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Laboratory study highlights the key influences of stormwater sediment thickness and bioturbation by tubificid worms on dynamics of nutrients and pollutants in stormwater retention systems

F. Mermillod-Blondin *, G. Nogaro, F. Vallier, J. Gibert

UMR-CNRS 5023, Laboratoire d'Ecologie des Hydrosystèmes Fluviaux, Université Claude Bernard Lyon I, Domaine Scientifique de la Doua, 6 rue Dubois, Batiment Forel, 69622 Villeurbanne, France

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Abstract

In urban area, the accumulation of polluted stormwater sediments (SWS) in retention ponds may be a source of dissolved pollutants and nutrients for the aquatic ecosystems. Our objective was to quantify the influence of the thickness of SWS layer and the occurrence of tubificid worms on organic matter processing (O_2 uptake and fluxes of NH_4^+, NO_3^-, PO_4^{3-}, and dissolved organic carbon between sediment and water), releases of 17 PAHs and 4 heavy metals, and microbial characteristics. Results showed that oxidation of SWS organic matter (O_2 and NO_3^- uptakes) and releases of nutrients were significantly increased by the quantity of accumulated SWS and the worm bioturbation. Releases of acenaphtene and naphthalene from sediments were significantly increased by the thickness of the SWS layer. In contrast, tubificid worms did not promote the mobilization of pollutants. In conclusion, biological activities and stormwater sediment characteristics need to be assessed to quantify the fate of pollutants and nutrients in stormwater retention ponds.

Keywords: Urban pollution; Organic matter; Heavy metals; PAHs; Microbial activities

1. Introduction

The increase in urbanization poses several environmental concerns such as air pollution and degradation of surface water and groundwater resources. The growth of impermeable surfaces limits the natural infiltration of storm water and increases the risk of urban floods. Current management practices consist in collecting and discharging storm water into rivers, ponds or infiltration basins (Marsalek and Marsalek, 1997; Barraud et al., 2002). Among these management practices, stormwater management ponds are used to reduce runoff peaks and to enhance stormwater quality by physical, chemical and biological processes. However, such systems retain large amount of polluted sediments (Marsalek and Marsalek, 1997) that could act as a source of pollution for the associated aquatic ecosystems (Marsalek et al., 2002; Datry et al., 2003a). For instance, it has been shown that several solutes (NH_4^+, PO_4^{3-} and dissolved organic carbon, DOC) could be released from stormwater sediments (SWS) to the water in contact with these sediments (Datry et al., 2003a; Mermillod-Blondin et al., 2005). The deposition of SWS in stormwater retention systems depends strongly on hydrodynamics (Marsalek and Marsalek, 1997), and the thickness of accumulated sediments may be highly variable in the collecting systems (Winiarski et al., 2006). However, the impact of the thickness of the SWS deposit in retention ponds has never been taken into account to assess the fluxes of pollutants between SWS and water.

Moreover, invertebrates like tubificid worms can pullulate in organic matter-rich SWS (Bishop et al., 2000; Lafont et al., 2000; Datry et al., 2003b). These invertebrates can have a very significant influence on the degradation of organic matter and the export of nutrients (and pollutants)

* Corresponding author. Tel.: +33 4 72 43 13 64; fax: +33 4 72 43 15 23.
E-mail address: mermillo@univ-lyon1.fr (F. Mermillod-Blondin).

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in polluted sediments (Reible et al., 1996; Mermillod-Blondin et al., 2005; Ciutat et al., 2007).

Despite several studies dealing with the role of bioturbation in SWS (Datry et al., 2003c; Mermillod-Blondin et al., 2005; Nogaro et al., 2007a), no work assessed the influence of the amount of SWS accumulated and its interaction with the bioturbation process in retention systems. Because of this interaction, we employed a factorial experimental approach in which bioturbation and SWS thickness were manipulated to address how these features may interact to influence organic matter and pollutant fluxes. As the thickness of SWS layer determines the quantity of pollutant and organic matter present at the water sediment interface, we hypothesized that the mineralization of the organic matter (respiration rates) and the releases of solutes (nitrogen, phosphorus, organic carbon, heavy metals, PAH) from SWS would increase with the thickness of SWS layer. Due to these expected differences, we also hypothesized that the stimulation of organic matter and pollutant fluxes by worms would be positively linked to the thickness of SWS deposits.

2. Materials and methods

2.1. Collection of sediment and tubificid worms

SWS and tubificid worms (*Tubifex tubifex*) were collected in a partially clogged stormwater infiltration basin of which the hydrological, physico-chemical, and biological characteristics were investigated in detail earlier (Django Reinhardt basin, Barraud et al., 2002; Winiarski et al., 2006). SWS were collected in a deposition area of the basin. They were sieved through a 0.5-mm mesh aperture sieve to remove resident animals and coarse particles. The pollutant contents of SWS reported on Table 1 were in the range of values reported in other stormwater infiltration/retention basins (Kamalakkannan et al., 2004; Clozel et al., 2006).

2.2. Experimental setup

Sediment columns were prepared by transferring the homogenised SWS (<0.5 mm) into 18 Plexiglas® columns (20-cm long and 5.5-cm internal diameter) previously filled with fluvial sediments characterizing the native bed of the stormwater retention–infiltration basins of the Lyon area (Winiarski et al., 2006). Three thicknesses of SWS layer were applied in the columns: 6 columns with a 2-cm thick SWS layer, 6 columns with a 5-cm thick SWS layer, and 6 columns with a 8-cm thick SWS layer. The upper part of the columns was filled with synthesized water (96 mg l^{-1} of $NaHCO_3$, 60 mg l^{-1} of $CaSO_4 \cdot 2H_2O$, 60 mg l^{-1} of $MgSO_4 \cdot 7H_2O$, and 4 mg l^{-1} of KCl) to simulate a water–sediment interface and was renewed with a peristaltic pump to maintained high oxygen concentrations in the overlying water (turnover rate of overlying water: 0.5 h). Experiments were done in a dark room at 15 °C for 24 d (10 d before and 14 d after introduction of tubificid worms).

For each SWS layer thickness (6 identical columns), two treatments were performed with three replicates per treatment: (1) without invertebrates (control) and (2) with 50 *T. tubifex*. The number of tubificid worms per column (density of 21 000 individuals m^{-2}) simulated the densities observed in the aquatic ecosystems receiving stormwater sediments (27 000 individuals m^{-2} was a mean density reported in periurban systems by Vivier (2006)). For acclimation to experimental conditions (as temperature), tubificid worms collected in the field were kept in the laboratory for 3 weeks before use in columns. Tubificids were introduced in the columns 10 d after the sediment installation (day 0 of the experiment). Before worm introduction, chemical measurements were performed to ensure that O_2 uptake, nutrient releases from the sediment, and microbial variables were not significantly different between control and tubificid columns. Once introduced into the columns, worms dug rapidly into the sediment (<2 h).

