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Interactive comment on "Functional classification of bioturbating macrofauna in marine sediments using time-resolved imaging of particle displacement and multivariate analysis" *by* Stina Lindqvist et al.

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Response from the authors to the interactive comment on the manuscript "Functional classification of bioturbating macrofauna in marine sediments using time-resolved imaging of particle displacement and multivariate analysis" (#bg-2016-411) by S. Lindqvist et al. for publication in Biogeosciences.

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Summary of the response

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We thank the reviewer for the thorough comments that significantly helped to improve the revised version of our manuscript. We are grateful for the detailed insight and acknowledge that some information appeared implicit and needed further clarifications. In accordance with comments provided by the reviewer and to improve the transparency of the raw data quality as well as to clarify arguments included in the manuscript, supplementary material has been included. This material includes videos with time-laps sequences of luminophore distributions and figures that illustrate how well experimental results fit to the gallery diffusion model. We have in the revised version also tried our best to further clarify our aims for the study. Further, variables used during the multivariate evaluation have been described in more detail and biogeochemical consequences from our study have been addressed in the discussion of the revised version of the manuscript. We have also emphasized to clarify the usefulness of the applied method in a broader context as the overriding principles are general and can be used in a wide range of analytical and methodological settings.

We have to the best of our ability tried to meet the comments provided by the reviewer and we now hope that our manuscript will be accepted for publication as a regular article in Biogeosciences.

Yours sincerely

Stina Lindqvist /on behalf of all authors

âĂČ General comments:

Reviewer: . . . However, in my view, the scope of the paper is rather specialized. As I explain in more detail below, I think the paper has a few deficiencies: 1. the aim of the study as well as the presentation and meaning of the data is not sufficiently clear; 2. The implications of the findings are insufficiently explained in the broader biogeochemical context; 3. the statistical approach used seems to me not fully appropriate as it mixes a subject-independent method (PCA) with a subject-dependent method (choice of specific, possibly not robust, variables *derived* from the raw dataset) to arrive at

conclusions.

Response: These three main concerns are addressed in detail under the bolded headlines below.

Reviewer: I fully agree that bioturbating macrofauna play an important role in the biogeochemistry of sediments. Also I can understand that dividing bioturbating macrofauna into functional groups is useful when dealing with complex communities and their impact on sediment biogeochemistry. All this is well explained in the introduction. However, what I do not really understand is the actual *aim* of this study within this context. If the aim was to classify eight specific species, then the study is rather specialized and perhaps more suitable for a more biological journal. In this context, it is also not clarified why these particular species were selected. If the aim was to demonstrate the utility of the method, then I find the description of the method and the data obtained insufficient to understand (i) what was really done, (ii) why the raw dataset was reduced to the specific variables, and (iii) what the differences between the studied species really mean.

Response: These comments are addressed under the Aim and Method-headlines below.

Reviewer: Personally, I prefer to look at raw data first and only then explore how they were processed, analyzed and interpreted by others. However, this study does not make it possible to do this as no measured data is presented. What is presented are only variables *derived* from the raw data based on a specific choice of the authors. This specific choice may be biased towards the aim, possibly influencing data interpretation. However, there is no way to find out. These days there is plenty of room for including images or videos as supplementary information, and I encourage the authors to do this. Specifically, it would be really useful if they present their raw data as videos showing frames with the luminophores distributions as a function of time. Application of the threshold would be fine, and the replicate images for each species

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could be combined into one frame. They don't have to be in the full resolution, but at least an impression of "how the distributions looked like" would be useful. Then we can understand the complexity of the dataset as well as fully appreciate the need to reduce it. Additionally, we can better judge whether the approach to reduce the dataset, as chosen by the authors, was appropriate.

Response: We agree to the overriding principles that raw data, whenever possible, should be included along with more detailed calculations and modelling of the data. As suggested by the reviewer we have as supplementary information along with this response to the reviewer's comments enclosed time-frame videos of raw data that illustrate the distribution of luminophores as a function of time for the various treatments. Videos were made up from separate images of luminophore distributions.

