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# Influence of *Chironomus riparius* (Diptera, Chironomidae) and *Tubifex tubifex* (Annelida, Oligochaeta) on oxygen uptake by sediments. Consequences of uranium contamination

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This study highlights the ecological importance of bioturbation in metal-contaminated sediments.

## A B S T R A C T

The diffusive oxygen uptake (DOU) of sediments inhabited by *Chironomus riparius* and *Tubifex tubifex* was investigated using a planar oxygen optode device, and complemented by measurements of bioturbation activity. Additional experiments were performed within contaminated sediments to assess the impact of uranium on these processes. After 72 h, the two invertebrate species significantly increased the DOU of sediments (13–14%), and no temporal variation occurred afterwards. Within contaminated sediments, it was already 24% higher before the introduction of the organisms, suggesting that uranium modified the sediment biogeochemistry. Although the two species firstly reacted by avoidance of contaminated sediment, they finally colonized it. Their bioturbation activity was reduced but, for *T. tubifex*, it remained sufficient to induce a release of uranium to the water column and an increase of the DOU (53%). These results highlight the necessity of further investigations to take into account the interactions between bioturbation, microbial metabolism and pollutants.

### Keywords:

Bioturbation  
Freshwater macroinvertebrates  
Diffusive oxygen uptake  
Sediments  
Heavy metals

## 1. Introduction

The oxygen uptake rate at the sediment-water interface is the main parameter used to estimate the benthic mineralization of organic matter occurring in the early diagenesis of sediments (Thamdrup and Canfield, 2000). It is considered as a relevant indicator of the biogeochemical functioning of sediments. Oxygen consumption by sediments results both from abiotic and biotic processes. Molecular diffusion from the water column and advection forces induce oxygen penetration into sediments of a few millimeters or centimeters (Jorgensen and Revsbech, 1985). The thickness of the oxic layer is negatively correlated to amount and flux of organic matter coming from the overlying water. An increase of organic matter input in surface sediments will lead to the increase of biological oxygen demand and thus to the reduction of the thickness of the oxygenated layer. The sediment-water interface constitutes a dynamic zone with intense oxygen consumption by heterotrophic and lithoautotrophic organisms but also production

by benthic photosynthetic communities. Sediment-dwelling macrofauna, in addition to its own respiration, exerts a strong influence on sediment properties that can enhance oxygen penetration and uptake rate (e.g. Heilskov and Holmer, 2001; Karlson, 2007). Particle mixing and solute transport induced by macrofauna bioturbation lead to a three-dimensional structuring of sediment in a mosaic of microenvironments with different physical, chemical and biological properties (Kristensen, 2000). Bioturbation favors abiotic redox reactions and the growth and the development of some aerobic microbial communities and meiofauna (Aller and Aller, 1986). These organisms could in return influence chemical reactions in zones with variable redox conditions. Oxygen uptake rate (and it) has been used in many studies to assess the impact of macroinvertebrate bioturbation (e.g. resulting from burrowing, food foraging, defecation, respiration activities), particularly in marine ecosystems (e.g. Glud et al., 2003; Mermillod-Blondin et al., 2004; Wenzhöfer and Glud, 2004; Michaud et al., 2005; Zorn et al., 2006), where the benthic metabolism has been shown to increase from 25 to 271% (Kristensen, 2000).

Comparatively, for freshwater ecosystems, there are few studies dealing with the influence of macroinvertebrate bioturbation on

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oxygen consumption by sediments. Most of the time measurements were associated to studies related to nitrogen cycling, gases or nutrients fluxes at the sediment-water interface as well as in burrow walls. Most of them concerned sediment-dwelling insect larvae (Wang et al., 2001; Stief et al., 2004; Leal et al., 2007), principally Chironomid larvae (Frenzel, 1990; Svensson and Leonardson, 1996; Svensson, 1997; Kajan and Frenzel, 1999; Lewandowski et al., 2007), which irrigate their burrows more or less permanently. Some experiments were also conducted with Tubificid worms (Mc Call and Fisher, 1980; Matisoff, 1995; Pelegri and Blackburn, 1995; Svensson et al., 2001; Mermillod-Blondin et al., 2005; Nogaro et al., 2007). Precise measurements performed in the burrows of freshwater benthic macroinvertebrates and in the corresponding surrounding sediments, particularly through microsensor experiments, clearly demonstrated that these organisms, globally smaller than marine invertebrates, can also enhance oxygen and nutrient fluxes. Nevertheless, it remains difficult to perform microsensor profiles in highly bioturbated sediments and to obtain an integrative response of sediments by averaging local one-dimensional-profiles, and so, to compare efficiently sediments with and without bioturbation. Recent developments in two-dimensional O<sub>2</sub> sensors – planar optodes – now enable detailed analysis and quantification of the oxygen distribution dynamics into sediments at a high spatial and temporal resolution (Glud et al., 1996). Although there is an increasing use of optode measurements in bioturbation studies in marine ecosystems (e.g. Timmermann et al., 2006; Behrens et al., 2007), only one study involving freshwater macroinvertebrates is currently reported in the literature (Polerecky et al., 2006).

The main objective of the present study is to provide new insights relative to the influence of bioturbation of two freshwater macroinvertebrate species, at relative high densities, on the global oxygen uptake of sediments using a planar optode device through a 12-day laboratory microcosm experiment.

The species *Chironomus riparius* (Diptera, Chironomidae) and *Tubifex tubifex* (Annelida, Tubificidae) were chosen as biological models because of their widespread distribution and abundance in freshwater ecosystems and their belonging to two distinct bioturbation functional groups as defined by Gérino et al. (2003).

*C. riparius* larvae are surface deposit-feeders with a low burrowing activity mainly dependent of oxygen and organic matter availability and presence of predators in the water column (Rasmussen, 1984; Hölker and Stief, 2005). The intermittent ventilation of their tubes induces a slight downward transport of sediment particles and influences solute fluxes at the sediment-water interface (Stief and De Beer, 2002, 2006; Stief, 2007).

*T. tubifex* worms are 'conveyor-belt' subsurface deposit-feeders, living head-down oriented and partially submerged in the sediment, with the posterior section of the body free in the overlying water so as to ensure cutaneous respiration. Foraging galleries into the sediment, these worms ingest sediment particles in reduced sediment and excrete them at the surface within fecal pellets (Palmer, 1968). This results in a high and ordered mixing of sediment particles with a dominant upward transport and effects on solute distribution (Mc Call and Fisher, 1980; Matisoff, 1995; Pelegri and Blackburn, 1995; Svensson et al., 2001; Mermillod-Blondin et al., 2005; Nogaro et al., 2007).

