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

Elena Fernández-Pascual<sup>1</sup>, Marcus Bork<sup>1,2</sup>, Birte Hensen<sup>3</sup>, Jens Lange<sup>1</sup>

<sup>1</sup>Hydrology, Faculty of Environment and Natural Resources, University of Freiburg, Freiburg, Germany

<sup>2</sup> Soil Ecology, Faculty of Environment and Natural Resources, University of Freiburg, Freiburg, Germany
 <sup>3</sup> Institute of Sustainable and Environmental Chemistry, Leuphana University Lüneburg, Lüneburg, Germany

*Correspondence to:* Elena Fernández-Pascual (elena.fernandez@hydrology.uni-freiburg.de)

#### Answers to editor

-----

Dear Prof. Zehe,

We are very pleased to have the opportunity to review and resubmit our manuscript. We truly appreciate all the insightful observations. We have carefully considered all the comments and recommendations, and the manuscript has been accordingly revised.

We provide below a point-by-point response with the editor and reviewers' comments and our responses in blue. We have also indicated how the observations and corrections have been made (in the new revised version) detailing the corresponding page and line number where applicable.

We hope that these revisions improve the paper such that you and the reviewers now deem it worthy of publication in the Hydrology and Earth System Sciences journal.

Once again, thank you for taking the time and energy to help us improve the paper.

We look forward to the outcome of your assessment.

On behalf of all the authors,

Elena Fernández-Pascual

# Editor

# -----

#### **Comment:**

I am happy to let you know that both reviewers recommend publication of your manuscript in HESS. Reviewer 2 suggested a long list of technical improvements, which need to be addressed to improve the presentation quality of your work to the level that we expect for HESS.

**Response:** We want to thank both reviewers for the positive feedback. The corresponding technical improvements have been addressed in the manuscript. We hope our revision meets with your approval.

Answers to referee #1

#### \_\_\_\_\_

#### Comment:

After careful reading of the comments of the reviewers, the answers of authors and the new manuscript, I am fully convinced that the authors did a great job to improve their manuscript. To my opinion, all important issues regarding the generic character of the study, the lack of replicate experiments, difficulties to close mass balances and relationship between reaction and residence time have been thoroughly taken into account. The authors clearly did a great job and the manuscript is in a very good shape. My only comment is that now the manuscript could be again polished a bit for English language. Please, avoid long sentences whenever possible.

**Response:** We wish to acknowledge the constructive and thoughtful comments of the reviewer. Following your recommendation, we have asked some colleagues to correct the whole manuscript.

Answers to referee #2

#### Comment 1:

The authors have made some efforts to rewrite and clarify the questions raised in the first reviews. Some issues, however, still need to be resolved. The authors should again check their line of argument in critical appraisal of their data and the peculiarities of their setup, and the manuscript could be streamlined and condensed. The resulting paper would surely deserve publication as a valuable source for further studies.

**Response 1:** We thank the reviewer for the positive feedback, thoughtful comments and constructive suggestions that have helped us improve the manuscript. We next detail our

responses to the reviewer's comments and the explanation of how the observations and corrections have been addressed in the revised manuscript.

# Comment 2:

Perhaps the authors might want to revisit the objectives stated in the introduction, also in light of the comments made by the other referee. Maybe objectives i) and ii) are enough and can also be formulated as three bullet points (investigation of transport, investigation of dissipation, comparison of tracers and pesticides). The design of the experiment does include vegetation and alternating saturation but was perhaps not adequate to investigate the influence of vegetation and alternating saturation in great details, due to the lack of replica and controls. Of course, these things can still be discussed, and answering the remaining questions is still challenging enough.

**Response 2:** We appreciate the reviewer's suggestion and we agree that we may have formulated too many questions in our study. As a result, the interpretation of the findings has turned out to be quite challenging. However, if we remove the study of the influence of vegetation and alternating saturation conditions from the objectives, the whole approach will make no sense because the operation and design of the experiment was intended to answer those questions, and so, it will be very difficult to justify the choice of our particular setup. On the other hand, our aim was not to investigate the influence of the aforementioned factors in great detail, but in a very general way. Besides, the inclusion of vegetation and alternating saturation conditions also sought to promote the processes under study.

# Comment 3:

The authors refrain from including modeling in the paper, as they point out in their responses to the reviews. Without a quantitative analysis, however, large parts of the conclusions on transport, retardation, sorption and transformation remain rather speculative. A comprehensive numerical simulation would indeed be out of scope of the paper, but perhaps a first assessment of the results using solute transport theory (transfer functions) would have provided a more adequate means to analyze the transport behavior. Instead, the analysis of the data relies heavily on correlations between the breakthrough curves, but correlation ignores any shifts due to retardation. The analysis of lag correlation (which neglects any dispersion) was newly introduced in the revised version, but it is limited to the bromide BTC. Comparing the different tracers and assessing possible retardation and transformation is difficult, if not impossible, with this approach. Looking at lag correlations between the different BTCs (and reporting the significant findings) could possibly improve this weakness a bit, if further analyses should really be out of scope.

**Response 3:** We are aware of the limitations of the correlation analysis, but it has served as a first comparison between the behavior of the different compounds. As we have commented in the previous review, the evaluation of the results using solute transport theory has not been made due to the complexity and particular approach of our experiment. In any case, we have followed your suggestion and we have looked at more lag-correlations. The results are discussed in the next comments.

# Comment 4:

Any quantitative assessment requires getting a good description of the flow behavior. The presented perceptual model of the flow field is that tracers enter at the inlet, percolate downwards to the bottom gravel layer, from where they are transported to the upper layers by upwelling. The observations show that the middle layers are not participating in this transport, which is attributed to preferential flow. That water should have passed by all observation points in this layer is unlikely, but if all other explanations can be ruled out, this surprising finding would have to be considered in the interpretation. In my opinion, it is much more likely that lateral transport also occurred at or near the surface, perhaps on top of the saturated, "unconnected" middle layers. This would much better explain the higher concentrations near the surface compared to the bottom. Preferential flow could still be present in the second run, when solutes also appear in the middle layers. I encourage the authors to revisit this point.

**Response 4:** We thank the reviewer for the valuable opinion. We fully agree and also consider that some type of lateral transport at or near the surface may have occurred in our experiment. This hypothesis was contemplated from the beginning, but due to the type of injection we considered it unlikely. In any case, we cannot ignore this possibility because, as the reviewer has pointed out, it would much better explain part of the behavior of the solutes in our system. Other possible explanations, such as the influence of the roots, have also been taken into account. All these points are now included in the discussion of the results in the revised version.

# Comment 5:

The discussion and interpretation of the results should be checked critically, streamlined and condensed. The structure with 5 sub-sections is fine, but perhaps it can be rearranged to separate more clearly the interpretation of the experimental results from the inferences on wetlands and the referencing to the literature. For example, last paragraph of 3.2 and last half of 3.3 could go to the end; 3.3 and 3.4 could be combined, etc.

**Response 5:** Thanks for the suggestion. We agree with your assessment. As such, we have rearranged some of the paragraphs to separate the interpretation of the results from the implications of our findings for wetland systems. Only the subsections "Recoveries of solutes at the outlet" and "Final mass balance" have not been merged because we consider that they discuss two different points that must be treated separately. Also, if we combine them the resulting subsection will be too long.

#### **Specific Comments**

#### Comment 6:

*p* 2, *l* 29: Strictly speaking, it is not a control, because the difference is not only that on part is being planted, but also is located at the inlet.

**Response 6:** We thank the reviewer for pointing this out. Indeed, the definition of control is not very clear in this particular experiment since it is a single unit. Therefore, we will omit the term "control" from the text.

#### Comment 7:

p 4, ll 12 to 15: The tracer injection is not fully clear. It is stated that solutes were contained in 40 L of water. This solution was pumped into the system until saturation. According to Fig. 2, saturation was reached in 1-2 days. On page 3, l 41, it is stated that saturation with target substances was one week. In Tab. 2 (B) the pumping rate is given with 21.6 L h-1. Was the tracer solution injected in two hours or two days? Was other water used to further saturate the system after the 40 L were injected?

**Response 7:** We apologize for the confusion. The water level in Fig. 2 is only schematic and does not correspond to real water level measurements (as indicated in the caption). This means that saturation was not reached in 2 days but in about 2 hours. What we meant on p. 3, L 41 was that the system remained saturated for about a week, but no other water was used to further saturate the system after the 40 L were injected. We have clarified this issue on p. 4 L. 1-2 and on p. 4 L. 14-16 as follows:

 $\rightarrow$  During the first saturation phase, only one injection was made, whereas in the second saturation phase, the system was kept saturated by constant injections of tap water.

 $\rightarrow$  The inflow was held constant for about two hours, until the system became saturated and the upward flow formed a surface ponding of approximately two centimeters.

#### Comment 8:

*p* 6, *l* 11: Due to its conservative and non-sorbing character, Br– can hardly serve for investigating retardation, which is commonly caused by reversible adsorption/desorption.

**Response 8:** Thanks for raising this important point. The cross-correlations between  $Br^-$  time series were intended to provide information about the arrival of the solutes to the different layers. Indeed, given the conservative character of  $Br^-$  the delay could not be related to sorption processes but rather to other causes, such as low pore connectivity, as has been discussed on p. 7 L. 25-27:

 $\rightarrow$  In contrast, the delayed peak of the uppermost layer was associated with possible low pore connectivity in the vicinity of the sampling ports.