Response: Comments related to the aim of the study Our main objective was indeed to investigate functionality of benthic macrofauna in a broad perspective and to describe the utility of the multivariate approach using a wide range of variables that describe a function essential for the system (particle transport). It was not, however, a primary and a priori goal to classify these specific species in accordance to the obtained variables by multi variate modelling. As pointed out by the reviewer, this distinction may not have been clearly stated in the manuscript. The revised version of the manuscript has been changed in accordance to this general intention of clarification (revised version p.3 l.14-21).

In the original manuscript, section 4.2 was included in part to compare this new approach of classification of fauna according to experimentally derived variables for functionality (particle transport) with previous classifications of these (or similar) species from a quantitative and mechanistic perspective. Overall, there is a general deficiency of studies with detailed investigations of particle reworking by single- or multi-species communities of benthic macrofauna. In response to the reviewer's comments and to clarify the distinction discussed above, we have shorted this section of the manuscript that describes the species of macrofauna used. Specific details of species behaviour

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have been removed (revised version p.11-13).

The motivation for selecting each of the eight species of macrofauna was already included in the original manuscript (p.4, l.1-3). We believe these grounds should be considered sufficient, particularly as the overall aim was to experimentally investigate functionality of benthic macrofauna in a broad perspective. No changes made in the revised version of the manuscript.

Response: Comments related to biogeochemical consequences Although quantification of biogeochemical parameters and detailed investigations on the effects of particle transport by various species of macrofauna for element cycling were considered outside the scope of this study, we agree that a section that describes this aspect would further emphasize the generally broad approach. Text in accordance to this aspect was therefore added to the discussion, section 4.2, in the revised version.

Response: Comments related to the method

Reviewer: . . .(i). What was really done. (ii). Why the raw dataset was reduced to the specific variables

. . .What is presented are only variables *derived* from the raw data based on a specific choice of the authors. This specific choice may be biased towards the aim, possibly influencing data interpretation.

. . . However, I am curious why PCA was not used on the raw data itself. The whole point of PCA is to reduce complexity of the dataset about a system into variables "that matter" most in its description. However, a priori reduction of the raw data into derived variables risks that this analysis is biased, because it gives higher statistical weight to some data (in the raw dataset) compared to others. Therefore, I think the authors need to dedicate some space justifying their statistical analysis approach. In my view, the classification of sediment reworking types/modes that the authors present should *emerge* from the PCA analysis of the raw data. Instead, the authors *bundle* the

raw data in such a way that the sought after classification of a specific species is either accepted (with some caveats) or rejected.

. . .the statistical approach used seems to me not fully appropriate as it mixes a subject-independent method (PCA) with a subject-dependent method (choice of specific, possibly not robust, variables *derived* from the raw dataset) to arrive at conclusions.

Response: In accordance with the reviewer's comments, the methodology and general principles of PCA has now been described in more detail (section 2.5.) to clarify and justify the statistical approach (revised version p.6 l.12-22). Further, we have explained the calculation of the 2D reworking variables in more detail (revised version p.5 l.19-31).

The PCA approach was applied as a means to evaluate the broad selection of variables in a general sense and to facilitate the description of a phenomenon (i.e. particle transport induced by benthic macrofauna), not to simplify the set of raw data. Rather than reducing the binarized images into principal components, we chose to reduce a larger number of reworking variables derived from the images into principal components. Variables used in the final PCA model were chosen based on increasing R2 and Q2 in the PCA. As was also pointed out by the reviewer, experimental variables that describe particle reworking were selected in a rather subjective manner. However, as a general principle, the selection was based on i) previous studies by others (see references in the manuscript), ii) some 20 years of experience working with various aspects of bioturbation by benthic macrofauna, iii) relevance and coupling to biogeochemical processes, and (iv) a judgement that the selection of variables could be extracted from the captured images. Which properties that are actually quantified and later also used during modelling are always up for a subjective decision in some sense.