To complete our analysis, measurements were additionally performed within uranium-contaminated sediments. Uranium is a natural radioactive heavy metal whose content in the environment has increased due to human activities, particularly in freshwater ecosystems (e.g. Baborowski and Bozau, 2006) where it can accumulate in sediments. Natural uranium concentrations considered as the 'background level' for freshwater sediments range below 10 µg U g<sup>-1</sup> dry weight (Kurnaz et al., 2007 and references

therein), but concentrations exceeding several hundreds to several thousands of µg U g<sup>-1</sup> dry wt have been registered in rivers and lakes closed to mining sites in Canada, Spain or Australia (Hart et al., 1986; Lozano et al., 2002; Lottermoser et al., 2005). Given that uranium can negatively affect benthic macroinvertebrates (Environnement Canada, 2003; Dias et al., 2008; Lagauzère et al., 2009), and influence microbial community metabolism (for reviews see: Wall and Krumholz, 2006; Wilkins et al., 2006; Renshaw et al., 2007), we have studied here the potential consequences of sediment contamination on oxygen fluxes at the sediment-water interface.

## 2. Materials and methods

### 2.1. Sediment and water preparation

Sediment and water used in our experiments were sampled from a closed channel of a lake on the Verdon River (Lac d'Esparron, south-eastern France). This sampling site was chosen because of the nature of the sediment (fine mud) and the quality of water (low turbidity, no pollution). Sediments were sieved through a 2-mm mesh to remove coarse fragments (e.g. stones, leaves, and wastes) and macrofauna, and kept frozen at -20 °C for a week in order to kill most of organisms that may have been present. After thawing and homogenization (mixing by mechanical stirring), they were kept at 4 °C until setting up the microcosms. The water was filtered through a 20-µm filter and then stored at 4 °C.

### 2.2. Microcosm setting-up

Two beakers of sediment were prepared: one non-contaminated hereafter referred to as 'control' and a second one that was spiked with a solution of uranyl nitrate UO<sub>2</sub>(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O (Sigma-Aldrich, France) to obtain end concentration of 600 µg U g<sup>-1</sup> of dry sediment. Previous work performed in the same experimental conditions has demonstrated that this concentration was sublethal for the two studied species with an LC<sub>50</sub> of 851 µg U g<sup>-1</sup> wt for *Chironomus riparius* and 2320 µg U g<sup>-1</sup> wt for *Tubifex tubifex*, respectively (Lagauzère et al., 2009). The beakers of sediment were hand-shaken for 10 min each day for 2 weeks to ensure that the contamination was homogeneous.

Five separate microcosms, constituted of transparent aquaria (10 × 10 × 20 cm; length × width × height) equipped with oxygen optodes on each face, were settled. In order to restrict the organism distribution to the microcosm side, a PVC cube was inserted inside the microcosm that reduced the sediment thickness to 1 cm in front of the optodes. Microcosms were then filled with 10 cm height of sediment and 10 cm height of water. As each microcosm side was isolated from the others, it was then considered as a replicate (i.e. four replicates/microcosm). Five different experimental conditions were considered: contaminated sediment/with Chironomid larvae [U-Chir], contaminated sediment/with Tubificid worms [U-Tub], control sediment/with Chironomid larvae [C-Chir], control sediment/with Tubificid worms [C-Tub], and control sediment/without Tubificid worms nor Chironomid larvae [C-no].

All microcosms were placed in a closed dark room with a constant temperature of 21 °C and received a gentle continuous ambient air pumping through the water column. Losses due to evaporation and sampling were systematically compensated by addition of filtered lake water. Prior to inoculation, microcosms were left to equilibrate for 4 weeks.

### 2.3. Organism acclimatization and addition

The Tubificid worms (*Tubifex tubifex*) came from a commercial breeding (GREBIL & Fils, Arry, France) whereas the Chironomid larvae (*Chironomus riparius*) were already reared in the laboratory. For each species, three aquaria (50 × 25 × 25 cm; length × width × height) were previously maintained for several months in the same conditions than those used for the experiments (e.g. 10 cm of sediment, 10 cm of water, 21 °C, constant air bubbling). Half of the water column was renewed each month and the organisms were fed by addition of Tetramin<sup>®</sup> (20 mg per aquarium) twice a week. Exactly 216 Tubificid worms and 51 Chironomid larvae (third and fourth instars, 5–12 mm body length) were introduced per microcosm allocated for their addition, resulting in initial densities of 60,000 and 14,000 ind m<sup>-2</sup>, respectively. These are typical densities observed under natural conditions (Palmer, 1968; Rasmussen, 1984). After the introduction of organisms, a series of oxygen measurements and corresponding sediment structure images were made daily during the experimental period (12 days).

### 2.4. Oxygen optode measurements

#### 2.4.1. Oxygen optode

The two-dimensional oxygen distribution in sediment and overlying water was measured with semi-transparent planar oxygen optodes. Oxygen measurement was

based on the dynamic quenching of oxygen on an immobilized fluorophore (Kautsky, 1939). The optical sensor was composed of two thin layers, the transparent polyester support foil (HP transparency, C2936A, ~150  $\mu\text{m}$  thick) and the sensing layer where the oxygen-quenchable fluorophore, the platinumium (II) meso-tetra (pentafluorophenyl) porphyrin (Frontier Scientific Inc.) was embedded in a polystyrene matrix (~20  $\mu\text{m}$ ) (Papkovsky et al., 1992; Liebsch et al., 2000). Sensing layer mixture was composed of 3 mg (1 mg mL<sup>-1</sup>) of Pt-PFPP dissolved in 3 ml of toluene (Rathburn Chemicals Ltd) and 0.65 g (5%) of polystyrene pellets (Acros Organics) dissolved in 15 ml of toluene. The two solutions were mixed and spread on the polyester support foil (300 cm<sup>2</sup>). The solvent was let to evaporate slowly until the membrane became completely dry. Optodes were further cut to fit inside the different microcosms (one per face).

#### 2.4.2. Calibration and measurements

For the oxygen measurement, each microcosm replicate was placed in front of the optical system which was controlled by the Image Pro Plus – Scope Pro package. The optode was excited by a Xenon lamp light (Perkin–Elmer, 300 Watts) passing through a shutter and a glass filter (405  $\pm$  10 nm, Omega Optical). The fluorescence emitted by the optode passed through another glass filter (654  $\pm$  24 nm) and was collected by a Peltier cooled 12 bit monochrome CCD camera (KAI 2000, 1600  $\times$  1200 pixels, 7.4  $\times$  7.4  $\mu\text{m}$ ). The oxygen optodes were calibrated before and after each experiment by a 3-points calibration method. For the two intermediate calibration points (90%, air bubbling and 50%, N<sub>2</sub> bubbling) the oxygen concentration was first measured just behind the optode with an oxygen probe (LDO HQ10, Hach) and immediately followed by the capture of the oxygen image. The 0% saturation was taken in the deeper non-bioturbated sediments.