## Comment 9:

# *p* 6, *l* 18: Correlating BTC between the vegetated and non-vegetated zones could also be interesting, especially for comparison of the zones

**Response 9:** Thank you for this suggestion. We have performed additional correlations between the time series of the solutes of the vegetated and non-vegetated zone distinguishing between the first and second run. These results are presented now in a new table (Table. 5, see below) and they are discussed in subsection 3.1, on p. 7 L. 29-32:

 $\rightarrow$  If we compare the performance of the vegetated and the non-vegetated zone by means of correlations (Table 5), we observe stronger correlations in the lower layers (at 27 and 39 cm depth) than in the upper layers (at 15 and 3 cm depth), especially in the uppermost during the first run. These results suggested a greater influence of the plants and/or other causes (e.g. transport along the surface) on solute transport in upper layers.

Depth		First run		Second ru	n
(cm)		rho	p-value	rho	p-value
3	Br <sub>nv</sub> : Br <sub>v</sub>	0.43	0.09	0.53	0.05*
	UR <sub>nv</sub> : UR <sub>v</sub>	0.30	0.26	0.33	0.25
	SRB <sub>nv</sub> : SRB <sub>v</sub>	0.79	0.26	0.32	0.26
	Bos <sub>nv</sub> : Bos <sub>v</sub>	0.79	0.06	0.76	0.03*
	$Pen_{nv}$ : $Pen_{v}$	0.46	0.35	-	-
	Met <sub>nv</sub> : Met <sub>v</sub>	0.59	0.22	0.58	0.13
	Met-ESA <sub>nv</sub> : Met-ESA <sub>v</sub>	0.40	0.43	-	-
	Met-OA <sub>nv</sub> : Met-OA <sub>v</sub>	-	-	-	-
15	Br <sub>nv</sub> : Br <sub>v</sub>	0.49	0.06	0.63	0.02*
	UR <sub>nv</sub> : UR <sub>v</sub>	0.53	0.04*	0.05	0.86
	SRB <sub>nv</sub> : SRB <sub>v</sub>	0.51	0.04*	-0.01	0.98
	Bos <sub>nv</sub> : Bos <sub>v</sub>	-	-	-	-
	Pen <sub>nv</sub> : Pen <sub>v</sub>	-	-	-	-
	Met <sub>nv</sub> : Met <sub>v</sub>	-	-	-	-
	Met-ESA <sub>nv</sub> : Met-ESA <sub>v</sub>	-	-	-	-
	Met-OA <sub>nv</sub> : Met-OA <sub>v</sub>			-	-
27	Br <sub>nv</sub> : Br <sub>v</sub>	0.58	0.02*	0.97	< 0.001***
	$UR_{nv}$ : $UR_v$	0.85	< 0.001***	0.78	< 0.001***
	SRB <sub>nv</sub> : SRB <sub>v</sub>	0.67	0.004 **	0.64	0.01**
	Bos <sub>nv</sub> : Bos <sub>v</sub>	-	-	-	-
	Pen <sub>nv</sub> : Pen <sub>v</sub>	-	-	-	-
	Met <sub>nv</sub> : Met <sub>v</sub>	-	-	0.76	0.03*
	Met-ESA <sub>nv</sub> : Met-ESA <sub>v</sub>	-	-	-	-
	Met-OA <sub>nv</sub> : Met-OA <sub>v</sub>	-	-	-	-
39	Br <sub>nv</sub> : Br <sub>v</sub>	0.53	0.03*	0.27	0.35
	$UR_{nv}$ : $UR_v$	0.84	< 0.001***	0.95	< 0.001***
	SRB <sub>nv</sub> : SRB <sub>v</sub>	-0.06	0.83	0.73	0.003**
	$Bos_{nv}$ : $Bos_v$	-	-	-	-
	$Pen_{nv}$ : $Pen_v$	-	-	-	-
	Met <sub>nv</sub> : Met <sub>v</sub>	0.40	0.44	0.76	0.03*
	Met-ESA <sub>nv</sub> : Met-ESA <sub>v</sub>	-	-	-	-
	Met-OA <sub>nv</sub> : Met-OA <sub>v</sub>	-	-	-	-

**Table 5.** Spearman rank correlation of the breakthrough curves between the vegetated and non-vegetrated zones for the different solutes and the first and second run.

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

nv = non-vegetated; v = vegetated

#### Comment 10:

# *p* 6, *l* 24: As stated in the next paragraph, an overall mass balance for pesticides was not possible.

**Response 10:** You raise a very valid point here. We have corrected the sentence on p. 6 L. 20-22 as follows:

 $\rightarrow$  The fate of the tracers and their main dissipation pathways were examined with a final overall mass balance that accounted for five different compartments (pore water, outlet water, sediments, stems + leaves and roots).

# Comment 11:

p 7, ll 8 to 18: Why can these observations not be due to flow along the surface? Higher values in the upper layers than in the bottom are hardly possible if solute flow was first to the bottom and then up - or you missed important parts of the breakthrough with your sampling frequency

**Response 11:** We thank the reviewer for pointing this out. As already stated in comment 4, lateral transport at or near the surface is now included as a possible explanation of the results of our study. Yet, we consider that the presence of plants may have also favored the transport of solutes towards the vegetated surface. This point is discussed in section 3.1, on p. 7 L. 10-15 as follows:

 $\rightarrow$  Hence, it can be speculated that lateral transport at or near the surface may have occurred during the injections causing augmented transport of solutes towards the vegetated surface. However, other possible explanations could not be ruled out. These include the likely influence of the plants by means of water uptake and the possible contribution of the roots to the formation of channels through which preferential flow took place. Other mechanisms, not necessarily related to plants (e.g. fingering), may have been involved in the transport of solutes to the vegetated area, too.

# Comment 12:

p 7, ll 19 to 24: What are the time units of the lag correlation? If it is days, why are only lags up to 7 days tested/displayed? From the BTC it appears that lag could be in the range of weeks – months. Why were only Br-BTC analysed?

**Response 12:** The time units of the lags are days but referring to the observation dates, and this may vary between several days to weeks. This information has been specified in Fig. 6.

We computed the cross-correlation of two univariate series. The number of lags displayed in the plot are calculated by default as  $10*\log_{10}(N/m)$  where N is the number of observations and m the number of series. In our case, given that we had from 8 to 16 number of observations and 2 series, the number of lags displayed were from 8 to 5.

We have analyzed Br<sup>-</sup> because due to its more conservative character, it serves for investigating transport. Unfortunately, the lag analysis performed to the other BTCs did not yield conclusive information.

#### Comment 13:

p7, ll 24/25: That is difficult to understand. Do you argue that transport was from the bottom to the top, but delayed due to low connectivity? Why was there a transport then at all? How fast would a transport have been that was not delayed? How do you rule out transport along the surface? Isn't that much more likely in view of the higher concentrations at the top compared to the bottom?

**Response 13:** Once again, thank you for pointing this out. As we have explained in the response to comments 4 and 11, transport along the surface is now included in our explanation of transport in the experiment.

What we argue here is that the system was filled from the bottom to the top and that due to the conditions previous to the injection in the sediments, the solutes distributed heterogeneously and reached the sampling ports at different times. It should be noted that the values obtained at the different sampling ports were only representative for the nearest areas. Considering this, we hypothesized that the delayed measurement of solutes in the uppermost layer was due to low pore connectivity in the vicinity of the sampling ports. Probably the solutes arrived to this layer earlier, but we couldn't measure them at the time of the arrival because of the presence of water-filled pores in the surroundings of the sampling ports. We apologize because we have not made this point clear enough. Therefore, we have better explained this question in the text on p. 7 L. 25-28 as follows:

 $\rightarrow$  In contrast, the delayed peak of the uppermost layer was associated with possible low pore connectivity in the vicinity of the sampling ports. Here the solutes probably arrived earlier, but we could not measure them at the time of the arrival because of the presence of water-filled pores in the surroundings of the sampling ports.

#### **Comment 14:**

p 7, l 29: The contrast between "strong" correlation and "not any" correlation sounds greater than it actually is (0.77 vs. 0.55). More important, that does not tell much about the transport characteristics other than that they are different. How about lag correlation here?

**Response 14:** We thank the reviewer for this correction. While it is true that some of the values indicated no correlation (e.g. 0.14 or 0.26) other values pointed out that the correlations were not significant (e.g. 0.55 with p-value 0.15). The sentence has been corrected on p. 7 L. 33-36 as follows:

 $\rightarrow$  With the exception of the uppermost layer, the non-vegetated zone showed strong correlation between the two runs regardless of the layer, whereas in the vegetated zone some layers did not show any correlation (layers at 3 and 27 cm depth) or displayed correlations that were statistically non-significant (layers at 15 and 39 cm depth).

As suggested, we have performed the lag correlation analysis to the Br- breakthrough curves between the first and second run. The results have been included in the supplementary material (Fig. S3, see below). This new information has been added to the text on p. 7 L. 38-40 as follows:

 $\rightarrow$  Lag correlations between the first and second run were also analyzed (Fig. S3 of the supplementary material). A significant value (at time t=-3) was found in the vegetated zone at 15 cm depth.



**Figure S3.**: Lag analysis performed to the Br<sup>-</sup> breakthrough curves between the first and second run for the vegetated and non-vegetated zones and the sampling depths: 1) 3cm; 2) 15cm; 3) 27cm and 4) 39cm.

#### Comment 15:

p 7, ll 30 to 33: Why not mentioning that Br also has experienced plant uptake? For example, S Xu et al., Environ. Sci. Technol. 2004, 38 (21), 5642-5648

**Response 15:** Thanks for raising this important point. We completely agree, and the possible influence of Br- uptake by the plants has been detailed on p. 8 L. 1-2:

 $\rightarrow$  However, other causes, such as the influence of flushing between runs and Br- uptake by the plants (Xu et al., 2004) could not be ruled out.

