Interactive comment on "Biological and biogeochemical methods for estimating bio-irrigation: a case study in the Oosterschelde estuary" *by* Emil De Borger et al.

Anonymous Referee #2

Received and published: 9 December 2019

This is a really nice study and a very interesting dataset. I have a few concerns but I think if these are addressed that this manuscript will be a really nice contribution to our understanding of bioirrigation.

Major concerns:

The manuscript starts off with a really nice, clearly written introduction to the importance of bioirrigation. However, the authors don't give a clear question or hypothesis at the end of the introduction, and the lead-in to the goal of the paper is a little confusing. To me, the question should be whether the bioirrigation potential calculated from community structure actually predicts the measured bioirrigation. I am somewhat skeptical

of this, and the data presented seems to indicate that the bioirrigation potential explains only a small amount of the variability in bioirrigation at most. The authors present this concept as if it is well established and a valid metric for characterizing bioirrigation, when it was introduced in 2018 and has not to my knowledge really been tested.

This lack of a clear question and testable hypothesis at the beginning leads to a number of other problems in the paper – the methods section is confusing because it consists of a list of methods without justifying exactly why the authors are doing these things. For example, there are measurements of chl a, C, N, grain size, but how do these relate to bioirrigation? What does it mean if these values are high or low? They clearly do relate to bioirrigation through sediment permeability, but this needs to be explicitly stated and justified. There was actually surprisingly little discussion of permeability, which is likely very important in bioirrigation.

More problematic, section 2.5 "Statistical analysis" was extremely difficult to follow, which made understanding and interpreting the results (Fig. 4) extremely difficult. These tests need to be tied to an explicit hypothesis and justified. Why were data on chl, grain size, etc., categorized rather than left linear? Why were absolute species abundances transformed to relative abundances? This doesn't make sense when considering the effect on bioirrigation, but it's not actually clear what this test was for. I understood very little of Fig. 4, it was confusing which datasets were used or what this figure is supposed to show, and what is the inset in fig. 4a?

The conclusions section was confusing and hard to follow since I didn't really follow the community analysis, need to revise around a clear question/hypothesis.

I was a little confused at times about why the authors were using abundance versus biomass – it seems to me that biomass is much more appropriate for predicting bioirrigation, so I don't understand, e.g., why they calculated an individual irrigation rate or what that is supposed to mean. Wouldn't the individual irrigation rate depend strongly on the size of the individual? It would make more sense to have an irrigation rate

normalized to the biomass, if normalization is useful.

Why were the data from subtidal and intertidal sites averaged? From Fig. 2, there are fairly big differences among sites, e.g., density among the intertidal sites, so this seems questionable.

I recommend re-framing the manuscript around the question of whether variability in community structure drives variability in bioirrigation or if other factors, e.g., season or temperature, sediment properties, subtidal vs intertidal, etc., are more important in driving bioirrigation. It's possible that this or something like this is what the community analysis was trying to get at, but it didn't come across clearly. Perhaps a generalized linear model predicting the measured r and a parameters from IPc and other variables would be more appropriate?

I think the high temporal resolution data on bioirrigation is really exciting and the analysis of those experiments is very interesting. While I haven't exhaustively read the literature on bioirrigation, these methods seem novel and exciting to me, and I encourage the authors to be a little more clear in taking credit for this, or at least be a little more explicit about whether this approach is new or how they built on previous studies. E.g., more detail on the models mentioned in line 46 and how this approach builds on those would be interesting to know. I really like the model presented in eqs 1-3, and encourage the authors to present more detail about the model results. For example, is it possible to separate the relative contribution of diffusion, advection, and adsorption (the 3 components in eq. 1) to the change in tracer concentration? In line 202, the authors mention that k (in the adsorption component) has much less impact than r and a, but I wasn't really sure how to interpret that. If I wanted to use this model to do a similar study, could I remove the adsorption term altogether or would I need to go to the trouble to measure k? Under what circumstances would it be okay to ignore that term? Similarly, diffusion is probably much smaller than advection – are there any circumstances in which diffusion is important?

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I really like Fig. 3 and think that Fig. 3b does a great job of explaining the model output. It might be useful to show a few different examples in addition to the one in 3a to better illustrate the range of variability in bioirrigation among the samples. It seems to me that the attenuation coefficient reflects the volume of burrows rather than the depth of burrows. It's possible I'm not understanding this correctly, and certainly depth and volume would be correlated, but it makes more sense to me to think about volume rather than depth when thinking about dilution of tracer in the volume of overlying water.

Minor comments:

Line 12 – I disagree that using biological traits is "quantification" Line 42 – should explicitly state assumptions in the bioirrigation potential calculation Line 60 – was this calibration with bromide done using the linear fit or mechanistic model? Line 70 – were the two years averaged for the fall season? Interannual variability can be substantial. Line 77 – don't understand "70 samples" – is this pairs of cores or individual cores? Line 81 – should give data for average water temperatures, I suggest including this in the model of factors that affect irrigation Line 157 – Do you mean supplementary table 2? Line 241 – there is no Fig. 5 – do you mean 4? Discussion 4.1 – I don't understand the term "Bio-irrigation shape" – rephrase? Fig. 3 – it took me a minute to figure out that the y-axis in b was also ug L-1. Since the numbers are different, it would be useful to label this. I also suggest making the axis scales more similar - even though the starting points are different, the scale could be the same. Table 2 – suggest using "d50" instead of "MGS"

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