Three measurements were taken for each replicate (side of microcosm) before introduction of organisms (time 0) and repeated after 0.5, 72, 120, 216 and 288 h. Images of the sediment structure were obtained without the use of filters. Their acquisition was performed in darkness during an exposure time of 30 s and 1 s for oxygen and sediment structure, respectively. Interval between the two image acquisitions was 30 s. The digital images were then stored in 12 bit gray scale (0–4095). For each time series, the acquisition and storage of images were automatized with a custom-made script. Final image pixel resolution was 56  $\mu\text{m}$ .

Pixel intensity on the recorded images was then converted in oxygen concentration by the use of a non-linear relation, slightly modified from Stern–Volmer equation (Klimant et al., 1995), allowing to take into account the oxygen quenching constant and the non-quenchable fraction of the luminescence:

$$I = I_0[\alpha + (1 - \alpha) \cdot (1 / (1 + K_{sv} \cdot C))] \quad (1)$$

where  $I_0$  is the fluorescence intensity in the absence of oxygen,  $K_{sv}$  is the quenching constant expressing the quenching efficiency,  $C$  is the oxygen concentration and  $\alpha$  is the non-quenchable fraction of the luminescence including scattered stray light. The constants  $\alpha$  and  $K_{sv}$  were determined from the two intermediate calibration points with oxygen concentration  $C_1$  and  $C_2$  corresponding to  $I_1$  and  $I_2$  intensities respectively, and integrated in Eq. (1):

$$K_{sv} = [I_0(C_2 - C_1) - (I_1 C_2 - I_2 C_1)] / [(I_1 - I_2) C_1 C_2] \quad (2)$$

$$\alpha = [I(1 + K_{sv} C) - I_0] / (I_0 K_{sv} C_1) \quad (3)$$

Having estimated the  $\alpha$ ,  $K_{sv}$  and  $I_0$ , oxygen concentration was obtained by rearranging Eq. (1):

$$C = (I_0 - I) / (K_{sv}(I - I_0 \cdot \alpha)) \quad (4)$$

The applied oxygen optode were custom-made and were homogenous enough, it was therefore possible to use average constants of  $\alpha$  and  $K_{sv}$  rather than performing pixel to pixel calibration as in some earlier planar optode studies (Glud et al., 1996).

Oxygen flux (O<sub>2</sub> uptake rate) and penetration depth (pdO<sub>2</sub>) into the sediment were measured from the obtained images whereas the length of the sediment–water interface ( $L_{SWI}$ ) was measured on the sediment structure images.

#### 2.4.3. Diffusive oxygen flux calculation

Vertical oxygen profiles extracted from images (Fig. 1) make it possible to determine diffusive oxygen flux  $J_{(z)}$  which was calculated from Fick's first law of diffusion (Berner, 1980; Jorgensen and Revsbech, 1985; Rasmussen and Jorgensen, 1992):

$$J_{(z)} = -\Phi D_s \frac{\partial C_{(z)}}{\partial z},$$

where  $\Phi$  is the porosity,  $D_s$  is the oxygen diffusion coefficient in sediments (cm<sup>2</sup> s<sup>-1</sup>),  $C$  is the oxygen concentration ( $\mu\text{mol m}^{-3}$ ),  $z$  is the depth (cm) and  $\partial C_{(z)} / \partial z$  is the oxygen gradient. This approach works on the assumption that molecular diffusion is the main oxygen transport mechanism. Oxygen fluxes were calculated by using the software PROFILE (Berg et al., 1998) which fits an overall profile between the sediment–water interface and within the sediment down to the oxygen 0% level. To make these measurements, we provide to PROFILE some profiles including few points in the overlying water. Three mean oxygen fluxes were calculated per image, each of them based on the average of six to 11 neighboring vertical oxygen profiles from oxygen images (2 cm total height, centered on the SWI).

#### 2.5. Bioturbation activity measurement

The bioturbation activity of *Tubifex tubifex* worms and *Chironomus riparius* larvae was assessed using green luminophores which are inert sand particles coated with a fluorescent paint ( $\Phi = 63 \mu\text{m}$ ,  $\lambda_{\text{excitation}} = 450 \text{ nm}$ ,  $\lambda_{\text{emission}} = 520 \text{ nm}$ , Geologisch-paleontologisches institute and Museum of Kiel University, Germany). One day before introduction of organisms, 2 g of luminophores were gently deposited on the top of the sediment of each microcosm.

Destructive sampling took place after 12 days of exposure, i.e. 288 h. The overlying water was removed and the sediment core was carefully sliced in fourteen layers of 0.5-cm thickness from 0 to 4 cm of depth and 1-cm thickness from 4 to 10 cm of