#### **Comment 16:**

p 8, ll 7/8: These parts are more or less the beginning and the end of the transport regime through the tank, right?

**Response 16:** That is right. The information has been incorporated on p. 8 L. 17-19 as follows:

 $\rightarrow$  According to the correlations performed to the solute time series (Fig. 8), two spots exhibited the strongest relationships: the non-vegetated part of the lowermost layer and the vegetated part of the uppermost layer. These spots coincided with the beginning and end of the transport regime through the system.

# Comment 17:

*p* 8 *l* 17: "confirmed creation of preferential flow paths" - Or maybe it was only high conductivity and low sorption in the gravel layer, and overland/near-surface flow?

**Response 17:** Thank you for pointing this out. We have rewritten the sentence on p. 8 L. 27-30 as follows:

 $\rightarrow$  This was not the case for SRB, which only displayed strong positive correlation with Br- in the lower- and uppermost layers. The former was explained by possible high conductivity and low sorption in the gravel. The results from the uppermost layer were associated with the likely promotion of transport towards the vegetated surface, given the strong sorptive character of SRB.

#### Comment 18:

p 9, l 40: "evidencing their great mobility and persistence" – But the total recovery of metazachlor and TPs was low, < 20 %. This is a contradiction.

**Response 18:** Here, we wanted to emphasize that the TPs were still coming out from the system at the end of the experiment when we flashed it. The fact that we recovered TPs at the outlet, even if it was a small amount, was an indication that they were not further degraded and/or retained in the system. We consider that this is an important finding. However, since the sentence can lead to confusion, we have rewritten it on p. 9 L. 41-42 and p.10 L. 1-2 as follows:

 $\rightarrow$  Even though small amounts of TPs were obtained, it was an indication that they were not further degraded or retained in the system, which was in agreement with the findings of other studies (Mamy et al., 2005; European Food Safety Authority (EFSA), 2008). Higher amounts of met-ESA were recovered compared to met-OA.

#### Comment 19:

# p 10, ll 9 to 14: These sentences are directly contradicting each other: Either DT50 values let expect higher recoveries, or DT50 values lead to lower recoveries

**Response 19:** We thank the reviewer for raising this point. We realize that there is a contradiction in our argument regarding the leaching potential and therefore it will be removed from the text. From the DT50 values, we would have expected higher recoveries, because the duration of the experiment was below those values. Since the recoveries were low, we think that both, the high sorption potential of these solutes and the possible incidence of plant uptake were the cause. We have corrected the sentence on p. 10 L. 15-16 as follows:

 $\rightarrow$  Hence, we have hypothesized that the cause of the low recoveries of boscalid and penconazole could have been their high sorption potential and possible plant uptake.

## Comment 20:

p 10, l 24 to p 11, l 2: This part not only related to recovery, perhaps put it towards the end (discussion of meaning of results for pesticide removal in wetlands in general).

**Response 20:** We appreciate this suggestion. The indicated part has been moved to the end of the discussion to the new subsection called: "Implications for pesticide mitigation in wetland systems"

# Comment 21:

p 11, 135 to p 12, 16: No plant uptake, no transformation, no recovery, no mineralization, no volatilization - where did it go? Is it really plausible that the bulk of the substances was sorbed on sand and gravel particles? What would that imply for future applications of these substances – would sorbing capacities be depleted?

**Response 21:** We are not arguing here that plant uptake or transformation did not take place at all. They probably occurred but to a lesser extent compared to sorption. The retention of these substances has definitely played a fundamental role (at least for boscalid and penconazole) and it should be considered when assessing the mitigation capacities of these pesticides in wetlands. In these systems, the depletion of the sorption capacities will depend on both, the concentration of the adsorbing substances and the number of sorption places. These factors should be taken into account for future applications of strongly sorbing pesticides such as boscalid and penconazole.

This conclusion has been included in the manuscript on p. 11 L. 27-31:

 $\rightarrow$  With this in mind, it can be concluded that retention has played a fundamental role in our study (at least for boscalid and penconazole). Therefore, special attention should be given to retention processes when assessing the mitigation capacities of strongly sorbing pesticides such as boscalid and penconazole in wetlands. In these systems the depletion of the sorption capacities will depend on both, the concentration of the adsorbing substances and the number of sorption places.

# Comment 22:

p 12, ll 7/8: Is this difference between vegetated and non-vegetated for Br and UR really significant? Can it only be attributed to the vegetation? How does the distance to the inlet influence this finding?

**Response 22:** We thank the reviewer for underscoring these questions. We have checked the differences in the values between the vegetated and the non-vegetated area and in fact they are not significant. In view of the results, we don't think that the distance to the inlet have had any influence on this finding. We have corrected the sentence on p. 11 L. 32-34 and now it can be read as follows:

 $\rightarrow$  Lower amounts of UR and Br- were recovered from the pore water of the vegetated compared to the non-vegetated zone (Fig. 10-A), however, the differences were not significant.

## Comment 23:

p 12, ll 10/11: How do you rule other possibilities? Couldn't preferential flow paths also be due to the flushing in between runs that contributed to a change in pore space? Which effect would a stronger hydraulic gradient invoked by root water uptake in the vegetated zone have?

**Response 23:** Indeed, it is possible that transport in the second run may have been influenced by the flushings performed at the end of the first run. This important information has been added to the discussion about the different performances of the system between runs on p. 8 L. 1-2:

 $\rightarrow$  However, other causes, such as the influence of flushing between runs and Br- uptake by the plants (Xu et al., 2004) could not be ruled out.

Root water uptake could have also facilitated transport of solutes towards the vegetated surface, although probably to a lesser extent in view of the results of the second run. Nevertheless, this possibility has also been included in the explanation of transport on p. 7 L. 12-14:

 $\rightarrow$  However, other possible explanations could not be ruled out. These include the likely influence of the plants by means of water uptake and the possible contribution of the roots to the formation of channels through which preferential flow took place.

## Comment 24:

# p12, ll 26/27: From Fig 8 and 9 (A1 and B1) I would rather argue that SRB and the two pesticides are not behaving that similar.

**Response 24:** We thank the reviewer for pointing this out. Obviously, we have not made our point clear enough. We have not stated that SRB and the other two pesticides (boscalid and penconazole) have behaved similarly in the pore water, as their breakthrough curves were different. Instead, we have argued that compared to the other solutes (Br, UR and metazachlor), they have shown a greater tendency to sorption. And this hypothesis has been based on their similar rapid decrease in their concentrations after the injections and gradual increase in accumulated mass recovery during the flushing phase. Regarding the recovery curves, what we wanted to express was that they still exhibited increases even after the systems was repeatedly flashed. To avoid misunderstandings, we have removed the sentence "Analogous recovery curves for SRB, boscalid and penconazole were observed" from the text.

# Comment 25:

p12, ll 33/34: I do not see where you have shown unequivocally that UR was transformed bio-chemically - you assume that, because there is a large part missing in the mass balance (cf. p 10, l19/20).

**Response 25:** Yes, that is correct. Unfortunately, biological degradation of UR has not been demonstrated unequivocally in this study. Rather, the results suggested that this process may have also occurred. In a recent study (Lange et al., 2018) biochemical

transformation of UR has been shown in arable soils, if this process happens also in saturated wetlands still needs to be shown.

# Comment 26:

p12, l 38: How realistic / relevant is that long time period? Elsewhere, you have reported mean residence times of 6 days for typical wetland systems.

**Response 26:** Obviously in natural wetland systems the period that we have simulated is probably not realistic. But we have shown that the promotion of long periods of stagnation may be beneficial to eliminate pesticides. This parameter could be taken into account when constructing engineered systems designed to remove contaminants from the water. This conclusion has been included in the revised manuscript on p. 12 L. 18-20 as follows:

 $\rightarrow$  According to the findings, promoting solute contact with the medium through long periods of stagnation should be taken into account when constructing engineered systems designed to remove contaminants from the water.

# Comment 27:

# p13, l 12 to 14: Absence in middle layers because of the flow regime (local effect of this particular setup)? (related to comments above )

**Response 27:** We wanted to highlight that boscalid and penconazole were not detected in the middle layers, while all the other compounds were detected (particularly in the nonvegetated zone). Therefore, we do not think that their absence was due to a local effect because, if that had been the case, it would have affected all solutes.

Nevertheless, it is true that the particular setup has had an effect on the results, especially the type of injection which has conditioned the way the solutes entered the system. This has been discussed in the manuscript, for instance on p. 8 L. 34-35:

 $\rightarrow$  Spatial and temporal variability of transport and dissipation processes were associated with the conditions prior to injection, the way the solutes entered the system, the presence of plants and the promotion of aeration during the drying phase.

# Comment 28:

#### p 19, Table 2(A): Mean initial organic carbon content - how is zero error achieved?

**Response 28:** This is because we rounded to one decimal place. The actual value is:  $0.2 \pm 0.02$ . We have added a second decimal place to avoid misunderstandings. The same has been done for the mean initial dithionite-extractable Fe (Fed).

#### Comment 29:

*p* 19, Table 2(A): Conductivity: Is Br breakthrough detectable in the conductivity measurements with the 5 TE sensors?

