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

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Answers to editor

Dear Prof. Zehe,

We are very grateful to have been given the opportunity to revise and resubmit our manuscript. We greatly appreciate your comments and suggestions. All of them have been taken into account and we now trust that the quality of the manuscript has improved considerably. We would also like to acknowledge the two reviewers for their constructive and helpful comments.

Below we indicate your evaluation of the manuscript and a point by point explanation (in blue) of how we have addressed your comments and those of the reviewers. For the latter ones, we have specified in some of the comments the corrections performed and where in the revised manuscript (in the not marked version) they have been made, detailing the corresponding page and line number where applicable. Additionally, we have indicated the new literature included in the revised version. The marked-up manuscript version is provided at the end of this document.

We hope you find these revisions rise to your expectations. Thank you once again for taking the time and energy to help us improve the paper.

On behalf of all the authors,

Elena Fernández-Pascual

Editor

Comment: After reading your study again, I am, in line with both reviewers, impressed by the high quality of the underlying experiments. Nevertheless, both reviewers came up with a long list of critical and constructive comments which need to be thoroughly addressed in a round of major revisions.

1. Reviewer 1 recommended rejection – her/his main critique is that it is not clear how the findings of your experiments can be generalized and the lack of replication. In your reply you better explained that the overall objective of your study is to introduce the approach and more specifically to show the potential of fluorescent tracers to explore the interplay of transport and transformation of reactive compounds. Seems like a good idea, however, this implies that the story line of the manuscript needs to be strongly revised. Moreover, I think that it is very much worth to consider her/his suggestion of "Putting the characteristics of the artificial wetlands (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."

2. I see that the lack of replicates is a weakness and that the abundance/absence of a single macropore will at this scale drastically change outcome of the experiment (you operate below the REV!). A proper design would need at least three replicates. But I do not think that it is a killing argument, if the story line will be revised as you proposed. An alternative is the combination with modelling to overcome this limitation. Solute transport has a theory, means that we are not thrown to a pure statistical learning paradigm.

3. While reviewer 2 recommended minor revision, her/his main critique points deserve much attention as well. A mass balance closure of 30% leaves us with much uncertainty about the entire concert of transport and transformation. In a field study this would be a nice achievement, but for a controlled environment it is poor, and this puts the value of these experiments into question (also with respect to the issue of being representative and working below the REV). This needs to be discussed.

4. Given coarse texture of the soil, a rapid reaction does not necessarily speak for preferential flow! This is surely related to fuzzy definition of preferential flow. Is transport in the "near field" preferential flow (transport times < than lateral mixing times) or is it a non-Gaussian residence time distribution? I think the manuscript will benefit from being precise in this respect, maybe a straight forward model exercise with could help to quantify the degree of non-normality of the breakthrough curves. Last but not least I think it is worth to discuss the role of retardation (which introduces a time lag) when doing a correlation analysis of breakthrough curves. One could thus also infer on the retardation using lag correlations?"

Response:

1. As suggested, the story line of our manuscript has been thoroughly revised. Some subsection have been completely rewritten (3.2 and 3.3) and new ones have been

created (3.1 and 3.5). Likewise, the results have been compared with similar investigations and the characteristics of our experimental system have been put into the context of real-world wetlands. In this regard, we have detailed under which circumstances our results would be valid and what would be the implications for the mitigation capacities of real-world wetlands. All this is detailed below in the responses to the reviewers' comments.

2. We still believe that the realization of two identical runs provides equally valid results. Moreover, our system was not the typical laboratory experiment, but rather a system halfway between controlled laboratory and field conditions. In addition, performing more replicates of such a complex experiment was beyond our financial possibilities.

3. We agree that the recoveries of some of the tracers were small. But we don't think it was due to uncertainty but rather due to the reactive character of such tracers and the type of experiment (long duration and stagnant conditions) since the most conservative tracer (bromide) was recovered almost entirely (98.3%). In fact, and as detailed in the revised version, recoveries of the same tracers in other studies were similar. Therefore, we believe in the validity of our results. We have widely discussed this point and the possible reasons for such recoveries in the revised version.

4. We also concur with the view of the editor in his opinion about the occurrence of preferential flow in our system. Indeed, considering the type of sediment used in our experiment, it is very unlikely that preferential flow towards the bottom during the injection will occur. That is why we have eliminated such statement. However, we still believe that transport of solutes may have been favored towards the vegetated surface since there are strong indications that support it. This point has also been discussed extensively in the revised version. In addition, after carefully reviewing the data, we have concluded that the conditions prior to injection (i.e., system at field capacity) have influenced the transport of solutes as well. Specifically, we believe that the delay in the middle layers has been due to the existence of water-filled pores, which has been associated with a possible lack of connectivity. These statements have been discussed in the revised version and are supported by additional information (new Table 4 and new Fig. 4, see below), including a lag correlation analysis, as suggested (new Fig. 6, see below).

   Finally, we would like to point out that we have not performed the classical tracer test under steady state conditions but rather a more complex experiment with different phases of variable duration, stagnant conditions and separate measurements over time. We believe that a simple modeling exercise cannot be applied but rather a more specific model capable of explaining the complexity of our system. Therefore, and as already stated in the response to the reviewers comments, although we are aware of the importance of modelling (it is intended to be carried out in the future), we think that including it in the manuscript would enlarge it too much and would go beyond the scope of the study.

Table 4. Selected relative concentrations of Br^- measured during the: 1) first and 2) second run for the different zones, phases (saturation and drying) and depths.

1)	Depth (cm)	Saturation			Drying					Saturation				
		09/03	13/03	16/03	20/03	21/03	23/03	27/03	04/04	10/04	12/04	02/05	04/05	09/05
Non-vegetated	3	0.08	0.17	0.19	0.34	0.41	-	-	-	0.33	0.35	0.21	0.17	0.03
	15	0	0	0	0.01	0.02	0.04	0.09	0.23	0.15	0.18	0.06	0.04	0.01
	27	0	0	0.02	0.03	0.04	0.04	0.05	0.08	0.08	0.07	0.01	0.01	0.05
	39	0.30	0.21	0.15	0.09	0.11	0.11	0.12	0.11	0.01	0.01	0.01	0.02	0.01
Vegetated	3	0.20	0.70	0.79	0.76	0.79	-	-	-	0.48	0.55	0.45	0.39	0.08
	15	0	0	0.01	0.03	0.04	0.06	0.09	0.06	0.02	0.00	0.06	0.05	0.03
	27	0	0	0	0	0	0	0	0.01	0.02	0.02	0.02	0.02	0.08
	39	0.10	0.02	0.02	0.03	0.04	0.05	0.06	0.06	0.01	0.02	0.04	0.05	0.00

2)	Depth (cm)	Saturation			Drying					Saturation				
		01/08	04/08	08/08	10/08	14/08	18/08	22/08	25/08	29/08	05/09	13/09	27/09	03/10
Non-vegetated	3	0.07	0.20	0.46	-	-	-	-	-	0.43	0.53	0.62	0.35	0.03
	15	0.01	0.01	0.02	0.06	0.18	0.31	0.42	-	0.39	0.41	0.30	0.15	0.02
	27	0.01	0.01	0.03	0.02	0.04	0.07	0.11	0.15	0.14	0.10	0.07	0.05	0.00
	39	0.29	0.19	0.19	0.19	0.20	0.21	0.21	0.20	0.02	0.02	0.02	0.02	0.00
Vegetated	3	0.18	0.59	0.53	-	-	-	-	-	0.09	0.16	0.18	0.08	0.01
	15	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.01	0.03	0.04	0.04	0.02
	27	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.03	0.06	0.04	0.03	0.02	0.00
	39	0.02	0.01	0.01	0.03	0.06	0.10	0.10	0.09	0.01	0.02	0.02	0.02	0.00

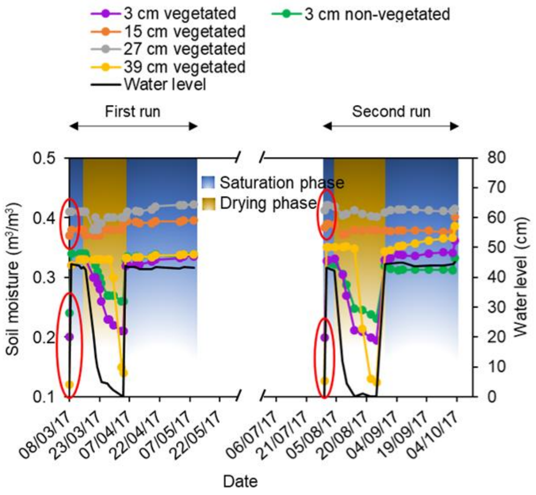

Figure 4: Soil moisture values measured in the pore water during the first and the second run for the different zones, phases (saturation and drying) and depths. Water level is displayed in the second y-axis. The missing data from the sampling depths at 15, 27 and 39 cm in the non-vegetated zone is due to failures in the sensors. Red circles indicate the values previous to the injection.

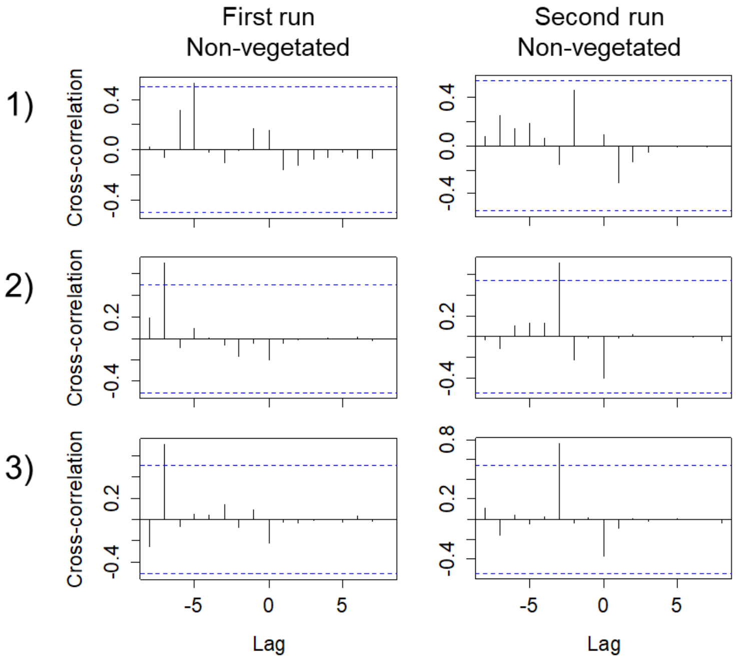

Figure 6: Lag analysis performed to the Br- breakthrough curves for the first and second run between the sampling depths: 1) 39cm and 3cm; 2) 39cm and 15cm; and 3) 39cm and 27cm. Only significant lag correlations are displayed.

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).

Notes on response 1: This comment has been addressed in the responses below (2, 3, 4, 5 and 6).

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.

Notes on response 2: We have addressed this point on p. 9 L. 12-20 as follows:

"Although our experiment has been carried out in a laboratory environment, the replicated conditions may resemble those of a groundwater-fed wetland that undergo wet-dry cycles and that intercepts pesticide-contaminated water during groundwater discharge. Similar systems have already been investigated with the same multi-tracer approach under laboratory (Durst et al., 2013) and field conditions (Maillard et al., 2016) and the results were analogous to our findings. For instance, Durst et al. (2013) found that preferential flow along the roots took place in the vegetated part of the wetland resulting in greater solutes recoveries, whereas Maillard et al. (2016) demonstrated that the alternation of oxic-anoxic conditions enhanced the dissipation of solutes. Other field studies in wetland systems have pointed out that the presence of vegetation greatly increases contact time and surface area for adsorption (Moore et al., 2006; Liu et al., 2018), which may also be enhanced when organic matter content is high (Passeport et al., 2011)."

Comment 3: *How to generalise the findings related to the different behavior 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.

Notes on response 3: This point has been discussed in the new subsection "3.5 Potential of hydrological tracers to evaluate transport and dissipation processes of pesticides in constructed wetlands" on p. 12 L. 18-39 as follows:

“In view of the results obtained in the present study, some conclusions could be drawn regarding the use of Br-, SRB and UR to evaluate transport and dissipation processes of pesticides in constructed wetlands. In particular, we have corroborated that Br- can be used to elucidate non-reactive transport of solutes in constructed wetlands, as already reported in the literature (Lin et al., 2003; Małoszewski et al., 2006). But it can also be applied to identify plant uptake, although to a lesser extent. As for SRB, despite the fact that it has been extensively used to identify sorption processes in wetland systems (Passeport et al., 2010; Lange et al., 2011; Schuetz et al., 2012), its special sorptive character makes it difficult to be compared with a certain type of pesticide. In this regard, while Dollinger et al. (2017) stated that SRB could be used as a good proxy for hydrophilic and strongly sorbing pesticides, Lange et al., 2018 demonstrated that the same tracer closely mimicked the gradual recession of a moderately hydrophobic pesticide in the top soil of an agricultural field. As for our results, we found that SRB could describe well the behavior of the pesticides boscalid and penconazole (moderately and highly hydrophobic, respectively) in terms of retention and retardation in the pore water and in the water at the outlet when the constructed wetland is repeatedly flushed. However, it may not be suitable to evaluate overall recoveries of boscalid and penconazole at the outlet given that greater amounts of SRB may be recovered compared to such pesticides, possibly due to its greater leachability and/or lower susceptibility to be taken up by the plants. Regarding UR, in terms of transport our results suggested that it may illustrate well the behavior of mobile and non-persistent pesticides, such as metazachlor, which is in agreement with the findings of other studies (Durst et al., 2013; Maillard et al., 2016; Torrentó et al., 2018). At the same time, our results have underlined that UR may experience not only photodegradation, but also (bio-)chemical transformation, which is consistent with the results of recent investigations (Maillard et al., 2016; Lange et al., 2018, Fernández-Pascual et al., 2018). Yet, UR biodegradation might be limited in the presence of preferred substrates for microorganisms. In any case, it should be noted that the conclusions presented here are only valid if these tracers are used in studies under similar conditions as those of our experiment. That is, constructed wetlands that undergo long periods of stagnation (> 2 months), with drying periods in between, sorbing material with low organic carbon content, similar vegetation and subject to analogous dominant processes.”

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.

Notes on response 4: This point has been addressed on p. 8, L. 40-41 and p. 9, L. 1-11 as follows:

"The results of our study underlined the importance of plants in promoting dissipation processes in constructed wetlands. Indeed, plants have already been attributed the ability to facilitate elimination, degradation and retention of pesticides in wetland systems (Liu et al., 2018). However, our findings also suggested that plant roots may be involved in the formation of preferential flow paths, which could result in a rapid transport of contaminants and decrease in the interactions between solutes and sediments (Durst et al., 2013). In fact, plant roots have been related to the creation of discontinuities in the soil profile, greater presence of macropores and occurrence of bypass flow (Ghestem et al., 2011). Therefore, the beneficial impact of plants in terms of elimination, degradation and retention may be reduced by the occurrence of preferential flows.

Our results have also indicated that the promotion of aeration has facilitated the degradation of some substances. This was in agreement with recent studies that have demonstrated that intermittent flow regimes support aerobic microbial populations and boost degradation rates of pesticides (e.g. Karpuzcu et al., 2013; Maillard et al., 2016). Other authors also found that by alternating drainage with no drainage periods in constructed wetlands, these systems are capable of reducing non-point pollution (Vallée et al., 2015). Hence, it could be generalized that the mitigation capacities of constructed wetlands might be improved if aerated conditions in the system are fostered."

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.

Notes on response 5: In order to better reflect the objectives of our study and how it can provide generalizable answers, we have modified the introduction on p. 2, L. 10-27 as follows:

"The mitigation capacities of buffer zones have recently been studied by using hydrological tracers as a low-cost approach. In this context, fluorescent tracers (e.g. uranine (UR), sulforhodamine B (SRB)) have often been chosen to study transport and fate of pesticides because they are organic molecules, non-toxic and easy to be analysed. For instance, some authors have used them in wetlands (Passeport et al., 2010; Lange et al., 2011; Durst et al., 2013; Maillard et al., 2016) and farm ditches (Dollinger et al., 2017). Yet, in most cases where this approach has been applied the system under study has been treated as a "black box" where the time scales were typically limited to the time spans of the tracers breakthroughs at the systems outlet. Hence, internal temporal and spatial mechanisms that dominate pesticides transport and dissipation (e.g. sorption, transformation, plant uptake) are still not fully clear. Moreover, information on the fate and, particularly, transformation of pesticides inside wetland sediments is still limited.
Therefore, the objectives of this study are i) to apply a multi-tracer approach together with high vertical-resolution sampling and monitoring to identify transport patterns and dissipation processes of three pesticides selected as test substances inside a model constructed wetland system; ii) to compare the temporal and spatial behavior of the applied tracers with the pesticides and evaluate their main dissipation pathways; and iii) to assess the influence of vegetation and alternating different hydrologic conditions (saturated and unsaturated) on transport and dissipation processes.
Our study is one of the first to look at the solutes behavior inside a constructed wetland on a long-term basis and detailed spatial scale. With this experiment we expect to provide new insights about the potential of hydrological tracers to evaluate transport and dissipation processes of pesticides. Likewise, we seek to extend the knowledge on the mitigation capacities of constructed wetlands with our approach."

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.

Notes on response 6: This point has been addressed on p. 10, L. 24-36 as follows:

"In principle, we expect to obtain analogous results in wetland systems if similar conditions are met. In this regard, if we compare the characteristics of our experiment (see Table 2) with those of other wetland studies (e.g. Catallo, 1999; Seybold et al., 2002; Maillard et al., 2011; Gardiner et al., 2012; Passeport et al., 2013; Vallée et al., 2016; Gikas et al., 2018) we find similar values in terms of sediment texture (values ranging from 4 to 89.5/6.2 to 55/3.8 to 44 for % Sand/Silt/Clay, respectively), sediment pH (values ranging from 6 to 8), conductivity (values ranging from 0.45 to 0.9 dS/m) and redox potential (values ranging from -500 to +500 mV). However, there are some discrepancies regarding organic carbon content (values ranging from 2.6 to 32.7 %) and mean residence time (values ranging from 0.5 to 8 days). In this case, the values of our experiment were either below (for the organic carbon content) or above average (mean residence time). Yet, the overall removal rates obtained in our experiment (see Table 6) were within the same range of those of the wetland studies. For instance, Vallée et al. (2015) found that the removal rates of boscalid in two pilot-scale wetlands ranged from 38 to 67%, whereas Gikas et al. (2018) obtained removal rates for S-metolachlor (pesticide from the same group as metazachlor) that reached up to 92.6% in a constructed wetland planted with Phragmites australis. Other authors have reported removal rates of 45%–90% for tebuconazole (a triazole fungicide similar to penconazole) in wetland systems (Passeport et al., 2013; Tournebize et al., 2013)."

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.

Notes on response 8-(2): The value of the equivalent hydraulic retention time has been specified in Table 2B (see below).

B

Parameter	**Unit**	**Value**
Inlet/outlet pumping rate	L h^{-1}	21.6
Peristaltic pumping rate	L h^{-1}	0.1
Volume of tracers and pesticides injected	L	40
Volume of clean water injected at the end of the drying phase	L	34.1 ± 3.1
Volume of total clean water injected in the flushings	L	355.1 ± 20.5
Hydraulic retention time	Days	62.5 ± 2.12

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.

Notes on response 10: The term "spatial and temporal mechanisms" has been clarified throughout the text as follows:

“…internal temporal and spatial mechanisms that dominate pesticides transport and dissipation (e.g. sorption, transformation, plant uptake)…”

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.

Notes on response 11: The information regarding the selection of pesticides (see below) has been included on p. 2, L. 33-36:

“Three pesticides were selected as test substances according to their different physicochemical properties and frequent detection in a field-based constructed wetland where other studies within the same project were carried out: boscalid (2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide), penconazole ((RS)-1-[2-(2,4-dichlorophenyl) pentyl]-1H-1,2,4-triazole) and metazachlor (2-chloro-N-(pyrazol-1-ylmethyl) acet-2',6'-xylidide).”

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.

Notes on response 12: The statement "transport dominated for some compounds" has been changed by “The strong temporal and spatial correlation found between Br-, UR and metazachlor indicated that these solutes experienced more transport than SRB, boscalid and penconazole” on p. 1, L. 21-22.

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.

Notes on response 13: The statement has been changed on p. 1, L. 24-25 and p. 13, L. 4 as follows:

"The overall tracer mass balance allowed us to identify three dissipation pathways: sorption, transformation and plant uptake."

>> 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.

Notes on response 14: We have added the following references on p. 1, L. 36-37: von der Ohe et al., 2011 and Casado et al., 2019.

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"

Notes on response 15: The corresponding change has been made on p. 1, L. 37-39 as follows:

"…transformation products (TPs), whose behavior is unknown, and toxicity or persistence may be in some cases greater than the parent compounds."

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.

Notes on response 16: The question regarding mitigation capacities of constructed wetlands has been addressed throughout the manuscript. For instance:

On p. 10, L. 41-43 and p. 11, L. 1-2: "These observations highlight the importance of certain factors in the elimination of pesticides, namely the presence of adequate vegetation, suitable matrix materials, long residence times, low flow rates, intermittent flow conditions, among others (Vymazal et al., 2015; Liu et al., 2018). When these factors are promoted, the elimination rates tend to increase, and therefore, the mitigation capacities of constructed wetlands."

On p. 13, L. 23-26: Our findings pointed out that the presence of plants and the alternation of different hydrological conditions (saturation and drying periods) may favor dissipation processes. The combination of these factors together with others (e.g. suitable matrix materials, long residence times, etc.) could increase the mitigation capacities of wetland systems. Yet, plants might also be involved in the creation of preferential flow paths with the consequent risk of rapid transport of contaminants."

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.

Notes on response 17: As already answered in comment 3, a new subsection (3.5) has been created in order to discuss the insight that we have gained from the selection of the hydrological tracers Br^-, UR and SRB.

>> **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).

Notes on response 19: This information has been clarified on p. 4, L. 27-30 as follows:

"Previously, a volume of 10 mL was transferred to 15 mL Polypropylene tubes and stored at -20°C for the subsequent pesticide and TPs analysis. Polypropylene was chosen instead of glass because the samples had to be frozen immediately after their collection. Such 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.

Notes on response 20: The following information has been added on p. 5, L. 12-13:

"The entire fluorescent spectrum (from 350 to 600 nm) was analyzed in order to identify different background fluorescent levels and subtract them."

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

Notes on response 21: The corresponding information has been included in Table 3

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.

Notes on response 23: The sentence has been reformulated on p. 6, L. 11-15 as follows:

"The predominance of transport processes among the solutes was examined by looking at the relationship between Br- and the other solutes via correlation matrices of the measured concentration time series. Here, it was assumed that a weak correlation would be due to the prevalence of sorption and transformation rather than transport. This was based on the premise that the solutes would experience retardation due to sorption or attenuation due to transformation."