During the 14 d of the experiment, chemical measurements (O_2 uptake, NH_4^+, NO_3^-, PO_4^{3-}, and DOC releases from SWS) were made at days 6 and 12 in columns to quantify the influence of SWS layer thickness and the role of tubificid worms. Bacteria attached to the sediment were analyzed on sediment samples collected in the columns at the end of the experiment (day 14). Concentrations of nitrogen, organic carbon, phosphorus, PAH, and heavy metals in the sediment were measured at the end of the experiment (day 14).

2.3. Chemical and bacterial measurements

2.3.1. Oxygen uptake

For each day of measurement (days 6 and 12), each column was individually sealed during oxygen measurements by stopping the water renewal in the overlying water. An oxygen sensor (UNISENSE, Denmark) coupled with a peristaltic pump was adapted to the columns to measure the O_2 decrease in the overlying water of each column for 2.5 h (O_2 concentrations were recorded every minute). The use of a peristaltic pump enabled a continuous mixing of the water column, thereby preventing the occurrence of a vertical gradient of O_2. Hourly based oxygen uptakes were determined from changes over time in the concentration of O_2 in the water column (Kristensen and Hansen, 1999). Oxygen uptake was expressed as μmol of O_2 h^{-1} m^{-2} of water–sediment interface.

2.3.2. Water–sediment fluxes of nutrients

On days 6 and 12, each column was isolated by stopping the water renewal in the overlying water. Water samples (20 ml) were collected in the overlying water of each column, 0, 6, and 12 h after isolation. Water samples for NH_4^+, NO_3^-, and PO_4^{3-} were taken using acid-washed 100 ml syringes, filtered through Whatman GF/F filters, and analyzed within 24 h. Water samples for DOC were

Table 1
Physico-chemical characteristics of stormwater and fluvial sediments at the start of the experiment and characteristics of stormwater sediments at the end of the experiment for all treatments

Release rates	Start of the experiment		Stormwater sediments at the end of the experiment					
	Stormwater sediments	Fluvial sediments	2 cm		5 cm		8 cm	
			Control	Tubificids	Control	Tubificids	Control	Tubificids
Particulate organic carbon (g kg dry sediment)	197.7 ± 8.1	1.18 ± 0.18	163.2 ± 12.7	158.3 ± 31.1	172.6 ± 11.5	171.0 ± 7.2	190.2 ± 1.4	190.0 ± 8.4
Particulate nitrogen (g kg dry sediment)	5.67 ± 0.26	0.17 ± 0.02	4.05 ± 0.83	4.41 ± 0.14	4.68 ± 0.22	5.12 ± 0.92	5.24 ± 0.13	4.98 ± 0.1
Particulate phosphorus (g kg^{-1} dry sediment)	3.56 ± 0.05	0.38 ± 0.1	2.07 ± 0.12	2.00 ± 0.35	2.67 ± 0.12	2.65 ± 0.14	2.87 ± 0.12	2.90 ± 0.1
Cd (mg kg dry sediment)	19.1 ± 0.4	<QL	19.4 ± 1.1	18.9 ± 2.4	18.7 ± 1.3	18.9 ± 1.1	19.1 ± 1.8	17.8 ± 3.3
Cu (mg kg dry sediment)	257 ± 4	<QL	257 ± 9	246 ± 36	238 ± 15	243 ± 9	245 ± 28	228 ± 46
Pb (mg kg dry sediment)	533 ± 12	<QL	532 ± 22	507 ± 71	510 ± 35	507 ± 22	518 ± 53	486 ± 89
Zn (mg kg dry sediment)	1168 ± 14	<QL	1181 ± 25	1161 ± 137	1110 ± 70	1127 ± 53	1147 ± 17	1068 ± 187
PAH (mg kg dry sediment)								
Fluoranthene	1.20 ± 0.36	<QL	1.14 ± 0.03	1.04 ± 0.26	1.39 ± 0.16	1.33 ± 0.12	1.24 ± 0.13	1.35 ± 0.15
Benzo(*b*)fluoranthene	1.10 ± 0.04	<QL	1.11 ± 0.11	0.93 ± 0.31	1.23 ± 0.16	1.18 ± 0.07	1.09 ± 0.13	1.15 ± 0.13
Benzo(*k*)fluoranthene	0.45 ± 0.02	<QL	0.44 ± 0.01	0.39 ± 0.10	0.48 ± 0.03	0.46 ± 0.03	0.45 ± 0.06	0.47 ± 0.05
Benzo(*a*)pyrene	0.69 ± 0.03	<QL	0.60 ± 0.34	0.51 ± 0.37	0.77 ± 0.16	0.81 ± 0.05	0.62 ± 0.16	0.80 ± 0.09
Benzo(*ghi*)perylene	1.03 ± 0.06	<QL	1.07 ± 0.14	0.94 ± 0.34	1.18 ± 0.10	1.19 ± 0.08	0.82 ± 0.62	1.15 ± 0.11
Indéno (1.2.3 cd) pyrene	0.70 ± 0.03	<QL	1.05 ± 0.59	0.62 ± 0.16	0.74 ± 0.06	0.74 ± 0.06	0.77 ± 0.06	0.78 ± 0.07
Anthracene	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL
Acénaphtene	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL
Chrysene	0.99 ± 0.03	<QL	0.88 ± 0.15	0.75 ± 0.18	1.01 ± 0.08	0.95 ± 0.06	0.87 ± 0.06	0.99 ± 0.11
Dibenzo(*a·h*)anthracene	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL
Fluorene	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL
Naphtalene	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL
Pyrene	1.63 ± 0.06	<QL	1.76 ± 0.22	1.60 ± 0.55	1.66 ± 0.03	1.63 ± 0.14	1.54 ± 0.19	1.56 ± 0.18
Phenanthrene	0.53 ± 0.03	<QL	0.47 ± 0.11	0.44 ± 0.10	0.51 ± 0.06	0.51 ± 0.04	0.53 ± 0.07	0.53 ± 0.06
2-Methyl naphtalene	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL
2-Methyl fluoranthene	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL
Benzo(*a*)anthracene	0.48 ± 0.02	<QL	0.47 ± 0.05	0.37 ± 0.09	0.53 ± 0.07	0.52 ± 0.06	0.49 ± 0.03	0.52 ± 0.06
Sediment grain sizes (% of volume)								
0–10 μm	59.9 ± 3.0	1.5 ± 0.5						
10–100 μm	34.6 ± 1.7	4.8 ± 0.4						
100–200 μm	3.6 ± 0.2	7.0 ± 0.1						
200–500 μm	2.0 ± 0.1	61.4 ± 0.9						
500–1000 μm	0.0 ± 0.0	25.3 ± 0.3						
Water content (%)	76.6 ± 6.7	28.7 ± 4.2						