Using the binary images directly would in our opinion not be an effective means to meet the aim of this manuscript. There would be too much data overshadowing transport processes of significance for biogeochemical processes in bioturbated deposits. We find it more relevant to identify variables directly coupled to transport processes

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that have previously been described, for example, events deep in the sediment, events occurring over time and events related to the magnitude of sediment that is displaced by macrofaunal activities. By using variables that previously have been described as important for studies of particle reworking, there is an opportunity to evaluate the variables in accordance with the present experimental settings and compare observations with previous research.

For a general discussion of available techniques to quantify particle reworking, please see e.g. Maire et al. (2008) and references therein. The variables used to describe particle transport in the present study have previously been used by us (e.g. Gilbert et al., 2003; Lindqvist et al., 2013) and by others (e.g. Hedman et al., 2011; Maire et al., 2006; Murray et al., 2014) to quantify reworking by benthic macrofauna.

In the iterative process of finding optimal variables for the data set, the variables were found to consistently group according to their representation of "bulk" or "depth" transport of particles. Additionally, the treatments of benthic macrofauna clustered similarly during the initial screening process as in the final model, which indicated robustness in the model. One of the points made in our manuscript is that reworking variables can be grouped according to whether they quantify sediment reworking/transport in terms of "bulk" (quantity) or "depth" (distance). Such quite simple distinction enables a general classification of benthic macrofauna according to these mechanisms of transport in almost any type of experimental set-up designed to quantify particle reworking.

Reviewer: I am particularly concerned about the maximum penetration depth (MPD) variable. As I understand it from the text, this variable is essentially a measure of rare events. That is, if a particle from the surface is by chance deposited at some depth in the sediment, it can remain there for a long time. This will lead to a constant value of MPD over extended periods of time (as indeed shown in Fig. 2), giving this one (likely rare) event a disproportionately large weight in the final PCA analysis of the (reduced) data.

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Response: While vertical events of particle transport to deeper sediment layers may be less frequent and less intense for most species than events of horizontal particle transport at the surface, they are not necessarily less important from a biogeochemical perspective. Patterns of vertical transport of both particles and solutes may not at all be stochastic as some species actually depend on such cross-boundary pathways for their metabolism (e.g. Thyasira sarsii). There are also species that create semi-permanent or permanent burrows into which organic material is transported for feeding. The burrow depth and maximum depth of particle reworking have implications for reaction pathways during element cycling (e.g. for redox reactions) because of the supply of oxidants (either dissolved, e.g. O2 and NO3-, through ventilation of pore water, or particulate material, e.g. MnO2 and FeOOH, through particle relocation) and surficial organic material that would not have been transported to these often anoxic sediment layers in the absence of bioturbation. There is also a potential for inverse transport of reduced particulate material (e.g. feaces) defecated at depth upwards to oxic surficial layers. The use of depth variables that describe temporal and spatial characteristics of vertical particle transport is quite common and often necessary within this line of research.

Reviewer: Also, it is not entirely clear how certain the estimates of the parameters shown in Fig.3 are. One can always fit a dataset with a model and obtain a process parameter such as r or Db. But the authors do not provide any clues as to the quality of their data fits and uncertainties of the estimated parameters. It would help if a few examples (best and, possibly, worse) of the data together with the fits are shown and the above points are discussed.

Response: To illustrate the coupling between raw data and results from the gallery diffusor model, we have included figures of luminophore distributions with depth (2-D) along with model fit describing the specific distributions as supplementary material. Additionally, model fits were presented in section 3.3 (p.8 l.6-7) in the revised version of the manuscript.

