

We thank the reviewer for taking the time to read the manuscript and provide thoughtful and constructive feedback. This feedback has highlighted areas which needed improvement in the form of additional references, or further clarification, and we were happy to improve these in the manuscript. The changes were applied in track changes in the manuscript, and copied in this reply in our answers. We also hope to have clarified the additional remarks about the methodology in a satisfactory manner.

1. Reviewer's comment: General comments: - My major concern deals with the use of uranine as dissolved tracer to quantify bioirrigation rates. Indeed, it is well known that uranine easily adsorbs to organic material so that bioirrigation rates can be severely overestimated if this process is not accurately quantified. To this end, the authors performed batch experiments to estimate the adsorption of uranine to the mud (i.e. sedimentary organic matter) but, if I understand well, they did not take into account the capacity of suspension-feeding bivalves to decrease uranine concentration in the overlying water through their filtration activity. I know from my own experience that bivalves such as *Cerastoderma edule*, *Scrobicularia plana*, *Mytilus edulis* or the filter-feeding gastropod *Crepidula fornicata* are able to rapidly trap a large amount of uranine in their mantle cavity (on the gill surfaces). Given that these species were very abundant in some sampling stations (see Table 3), what is the level of accuracy of bioirrigation rates measured in the corresponding experimental cores?

Reply: We had a similar concern during the initial testing of this method, therefore we performed an experiment. Six live *C. edule* were placed into incubation cores (performed in duplo, same controlled setup as in the manuscript) without sediment, and the tracer concentrations were monitored over time. Figure R1(a) shows the results of that trial, and from this we concluded that there was no obvious interaction between the organisms, and the uranine concentration. We also tested whether the cores, stirring devices, or bubbling stones interacted with the uranine, by monitoring the tracer profile in cores filled with only water with- and without a bubbling stone (Figure R1(b)). No reports of uranine adsorption/trapping by organisms were found in literature to warrant further testing. However, we would be interested in seeing results of your experiments for future reference. We added this figure in the supplement, and referred to it on line 87 of materials and methods: "Short experiments were performed to assess possible interactions between the tracer, and the used setup (Supplement)".

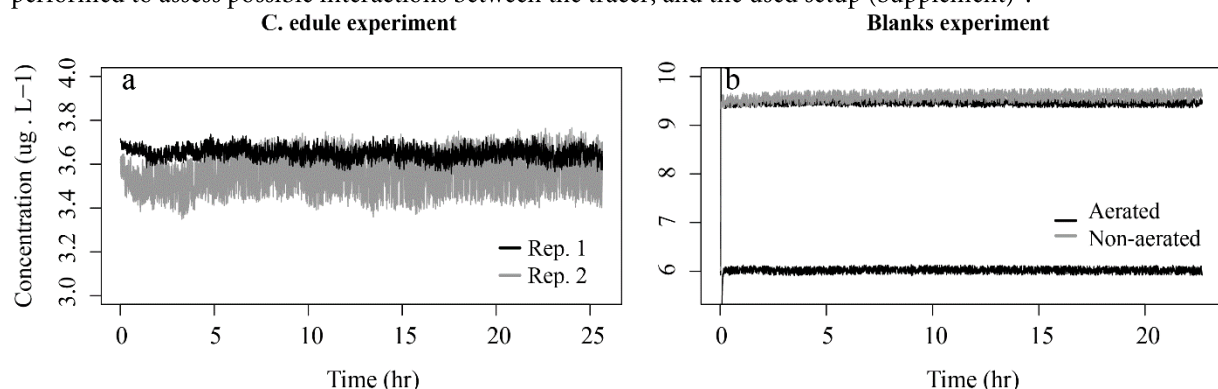


Figure R1: a. Concentration of uranine over time in duplicate cores containing six live cockles each. b. Two cores aerated with a bubbling stone (black), and one non-aerated core (gray). For the aerated core two concentrations were tested to assess whether this would have any impact.

2. Reviewer's comment: The comparison between experimentally measured bioirrigation rates and traitsbased ecological indices should be discussed a little bit further.