>> 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.

Notes on response 24: Results and discussion section has been rewritten to present the results in a more understandable way. Here, it has been decided to eliminate the term "early breakthrough" because it can be misleading.

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.

Notes on response 25: As indicated in notes on response 24, the results section has been rewritten and restructured in order to present the results in a clearer and more logical way. This has included the creation of a new subsection (3.1 Transport processes in the pore water according to Br- behavior).

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.

Notes on response 26: This information has been clarified on p. 7, L. 37-39 as follows:

"Here in the middle layers, dominant processes most likely differed between zones, as was also evidenced by the different redox potentials measured in the vegetated and the non-vegetated zone (See Fig. 5, second y-axis)."

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.

Notes on response 27: This information has been detailed on p. 8, L. 37-39 as follows:

"Given the rapid decrease in relative concentrations shortly after the injections, it was assumed that sorption velocity was higher at the beginning of the experiment compared to later phases, when it probably decreased due to a decline in the number of free sorption places."

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).

Notes on response 29: As indicated in comment 25, the results have been rewritten to better illustrate the findings. Transport processes have been now discussed according to Br- behavior in subsection 3.1. Here, on p. 7, L. 26-33 we have inserted the information related to the role of plants in the possible modification of transport processes as follows:

"The different behavior of Br- in terms of transport observed in the first and second run was explained by a possible development of the root system. In fact, when correlating the breakthrough curves of Br- of the first and the second run, differences between the vegetated and non-vegetated zones were observed (Table 5). With the exception of the uppermost layer, the non-vegetated zone showed strong correlation between the two runs regardless of the layer, whereas the vegetated did not show any correlation. This meant that the performance of the non-vegetated zone was similar in both runs, whereas the vegetated behaved differently. Hence, changes in root density and/or spatial distribution most likely occurred during the experiment. As a result, presumably both, transport processes and dissipation varied over time (Goss et al., 1993). This assumption was supported by visual observations of the sediment (Fig. 7)."

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.

Notes on response 30: See notes on responses 25 and 29

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.

Notes on response 32: This question has been discussed in the new subsection 3.5 on p. 12, L. 21-31 as follows:

"As for SRB, despite the fact that it has been extensively used to identify sorption processes of pesticides in wetland systems (Passeport et al., 2010; Lange et al., 2011; Schuetz et al., 2012), its special sorptive character makes it difficult to be compared with a certain type of pesticide. In this regard, while Dollinger et al. (2017) stated that SRB could be used as a good proxy for hydrophilic and strongly sorbing pesticides, Lange et al., 2018 demonstrated that the same tracer closely mimicked the gradual recession of a moderately hydrophobic pesticide in the top soil of an agricultural field. As for our results, we found that SRB could describe well the behavior of the pesticides boscalid and penconazole (moderately and highly hydrophobic, respectively) in terms of retention and retardation in the pore water and in the water at the outlet of constructed wetlands when the system is repeatedly flushed. However, it may not be suitable to evaluate overall recoveries of boscalid and penconazole at the outlet given that greater amounts of SRB may be recovered compared to such pesticides, possibly due to its greater leachability and/or lower susceptibility to be taken up by the plants.”

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.

Notes on response 33: The statement has been integrated into the text on p. 9, L. 39-41 as follows:

“Cumulative recovery curves of metazachlor TPs were also obtained at the outlet of the system during the flashings (Fig. 9), thereby evidencing their great mobility and persistence in the environment (Mamy et al., 2005; European Food Safety Authority (EFSA), 2008). In this case, higher amounts of met-ESA were recovered compared to met-OA.”

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.

Notes on response 34: We have rewritten the statements on p. 12, L. 11-15 as follows:

“On the other hand, most of SRB was found sorbed in the sediment of the vegetated zone, where the highest concentration of organic carbon was located (Fig. 11). This may be explained by the susceptibility of SRB to sorption on nonpolar sorption sites of organic matter (Polat et al., 2011). Moreover, it has been recently demonstrated that SRB has high sorption affinity for litters in wetlands (Dollinger et al., 2017). Thus, probably the presence of dead leaves and decaying plant residues in the uppermost layer enhanced sorption of SRB.”

>> **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.
>
> Notes on response 35: The new figure (Fig. 5) is shown below

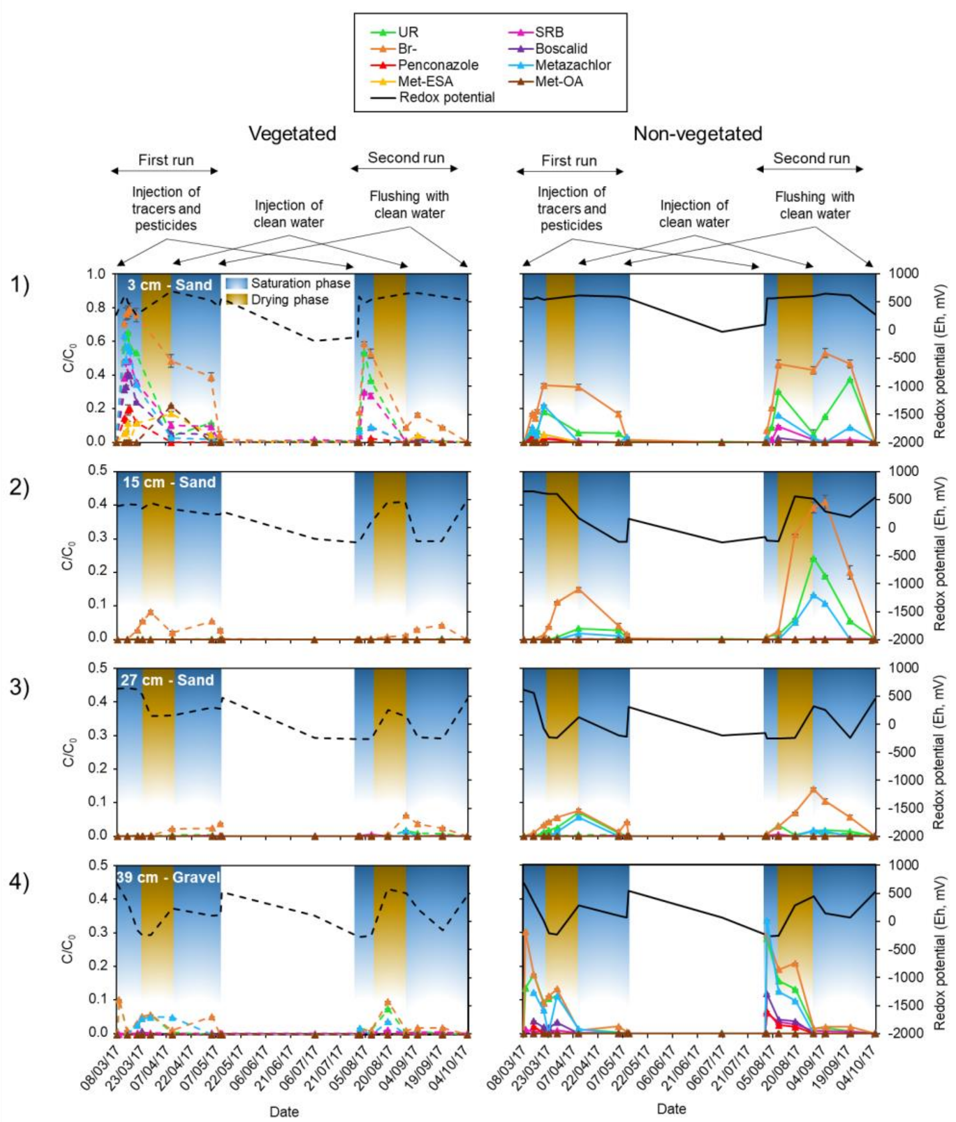

Figure 5: Breakthrough curves of the different tracers, pesticides and their TPs in terms of relative concentrations (C/C_0) (obtained by scaling with the input concentrations) measured in the pore water during the first and the second run for the different zones, phases (saturation and drying) and depths: 1) 3cm; 2) 15cm; 3) 27cm and 4) 39cm. Changes in redox potential are displayed in the second y-axis (Eh in mV). The different injections performed during each run are displayed on top of the figure. Note that the scale of the relative concentrations corresponding to the sampling depth of 3 cm is extended.

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.

Notes on response 38: See notes on responses 32 and 34.

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).

von der Ohe, P. C., Dulio, V., Slobodnik, J., De Deckere, E., Kühne, R., Ebert, R. U., ... & Brack, W. (2011). A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive. Science of the Total Environment, 409(11), 2064-2077.

Casado, J., Brigden, K., Santillo, D., & Johnston, P. (2019). Screening of pesticides and veterinary drugs in small streams in the European Union by liquid chromatography high resolution mass spectrometry. Science of The Total Environment, 670, 1204-1225.

Answers to referee #2

Comment 1: *The authors present an interesting and well written paper on transport and dissipation processes of different substances in a constructed wetland at the lab scale. This is a highly relevant topic that is within the scope of HESS and of interest for a broader audience. The experiments and results are highly interesting and are largely presented clearly. A few points in the analysis and interpretation, however, should be revised to be less speculative and more supported by the results. This requires mainly further elaboration of the discussion, as detailed in the specific comments below.*

> **Response 1:** We appreciate the positive feedback, thoughtful comments and constructive suggestions from the reviewer that will help us improve the manuscript. We next detail the reviewer's comments (in italics) and our answers on how we will address the comments in the revised manuscript. Some long comments have been subdivided into several comments

General comments

1. Recovery

Comment 2: *A main concern is the low recovery of most of the substances. Except for bromide and SRB, less than a third of the applied masses were detected in the investigated compartments (the data on SRB suggests a recovery between 48 and 105% - Fig. 8). Because it is ambiguous to judge the parts that have not been observed, care needs to be taken in drawing conclusions on transport and dissipation from the data. The authors often did well in this regard and addressed possible pathways of the substances' fates by deduction and use of available literature. Sometimes they overachieved a bit, and some aspects deserve further clarification.*

> **Response 2:** We thank the reviewer for pointing this out. We are aware of the difficulty of drawing conclusions on transport and dissipation from the data when some observations in certain parts could not been made. That is why we have been very careful when interpreting the results. In any case, we agree with the comments of the reviewer and further clarification of the results in terms of transport and dissipation will be done in the revised version.

Notes on response 2: As already mentioned in the notes to the responses of referee 1, a new subsection has been created within the results (3.1) to address only transport processes. Regarding dissipation, a better clarification has been made based on the results obtained and similar studies found in the literature. This last point has been developed further and additional literature has been included in the revised manuscript.

Comment 3: *I would appreciate if the authors discussed possible reasons for low recovery in more detail.*

Response 3: Thank you for this suggestion. The low recoveries of the solutes is precisely a key point in our study and despite the limitations of the experiment, we have very detailed data that can help us better address this question. We agree with your assessment. As such, we will discuss it in greater depth in the revised version: in particular we will discuss recent scientific studies that have shown transformation of UR in contrast to SRB. Br as a salt can anyhow be treated to be chemically inert. Pesticides are known to be affected by biochemical degradation, however most knowledge stems from unsaturated soil and not from wetland sediments.

Notes on response 3: The discussion about the overall low recoveries of solutes at the outlet has been extended on p. 10, L. 16-23 as follows:

“As for the tracers, as expected Br- recovery was the highest, given its most conservative character. Following Br-, SRB showed the greatest recoveries, presumably because this tracer was mostly subject to sorption processes, as evidenced by its behavior in the pore water, and probably because it was more resistant to degradation, as already evidenced in a recent study (Fernández-Pascual et al., 2018). The lowest recovery among the tracers was for UR. In this case, it was assumed that both retention and especially degradation processes were involved in its dissipation. Overall, these results were in agreement with field studies performed in wetland systems where recoveries for UR were lower than for SRB and were explained by a higher incidence of degradation processes (i.e., photodegradation) on UR dissipation compared to SRB (Passeport., 2010; Lange et al., 2011; Schuetz et al., 2012).”

And on p. 10, L. 37-40 as follows:

“Overall, a possible explanation for the high elimination rates obtained in our experiment could be the fact of having promoted the contact of solutes with the medium through a long period of stagnation (i.e., about two months in each run). In this regard, a recent study performed by Gaullier et al. (2019) has reported almost total mitigation of pesticides and their TPs during stagnation (over 50 days) in constructed wetlands.”

Comment 4: *What about the formation of other transformation products – is this likely, are other TP known that might be formed under the given circumstances?*

Response 4: We are grateful for this comment. Indeed, although met-ESA and met-OA are reported to be the major metabolites of metazachlor, it is possible that other transformation products formed in our system. However, such minor compounds were most likely below the limit of detection and therefore could not be identified. This information will be mentioned in the revised version.

Notes on response 4: This point has been clarified on p. 7, L. 40-41 and p. 8, L. 1-2 as follows:

"It should be noted that other transformation products may have been formed in our system. However, such compounds were most likely below the limit of quantification (≤ 9.29 and ≤ 10.28 µg L-1 for p-Chlorobenzoic acid and 1,2,4-Triazole, respectively), and therefore, they could not be identified."

Comment 5: *If sorption is a major pathway, why have the substances not been detected in sediment/plants?*

Response 5: We are sorry for not making this point clear enough. The hydrological tracers were detected in the sediment/plants. Only the pesticides and their TPs could not be measured in this compartment because a quantitative method was lacking. We are aware of the importance of such information in unveiling the fate of the solutes. However, this again points to the advantage of using tracers instead of pesticides, as they are easier to be measured. The data provided in our study allowed us to build an overall view of the solutes behavior with great spatial and temporal detail. Moreover, we believe that our study represents a first approximation in this regard, and further experiments need to be done. We will enlarge the discussion in this point.

Notes on response 5: We have better explained this point on p. 6, L. 30-34 as follows:

"In the present study pesticides and their TPs could not be measured in the sediment and plants because a quantitative method was lacking. This issue pointed to the advantage of using tracers instead of pesticides because they are generally easier to be measure. Statements on the behavior of pesticides in the compartments where they could not be measured were made according to their physicochemical properties, the results of the breakthrough and recovery curves, their comparison with the tracers and the findings of similar studies."

Comment 6: *Which other pathways are possible, especially for the substances that are not likely to be adsorbed or degraded?*

Response 6: We thank the referee for this important comment. In our study, we have speculated that plant uptake could be an important dissipation pathway. Mineralisation of the compounds to CO_2 may be another possible pathway, although according to literature (EFSA, 2008) the mineralisation of the compounds is generally minimal and slow. As for possible volatilisation from aqueous systems/soil water, only limited losses can be expected, based on the same literature. This information will be discussed more in depth in the revised version.

Notes on response 6: The information has been included and discussed on p. 12, L. 4-6 as follows:

"Other dissipation pathways, such as mineralisation of the compounds to CO2 or volatilisation from aqueous systems/soil water were not ruled out. However, they were considered to be minimal according to literature (EFSA, 2008)."

Comment 7: *Can the expected degradation/mineralisation be quantified using literature values, and contrasted with the measurements?*

Response 7: We appreciate the observation, but an exact quantification would imply the application of modeling approaches. As already mentioned in the response to comment 8 of reviewer 1, modeling the data to do predictions is a distinct topic and not the purpose of the present manuscript. Therefore, it will be treated separately in a forthcoming study.

Comment 8: *With regard to the transformation/degradation of UR:*

(1) How much of the degradation was possibly due to photolysis in the inlet container or at the system's surface?
(2) Could you estimate photolysis rates quantitatively?
(3) Microbial degradation of UR seems not to be enhanced after the first application, as illustrated by the similar recovery rate in the second part. If the system has not been exposed to UR before, and microbial decay was a major pathway, the first application of tracer probably would have fostered the microbial community capable of degrading the dye (Käss 1998), which would have enhanced microbial decay in the second run.
(4) Can alteration of fluorescent properties be a reason for apparent loss of UR (pH values are given in Table 2, but apparently only one measurement)?

Response 8-(1): We thank the reviewer for raising this important point. Possible photolysis of the compounds in the inlet container was discarded, because this part of the system was covered to avoid exposure to light. As for the system's surface, we have assumed that photolysis of UR most likely took place. We will try to estimate a possible loss in the revised version.
Response 8-(2): One could estimate how much UR was lost during the first saturation phase according to the concentrations measured at the vegetated part of the uppermost layer. Light decay can be estimated assuming first order loss and half-lives from literature. This information will be included in the revised version.

Notes on response 8-(2): The estimated values are detailed and discussed on p. 11, L. 27-31 as follows:

"Assuming first-order decay, we obtained degradation coefficients of 0.05 and 0.17 days-1, and half-life times of 13 and 4 days for the first and second run, respectively. These values were comparatively lower than the half-life times reported in the literature, that are in the range of 11 hours (Leibundgut et al., 2009). However, natural light conditions could not be achieved in the laboratory and this could have limited UR photodegradation."

Response 8-(3): Regarding the possible microbial degradation of UR, we speculated that the missing percentage of the final mass balance was mostly due to abiotic degradation (i.e., photodegradation). Nevertheless, we have also hypothesized that possible microbiological degradation of UR took place, but to a lesser extent. The fact that it was not enhanced after the first application could be due to the probable existence of other preferred substrates for microbial degradation. These preferentially utilized compounds would have limited the degradation of alternative substrates such as UR. This is an important point that will be enlarged in the discussion of the revised version, since we also expected a more intense biodegradation of UR during the second execution of our experiment.

Notes on response 8-(3): The discussion about microbial degradation has been addressed on p. 11, L. 15-23 as follows:

"UR, on the other hand, displayed comparatively lower recoveries, and based on the small amounts found in the sediments, sorption processes were not relevant for its dissipation. Thus, photodegradation and, to a lesser extent, (bio-)chemical transformation were most likely the major dissipation pathways for UR. Indeed, the contribution of (bio-)chemical transformation to UR dissipation has already been reported in other long-term studies (Maillard et al., 2016; Fernández-Pascual et al., 2018; Lange et al., 2018). Due to the likely adaptation of microorganisms to UR degradation after being exposed in the first run, we would have expected lower recovery rates in the second run (Käss 1998). However, the final recovery values of UR were similar in both runs (31.71 and 29.82% for the first and second run, respectively). Hence, we hypothesized that other substrates for microbial degradation were present in the system and were preferentially utilized limiting the degradation of alternative substrates such as UR."

Response 8-(4): The possible alteration of the fluorescent properties of UR is ruled out because the pH of the samples was always raised with buffer solution during the measurements. Unfortunately, the pH of the sand could only be measured at the end of the experiment when the sediment samples could be extracted. That is why we only provide one measurement.

Comment 9: *The different substances were mixed in one solution for application, but possible interactions of the applied substances are not discussed.*

(1) Can interactions of the different organic compounds be ruled out?

(2) Testing a reference sample of the injection solution repeatedly over time could give further hints on interactions or degradation rates of the mixture.

Response 9-(1): It is true that the possible interaction between the applied substances is not discussed in the manuscript and cannot be completely ruled out. The solution was prepared immediately prior to the injection. Tracer concentrations were measured inside the solution during the same day and a couple of days after and no significant changes were observed. On the other hand, the pesticides were mixed in one solution in the laboratory and no interaction of the substances was detected. We will discuss this point in the revised version.

Notes on response 9-(1): This point has been indicated on p. 4, L. 2-4 as follows:

"The solution of tracers and pesticides was prepared immediately before the injection. To control possible interactions between substances, the concentrations in the solution were measured on the day of the injection and a couple of days after."

Response 9-(2): Testing the injection solution over a long time was not considered. On the one hand because it was not possible to prevent the solution from being degraded by unknown microorganisms, and on the other hand, because the results of a possible degradation/interaction in a bottle could not be transferred to a system with different conditions and greater complexity.

2. Flow paths and preferential flow

Comment 10: *The flow paths during the tracer application are not completely clear to me.*

(1) From Fig. 3 it appears as if the tracer solution was applied to the surface near the inlet container by letting the inlet container overflow.
(2) The arrows indicate vertical movement downwards near the inlet, and vertical movement upwards elsewhere. Is the ponding on the surface from surface flow from the inlet, or from upward flow through the soil? In case of the former: Has air been entrapped in the system?

Response 10-(1): We apologize for the confusion. In fact, the arrows on the surface of Fig. 3-1) only indicate the direction of the flow but do not represent the actual movement of the water during the injection and therefore can be misleading. These arrows will be eliminated in the revised version.

Notes on response 10-(1): The arrows in Fig. 3-1) have been corrected (see below).

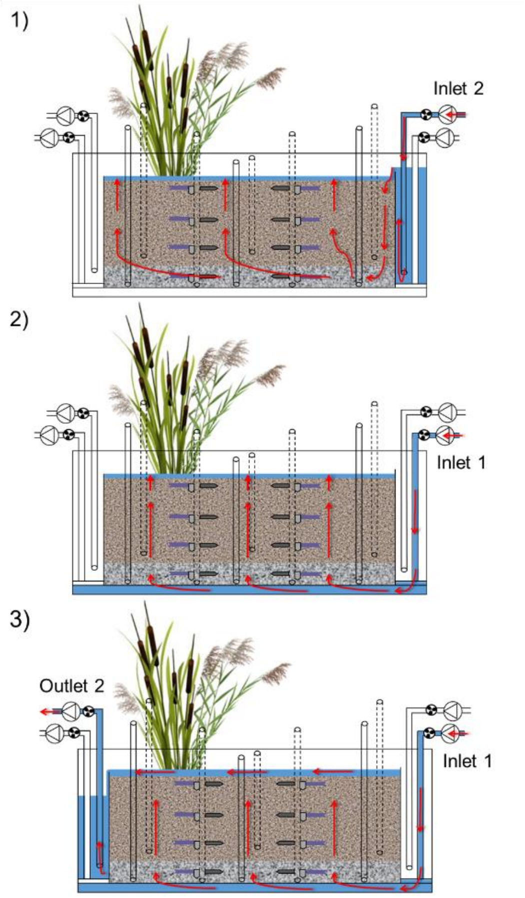

Figure 3: Front view of the model constructed wetland system showing the execution of the injections (red arrows indicate the direction of the water flow): (1) surface injection of tracers and pesticides, (2) injection of clean water (tap water) from the bottom, (3) flushing of the system with clean water (tap water) from the bottom.

Response 10-(2): That is correct, the injection solution was applied to the surface near the inlet by letting the inlet container overflow. Due to the low flow rate the solution moved first downward near the inlet and then upward as the system was filling up. The ponding on the surface is coming from the upward flow. Therefore, possible entrapment of air in the system can be ruled out. We will clarify the flow paths by a more detailed description in the revised version.

Notes on response 10-(2): The description of the surface injection has been detailed on p. 4, L. 12-16 as follows:

"The surface injection (i) was performed after having drained the system. The solution was constantly pumped into the inlet reservoir. Then, it overflowed it and enter the sediments bed. Due to the low flow rate the solution moved first downward near the inlet and then upward as the system was filling up. The inflow was maintained until the system became saturated and the upward flow formed a surface ponding of approximately two centimeters height. In this way, possible entrapment of air in the system was avoided."