Reviewer: Last but not least, the quantities characterizing the 2D redistribution of the particles shown in Fig. 5 are unclear. As I understand it, redistribution of particles is a 2D image. But how is it reduced to a number in cmËĘ2? Obviously, there is an enormous data reduction involved. If there is no other metric used to characterize it (e.g., some sort of variance), then again this data points will have disproportionately large weight in the final PCA analysis.

Response: The 2D reworking variables (i) 2D redistribution (ii) daily transport and (iii) transport rate were calculated from the time series of images. They represent a measure of 2-D transport but are not in 2D as such. The manuscript was revised to further clarify how variables for 2-D redistribution were estimated from images of luminophore redistribution in section 2.4 (revised version p.5 l.19-29)

All replicate data points are plotted in Fig. 4 and 5 to facilitate transparency in the quality of the dataset.

Reviewer: Specific comments/questions: p.3 l.16: you make a distinction between functional groups and functional modes. In the introduction these subtleties are not clearly explained.

Response: Functional mode is how a functional outcome or performance is achieved. Functions take place in a mode at a performance level (intensity) by a portion of the members of a species (could be different at different life stages). A functional group is a group of organisms that perform similar functional modes that make it possible to classify them into a fairly coherent group of bioturbators that have a similar effect on the sediment transport properties. A difference in functional mode of particle reworking would facilitate grouping into functional groups. Based on the comment by the reviewer, we have in this context replaced functional group with functional mode in the revised version, p.2 l.19.

Reviewer: l.21: After reading the introduction, I do not understand the aim of the study, why the specified animals were chosen, and how this choice was related to the classi-

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fication mentioned in the preceding text.

Response: Response to this comment on the aim of the study is provided above and revisions have been made in the revised manuscript.

Reviewer: l.25: what's the point of (n=4) here?

Response: This refers to the number of replicates for each treatment i.e. number of aquaria. 10 treatments were examined and they were replicated 4 times = 40 aquaria. The sentence was slightly modified to clarify (revised version p.3 l.24).

Reviewer: l.32: what do you mean by "gallery-diffusor model could be *evaluated*"?

Response: Typo. The model was not evaluated. The sentence has been revised to clarify (revised version p.3 l.32).

Reviewer: l.35: I am quite confused about terminology used with respect to functional group vs. behavior, or reworking mode vs. behavior.

Response: As mentioned above, the text has been revised to clarify where appropriate.

Reviewer: p.5 l.29: unclear formulation: for each column? or for two luminophore particles?

Response: The description of the variable rugosity has been revised to further clarify that distances were calculated for each column and then summed (revised version p.5 l.30-32).

Reviewer: l.31: incorrect use of "summarized". perhaps "summed"?

Response: We agree. Summarized has been changed to summed (revised version p.5 l.33).

Reviewer: l.35: No results of this evaluation shown. Hard to get a feeling for it, and numbers don't tell much.

Response: As mentioned above, we have included figures on luminophore distributions

along with modeled data and error values as supplementary material. By providing this additional information the coupling between presented data and results from the model description can be further evaluated.

Reviewer: I p.7 l.15-25: The results are presented in a very inaccurate way, making it hard to decide how to understand them. Check out the following words that are scattered throughout the paragraph and be more specific about their meaning: occasionally, a significant downward transport, sporadically observed, suggested, specific sampling occasions, mainly observed, clear tendency of similar patterns.

Response: To meet this comment we have tried to be more specific and stringently when describing observations. Descriptions of individual aquaria have been removed from the text with more focus on the general trends. Revisions were made in section 3.2, revised version p.7 l.21-32.

Reviewer: l.26: why do you exclude G. alba and B.lyrifera from this list? Their values are also quite large, and, looking at the STD, probablz not significantlz different from the other three mentioned species.

Response: Results for individual species are summarized in Table 4. In accordance with the ambition to reduce the focus on observations for individual species, we have tried to highlight species that were particularly important for the respective variable. The impact from activities related to G. alba and B.lyrifera was considered as “intermediate” for the variable 0.5 cm in the comparison between species. No changes made.

