controls	Tubificids	Controls	Tubificids
$N\text{-}NH_4^+$	0.42 ± 0.05	0.52 ± 0.03	0.49 ± 0.01	0.69 ± 0.08	0.11 ± 0.02	0.17 ± 0.05
DOC	13.0 ± 1.55	12.9 ± 1.66	16.0 ± 2.18	21.2 ± 1.04	3.0 ± 0.14	3.5 ± 0.31

Abbreviation: DOC = Dissolved Organic Carbon.

Table 3
Mean release from the STORM deposit to water of heavy metals and polycyclic aromatic hydrocarbons in the control and the tubificid columns at days 1, 8 and 15 (mean ± SD, n = 3)

Pollutant release from STORM deposit to water	DAY 1		DAY 8		DAY 15	
	Controls	Tubificids	Controls	Tubificids	Controls	Tubificids
Heavy metals (µg L^{-1})						
Cadmium	< 4.6	< 4.6	< 4.6	< 4.6	< 4.6	< 4.6
Copper	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1
Lead	17.3 ± 1.5	13.3 ± 3.1	18.0 ± 4.4	21.7 ± 2.5	15.0 ± 4.4	13.3 ± 4.2
Zinc	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
PAHs (ng L^{-1})						
Acenaphtene	14.3 ± 2.1	15.0 ± 13.2	17.0 ± 2.0	19.3 ± 3.5	16.3 ± 2.1	16.0 ± 1.0
Anthracene	< 10	< 10	< 10	< 10	< 10	< 10
Benzo (a) anthracene	< 10	< 10	< 10	< 10	< 10	< 10
Benzo (a) pyrene	< 10	< 10	< 10	< 10	< 10	< 10
Benzo (b) fluoranthene	< 10	< 10	< 10	< 10	< 10	< 10
Benzo (k) fluoranthene	< 10	< 10	< 10	< 10	< 10	< 10
Benzo (ghi) perylene	< 10	< 10	< 10	< 10	< 10	< 10
Chrysene	< 10	< 10	< 10	< 10	< 10	< 10
Dibenzo (a,b) anthracene	< 10	< 10	< 10	< 10	< 10	< 10
Fluoranthene	< 10	< 10	< 10	< 10	< 10	< 10
Fluorene	< 10	< 10	< 10	< 10	< 10	< 10
Indeno (1,2,3 cd) pyrene	< 10	< 10	< 10	< 10	< 10	< 10
Naphtalene	59.0 ± 30.1	87.3 ± 78.2	60.7 ± 9.0	64.0 ± 9.6	65.7 ± 5.8	57.3 ± 9.0
Phenanthrene	11 ± 1.7	14.7 ± 1.2	< 10	< 10	< 10	< 10
Pyrene	< 10	< 10	< 10	< 10	< 10	< 10
2-méthyl naphtalene	< 10	< 10	< 10	< 10	< 10	< 10
2-méthyl fluoranthene	< 10	< 10	< 10	< 10	< 10	< 10

Abbreviation: PAHs = Polycyclic Aromatic Hydrocarbons.