QL = quantification limit: $QL_{Cd} = 0.2$ mg kg^{-1}, $QL_{Cu} = 10$ mg kg^{-1}, $QL_{Pb} = 10$ mg kg^{-1}, $QL_{Zn} = 30$ mg kg^{-1}, and $QL_{PAHs} = 0.13$ mg kg^{-1}.

filtered through Whatman GSWP filters and immediately stored at 4 °C for later analysis. The release rate of NH_4^+, NO_3^-, PO_4^{3-}, and DOC across the sediment–water interface was calculated from changes over time in the concentration of each species in the water column. The release rates were expressed as μmol h^{-1} m^{-2} of water–sediment interface.

2.3.3. Exchanges of PAH and heavy metals between sediment and water

Exchange rates of PAH and heavy metals were measured on day 13 of the experiment. All columns were isolated and water samples were collected 0 and 10 h after isolation. Samples for heavy metals (10 ml) were filtered through HAWP membrane filters (0.45 μm, Millipore) and acidified with nitric acid (pH < 2). Samples for PAH (300 ml) were collected in brown bottles and stored at 4 °C before analyses. The release rates of heavy metals and PAH were expressed as μg (or mg) h^{-1} m^{-2} of water–sediment interface based on differences in concentrations measured after 10 h of incubation.

2.3.4. Sediment analyses

During column dismantling (day 14), SWS samples were collected in the top sediment layer (0–1 cm) and were homogenised by sieving (0.5 mm mesh aperture) with deionized water. These samples were used to measure the concentrations of particulate organic carbon, particulate nitrogen, and particulate phosphorus, PAHs, and heavy metals (Cd, Cu, Pb, and Zn) in top sediments.

2.3.5. Chemical analyses

Particulate organic carbon (POC) was determined by high-temperature combustion of pre-acidified sediment samples and subsequent measurement of CO_2 by infrared detectors (Analytik Jena). Particulate nitrogen (PN) was analyzed by high-temperature combustion and subsequent measurement of N_2 by thermal conductometry (FlashEA, Thermo Electron Corporation). Particulate phosphorus (PP) was measured with the ascorbic acid method after microwave-assisted digestion with HNO_3 and $HClO_4$ and analyzed by colorimetry (Grasshoff et al., 1983). Analyses of hydrocarbons and heavy metals in sediment and water were performed by the Health and Environmental Laboratory of Lyon following standard methods (APHA, 1998; AFNOR, 1999). Metals (Cd, Cu, Pb, and Zn) in sediment were extracted using microwave-assisted digestion with HNO_3 and HCl. Extracted metals from sediment and metals contained in water samples were analyzed by inductively coupled plasma MS. PAH analyses were performed using High-Performance Liquid Chromatography with fluorescence detectors (Agilent 1100 HPLC). Quantification limits of heavy metals and PAHs given by the Health and Environmental Laboratory of Lyon were indicated on Table 1.

Analyses of NH_4^+, NO_3^- ($NO_3^- + NO_2^-$), and PO_4^{3-} in water were performed using an automatic analyzer Easychem Plus (Systea, Italia) based on standard colorimetric methods (Grasshoff et al., 1983). DOC concentration was measured with an Analytik Jena total carbon analyzer based on high-temperature combustion after removing inorganic C with hydrochloric acid and CO_2 stripping under O_2 flow.

2.3.6. Bacterial measurements

One molecular probe was used on sediment samples to detect the Domains Bacteria (probe EUB 338, eubacteria). The use of this labelled rRNA-targeted nucleic acid probe allows an in situ identification of active microbial cells in their natural habitats (Amann et al., 1997). During column dismantling (day 14), 2 g of wet sediment were immediately collected at the surface (0–1 cm) of SWS. Sediment samples were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 h. Fixed samples were subsequently washed twice in PBS and were stored in ethanol and PBS (50:50) at –20 °C. After storage (1 month), 0.5 g of fixed samples was homogenized in 4 ml of 0.1% pyrophosphate in PBS using a sonicator with a 2-mm-diameter probe (Sonicator XL 2020) at 100 W during 2 periods of 60 s. All homogenised samples were finally supplemented with the detergent NP-40 (Flucka, Buchs, Switzerland) to a final concentration of 0.01%. Aliquots (10 μl) of homogenised samples were spotted onto gelatine-coated slides and were hybridized with Cy3-labeled oligonucleotide probe (EUB 338) and concomitantly stained with the DNA intercalating dye DAPI (4′,6-diamidino-2-phenylindole, 200 ng μl^{-1}, Sigma, Buchs, Switzerland). Hybridizations were performed in 15 μl of hybridization buffer (0.9 M NaCl, 20 mM Tris/HCl, 5 mM EDTA, 0.01% sodium dodecyl sulphate; pH 7.2) in the presence of 30% formamide, 1 μl of DAPI, and 1 μl of the probe (25 ng μl^{-1}) at 37 °C for 2 h. After hybridization, the slides were washed in buffer at 48 °C for 20 min, rinsed with distilled water and air-dried. Slides were mounted with Citifluor solution (Citifluor Ltd., London, U.K.) and the preparations were examined at 1000× magnification with a BH2-RFCA Olympus microscope fitted for epifluorescence with a high-pressure mercury bulb (50 W) and filter sets BP 405 (for DAPI) and BP 545 (for Cy3). Bacteria from the samples were analyzed in 20 fields per sample with up to 30 cells per field. Numbers of DAPI- and Cy3-bacteria were counted separately from the same field in order to calculate the percentages of active bacteria (EUB/DAPI) from each analyzed field.

Hydrolytic activity was measured using fluorescein diacetate (FDA) as substrate for hydrolases (Fontvieille et al., 1992). Wet sediment (0.4–0.6 g) was incubated in the dark with 3 ml of phosphate buffer (pH 7.6) and 0.1 ml of FDA solution (2 mg ml^{-1}) at 15 °C until the green colour of fluorescein became visible (0.5–1.5 h). Blank samples were prepared by using 6 sediment samples treated with 1.5 ml of acetone and 1.5 ml of phosphate buffer 40 min prior to the addition of the FDA solution. For all samples and blanks, the reaction was stopped by freezing the sediment after addition of 3 ml of mercuric chloride solution

(200 mg l^{-1}). The absorbance of solutions was measured at 490 nm after filtration through a HAWP filter (mean pore size: 0.45 μm). Results were expressed as μmoles of hydrolysed FDA h^{-1} g^{-1} sediment dry weight. The average absorbance of blank samples was subtracted from that of the samples in order to obtain values corresponding to the hydrolytic activity mediated by microbes.

