Response to the review of the manuscript: Hydrological tracers for assessing transport and dissipation processes of pesticides in a model constructed wetland system

Elena Fernández-Pascual [a] *, Marcus Bork [a, b], Birte Hensen [c], Jens Lange [a]*

Hydrology, Faculty of Environment and Natural Resources, University of Freiburg, Freiburg, Germany [a]
Soil Ecology, Faculty of Environment and Natural Resources, University of Freiburg, Freiburg, Germany [b]
Institute of Sustainable and Environmental Chemistry, Leuphana University Lüneburg, Lüneburg, Germany [c]

* corresponding author, elena.fernandez@hydrology.uni-freiburg.de

Answers to referee #1

We wish to acknowledge the constructive and thoughtful comments of the reviewer. The following explains point by point how we will address the reviewer comments (in italics). We appreciate the efforts of the reviewer and the valuable suggestions that we will consider when revising our manuscript. Some long comments have been subdivided into several comments.

Scientific significance

Comment 1: *The manuscript aims at improving the understanding of the fate of pesticides in constructed wetlands, which are implemented to mitigate pesticide pollution of surface water bodies. To that end, the authors describe in quite some detail findings from a complex laboratory experiment simulating the fate of different (organic) chemicals and Br- as a conservative tracer (except for plant uptake) in a constructed wetland. To improve with regard to previous studies, the authors have put a lot of effort in obtaining spatial and temporal resolution of the concentrations of their model compounds in the experimental wetland.*
Despite the fact that constructed wetlands have some practical relevance as mitigation measures, the scientific relevance of the manuscript seems to be limited. On p. 2, L. 5 – 9, the authors describe their objectives. However, in the current form they are very specific to the experimental design and it remains unclear (also subsequently in the manuscript, see also comments below) how answers to the posed question can be generalised:

> **Response 1:** We thank the referee for pointing this out. Indeed, we have not clearly stated in the manuscript how the specific findings of this experiment can be generalised. Primarily, we wanted to highlight the usefulness of the experimental method, namely that fluorescent tracers (which are organic molecules, non-toxic and easy to be analysed) can be used to highlight the fate of pesticides inside wetland systems (mostly considered as black boxes so far). We apologize for the lack of clarity in this regard. While we think that a generalization of the results of our study to real-world wetlands cannot be made without validation in the field by additional experiments, it is true that the generality could be improved in the manuscript. In the revised version we will be

clearer about this question in order not to limit the scientific relevance of the study (see also the responses to the comments below).

Comment 2: *How to gain general insights if one knows in detail the spatial and temporal patterns of pesticide fate processes in this particular wetland at the lab scale (refers to objective i))?*

Response 2: The first objective of the study (objective i)) was to find out whether the use of a multi-tracer approach together with high vertical-resolution sampling and monitoring would allow to identify spatial and temporal patterns of pesticide fate processes. Our experiment aimed at providing a new methodology to better understand the behavior of pesticides in constructed wetlands. The level of detail of the data obtained made it possible to link more accurately the response of the target compounds with the different variables. If we know these relationships, we can extrapolate the results of our particular lab-scale experiment to real-world systems, provided that the same conditions take place. Furthermore, we found important state variables that should be monitored in field experiments.
A better explanation about how to generalise our particular results will be provided in the revised version. This will include a comparison of our system with real-world wetlands.

Comment 3: *How to generalise the findings related to the different behaviour of the model compounds (refers to objective ii))? Tracers-versus pesticides*

Response 3: The second objective of the study (objective ii)) was to compare the temporal and spatial behavior of the selected pesticides with reference tracers. In this case, a generalization could be made by comparing our results with those of other similar studies where the same or comparable tracers and pesticides have been used in wetland/buffer systems. One example is the study of Maillard et al., 2016. This information will be included in the revised version.

Comment 4: *How to generalise the results regarding vegetation and hydrologic conditions (refers to objective iii))?*

Response 4: The third objective of the study (objective iii)) was to assess the influence of vegetation and the alternation of different hydrologic conditions on pesticide transport and dissipation processes. The results of our study regarding vegetation and hydrologic conditions can be generalised by establishing parallels between the conditions simulated in the laboratory and those that occur in real wetlands. In particular we will discuss effects of temporary flooding and different kinds of groundwater surface water interactions. These questions will be addressed in the revised version.

