Answer to anonymous referee #1

General comments:

1) Advice to employ an English language service to provide grammatical editing

➔ The new and present revised manuscript was sent to such a service and entirely corrected.

2) Focus on uranium biogeochemistry

➔ We perfectly understand this point and we agree with the referee. Nevertheless, if we cut parts relative to uncontaminated sediment we lose crucial information about the influence of bioturbation on general biogeochemistry (such data are still scarce in freshwater sediments compared to their marine counterparts). Then it becomes hard to explain what happens when the sediment is contaminated. So, we think it is important to keep this information for the reader. In order to satisfy the referee, we nonetheless significantly shortened several paragraphs (see below).

3) Reworking of the discussion

➔ Here, the referee points out the same problem as in the previous comment. It is true that we start the discussion with the non-contaminated aquaria but it represents only 2 pages on 8 pages of discussion (1/4 is not so much!) and these results are also part of the study. This information is crucial to have a good view of what happens in bioturbated sediment without contamination. And to make a link between our previous works – which were made in exactly the same experimental conditions (assessment of uranium toxicity on worms, estimation of oxygen uptake, measurement of bioturbation activity) – and the main line of the present study, e.g. influence on uranium biogeochemistry, which is described in detail in the following paragraphs. Finally, we effectively reduced and reworked this part (e.g. 3 full sentences were removed) but we let it at the beginning of the discussion to keep a logical order in our results interpretation.

Specific comments:

1) Section from page 17014 line 26 to page 17015 line 10 (paragraph 3.2.1)

➔ The text has been modified to be more concise and clearer.

2) Combination of Figures 3 to 6

➔ If the Editor agrees, we would prefer keeping the figures as they are. If we combine all on 2 large figures, each graph will be difficult to read (probably too small) and it won't follow their comment order in the text (in both results and discussion sections, the different measured parameters are commented one after the others). Moreover, for each figure (i.e. for each element), the profiles (a and b) were used to obtain the two other graphs (c, for the variation in the water; d, for the diffusive fluxes). So, it makes sense to have them together.

Answer to anonymous referee #2

General comments:

1) Better description of DET probes

➔ We understand the difficulty for someone not familiar with this device but DET gel probes have been already described by many authors and are now used in routine (first uses in 1991). Nice pictures are available on the manufacturer's website. Compared to other recent publications, included in *Biogeosciences* Journal, we even provided quite a long description of the probes… Depending on the Editor's considerations, we could try to give a better description but we think it is sufficient in the present form. (And YES, "open window" and "aperture" mean the same thing).

2) Statistical analyses

➔ The RM-ANOVAs concerned the parameters measured in the water column (total uranium, temperature, pH, dissolved oxygen) in the course of the experiment, i.e. one day before introduction of the worms (day -1), and at days 2, 4, 6, 8 and 11. It makes in total 6 times of sampling. This information appears already in the 'Material and methods' section at the paragraph 2.4.1. But it was added in the 'statistical analyses' section (paragraph 2.5).

➔ Concerning the T-test, the referee is right, we compared the U-contaminated treatments with and without worms. This information was added.

➔ As recommended by the referee, we re-analyzed our data (both one-way ANOVAs for diffusive fluxes and 2-ways ANOVAs for net accumulation rates) by using Bonferroni, Scheffé or Tukey's post-hoc tests. We finally present the results with Bonferroni corrections. Visually, it made only minor changes on graphs and it did not fundamentally change the interpretation of results.

3) NH4 data

➔ We are sorry but we do not have data relative to ammonium that we could present in this study. Nevertheless, we did not forget to consider this point in the discussion and we reported the information available in the literature.

Specific comments:

1) The sentence "previous studies have demonstrated that" was removed from the abstract.

2) Paragraph 2.1

➔ "a dead-arm" was removed and "grosser" was replaced by "coarse" (1st and 3rd sentences).