Reply: On your suggestion we expanded the discussion on lines 348-351, with the inclusion of predator – prey interactions: "Species also compete in the form of predator prey interactions, which have also been shown to alter behavior. For example, the presence of *Crangon crangon* reduced the food uptake of *L. conchilega* (De Smet et al., 2016), and altered the sediment reworking mode of *L. balthica* (Maire et al., 2010), in both cases because *C. crangon* predares on the feeding apparatus of these species protruding from the sediment."; and on lines 365-371 with macrofaunal responses to seasonal temperature variations: "Furthermore, Wrede et al. (2018) suggested to include

a temperature correction factor (Q_{10}) in the calculations to account for the expected metabolic response of macrofauna to increasing water temperatures (Brey, 2010). This temperature effect on benthic activity has indeed been noticed in similar works (Magni and Montani, 2006; Rao et al., 2014), but in this study and others the highest temperatures were not clearly associated with highest functional process rates (Schlüter et al., 2000; Braeckman et al., 2010; Queirios et al., 2015). The reasons for this ranged from a mismatch between food availability and the temperature peak, upward migration in the sediment to escape hypoxia, or the presence of confounding factors in the analysis such as faunal abundances and behavior (Forster et al., 2003).

3. Reviewer's comment: Specific comments: - Abstract: Line 11-12: Biological traits do not really allow for the quantification of bioirrigation. This trait-based index (IPc) only give a more or less "rough" prediction of bioirrigation depth and rate. The sentence line 42 seems more correct.

Reply: Agreed, we added the word "estimate" to indicate this, line 11-12 now reads: "Quantification of bioirrigation is done either through measurements with tracers, or more recently, estimated using biological traits to derive the community (bio-) irrigation potential (IPc)."

4. Reviewer's comment: Line 16-17: I well understand that irrigation rates can be significantly affected by bioirrigator densities but it is not clear to me how higher densities could impact the bioirrigation depth.

Reply: Please see our reply to your similar comment no. 13.

5. Reviewer's comment: Introduction: Line 27: How do bioirrigation increase the exchange surface?

Reply: When there are no burrowing organisms, the sediment-water interface (SWI) can be considered flat with a certain surface area per m² of seafloor. When organisms construct burrows connected to the SWI, the surface area is extended in the vertical dimension, as the burrow walls are in connection with the overlying water. In the cited study (Quintana et al., 2007), the effect of two burrowing polychaetes on solute transport was investigated. One species (*M. viridis*) caused higher exchange through increasing diffusion rates, the other (*H. filiformis*) caused higher exchange rates through nonlocal exchange. The flux of oxygen is increased as the polychaetes consume it, but the burrow walls are an additional (anoxic) surface over which oxygen can be taken up, hence an extension of the exchange surface. To clarify this matter, we partially integrated this explanation in the introduction on lines 26 – 29: "By extending the otherwise horizontal sediment water interface in the vertical dimension, burrowing organisms extend the exchange surface, especially when the burrow water is refreshed by ventilation activities. This enhances nutrient exchange (Quintana et al., 2007), and increases degradation rates (Na et al., 2008)."

6. Reviewer's comment: Line 40: The term "pumping activity" is usually employed to describe the "filtration activity" suspension-feeding bivalves. May "ventilation activity" be more appropriate?

Reply: In reading a significant part of the literature concerning bio-irrigation, we have not encountered this distinction, but it's an interesting remark. There seems to be no real consistency in terminology, and the preference of "pumping" vs. "ventilation" differs between authors (just a few examples: pumping: Berg et al. (2003), Forster and Graf, (1995); ventilation: Stief and de Beer (2006), Kristensen et al. (2012), or both: Renz et al. (2018), Roskosch et al. (2011)). To us the most "correct", based on consensus, is that bio-irrigation is the effect (enhanced pore-water transport and exchange etc.), and that it is caused by ventilation activity in general, with pumping activity as the main mechanism (Kristensen et al., 2012; Meysman et al., 2006). In this manuscript the terminology ("pumping rate") used in the rest of the text, reflects the initial modelling of *A. marina* as a "pump" by Meysman et al. (2006), we would like to keep it as such.

7. Reviewer's comment: Line 42: Yes but over very different spatial scales.

Reply: If we interpreted your comment correctly (that indices are most valid to compare bio-irrigation over larger spatial scales), we agree with you. However, the methodology in Wrede et al. (2018) rightly compares index values to measured irrigation rates from the same sediment cores, thus on the same scale.

8. Reviewer's comment: Materials and methods Line 74: I am wondering whether the sampling method (i.e. small sediment cores <20 cm) really allows for the collection all bioirrigators inhabiting intertidal mudflats of the Oosterschelde estuary. It is clear that large and/or deep infaunal species such as burrowing mud shrimp could not be properly captured, thus leading to strong underestimation of bioirrigation rates.