2.4. Vertical distribution of tubificid worms at the end of the experiment

The living worms were recovered at the end of the experiment (day 14) to estimate mortality and vertical migration in each treatment. The top 10 cm of sediments were sliced (the top 3 cm in slices of 0.5 cm thickness and lower in slices of 1 cm thickness), sieved though a 630 μm mesh, and preserved in alcohol (96%) before counting. For each column, invertebrates were counted within the different slices under a dissecting microscope. At the end of the experiment, we recovered more than 92% of the animals added initially. Vertical profiles of living invertebrates in the sediment at the end of experiment were expressed in percentage of organisms found in each column.

2.5. Statistical analyses

The concentrations of heavy metals, PAHs, particulate organic carbon, total nitrogen, and total phosphorus in SWS were compared between treatments (animal × SWS layer thickness) using a two-way analysis of variance (ANOVA). The effects of tubificid worms and SWS layer thickness on O_2 uptake and nutrient release were tested by means of two-way repeated measures (RM)-ANOVA (2 days: days 6 and 12) using Statistica5TM (Statsoft, Tulsa). One-way ANOVA were used to test for differences in microbial parameters between treatments (animal × SWS layer thickness). If significance was detected, Scheffé post hoc tests were performed to determine which treatments differed. Data were log or square-root transformed to homogenize variances when homoscedasticity was not observed. Percentages of active bacteria were arcsine transformed before statistical analyses to meet the assumption of normality.

3. Results

3.1. Nutrient and pollutant concentrations in SWS

SWS had high concentrations of PAHs, heavy metals, and nutrients and were characterized by a high percentage of particles finer than 10 μm (Table 1). In contrast, fluvial sediments below the SWS layer were coarser, less rich in nutrients, and uncontaminated. The concentrations of pollutants (heavy metals and PAH) in SWS did not significantly evolve from the beginning to the end of the experiment in the different treatments (Student's *t*-tests, $p > 0.05$, time effect). The concentration of particulate organic carbon, particulate nitrogen, and particulate phosphorus significantly decreased from the start to the end of the experiment in most treatments (Table 1). At the end of the experiment, there were no significant effects of animal treatment on organic carbon, nitrogen and phosphorus in SWS (two-way ANOVAs, animal effect, $p >, 0.7$, "animal × SWS layer thickness" effect, $p > 0.5$). However, the concentrations of particulate organic carbon and particulate phosphorus were significantly lower in the 2-cm thick SWS layer than in thicker SWS layers (two-way ANOVAs, "SWS layer thickness" effect, $p < 0.02$).

3.2. Distribution of worms and visual observations

During the experiments, tubificid worms dug galleries in the SWS layer (Fig. 1). For all treatments, visual observations showed that galleries of tubificid worms were restricted to the SWS layers. This observation was supported by the vertical distributions of tubificid worms obtained at the end of the experiments (Fig. 2): tubificid worms occurred in the top 2 cm of sediment with the 2-cm thick SWS layer and occurred in the top 4 cm of sediment with the 5-cm and 8-cm thick SWS layers. Vertical distributions of worms significantly increased with the thickness of the SWS layer (Fig. 2, two-way ANOVA, interaction "SWS layer thickness × vertical distribution of worms", $p < 0.001$).

3.3. O_2 uptake and release of nutrients, DOC, heavy metals, and PAHs

Uptake (influx from overlying water to SWS) rates of O_2 and NO_3^- were significantly influenced by SWS layer thickness and the occurrence of worms (Fig. 3), two-way RM ANOVA, SWS layer thickness and animal treatments, $p < 0.001$). O_2 and NO_3^- uptake rates significantly increased with the thickness of the SWS layer (Scheffé post hoc tests,

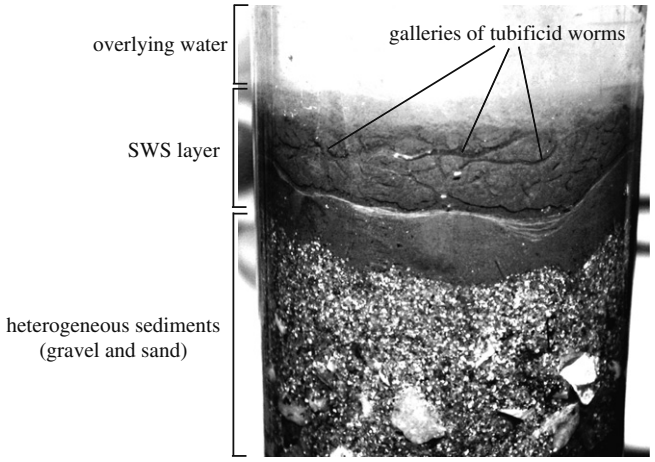

Fig. 1. Picture from the experimental system showing the galleries produced by tubificid worms in the top centimeters of the stormwater sediment layer (2-cm thick).

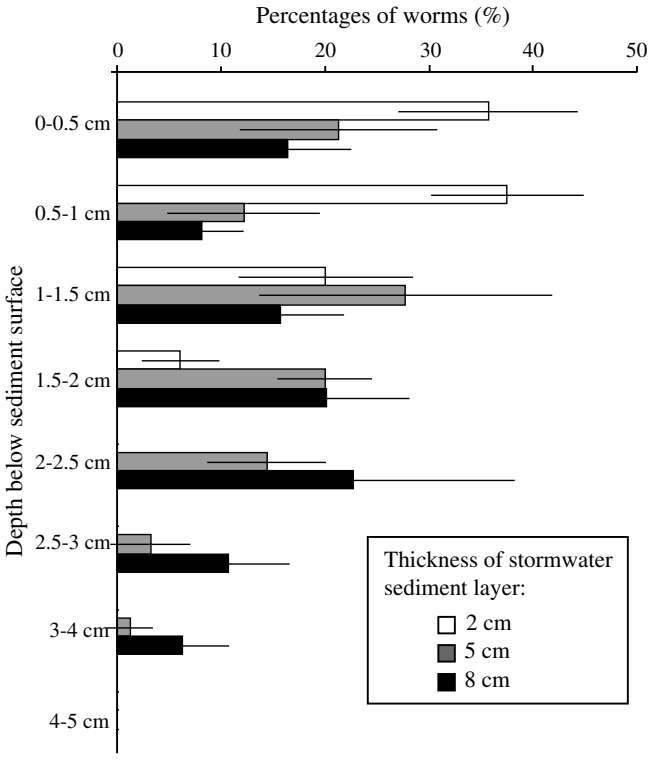

Fig. 2. Vertical distributions of tubificid worms obtained for the 3 thicknesses of stormwater sediment layer.