**Response 29:** Yes, some of the sensors captured the trend of Br<sup>-</sup> but not a complete breakthrough. Strong correlations between conductivity and Br<sup>-</sup> have been observed,

particularly in the non-vegetated area at 15 cm depth for the first run and the vegetated area at 15 and 27 cm depth for the second run. The information has been added to the text on p. 7 L. 19-22 as follows:

 $\rightarrow$  The delayed peak of Br- observed at 15 cm depth in the non-vegetated area for the first run was also detected by the conductivity probe located at the same depth. The complete breakthrough curve could not be capture by the sensor, but a strong correlation (Spearman's rho=0.83 and p-value<0.001) between Br and the conductivity values was found (see Table S2 of the supplementary material).

**Table S2.** Spearman rank correlation between the breakthrough curves of Br<sup>-</sup> and the conductivity values of the probes located at the same depth for the first and second experimental run and the different zones.

Zonas	First run		Second run	
Zones	rho	p-value	rho	p-value
Non-vegetated	-0.34	0.19	0.60	0.02 *
Vegetated	0.07	0.80	-0.2	0.49
Non-vegetated	0.83	<0.001 ***	-	-
Vegetated	-0.39	0.13	0.84	<0.001 ***
Non-vegetated	-	-	-	-
Vegetated	-0.18	0.51	0.82	<0.001 ***
Non-vegetated	-0.07	0.81	0.05	0.86
Vegetated	0.32	0.23	0.54	0.04 *
	Zones Non-vegetated Vegetated Non-vegetated Non-vegetated Vegetated Non-vegetated Vegetated	ZonesFirst run rhoNon-vegetated-0.34Vegetated0.07Non-vegetated-0.39Non-vegetated-Vegetated-0.18Non-vegetated-0.07Vegetated0.32	$\begin{tabular}{ c c c } \hline First run \\ \hline rho & p-value \\ \hline rho & 0.19 \\ \hline rho & 0.19 \\ \hline rho & 0.80 \\ \hline rho & 0.83 & <0.001 *** \\ \hline rho & 0.83 & <0.001 *** \\ \hline rho & -0.39 & 0.13 \\ \hline rho & -0.18 & 0.51 \\ \hline rho & -0.07 & 0.81 \\ \hline rho & 0.32 & 0.23 \\ \hline ext{tabular}$	$\begin{tabular}{ c c c c c } \hline Pirst run & P-value & rho & rho & rho & rho & rho & 0.19 & 0.60 & 0.07 & 0.80 & -0.2 & 0.01 & *** & - & 0.20 & 0.13 & 0.84 & 0.07 & 0.13 & 0.84 & 0.07 & 0.13 & 0.84 & 0.07 & 0.13 & 0.84 & 0.07 & 0.13 & 0.82 & 0.07 & 0.81 & 0.05 & 0.05 & 0.02 & 0.23 & 0.54 & 0.05 & 0.25 & 0.23 & 0.54 & 0.05 & 0.25 $

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

#### Comment 30:

*p* 21, Table 6: is this really "removal "- in light of the low probability of degradation stated in your text? Or just "trapping" in the sediments with the potential of being slowly released?

*In case of Met it is obviously wrong – it was not removed by 92.6 %, but at least another 6 % were transformed.* 

**Response 30:** Thank you for this interesting point. Sorption is also included in the processes and mechanisms involved in the removal of pesticides from contaminated waters in constructed wetland systems. Therefore, we still think that "removal" is the correct term. However, the adsorption of solutes by the substrates could imply their temporary removal from the water, as some of them may be released back into the soil solution. This has been specified in the definition of "removal" on p. 10 L. 5-7 as follows:

 $\rightarrow$  Several processes, mostly adsorption by substrates, transformation and plant uptake, were responsible for the removal of tracers and pesticides from the water in our system. Here, it should be noted that the adsorption of the solutes by the substrates could also involve their "temporary removal" from the water, since some of them may be released back into the soil solution.

#### Comment 31:

p 24, Fig. 3-1): Why is there no flow on surface? This was changed from the earlier version

**Response 31:** We removed the arrow from the last version to avoid misunderstandings since it could imply that the ponding on the surface was formed from surface flow

originated at the very beginning of the injection. This could only have happened when the system was already saturated and the surface water had formed. Fig. 3 has been modified to include this new explanation (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 (A) corresponds to the upward vertical filling and B) to the flow on the surface while the ponding was forming), (2) injection of clean water (tap water) from the bottom, (3) flushing of the system with clean water (tap water) from the bottom.

#### **Comment 32:**

p 25, Fig 4: The soil moisture sensors probably were not calibrated for the specific material? Gravel is reported to have 45% porosity, but does not exceed 33% moisture during saturated conditions?

Even though soil moisture is not calibrated - could simple mass balance shed light on the proportion of stagnant zones in the system? 40 Liters in a given volume with given porosity require a certain antecedent soil moisture to effect saturation as shown here.

**Response 32:** We thank the reviewer for highlighting this point. Unfortunately, the soil moisture sensors could not be calibrated. Moreover, some of them failed or stopped measuring since the beginning of the experiment (see Fig. 4). Therefore, even if we had made simple mass balances, we would still lack information from a large proportion of the system.

# Comment 33:

p 27, Fig 6: Time units? Why only +-7?

**Response 33:** This question has already been answered in comment 12.

## Comment 34:

p 28, Fig 8: In my opinion, this figure shows that all substances were transported with surface flow and vertically downwards from the inlet. Only after that first period of transport, behavior seems to differ.

**Response 34:** Once again we appreciate the feedback from the reviewer. If the assumption you propose were true, the breakthrough peaks would have reached the lowermost layer later than what actually happened, and yet, the solutes arrived first to the bottom (see Fig. 5 and 6). Besides, as already indicated in the manuscript, the system was filled from the bottom to the top, and thus, the solutes were expected to be transported in the same way.

# Comment 35:

p 29, Fig 9: Check caption.

**Response 35:** The caption has been corrected.

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

Elena Fernández-Pascual<sup>1</sup>, Marcus Bork<sup>1,2</sup>, Birte Hensen<sup>3</sup>, Jens Lange<sup>1</sup>

<sup>1</sup>Hydrology, Faculty of Environment and Natural Resources, University of Freiburg, Freiburg, Germany

<sup>2</sup>Soil Ecology, Faculty of Environment and Natural Resources, University of Freiburg, Freiburg, Germany

<sup>3</sup> Institute of Sustainable and Environmental Chemistry, Leuphana University Lüneburg, Lüneburg, Germany

Correspondence to: Elena Fernández-Pascual (elena.fernandez@hydrology.uni-freiburg.de)

Abstract. Studies that have used hydrological tracers to investigate the fate and transport of pesticides in constructed wetlands

- 10 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 <u>model constructed</u> wetland system on
- 15 a long term basis and detailed spatial scale. Moreover, the influence of vegetation and alternating different hydrologic conditions on transport and dissipation processes has been evaluated by comparing a vegetated with a non-vegetated section of the wetland system and by alternating periods of saturation and drying, respectively. Breakthrough curves obtained at different sampling depths pointed out that the solutes were not equally distributed within the constructed wetland. Data revealed that a higher mass of solutes was transported to the vegetated part of the uppermost layer, which was associated with possible lateral
- 20 transport at or near the surface and/or a possible shortcut effect produced by the roots. In contrast, the middle layers showed retardation, possibly most likely due to the presence of water filled pores before the injections and low pore connectivity in the vicinity of the sampling ports. Transport of solutes was driven by the injections. The strong temporal and spatial correlation found between Br<sup>-</sup>, UR and metazachlor indicated that these solutes experienced more transportless retention than SRB, boscalid and penconazole, which most likely underwent sorption, as evidenced by their absence in the middle layers, rapid
- 25 decrease in their concentrations after the injections and similar-gradual increase in accumulated mass recovery at the outlet. The overall tracer mass balance allowed us to identify three dissipation pathways: sorption, transformation and plant uptake. The detection of metazachlor transformation products (TPs) confirmed the contribution of transformation to metazachlor dissipation, whereas no TPs for boscalid and penconazole were detected. 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
- 30 at the beginning of the experiment for sorption and after promoting aerated conditions for transformation. The use of hydrological tracers 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. This study represents a first approximation, and further experiments need to be done under field conditions together with mathematical-modeling.

#### **1** Introduction

5

- 35 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, 2015). 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 <u>ifwhen</u> the <u>incomplete degradation of</u> pesticides produce-leads to a variety-large number of transformation products (TPs), whose behavior is <u>often</u> unknown, and <u>whose</u> toxicity
- 40 or persistence may be-in some cases greater than that of the parent compounds. In fact, the presence of TPs in water bodies has 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. 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

- 5 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).
- 10 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). HoweverYet, in most cases where this approach has been applied, the system under study has been treated as a
- 15 "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.

The 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

- 30 sampling and monitoring on a long-term basis. Three hydrological tracers were chosen as reference substances according to their reactive nature: bromide (Br<sup>-</sup>) as a non-adsorbing tracer (Whitmer et al., 2000), UR as a photosensitive tracer (Gutowski et al., 2015) that can undergo processes of (bio-) chemical transformation (Lange et al., 2018) and SRB as a highly sorptive tracer (Kasnavia et al., 1999). 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
- 35 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<sup>-</sup> 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

- 5 Terbutryn-D5 (98.5 %) already dissolved in acetonitrile (100  $\mu$ g 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<sup>-1</sup> for UR and SRB and of 10 g L<sup>-1</sup> for Br<sup>-</sup> were prepared in MilliQ water. Pesticides (0.1 g L<sup>-1</sup>) dissolved in acetonitrile were directly mixed with the injection solution. The concentration of tracers and pesticides in the injection solution was 100 mg L<sup>-1</sup>, 50  $\mu$ g L<sup>-1</sup> and 100  $\mu$ g L<sup>-1</sup>, for sodium bromide, UR and SRB, respectively; and 50  $\mu$ g
- 10  $L^{-1}$  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

- 15 (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. They were purchased from a local garden center. The whole experiment was carried out indoors in a laboratory assembly, therefore. Therefore, 64 OSRAM SSL 3W light-emitting
- 20 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
- 25 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 hHalf of the pipes were located at the gravel layer and the other half in the sand. With a viewIn order to obtaining pore water samples at awith high vertical resolution, a multi-level pipe with a sampling
- resolution of 12 cm was designed-with a sampling resolution of 12 cm. This ereated-resulted in 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<sub>15</sub> 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
- 35 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).