Reply: This is a valid remark on the limitations of this study as larger individuals of *A. marina* and *N. latericeus* were possibly omitted from the sampling. The chosen cores were a trade-off between the sediment surface that could be sampled, and how deep it could be sampled, as cores that are both deep (50 cm), and wide enough (20 cm) would compromise the feasibility of successfully collecting samples in the field. We have added an acknowledgment of this fact in the discussion on lines 266-269: "It should be noted that given the maximal depth of the incubation chambers was 20 cm of sediment, individuals of some species living deeper than this were not included in the incubations, and thus measurements of bio-irrigation (e.g. larger *A. marina*, or *N. latericeus*). This means that the patterns described previously can only be applied with certainty to the upper 20 cm of the sediment."

9. Reviewer's comment: Line 80: How long have experimental cores been kept in buffering seawater tanks before the beginning of the experiments?

Reply: As stated in materials and methods (line 83), the experimental cores were kept for 24 – 48 hours, in buffering water tanks before the start of the experiment.

10. Reviewer's comment: Line 81: What was the average temperature at each studied season? That's a very important factor, which greatly determines the activity level of benthic invertebrates.

Reply: The reviewer is correct. Average temperatures of the water in the cores during measurements were added to table 1, and referred to in methods on line 81.

Season	Spring	Summer	Autumn	Winter
Months	Apr – Jun	Jul – Sep	Oct – Dec	Jan – Mar
Avg. Temperature (°C)	12.8	17.9	11.9	7.3
Dortsman	4	5	9	5
Zandkreek	4	6	9	6
Olzendenpolder	4	4	8	6
Lodijksegat	4	4	8	2
Hammen	4	4	8	2
Viane	3	0	6	2

11. Reviewer's comment: Line 128-129: Batch adsorption experiments have been performed with sediment cores collected from Zandkreek (%Corg=0.79) and Dortsman (%Corg=0.07). Why not with sediment cores from Viane where the proportion of organic carbon is the highest (potentially the highest adsorption rate).

Reply: The final methodology for determining the adsorption coefficient was established only very late during the sampling period. Previous trials were all based on batch adsorption experiments with dried sediment (Gerke et al., 2008). However, we figured out that these readings were very much affected by the pH dependence of the fluorescence of uranine (see Gerke et al. (2013), and Figure R2, our own measurements of uranine fluorescence under a pH range). The degradation of organic matter present in the dried sediment, in the enclosed small volumes of the batch adsorption experiments, lowered the pH of the water outside of the stable range for uranine. This prompted the shift to working with natural sediment cores, where the pH is stable within the range "safe to use"

for uranine. However, by this time we had no more sediment cores from the Viane station, and retrieving them was impossible as these subtidal samples were taken onboard of a vessel outside of the research institute. Given the large difference between organic carbon values acquired for Dortsman (%Corg = $0.07 \pm$) and Zandkreek (%Corg = 0.79 ± 0.33), and the similarity between Zandkreek and Viane in terms of MGS ($59 \pm 14 - 53 \pm 60 \mu\text{m}$), silt content ($51 \pm 7 - 63 \pm 19 \%$), and OM content ($0.79 \pm 0.33 - 1.16 \pm 0.36$), we believe that actual values measured in Viane would not significantly differ from those of Zandkreek. Incidentally chl *a* values (also a measure for organic matter content) were highest in Zandkreek ($20.60 \pm 4.19 \mu\text{g g}^{-1}$).

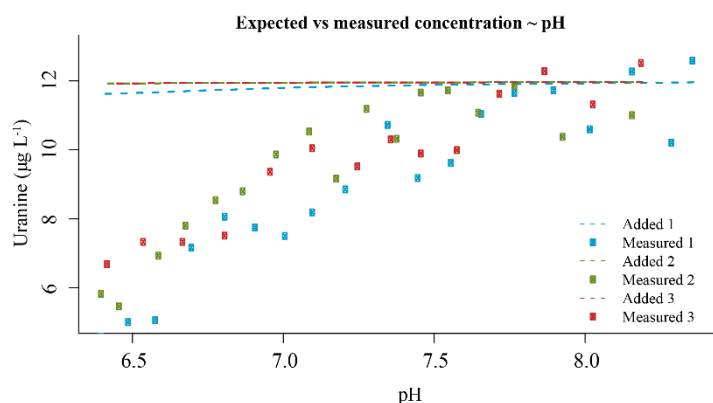


Figure R2: measurements of uranine concentration measured (points) vs. fluorescence predicted based on added uranine concentration. Note that the lines are not straight, as the added acidic solution (0.62 M HCl) was added dropwise, which eventually decreases the concentration of the tracer.