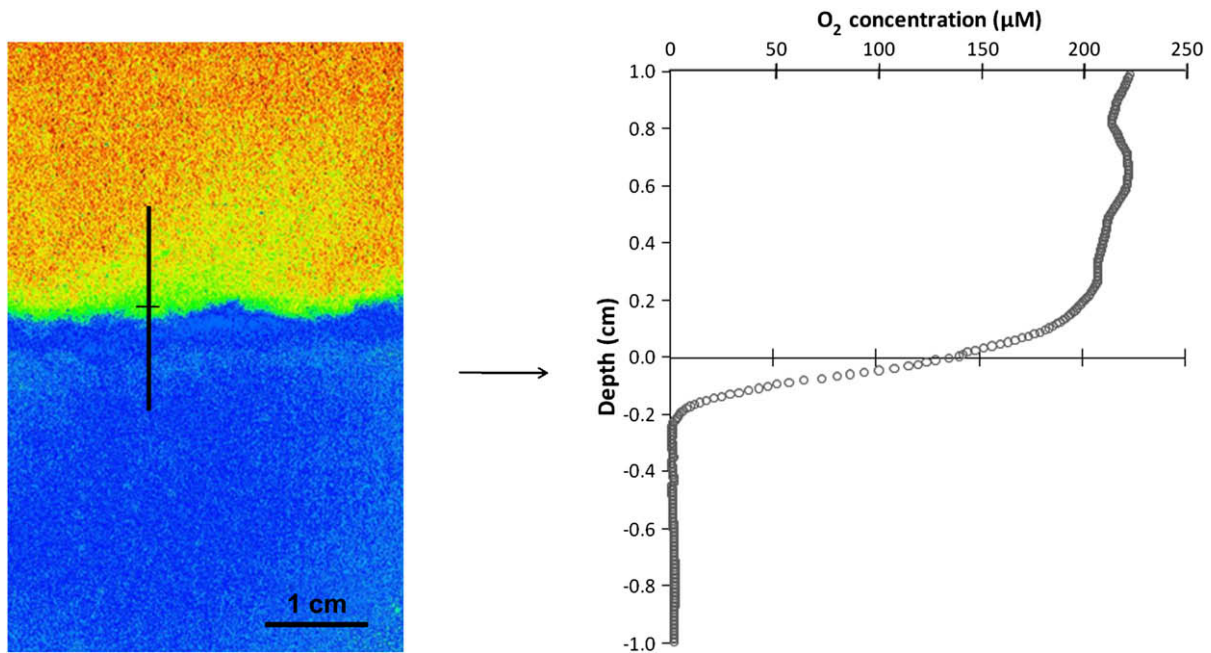


Fig. 1. Example of oxygen vertical profile extracted from a two-dimensional oxygen images (*Chironomus riparius* in uncontaminated sediment [C-Chir] after 192 h).

depth. Each layer was hand-homogenized and a sediment subsample was retrieved, weighted and dried 72 h at room temperature to evaluate the luminophore content. This counting was achieved by a fluorimetric technique after a calibration step with sediment samples of known luminophore concentrations (Lagauzère et al., submitted). From this, the number of luminophores per layer ( $n$ ) and the total number in the profile ( $N$ ) were obtained, and hence, the fraction ( $n/N$ ) of luminophores per layer could be determined. The luminophore concentration was estimated as  $C = n / (z \times A \times N)$ , where  $z$  (cm) is the thickness of the sampling layer and  $A$  the core area. To estimate bioturbation parameters, biodiffusion coefficient  $D_b$  and bioadvective rate  $V$ , the profiles were simulated using the classical biodiffusion-bioadvection model in non-steady state conditions (Officer and Lynch, 1982; Gérino et al., 1994). The maximal depth where luminophores were qualitatively detected by epifluorescence microscopy was also reported as maximal depth of bioturbation (MDB).

## 2.6. Physico-chemical measurements

The temperature, pH, and concentration of dissolved oxygen in the overlying water of the microcosms were measured at days  $-2$ ,  $0$  (introduction of organisms),  $4$ ,  $7$  and  $12$  (end of the experiment). In order to indirectly estimate the release of uranium from the sediment to the overlying water, total uranium concentration was assessed by ICP-AES (Optima 4300 DV, Perkin-Elmer, USA) from acidified (2%  $\text{HNO}_3$ ) water samples collected when the aforementioned measurements were taken.

## 2.7. Statistical analyses

All statistical analyses were performed using the STATISTICA® software package (StatSoft, Inc., Tulsa, OK, USA). Before each analysis, the normality (Shapiro-Wilk test) and homogeneity of data variance (Levene test) were tested. It was repeated after transformation of data when these assumptions were not first found. A significance level of 5% was applied to all analyses.

The physico-chemical parameters, the oxygen uptake rate, the oxygen penetration depth, and the length of the sediment-water interface were analyzed by repeated-measures ANOVAs (RM-ANOVA), both with all the data to test effect of treatment, time, and time \* treatment; and with data from 72 to 288 h to compare treatments after equilibration. These analyses of variance were followed by Newman-Keuls multiple-comparisons tests.

For each macroinvertebrate species, the effects of uranium on bioturbation parameters (bioadvective rate  $V$  and biodiffusive rate  $D_b$ ) were analyzed using one-way ANOVAs, including the control treatment [C-no], followed by Tukey's post hoc comparison tests.

## 3. Results

### 3.1. Oxygen uptake rate

Analysis of oxygen data on times 0, 0.5, 72, 120, 216 and 288 h, revealed significant effects of both time, treatment and time \* treatment (RM-ANOVA 'time', 'treatment', 'time \* treatment':  $F_4 = 6.44$ ,  $F_{4,13} = 4.86$ ,  $F_{16,52} = 3.20$ , respectively,  $p < 0.05$ ). These differences mainly came from the two first series of data. The analysis of data from 72 to 288 h, showed only a significant effect of treatment (RM-ANOVA 'treatment':  $F_{4,15} = 10.58$ ,  $p < 0.05$ ) and these data were averaged in order to consider the systems after equilibration (Fig. 2). Three days after introduction of *Chironomus riparius* and *Tubifex tubifex* in the microcosms, this parameter has increased of 13 and 14%, respectively, and remained stable until the end of the experiment. Compared to control treatment [C-no], the oxygen uptake between 72 and 288 h was 27 and 20% much higher in *C. riparius* [C-Chir] and *T. tubifex* [C-Tub] treatments, respectively.

At the beginning of the experiment, i.e. before introduction of organisms and after 4 weeks of equilibration (time 0), the two microcosms corresponding to the uranium-contaminated experimental treatments, [U-Chir] and [U-Tub], had a higher diffusive oxygen uptake rate than uncontaminated microcosms, [C-no], [C-Chir] and [C-Tub] (Newman-Keuls test:  $p < 0.05$ ). With an oxygen flux at the sediment-water interface of  $0.33 (\pm 0.08) \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$  in uncontaminated treatments and  $0.41 (\pm 0.06) \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$  in contaminated treatments, that corresponded to an increase of 24% in presence of uranium.

Thirty minutes after introduction of organisms in microcosms, oxygen uptake rate was similar for all the treatments, except for [U-Tub] with a lower value (Newman-Keuls test:  $p < 0.05$ ).

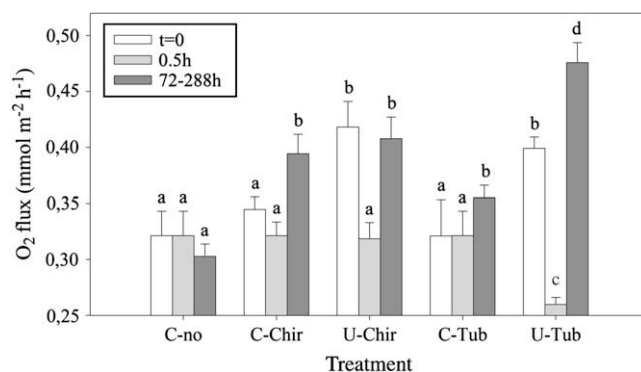


Fig. 2. Oxygen fluxes at the sediment/water interface in the different treatments (C: uncontaminated, U: contaminated, Chir: presence of *Chironomus riparius*, Tub: presence of *Tubifex tubifex*, no: no organism) before the introduction of organisms (white bars), after 0.5 h (gray bars), and after 72 h to the end (black bars). Means  $\pm$  SD ( $N = 4$ ). Different letters indicate significant differences.