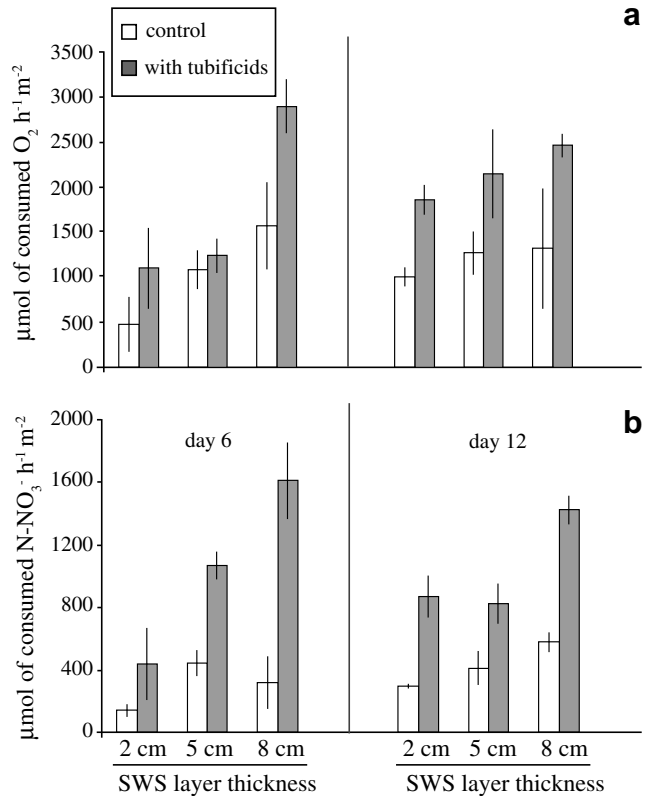

Fig. 3. Influxes of dissolved oxygen (a) and nitrate (b) from overlying water to SWS measured at two dates for the 6 treatments (SWS layer thickness × tubificid worms).

$p < 0.05$). The effect of tubificid worms also depended on the thickness of the SWS layer (two-way RM ANOVA, "SWS layer thickness × animal" effect, $p < 0.05$), the highest stimulation of uptake rates by tubificid worms being observed with the 8-cm thick SWS layer (Fig. 3, mean increases of 85% and 230% for O_2 and NO_3^- uptakes, respectively).

The release (efflux) rates of NH_4^+, PO_4^{3-}, and DOC from the SWS to the overlying water were significantly influenced by the SWS layer thickness (Fig. 4, two-way RM

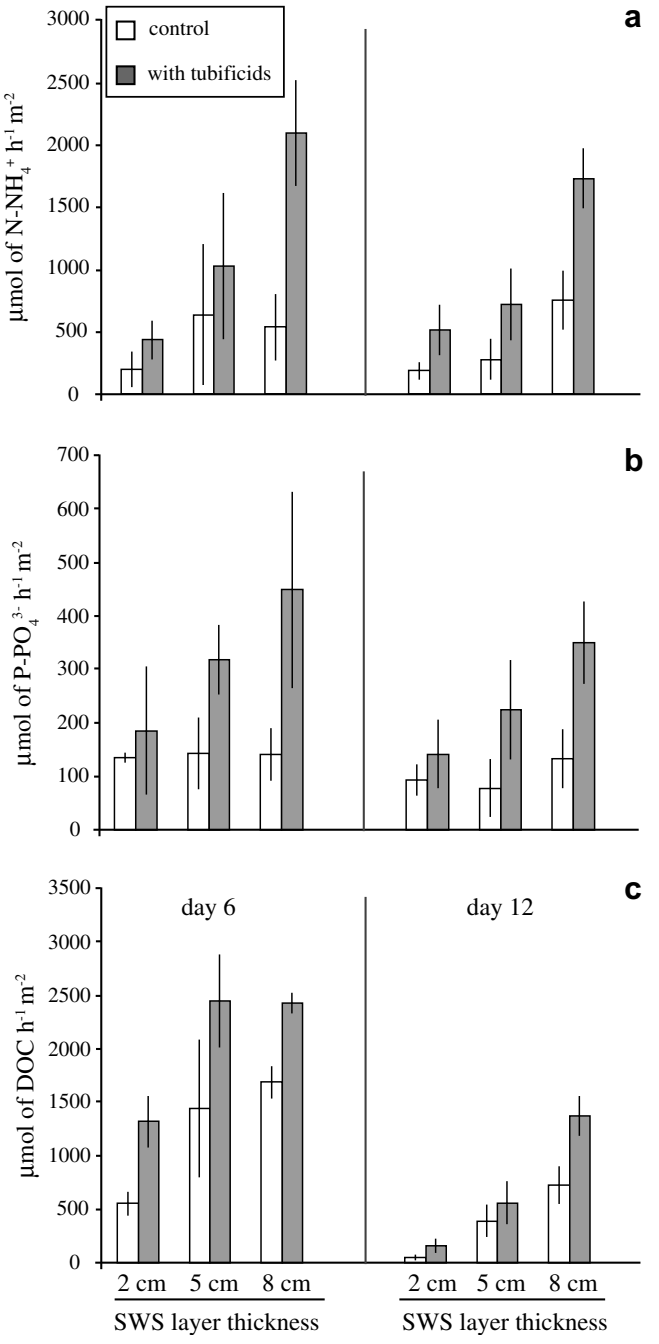

Fig. 4. Effluxes of ammonium (a), phosphate (b), and dissolved organic carbon (c) from SWS to overlying water measured at two dates for the 6 treatments (SWS layer thickness × tubificid worms).

ANOVA, SWS layer thickness effect, $p < 0.001$) and were stimulated by the occurrence of tubificid worms (Fig. 4, two-way RM ANOVA, animal effect, $p < 0.001$). The three fluxes significantly increased with the thickness of the SWS layer (Scheffé post hoc tests, $p < 0.05$). The stimulation of the release rates of NH_4^+ by tubificid worms was positively linked to the thickness of the SWS layer (Fig. 4a, two-way RM ANOVA, "SWS layer thickness × animal" effect, $p < 0.02$). For PO_4^{3-} release rates, highest stimulation of fluxes was also measured in the 8-cm thick SWS layer (Fig. 4b), despite no statistical significance at a p-level of 0.05 (two-way RM ANOVA, "SWS layer thickness × animal" effect, $p = 0.054$).