➔ "a bioturbation activity though diminished that generates…". Sorry if this sentence did not sound clear… probably a problem with English language. We would like to say that at this concentration of U in the sediment (around 600 ppm), the bioturbation activity of worms is significantly reduced but it remains sufficient to induce a high release of uranium from the sediment to the water column. The text was modified to be more understandable.

3) Paragraph 2.3

➔ The referee is right concerning the high density of worms for such low-organic sediment. But the idea was to point out the effect of bioturbation and we intentionally used a higher density as the density naturally observed in the environment for such organic-matter conditions. However, for our global project (almost entirely yet published) we always used this density of 60,000 ind m^{-2} and we tested different densities before our first experiments. We chose this density as a trade-off between the survival capacity of the worm's populations in our experimental set-ups and the observation framework necessary to see the effects of bioturbation (and, last but not least, the laboratory safety constraints due to radioactive element handling).

➔ "any organism" has been corrected.

4) Paragraph 2.4.2

➔ As already reported in the general comments, we would rework this paragraph depending on the Editor's opinion. As regard to recent works in which DET are used, our text is not necessarily difficult to understand, except for English – we apologize – but it has been corrected.

5) Paragraph 2.4.4: Porosity of the sediment

➔ The porosity of the sediment in presence or absence of worms was estimated in one of our previous work (Lagauzère et al. 2009a) by drying/weighing of sediment samples (depth profiles). The reference was added in the text.

6) Results: general

➔ All P-values were corrected in the text (e.g. $P<0.05$ or $P<0.0001$).

➔ In all the section, the text was corrected to have only past tenses.

7) Results: paragraph 3.2.1

➔ All measurements made with DET gel probes give concentrations of **dissolved** forms. Then the profiles correspond to dissolved uranium (in water column for the part of the probe out of the sediment, and in porewater for the part of the probe inside the sediment). Anyway, we added "dissolved" before "uranium" to be clear.

8) Results: paragraph 3.2.2

➔ "undisturbed aquaria" was replaced by "non-bioturbated", here but also 6 other times throughout the manuscript.

9) Results: paragraph 3.3, bioconcentration factor (BCF)

➔ As we measured BCF only at the end of the experiment, we cannot answer the question of the referee. There is no evidence that the BCF depends on the exposure time and we cannot say what would happen if the experiment was longer. Based on personal observations, we can simply speculate that the worms would continue feeding and egesting fecal pellets with the same rate, without accumulating more uranium from sediment during the transit of sediment particles in their digestive tract. But the uranium concentration in water increased with time… until a steady-state was reached (but we don't know how much time it would take), it can be assumed that the worms would be more and more exposed to uranium from

the water and then would accumulate it (and then the BCF relative to water concentration would probably increase with time)… further experiments are obviously necessary to finally answer this question (no relevant information was found in the literature). These considerations were already present in the discussion but we added some precisions.

10) Discussion: paragraph 4.1

➔ This paragraph was shortened and reworked according to advices of referees #1 and #2.

11) Discussion: paragraph 4.2

➔ Page 17020: "higher" was replaced by "high", and "enhanced of 10%" was replaced by "enhanced by 10%"

➔ Page 17022: "Nevertheless… denitrification". This sentence was reworked to be more understandable. We would like to say that the nitrate supply due to uranyl-nitrate used for contamination (compared to non-contaminated sediment) did not increase the denitrification.

➔ Page 17022: "capable of conserve energy"… The sentence was modified to be clear.

➔ Page 17023: depth of maximal ingestion rate (2 cm). This value was determined in a previous work in which the sediment particle reworking induced by Tubifex's bioturbation was checked by tracking fluorescent particles (Lagauzère et al. 2009a). The reference was added in the text.

➔ Page 17024: DOU was not replaced by "diffusive oxygen uptake" since it was previously made in the same paragraph (the first time DOU was used, the term was written into brackets).