Compared to initial conditions, the oxygen uptake decreased significantly in uranium-contaminated microcosms (Newman-Keuls test:  $p < 0.05$ ).

During the rest of the experiment, oxygen uptake rate was constant in each treatment (data not shown, RM-ANOVA 'time':  $F_{4,15} = 1.16$ ,  $p > 0.05$ ). It was significantly higher in all inhabited microcosms than in control microcosm, particularly in the [U-Tub] treatment which was significantly different from all the others (RM-ANOVA 'treatment':  $F_{4,15} = 10.58$ ,  $p < 0.05$ ; Newman-Keuls test:  $p < 0.05$ ). Compared to initial conditions, this rate increased during the experiment, except in the [U-Chir] treatment (RM-ANOVA 'time \* treatment':  $F_{16,52} = 3.20$ ,  $p < 0.05$ ; Newman-Keuls test:  $p < 0.05$ ).

### 3.2. Oxygen penetration depth ( $pdO_2$ )

As for oxygen uptake rate, RM-ANOVA of all the data concerning  $pdO_2$  depth, showed significant effects of time, treatment and time \* treatment; while only the treatment had a significant effect with data from 72 to 288 h (Fig. 3). However, given that any significant difference exist between initial conditions and after 30 min for each microcosm (RM-ANOVA 'time \* treatment':  $F_{16,52} = 1.33$ ,  $p > 0.05$ ), only the averaged data from 72 to 288 h

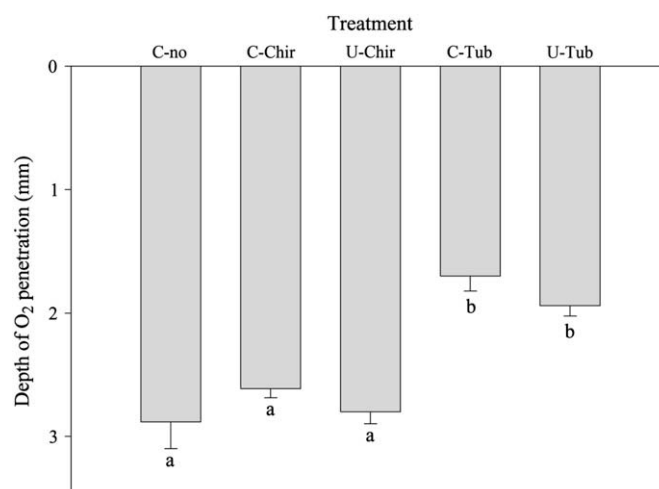


Fig. 3. Depth of oxygen penetration  $pdO_2$  into the sediments of the different treatments (C: uncontaminated, U: contaminated, Chir: presence of *Chironomus riparius*, Tub: presence of *Tubifex tubifex*, no: no organism). Means  $\pm$  SD ( $N = 4$ ). Different letters indicate significant differences.

were represented in Fig. 2. Compared to non-inhabited control treatment, the  $\text{pdO}_2$  was similar in *C. riparius* treatments, while it was reduced in *T. tubifex* treatments, independently of the uranium contamination (RM-ANOVA 'treatment':  $F_{4,15} = 16.932$ ,  $p < 0.05$ ; Newman-Keuls test:  $p < 0.05$ ).

### 3.3. Length of the sediment-water interface ( $L_{\text{SWI}}$ )

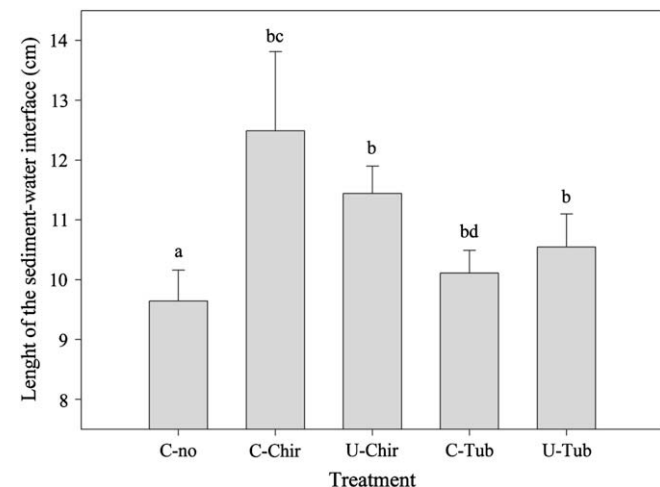
For the same reasons than for oxygen penetration, only the averaged  $L_{\text{SWI}}$  measurements from 72 to 288 h are represented on Fig. 4. These data showed a significant effect of treatment (RM-ANOVA 'treatment':  $F_{4,15} = 10.65$ ,  $p < 0.05$ ). In all inhabited treatments, the  $L_{\text{SWI}}$  was higher than in the non-inhabited control treatment, and there was a significant difference between [C-Chir] and [C-Tub] treatments (Newman-Keuls test:  $p < 0.05$ ).

### 3.4. Bioturbation activity

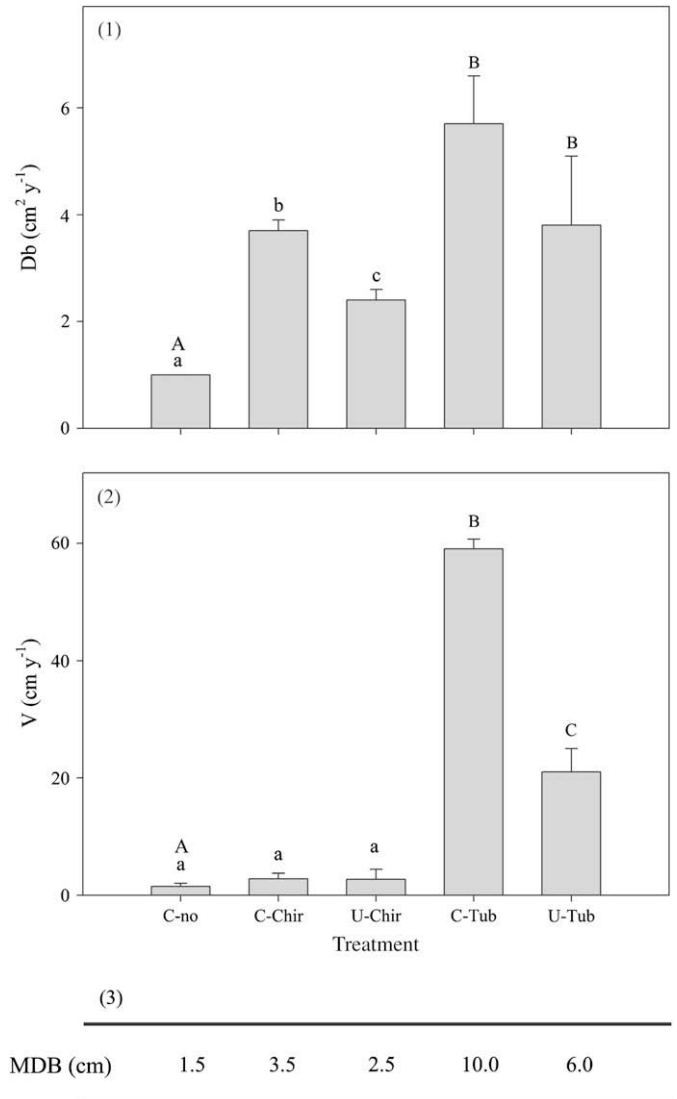
Bioadvective rate  $V$  and biodiffusive rate  $Db$  estimates from fitting the luminophore profiles after 12 days showed significant effect of uranium on both *Chironomus riparius* and *Tubifex tubifex* bioturbation activities (Fig. 5).