From the 4 heavy metals (Zn, Pb, Cd, and Cu) and the 17 PAHs studied, quantifiable release rates from SWS to the overlying water were only measured for 1 heavy metal (Zn) and 2 PAHs (acenaphtene and naphtalene) in all experimental treatments (Fig. 5). Release rates of fluoranthene, pyrene, phenanthrene, and 2-methyl-naphtalene were also quantified but only in 1 or 2 experimental columns (Fig. 5). Although mean release rates of Zn tended to be higher in columns with tubificids (5.13–5.38 mg of Zn $h^{-1} m^{-2}$) than values reported in control columns (3.24–4.19 mg of Zn $h^{-1} m^{-2}$), there were no significant differences due to animal treatment (two-way ANOVA, animal effect, $p > 0.13$). For acenaphtene and naphthalene release rates, no significant influence of tubificid worms was detected (two-way ANOVA, animal effect, $p > 0.3$) but released rates were significantly lower in columns with a 2-cm thick SWS layer than in columns with 5-cm and 8-cm thick SWS layers (two-way ANOVA, SWS layer thickness effect, $p < 0.02$).

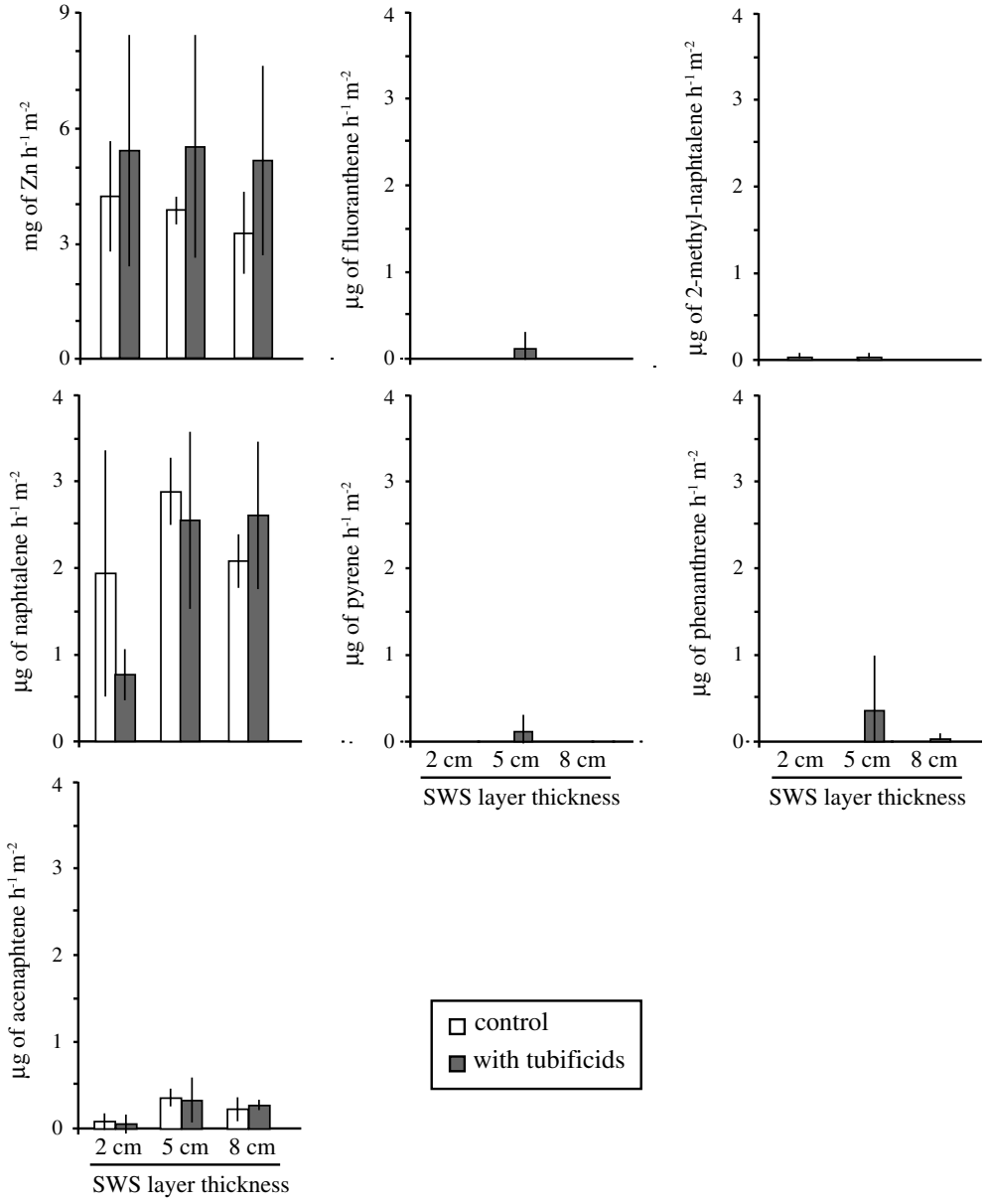

Fig. 5. Effluxes of pollutants from SWS to overlying water measured for the 6 treatments (SWS layer thickness × tubificid worms).

3.4. Microbial characteristics

The total numbers of bacteria in the SWS layer were not significantly influenced by the SWS layer thickness and the presence of tubificid worms (Fig. 6a, two-way ANOVA, SWS layer thickness and animal effects, $p > 0.05$). Despite no influence on total numbers of bacteria, tubificid worms increased significantly the percentages of active bacteria in SWS layer (Fig. 6b, two-way ANOVA, animal effect, $p < 0.001$). The percentages of active bacteria were also influenced by the SWS layer thickness (two-way ANOVA, SWS layer thickness effect, $p < 0.005$), highest percentages being measured in the 2-cm thick SWS layer (Scheffé post hoc tests, $p < 0.03$). Hydrolytic activity was not influenced by SWS layer thickness and occurrence of tubificid worms (Fig. 6c, two-way ANOVA, SWS layer thickness and animal effects, $p > 0.25$).