12. Reviewer's comment: Results Bioirrigation rates: The “pumping” rate and the irrigation attenuation coefficient were estimated by fitting a mathematical model to the tracer data. However, I’m wondering if the first part of the experiments (10-20 min) should be considered for the estimation of these parameters as the initial decrease in uranine concentration may mainly result from its rapid adsorption onto surficial organic particles as well as the mixing between overlying and burrow waters (which is not really sediment bioirrigation from my point of view).

Reply: The way the stirring device was set up, mixing of the uranine in the water column took place in about two minutes of injection of the tracer (Fig. R3). This period was always cut out of the analysis. However, we argue that from then onwards we see the effects of burrow ventilation. Water in burrows is exchanged through organism activities in this timeframe, as diffusive exchange would take much longer. Additionally, often cores showed a flat profile from the start onwards, regardless of the organic matter or mud content.

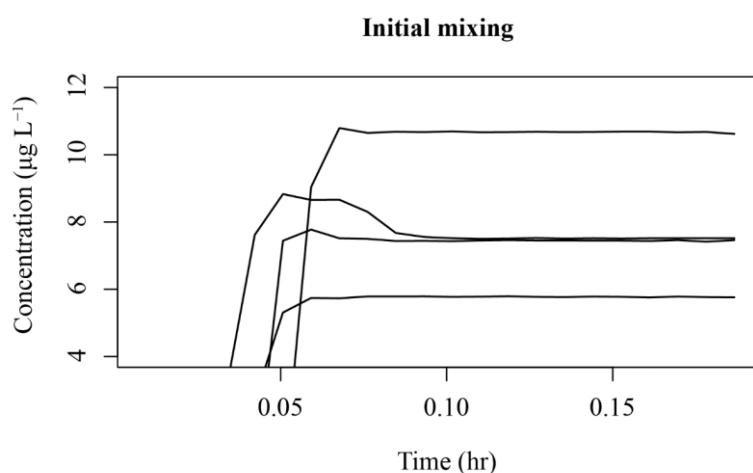


Figure R3: First minutes of an incubation, after adding the uranine spike. Mixing takes place within 2-3 minutes, after which the starting concentrations is considered stable, and when the incubation starts.

13. Reviewer's comment: Discussion Line 281: I don't really understand the conclusion stating that the density of bioirrigating species would affect more the bioirrigation depth than the bioirrigation rates. I would have believed the opposite. Indeed, it has been reported that faunal activities (e.g. feeding, burrowing) can be altered by intense intraspecific interactions leading to lower bioturbation rates. Is there any references showing that increasing invertebrate densities result in increasing bioirrigation depths?

Reply: We could not find studies where the depth of bio-irrigation is directly measured in combination with varying densities and species compositions. However, in Braeckman et al. (2010), increasing densities of *L. conchilega* tended to increase oxygen penetration, likely due to bio-irrigation. Our conclusion is twofold: in the intertidal, species that are assumed to be "deep" irrigators (> 5-10 cm, in terms of our model) are present in higher densities, and despite their smaller individual sizes (intertidal: higher densities, for similar biomass), we expect the effect of deeper burrows to still be noticeable in the tracer profiles. In addition to this, species in the intertidal have been found to reside deeper in the sediment, to escape the different pressures associated with this habitat (references in manuscript on lines 318-320).

14. Reviewer's comment: Line 290-292: Another hypothesis is that high densities of *C. fornicata* may induce a rapid deposition of fine particles at the sediment-water interface (i.e. biodeposition) thus decreasing the permeability of upper sediment layers.

Reply: This is indeed an interesting hypothesis, we have added it to the text with the appropriate references (line 298 - 300): "*C. fornicata* is also known to cause significant biodeposition of fine particles on the sediment surface (Ehrhold et al., 1998; Ragueneau et al., 2005). This could decrease the permeability of the surface layers and as such decrease the extent of possible bio-irrigation."

15. Reviewer's comment: Line 293: Burrows of *N. latericeus* can extend down to 40 cm, yet experimental chambers were only 20 cm long. Thus, the ventilation activity of the worms may have been biased due to a constrained (shallow) benthic habitat.

Reply: We acknowledge this, and as discussed in a previous comment, a paragraph has been added on limitations of the study.

Lines 266 – 269: It should be noted that given the maximal depth of the incubation chambers was 20 cm of sediment, individuals of some species living deeper than this were not included in the incubations, and thus measurements of bio-irrigation (e.g. larger *A. marina*, or *N. latericeus*). This means that the patterns described previously can only be applied with certainty to the upper 20 cm of the sediment.

16. Reviewer's comment: Lines 307-309: See comment Line 281. Why organisms of the same species (same stage) would irrigate over different depth ranges at high densities?

Reply: Please see our reply to comment 13

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