Comment 5: *My statement does not imply that no such general insight could be gained from the experiment. However, in order to do so, one would need to ask first general scientific questions and subsequently demonstrate how the experiment can provide such generalizable answers. Such questions however are missing. The sentence on p. 2, L. 3 – 4, is too vague in this respect. This limitation is subsequently reflected in the Conclusion section. There is a lack of novelty and the statements are either very general or too speculative.*

Response 5: We apologize for the overall lack of clarity and agree that our general scientific questions should be better defined in order not to limit the conclusions, and we are grateful to the reviewer for pointing this out. As stated above, we will address this point in the revised version. We will follow two main lines: (i) we will compare existing (black-box) field results with our findings and (ii) we will further emphasize which conditions in natural wetland systems were actually mimicked in our experiments. This way, our experiment will provide original and relevant data that can help improve the understanding of complex phenomena related to transport and dissipation of pesticides observed in real-world systems.

Comment 6: *One way how the generality could be improved would for example be to put the characteristics of the study wetland (texture, organic carbon content, water residence time, redox conditions etc.) into the context of real-world wetlands, to reflect – based on scientific theory – what follows for pesticide retention in such wetland and to demonstrate respective insights that go beyond prior knowledge. I missed such information in the manuscript.*

Response 6: We appreciate this comment and we agree that we have to improve the explanation about the insights we have gained from of our study. As stated in Responses 2, 3 and 4, the characteristics of our lab-scale constructed wetland will be better addressed and put into the context of real-world wetlands.

Scientific quality

Comment 7: *Overall, the manuscript indicates that the experiments were carefully planned and executed. There are few technical questions that are listed below.*
However, there are conceptual limitations that also relate to the comments on the scientific significance above. A major issue is the lack of replication. There is only one vegetated and one non-vegetated chamber of the experimental tank. I am aware of the effort needed to carry out such experiments and to build such experimental facilities. Nevertheless, the results and conclusions hinge solely on single realisations of two experimental treatments. Especially in the context of preferential flow phenomena, this may be very critical because a single connected flow paths may exert a strong effect on the overall outcome. Without replication, it is very difficult to judge the robustness of the differences observed between the two treatments

Response 7: We thank the reviewer for pointing this out. While it is true that we only had one experimental unit with one vegetated and one non-vegetated zone, the results and conclusions did not depend solely on one single experimental run. In fact, we performed two experimental runs. We think that two identical runs of a dynamic system (the vegetation with its root system was constantly developing and hence also modified preferential flowpaths) may be treated as a replication. To build replicates of such a complex experiment was beyond our financial possibilities.

Comment 8: *Another limitation is the lack of quantitative analyses that could link the different pieces of information.*

(1) *The authors report for example Koc-values for the different compounds from the literature but do not provide quantitative analyses how transport and concentrations levels were expected based on this information.*
(2) *I also missed key features such as expected hydraulic residence time in the system etc. One could probably calculate such things from the information in the text and Tab. 2, but it would be useful for readers to directly get such information.*

Response 8-(1): Thanks for raising this important point. The information regarding Koc-values has been given in the text primarily as a guiding reference to interpret the behavior of the different solutes in terms of persistence and mobility. The use of parameters such as Koc-values to do predictions on transport and concentrations levels may be possible by applying modeling approaches. We are aware of the importance of modelling and we plan to carry out modelling in the future. However, we believe that this would go beyond the scope of the present study and would enlarge the manuscript too much.
Response 8-(2): Our system has not worked like a conventional constructed wetland. That is, the solutes were injected in the system and principally remained there throughout the experiment. We only sampled very small fractions of pore water. That is why, the hydraulic residence time would largely be equivalent to the duration of the experiment. We understand that this has to be made clear in the revised version and we will take care of this point.

Presentation quality

Comment: In general, the paper is clearly written, and the findings are carefully presented in the figures and tables.

Detailed comments

>> Title:

Comment 9: *Use of tracers: Why do you distinguish between tracers and pesticides? Uranine and sulforhodamine B are organic chemicals as are the three pesticides used in the study. Of course, there is a difference in the use of the compounds, but why is this distinction relevant for elucidating the fate of the pesticides (given the fact that also these tracers undergo sorption plant uptake and degradation)?*

Response 9: We appreciate your comment. It is true that both the tracers (Uranine (UR) and sulforhodamine B (SRB)) and the pesticides are organic chemicals. We have made a distinction between them because the hydrological tracers are the instrument that we expect to be a reference to study pesticide transport and dissipation processes. The present study seeks to confirm the feasibility of these tracers to investigate processes that dominate the behavior of pesticides in constructed wetlands. To do that we need to make comparisons between them, and therefore a distinction was made.