#### 40 2.3 Operation of the model constructed wetland system

The model constructed wetland system was designed to alternate saturated <u>andwith</u> unsaturated conditions (long periods of aeration). <u>In totalA total of</u> three phases were created (Fig. 2): 1) saturation with target substances (one week), 2) drying by evapotranspiration (three weeks) and 3) saturation with <u>tapelean</u> water (one month). <u>During the first saturation phase, only one injection was made, whereas in the second saturation phase, the system was kept saturated by constant injections of eleantap</u>

- 5 water. The saturation phases were preceded by the injection of solutes or tapclean 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\_check possible interactions between substances, the concentrations in the solution were measured on the day of the injection and a couple of days after. Prior to the injection, 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
- 10 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 <del>clean water (tap</del> water) from the bottom of the system and (iii) flushing of

- 15 the sediments with <u>clean watertap water</u> 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 overflowed <u>the reservoirit</u> 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 <u>held constant for about two hours-maintained until until-when</u> the system became saturated and the upward flow formed a surface ponding of approximately two centimeters-<u>height</u>. 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 <del>clean water (tap water)</del> from
- the bottom. The water flowed evenly through the sediment in vertical upward direction. <u>Again</u>, <u>T</u>the inflow was maintained until the system became saturated and a surface ponding of approximately two centimeters <u>height</u>-was formed. 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, <u>clean water (tap water)</u> 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 30 water was performed simultaneously in order to prevent <u>the</u> mixing of waters. A volume of 60 mL of pore water was transferred to 100 mL brown glass bottles and stored at 4°C for major ions and tracers analysis. Previously, a volume of 10 mL was transferred to 15 mL Ppolypropylene 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
- 35 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°C for the subsequent analysis of major ions and tracers and -20°C for the analysis of pesticides and TPsFollowing this, the samples were stored at 4°C, in case of major ions and tracer analysis orand -20°C depending on the type of analysis performed afterwards (major ions and tracers analysis orin case of pesticide and TPs, respectively) analysis.
- 40 At the end of the experiment, the sediment bed was emptied of its gravitational water. Following this Then, 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. Following thisThen, 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 60°C for approximately 24 hours and stored in the dark for subsequent measurements of tracers. Biomass was determined on a dry matter basis.

5 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

35

#### 10 2.5.1 Major ions and tracers in the pore- and outlet-water

Pore- and outlet-water samples were measured for major ions (Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Br<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup>) by ion chromatography (Dionex ICS-1100, Thermo Scientific, USA). All samples were previously filtered with a 0.45  $\mu$ m 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

15 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<sup>-1</sup> for UR and 0.1 µg L<sup>-1</sup> 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<sup>-1</sup>. 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,
- 30 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.

Germany) was used as stationary phase with a and oven temperature was set oven temperature of  $T = to 30^{\circ}C$ . Limits of

#### 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 ehelen for 20 minutes at 240 mm and stored at 4%C in the artificentee for at least 24 hours. Afterwards, supermeteet use

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  $\mu$ m) and measured for the tracers. The resulting 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<sup>-</sup> 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

5 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<sub>d</sub>) 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

10

Spatial and temporal dynamics of transport processes in the pore water were investigated by analyzing soil moisture data and Br<sup>-</sup> breakthrough curves. Here, Br<sup>-</sup> was chosen as reference due to its most conservative character. The occurrence and role of

- 15 retardation was studied by performing cross-correlations between Br<sup>-</sup> time series. The predominance of transport processes among the solutes was examined by looking at the relationship between Br<sup>-</sup> 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
- 20 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<sub>s</sub>) 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. The influence of the vegetation and hydrologic conditions on transport and dissipation processes wasere evaluated through the comparison of by comparing the results of the vegetated with the non-vegetated zones and the results of the different phases. In addition, tThe
- 25 performance of the two experimental runs was assessed by making-correlations between Br<sup>-</sup> breakthrough curves of the first and the second run.

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 tracers and their main dissipation pathways were examined with a final overall mass balance that accounted for five different compartments (pore water, outlet water, sediments, stems +

- 30 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.
- 35 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, and their comparison with the tracers-and the findings of similar studies.

#### 40 **3** Results and discussion

#### 3.1 Transport processes in the pore water according to Br<sup>-</sup> behavior

The relative concentrations of Br<sup>-</sup> measured at different depths (Table 4) indicated that similar values were reached in the lowerand uppermost layers right after the injection. In contrast, no Br<sup>-</sup> 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

- 5 pores may have caused heterogeneities resulting in an uneven distribution of solutes within the system. Indeed, soil moisture values (Fig. 4) measured prior to <u>the</u> injections (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, thus suggesting which suggested that the water holding capacity of the middle sections was higher compared to the other layers (at least in the vegetated zone, since there is no data
- 10 from the non-vegetated). These conditions results 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<sup>-</sup> (Table 4) and subsequent delay of the breakthrough peaks (see Fig. 5).

On the other hand, given that Br<sup>-</sup> 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 in the middle layers were only located in some areas parts of

- 15 the middle layers including the surroundings of the sampling ports. In contrast, in the other areas rest of the system probably matrix flow dominated. <u>AYet, according to Fig. 5</u>, the uppermost layer displayed a delayed breakthrough peak with relative concentrations of Br<sup>-</sup> 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 can be speculated that lateral transport at or
- 20 near the surface may have occurred during the injections causing augmented transport of solutes towards the vegetated surface. However, other possible explanations could not be ruled out. These include the likely influence of the plants by means of water uptake and the possible contribution of the roots to the formation of channels through which preferential flow took place. Other mechanisms, not necessarily related to plants (e.g. fingering), may have been involved in the transport of solutes to the vegetated area, tooHence, it was speculated that the plants, and more specifically the rootscontributed to the formation of channels through
- 25 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<sup>-</sup> breakthrough curves (Fig. 6) confirmed that a the delayed arrival 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

- 30 zone. Specifically, the delays obtained in the middle layers (at time t = -7 and t = -3 in the first and the second run, respectively) were greater than those obtained in the uppermost layer (at time t = -5 and t = -2 in the first and the second run, respectively). The delayed peak of Br- observed at 15 cm depth in the non-vegetated area for the first run was also detected by the conductivity probe located at the same depth. The complete breakthrough curve could not be capture by the sensor, but a strong correlation (Spearman's rho=0.83 and p-value<0.001) between bromide and the conductivity values was found (see Table S2 of the
- 35 supplementary material). Similar results could not be observed in the non-vegetated layer at 27 cm depth due to the failure of the sensor. Overall, tThe delayed peak of solutes in the middle layers coincided in time with the end of the drying phase and beginning of the second saturation phase., and hHence, it was related to the likely migration of solutes during the drop and subsequent rise of the in water table from the surface. In contrast, the delayed peak of the uppermost layer was associated with possible low pore connectivity in the vicinity of the sampling ports. Here the solutes probably arrived earlier, but we could not

measure them at the time of the arrival presumably because of the presence of water-filled pores in the surroundings of the sampling ports.

If we compare the performance of the vegetated and the non-vegetated zone by means of correlations (Table 5), we observe stronger correlations in the lower layers (at 27 and 39 cm depth) than in the upper layers (at 15 and 3 cm depth), especially in

- 5 the uppermost during the first run. These results suggested a greater influence of the plants and/or other causes (e.g. transport along the surface) on solute transport in the upper layers. These findings also indicated that the system did not behave the same way in each run. In fact, if we evaluate its performance over timeThe different behavior of Br<sup>-</sup> 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<sup>-</sup> of the first and the second run-(Table <u>65</u>), differences between the vegetated and non vegetated
- 10 zones-were observed. With the exception of the uppermost layer, the non-vegetated zone showed strong correlation between the two runs regardless of the layer, whereas in the vegetated zone some layers the vegetated did not show any correlation (layers at 3 and 27 cm depth) or displayed correlations that were statistically non-significant (layers at 15 and 39 cm depth). This meant-These results indicated that the overall performance of the non-vegetated zone was similar in both runs, whereas the vegetated behaved differently. This was not applicable to the uppermost layer because it was probably influenced by the
- 15 surface water and/or the presence of plants. Lag correlations between the first and second run were also analyzed (Fig. S3 of the supplementary material). A significant value (at time t=-3) was found in the vegetated zone at 15 cm depth. In general, these results were related to possible changes in root density and/or spatial distribution in the system over time most likely occurred during the experiment. This assumption was supported by visual observations of the sediment (Fig. 7). However, other causes, such as the influence of the flushings between runs and Br uptake by the plants (Xu et al., 2004) could not be ruled out.
- 20 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).