Comment 11: *A great part of the transport in the experiment has been attributed to preferential flow in the upper and lowermost layer. Given the coarse texture of the soil, hydraulic conductivity will be high and lead to fast regular flow rates already.*

(1) Is the observed breakthrough still considered preferential when compared with expected flow rates using conductivity and hydraulic gradient?

(2) If preferential flow is an issue, how would that influence the spatial distribution of substances in the sediment, and in turn the recovery of substances from sampling sediment?

Response 11-(1): We thank the referee for this important comment. Yes, we consider that the obtained breakthrough curves are due to non-uniform movement of water through the soil as a consequence of the system design and the presence of plants. This statement is based on the obvious differences in concentrations observed and the faster arrival of solutes to the lower and uppermost layers compared to the middle layers.
Mean flow velocity can be estimated if we assume uniform flow, but a comparison with expected velocities for each curve cannot be made without modeling the data. And, as already indicated in previous comments, this was not the purpose of the present manuscript.

Response 11-(2): Indeed, the observed distribution of substances in the sediment and their recovery is in agreement with the formation of preferential flow in the upper and lowermost layers.

Notes on response 11-(2): After carefully reviewing the data we have realized that certainly with the type of sediment we have used in our experiment it is very unlikely that preferential flow towards the bottom will occur. That is why we have eliminated such statement from the manuscript. However, we still believe that transport of solutes may have been favored towards the vegetated surface as there are strong indications that support it.

3. Correlation analysis

Comment 12: *Much of the interpretation is based on a correlation analysis.*

(1) *Please describe in bit more detail what was correlated – I expect you used measured concentration time series?*

(2) *Sorption is significant for some of the substances. How would retardation affect the results of the correlation analysis?*

Response 12-(1): We apologize for the lack of detail in this section and the revised version will include a better description. That is correct, we used measured concentration time series for the correlations.

Response 12-(2): We have hypothesized that the shape of the breakthrough curves will be affected by the retardation of the solutes resulting in non-significant or non-existent correlations particularly with Br. We will clarify this dependence in the revised version.

Notes on response 12-(2): This point has already been commented in the notes on response 23 of referee 1.

Specific comments

Comment 13: *P6, LL 33-35: This part is unclear.*

(1) *How does the design of the inlet cause preferential flow towards the bottom?*

(2) *And what is meant with "plants channel flow to the surface" – flow from lower layer to the soil surface? Or do you mean enhanced infiltration from the surface?*

Response 13-(1): We appreciate this comment. Obviously, we did not make this point clear enough and will clarify this in the revised version. The inflow system (by overflowing the inlet reservoir) and the low flow rate are believed to be the origin of preferential flow. Such design caused the injection solution to slide down the inlet glass wall channeling the water towards the bottom.

Notes on response 13-(1): See above notes on response 11-(2).

Response 13-(2): As for the plants, we hypothesized that they likely facilitated the transport of solutes along the root channels from the bottom to the surface layer. Besides this, the plants may have also introduced heterogeneities in the medium that have contributed to the formation of preferential flows. However, in agreement with comment 16 (see below), there could be other explanations for this phenomenon that are not necessarily related to the presence of plants. This will be addressed in the revised version.

Notes on response 13-(2): We have addressed this statement on p. 7, L. 11-18 as follows:

"Yet, according to Fig. 5, the uppermost layer displayed a delayed breakthrough peak with relative concentrations of Br- about three times higher than the maximum detected in the bottom (see also Table 4). In addition, the maximum values reached in the vegetated zone of the uppermost layer were twice as high as those of the non-vegetated, although these differences were not that pronounced in the second run. Hence, it was speculated that the plants, and more specifically the roots contributed to the formation of channels through which the transport of solutes was favored towards the vegetated surface. In this context, transport by preferential flow may have occurred along the macropores formed by the root system. However, the results were inconclusive and other mechanisms, not necessarily related to plants (e.g. fingering), may have been involved too."

Comment 14: *P6, LL39-40: Consider rewriting sentence. Br- had almost complete recovery and was found in plants and roots, so you may delete "possibly", and refer to Fig. 8 and not only the lack of measured Br- in pore water.*

Response 14: Thank you for this suggestion. We agree with your assessment. As such, we will rewrite the sentence as proposed.

Comment 15: *P7, L4: "Early Breakthrough" – compare with expected flow velocity (see comment above)*

Response 15: We appreciate this remark. Yet, as stated in comment 11-(1), a comparison between the estimated mean flow velocity and the expected flow velocities for each curve is not possible without applying modeling approaches.

Notes on response 15: This point has already been commented in the notes on response 24 of referee 1. Here, we have clarified that the term "early breakthrough" has been removed from the text because it can be misleading.

Comment 16: *P7 L12: "absence of BTC in middle layer" and "early BTC in uppermost layer" "confirmed the influence of plants" is too strong as a statement. Other explanations are possible for these observations – preferential flow without the influence of plants (fingering), bias in the observations, etc.*

Response 16: We agree, and the statement will be corrected so that other possible explanations to the observations will be discussed.

Notes on response 16: See above notes on response 13-(2).

Comment 17: *P7 L 14 "evidenced" – too strong as well. It might be a hint but could also be that the degradation is just a function of time, and transport over that time ended in the vegetated part, opposite from the inlet.*

Response 17: We thank the reviewer for pointing this out. This sentence will be corrected too.

Notes on response 17: The sentence has been corrected on p. 8, L. 28-32 as follows:

"Metazachlor TPs were only found in the uppermost layer and their maximum relative concentrations were measured in the vegetated part after the promotion of aerated conditions. It should be noted, however, that the process of transformation may have been a function of time, and transport over that time ended in the vegetated part of the uppermost layer. Hence, the uppermost layer (possibly the vegetated part) and the end of the drying phase may have constituted hot spots and hot moments for transformation processes, respectively."

Comment 18: *P7 L23-27/Table 4: How significant are the differences in recovery of Br- given in Table 4, which is the basis for your argumentation here? The differences do not appear large enough to justify the conclusion.*

> **Response 18:** We are grateful for this comment. The percentage of total Br recovered from the different depths was used to support the statement about the possible affectation of the system's performance due to changes in the density of the roots and/or spatial distribution. However, we agree that the differences in recovery between the first and second run are not big enough to justify such conclusion. Therefore, we will refrain from this conclusion in the revised version.

Comment 19: *P8, L 36: Please explain "low leaching potential" as a property of a substance – does that mean high sorption?*

> **Response 19:** We appreciate this comment. "low leaching potential" means that the substance is less likely to move through the soil, but not only because of sorption, as this index is based on the chemical's adsorption (Koc) and persistence (DT50) in the soil. We will clarify this in the revised version.
>
> Notes on response 19: This information has been clarified on p. 10, L. 11-15 as follows:
>
> "Hence, we have hypothesized that the cause of the lower recoveries of boscalid and penconazole could have been their low leaching potential (USEPA, 2003; European Food Safety Authority (EFSA), 2008; Marín-Benito et al., 2015), which is based on their chemical's adsorption (Koc) and persistence in the soil (DT50). Yet, other causes, such as a higher incidence of plant uptake could not be ruled out."

Comment 20: *P9, L 38: "could be identified" – an unambiguous identification was unfortunately not possible in the experiment, but valuable hints / indications were collected*

> **Response 20:** We thank the reviewer for this suggestion. The sentence in the revised version will be changed as proposed.
>
> Notes on response 20: The corresponding change has been made on p. 13, L. 5-6.

Comment 21: *P10, L 5: "biochemical transformation had a major contribution" – only <10 % of the parent substance were found as TP, so it is not possible to say which was a major contribution*

> **Response 21:** While it is true that only <10 % of metazachlor was found as TPs; the recoveries of this pesticide were the lowest among the solutes. This result, together with the physicochemical properties of metazachlor could be a hint that transformation/mineralisation might have played an important role in its dissipation. Nevertheless, it is true that we do not have enough information to justify such statement. Therefore, we will be more careful with this statement in the revised version.
>
> Notes on response 21: This statement has been rewritten on p. 13, L. 18-20 as follows:

"The detection of metazachlor TPs, namely met-ESA and met-OA demonstrated that biochemical transformation played an important role in metazachlor dissipation,"

Comment 22: *Fig 4: How do you explain the obvious differences in Br- breakthrough between the first and second run?*

Response 22: We thank the referee for pointing this out. This difference has been attributed to possible changes in the density of the roots and/or spatial distribution over the experiment. This statement is based on the greatest development of the roots observed in the system at the end of the study (see Fig. 5 below). We will include this discussion in our revised version.

Notes on response 22: This point has already been commented in the notes on response 29 of referee 1.

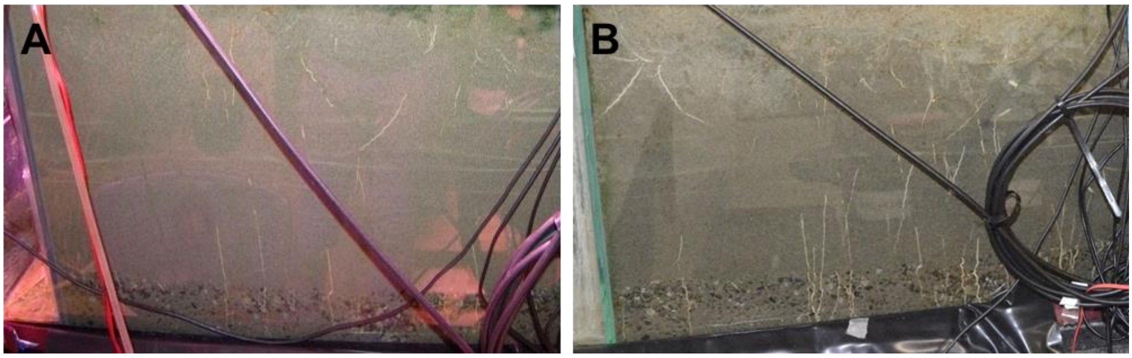

Figure. 5: Front view of the root system in the vegetated part of the model constructed wetland before the first run A) and at the end of the second run B).

Comment 23: *Fig 7: Recovery of TP in % - how can the total amount be known?*

Response 23: We apologize for the lack of clarity in this case. The recovery of TPs has been calculated according to the total amount of parent compound injected. This will be defined in the revised paper.

Notes on response 23: This information has been clarified in Fig. 10 as follows:

"The mass balance for the TPs was calculated according to the total amount of parent compound injected."

Comment 24: *Fig. 8: Please comment on the large error bar for SRB:*
(1) Which indicates recovery to be between 48 and 105 %.
(2) Would more sediment samples have reduced this uncertainty?
(3) How does this uncertainty influence your interpretation?

Response 24-(1): We appreciate this comment. The recovery measured in the sediment of the vegetated part has a large error bar due to the heterogeneous distribution of the tracer. That is, almost 99% of the tracer measured in the vegetated part is located in the uppermost layer. This heterogeneous distribution indicates that the tracer was transported preferentially to this layer, as discussed in the manuscript.

Response 24-(2): We thank the referee for raising this important question. In fact, we collected a great number of sediment samples to reduce the uncertainty**:** A total of 16 sediment cores (four per longitudinal and four per lateral transect) that were divided into four fractions, each representing a different sampling depth (0-8 cm, 9-20 cm, 21-32 cm, 33-42 cm). This gives a total number of 64 sediment samples. We think that this number is adequate for the system.

Response 24-(3): In our case, we believe that it does not constitute a major factor of uncertainty. As indicated in the previous response, we have a measurement for each longitudinal/lateral transect and sampling depth, covering practically the whole sediment. This gives us a detailed picture of the distribution of the tracers in the system.

Technical comments

Comment 25: *P2, L32: 100 mg L -> 100 mg* L^{-1}

Response 25: The indicated change will be made in the revised version.

Notes on response 25: The corresponding change has been made on p. 3, L. 9.

Comment 26: *P2, L35: Please give the dimensions of the constructed wetland system also without inlet/outlet.*

Response 26: The dimensions will be provided in the revised version.

Notes on response 26: This information has been provided in Fig. 1 (see below).

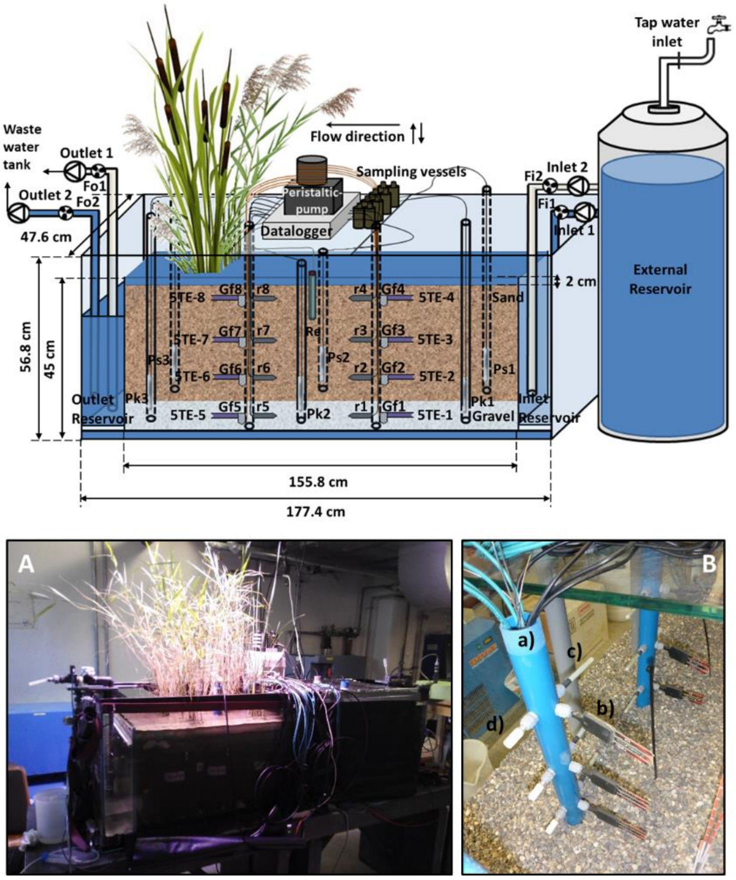

Figure 1: Schematic representation of the model constructed wetland system (not to scale). Fi1 and Fi2 indicate the flowmeters at the inlet; Fo1 and Fo2, the flowmeters at the outlet; Ps(n), piezometer in the sand; Pk(n), piezometer in the gravel; 5TE-(n), soil moisture, temperature and electrical conductivity sensor; r(n), platinum redox electrode; Re, reference electrode (Ag:AgCl); Gf(n), glassfilter. For the piezometers, n indicates the position with respect to the inlet; n=1, close to the inlet; n=2, in the middle of the sediment bed and n=3, close to the outlet. For the sensors installed in the multi-level pipes, n indicates the zone and the depths where they are located; n = 1, 2, 3 and 4, non-vegetated zone at a depth of 39, 27, 15 and 3 cm, respectively; n = 5, 6, 7 and 8, vegetated zone at a depth of 39, 27, 15 and 3 cm, respectively.

(A) front view photograph of the model constructed wetland system; (B) detail of the multi-level pipes: (a) multi-level pipe at the vegetated zone, (b) 5TE sensor, (c) redox electrode and (d) glass filter.

Comment 27: *P5, L11: resulted curves -> resulting curves*

Response 27: The indicated change will be made in the revised version.

Notes on response 27: The corresponding change has been made on p. 5, L. 33.

Comment 28: *P5, L13, and elsewhere throughout the text: Br -> Br^-*

Response 28: The indicated change will be made in the revised version.

Notes on response 28: The corresponding change has been made throughout the manuscript.

Comment 29: *P7, L26: "was most likely" -> "were most likely"*

Response 29: The indicated change will be made in the revised version.

Notes on response 29: The corresponding change has been made on p. 7, L. 41.

Comment 30: *P8, L28: "were classified": classified for what (recovery rate, I presume?)*

Response 30: Yes, that is right, the classification is for the recovery rate. We apologize for the confusion. The sentence will be improved.

Notes on response 30: The sentence has been improved on p. 10, L. 1-2 as follows:

"According to the total amount of tracers and pesticides recovered at the outlet after the flushings (Table 6), the solutes were classified as follows (from highest to lowest recovery rate): Br- >> SRB >> UR >> Boscalid >> Penconazole >> Metazachlor."

Comment 31: *Fig 4: Consider duplicating the figure and display vegetated and non-vegetated parts separately, which would make distinguishing these parts a lot easier*

Response 31: Thanks for the suggestion. We agree with your assessment, and as already indicated in the response to the comment 35 from reviewer 1, we will duplicate the figure to show separately the vegetated and non-vegetated parts.

Notes on response 31: This point has already been commented in the notes on response 35 of referee 1.

References

Comment 32: Käss, W. (1998): Tracing technique in geohydrology, 581 pp., Balkema, Rotterdam, The Netherlands.

Response 32: The indicated change will be made in the revised version.

Notes on response 32: The corresponding change has been done on p. 15, L. 29.

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Hydrological tracers for assessing transport and dissipation processes of pesticides in a model constructed wetland system

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Abstract. ~~Hydrological tracers have been recently used as a low-cost approach to study the fate and transport of pesticides in constructed wetlands. Yet, internal temporal and spatial mechanisms that dominate their transport and dissipation in such environments are still not fully understood~~Studies that have used hydrological tracers to investigate the fate and transport of pesticides in constructed wetlands have often considered such systems as a "black box". Consequently, internal temporal and spatial mechanisms that dominate pesticides transport and dissipation (e.g. sorption, transformation, plant uptake) are still not fully understood. Here we present a novel approach that combines the use of tracers with different sorptive and reactive properties (i.e., bromide (Br^-), uranine (UR) and sulforhodamine B (SRB)) with high vertical-resolution sampling and monitoring to evaluate transport and dissipation processes of three selected pesticides (boscalid, penconazole and metazachlor) inside a wetland system on a long term basis and detailed spatial scale.~~We have applied hree tracers with different sorptive and reactive properties bromide (Br), uranine (UR) and sulforhodamine B (SRB)) to investigate dominant temporal and spatial transport and dissipation processes of three selected pesticides: boscalid, penconazole and metazachlor in a model constructed wetland system designed to perform high vertical-resolution sampling and monitoring on a long-term basis.~~ Moreover, the influence of vegetation and alternating different hydrologic conditions on transport and dissipation processes were evaluated by comparing a vegetated with a non-vegetated section of the wetland system and by alternating periods of saturation and drying, respectively. ~~The experimental observations revealed that two different preferential flow paths developed, one due to the constructional design of the inflow and the other one due to the influence of the free water at the surface along with the plants.~~ Breakthrough curves obtained at different sampling depths pointed out that the solutes were not equally distributed within the constructed wetland, Data also revealed that a higher mass of solutes was transported to the vegetated part of the uppermost layer, which was associated with a possible shortcut effect produced by the roots, whereas the middle layers showed retardation possibly due to the presence of water filled pores before the injections and low pore connectivity. Transport of solutes was driven by the injections. ~~and dominated for~~ The strong temporal and spatial correlation found between Br^-~~Br~~, UR and metazachlor indicated that these solutes experienced more transport than SRB, boscalid and penconazole, which most likely underwent sorption, as evidenced by their absence in the middle layers, rapid decrease in their concentrations after the injections and similar gradual increase in accumulated mass recovery at the outlet. The overall ~~final~~ tracer mass balance allowed us to identify three dissipation pathways: sorption, transformation and plant uptake~~highlighted that the main dissipation pathways were sorption, transformation and plant uptake~~. The detection of metazachlor transformation products (TPs) confirmed the contribution of this process ~~transformation~~ to metazachlor dissipation, whereas no TPs for boscalid and penconazole ~~mainly experienced sorption processes.~~were found. Yet, their transformation could not be ruled out in the present study. Hot spots of sorption and transformation were found in the uppermost layer, whereas hot moments were detected at the beginning of the experiment for sorption and after promoting aerated conditions for transformation. ~~Sorption was detected immediately after the injection of solutes, while transformation was enhanced by the presence of plants and the promotion of aerobic conditions.~~ The use of hydrological tracers ~~together with selected pesticides and~~ coupled with high vertical-resolution sampling and monitoring proved to provide valuable information about transport vectors and dissipation processes of pesticides inside a constructed wetland ~~in a vegetated redox-dynamic environment on a long-term basis and detailed spatial scale~~. This study represents a first

approximation, and further experiments need to be done under field conditions together with mathematical modeling in order to understand the complex processes that undergo pollutants inside constructed wetlands.

1 Introduction

Pesticides are widely used to protect crops and increase their yields around the world. It is well known that their use might result in ecotoxicological effects in non-target environments (Stehle and Schulz, 2015a). Chemical analysis performed in the waters of European countries revealed that pesticides are often detected in surface waters (Müller et al., 2002; von der Ohe et al., 2011; Casado et al., 2019). This problem becomes even more severe when the pesticides produce a variety of transformation products (TPs), whose behavior is unknown and toxicity or persistence may be,~~is~~ ~~unknown~~in some cases, greater than the parent compound. In fact, the presence of TPs in water bodies has ~~also~~ already been reported in numerous studies (Kolpin et al., 2004; Eurostat, 2012; Reemtsma et al., 2013).

Buffer zones emerged as a measure for controlling water pollution. Constructed wetlands are one example of buffer zones, where the removal of pesticides takes place. Indeed, constructed wetlands are designed to simulate and take advantage of processes that occur in natural wetlands (Vymazal et al., 2005), such as sedimentation, photolysis, hydrolysis, adsorption, microbial degradation and plant uptake (Vymazal et al., 2015). In these systems vegetation plays an essential role promoting sedimentation by reducing the current velocities of the water (Petticrew and Kalff, 1992), providing a substrate for microorganism in the roots and rhizomes (Hofmann, 1986) and creating oxidized conditions in the roots that stimulate aerobic decomposition (Brix, 1997). Removal processes in constructed wetlands may also be promoted through intermittent water flows by enhancing aeration and by providing different redox conditions suitable for the growth of different microbiological communities (Ong et al., 2010; Maillard et al., 2011; Fan et al., 2013).

The mitigation capacities of buffer zones have recently been studied by using hydrological tracers as a low-cost approach. ~~Indeed, several studies have investigated transport and dissipation of pesticides~~ In this context, fluorescent tracers (e.g. uranine (UR), sulforhodamine B (SRB)) have often been chosen to study transport and fate of pesticides because they are organic molecules, non-toxic and easy to be analysed. For instance, some authors have used them in wetlands (Passeport et al., 2010; Lange et al., 2011; Durst et al., 2013; Maillard et al., 2016) and farm ditches (Dollinger et al., 2017). Yet, in most cases where this approach has been applied, ~~studies~~ the system under study has~~ve~~ been treated ~~the systems~~ as a "black box" ~~es~~ ~~and~~ where the time scales were typically limited to the time spans of the tracers breakthroughs at the systems outlet. Hence, internal temporal and spatial mechanisms that dominate pesticides transport and dissipation (e.g. sorption, transformation, plant uptake) ~~in buffer zones~~ are still not fully clear~~,~~. ~~especially when it comes to different redox regimes, long time scales and the presence of vegetation~~. Moreover, information on the fate and, particularly, transformation of pesticides inside wetland sediments is still limited.