>> Abstract:

Comment 10: *p. 1, L. 10: What are spatial and temporal mechanisms?*

Response 10: Here we refer to those "processes" that may dominate pesticide transport and dissipation in constructed wetlands over time and space (e.g. sorption, transformation, plant uptake). We will make this clear in the revised version.

Comment 11: *p. 1, L. 13: What was the rationale behind the selection of these compounds?*

Response 11: We thank the referee for pointing this out. Boscalid, penconazole and metazachlor were selected because these pesticides were the most frequently detected in a field-based constructed wetland where other studies within the same project were carried out. We apologize for the omission of this information, which will be duly included in the revised version.

Comment 12: *p. 1, L. 16 – 17: What do you mean by the statement that transport dominated for some compounds?*

Response 12: Obviously, we did not make this point clear enough. Here, we mean that transport was more significant for Br, UR and metazachlor compared to SRB, boscalid and penconazole. That is, according to the results Br, UR and metazachlor experienced more transport than the other solutes during the experiment. This will be clarified in the revised version.

Comment 13: *p. 1, L. 17 –18: What other dissipation processes could be expected? This statement is not very informative.*

Response 13: This is a very important remark. We agree that the statement may not be clear enough. Our intention was to show that the mass balance has allowed us to identify the processes of sorption, transformation and plant uptake. So, we still believe that this statement should be kept in the text, but it will be better explained.

>> Introduction:

Comment 14: *p. 1, L. 27: The reference is not very recent. Many others are available representing more current findings.*

Response 14: We agree, and the reference "Müller et al., 2002" will be replaced by more recent studies.

Comment 15: *p. 1, L. 28 – 29: Generally, transformation products are less toxic. There are exceptions but the wording may be misleading.*

Response 15: We thank the referee for this comment. The sentence "transformation products (TPs), whose toxicity or persistence is unknown." will be changed to "transformation products (TPs), which in some cases, could be more persistent and toxic than the parent compound"

Comment 16: *p. 1, L. 40: This is an important aspect. Unfortunately, this manuscript does not really elaborate any further on this topic. It would be interesting to learn how the results reported here relate to other studies and what the results imply for mitigation capacities.*

Response 16: Thanks for raising this important point. It is true that the possible implications of our results for the study of the mitigation capacities of constructed wetlands have not been discussed thoroughly enough. In this sense, we believe that our findings are relevant and make an important contribution for the evaluation of the mitigation capacities of buffer zones. Therefore, the revised version will provide a more in-depth discussion on this topic. This also refers to the general comments above: we will provide comparisons to existing wetland field studies that have used the same or similar components.

Comment 17: *p. 2, L. 13 – 18: The critical question about the compound selection is what insight can be gained. In the result section (p. 9, L. 1 – 20), the results about the compound-specific differences are summarised. The reported findings basically reflect the knowledge already used for making the compound selection. Hence, the authors miss to derive more general insight that goes beyond the prior knowledge.*

Response 17: You raise a very valid point about the fact that we have not added enough information about the insight that we have gained from the selection of the hydrological tracers Br, UR and SRB. Our study is relevant because it has corroborated previous knowledge about these hydrological tracers with an experiment that had not been done before. We agree that more general statements about the use of these tracers for studying transport and dissipation processes of other pesticides can be made. To do this, a more exhaustive review of the bibliography on this topic will be included in the revised version.

>> Methods:

Comment 18: *p. 2, L. 28: How reliable is terbutryn as an internal standard for the other pesticides?*

Response 18: In fact, as stated in the manuscript, we used Terbutryn-D5 as an internal standard for the measurement of environmental water samples due to the possible occurrence of Terbutryn. Measurements of a variety of samples (about 1000 samples) determined that this internal standard was reliable for the detection of the substances in the water. Reliability was proved by the determination of recovery rates of substances. Here, a certain concentration was spiked into the environmental water samples where matrix effects could suppress the signal of the substance. Recoveries were found to be about 100 % by the correction of the internal standard.

Comment 19: *p. 4, L. 8: Generally, glass bottles are used for storing pesticide samples.*

Response 19: We used polypropylene tubes instead of glass bottles to store the pesticides because the samples had to be frozen immediately after their collection in order to preserve them before their shipment to the laboratory. This type of material has already been used to store pesticides in other studies (e.g. Joseph, 2015).