# 3.2 <u>Relationship between solutes and their b</u>Behavior in the pore water throughout the experiment and relationship between solutes throughout the experiment

- Overall, the injected solutes have followed the same trend as Br<sup>-</sup> in the lower- and uppermost layers (Fig. 5). Conversely, in the middle sections, only UR and metazachlor behaved similar to Br<sup>-</sup>, although this was only observed in the non-vegetated part. In fact, the vegetated zone only displayed observable amounts of Br<sup>-</sup>. 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-nonvegetated 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 30 be noted that other transformation products may have been formed in our system. However, such compounds were most likely below the limit of quantification ( $\leq$  9.29 and  $\leq$  10.28 µg L<sup>-1</sup> 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
- 35 vegetated uppermost layer 32 days after the initial injection during the first run. Whereas-However, 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), two spots exhibited the strongest relationships: the non-vegetated part of the lowermost layer and the vegetated <u>part of the uppermost layer</u>. The other respective halves also showed high correlations. These spots coincided with the beginning and end of the transport regime through the system.
 40 however, <u>B</u>boscalid and penconazole did not correlate with Br<u>in the vegetated part of the lowermost layer and the non-vegetated of the uppermost layer</u>. These results pointed out that probably other processes besides transport (e.g. sorption or

plant uptake) dominated the dissipation of <u>these compounds boscalid and penconazole</u> during the experiment. In the middle layers only UR and metazachlor exhibited significant correlations with Br<sup>-</sup>, although metazachlor did not show any correlation in the vegetated zone. These <u>findingsresults</u> suggested that transport was the dominant process for metazachlor in the absence of vegetation, whereas under the influence of plants, <u>itmetazachlor</u> most likely experienced <u>other processes (i.e., plant uptake</u>,

- 5 mineralization in the roots, sorption and/or transformation). In contrast, UR, on the other hand, correlated with Br<sup>-</sup> in all layers regardless of the presence of vegetation. Therefore, transport was almost certainly a dominant process for this tracer in the pore water. This was not the case for SRB, whose-which only displayed strong positive correlation with Br<sup>-</sup> in the upperlower- and upperlowermost layers. The former was explained by possible high conductivity and low sorption in the gravel. The results from the uppermost layer were associated with the likely promotion of transport towards the vegetated surface, given the strong
- 10 sorptive character of SRB. 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.

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

- 15 Spatial and temporal variability of transport and dissipation processes were associated with the conditions prior to injection, the way the solutes entered the system, the presence of plants and the promotion of aeration during the drying phase. Most of the solutes went to the lower- and uppermost layers. However, <u>T</u>the highest concentrations were recorded in the vegetated part of the uppermost layer soon after the injection. <u>This suggested thus suggesting</u> 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,
- 20 <u>we speculated it is believed</u> 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

- 25 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<sup>-</sup>, UR and metazachlor) were transported to the non-vegetated part of the middle layers during the drop and subsequent rise of the water tablethe drop in water table. However, that was only true in the non-vegetated part of the middle layers, as-In the vegetated
- 30 part of the middle layers in the vegetated only Br<sup>-</sup> was detected displayed observable amounts (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. This hypothesis was in agreement with as evidenced by the behavior amount of SRB found in the sediment (see Fig. 11). It was assumed that sorption velocity was highest at the beginning of the experiment gGiven the rapid decrease in relative concentrations of SRB, boscalid and penconazole shortly after the injections,. In the later phases, sorption it was assumed that
- 35 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

40 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, Pplant 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

5 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

15 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).

#### **3.43** Recoveries of solutes at the outlet

The results of the cumulative recovery curves obtained at the outlet of the system during the flushings are displayed in Fig. 9.

- 20 <u>During the first flushings</u>Generally, we observed a rapid increase in accumulated mass recovery for 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<sup>-</sup>, 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<sup>-</sup>, UR and metazachlor. Indeed, the sorptive character of SRB, boscalid and penconazole has already been reported in the literature (Long
- 25 <u>et al., 2005; Vallée et al., 2014; Dollinger et al., 2017</u>). Indeed, In the fourth flushing, 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-A1 and B1). Yet, this was only true after the system was repeatedly flushed, even though SRB has different physico-chemical properties and SRB showed higher mass recoveryies than boscalid and penconazole (Table 67). Yet, this was only true after the system was repeatedly flushed. A possible explanation
- 30 for these similarities is that SRB, boscalid and penconazole 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 These findings were associated with the different physico-chemical properties of boscalid and penconazole compared to SRB<sub>2</sub>, According to this, 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
- 35 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 systems.

Cumulative recovery curves of metazachlor TPs were also obtained detected at the outlet of the system during the flushings
 (Fig. 9). Even though small amounts of TPs were obtained, it was an indication that they were not further degraded or retained in the system, which was in agreement with the findings of other studies thereby evidencing their great mobility and persistence

in the environment (Mamy et al., 2005; European Food Safety Authority (EFSA), 2008). In this case, <u>Hh</u>igher 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  $\frac{67}{}$ ), the solutes were classified as follows (from highest to lowest recovery rate): Br<sup>-</sup> >> SRB >> UR >> Boscalid >> Penconazole >> Metazachlor.

- 5 It is believed that <u>S</u>several processes in our system, mostly adsorption by substrates, transformation and plant uptake, were responsible for the removal of tracers and pesticides from the water in our system. Here, it should be noted that the adsorption of the solutes by the substrates could also involve their "temporary removal" from the water, since some of them may be released back into the soil solution. In this context, <u>T</u>the physico-chemical properties of the compounds have most likely been a determining factor for their dissipation. Vallée et al. (2014) found that a greater retention of pesticides in the soil was related
- 10 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 boscalid and penconazole, transformation processes played an important role in the dissipation of metazachlor, as demonstrated by the <u>detection</u>measurement\_of its TPs. On the other hand, given the greater persistence of boscalid and penconazole in the soil (DT<sub>50</sub> values of 200 and 117 days, respectively), we would have anticipated higher recoveries of these solutes after the
- 15 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 high sorption potential 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<sub>50</sub>). Yet, other causes, such as and possible higher incidence of plant uptake could not be ruled out.
- 20 As for the tracers, as expected Br<sup>-</sup> recovery was the largest highest, given which was in agreement with its most conservative character. Following Br<sup>-</sup>, The recovery of SRB was also high recoveries, compared to the other solutes. 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 and plant uptake.<sup>5</sup> This behavior has already been evidenced reported 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
- 25 retention and especially degradation processes were involved in its dissipation. Overall, <u>T</u> 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).
- In principle, we expect to obtain analogous results in wetland systems if similar conditions are met. In this regard, if we compare
- 30 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
- 35 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
- 40 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

5 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.

#### 3.54 Final mass balance

40

The overall mass balance performed at the end of the experiment is shown in Fig. 10. According to the tracer results, Br was 10 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 in their wetland experiment under batch conditions. Discrepancies with SRB were associated with the large uncertainty in the measurements performed in the sediment, These results were-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 15 the vegetated part was located in the uppermost layer.

According to the different compartments, Br and UR showed the highest recoveries in the outlet water which highlighted their higher mobility-whereas.-In contrast, SRB was mainly found in the sediment. These results pointed out the different behavior of the tracers when it comes to dissipation. As already evidenced by the breakthrough and cumulative recovery curves, Br displayed the most conservative character, although some dissipation was observed via plant uptake (16.76% of the total

- 20 recovered). In contrast, Tthe 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, 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
- 25 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
- 30 substrates such as UR. Photodegradation of UR was evidenced by the decrease in its concentration during saturation in the vegetated part (exposed to light) of the uppermost layer that was exposed to light (see Fig. 5). In contrast, no decrease was observed in the part of the same layer that was not exposed to light. Differences were also observed between runs. While in the first run a decrease in the concentration of UR from 17.44 to 12.26 µg/l was detected, 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<sup>-1</sup>, and half-life
- 35 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 could not be excluded eannot be ruled out. As for the pesticides-mass balance, while the measurement of metazachlor TPs confirmed that biochemical transformation contributed to its dissipation, the transformation of boscalid and penconazole could not be proven. Yet, it was not could not be

ruled out since the concentration of their metabolites may have been below the limit of quantification. However, considering the duration of the experiment ( $62.5 \pm 2.12$  days each run) and the DT<sub>50</sub> values of boscalid and penconazole reported in the literature (Table 1), probably their transformation was <u>minimalnegligible</u>. Therefore, and based on the overall results, sorption most likely played the main role in the dissipation of boscalid and penconazole. Plant uptake, on the other hand, could not be

- 5 determined for the pesticides in the present study... Yet, it was assumed that it took place given that the results of Br in the present study and thoseproved its occurrence and it has been reported in of previous investigations. 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). Traces of metazachlor and its metabolites were also detected in the roots and
- 10 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<sub>2</sub> or volatilisation from aqueous systems/soil water were-could not be ruled out. However, they were considered to be minimal according to literature (EFSA, 2008). With this in mind, it can be concluded that retention has played a fundamental role in our study (at least for boscalid and penconazole). Therefore, special attention should be given to retention processes when assessing the
- 15 mitigation capacities of strongly sorbing pesticides, such as boscalid and penconazole, in wetlands. In these systems the depletion of the sorption capacities will depend on both, the concentration of the adsorbing substances and the number of sorption places.

The <u>contribution of vegetation to dissipation has also been evaluated in the</u> final mass balance (Fig. 10). <u>also confirmed the</u> contribution of vegetation to uptake and breakdown. Indeed, <u>IL</u> ower amounts of UR and Br<sup>-</sup> were recovered from the pore

- 20 water of the vegetated compared to the non-vegetated zone (Fig. 10-A), although the differences were not significant. 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 promotion of transport towards the vegetated surface. creation of preferential flow paths induced by the plants. On the other hand, <u>M</u>most of SRB was found sorbed in the sediment of the vegetated zone, where the highest concentration of
- 25 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.