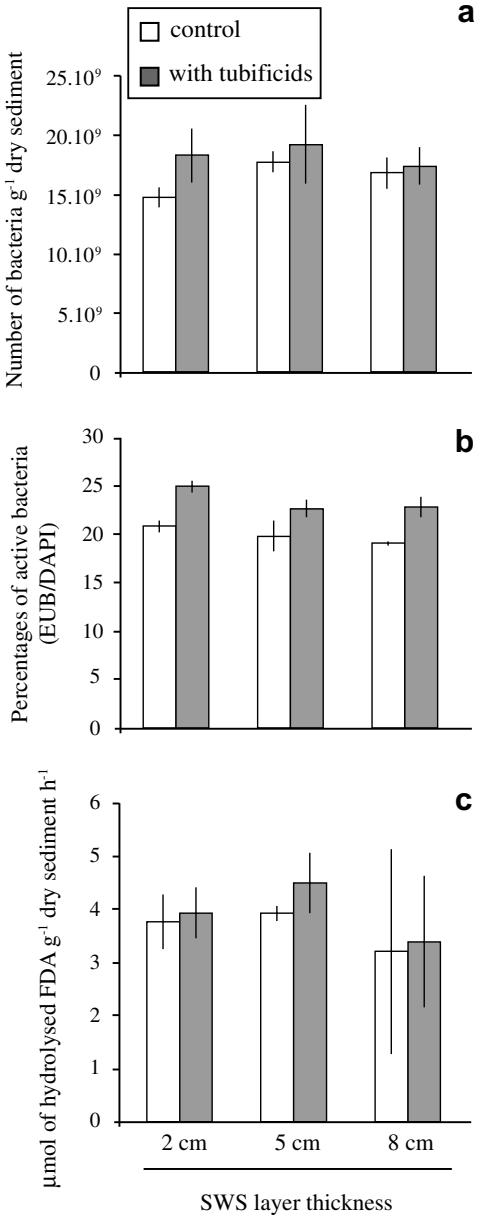

Fig. 6. Number of bacteria (a), percentages of active bacteria (b), and hydrolytic activity (c) measured in stormwater sediments for the 6 treatments (SWS layer thickness × tubificid worms).

4. Discussion

4.1. Influence of the thickness of SWS layer on biogeochemical processes and pollutant fluxes

According to our hypothesis, the mineralization of the organic matter (respiration rates) and the releases of solutes (ammonium, phosphorus, organic carbon, heavy metals, PAH) from SWS increased with the thickness of SWS layer: higher mean O_2 uptake rates (+120%) and mean release rates of ammonium (+230%), DOC (+300%), and acenaphtene (+160%) were measured in control columns with 8-cm thick SWS layer than in control columns with a 2-cm thick SWS layer. As benthic metabolism may be strongly stimulated by supply of particulate organic matter (Crenshaw et al., 2002; Nogaro et al., 2007b), the influence of the SWS layer thickness on water–sediment functioning was likely due to the increase of the total quantity of POC, PN, and PP with the quantity of stormwater sediments. As commonly measured in diffusion-dominated (stagnant) systems (Kristensen and Hansen, 1999), the concentrations of several solutes (ammonium, DOC) probably increased with depth in stormwater sediments. In such condition, the thickness of the SWS layer was positively linked with solute concentrations and solute effluxes from sediments. Moreover, we observed a compacting of the stormwater sediment at the interface with the heterogeneous (sand and gravel) sediments. This phenomenon could explain the fact that tubificid worms were restricted to the SWS layer and produced horizontal burrows in sediment (Fig. 1) whereas Nogaro et al. (2006) showed in a comparable system (with a layer of SWS of a coarser grain size) that tubificid worms produced vertical burrows and lived in the whole sediment column (both in SWS and deep fluvial sediments). Due to this compacting process, the molecular diffusion which often determines solute fluxes at the water–sediment interface (Aller, 1983; Kristensen, 2000) was restricted to the thickness of SWS layer. Thus, the thickness of the SWS layer determined the diffusion zone and the efflux of several solutes in our experimental system. Because benthic metabolism and release of nutrients and pollutants were positively linked to the quantity of deposited SWS, management of stormwater retention ponds needs to regulate of the accumulation of SWS to reduce nuisance linked to efflux of nutrients and pollutants. We could however note that only the most soluble pollutants (Zn for heavy metals, Welp and Brümmer, 1999; naphthalene and acenaphtene for PAHs; Srogi, 2007) were significantly released from SWS to overlying water under our experimental conditions. According with field studies (e.g., Datry et al., 2004), these results indicate that organic SWS have very high adsorption capacities for pollutants and thus are less

a source of contaminants than a source of nutrients for aquatic ecosystems.

4.2. Influence of the tubificid worms on biogeochemical processes and pollutant fluxes

According to a previous study performed with another SWS (Mermillod-Blondin et al., 2005), tubificid worms strongly stimulated the organic matter processing in SWS. Considering the 8-cm thick SWS layers, tubificid worms increased by 85% the oxygen uptake, by 230% the nitrate uptake, and by 200%, 190%, and 55% the release rates of NH_4^+, PO_4^{3-}, and DOC, respectively. The influence of tubificid worms on O_2 uptake, and nutrient release could result from indirect (bioturbation) and/or direct (respiration and feeding activities) effect of animals in the sediment. According to the measurements of NH_4^+ excretion rate and O_2 respiration rate of tubificid worms performed by Datry et al. (2003b), the NH_4^+ excretion and respiration rates of tubificid worms were estimated to 0.7 μmol of NH_4^+ h^{-1} m^{-2} and 25 μmol of O_2 h^{-1} m^{-2} in our experimental conditions. These estimates clearly indicated that direct effects of worms accounted for a very small proportion of the increase in NH_4^+ release (>400 μmol h^{-1} m^{-2}) and O_2 uptake (>1000 μmol h^{-1} m^{-2}) measured in columns with worms. Therefore, bioturbation by tubificid worms had a major influence on solute fluxes at the water sediment interface. As observed in other studies (Pelegri and Blackburn, 1995; Svensson et al., 2001), the production of galleries by tubificid worms promoted the exchange of solutes between the sediment and the water column, thereby increasing the supply of oxygen and other electron acceptors (e.g., NO_3^-) at depth into the sediment and the release of metabolites (e.g., NH_4^+, DOC). The enhanced transport of O_2 and NO_3^- from the overlying water to the stormwater sediments highly colonised by bacteria (more than 10^{10} bacteria per gram of dry sediment) increased by O_2 and NO_3^- uptakes in tubificid columns. These supplies of electron acceptors could also explain the stimulation of the percentage of active bacteria in the bioturbated zone (the top 3 cm of the sediment). If worms significantly increased the release of nutrients and DOC at the water–sediment interface, they did not significantly enhance the releases of PAHs and heavy metals from the sediment. Several studies (Karickhoff and Morris, 1985; Reible et al., 1996) demonstrated that tubificid worms could increase the release rates of hydrophobic compounds such as pyrene and phenanthrene from freshwater sediments to the water column. However, the characteristics of SWS appeared to limit the influence of worms on the mobility of pollutants from the sediment to overlying water in our experiment.