Therefore, the objectives of this study are i) to apply a multi-tracer approach together with high vertical-resolution sampling and monitoring to identify ~~temporal and spatial~~ transport patterns and dissipation processes of three pesticides selected as test substances inside a model constructed wetland system ~~by applying a multi-tracer approach together with high vertical-resolution sampling and monitoring on a long-term basis~~; ii) to compare the temporal and spatial behavior of the applied tracers with ~~three~~ the pesticides ~~selected as test substances~~ and evaluate their main dissipation pathways; and ii~~i~~) to assess the influence of vegetation and alternating ~~the alternation of~~ different hydrologic conditions (saturated and unsaturated) on transport and dissipation processes.

Our study is one of the first to look at the solutes behavior inside a constructed wetland on a long-term basis and detailed spatial scale. With this experiment we expect to provide new insights about the potential of hydrological tracers to evaluate transport and dissipation processes of pesticides. Likewise, we seek to extend the knowledge on the mitigation capacities of constructed wetlands with our approach.

The ~~present~~ experiment was conducted in a constructed wetland with one half planted with two common wetland plants and the other half unplanted (control). The constructed wetland was equipped with a system designed to perform high vertical-resolution sampling and monitoring on a long-term basis. Three hydrological tracers were chosen as reference substances according to their reactive nature: bromide (Br^-~~Br~~) as a non-adsorbing tracer (Whitmer et al., 2000), ~~uranine (~~UR~~)~~ as a photosensitive tracer (Gutowski et al., 2015) that can undergo processes of (bio-) chemical transformation (Lange et al., 2018) and ~~sulforhodamine B (~~SRB~~)~~ as a highly sorptive tracer (Kasnavia et al., 1999).~~ Whereas,~~ T~~t~~hree ~~commonly applied~~ pesticides were selected as test substances according to ~~with~~ their different physicochemical properties~~ were selected as test substances~~ and frequent detection in a field-based constructed wetland where other studies within the same project were carried out: boscalid (2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide), penconazole ((RS)-1-[2-(2,4-dichlorophenyl) pentyl]-1H-1,2,4-triazole)~~,~~ and metazachlor (2-chloro-N-(pyrazol-1-ylmethyl) acet-2',6'-xylidide).

2 Materials and Methods

2.1 Chemicals

The physio-chemical properties of tracers and pesticides are summarized in Table 1. UR was purchased from Simon & Werner GmbH (CAS-no. 518-47-8), SRB from Waldeck GmbH & Co KG (CAS-no. 3520-42-1) and Br^-~~Br~~ was obtained as sodium bromide from Carl Roth GmbH & Co KG. Boscalid (99.8%), penconazole (99%) and metazachlor (99.7%) already dissolved in acetonitrile (99.9%) were purchased from Neochema (Bodenheim, Germany). The analytical standards of boscalid (99.9 %), penconazol (99.1 %), metazachlor (99.6 %) and p-Chlorobenzoic acid (99%) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). 1,2,4-Triazole (99.5%) was purchased from LGC Standards (Wesel, Germany). Metazachlor-ESA (95 %) and metazachlor-OA (98.8 %), hereunder named as met-ESA and met-OA, respectively, and the internal standard Terbutryn-D5 (98.5 %) already dissolved in acetonitrile (100 µg mL-1) were received from Neochema (Bodenheim, Germany).

The target injection masses of tracers and pesticides for an injection volume of 40 L were calculated according to Durst et al. (2013). Standard stock solutions of 1 g L^{-1} for UR and SRB and of 10 g L^{-1} for Br^-~~Br~~ were prepared in MilliQ water. Pesticides (0.1 g L^{-1}) dissolved in acetonitrile were directly mixed with the injection solution. The concentration of tracers and pesticides in the injection solution was 100 mg L^{-1}, 50 µg L^{-1} and 100 µg L^{-1}, for sodium bromide, UR and SRB, respectively; and 50 µg L^{-1} for boscalid, penconazole and metazachlor.

2.2 Design of the model constructed wetland system

The model constructed wetland system consisted of a glass tank 177.4 cm long, 47.6 cm wide and 56.8 cm deep (Fig. 1). The system was divided into three parts, two of them inlet and outlet reservoirs located at both ends and separated by two glass walls and a third part located in the middle consisting of the main bed of sediments. The bottom was filled with 10 cm of gravel (grain size 4-8 mm) and topped with 32 cm of sand (grain size 0.01-2 mm). The characteristics of the system are given in Table 2A. One half of the system was left unplanted (control), while the other half was planted with two species of widespread and ubiquitous wetland plants (*Typha latifolia* and *Phragmites australis*) that were purchased from a local garden center with an average initial height of 79.8 cm ± 18.6 and 76.9 cm ± 10.1, respectively. The whole experiment was carried out indoors in a laboratory assembly, therefore 64 OSRAM SSL 3W light-emitting diode lamps for plant growth (Purple Alien 2.0, LED Grow Shop, Germany) were installed with daily photoperiods of 11 hours.

The inlet and outlet were intended to create vertical water flows. This was achieved through the installation of two pairs of peristaltic pumps coupled to Plexiglas pipes (15 mm diameter) that were connected to the bottom of the system; and another two pairs of peristaltic pumps coupled to Plexiglas pipes that channelled directly into the inlet and outlet reservoirs, respectively. A tank with a capacity of 350 L that was connected to the tap water served as external inlet reservoir, while a second tank with a capacity of 1000 L received the waste water. In order to monitor the water level, three pairs of PVC observation pipes (DN:

35-40 mm, STÜWA Konrad Stükerjürgen GmbH, Germany) with a length of 50 cm, were arranged symmetrically on both sides at the center line of the system. One half of the pipes were located at the gravel layer and the other half in the sand.

With a view to obtaining pore water samples at a high vertical resolution, a multi-level pipe was designed with a sampling resolution of 12 cm. This created a total of four sampling depths that ranged from the gravel to the uppermost layer of the sand. Two multi-level pipes were installed in the sediment bed, one at the non-vegetated and the other one at the vegetated half of the system. Small glassfilters (12.5 mm diameter, porosity 2, ROBU, Germany) were installed in both multi-level pipes at each sampling depth. The filters were connected to a multichannel peristaltic pump (Pulse-free flow peristaltic pump, Gilson, France) via capillaries made of stainless steel (1/16" inner diameter, Swagelok, Germany) that were directly inserted into TYGON tubes (ID: 1.02 mm, Proliquid, Germany). In addition, 5TE sensors (Decagon Em50 serie, Campbell Scientific) and redox probes (Paleo terra, Amsterdam, The Netherlands) were installed at the same depths in both multi-level pipes. Glassfilters, sensors and probes were separated from each other at an angle of 90 degrees at each sampling depth (Fig. 1B). Furthermore, a reference electrode (Ag:AgCl) connected to the redox probes, was inserted in the sediment between the multi-level pipes. All sensors and probes were connected to a datalogger (CR1000, Campbell Scientific).

2.3 Operation of the model constructed wetland system

The model constructed wetland system was designed to alternate saturated with unsaturated conditions (long periods of aeration). In total three phases were created (Fig. 2): 1) saturation with target substances (one week), 2) drying by evapotranspiration (three weeks) and 3) saturation with clean water (one month). The saturation phases were preceded by the injection of solutes or clean water (tap water). The operation of the constructed wetland is summarized in Table 2B. The solution of tracers and pesticides was prepared immediately before the injection. To control possible interactions between substances, the concentrations were measured inside the solution during the injection day and a couple of days after. Prior to the injection ~~of tracers and pesticides~~, the system was drained until field capacity was reached.

The whole experiment lasted seven months (from March 2017 to October 2017), during which two identical experimental runs were performed. The first run (from March 9 to May 9, 2017) was followed by a resting period of about three months (from May 9 to August 1, 2017), during which occasional water additions to maintain the vegetation were carried out. After this, the second run (from August 1 to October 3, 2017) was conducted.

The execution of the injections is shown in Fig. 3. Three injections took place in each run of the experiment: (i) initial surface injection of tracers and pesticides, (ii) injection of clean water (tap water) from the bottom of the system and (iii) flushing of the sediments with clean water from the bottom. The surface injection (i) was performed after having drained the system.

The solution was constantly pumped into the inlet reservoir. Then, it ~~began to~~ overflowed ~~the inlet reservoir~~ it and enter the sediments bed ~~in descending direction~~. Due to the low flow rate the solution moved first downward near the inlet, and then upward as the system was filling up. ~~As the inflow progressed, the solution extended to the rest of the sediments in an upward direction~~. The inflow was maintained until the system became saturated and the upward flow formed a surface ponding ~~surface layer~~ of approximately two centimeters height ~~was formed on top of the sediments~~. In this way, possible entrapment of air in the system was avoided. The second injection (ii) was performed at the end of the drying phase by pumping clean water (tap water) from the bottom. The water flowed evenly through the sediment in vertical upward direction. The inflow was maintained until the system became saturated and a surface ponding ~~a surface layer~~ of approximately two centimeters height was formed ~~on top of the sediment~~. This injection was repeated throughout the second saturation phase in order to keep the system constantly saturated. The flushing of the system (iii) was performed at the end of the second saturation phase and it was intended to recover all mobile fractions of the target compounds. To do this, clean water (tap water) was injected from the bottom and was allowed to flow into the system continuously. Water overflowed the main bed and exited towards the outlet reservoir, from where it was pumped to the waste tank.

2.4 Sampling and monitoring

Pore water samples were collected from different depths twice a week during the experimental runs. The sampling of pore water was performed simultaneously in order to prevent mixing of waters. A volume of 60 mL of pore water was transferred to 100 mL brown glass bottles and stored at 4ºC for major ions and tracers analysis. Previously, a volume of 10 mL was transferred to 15 mL Polypropylene tubes and stored at -20°C for the subsequent pesticide and TPs analysis. Polypropylene was chosen instead of glass because the samples had to be frozen immediately after their collection. Such material has already been used to store pesticides in other studies (e.g. Joseph, 2015). Additional pore water samples were taken before and after the initial injections of tracers and pesticides to account for the background. During the flushing of the system, surface water samples were collected at the outlet and transferred to 100 mL brown glass bottles. Following this, the samples were stored at 4 or -20ºC depending on the type of analysis performed afterwards (major ions and tracers analysis or pesticide and TPs, respectively).

At the end of the experiment, the sediment bed was emptied of its gravitational water. Following this, 16 sediment cores (four per longitudinal and four per lateral transect) were collected by inserting plastic pipes into the sediment. Sediment cores were divided into four fractions, each representing a different sampling depth (0-8 cm, 9-20 cm, 21-32 cm, 33-42 cm). The sediment samples were dried at room temperature for 24 h and stored in the dark for subsequent measurements of tracers, total organic carbon and iron oxides. Then, the plants were removed from the vegetated zone and separated into aerial parts (stems and leaves) and roots. Immediately after they were oven dried at 60ºC for approximately 24 hours and stored in the dark for subsequent measurements of tracers. Biomass was determined on a dry matter basis.

Temperature, soil moisture, conductivity and redox potential were constantly monitored by means of the datalogger with an interval of two minutes throughout the entire experiment. Redox potential was calculated by adding the potential from the reference electrode (Ag/AgCl) to the measured potential (Vorenhout et al., 2011). The final result was corrected for differences in temperature according to Bard et al. (1985).

2.5 Laboratory analysis

2.5.1 Major ions and tracers in the pore- and outlet-water

Pore- and outlet-water samples were measured for major ions (Na^+, NH_4^+, K^+, Ca^{2+}, Mg^{2+}, Br^-, SO_4^{2-}, Cl^-, NO_3^- and NO_2^-) by ion chromatography (Dionex ICS-1100, Thermo Scientific, USA). All samples were previously filtered with a 0.45 µm filter.

Concentrations of the tracers UR and SRB in pore and outlet water samples were measured by fluorescence spectrometry (Perkin Elmer LS 50 B) as previously described (Leibundgut et al., 2009). Briefly, a synchronous scan method was applied with an excitation/emission wavelength difference of 25 nm and target wavelengths of 488 nm and 561 nm for UR and SRB, respectively. Detection limits were 0.05 µg L^{-1} for UR and 0.1 µg L^{-1} for SRB. The entire fluorescent spectrum (from 350 to 600 nm) was analyzed in order to identify different background fluorescent levels and subtract them.

2.5.2 Pesticides and TPs in the pore- and outlet- water

Pore- and outlet-water samples were analyzed for the pesticides boscalid, penconazole, metazachlor and their known TPs (metazachlor-ESA and -OA, p-Chlorobenzoic acid (boscalid), and 1,2,4-Triazole (penconazole)). Acetonitrile (LC-MS grade; VWR International GmbH, Darmstadt, Germany) was used as organic mobile phase in chromatography and for the preparation of stock solutions. Aqueous mobile phase was prepared with ultrapure water (Membra Pure, Germany; Q1:16.6 MΩ and Q2: 18.2 MΩ). Samples were filtered using syringe filter units (CHROMAFIL® Xtra RC-20/25; Macharey-Nagel, GmbH & Co. KG, Germany). Each sample (990 µL) was spiked with 10 µL Terbutryn-D5 as internal standard. Analysis of 5 µL of each sample was done by LC-MS/MS (Agilent Technologies, 1200 Infinity LC-System and 6430 Triple Quad, Waldbronn, Germany). Mobile phases were 0.01 % formic acid (A) and acetonitrile (B) with a flow of 0.4 mL min^{-1}. Gradient was as follows: 0-1 min (10% B), 1-11 min (10-50% B), 11-18 min (50-85% B), 18-21 min (85-90% B), 21-24 min (90% B), 24-26 min (90-10% B) and 26-30 (10% B). A NUCLEODUR® RP-C18 (125/2; 100-3 µm C18 ec) column (Macherey Nagel, Düren, Germany) was used as stationary phase with a set oven temperature of T = 30°C. Limits of detection (LOD) and quantitation (LOQ) were calculated with DINTEST (2003) according to DIN 32645. LOQ/LOD values for pesticides and transformation products are provided in Table 3.

2.5.3 Extraction and measurement of tracers in the sediments and plants

UR and SRB in the sediment (sand) and plants were extracted as described by Wernli (2009). In brief, two grams of the dried material were mixed with 10 mL of ammonia-ethanol solution (40:60, v/v). Dried stems, leaves and roots were previously grinded with a vibratory disc mill (Siebtechnik GmbH, Germany). All samples were shaken on an IKA HS 250 reciprocating shaker for 30 minutes at 240 rpm and stored at 4°C in the refrigerator for at least 24 hours. Afterwards, supernatant was collected, filtered (< 0.45 µm) and measured for the tracers. The resulting~~resulted~~ curves were corrected through interpolation and subtraction of the background signal from the peak intensity as described by Leibundgut et al. (2009).

A different methodology based on McMahon et al. (2003) was used to measure Br^-~~Br~~ in the sediment (sand) and plants to avoid the interference of the ammonia-ethanol solution with the ion chromatograph. Samples were prepared in the same way as previously described, but they were mixed with 20 mL of deionized water instead. Following this, they were shaken on an IKA HS 250 reciprocating shaker for 1 hour at 240 rpm and later centrifuged at 3000 rpm for 30 minutes (Megafuge 1.0R; Heraeus Instruments). Supernatant was then taken, filtered (< 0.45 µm) and measured by ion chromatography (Dionex ICS-1100, Thermo Scientific, USA).

2.5.4 TOC and iron oxides in the sediment

Total organic carbon (TOC) was measured in the sediment (sand) with a CNS-analyser (Vario El Cube, Elementar, Germany) after grinding the dried samples with a vibratory disc mill (Siebtechnik GmbH, Germany). Dithionite-extractable Fe (Fe_d) in the sediment (sand) was extracted according to Mehra and Jackson (1960) and measured using inductively coupled plasma - optical emission spectrometry (Spectro Ciros CCD, Spectro Analytical Instruments GmbH, Germany).

2.6 Data Analysis

Spatial and temporal dynamics of transport processes in the pore water were investigated by analyzing soil moisture data and Br^- breakthrough curves. Here, Br^- was chosen as reference due to its most conservative character. The occurrence and role of retardation was studied by performing cross-correlations between Br^- time series. The predominance of transport processes among the solutes was examined by looking at the relationship between Br^- and the other solutes via correlation matrices of the measured concentration time series. Here, it was assumed that a weak correlation would be due to the prevalence of sorption and transformation rather than transport. This was based on the premise that the solutes would experience retardation due to sorption or attenuation due to transformation. Transformation processes were examined through the detection of TPs. The calculated correlation matrices were also used to analyze the general behavior of the solutes and their relationship in the pore water. Spearman rank correlation coefficients (rho_s) were applied since the data did not fit a normal distribution. Correlations were calculated individually for the vegetated and the non-vegetated zone and the different depths. ~~Spatial and temporal dynamics of transport and dissipation of solutes were studied by analysing their breakthrough curves obtained in the pore water at different depths.~~ The influence of the vegetation and hydrologic conditions on transport and dissipation processes were evaluated by comparing the results of the vegetated with the non-vegetated zones and the results of the different phases~~saturation with the drying phases~~. The performance of the two experimental runs was assessed~~evaluated~~ by ~~means of the~~ making correlations between the Br^-~~Br~~ breakthrough curves of the first and the second run. ~~In addition, transport and plant~~

~~uptake were assessed with the total percentage of Br recovered from the pore water of each experimental run. Here, a distinction between the different zones and depths was made.~~

~~The general behavior of the solutes and their relationship in the pore water throughout the experiment were analyzed by correlation matrices. Spearman rank correlation coefficients (rho$_s$) were applied since the data did not fit a normal distribution. Correlations were calculated individually for the vegetated, the non-vegetated zone and the different depths. Additionally, the predominance of transport processes in the pore water was examined by looking at the correlation between the solutes and Br. Here, a strong correlation with Br was assumed to be due to a prevalence of transport over other processes in the pore water, given the most conservative character of Br.~~

Further comparisons between tracers, pesticides and their TPs were made by analyzing their cumulative recovery curves obtained at the outlet after the flushing phases. The fate of the solutes and their main dissipation pathways were examined~~analyzed~~ with a final overall mass balance that accounted for five different compartments (pore water, outlet water, sediments, stems + leaves and roots). The mass of tracers and pesticides recovered in the pore water was calculated as the sum of the weekly dissolved concentrations multiplied by the volume sampled. The mass of tracers and pesticides recovered in the outlet water was calculated based on the recovery curves obtained during the flushing phases. The mass of tracers and pesticides recovered in the sediments and plants was determined as the concentrations measured in their corresponding compartments multiplied by the total amount of sediments and plants in the system, respectively.

In the present study the pesticides and their TPs could ~~only be measured in the pore water and the outlet water~~ not be measured in the sediment and plants because a quantitative method was lacking. This issue pointed to the advantage of using tracers instead of pesticides because they are generally easier to measure. Statements on the~~ir~~ behavior of pesticides ~~in~~ in the compartments where they could not be measured ~~the remaining compartments~~ were made according to their physicochemical properties, the results of the breakthrough and recovery curves, their comparison with the tracers and the findings of similar studies.~~ reported in the literature~~.

3 Results and discussion

3.1 ~~Breakthrough curves of~~ Transport processes ~~solutes~~ in the pore water according to Br$^-$ behavior

The relative concentrations of Br$^-$ measured at different depths (Table 4) indicated that similar values were reached in the lower- and uppermost layers right after the injection. In contrast, no Br$^-$ was detected in the middle sections. These results were attributed to the conditions previous to the injection (i.e., system at field capacity). In such context, the presence of water-filled pores may have caused heterogeneities resulting in an uneven distribution of solutes within the system. Indeed, soil moisture values (Fig. 4) measured prior to injection (indicated with a red circle) were three to two times higher in the middle sandy layers (15 and 27 cm) compared to the lower- and uppermost layers (39 and 3 cm, respectively). Moreover, such values barely changed over the experiment, not even during the drying phase, which suggested that the water holding capacity of the middle seccions was higher compared to the other layers (at least in the vegetated zone, since there is no data from the non-vegetated). These conditions were associated with a possible lack of connectivity, as already suggested by Nimmo (2012). Consequently, water flow through these sections was most likely inhibited and/or delayed, as evidenced by the initial absence of Br$^-$ (Table 4) and subsequent delay of the breakthrough peaks (see Fig. 5). On the other hand, given that Br$^-$ was detected in the uppermost layer right after the injection (see Table 4), it was assumed that the heterogeneities due to the presence of poorly connected pores were only located in some parts of the middle layers including the surroundings of the sampling ports. In contrast, in the other areas of the system probably matrix flow dominated. Yet, according to Fig. 5, the uppermost layer displayed a delayed breakthrough peak with relative concentrations of Br$^-$ about three times higher than the maximum detected in the bottom (see also Table 4). In addition, the maximum values reached in the vegetated zone of the uppermost layer were twice as high as those of the non-vegetated, although these differences were not that pronounced in the second run. Hence, it was speculated that the plants, and more specifically the roots contributed to the formation of channels through which the transport of solutes was favored towards the vegetated surface. In this context, transport by preferential flow may have occurred along the macropores formed by the root system. However, the results were inconclusive and other mechanisms, not necessarily related to plants (e.g. fingering), may have been involved too.

Lag correlations performed to the Br^- breakthrough curves (Fig. 6) confirmed that a delay in the arrival of solutes to the middle and uppermost layers took place. A significant lag correlation could only be observed in the non-vegetated zone. Specifically, the delays obtained in the middle layers (t = -7 and t = -3 in the first and the second run, respectively) were greater than those obtained in the uppermost layer (t = -5 and t = -2 in the first and the second run, respectively). The peak of solutes in the middle layers coincided in time with the end of the drying phase, and hence, it was related to the likely migration of solutes during the drop in water table from the surface. In contrast, the delayed peak of the uppermost layer was associated with possible low pore connectivity.