*C. riparius* led to a low sediment particle reworking as illustrated by the slight downward transport of luminophores within the sediment (<3.5 cm of depth). This particle burial was lower within uranium-contaminated sediment (<2.5 cm of depth). Comparison of [C-no], [C-Chir] and [U-Chir] treatments, showed that the bioturbation of *C. riparius* was mainly limited to biodiffusion processes (quantified by the  $Db$ ), and that this parameter was reduced in presence of uranium (ANOVA:  $F_{2,6} = 273.5$ ,  $p < 0.05$ ; Tukey test:  $p < 0.05$ ), while any significant difference was detected for bioadvective rate  $V$  (ANOVA:  $F_{2,6} = 0.98$ ,  $p > 0.05$ ).

On the other hand, *T. tubifex* led to a strong burial of luminophores as attested by the presence of these tracers at the bottom of the uncontaminated microcosm (10 cm). In uranium-contaminated sediment, no luminophore was detected below 6 cm. Compared to control treatment [C-no], both bioadvection and biodiffusion rates were enhanced in *T. tubifex* treatments, [C-Tub] and [U-Tub] (ANOVA:  $F_{2,6} = 396.97$  and  $F_{2,6} = 13.8$ , respectively,  $p < 0.05$ ; Tukey test:  $p < 0.05$ ), but only the bioadvective rate was affected by uranium (Tukey test:  $p < 0.05$ ).



**Fig. 4.** Length of the sediment/water interface  $L_{\text{SWI}}$  of the different treatments (C: uncontaminated, U: contaminated, Chir: presence of *Chironomus riparius*, Tub: presence of *Tubifex tubifex*, no: no organism). Means  $\pm$  SD ( $N = 4$ ). Different letters indicate significant differences.



**Fig. 5.** Biodiffusion rate  $Db$  (1), bioadvection rate  $V$  (2), and maximal depth of bioturbation  $MDB$  (3) in the different treatments (C: uncontaminated, U: contaminated, Chir: presence of *Chironomus riparius*, Tub: presence of *Tubifex tubifex*, no: no organism). Means  $\pm$  SD ( $N = 4$ ). Different letters indicate significant differences (small letters, in *C. riparius* experiments; capital letters, in *T. tubifex* experiments).

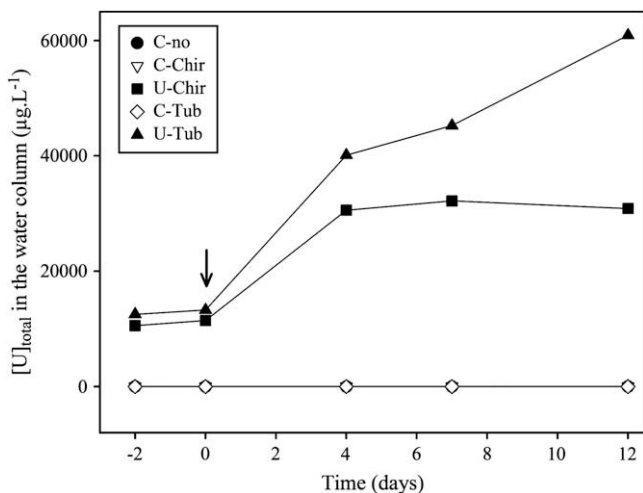
### 3.5. Physico-chemical measurements

The data set from all of the microcosms showed that the temperature was maintained at  $21.1 (\pm 0.1)^\circ\text{C}$ , the dissolved oxygen concentration at  $7.7 (\pm 0.3) \text{mg L}^{-1}$  and pH at  $8 (\pm 0.2)$  throughout the experiment, without any significant difference between treatments (RM-ANOVA:  $p > 0.05$ ). The total uranium concentration in the water column of microcosms gradually increased over time in both [U-Chir] and [U-Tub] treatments, with a factor of 2.7 and 4.6, respectively (Fig. 6).

## 4. Discussion

### 4.1. Effects of bioturbation

As previously demonstrated both in marine and freshwater ecosystems, the present results confirmed that benthic macro-invertebrates enhance the diffusive oxygen uptake (DOU) of sediments. For instance, the same trend has already been observed for



**Fig. 6.** Evolution of the uranium concentration in the water column of the different microcosms during 12 days (C: uncontaminated, U: contaminated, Chir: presence of *Chironomus riparius*, Tub: presence of *Tubifex tubifex*, no: no organism).

*Chironomus riparius* by Stief and De Beer (2002) and for *Tubifex tubifex* by Pelegri and Blackburn (1995), by using microsensors measurements. Yet the only experiment using oxygen optodes in freshwater sediments only focuses on local oxygen fluxes in the burrow wall of *Chironomus plumosus* (Polerecky et al., 2006). The behavior of organisms was the same in our case than in the latter cited experiment, i.e. a rapid burial of organisms into the sediment after their introduction in microcosms. However, these authors measured significantly higher oxygen uptake rates in the sediment surrounding the burrows during 16 min after the introduction of organisms in the sediments; whereas no changes in the DOU was observable 30 min after the introduction in our study (Fig. 2). Although local changes probably occur very rapidly during the settling of macroinvertebrates into the sediments, changes of the DOU at the benthic interface seem to become visible later.