Comment 20: *p. 4, L. 30: What about possible inferences with the fluorescence of the background matrix?*

Response 20: We always analysed the entire fluorescent spectrum from 350 to 600 nm. This way, we could identify different background fluorescent levels and were able to subtract them. We will state this detail in the revised version.

Comment 21: *p. 5,L. 4: Where are LOQ/LOD provided?*

Response 21: Thanks for pointing this out. LOQ/LOD values (see below) for the pesticides and transformation products will be provided in Section 2.5.2 “Pesticides and TPs in the pore- and outlet- water” of the manuscript.

Substance	**LOD [ng L^{-1}]**	**LOQ [ng L^{-1}]**
Boscalid	0.35	1.27
Penconazole	0.35	1.29
Metazachlor	0.35	1.27
Metazachlor-ESA	2.78	10.35
Metazachlor-OA	0.54	1.90

Comment 22: *p. 5, L. 12: How can an independent background be determined?*

Response 22: We extracted the background signal according to the method described by Leibundgut et al. (2009). Such method does not use an independent background. Instead, it uses an equation that is based on the geometry of the curve from which the background is to be removed.

Comment 23: *p. 5, L. 35 – 36: This sentence sounds strange because transport processes affect all compounds irrespective whether or not they are sorbed or not (or degraded or not).*

Response 23: We agree with your statement. However, what we claim here is that if other processes such as sorption or transformation dominate, they will have an influence on the behavior of solutes in terms of transport (e.g. retardation by sorption, attenuation by degradation). Therefore, we have assumed that in those cases a strong correlation with Br (considered as the most conservative tracer) will not be observed. We will reformulate the sentence to make it more clear.

>> **Results and discussion:**

Comment 24: *p. 6, L.10: What means an early breakthrough? Early compared to what?*

Response 24: We thank the referee for this important comment. “Early breakthrough peaks” means that they were detected in the first place. This is a common expression that has been used in other studies (e.g. Torrentó et al., 2018). We will clarify this in the revised version.

Comment 25: *p. 6, L.10– 30: These paragraphs list different findings without a clear structure and logic.*

Response 25: We apologize for the lack of clarity. Our intention in this subsection was to explain the arrival of the breakthrough peaks of the solutes (including the TPs) to the different zones and depths in chronological order. The structure of these paragraphs will be improved in the revised version so that the ideas are presented in a more clear way.

Comment 26: *p. 6,L. 37: Where can one see these redox conditions?*

Response 26: The graphs of the redox conditions can be found in Fig. 4 (black line, second y-axis). We will better indicate this information in the revised version.

Comment 27: *p. 7, L. 6: I assume that sorption takes place all the time and not only during the initial phase.*

Response 27: That is correct. What we have stated here is, that sorption velocity was most likely higher at the beginning of the experiment compared to later phases when it probably decreased, given that the number of free sorption places became smaller. This will be clarified in more detail in the revised version.

Comment 28: *p. 7, L. 18: Where can one see this correlation?*

Response 28: The correlation between the breakthrough curves of Br is shown in Table 3. We will clarify this.

Comment 29: *p. 7, L. 20 – 30: These sentences are confusing.*

Response 29: We apologize for the confusion. These sentences provide evidence (through correlations) that the performance of the experiment in the vegetated zone during the first run was different compared to the second run. This information supports the idea that the plants possibly played an important role in our experiment and possibly modified flowpaths, etc. Therefore, it has been included in this subsection. The sentences will be rewritten to better express the main message in the revised version. This also has a bearing on why we regarded the second execution as a kind of replication (see general comments above).

Comment 30: *p. 7, L. 30 – 33: This paragraph is not well linked into the structure.*

Response 30: We are grateful for this observation. The information provided in this paragraph is relevant because it justifies the role of the hydrologic conditions in transport and dissipation of pesticides. This paragraph will be better explained and integrated into the text in the revised version.

Comment 31: *p. 8, L. 11 – 13: Please be aware that different transformation products may have different source terms because they are generally formed at different rates and possibly in different parts of the subsurface.*

Response 31: We thank the reviewer for this comment. We totally agree, and the sentences regarding the possible transport of metazachlor TPs based on their comparison with Br will be removed from the text, as we cannot accurately determine where and when they were formed.

Comment 32: *p. 8, L. 22 – 24: This is very qualitative. What were the expected compound-specific differences solely based on the Koc-values?*

Response 32: We thank the referee for raising this important issue. We have stated in the manuscript that, according to our results, SRB, boscalid and penconazole experienced more sorption than the other compounds (Br, UR ad metazachlor), which may be explained by their sorption properties. While it is true that the Koc-values may help interpreting these results from a qualitative point of view, the amount of compounds adsorbed and/or the type of interaction behind the adsorption cannot be explained only with Koc-values. Hence, a more detailed discussion based on substrate properties and additional parameters (e.g. Kd-values, aqueous solubility) will be done in the revised version.