The different behavior of Br^- in terms of transport observed in the first and second run was explained by a possible development of the root system. In fact, when correlating the breakthrough curves of Br^- of the first and the second run, differences between the vegetated and non-vegetated zones were observed (Table 5). With the exception of the uppermost layer, the non-vegetated zone showed strong correlation between the two runs regardless of the layer, whereas the vegetated did not show any correlation. This meant that the performance of the non-vegetated zone was similar in both runs, whereas the vegetated behaved differently. Hence, changes in root density and/or spatial distribution most likely occurred during the experiment. As a result, presumably both, transport processes and dissipation varied over time (Goss et al., 1993). This assumption was supported by visual observations of the sediment (Fig. 7). ~~Early breakthrough peaks of tracers and pesticides (Fig. 4) were reached in the non-vegetated zone (represented by solid lines) of the lowermost layer (39 cm) after the surface injection. By contrast, in the vegetated zone of the same layer (represented by dashed lines) only low relative concentrations of Br, UR and metazachlor could be detected. Similar early breakthrough peaks were reached in the vegetated part of the uppermost layer (3 cm), although the tracers and pesticides displayed more than double the relative concentration measured in the lowermost layer. Conversely, in the non-vegetated part of the uppermost layer, the tracers and pesticides showed a delayed breakthrough peak with lower relative concentrations.~~

~~In the following days, two of the major metabolites of metazachlor (met-ESA and met-OA) were detected in the uppermost layer. In the vegetated zone, met-ESA peaked first (day 6). Five days later met-ESA appeared in the non-vegetated zone of the same layer with half of the relative concentration that was measured in the vegetated zone. Yet, during the same phase of the second run no TPs were found. A second peak of both met-ESA and met-OA, with about double the relative concentration measured before, was observed in the vegetated uppermost layer 32 days after the initial injection during the first run. Whereas in the same period of the second run only met-ESA peaked displaying residual amounts.~~

~~A delay of about one month in the breakthrough peaks during both runs was observed in the middle sediment layers (15 and 27 cm). The maximum relative concentrations were reached at the end of the drying phase. This was related to the possible migration of solutes during the drop in the water table from the surface to lower layers. In this case, while in the non-vegetated zone clear breakthrough peaks of Br, UR and metazachlor could be detected, in the vegetated zone only Br displayed observable amounts. Yet, a clear breakthrough peak of Br could only be measured during the second run at the sampling depth of 27 cm.~~

~~The relative concentration of tracers and pesticides experienced an overall decline during the second saturation phase until the end of the experiment in both runs. However, a late second peak of Br, UR and metazachlor was observed in the non-vegetated part of the uppermost layer during the second run. This result was related with possible upward migration of solutes from lower layers during the injection of clean water from the bottom.~~

3.2 Behavior and ~~R~~relationship between solutes throughout the experiment

Overall, the injected solutes have followed the same trend as Br^- in the lower- and uppermost layers (Fig. 5). Conversely, in the middle sections, only UR and metazachlor behaved similar to Br^-, although this was only observed in the non-vegetated part. In fact, the vegetated zone only displayed observable amounts of Br^-. Here in the middle layers, dominant processes most likely differed between zones, as was also evidenced by the different redox potentials measured in the vegetated and the non-vegetated zone (See Fig. 5, second y-axis).

Two of the major metabolites of metazachlor, namely met-ESA and met-OA, were measured in the uppermost layer. It should be noted that other transformation products may have been formed in our system. However, such compounds were most likely below the limit of quantification (≤ 9.29 and ≤ 10.28 µg L^{-1} for p-Chlorobenzoic acid and 1,2,4-Triazole, respectively) and therefore they could not be identified. Met-ESA peaked first (day 6) in the vegetated zone. Five days later it appeared in the non-vegetated zone with half of the relative concentration. Yet, during the same phase of the second run no TPs were found. A second peak of both, met-ESA and met-OA, with about double the relative concentration measured before, was observed in the vegetated uppermost layer 32 days after the initial injection during the first run. Whereas in the same period of the second run only met-ESA peaked displaying residual amounts.

According to the correlations performed to the solute time series (Fig. 8), ~~T~~two spots ~~layers~~ exhibited the strongest relationships ~~correlations between solutes (Fig. 6):~~ the non-vegetated part of the lowermost layer- and the vegetated of the uppermost layer. ~~These findings were consistent with the hypothesis that transport by preferential flow was the dominant process in these layers, given the strong correlation shown between the solutes and Br.~~ The other respective halves also showed high correlations, ~~Yet, this was not true for all pesticides, as~~, however, boscalid and penconazole did not correlate with Br^- ~~Br in the vegetated zone of the lowermost layer and the non-vegetated zone of the uppermost layer. For these pesticides,~~ These results pointed out that probably other processes besides transport ~~, such as~~ (e.g. sorption or plant uptake) dominated the dissipation of boscalid and penconazole during the experiment.

In the middle layers only UR and metazachlor exhibited significant correlations with Br^- ~~Br~~, although metazachlor did not show any correlation in the vegetated zone. ~~no correlation was found between metazachlor and Br.~~ These results suggested that transport was the dominant process for metazachlor in the absence of vegetation, ~~, while~~ whereas under the influence of plants, ~~in the vegetated zone other processes,~~ metazachlor most likely experienced plant uptake, mineralization in the roots, sorption and/or transformation. ~~predominated. In contrast,~~ UR, on the other hand, correlated with Br^- ~~Br~~ in all layers regardless of the presence of vegetation. Therefore, transport was almost certainly a~~the~~ dominant process for this tracer ~~UR in the pore water during the experiment~~. This was not the case for SRB, whose strong positive correlation with Br^- ~~Br~~ in the upper- and lowermost layers further confirmed the creation of preferential flow paths, given its strong sorptive character.

Met-ESA also displayed strong positive correlation with the tracers and pesticides in the vegetated zone of the uppermost layer. On the contrary, met-OA did not show any statistically significant correlation. Yet, no conclusion could be drawn for the TPs, given their overall lower amounts detected in the present study.

~~As for the TPs of metazachlor, Met-ESA, displayed strong positive correlation with the tracers and pesticides in the vegetated zone of the uppermost layer, whereas met-OA did not show any statistically significant correlation. These results may indicate that met-ESA experienced transport processes in the uppermost layer after it was formed in the system. Yet, for met-OA no conclusion could be drawn, given the lower amounts detected in the present study.~~

3.3~~1.1~~ Spatial and temporal dynamics of transport and dissipation processes: role of vegetation and hydrologic conditions

Spatial and temporal variability of transport and dissipation processes were associated with the conditions prior to injection, presence of plants and promotion of aeration during the drying phase. Most of the solutes went to the lower- and uppermost layers. However, the highest concentrations were recorded in the vegetated part of the uppermost layer soon after the injection, thus suggesting that transport of solutes was favored towards the vegetated surface, what has already been observed in other studies (Holden, 2005; Durst et al., 2013). On the other hand, it is believed that transport was retarded in the middle layers because of the presence of water filled pores before the injection and low pore connectivity.

Metazachlor TPs were only found in the uppermost layer and their maximum relative concentrations were measured in the vegetated part after the promotion of aerated conditions. It should be noted, however, that the process of transformation may have been a function of time, and transport over that time ended in the vegetated part of the uppermost layer. Hence, the uppermost layer (possibly the vegetated part) and the end of the drying phase may have constituted hot spots and hot moments for transformation processes, respectively. Higher sorption activity was attributed to the same layer since a migration of the most sorptive solutes (SRB, boscalid and penconazole) was not observed. In contrast, the most mobile ones (Br^-, UR and metazachlor) were transported to the middle layers during the drop in water table. However, that was only true in the non-vegetated part of the middle layers, as in the vegetated only Br^- was detected (see Fig. 5). This pointed out that besides plant uptake and transformation, retention processes may have also played a major role in the vegetated zone, as evidenced by the behavior of SRB in the sediment (see Fig. 11). Given the rapid decrease in relative concentrations of SRB, boscalid and penconazole shortly after the injections, it was assumed that sorption velocity was higher at the beginning of the experiment compared to later phases, when it probably decreased due to a decline in the number of free sorption places.

The results of our study underlined the importance of plants in promoting dissipation processes in constructed wetlands. Indeed, plants have already been attributed the ability to facilitate elimination, degradation and retention of pesticides in wetland systems (Liu et al., 2018). However, our findings also suggested that plant roots may be involved in the formation of preferential flow paths, which could result in a rapid transport of contaminants and decrease in the interactions between solutes and sediments (Durst et al., 2013). In fact, plant roots have been related to the creation of discontinuities in the soil profile, greater presence of macropores and occurrence of bypass flow (Ghestem et al., 2011). Therefore, the beneficial impact of plants in terms of elimination, degradation and retention may be reduced by the occurrence of preferential flows.

Our results have also indicated that the promotion of aeration has facilitated the degradation of some substances. This was in agreement with recent studies that have demonstrated that intermittent flow regimes support aerobic microbial populations and boost degradation rates of pesticides (e.g. Karpuzcu et al., 2013; Maillard et al., 2016). Other authors also found that by alternating drainage with no drainage periods in constructed wetlands, these systems are capable of reducing non-point pollution (Vallée et al., 2015). Hence, it could be generalized that the mitigation capacities of constructed wetlands might be improved if aerated conditions in the system are fostered.

Although our experiment has been carried out in a laboratory environment, the replicated conditions may resemble those of a groundwater-fed wetland that undergo wet-dry cycles and that intercepts pesticide-contaminated water during groundwater discharge. Similar systems have already been investigated with the same multi-tracer approach under laboratory (Durst et al., 2013) and field conditions (Maillard et al.,2016), and the results were analogous to our findings. For instance, Durst et al. (2013) found that preferential flow along the roots took place in the vegetated part of the wetland resulting in greater solutes recoveries, whereas Maillard et al. (2016) demonstrated that the alternation of oxic-anoxic conditions enhanced the dissipation of solutes. Other field studies in wetland systems have pointed out that the presence of vegetation greatly increases contact time and surface area for adsorption (Moore et al., 2006; Liu et al., 2019), which may also be enhanced when organic matter content is high (Passeport et al., 2011).

Overall, our study provides important hints about the role of plants and promoting aerated conditions in increasing the mitigation capacities of wetland systems. However, the results also suggest that plant roots may be involved in the formation of preferential flow paths, which could result in a rapid transport of contaminants and decrease in the interactions between solutes and sediments, as already observed by Durst et al. (2013). Therefore, the beneficial impact of plants in terms of retention, transformation and removal of contaminants may be reduced by the occurrence of preferential flows.

~~From a spatial point of view, both the lower- and the uppermost layers were affected by preferential flow paths, as evidenced by the measurement of early breakthrough peaks of tracers and pesticides. The constructional design of the inflow most likely influenced the transport of solutes causing preferential flow towards the bottom. In addition, the free water at the surface and the channeling effect of the vegetation caused preferential flow paths towards the surface, similar to the findings of Durst et al., 2013This was corroborated by the highest relative concentrations of tracers and pesticides measured in the vegetated zone of the uppermost layer. In the middle layers, the dominant processes differed between zones as evidenced by the different redox potentials measured in the vegetated and the non-vegetated zone. In this case, transport from the upper layers dominated in the non-vegetated zone, as confirmed by the delayed breakthrough peaks. Conversely, plant uptake, mineralization in the roots and sorption processes possibly played major roles in the vegetated zone, since overall small amounts of Br were measured~~

~~The uppermost layer was also a hot spot for sorption and degradation processes, regardless of the presence of vegetation. This conclusion is based on the fact that the most sorptive solutes (boscalid and penconazole) did not migrate to lower layers during the drop of the water table. Besides, the TPs of metazachlor were only detected in the uppermost layer.~~

~~From a temporal point of view, preferential flow took place at the beginning of the experiment according to the early breakthrough peaks detected. Later, vertical transport processes dominated during the drying phase, as demonstrated by the temporal dynamics of the most mobile solutes (Br, UR and metazachlor). Sorption most likely ocurred shortly after the injection of tracers and pesticides, since the relative concentration of the most sortive solutes decreased rapidly in the areas where they were detected. Likewise, degradation processes were observed at early stages and dominated during the drying period, when the maximum relative concentration of metazachlor TPs were measured.~~

~~3.1.2 Role of vegetation and hydrologic conditions in transport and dissipation processes~~

~~The overall absence of breakthrough curves in the vegetated zone of the middle layers and the early breakthrough curves measured in the vegetated zone of the uppermost layer confirmed the influence of the plants, in both, dissipation processes and the promotion of preferential flows, respectively. In addition, the increased occurrence of metazachlor TPs in the vegetated zone of the uppermost layer evidenced the role of the plants in enhancing transformation processes.~~

~~The performance of the system over time in terms of transport and dissipation was also affected by the presence of plants. When comparing the correlation between the breakthrough curves of Br (chosen for its most conservative character) of the first and the second run, differences between the vegetated and non-vegetated zones were observed (Table 3). Indeed, with the exception of the uppermost layer, the non-vegetated zone showed strong correlation between the two runs regardless of the layer, whereas the vegetated did not show any correlation. This means that the performance of the non-vegetated zone was similar in both runs, whereas the vegetated behaved differently. We hypothesize that possible changes in root density and/or spatial distribution occurred during the experiment. This assumption was supported by visual observations of the root system in the sediment (Fig. 5). As a result, presumably both transport processes and plant uptake varied over time (Goss et al., 1993). The magnitude of these changes was analyzed by the total percentage of bromide recovered from the pore water (Table 4). Here, similar values were observed in both runs for the vegetated zone at 39 and 27 cm depth. Consequently, the different breakthrough curves measured in each run was most likely due to changes in transport processes rather than plant uptake. In contrast, the vegetated part at 15 cm depth showed different Br recoveries between the two runs. Therefore, plant uptake was probably more important than transport in this layer. The uppermost layer also displayed different Br recoveries in the vegetated zone, but in this case given that no correlation was found between the first and the second run, the differences were most likely due to the strong influence of preferential flow~~.

~~3.2 Relationship between solutes throughout the experiment~~

~~Two layers exhibited the strongest correlations between solutes (Fig. 6): the lower- and the uppermost layer. These findings were consistent with the hypothesis that transport by preferential flow was the dominant process in these layers, given the strong correlation shown between the solutes and Br. Yet, this was not true for all pesticides, as boscalid and penconazole did not correlate with Br in the vegetated zone of the lowermost layer and the non-vegetated zone of the uppermost layer. For these pesticides, probably other processes, such as sorption or plant uptake dominated during the experiment.~~

~~In the middle layers only UR and metazachlor exhibited significant correlations with Br, although in the vegetated zone no correlation was found between metazachlor and Br. These results suggested that transport was the dominant process for metazachlor in the absence of vegetation, while in the vegetated zone other processes, most likely plant uptake, mineralization in the roots, sorption and transformation predominated. In contrast, UR correlated with Br in all layers regardless of the presence of vegetation. Therefore, transport was almost certainly the dominant process for UR in the pore water during the experiment. This was not the case for SRB, whose strong positive correlation with Br in the upper- and lowermost layers further confirmed the creation of preferential flow paths, given its strong sorptive character.~~

~~As for the TPs of metazachlor, Met-ESA, displayed strong positive correlation with the tracers and pesticides in the vegetated zone of the uppermost layer, whereas met-OA did not show any statistically significant correlation. These results may indicate that met-ESA experienced transport processes in the uppermost layer after it was formed in the system. Yet, for met-OA no conclusion could be drawn, given the lower amounts detected in the present study.~~

3.3 Recoveries of solutes at the outlet

The results of the cumulative recovery curves obtained at the outlet of the system during the ~~first~~ flushings ~~(~~are displayed in Fig. 9~~7) showed~~. Generally, we observed ~~-~~a rapid increase in accumulated mass recovery for Br$^-$~~Br~~ during the first flushings, whereas the rest of the solutes displayed comparatively slower increases. In the following flushings, the accumulated mass recovery of SRB, penconazole and boscalid gradually increased, while for Br$^-$~~Br~~, UR and metazachlor it stabilized. These results suggested that the retained fractions of SRB, boscalid and penconazole in the soil were greater than for Br$^-$~~Br~~, UR and metazachlor. Indeed, SRB, boscalid and penconazole still exhibited increases in their accumulated mass recoveries weeks or even months after the first flushing. Analogous recovery curves for SRB, boscalid and penconazole were observed (Fig. 9~~7~~-A1 and B1) even though SRB has different physico-chemical properties and showed higher mass recoveries than boscalid and penconazole (Table 6~~5~~). Yet, this was only true after the system was repeatedly flushed. A possible explanation for these similarities is that SRB, boscalid and penconazole ~~have~~ are equally resistant to degradation, and therefore remain in the soil longer. In addition, these solutes are more strongly retained in the soil matrix than the others, as already reported in the literature (Long et al., 2005; Vallée et al., 2014; Dollinger et al., 2017). However, due to the different physico-chemical properties of boscalid and penconazole compared to SRB, different mechanisms are expected to be involved in their sorption, which will ultimately affect their fate in the environment. In fact, SRB has, besides a non-polar region, both charged groups (cationic and anionic). Hence, it is susceptible to sorption onto positive and negative charged mineral sites, OH-groups of hydroxides, clay minerals and organic matter (Kasnavia et al., 1999; Polat et al., 2011). This particular characteristic of SRB must be taken into account when using the tracer to investigate sorption processes of pesticides inside wetland system.

~~K_{oc} values, which means that these compounds are strongly retained in the soil matrix (Long et al., 2005; Vallée et al., 2014; Small amounts~~ Cumulative recovery curves of metazachlor TPs were also obtained at the outlet of the system during the flashings (Fig. 9) ~~Especially met-ESA, were also recovered at the outlet of the system~~, thereby evidencing their great mobility and persistence in the environment (Mamy et al., 2005; European Food Safety Authority (EFSA), 2008). In this case, higher amounts of met-ESA were recovered compared to met-OA.

According to the total amount of tracers and pesticides recovered at the outlet after the flushings (Table 6~~5~~), ~~the tracers and pesticides~~ the solutes were classified as follows (from highest to lowest recovery rate): Br$^-$~~Br~~ >> SRB >> UR >> Boscalid >> Penconazole >> Metazachlor. It is believed that several processes in our system, mostly adsorption by substrates, transformation and plant uptake, were responsible for the removal of tracers and pesticides. ~~Nevertheless, t~~In this context, the physico-chemical properties of the compounds have most likely been ~~the~~ a determining factor for their dissipation. ~~In this regard,~~ Vallée et al. (2014) found that a greater retention of pesticides in the soil was related to higher hydrophobic properties (low solubilities and high K_{oc} values). Based on this assumption, we would have expected higher recoveries for metazachlor than for penconazole and boscalid, given its less hydrophobic character. However, unlike ~~for~~ boscalid and penconazole, transformation processes ~~predominated~~ played an important role in the dissipation of ~~for~~ metazachlor, as demonstrated by the measurement of its TPs. On the other hand, given the greater persistence of boscalid and penconazole in the soil (DT_{50} values of 200 and 117 days, respectively), we would have anticipated higher recoveries of these solutes after the flushings. However, only 26.4 and 19% of boscalid and penconazole, respectively, were recovered. Hence, we have hypothesized that the cause of the lower recoveries of boscalid and penconazole could have been their low leaching potential (USEPA, 2003; European Food Safety Authority (EFSA), 2008; Marín-Benito et al., 2015), which is based on their chemical's adsorption (Koc) and persistence in the soil (DT_{50}). Yet, other causes, such as a higher incidence of plant uptake could not be ruled out.

As for the tracers, as expected Br^-~~Br~~ recovery was the highest, ~~as expected~~ given its most conservative character. Following Br^-, SRB showed the greatest recoveries, presumably because this tracer was mostly subject to sorption processes, as demonstrated by its behavior in the pore water, and probably because it was more resistant to degradation, as already evidenced in a recent study (Fernández-Pascual et al., 2018). The lowest recovery~~ies~~ among the tracers was for ~~of~~ UR. In this case, it was~~ere~~ assumed ~~to be due~~that ~~to~~ both retention and especially degradation processes were involved in its dissipation~~, as already reported by Lange et al. (2018). Whereas SRB mainly experienced sorption, as evidenced by its behavior in the pore water and the results of the cumulative recovery curves~~. Overall, these results were in agreement with field studies performed in wetland systems where recoveries for UR were lower than for SRB and were explained by a higher incidence of degradation processes (i.e., photodegradation) (Passeport., 2010; Lange et al., 2011; Schuetz et al., 2012).

In principle, we expect to obtain analogous results in wetland systems if similar conditions are met. In this regard, if we compare the characteristics of our experiment (see Table 2) with those of other wetland studies (e.g. Catallo, 1999; Seybold et al., 2002; Maillard et al., 2011; Gardiner et al., 2012; Passeport et al., 2013; Vallée et al., 2016; Gikas et al., 2018) we find similar values in terms of sediment texture (values ranging from 4 to 89.5/6.2 to 55/3.8 to 44 for % Sand/Silt/Clay), sediment pH (values ranging from 6 to 8), conductivity (values ranging from 0.45 to 0.9 dS/m) and redox potential (values ranging from -500 to +500 mV). However, there are some discrepancies regarding organic carbon content (values ranging from 2.6 to 32.7 %) and mean residence time (values ranging from 0.5 to 8 days). In this case, the values of our experiment were either below (for the organic carbon content) or above average (mean residence time). Yet, the overall removal rates obtained in our experiment (see Table 6) were within the same range of those of the wetland studies. For instance, Vallée et al. (2015) found that the removal rates of boscalid in two pilot-scale wetlands ranged from 38 to 67%, whereas Gikas et al. (2018) obtained removal rates for S-metolachlor (pesticide from the same group as metazachlor) that reached up to 92.6% in a constructed wetland planted with Phragmites australis. Other authors have reported removal rates of 45%–90% for tebuconazole (a triazole fungicide similar to penconazole) in wetland systems (Passeport et al., 2013; Tournebize et al., 2013).

Overall, a possible explanation for the high elimination rates obtained in our experiment could be the fact of having promoted the contact of solutes with the medium through a long period of stagnation (i.e., about two months in each run). In this regard, a recent study performed by Gaullier et al. (2019) has reported almost total mitigation of pesticides and their TPs during stagnation (over 50 days) in constructed wetlands.

These observations highlight the importance of certain factors in the elimination of pesticides, namely the presence of adequate vegetation, suitable matrix materials, long residence times, low flow rates, intermittent flow conditions, among others (Vymazal et al., 2015; Liu et al., 2018). When these factors are promoted, the elimination rates tend to increase, and therefore, the mitigation capacities of constructed wetlands. However, as stated before, the physico-chemical properties of the compounds will be a decisive factor in their elimination.