#### 3.6 Implications for pesticide mitigation in wetland systems

- 30 The replicated conditions in our experiment may resemble those of a groundwater-fed wetland that undergo wet-dry cycles. In principle, we can expect to obtain analogous results in natural systems if similar conditions to those of our study are met. If we compare the characteristics of our experiment (see Table 2) with those of other wetland investigations (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),
- 35 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 7) were within the same range as those of the wetland studies. For instance, Vallée et al. (2015)
- 40 <u>found that the removal rates of boscalid in two pilot-scale wetlands ranged from 38 to 67%, whereas Gikas et al. (2018) obtained</u> 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).

A possible explanation for the high removal 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

5 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. According to the findings, promoting solute contact with the medium through long periods of stagnation should be taken into account when constructing engineered systems designed to remove contaminants from the water.

Overall, these observations highlight the importance of certain factors in the removal of pesticides, namely the presence of

10 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 removal 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.

Although our experiment has been carried out in a laboratory environment, the replicated conditions may resemble those of a

- 15 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
- 20 <u>of solutes. Other field studies in wetland systems have pointed out that the presence of vegetation greatly increases contact time</u> <u>and surface area for adsorption (Moore et al., 2006; Liu et al., 2018), which may also be enhanced when organic matter content</u> <u>is high (Passeport et al., 2011).</u>

# 3.75 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 (Xu et al., 2004), although to a lesser extent. As for SRB, despite the fact that it has been extensively frequently used to identify sorption processes of pesticides in
- 30 wetland systems (Passeport et al., 2010; Lange et al., 2011; Schuetz et al., 2012). Yet., its special sorptive character makes it difficult to be compared with a specific eertain type of pesticide. In this regard, while Some authors (e.g. Dollinger et al., (2017)) stated that SRB could be used as a good proxy for hydrophilic and strongly sorbing pesticides, while others (e.g. Lange et al., 2018) demonstrated proved that the same tracer closely mimicked the gradual recession of a moderately hydrophobic pesticide in the top soil topsoil of an agricultural field. As for Oour results, demonstrated we found that SRB can serve to approximate
- 35 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. In this context, given that greater lower amounts of boscalid and penconazole SRB-may be recovered compared to SRBsuch pesticides, due to the greatest sorption potential of the pesticides and possibly due to its greater leachability and/or lower-susceptibility to be
- 40 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<sub>27</sub> which This 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

5 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<u>and the influence that the vegetation and alternating different hydrologic</u> eonditions have on such processes. By comparing tracers with selected pesticides, valuable hints about dominant transport vectors and main dissipation pathways have been collected.

Breakthrough curves obtained at different sampling depths suggested that solute transport was favored towards the vegetated

- 15 surfaceuppermost layer, probably due to lateral transport at or near the surface and/or a possible shortcut effect produced by the roots. Yet, other mechanisms not necessarily related to plants (e.g. fingering) could not be ruled out., while Conversely, it solute transport was retarded in the middle layers possibly most likely due to the presence of water filled pores before the injection and low pore connectivity in the vicinity of the sampling ports. Spatial and temporal variability of transport and dissipation processes were associated with the conditions prior to injection, the way the solutes have entered the system, the
- 20 presence of plants and the promotion of aeration during the drying phase. The strong positive correlation found between Br, UR and metazachlor highlighted the predominance of transport processes for these compounds. By contrast, SRB, boscalid and penconazole most likely experienced sorption as evidenced by their absence in the middle layers (in the case of boscalid and penconazole), rapid decrease in their concentrations after the injection and gradual increase in accumulated mass recovery during flushings. Yet, their lowerdifferent final recoveries of boscalid and penconazole compared to SRB suggested indicated
- 25 lower leaching potential a greater contribution of retention and and/or higher incidence of possibly plant uptake in their dissipation of boscalid and penconazole compared to SRB.

The overall tracer mass balance allowed us to identify three dissipation pathways: sorption, transformation and plant uptake. While Br<sup>-</sup> 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

30 of metazachlor TPs, namely met-ESA and met-OA demonstrated that biochemical transformation played an 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.

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<del>, etc.</del>) 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.

Overall, the complexity of the processes that take place inside constructed wetlands and the lack of sufficient data on a temporal and spatial scale highlights the need to adopt new methods to fully understand the behavior of pollutants in these systems. The

40 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. Yet, further experiments need to be done, especially under field conditions combined with modeling.

#### Acknowledgements

This research has been carried out in the framework of the MUTReWa-project (02WRM1366B) funded by the German Federal

5 Ministry for Education and Research. The authors wish to acknowledge Emil Blattmann and Britta Kattenstroth for their help with the construction of the experimental setup as well as Barbara Herbstritt, Jens Robertson, Felix Zimmermann and Maria Martin Pérez for sampling and analytical support. We would also like to thank Marit van Tiel and Sunanth Venkateshwaran for constructive criticism of the manuscriptWe would also like to thank and Christine Stumpp for her valuable advice and contribution to the design of the sampling setup.

#### 10 References

20

Aga, D. S., Thurman, E. M., Yockel, M. E., Zimmerman, L. R., & Williams, T. D.: Identification of a new sulfonic acid metabolite of metolachlor in soil, Environmental science & technology, 30(2), 592-597, 1996.

Aislabie, J., & Lloyd-Jones, G.: A review of bacterial-degradation of pesticides, Soil Research, 33(6), 925-942, 1995.

Bard, A.J., Parsons, R., Jordan, J.: Standard Potentials in Aqueous Solution. Marcel Dekker Inc., New York, 1985.

15 Birkigt, J., Stumpp, C., Małoszewski, P., & Nijenhuis, I.: Evaluation of the hydrological flow paths in a gravel bed filter modeling a horizontal subsurface flow wetland by using a multi-tracer experiment, Science of The Total Environment, 621, 265-272, 2018.

Bouldin, J. L., Farris, J. L., Moore, M. T., Smith Jr, S., Stephens, W. W., & Cooper, C. M.: Evaluated fate and effects of atrazine and lambda-cyhalothrin in vegetated and unvegetated microcosms, Environmental Toxicology: An International Journal, 20(5), 487-498, 2005.

Brix, H.: Do macrophytes play a role in constructed treatment wetlands?, Water science and technology, 35(5), 11-17, 1997.

Calderbank, A.: The occurrence and significance of bound pesticide residues in soil, in: Reviews of environmental contamination and toxicology (pp. 71-103). Springer, New York, NY, 1989.

Casado, J., Brigden, K., Santillo, D., & Johnston, P.: Screening of pesticides and veterinary drugs in small streams in the

25 European Union by liquid chromatography high resolution mass spectrometry, Science of The Total Environment, 670, 1204-1225, 2019.

Catallo, W. J: Hourly and Daily Variation of Sediment Redox Potential in Tidal Wetland Sediments, Geological Survey Reston Va, 1999.

Chen, Z., Chen, Y., Vymazal, J., Kule, L., & Koželuh, M.: Dynamics of chloroacetanilide herbicides in various types of mesocosm wetlands, Science of the Total Environment, 577, 386-394, 2017.

Dollinger, J., Dagès, C., & Voltz, M.: Using fluorescent dyes as proxies to study herbicide removal by sorption in buffer zones, Environmental Science and Pollution Research, 24(12), 11752-11763, 2017.

Đurović, R., Gajić-Umiljendić, J., & Đorđević, T.: Effects of organic matter and clay content in soil on pesticide adsorption processes, Pesticidi i fitomedicina, 24(1), 51-57, 2009.

35 Durst, R., Imfeld, G., & Lange, J.: Transport of pesticides and artificial tracers in vertical-flow lab-scale wetlands, Water Resources Research, 49(1), 554-564, 2013.

Ebert, D., & Harder, U.: Boscalid: The degradation behaviour of 14C-BAS 510 F in different soils (DT50/DT90), BASF AG, Agrarzentrum Limburgerhof, 2000.

Edwards, C. A.: Factors that affect the persistence of pesticides in plants and soils, in: Pesticide Chemistry-3 (pp. 39-56), 1975.

European Food Safety Authority (EFSA).: Conclusion regarding the peer review of the pesticide risk assessment of the active substance metazachlor, EFSA Journal, 6(7), 145r, 2008.

European Food Safety Authority (EFSA).: Conclusion regarding the peer review of the pesticide risk assessment of the active substance penconazole, EFSA Journal, 6(10), 175r, 2008.

Eurostat: Agri-environmental indicator-pesticide pollution of water, Statistics explained, European Commission, https://ec.europa.eu/eurostat/statistics-explained/index.php/Archive:Agri-environmental\_indicator\_

10 \_pesticide\_pollution\_of\_water, 2012.

5

Fan, J., Zhang, B., Zhang, J., Ngo, H. H., Guo, W., Liu, F., ... & Wu, H.: Intermittent aeration strategy to enhance organics and nitrogen removal in subsurface flow constructed wetlands, Bioresource technology, 141, 117-122, 2013.

Fernández-Pascual, E., Zaman, S., Bork, M., Lang, F., & Lange, J.: Long-term mesocosm experiments to investigate degradation of fluorescent tracers, Journal of Hydrology X, 100014, 2018.

15 Flury, M., & Wai, N. N.: Dyes as tracers for vadose zone hydrology. Reviews of Geophysics, 41(1), 2003.

Gardiner, D. T., & James, S.: Wet soil redox chemistry as affected by organic matter and nitrate, American Journal of Climate Change, 1(04), 205, 2012.