Comment 33: *p. 8, L. 25 – 26: Again, this statement appears rather isolated in the text.*

Response 33: We are sorry for the lack of clarity in this regard. Given that we consider that the observations on the recovery of metazachlor TPs at the outlet are an important finding of our study, they will be better integrated into the text to facilitate the reader's understanding.

Comment 34: *p. 9, L. 31: Here you contradict yourself: above you have argued that SRB is expected to be strongly sorbed because of its Koc-value (p. 8, L. 23)*

Response 34: We thank the referee for raising this important issue. The peculiarity of SRB is that it has both charged groups (cationic and anionic) and a non-polar region (Polat et al., 2011). This will make SRB susceptible to sorption on positive and negative charged mineral sites, OH-groups of hydroxides and clay minerals, but also on nonpolar sorption sites of organic matter. The latter would explain why we found large amounts of this tracer in the part of the sediment where the largest portion of organic carbon was observed. Considering the above, the use of Koc-values would probably not be appropriate to interpret the results of SRB as it may lead to misunderstandings. This will be taken into account and corrected in the revised version.

>> **Figures:**

Comment 35: *Fig. 4: - It is difficult to distinguish all the different lines. - What were the hypotheses, how the breakthrough would differ between the different depths and the different compounds?*

Response 35: We are grateful for this comment. In order to facilitate a better distinction of the curves, Figure 4 will be split in two graphs, one for the vegetated and one for the non-vegetated zone. What we wanted to show in this figure was, on the one hand, the evolution of the temporal and spatial concentration of the solutes in the pore water, and on the other, how the pesticides behave compared to the tracers.

Comment 36: *Fig. 7: Is there no differentiation between vegetated and non-vegetated treatments?*

Response 36: No, because the objective of this figure is to show how much solute in general is recovered at the outlet of the system after each flushing.

Comment 37: *Fig. 8: You might consider comparing the two treatment with separate bars.*

Response 37: We thank the referee for this suggestion. However, it is not possible to make a distinction between the treatments with two bars. Both zones (vegetated and non-vegetated) are part of the same unit and the percentages of recovery from each zone have been calculated with respect to the total amount of solutes injected. Therefore, the final percentage recovered is the sum of the percentages from the vegetated and the non-vegetated zone.

Comment 38: *Fig. 9: Is the sorption consistent with Koc-values known for SRB?*

Response 38: As stated in the responses to comments 32 and 34, the Koc-value for SRB itself would not explain the results obtained for this tracer. In this case, we have to look into its molecular structure and sorption properties in more detail to elucidate the performance of SRB in the sediment. Therefore, to avoid confusion, and as already mentioned in the previous comments, Koc-values will not be used in the revised version to interpret the behavior of SRB. We think that our findings provide some general insights into the ambivalent sorption behavior of the tracer SRB that has been reported in literature. We will discuss this in our revised version.

Literature

Joseph, G. (2015). Determination of sodium monofluoroacetate in dairy powders by liquid chromatography tandem mass spectrometry (LC-MS/MS): First Action 2015.02. Journal of AOAC International, 98(4), 1121-1126.

Leibundgut, C., Maloszewski, P., & Külls, C. (2009). Environmental tracers. Tracers in Hydrology, John Wiley&Sons, Ltd., Chichester, UK, 13-56.

Maillard, E., Lange, J., Schreiber, S., Dollinger, J., Herbstritt, B., Millet, M., & Imfeld, G. (2016). Dissipation of hydrological tracers and the herbicide S-metolachlor in batch and continuous-flow wetlands. Chemosphere, 144, 2489-2496.

Polat, B. E., Lin, S., Mendenhall, J. D., VanVeller, B., Langer, R., & Blankschtein, D. (2011). Experimental and molecular dynamics investigation into the amphiphilic nature of sulforhodamine B. The Journal of Physical Chemistry B, 115(6), 1394-1402.

Torrentó, C., Prasuhn, V., Spiess, E., Ponsin, V., Melsbach, A., Lihl, C., ... & Hunkeler, D. (2018). Adsorbing vs. nonadsorbing tracers for assessing pesticide transport in arable soils. Vadose Zone Journal, 17(1).