Reviewer: l.34: what do you mean by indications?

Response: There were somewhat contradictory observations from the distribution of luminophores (which affected e.g. the variable MPD) in the N. pernula treatmentand the non-local coefficients described by r in the model fit.

Reviewer: Table 1: since the values do not add up to 100%, I wonder where the rest of the luminophores goes. What about the wall effect, i.e., the transport of luminophores

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that are transported away from the wall and are thus invisible from the side?

Response: It is not clear if this comment refers to Table 1 or Table 4. We believe the reviewer refers to Table 4 rather than Table 1. The numbers are not meant to add up to 100% as they describe the relative amount (%) of total luminophores observed in the image that were found beneath a certain depth (0.5, 2 and 4 cm). Examples of luminophore distributions along with model fits are included as supplementary material in the revised version of the manuscript. No additional revisions were made.

Reviewer: p.8 l.1: Unclear what the "transport rate" shown in Fig. 3 really means. Also the meaning of the unit is unclear.

Response: As mentioned above, the description in the method section has been revised to further clarify transport rate and the unit used.

Reviewer: l.4: Unclear what you really show in Fig. 4. According to methods, 2D redistribution reflects Mt-M0, which is a matrix. But all I see in Fig. 4 points. It is unclear what these points have to do with the 2D character of this metric.

Response: Please see the response above. Revisions were made to clarify that the variable is derived from the 2D images, not a matrix itself. Variables derived from the luminophore distributions (1D) are also numbers – not vectors (e.g. MPD, Db, r, 0.5 cm).

Reviewer: l.8: I see this only for Glycera and Scalibregma, which is not "in general".

Response: We disagree. In the plot where particle redistributions are illustrated vs time (Fig. 4), the slope was steepest during the first couple of days for most aquaria. No changes made.

Reviewer: l.21: So this is the measure of bulk sediment transport? Please clarify how/why.

Response: The 2D redistribution and the daily transport describe the number of pixels

that represent particle transport with time. Transport rate is the sum of the daily transport of particles throughout the experimental period divided by time. Because “bulk transport” was first introduced in section 3.5, “bulk” was removed from section 3.4 in the revised version of the manuscript.

Reviewer: p.10 l.5: Could you not look at this aspect (frequency distributions) in your data? Or is this where the Db and r come from? Not clear how you got them.

Response: In order to evaluate fingerprints for particle mixing, particles must be tracked individually. Such approach would require high-frequency image acquisition (0.1 Hz was used in Bernard et al., 2012 and Bernard et al., 2016). Our experimental setup was focused on longer duration of particle reworking activities (days) and the frequency of image acquisition was therefore lower.

Reviewer: l.17-19: unclear what the difference is between diffusive-like mixing mode and diffusivelike transport over long time scales.

Response: Not clear what the reviewer mean.

Reviewer: p.13 l.21-24: Nice, but you still made an a priori choice of a model when you were choosing the variables derived from your raw data.

Response: See detailed discussion above under Method-headline

Reviewer: p.21 Table 1: Unclear meaning of terminology. What does it mean "Feeding mode = Subsurface deposit"? And if you say "variable mobility", variable between what? Or "semimobile" - what exactly is that?

Response: Occasionally, it is useful to distinguish between a quantitative measure of a function/mode and a qualitative description of the function/mode itself. The mode of mobility and its quantitative measure was originally extracted from different references. For this reason, the description of e.g. mobility was not stringent. To meet the comment provided by the reviewer, descriptions of mobility of fauna were supplemented with information from Queirós et al. (2013) (Table 1; revised version).

Additional comments from the authors:

During production of the supplementary material, an error was observed in Fig. 4. One data point in the Scalibregma inflatum treatment at t=12 h was erroneous. The 2D redistribution was recalculated and Fig. 4 corrected accordingly in the revised version of the manuscript.

References referred to in the response:

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Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/bg-2016-411/bg-2016-411-AC1-supplement.pdf

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