3.4 Final mass balance

The overall mass balance performed at the end of the experiment is shown in Fig. 10~~8~~. According to the tracer results, Br^-~~Br~~ was recovered almost fully (98.3 %), while SRB and UR displayed lower recoveries (76 % and 32.4 %, respectively). These findings were similar to those of Maillard et al. (2016) who recovered in their wetland experiment under batch conditions 97.2, 43.3 and 24.3 % of Br^-, SRB and UR, respectively. Discrepancies with SRB were associated with the large uncertainty in the measurements performed in the sediment, what was caused by the heterogeneous distribution of SRB in the system, as can be seen in Fig. 11. Indeed, almost 99% of the SRB measured in the vegetated part was located in the uppermost layer.~~Based on the~~ According to the different compartments, Br^-~~Br~~ and UR showed the highest recoveries in the outlet water which highlighted their higher mobility~~, followed by the stems and leaves~~, whereas SRB ~~and UR~~ was~~ere~~ mainly found in the sediment~~ and the outlet water, respectively~~. These results pointed out the different behavior of the tracers when it comes to dissipation. ~~highlighted different dissipation pathways of the solutes. Indeed,~~ A~~a~~s already evidenced by the breakthrough and cumulative recovery curves, Br^-~~Br~~ displayed the most conservative character, although some dissipation was observed via plant uptake ~~was proven to be an important dissipation pathway for this tracer~~(16.76% of the total recovered). In contrast, the main dissipation pathway of SRB was sorption, which was in agreement with its sorptive character (Kasnavia et al., 1999) and the results of other studies (Lange et al., 2011; Durst et al., 2013; Maillard et al.,2016). UR, on the other hand, displayed comparatively lower recoveries, and based on the small amounts found in the sediments, sorption processes were not relevant for its dissipation. Thus, ~~it is most likely that~~ photodegradation~~ via (bio-)chemical transformation~~ and, to a lesser extent, (bio-)chemical transformation were~~as~~ ~~the dominant process~~ most likely the major dissipation pathways for UR. Indeed, the contribution of (bio-)chemical transformation to UR dissipation has already been reported in other long-term studies (Maillard et al., 2016; Lange et al., 2018, Fernández-Pascual et al., 2018). Due to the likely adaptation of microorganisms to UR degradation after being exposed in the first run (Käss 1998), we would have expected lower recovery rates in the second run. However, the final recovery values of UR were similar in both runs (31.71 and 29.82% for the first and second run, respectively). Hence, we hypothesized that other substrates for microbial degradation were present in the system and they were preferentially utilized limiting the degradation of alternative substrates such as UR. Photodegradation of UR was evidenced by the decrease in its concentration in the vegetated part of the uppermost layer, which was the only section exposed to light. During the first run it was observed a decrease in the concentration of UR from 17.44 to 12.26 µg/l over one week, whereas in the second run the values decreased from 26.45 to 9.62 µg/l. Assuming first-order decay, we obtained degradation coefficients of 0.05 and 0.17 days^{-1}, and half-life times of 13 and 4 days for the first and second run, respectively. These values were comparatively lower than the half-life times reported in the literature that are in the range of 11 hours (Leibundgut et al., 2009). However, natural light conditions could not be achieved in the laboratory and this could have limited UR photodegradation. The differences between the first and second run were more difficult to explain, since according to literature the decay rate is inversely correlated to the tracer concentration (Leibundgut et al., 2009). The co-occurrence of photodegradation and microbial degradation could be a possible explanation of the higher decay rate obtained in the second run. However, other factors cannot be ruled out.

~~Likewise,~~ As for the pesticides mass balance~~b~~, while the measurement of metazachlor TPs confirmed that biochemical transformation contributed to its~~metazachlor~~ dissipation, ~~as was confirmed by the measurements of its TPs~~, ~~whereas~~ the transformation of boscalid and penconazole could not be proven. Yet, it was not ruled out ~~in the present study~~ since the concentration of their metabolites may have been below the limit of quantification~~ (≤ 9.29 and ≤ 10.28 µg L^{-1} for p-Chlorobenzoic acid and 1,2,4-Triazole, respectively)~~. However, considering the duration of the experiment (~~two months~~ 62.5 ± 2.12 days each run) and the DT_{50} values of boscalid and penconazole reported in the literature (Table 1), probably~~it was most likely that~~ their transformation was negligible~~did not take place~~. ~~In addition, their high sorption affinity probably made them less bioavailable.~~ Therefore, and based on the overall results, sorption most likely played the main role in the dissipation of boscalid and penconazole. ~~As for the~~ Plant uptake, on the other hand, could not be determined for the pesticides in the present study ~~plant uptake of pesticides, although it was not possible to measure it in the present study~~, yet, it was assumed that it took place given that the results of Br^- proved its occurrence and it has been reported in previous investigations ~~it was assumed that it took place during the experiment. Previous investigations have already reported plant uptake of pesticides~~. For instance, Papaevangelou et al. (2017) demonstrated that high amounts of boscalid accumulated in the tissue of *Phragmites australis* in constructed wetlands, although adsorption accounted as a main process as well. The same plant species was shown to take up, translocate and metabolize tebuconazole (Lv et al., 2017)~~, a triazole fungicide similar to penconazole~~. Traces of metazachlor and its metabolites were also detected in the roots and stems of *Glyceria maxima* in wetland mesocosms experiments (Chen et al., 2017), although plant uptake was reported to play a negligible role in their removal. Other dissipation pathways, such as mineralisation of the compounds to CO_2 or volatilisation from aqueous systems/soil water were not ruled out. However, they were considered to be minimal according to literature (EFSA, 2008).

The final mass balance ~~he~~ also confirmed the contribution of vegetation to uptake and breakdown ~~was also confirmed by the final mass balance~~. Indeed, lower amounts of UR and Br^- ~~Br~~ were recovered from the pore water of the vegetated compared to the non-vegetated zone (Fig. 10~~8~~A). Moreover, most of the TPs of metazachlor were recovered from the pore water of the vegetated zone. Contrary to expectations, the largest amounts of pesticides and SRB were recovered from the pore water of the vegetated zone. Yet, these results supported the hypothesis of the creation of preferential flow paths induced by the plants ~~(Durst et al., 2013)~~. On the other hand, most of SRB was found sorbed in the sediment of the vegetated zone, where the highest concentration of organic carbon was located (Fig. 11). This may be explained by the susceptibility of SRB to sorption on nonpolar sorption sites of organic matter (Polat et al., 2011). Moreover~~On the other hand, most of SRB was found sorbed in the sediment of the vegetated zone, where the highest concentration of organic carbon was located (Fig. 9), thereby contradicting the greatest tendency of SRB to sorption onto mineral surfaces (Kasnavia et al., 1999). Nevertheless~~, it has been recently demonstrated that SRB has high sorption affinity for litters in wetlands (Dollinger et al., 2017). Thus, probably the presence of dead leaves and decaying plant residues in the uppermost layer enhanced sorption of SRB.

3.5 Potential of hydrological tracers to evaluate transport and dissipation processes of pesticides in constructed wetlands

In view of the results obtained in the present study, some conclusions could be drawn regarding the use of Br^-, SRB and UR to evaluate transport and dissipation processes of pesticides in constructed wetlands. In particular, we have corroborated that Br^- can be used to elucidate non-reactive transport of solutes in constructed wetlands, as already reported in the literature (Lin et al., 2003; Małoszewski et al., 2006). But it can also be applied to identify plant uptake, although to a lesser extent. As for SRB, despite the fact that it has been extensively used to identify sorption processes in wetland systems (Passeport et al., 2010; Lange et al., 2011; Schuetz et al., 2012), its special sorptive character makes it difficult to be compared with a certain type of pesticide. In this regard, while Dollinger et al. (2017) stated that SRB could be used as a good proxy for hydrophilic and strongly sorbing pesticides, Lange et al., 2018 demonstrated that the same tracer closely mimicked the gradual recession of a moderately hydrophobic pesticide in the top soil of an agricultural field. As for our results, we found that SRB could describe well the behavior of the pesticides boscalid and penconazole (moderately and highly hydrophobic, respectively) in terms of retention and retardation in the pore water and in the water at the outlet of constructed wetlands when the system is repeatedly flushed. However, it may not be suitable to evaluate overall recoveries of boscalid and penconazole at the outlet given that greater amounts of SRB may be recovered compared to such pesticides, possibly due to its greater leachability and/or lower susceptibility to be taken up by the plants. Regarding UR, in terms of transport our results suggested that it may illustrate well the behavior of mobile and non-persistent pesticides, such as metazachlor, which is in agreement with the findings of other studies (Durst et al., 2013; Maillard et al., 2016; Torrentó et al., 2018). At the same time, our results have underlined that UR may experience not only photodegradation, but also (bio-)chemical transformation, which is consistent with the results of recent investigations (Maillard et al., 2016; Lange et al., 2018, Fernández-Pascual et al., 2018). Yet, UR biodegradation might be limited in the presence of preferred substrates for microorganisms. In any case, it should be noted that the conclusions presented here are only valid if these tracers are used in studies under similar conditions as those of our experiment. That is, constructed wetlands that undergo long periods of stagnation (> 2 months), with drying periods in between, sorbing material with low organic carbon content, similar vegetation and subject to analogous dominant processes.

4 Conclusions

The present study introduces a new approach that combines the use of hydrological tracers with different sorptive and reactive properties and high vertical-resolution sampling and monitoring to explore transport and dissipation processes of reactive compounds (i.e., pesticides) inside a wetland system and the influence that the vegetation and alternating different hydrologic conditions have on such processes.~~The use of hydrological tracers with different sorptive and reactive properties together with selected pesticides and coupled with high vertical-resolution sampling and monitoring in a model constructed wetland system provided valuable information about transport and dissipation processes on a long-term basis and detailed spatial scale~~. By comparing tracers with selected pesticides, valuable hints about dominant transport vectors and main dissipation pathways ~~could~~have been collected~~identified~~.

~~Preferential flow was a crucial component affecting the fate and transport of solutes in the system and was caused by both the constructional design of the inflow and the free water at the surface along with the plants.~~ Breakthrough curves obtained at different sampling depths suggested that solute transport was favored towards the vegetated area, while it was retarded in the middle layers possibly due to the presence of water filled pores before the injection and low pore connectivity. Spatial and temporal variability of transport and dissipation processes were associated with the conditions prior to injection, presence of plants and promotion of aeration during the drying phase. The strong positive correlation found between Br$^-$~~Br~~, UR and metazachlor highlighted the predominance of transport processes for these compounds. By contrast, SRB, boscalid and penconazole most likely experienced sorption~~only displayed significant correlations where preferential flow paths took place, which together with their absence or low detection in the middle sediment layers suggested high sorption affinity. These findings were supported~~as evidenced by their absence in the middle layers, rapid decrease in their concentrations after the injection and gradual increase in accumulated mass recovery ~~obtained for these solutes~~ during flushings. Yet, their different final recoveries indicated lower leaching potential and/or higher incidence of plant uptake in the dissipation of boscalid and penconazole compared to SRB.

The ~~final~~ overall tracer mass balance allowed us to identify three dissipation pathways: sorption, transformation and plant uptake~~indicated that plant uptake, transformation and sorption were the main dissipation pathways~~. While Br$^-$ was almost fully recovered (98.3 %), SRB and UR displayed lower recovery rates (76 % and 32.4 %, respectively). These results were explained by the greater occurrence of sorptive processes in SRB and transformation in UR. The detection of metazachlor TPs, namely met-ESA and met-OA demonstrated that biochemical transformation ~~had~~ played an ~~major contribution to~~important role in metazachlor dissipation, whereas no TPs for boscalid and penconazole were found. Yet, their transformation could not be ruled out in the present study. Likewise, plant uptake of pesticides could not be confirmed but it was assumed that it took place throughout the experiment. ~~Met-ESA and met-OA recoveries obtained at the outlet of the system during flushings confirmed their great mobility and persistence. In contrast, sorption was the main dissipation pathway for boscalid and penconazole, although their transformation could not be ruled out within the timeframe of the present study. Likewise, plant uptake of pesticides could not be confirmed but it was assumed that it took place throughout the experiment.~~

Our findings pointed out that the presence of plants and the alternation of different hydrological conditions (saturation and drying periods) may favor~~favored the transformation of certain pesticides~~ dissipation processes. The combination of these factors together with others (e.g. suitable matrix materials, long residence times, etc.) could increase the mitigation capacities of wetland systems. Yet, plants might also be involved in the creation of preferential flow paths with the consequent risk of rapid transport of contaminants.

~~Maximum relative concentrations of metazachlor TPs were measured after the promotion of aerobic conditions during the drying period. Similarly, the presence of plants was a key factor in boosting transformation processes, as evidenced by the increased occurrence of metazachlor TPs in the vegetated zone. Our study also revealed a different performance of the vegetated zone over time. In this case, unlike the non-vegetated, the vegetated zone did not show any correlation between the breakthrough curves of Br for each experimental run. This result was explained by possible changes in root density and/or spatial distribution during the experiment. As a result, different transport vectors and plant uptake rates took place in each experimental run.~~

Overall, the complexity of the processes that take place inside~~ buffer zones~~ constructed wetlands~~, such as constructed wetlands,~~ and the lack of sufficient data on a temporal and spatial scale highlights the need to adopt new methods ~~and~~to fully understand the behavior of pollutants in these systems. The application of a multi-tracer approach coupled with high vertical-resolution sampling and monitoring may assist in unveiling internal mechanisms that dominate transport vectors and dissipation of contaminants.~~ perform further experiments, especially under field conditions combined with mathematical modeling. In this regard, ~~Such approach has allowed us to build an overall view of the solutes behavior with great spatial and temporal detail. Yet, further experiments need to be done especially under field conditions combined with mathematical modeling in order to better understand the factors involved in pesticide transport and dissipation in constructed wetlands and ensure an optimal use of these systems. ~~the application of a multi-tracer approach coupled with high vertical-resolution sampling and monitoring may assist in unveiling internal mechanisms that dominate transport vectors and dissipation of contaminants in buffer zones.~~

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Tables and Figures

Table 1: Physico-chemical properties of the applied tracers and pesticides (20°C-25°C).

Property		***Unit***	***UR***	***SRB***	*Br^-* ~~**Br**~~	***Boscalid***	***Penconazole***	***Metazachlor***
Chemical formula			$C_{20}H_{10}O_5Na_2$	$C_{27}H_{29}N_2NaO_7S_2$	NaBr	$C_{18}H_{12}Cl_2N_2O$	$C_{13}H_{15}Cl_2N_3$	$C_{14}H_{16}ClN_3O$
Chemical family			Xanthene dye	Xanthene dye	Inorganic salt	Carboxamide	Triazole	Chloroacetamide
Molecular mass [a]		g mol^{-1}	376.3	580.7	102.89	342.033	283.064	277.098
Aqueous solubility		g L^{-1}	25.0 [b]	70.0 [b]	850 [c]	0.0046 [d]	0.073 [d]	0.450 [d]
Aqueous diffusion coefficient		cm^2 s	3.5 x 10^{-6} [c]	4.7 x 10^{-6} [c]	-	4.4 x 10^{-12} [g]	-	-
Soil degradation	DT_{50}	days	-	-	stable [c]	200 [d] (persistent)	117 [d] (persistent)	8.6 [d] (non-persistent
Dissipation rate on plant matrix	RL_{50}	days	-	-	-	6.9 [d]	65.6 [d]	-
Photolytic stability	DT_{50}	days	0.5 [c]	34 [c]	stable [c]	30 [d] (stable)	4 [d] (moderately fast)	stable [d]
Hydrolytic stability	DT_{50}	days	stable [c]	stable [c]	stable [c]	stable [d]	stable [d]	stable [d]
Water-sediment	DT_{50}	days	stable [i]	stable [i]	-	-	853[d] (stable)	20.6 [d] (fast)
Organic carbon - water partitioning	Koc	L kg^{-1}	0-62 [b]	147-498 [b]	-	772.0 [f]	2205 [f]	134.0 [f]
Octanol - water partitioning (at pH 7)	Log Kow		1.26-3.56 [h]	0.21-4.77 [h]	-	2.96 [d]	3.72 [d]	2.49 [d]

(-)*Information not available*

[a] *From ChemID database (2017)*

[b] *From Sabatini (2000)*

[c] *From Leibundgut et al. (2009)*

[d] *From Pesticide Properties DataBase, University of Hertfordshire.*

[e] *From Merck Millipore (http://www.merckmillipore.de)*

[f] *From PAN Pesticides Database (.pesticideinfo.org/Search_Chemicals.jsp)*

[g] *From Martin et al. (2017)*

[h] *From EPA Chemistry Dashboard*

[i] *From Smart and Laidlaw (1977)*

Table 2: (A) characteristics and (B) operation of the model constructed wetland system.

A

Compartment	***Parameter***	***Unit***	***sub-compartment***	***Value***
Sediments	Texture*	% Sand/Silt/Clay	Sand	97.8/2.3/0.1
	Mean initial organic carbon content *	%	Sand	0.2 ± 0.0

	Mean final organic carbon content **	%	Sand	0.8 ± 1.4
	Mean initial dithionite-extractable Fe (Fed)*	g Kg^{-1}	Sand	1.0 ± 0.0
	Mean final dithionite-extractable Fe (Fed)**	g Kg^{-1}	Sand	1.1 ± 0.2
	pH (H_2O)	-	Sand	9.1
	pH ($CaCl_2$)	-	Sand	8.1
	Diameter*	mm	Sand	0-2
			Gravel	4-8
	Bulk density*	Kg L^{-1}	Sand	1.5
			Gravel	1.6
	Porosity	%	Sand	42
			Gravel	45
	Height	cm	Sand	32
			Gravel	10
	Surface area	m^2	-	0.7
	Mass	Kg	Sand	430.8
			Gravel	124.0
	Redox potential***	mV	Sand and gravel	-328 ± 10.7 to +740 ± 25.6
	Conductivity***	dS/m	Sand and gravel	0 to 0.4 ± 0.1
Plants	Density	N°	*Typha latifolia*	4
			Phragmites australis	7
		Plants m^{-2}	*Typha latifolia*	10.8
			Phragmites australis	18.9
	Mean initial height	cm	*Typha latifolia*	79.8 ± 18.6
			Phragmites australis	76.9 ± 10.1

** Determined prior to planting*
*** Determined at the end of the experiment as the mean of all the values measured at the different depths*
**** Range of values (min. to max.) measured in the sediment during the experiment*

B

Parameter	**Unit**	**Value**
Inlet/outlet pumping rate	L h^{-1}	21.6
Peristaltic pumping rate	L h^{-1}	0.1
Volume of tracers and pesticides ~~solution~~ injected	L	40
Volume of clean water injected at the end of the drying phase	L	34.1 ± 3.1
Volume of total clean water injected in the flushings	L	355.1 ± 20.5
Hydraulic retention time	Days	62.5 ± 2.12

Values represent means ± standard deviation.

~~**Table 3:** Spearman rank correlation between the breakthrough curves of Br of the first and the second experimental run, distinguishing between the different depths and zones.~~

~~**Depth (cm)**~~	~~**Zone**~~	~~**rho**~~	~~**p-value**~~
~~3~~	~~Non-vegetated~~	~~0.29~~	~~0.56~~
	~~Vegetated~~	~~0.14~~	~~0.80~~
~~15~~	~~Non-vegetated~~	~~0.84~~	~~<0.01 **~~
	~~Vegetated~~	~~0.55~~	~~0.17~~
~~27~~	~~Non-vegetated~~	~~0.77~~	~~0.03 *~~
	~~Vegetated~~	~~0.26~~	~~0.53~~
~~39~~	~~Non-vegetated~~	~~0.73~~	~~0.04 *~~
	~~Vegetated~~	~~0.55~~	~~0.15~~

~~Signif. Codes: 0.001 '***'; 0.01 '**'; 0.05 '*'~~

~~**Table 4:** Percentage of total bromide recovered from the pore water for the first and second run, distinguishing between the different depths and zones.~~

~~**Depth (cm)**~~	~~**Zone**~~	~~**Recovery pore water (%)**~~ ~~**1º run**~~	 ~~**2º run**~~	~~**Mean recovery (%)**~~
~~3~~	~~Non-vegetated~~	~~0.6~~	~~0.7~~	~~0.6 ± 0.1~~
	~~Vegetated~~	~~1.3~~	~~0.4~~	~~0.8 ± 0.7~~
~~15~~	~~Non-vegetated~~	~~0.3~~	~~0.5~~	~~0.4 ± 0.1~~
	~~Vegetated~~	~~0.2~~	~~0.1~~	~~0.1 ± 0.1~~
~~27~~	~~Non-vegetated~~	~~0.2~~	~~0.2~~	~~0.2 ± 0~~
	~~Vegetated~~	~~0.1~~	~~0.1~~	~~0.1 ± 0~~
~~39~~	~~Non-vegetated~~	~~0.4~~	~~0.3~~	~~0.3 ± 0.1~~
	~~Vegetated~~	~~0.1~~	~~0.1~~	~~0.1 ± 0~~

~~Mean recovery represents means ± standard deviation.~~

Table 3: LOQ/LOD values for the pesticides and TPs.

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

Table 4. Selected relative concentrations of Br^- measured during the 1) first and 2) second run for the different zones, phases (saturation and drying) and depths.

1)	Depth (cm)	Saturation 09/03	 13/03	 16/03	Drying 20/03	 21/03	 23/03	 27/03	 04/04	Saturation 10/04	 12/04	 02/05	 04/05	 09/05
Non-vegetated	3	0.08	0.17	0.19	0.34	0.41	-	-	-	0.33	0.35	0.21	0.17	0.03
	15	0	0	0	0.01	0.02	0.04	0.09	0.23	0.15	0.18	0.06	0.04	0.01
	27	0	0	0.02	0.03	0.04	0.04	0.05	0.08	0.08	0.07	0.01	0.01	0.05
	39	0.30	0.21	0.15	0.09	0.11	0.11	0.12	0.11	0.01	0.01	0.01	0.02	0.01
Vegetated	3	0.20	0.70	0.79	0.76	0.79	-	-	-	0.48	0.55	0.45	0.39	0.08
	15	0	0	0.01	0.03	0.04	0.06	0.09	0.06	0.02	0.00	0.06	0.05	0.03
	27	0	0	0	0	0	0	0	0.01	0.02	0.02	0.02	0.02	0.08
	39	0.10	0.02	0.02	0.03	0.04	0.05	0.06	0.06	0.01	0.02	0.04	0.05	0.00

2)	Depth (cm)	Saturation 01/08	 04/08	 08/08	Drying 10/08	 14/08	 18/08	 22/08	 25/08	Saturation 29/08	 05/09	 13/09	 27/09	 03/10
Non-vegetated	3	0.07	0.20	0.46	-	-	-	-	-	0.43	0.53	0.62	0.35	0.03
	15	0.01	0.01	0.02	0.06	0.18	0.31	0.42	-	0.39	0.41	0.30	0.15	0.02
	27	0.01	0.01	0.03	0.02	0.04	0.07	0.11	0.15	0.14	0.10	0.07	0.05	0.00
	39	0.29	0.19	0.19	0.19	0.20	0.21	0.21	0.20	0.02	0.02	0.02	0.02	0.00
Vegetated	3	0.18	0.59	0.53	-	-	-	-	-	0.09	0.16	0.18	0.08	0.01
	15	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.01	0.03	0.04	0.04	0.02
	27	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.03	0.06	0.04	0.03	0.02	0.00
	39	0.02	0.01	0.01	0.03	0.06	0.10	0.10	0.09	0.01	0.02	0.02	0.02	0.00

Table 5. Spearman rank correlation between the breakthrough curves of Br^- of the first and the second experimental run and the different depths and zones.