12) Conclusion

The referee proposes an interesting hypothesis to explain the exchanges between porewater and overlying water due to biogenic structures and worm displacements within the sediment. This assumption was actually already discussed in our previous work based on the model we developed to describe the reworking of sediment particles by Tubifex worms (Lagauzère et al. 2009a). Rapidly… of course it plays a role, but compared to the relocation of sediment particle from the bottom of the aquaria directly at the sediment/water interface (bioadvection), these phenomena have not the same importance as for other macro-invertebrates with different modes of bioturbation (e.g. chironomid larvae living in U-shaped and ventilated burrows = biodiffusion is greater).

13) References

➔ The referee found an error for the reference Krantzberg et al. (1985). We checked it again and we always find the reference as:

Krantzberg, G. (1985). The influence of bioturbation on physical, chemical and biological parameters in aquatic environments: a review. *Environmental Pollution Series A, Ecological and Biological*, *39*(2), 99-122.

Then, we corrected the reference by adding *„ Series A, Ecological and Biological" after "Environmental Pollution".*

Answer to anonymous referee #3

General comments:

1) As already mentioned for answering Referee#1, the manuscript was sent to an English editing service and corrected.

2) The referee points out that the control conditions are missing on some graphs and tables. Actually it is only the case on Table 1 and Figures 1-2, which actually only refer to contaminated aquaria since no uranium was measured in control ones. We could add plots and values=0 to satisfy the referee but we would prefer keeping the figures in the present form. Then, we kindly ask for the Editor's opinion on this point. Anyway, the information was added in the legends.

Specific comments:

ABSTRACT

➔ The sentence of line 9 was corrected.

MATERIAL AND METHODS

1) The referee found that we should clearly specify which analyses were made for each experimental condition. Probably, this is also relative to his remark in the 'general comments' concerning control conditions. The text was entirely checked and reworked to take into account this remark.

2) Paragraph 2.2

➔ The worms were acclimated to the experimental conditions during 10 weeks. This information was added into the text.

3) Paragraph 2.4.1

➔ 6 times during the experiment (days -1, 2, 4, 6, 8 and 11), a volume of 10-ml of water was sampled in each aquarium. This information was added into the text.

4) The dissolved uranium concentration in water was actually measured. It corresponds to the top of the DET-profiles (part of the probes out of the sediment). And, we already explained it in the 'Material and methods' section (see, the penultimate sentence of paragraph 2.4.1) and in the 'Results' section (see, 2nd sentence of paragraph 3.2.1).

5) The DET were inserted in the sediment 48h before introduction of the worms. It is already in the text (see, paragraph 2.4.2).

6) Unfortunately, we did not check whether the DET insertion modified the worms' activity. But there was no apparent avoidance of sediment around the probes and there is no evidence of problems in using such devices in bioturbation experiments from the available literature. The

probe lateral dimension (4 cm) would certainly limit the horizontal mobility of Tubifex across the sediment surface by one third of the tube diameter (12 cm). However, the main mobility axis for Tubifex remains vertical (this study and others) and in this case the probe would have little effect.

7) DET measurements are actually an invasive method and the removal of probes from the sediment should create local perturbations, even when it is achieved very carefully and gently. In our study, the only measurement made after their removal is the analyses of the solid phases of sediment (after slicing, homogenization and subsampling for each layer). Compared to the volume of the total sediment core, the volume corresponding to the 2 probes is small (3.6%) and we can consider that the effects on final results are negligible. This consideration was added to the text.

8) Paragraph 2.4.5

➔ First sentence: "overlying" water was added.

➔ There is no available reference for the mineralization method. The protocol was adapted by one of our technician from a routine protocol used for soils in the laboratory. And it was checked previously with reference material to make sure the recovery was total.

➔ The vertical profile of uranium in the solid phase of the sediment were effectively made but are not presented because there did not provide relevant information. They were only used for the estimation of the global mass budget, as already explained in the text.