As the DOU measurements probably included the respiration of animals only at a minor level, the oxygen uptake rate enhancement in microcosms inhabited by *C. riparius* and *T. tubifex* can be related to the physical, chemical and biological modifications induced by their bioturbation activities (Fig. 5). Among physical disturbances, in bioturbated sediments, a longer sediment-water interface (Fig. 4) has been shown to favor the oxygen exchanges by increasing the diffusion surface (Pischedda et al., 2008). Advective transport can also be increased due to higher porosity of the uppermost layers of sediments. However, the measured oxygen penetration into the sediment was not higher in *C. riparius* treatment and was lower in *T. tubifex* treatment, comparatively to control treatment (Fig. 3). Although penetration of oxygen into the sediment due to bioturbation is effective as shown by microsensors measurements in the wall of burrows reported in previous studies (Wang et al., 2001; Polerecky et al., 2006), this result suggests that intense oxygen consumption occurred in subsurface sediments by stimulation of the microbial respiration. Through microcosm experiments, Van de Bund et al. (1994) demonstrated that the microbial production increased by a factor 4.4 and 1.4 in sediments inhabited by *C. riparius* and *T. tubifex*, respectively, despite of reduction of the bacterial abundance.

The consequences of *C. riparius* larvae bioturbation on the sediment biogeochemistry have already been well documented (Rasmussen, 1984; Van de Bund et al., 1994; Stief and De Beer, 2002; De Haas et al., 2005; Hölker and Stief, 2005; Stief, 2007). Larvae can exhibit two distinct behavioral modalities: (i) displacements at the

top of the sediment; and/or (ii) digging and irrigating of burrows. Their relative importance is mainly determined upon density of organisms, oxygenation of the overlying water, granulometry and organic content of sediments. In our experiment, both these two behaviors were observed, with no apparent dominance of one of them. At first sight, larvae roamed at the sediment surface where they could feed by grazing leading to the reduction of microbial biomasses. They could also act as deposit-feeders, resulting in the exposition of sediment-associated organic matter to variable oxic and redox conditions through alternative burial/rising and ingestion/egestion of particles. Stief (2007) demonstrated that this mechanism stimulates microbial hydrolytic exoenzyme production and thus the decomposition of organic matter. Furthermore, larvae randomly built burrows into the sediment and irrigate them through intermittent pumping of the overlying water. These burrows clearly enhance the exchange area at the sediment-water interface and as a consequence the fluxes of solutes and gases (Svensson, 1997; Kajan and Frenzel, 1999; Lewandowski et al., 2007). With supply of fresh organic matter linked to mucus and feces production, as well as availability of nutrients, these burrows provide privileged habitats for microbial communities in subsurface sediments (Stief and De Beer, 2002). Therefore, aerobic nitrification can be stimulated by concomitant ventilation and ammonium excretion in the burrows, and denitrification can be facilitated by higher nitrate penetration into periodically anoxic sediment (Svensson and Leonardson, 1996; Svensson, 1997; Stief and De Beer, 2002).

Comparatively, the influence of Tubificid worms on oxygen dynamics has received less attention, principally because they live in non-irrigated galleries. However, their behavior exert a strong influence on sediment reworking and thus on organic matter processing, all the more so their abundance can reach very high values in natural sediments, up to several millions  $\text{ind m}^{-2}$  (Palmer, 1968). Their conveyor-belt feeding activity leads to the transport of reduced materials from the bottom sediment to the surface and to the formation of a top layer mainly composed of mucus-bounded fecal pellets. Both abiotic and biotic oxidation reactions are then stimulated. For instance, Mc Call and Fisher (1980) demonstrated that, for a density of 100,000  $\text{ind m}^{-2}$ , oxygen uptake rate of sediments was doubled in presence of worms, with 50–70% relative to the oxidation of removed iron sulfates (Fe-S) from the bottom sediments, 10–30% relative to the stimulation of microbial activity, and only 20% relative to the own respiration of worms. The high porosity of the pelletized top layer, coupling with the higher exchange surface of the sediment-water interface due to the dense network of galleries dug into sediments; enhance diffusion and advection, and then the fluxes of solutes (Matisoff, 1995; Mermillod-Blondin et al., 2005; Nogaro et al., 2007). Therefore, aerobic respiration and denitrification can be stimulated by these worms (e.g. Svensson et al., 2001), proportionally to their density into the sediments (Mc Call and Fisher, 1980; Pelegri and Blackburn, 1995). On the other hand, Pelegri and Blackburn (1995) demonstrated that nitrification was stimulated at low densities ( $<20,000 \text{ ind m}^{-2}$ ) whereas it was inhibited at high densities (20,000–70,000  $\text{ind m}^{-2}$ ). These authors suggested that at high densities, the oxygen penetration into the sediments is reduced by the transport of reduced materials and the intense aerobic microbial activity in the feces layer. These anoxic conditions stimulate denitrification and limit nitrification to a very fine layer under the surface of sediments. The lower oxygen penetration measured in *T. tubifex* treatments (Fig. 3) fits well with this assumption.

Finally, despite their different ways of life, both *C. riparius* larvae and *T. tubifex* worms enhanced the oxygen utilization in subsurface sediments, with a quantitatively similar resultant oxygen flux at the sediment-water interface (Fig. 2). However, the density of *T. tubifex*

in microcosms was more than four times higher than the density of *C. riparius*. Given that oxygen uptake rate of sediments is correlated with the density of organisms (Pegri and Blackburn, 1995; Svensson and Leonardson, 1996), this result suggests that the bioturbation of Chironomid larvae has a more pronounced effect on oxygen distribution than the bioturbation of Tubificid worms. This probably reflects the higher oxygen demand of Chironomid larvae compared to Tubificid worms, and above all the higher impact of bioirrigation on oxygen distribution compared to bioconveying. Svensson et al. (2001) suggested the same interpretation for the influence of the bioturbation on denitrification. Be that as it may, the applied densities of organisms fall well within the range of abundances that are realistic for natural sediments.