Depth (cm)	**Zones**	**rho**	**p-value**
3	Non-vegetated	0.29	0.56
	Vegetated	0.14	0.80

15	Non-vegetated	0.84	<0.01 **
	Vegetated	0.55	0.17
27	Non-vegetated	0.77	0.03 *
	Vegetated	0.26	0.53
39	Non-vegetated	0.73	0.04 *
	Vegetated	0.55	0.15

Signif. Codes: 0.001 ‘***’; 0.01 ‘**’; 0.05 ‘*’

Table 6~~5~~: Percentage of tracers, pesticides and their TPs recovered from the outlet water after the flushings of the system for the first and second run.

	Recovery water outlet (%)		**Mean recovery (%)**
	1º run	**2º run**	
Br^-~~Br~~	76.5	76.7	76.6 ± 0.1
UR	30.3	28.8	29.6 ± 1.1
SRB	36.4	38.0	37.2 ± 1.1
Boscalid	27.9	24.9	26.4 ± 2.1
Penconazole	17.3	20.7	19.0 ± 2.4
Metazachlor	7.5	7.4	7.5 ± 0.1
Met-ESA	6.1	5.4	5.8 ± 0.5
Met-OA	0	0.8	0.4 ± 0.6

Mean recovery represents means ± standard deviation.

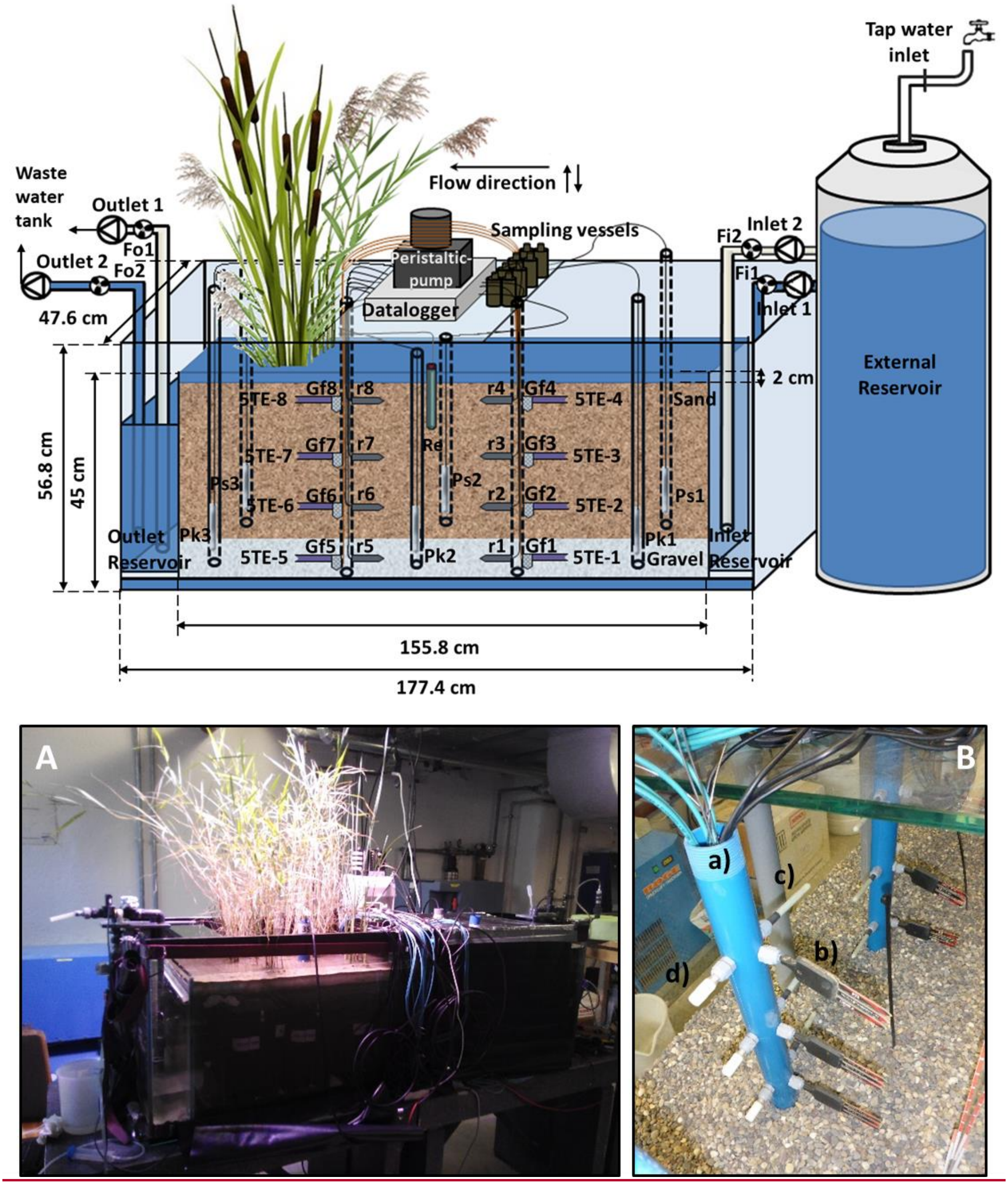


Figure 1: Schematic representation of the model constructed wetland system (not to scale). Fi1 and Fi2 indicate the flowmeters at the inlet; Fo1 and Fo2, the flowmeters at the outlet; Ps(n), piezometer in the sand; Pk(n), piezometer in the gravel; 5TE-(n), soil moisture, temperature and electrical conductivity sensor; r(n), platinum redox electrode; Re, reference electrode (Ag:AgCl); Gf(n), glassfilter. For the piezometers, n indicates the position with respect to the inlet; n=1, close to the inlet; n=2, in the middle of the sediment bed and n=3, close to the outlet. For the sensors installed in the multi-level pipes, n indicates the zone and the depths where they are located; n = 1, 2, 3 and 4, non-vegetated zone at a depth of 39, 27, 15 and 3 cm, respectively; n = 5, 6, 7 and 8, vegetated zone at a depth of 39, 27, 15 and 3 cm, respectively.

(A) front view photograph of the model constructed wetland system; (B) detail of the multi-level pipes: (a) multi-level pipe at the vegetated zone, (b) 5TE sensor, (c) redox electrode and (d) glass filter.

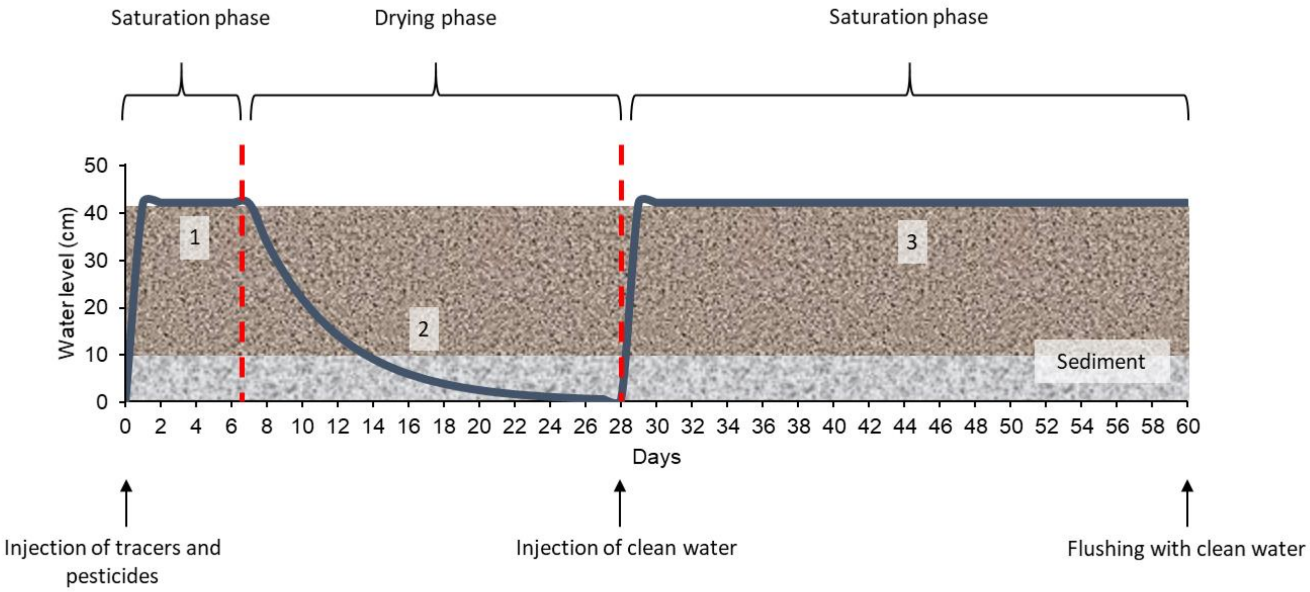
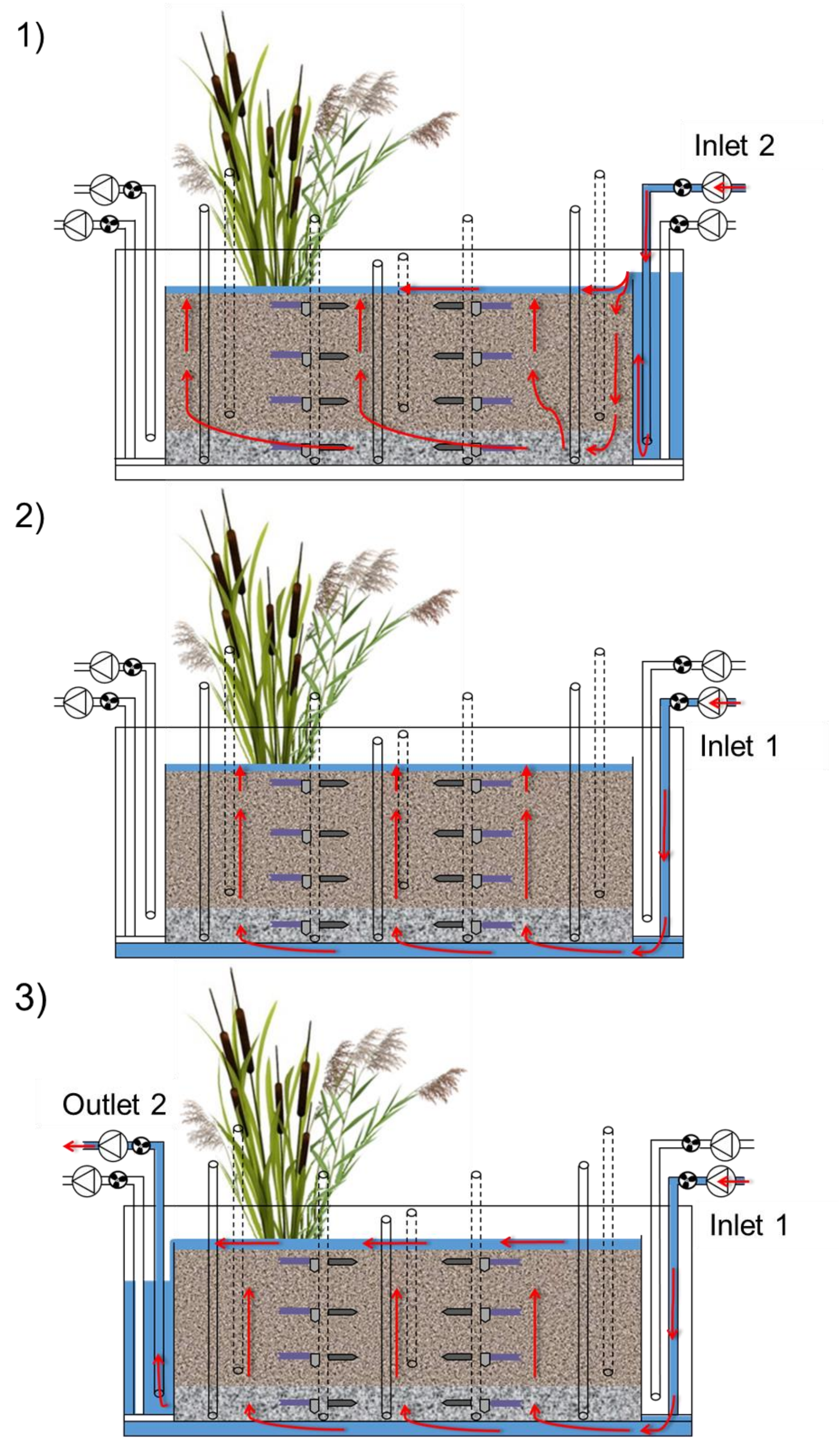


Figure 2: Experimental protocol with the different phases and injections performed during the experiment. The x-axis indicates the duration of the experiment and the y-axis the variation of the water level during the different phases.

Note Fig. 2: The water level curve (blue) is only schematic and does not correspond to real water level measurements.


1)
Inlet 2
2)
Inlet 1
3)
Outlet 2
Inlet 1

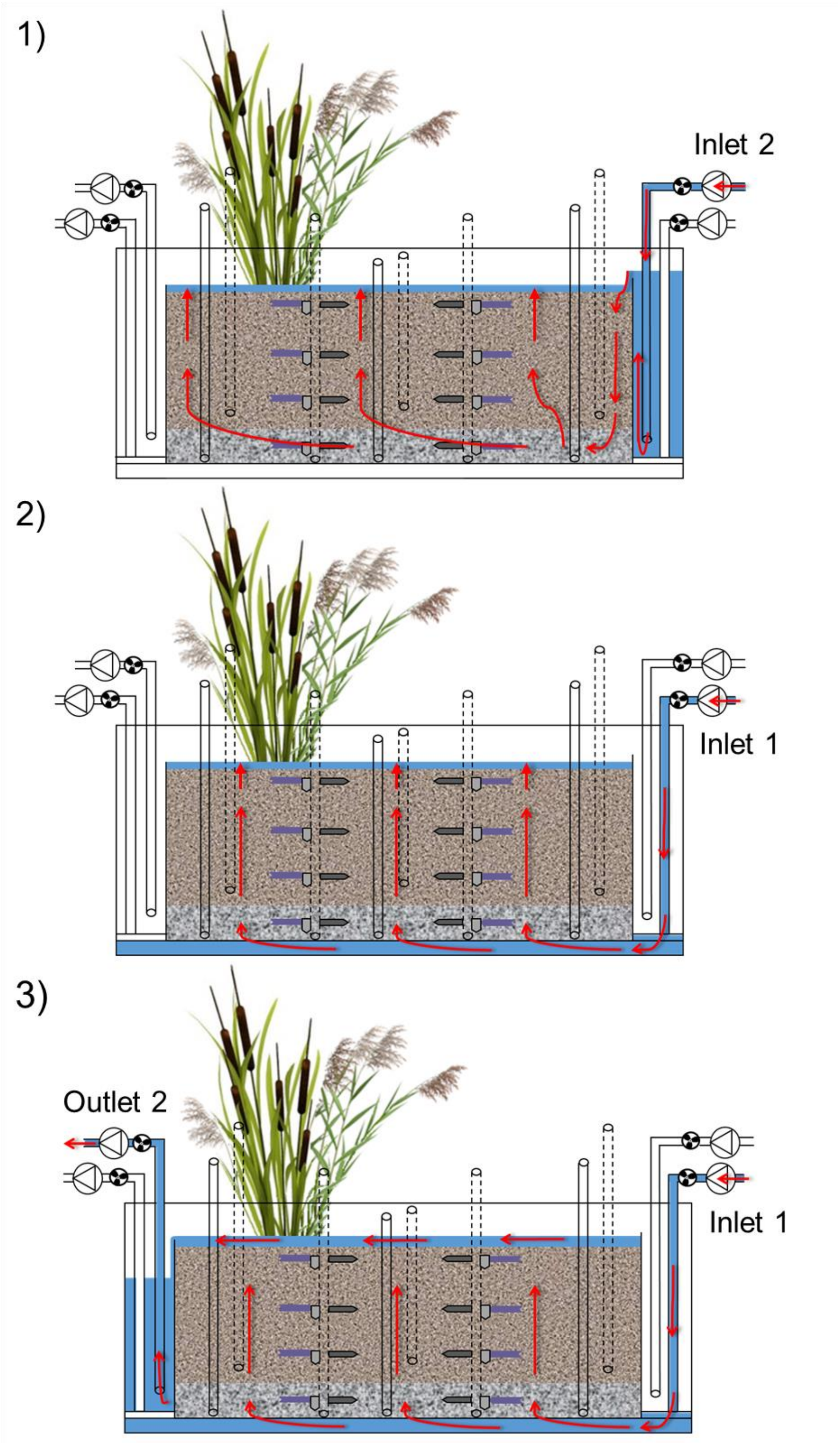


Figure 3: Front view of the model constructed wetland system showing the execution of the injections (red arrows indicate the direction of the water flow): (1) surface injection of tracers and pesticides, (2) injection of clean water (tap water) from the bottom, (3) flushing of the system with clean water (tap water) from the bottom.

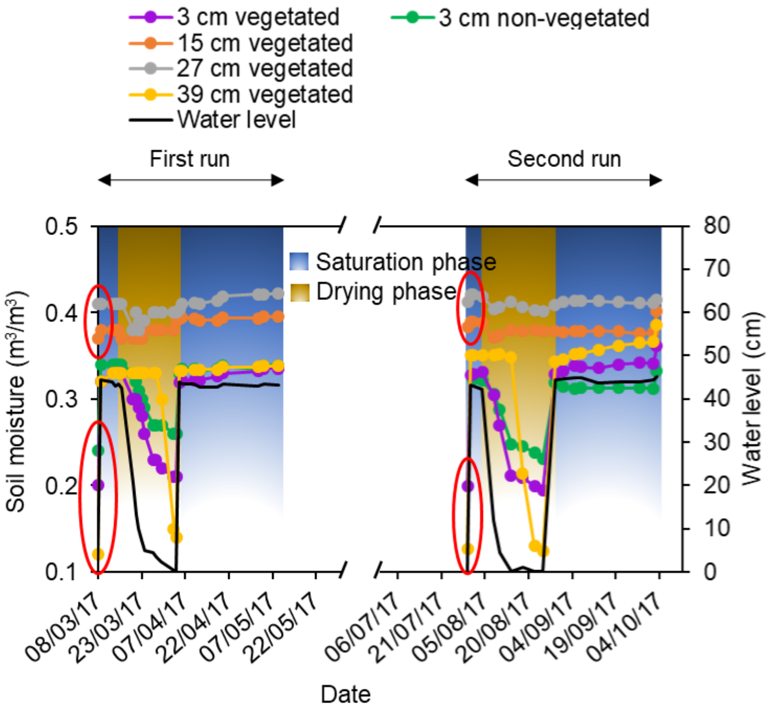
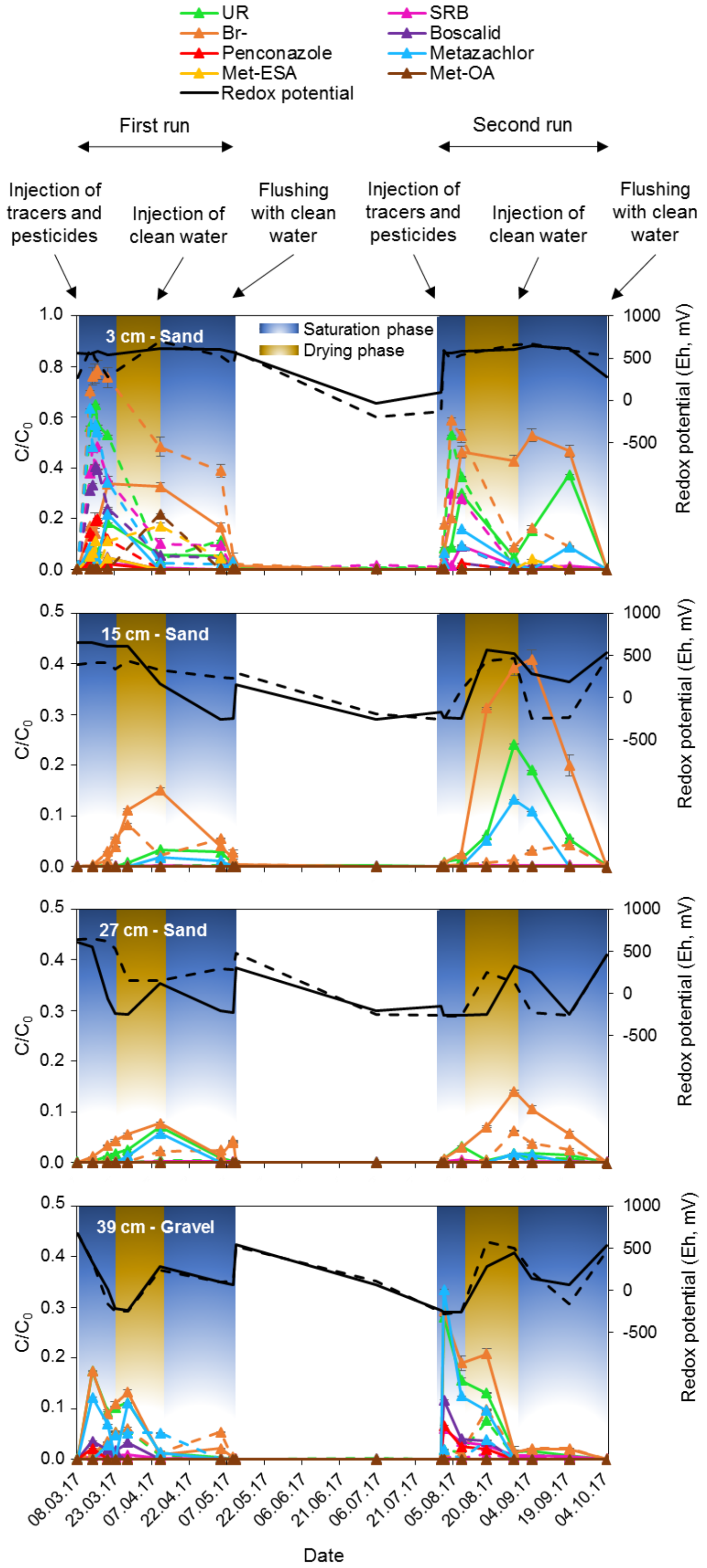


Figure 4: Soil moisture values measured in the pore water during the first and the second run for the different zones, phases (saturation and drying) and depths. Water level is displayed in the second y-axis. The missing data from the sampling depths at 15, 27 and 39 cm in the non-vegetated zone is due to failures in the sensors. Red circles indicate the values previous to the injection.