➔ The referee says "it would have been interesting to measure also the uranium dissolved in the porewater"… it is actually the exact goal of DET measurements!

9) Paragraph 2.4.6

➔ We did not measure the uranium bioaccumulation in worms coming from control sediment simply because it is a complicated procedure in our laboratories due to safety rule constraints. As we absolutely did not detect uranium in water and sediment of control aquaria (no crossed contamination), we reduced our efforts to avoid handling of materials. It was evident that we would not find uranium in worms coming from control sediment. On the other hand, a quality control sample of worms was prepared to have a reference for our measurements (as explained in the penultimate sentence of the paragraph).

10) The referee asked several questions to have information about the worms' conditions after 12 days in uranium-contaminated sediment. It is quite surprising because we referred many times to these observations in the present manuscript, all reported in our previous works. More precisely, the referee could find all data concerning (i) the ecotoxicity of uranium to worms in Lagauzère et al. 2009c (e.g. autotomy, effect on biomass) and (ii) the effects of uranium on bioturbation activity in Lagauzère et al. 2009a.

11) The referee doubts that we really worked with the species T. tubifex but we used the same worms for 4 years and before each experiment a sample was sent to Professeur Giani at the University of Toulouse (France) who is a specialist of oligochaetes and confirmed the taxonomic identification. But we omitted a detail in the present manuscript… the worms were retrieved from the sediment after some hours of hypoxia but we also warmed a little

bit the aquaria by placing them into a bain-marie at 25°C for 10 minutes, as already reported in Lagauzère et al. 2009c. This information was added into the text (paragraph 2.4.6).

12) Paragraph 2.4.6 – about the digestive tract content after 2h…

➔ We effectively verified that there was no more sediment in the digestive tract of the worms after 2 hours in clean water (binocular observations).

13) Before slicing the sediment for solid phase analyses, the progressive hypoxia (4h without air bubbling) was probably a disruptive factor for the top layer of sediment and it is possible that the uranium concentration of the top layer was then overestimated (if we consider that the kinetics permits to have already some uranium reduction and deposition/precipitation). However, it makes no fundamental difference in our interpretation of data because we compared all the treatments with the same protocol and these measurements were only used for the global mass budget calculations.

RESULTS

1) We are sorry but we cannot provide the data of bioaccumulation at time 0 and in control treatments simply because we did not measure them. As already mentioned, although it would be strictly scientifically rigorous to have done them, these measurements were not as necessary as the referee pretends. It is clear that there was not uranium at all in worms coming from non-contaminated sediment since we did not detect uranium in water and sediment of control aquaria.

2) Figure 1 – we did not plot the control conditions but we added the information in the legend.

3) The referee asked to present in a figure 1b the concentration of uranium in filtered water samples… We simply do not have these data… The water samples were not filtered to have the total uranium in water (Figure 1). Thanks to the DET gel probes (part which was out of the sediment), we have the dissolved uranium (Figure 2 A-B-C).

4) Figure 2 – we kindly ask the Editor to check the quality of this figure and we could modify it if it is really difficult to read.

5) As already explained in the 'Material and methods' section just above, we measured the uranium in the solid phase and we had profiles. But we did not present them because it does not provide relevant information and they were only used to calculate the mass balance budget.

DISCUSSION

In control sediment, we observed that the worms reached the bottom of the sediment, e.g. a depth of 10 cm. The referee asks whether this depth was not sufficient for the worms… well, it is a good question and if the aquaria were deeper the worms would probably reach a higher depth. But we think that the worms simply occupied all the sediment volume which is available for them, and there

is no problem of limitation. In natural environment, these worms can form very dense assemblages and it was not the case in our experiments. Some pictures are presented in Lagauzère et al. 2009c. We clearly distinguish individuals at the sediment/water interface as well as within the sediment showing that there is enough “space” for the worms’ population and there is no evidence of space limitation.