#### 4.2. Consequences of sediment uranium contamination

At initial conditions (time 0), the oxygen uptake rate of sediments was 24% higher in uranium-contaminated microcosms compared to uncontaminated microcosms (Fig. 2). Given that sediments were contaminated before introduction of *Chironomus riparius* and *Tubifex tubifex*, this result suggests that uranium directly influenced the benthic biogeochemistry. Two assumptions can be proposed: the oxidation of uranium into the sediment consumed oxygen and/or uranium modified the microbial community by directly or indirectly stimulating aerobic organisms. The first hypothesis can be consistent with the uranium concentration measured at initial conditions in the water column. Indeed, before the setting-up of microcosms, the sediments were spiked with uranium in a close beaker. Given the low oxygen availability, uranium contained in the sediments might be under its reduced form, at the redox state (+IV), which is not soluble (Markich, 2002). During the 4 weeks of equilibration of the microcosms, the exposure to a constantly aerated water column, probably favored the oxidation of uranium in U(+VI) in surface sediments, and thus its higher solubility (Markich, 2002). The relative high uranium concentration observed in the water column before the introduction of organisms may reflect the release of uranium from the sediments during this step of the experiment. The second hypothesis related to the stimulation of microbial respiration by uranium is more difficult to assess. Most of available literature dealing with the interactions between sedimentary micro-organisms and uranium focuses on immobilization of uranium through the bioreduction of U(+VI) in U(+IV) in the context of bioremediation of contaminated sites (Wall and Krumholz, 2006; Wilkins et al., 2006; Renshaw et al., 2007). The toxicity of uranium to micro-organisms has been so far poorly investigated, but it seems to be much lower than toxicity of other heavy metals (Nies, 1999). A case of resistance was also reported on an aerobic bacterium which can incorporate uranium in the form of intra-cytoplasmic polyphosphate-associated granules by a detoxification process (Suzuki and Banfield, 2004). Furthermore, uranium may be positive factor for some micro-organisms as it can be a potential substrate for anaerobic respiration (Lovley et al., 1991). Most of iron-reducing micro-organisms able to conserve energy by coupling H<sub>2</sub> and organic matter oxidation with the reduction of ferrous ions can also reduce uranium. Some sulphate-reducers bacteria can also enzymatically reduce ferrous ions and uranium without keeping energy or grow up with either ferrous ions or uranium as sole electron acceptor (Wilkins et al., 2006). In natural uranium-contaminated environments, it was demonstrated that anaerobic prokaryotes were easily cultivable on nuclear wastes, and that nitrate-reducers represent a dominant community (Akob et al., 2007). However, neither negative nor positive effects on aerobic microbial communities non-previously exposed to uranium were reported. Therefore, the present results demonstrated that more investigations are required to assess the interactions between

uranium and micro-organisms in a different context of bioremediation. Finally, although the preparation of sediments avoided the persistence of meiofauna in the microcosms, it can not be excluded that some organisms were maintained after all. Even there is no data in the literature concerning uranium toxicity to meiofauna living in sediments; several authors reported negative effects for other heavy metals (e.g. Gyedu-Ababio and Baird, 2006; Heining et al., 2007). Uranium could have affected some meiofauna, decreasing the grazing pressure on micro-organisms, and leading to the supply of labile organic matter which could have stimulated the microbial activity.

Thirty minutes after introduction of *C. riparius* larvae and *T. tubifex* worms into the contaminated microcosms, the oxygen uptake dramatically decreased in both cases. Such a result was not observed in uncontaminated microcosms indicating that the sudden exposure to uranium modified the behavior of organisms with a significant impact on oxygen uptake of sediments. In the contaminated sediments, it was noticed that organisms regrouped themselves and that their burial into the sediments was visibly reduced. It is probable that such a concentration of organisms at the sediment-water interface has limited the diffusion of oxygen into the sediments. Avoidance of sediment polluted with metals was previously described for Chironomid larvae (Wentzel et al., 1977) and Tubificid worms (Meller et al., 1998; West and Ankley, 1998). Moreover, the latter are known to congregate together in a form of a tightly packed mass when exposed to environmental perturbations (Palmer, 1968). This phenomenon was effectively observed at the time of the introduction of the worms in the microcosms which may explain why these organisms induced a strongest limitation on oxygen diffusion. However, after 24 h, the organisms have colonized the sediments, and the subsequent measurements of oxygen uptake rates have shown similar values as initial conditions (data not shown). Compared to uncontaminated treatments, the burial of organisms into the sediments was delayed but not inhibited, even if the maximal depth of burial during the rest of the experiment was lower (Fig. 5).

As in uncontaminated microcosms, oxygen measurements performed between 72 and 288 h did not show any significant temporal variation. Compared with initial conditions, *T. tubifex* increased the oxygen uptake of sediments by 18%, whereas *C. riparius* larvae did not induce significant difference. In the case of *C. riparius*, the negative effects of uranium have probably limited the effect of bioturbation on oxygen dynamics into the sediments. On the other hand, in the case of *T. tubifex*, such a conclusion can not be drawn as the oxygen uptake rate was surprisingly higher despite of the significant reduction of bioturbation intensity induced by uranium (Fig. 5). Compared to control treatment [C-no], the association of uranium contamination with the presence of *T. tubifex* lead to the increase of 53% of the oxygen consumption of sediments. Without additional investigations to understand the interactions between uranium, bioturbation and microbial communities into the sediment, we can only speculate that *T. tubifex* stimulated some micro-organisms already favored by uranium contamination, such as nitrate-reducers, metal-reducers or sulphate-reducers. However, these organisms have generally an anaerobic metabolism which can not totally explain the higher consumption of oxygen, even if, for instance, some sulphate-reducers can use O<sub>2</sub> as terminal electron acceptor. Likewise, reduced compounds (e.g. NH<sub>4</sub><sup>+</sup>, Fe<sup>2+</sup>, S<sub>2</sub><sup>-</sup>) produced by anaerobic metabolism can diffuse and be re-oxidized at the sediment surface. On the other hand, aerobic micro-organisms can be stimulated by the supply of fresh organic matter induced by the effect of uranium on *T. tubifex*. Indeed, it was demonstrated that, in the same experimental conditions and for the same level of contamination, the worms secreted more mucus to protect themselves and reacted by a caudal autotomy process

permitting their detoxification (Lagauzère et al., 2009). Additionally to the death of some individuals (~20%), these mechanisms may therefore induce a significant supply of organic matter all the more so the initial density of worms into the sediments was high. Finally, despite their more surficial distribution into uranium-contaminated sediment (<6 cm), *T. tubifex* continued to remove reduced materials from the bottom sediments, as attested by the significant bioadvection of particles (Fig. 5). This can be related to the increase of uranium concentration in the water column during the 12 days of exposure, which was probably due to the removal of uranium from the sediment through egestion of fecal pellets and its subsequent reoxidation.

## 5. Conclusion

This work confirmed the ecological importance of Chironomid larvae and Tubificid worms within freshwater benthic ecosystems. Despite of their different ways of life, the bioturbation of these two different taxonomic groups stimulated the microbial metabolism into the sediments. Although a lower influence of bioturbation was expected within uranium-contaminated sediment, it was demonstrated that this can be straight contradicted in the case of Tubificid worms, since their presence strongly increased the oxygen uptake of the sediments. This result raises fundamental questions concerning the interactions existing between bioturbation, microorganisms and metallic pollutants into freshwater sediments.

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