In relation with the variability in accumulation of SWS observed in retention ponds, we hypothesized that the stimulation of organic matter and pollutant fluxes by worms would be positively linked to the thickness of SWS layer. This hypothesis was validated by results concerning O_2 uptake rates, NO_3^- uptake rates, and release rates of nutrients and DOC. The positive relationship between worm influence on fluxes and SWS layer thickness was strongly linked to the space burrowed by tubificid worms in the different thickness treatments. Vertical distribution of worms collected at the end of the experiment showed that thicker was the SWS layer, deeper was the occurrence of tubificid worms. As demonstrated in marine studies (e.g., Mermillod-Blondin et al., 2004; Michaud et al., 2006), the solute fluxes induced by invertebrates at the water–sediment interface were linked to interactions between burrowing depth and depth distribution of pore water solutes. Because reduced compounds such as NH_4^+ increased with depth below the water–sediment interface (Mermillod-Blondin et al., 2005), the release rates of these compounds were the most stimulated by tubificid worms in columns where deepest distributions of worms were observed (with a 8-cm thick SWS layer). The vertical distribution of the tubificid worms also determined the volume of galleries in the sedimentary column. Several marine studies (Gilbert et al., 1995; Michaud et al., 2005) showed that the volume of biogenic structures produced by benthic fauna influenced the exchange area between sediments and overlying water and the fluxes at the water sediment interface. In our experiments, the highest increase of the water–sediment interface area due to worm galleries probably occurred in columns with 8-cm thick SWS layers, explaining the highest effects of worms on flux rates measured in the same columns. Therefore, our results highlight that environmental conditions affecting the burrowing activities of the invertebrates (the SWS layer thickness being the main environmental factor affecting the distribution of worms in the present study) can strongly affect the mineralization process in sediments and the nutrient fluxes at the water–sediment interface.

4.3. Functional significance of sediment accumulation and biological activity in stormwater retention basin

The biological degradation of organic SWS represents a net loss in a gaseous form (CO_2, CH_4) of organic matter stored in the stormwater retention systems. However, this degradation also produces dissolved forms of nitrogen, phosphorus and organic carbon, which contribute to the nutrient load of rivers and subsurface water. Based on results of the present experiment, we assessed the influence of the thickness of accumulated sediments and the role of tubificid worms in consuming organic carbon and releasing nutrients at the surface of a retention infiltration basin (Django Reinhardt, 7405 m^2, Winiarski et al., 2006), in which sediments and worms were collected. We assumed that the density of tubificid worms in the layer of SWS deposited in the bed of the retention basin resulted in similar respiration and nutrient release rates than those measured in laboratory experiments.

The O_2 uptakes of SWS (from 741 to 2681 μmol h^{-1} m^{-2}) were comparable to those measured in lake sediments having high organic matter content (for

instance, values from 1000 to 2600 μmol h^{-1} m^{-2} were reported from lake sediments containing 15–20% of organic matter per sediment dry mass, Leal et al., 2003). However, the N-NO_3^- uptake rates of SWS (from 217 to 1518 μmol h^{-1} m^{-2}) were very high in comparison with values reported in literature (for instance, uptake rates reported from muddy sediments enriched with macroalgal detritus did not exceed 150 μmol h^{-1} m^{-2}, Hansen and Kristensen, 1998), highlighting the very high biological activity in these urban sediments.

Assuming a consumption of 1 mol of carbon for 1 mol of O_2 consumed during the aerobic degradation of the organic matter and a consumption of 1 mol of carbon for 4/5 mol of NO_3^- consumed during the denitrification process (Hedin et al., 1998), the amount of organic carbon consumed per year in the infiltration bed (surface: 7405 m^2) was estimated to 1790 kg for a 2-cm thick SWS layer, 2240 kg for a 5-cm thick SWS layer, and 3560 kg for a 8-cm thick SWS layer. For the three SWS layer thicknesses, between 40% and 56% of the carbon consumption was attributed to the bioturbation activity of tubificid worms. However this estimate of organic matter degradation should be taken with care as it does not include the effect of fermentative processes in the deeper layers of the sediment and it varies probably in response to a number of factors naturally occurring in the field (e.g., seasonal variation in microbial activity, variation in water volume retained in the infiltration bed).

The release rates NH_4^+, PO_4^{3-}, and DOC from SWS were in the upper range of values reported in organic matter-rich sediments (Hansen and Kristensen, 1998; Christensen et al., 2000; Leal et al., 2003). For instance, NH_4^+ release rates reported with tubificid worms in columns with a 8-cm thick SWS layer (1900 μmol h^{-1} m^{-2}) was more than 2-fold higher than values reported from muddy sediments enriched with organic matter and bioturbated by worms (<850 μmol h^{-1} m^{-2}, Hansen and Kristensen, 1998; Christensen et al., 2000).

The amounts of ammonium, phosphorus, and dissolved organic carbon released per year in the infiltration bed were estimated to 430 kg N, 330 kg P, and 580 kg C with a 2-cm thick SWS layer, 790 kg N, 545 kg P, and 1170 kg C with a 5-cm thick SWS layer, and 1730 kg N, 800 kg P, and 1480 kg C with a 8-cm thick SWS layer. The bioturbation activity of worms would account, respectively for 48–67%, 30–66%, and 37–59% of the annual amounts of dissolved nitrogen, phosphorus, and organic carbon released by SWS to water of the retention basin. Despite uncertainty in data used for calculation, these rough estimates suggest that biological degradation of organic SWS in retention basins may constitute a significant source of dissolved nutrients, this source of nutrients depending on the quantity of accumulated SWS and the bioturbation process. Because export of the dissolved nutrients released from stormwater retention systems may influence the functioning of nearby ecosystems (e.g., Datry et al., 2004 for aquifers; and Lafont et al., 2006 for rivers), our results suggest that management of stormwater ponds needs a regulation (control) of SWS accumulations and biological activities to limit the export of pollutants and nutrients.

5. Conclusions

The oxidation of SWS organic matter and the release of nutrients in water were significantly increased by the quantity of accumulated SWS and the bioturbation process. The thickness of SWS layer also influenced the release of two PAHs (acenaphtene and naphthalene) from the sediment to the overlying water whereas the activities of tubificid worms did not appear to promote the mobilization of pollutants. The present study clearly suggests that the bed of stormwater retention systems should no longer be viewed only as physical systems retaining suspended solids transported by storm water but also as biological reactors. This also implies that management practices of retention basins can be oriented to regulate the quantity of accumulated SWS and the biological activities in sediment, thereby influencing the degradation of organic matter and export of nutrients and pollutants.

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