UR
SRB
Br-
Boscalid
Penconazole
Metazachlor
Met-ESA
Met-OA
Redox potential
First run
Second run
Injection of tracers and pesticides
Injection of clean water
Flushing with clean water
Injection of tracers and pesticides
Injection of clean water
Flushing with clean water
Saturation phase
Drying phase
3 cm - Sand
15 cm - Sand
27 cm - Sand
39 cm - Gravel
C/C0
Redox potential (Eh, mV)
1.0
0.8
0.6
0.4
0.2
0.0
0.5
0.4
0.3
0.2
0.1
1000
500
0
-500
08.03.17
23.03.17
07.04.17
22.04.17
07.05.17
22.05.17
06.06.17
21.06.17
06.07.17
21.07.17
05.08.17
20.08.17
04.09.17
19.09.17
04.10.17
Date

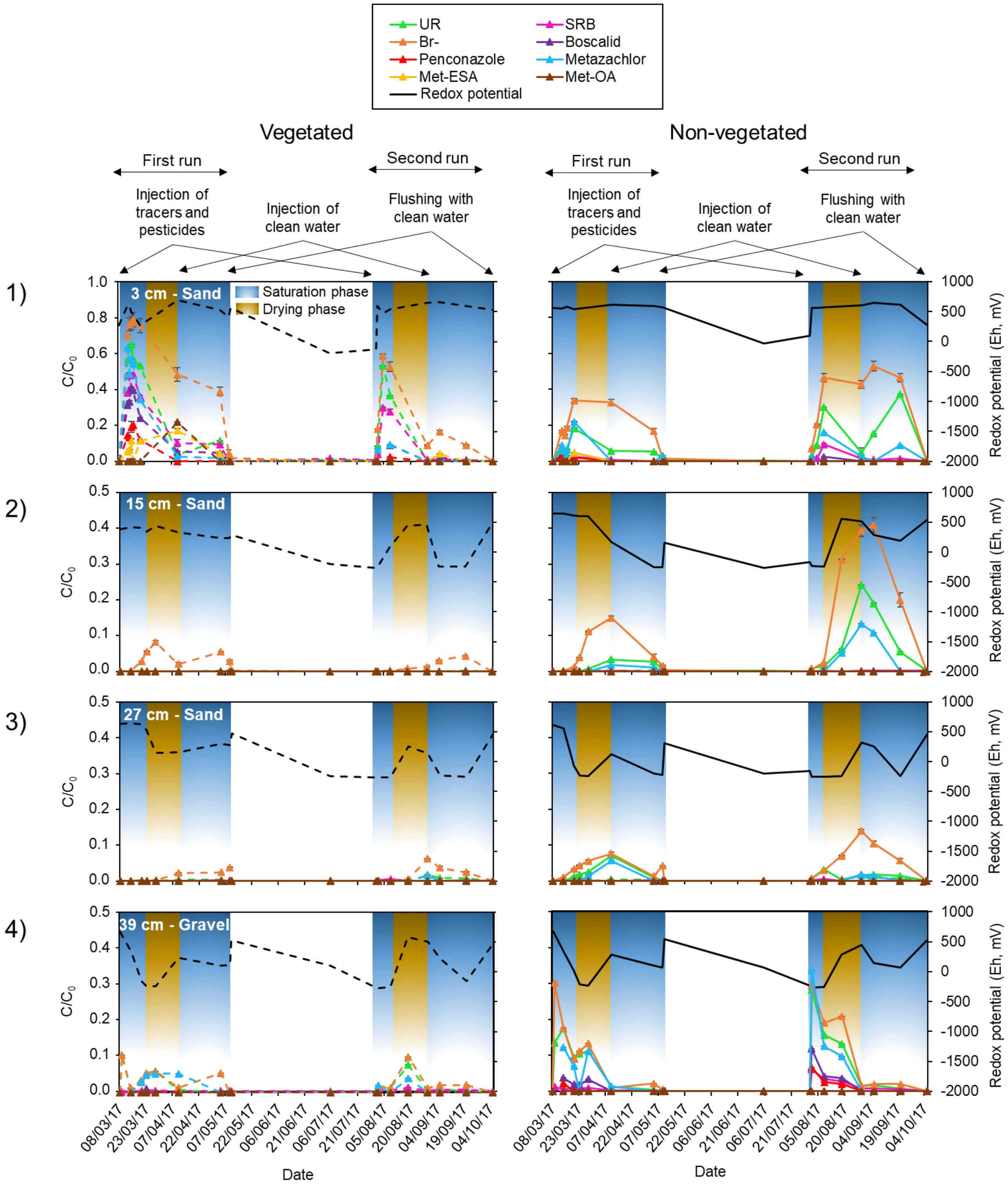


Figure 54: Breakthrough curves of the different tracers, pesticides and their TPs in terms of relative concentrations (C/C_0) (obtained by scaling with the input concentrations) measured in the pore water during the first and the second run for the different zones, phases (saturation and drying) and depths: 1) 3cm; 2) 15cm; 3) 27cm and 4) 39cm~~measured in the pore water during each run at different depths and phases (saturation and drying)~~. Changes in redox potential are displayed in the second y-axis (Eh in mV).~~ For both, the relative concentration and the redox potential, the solid lines represent the values measured in the non-vegetated zone and the dashed lines the values measured in the vegetated zone~~. The different injections performed during each run are displayed on top of the figure. Note that the scale of the relative concentrations corresponding to the sampling depth of 3 cm is extended.

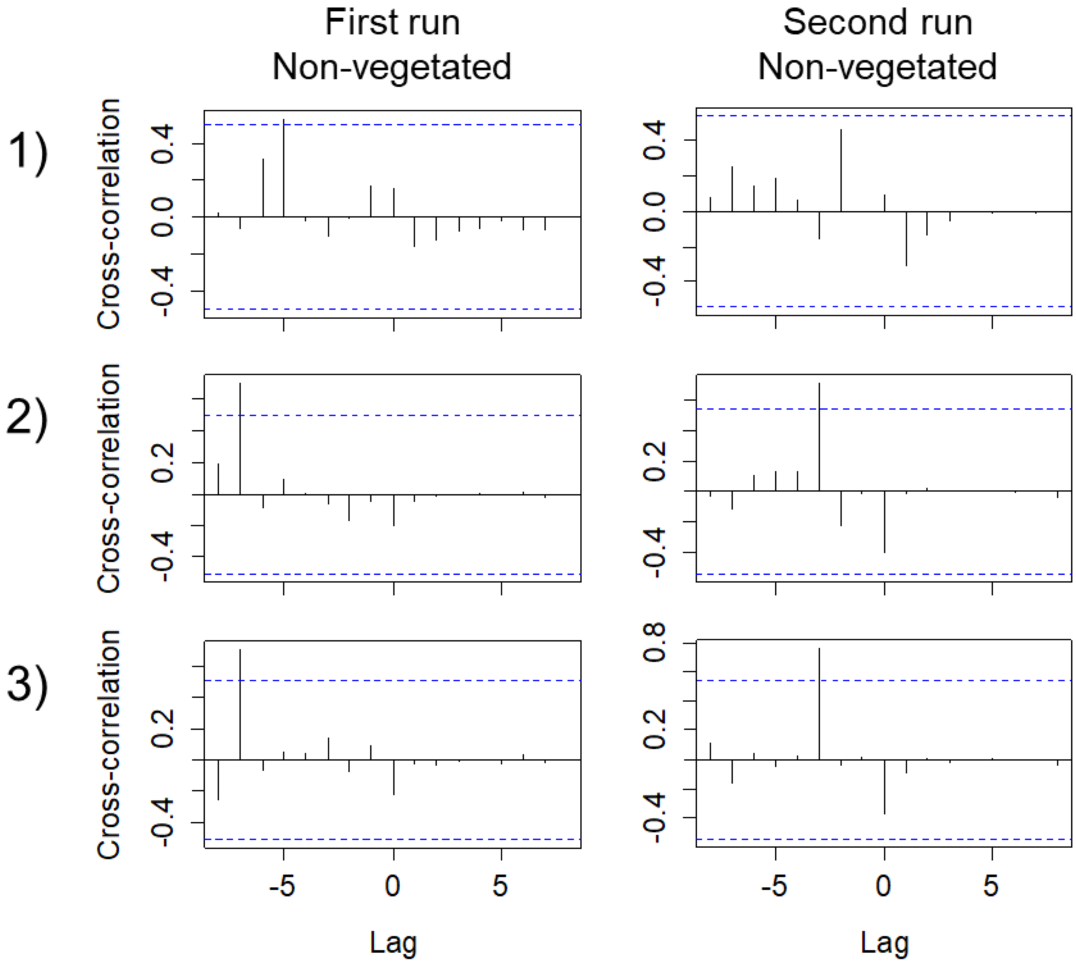


Figure 6: Lag analysis performed to the Br^- breakthrough curves for the first and second run between the sampling depths: 1) 39cm and 3cm; 2) 39cm and 15cm; and 3) 39cm and 27cm. Only significant lag correlations are displayed.

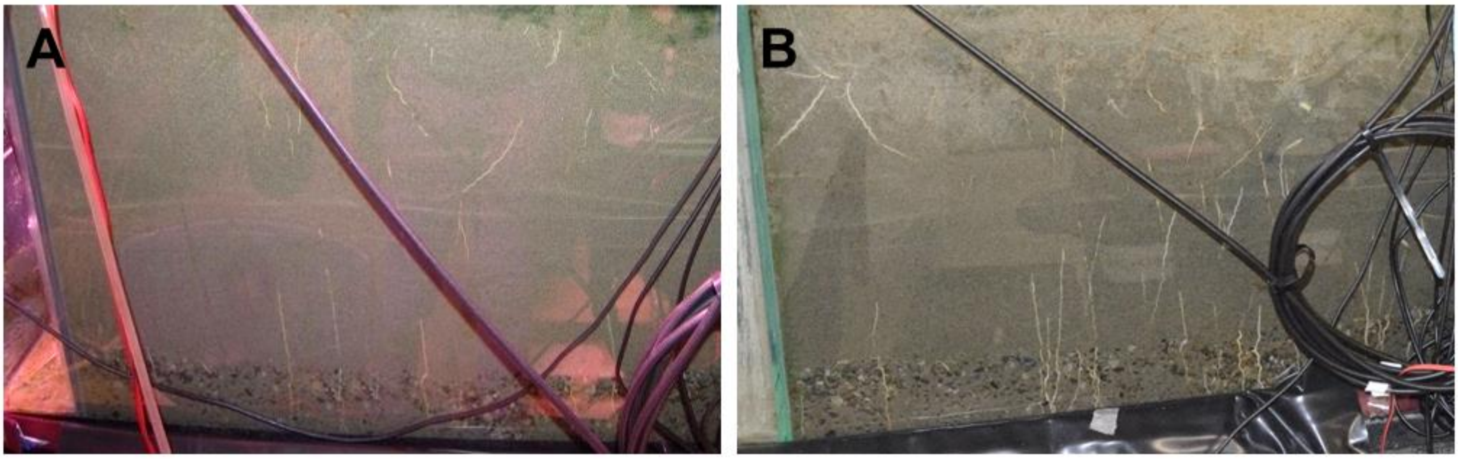


Figure 7~~5~~: Front view photograph of the root system in the vegetated part of the model constructed wetland for: A) before the first run and B) at the end of the second run.

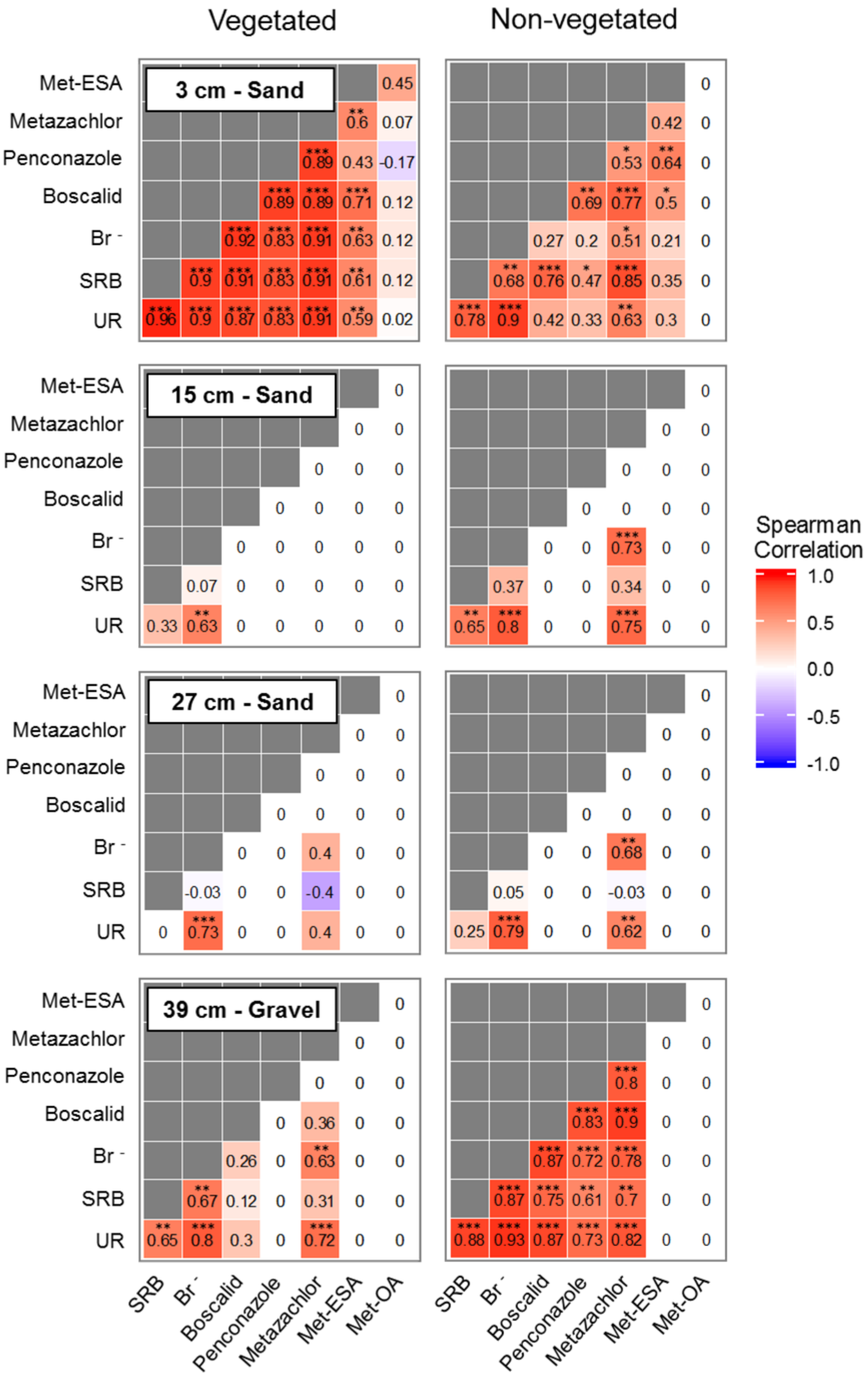


Figure 8~~6~~: Spearman correlation matrices between the relative concentration of tracers, pesticides and their TPs in the pore water during the whole experiment, distinguishing between the different depths and zones.

Signif. Codes: 0.001 ‘***’; 0.01 ‘**’; 0.05 ‘*’

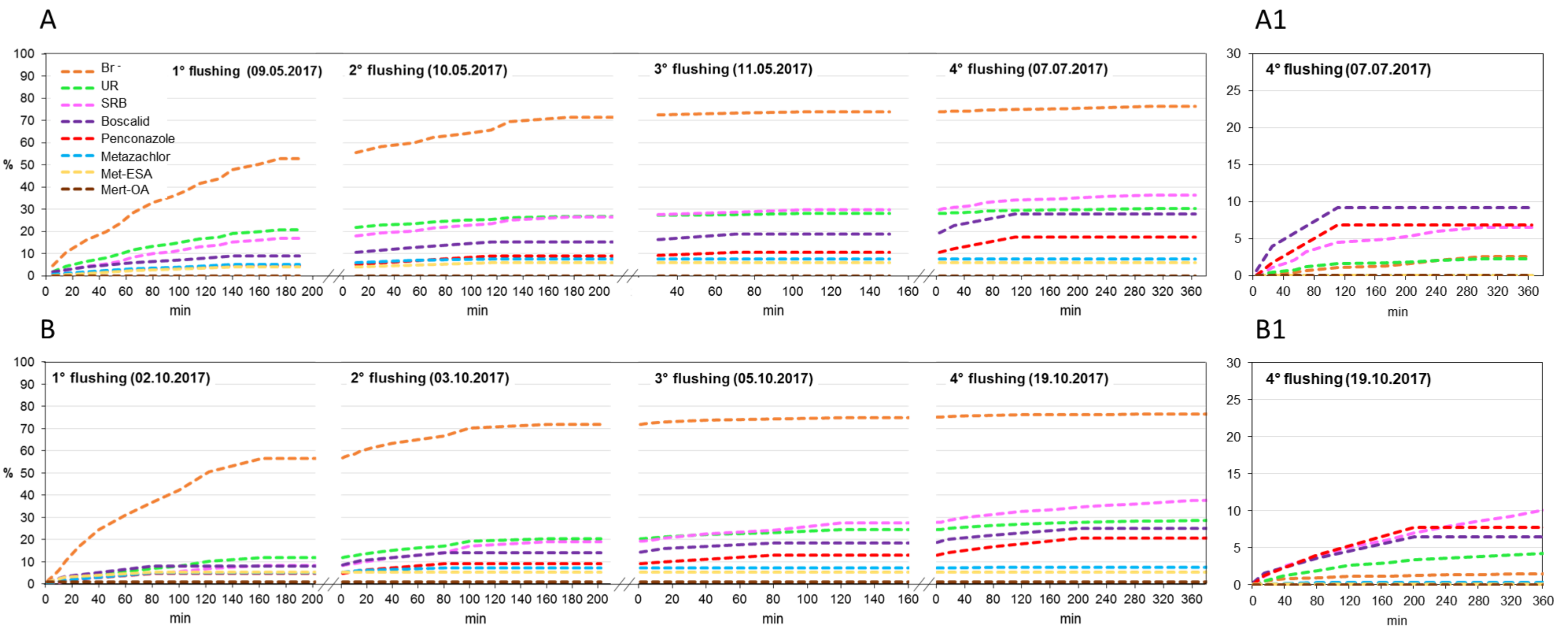


Figure 9~~7~~: Cumulative recovery curves of tracers, pesticides and their TPs during the four flushings for: A) first and B) second run. Recovery curves for the fourth flushing are detailed in: A1 and B1 for the first and the second run, respectively.

Recoveries of tracers, pesticides and their TPs at the outlet

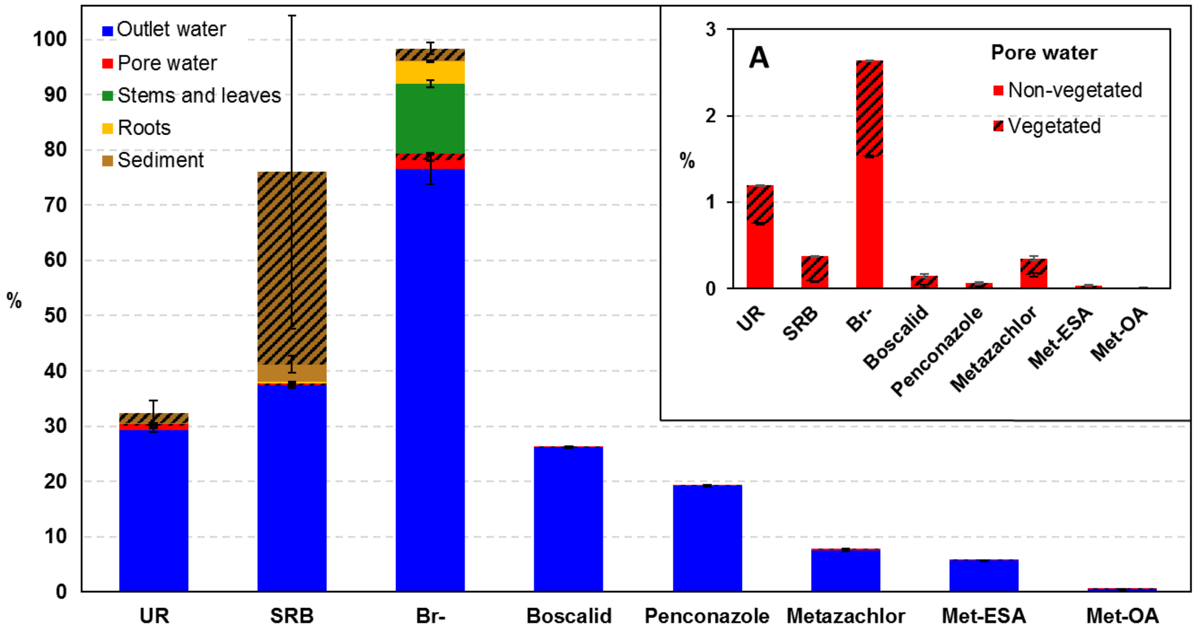


Figure 10~~8~~: Final mass balance conducted at the end of the experiment in the different compartments.

Note that the pesticides and their TPs could only be measured in the outlet and pore water compartments. The mass balance for the TPs was calculated according to the total amount of parent compound injected. The shaded area represents the percentage measured in the vegetated zone. The mass balance for the pore water compartment is detailed in the upper right portion of the graph (A).

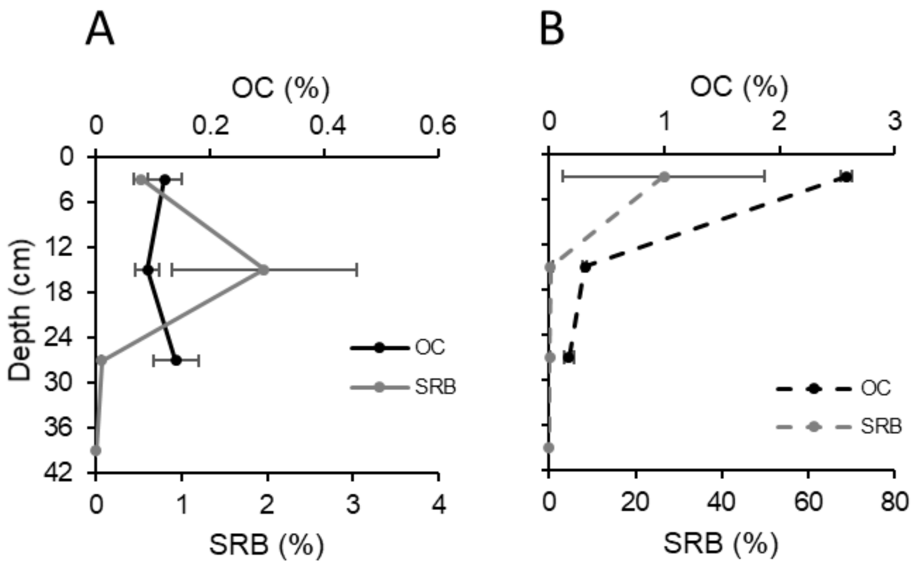


Figure 119: Selected vertical gradients of percentage of organic carbon content (OC) and SRB measured in the sediment at the end of the experiment for A) non-vegetated and B) vegetated zone. Values represent means of duplicates ± standard deviation.