Gaullier, C., Baran, N., Dousset, S., Devau, N., Billet, D., Kitzinger, G., & Coisy, E.: Wetland hydrodynamics and mitigation of pesticides and their metabolites at pilot-scale, Ecological Engineering, 136, 185-192, 2019.

20 Ghestem, M., Sidle, R. C., & Stokes, A.: The influence of plant root systems on subsurface flow: implications for slope stability, Bioscience, 61(11), 869-879, 2011.

Gikas, G. D., Vryzas, Z., & Tsihrintzis, V. A.: S-metolachlor herbicide removal in pilot-scale horizontal subsurface flow constructed wetlands, Chemical Engineering Journal, 339, 108-116, 2018.

Goss, M. J., Miller, M. H., Bailey, L. D., & Grant, C. A.: Root growth and distribution in relation to nutrient availability and 25 uptake, European Journal of Agronomy, 2(2), 57–67, 1993.

Gregoire, C., Elsaesser, D., Huguenot, D., Lange, J., Lebeau, T., Merli, A., ... & Schulz, R.: Mitigation of agricultural nonpointsource pesticide pollution in artificial wetland ecosystems–a review, in: Climate Change, Intercropping, Pest Control and Beneficial Microorganisms, 293-338, Springer, Dordrecht, 2009.

Gutowski, L., Olsson, O., Lange, J., & Kümmerer, K.: Photolytic transformation products and biological stability of the 30 hydrological tracer Uranine, Science of the Total Environment, 533, 446-453, 2015.

Hofmann, K.: Wachstumsverhalten von Schilf (Phragmites australis (Cav.) Trin. ex Steudel), in: klärschlammbeschickten Filterbeeten, Archiv für Hydrobiologie, 107(3), 385-409, 1986.

Holden, J.: Piping and woody plants in peatlands: Cause or effect? Water Resources Research, 41(6), 2005.

Joseph, G.: Determination of sodium monofluoroacetate in dairy powders by liquid chromatography tandem mass spectrometry 35 (LC-MS/MS): First Action 2015.02, Journal of AOAC International, 98(4), 1121-1126, 2015.

Käss, W., Behrens, H., & Behrens, H.: Tracing technique in geohydrology, Balkema, Rotterdam, The Netherlands, 581, 1998.

Karlsson, A. S., Weihermüller, L., Tappe, W., Mukherjee, S., & Spielvogel, S.: Field scale boscalid residues and dissipation half-life estimation in a sandy soil, Chemosphere, 145, 163-173, 2016.

Karpuzcu, M. E., Sedlak, D. L., & Stringfellow, W. T.: Biotransformation of chlorpyrifos in riparian wetlands in agricultural watersheds: Implications for wetland management, Journal of hazardous materials, 244, 111-120, 2013

5 Kasnavia, T., Vu, D., & Sabatini, D. A.: Fluorescent dye and media properties affecting sorption and tracer selection, Ground water, 37(3), 376-381, 1999.

Kolpin, D. W., Schnoebelen, D. J., & Thurman, E. M.: Degradates provide insight to spatial and temporal trends of herbicides in ground water, Groundwater, 42(4), 601-608, 2004.

Lange, J., Schuetz, T., Gregoire, C., Elsässer, D., Schulz, R., Passeport, E., & Tournebize, J.: Multi-tracer experiments to
 characterise contaminant mitigation capacities for different types of artificial wetlands, International Journal of Environmental and Analytical Chemistry, 91(7-8), 768-785, 2011.

Lange, J., Olsson, O., Sweeney, B., Herbstritt, B., Reich, M., Alvarez-Zaldivar, P., ... & Imfeld, G.: Fluorescent tracers to evaluate pesticide dissipation and transformation in agricultural soils, Science of The Total Environment, 619, 1682-1689, 2018.

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

Lin, A. Y. C., Debroux, J. F., Cunningham, J. A., & Reinhard, M.: Comparison of rhodamine WT and bromide in the determination of hydraulic characteristics of constructed wetlands, Ecological Engineering, 20(1), 75-88, 2003.

Liu, T., Xu, S., Lu, S., Qin, P., Bi, B., Ding, H., ... & Liu, X.: A review on removal of organophosphorus pesticides in constructed wetland: performance, mechanism and influencing factors, Science of The Total Environment, 2018.

Lv, T., Carvalho, P. N., Bollmann, U. E., Arias, C. A., Brix, H., & Bester, K.: Enantioselective uptake, translocation and degradation of the chiral pesticides tebuconazole and imazalil by Phragmites australis, Environmental pollution, 229, 362-370, 2017.

25 201

Maillard, E., Payraudeau, S., Faivre, E., Grégoire, C., Gangloff, S., & Imfeld, G.: Removal of pesticide mixtures in a stormwater wetland collecting runoff from a vineyard catchment, Science of the Total Environment, 409(11), 2317-2324, 2011.

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

30 Małoszewski, P., Wachniew, P., & Czupryński, P.: Study of hydraulic parameters in heterogeneous gravel beds: Constructed wetland in Nowa Słupia (Poland), Journal of Hydrology, 331(3-4), 630-642, 2006

Mamy, L., Barriuso, E., & Gabrielle, B.: Environmental fate of herbicides trifluralin, metazachlor, metamitron and sulcotrione compared with that of glyphosate, a substitute broad spectrum herbicide for different glyphosate-resistant crops, Pest Management Science: formerly Pesticide Science, 61(9), 905-916, 2005.

35 Martin, A., Margoum, C., Jolivet, A., Assoumani, A., El Moujahid, B., Randon, J., & Coquery, M.: Calibration of silicone rubber rods as passive samplers for pesticides at two different flow velocities: Modeling of sampling rates under water boundary layer and polymer control, Environmental toxicology and chemistry, 37(4), 1208-1218, 2018.

Long, R., Gan, J., & Nett, M.: Pesticide choice: Best management practice (BMP) for protecting surface water quality in agriculture, 2005.

Marín-Benito, J. M., Rodríguez-Cruz, M. S., Sánchez-Martín, M. J., & Mamy, L.: Modeling fungicides mobility in undisturbed vineyard soil cores unamended and amended with spent mushroom substrates, Chemosphere, 134, 408–416, 2015.

McMahon, P. B., Dennehy, K. F., Michel, R. L., Sophocleous, M. A., Ellett, K. M., & Hurlbut, D. B.: Water movement through thick unsaturated zones overlying the central High Plains aquifer, southwestern Kansas, 2000–2001, USGS Water Resources Investigation Papert 3(4171) 32, 2003

5 Investigation Report, 3(4171), 32, 2003.

Mehra, O. P., & Jackson, M. L.: Iron oxide removal from soils and clays by a dithionite–citrate system buffered with sodium bicarbonate, in: Clays and clay minerals: proceedings of the Seventh National Conference, 317-327, 1960.

Moore, M. T., Bennett, E. R., Cooper, C. M., Smith Jr, S., Farris, J. L., Drouillard, K. G., & Schulz, R.: Influence of vegetation in mitigation of methyl parathion runoff, Environmental Pollution, 142(2), 288-294, 2006.

10 Müller, K., Bach, M., Hartmann, H., Spiteller, M., & Frede, H. G.: Point-and nonpoint-source pesticide contamination in the Zwester Ohm catchment, Germany, Journal of environmental quality, 31(1), 309-318, 2002.

Nimmo, J. R.: Preferential flow occurs in unsaturated conditions, Hydrological Processes, 26(5), 786-789, 2012.

Ong, S. A., Uchiyama, K., Inadama, D., Ishida, Y., & Yamagiwa, K.: Performance evaluation of laboratory scale up-flow constructed wetlands with different designs and emergent plants, Bioresource technology, 101(19), 7239-7244, 2010.

15 Papaevangelou, V. A., Gikas, G. D., Vryzas, Z., & Tsihrintzis, V. A.: Treatment of agricultural equipment rinsing water containing a fungicide in pilot-scale horizontal subsurface flow constructed wetlands, Ecological engineering, 101, 193-200, 2017.

Passeport, E., Tournebize, J., Jankowfsky, S., Prömse, B., Chaumont, C., Coquet, Y., & Lange, J.: Artificial wetland and forest buffer zone: hydraulic and tracer characterization, Vadose Zone Journal, 9(1), 73-84, 2010.

20 Passeport, E., Tournebize, J., Chaumont, C., Guenne, A., & Coquet, Y.: Pesticide contamination interception strategy and removal efficiency in forest buffer and artificial wetland in a tile-drained agricultural watershed, Chemosphere, 91(9), 1289-1296, 2013.

Petticrew, E. L., & Kalff, J.: Water flow and clay retention in submerged macrophyte beds, Canadian Journal of Fisheries and Aquatic Sciences, 49(12), 2483-2489, 1992.

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

Reemtsma, T., Alder, L., & Banasiak, U.: Emerging pesticide metabolites in groundwater and surface water as determined by the application of a multimethod for 150 pesticide metabolites, Water research, 47(15), 5535-5545, 2013.

Rose, M. T., Crossan, A. N., & Kennedy, I. R.: The effect of vegetation on pesticide dissipation from ponded treatment wetlands: Quantification using a simple model, Chemosphere, 72(7), 999-1005, 2008.

Sabatini, D. A.: Sorption and intraparticle diffusion of fluorescent dyes with consolidated aquifer media, Groundwater, 38(5), 651-656, 2000.

Sanchez-Martin, M. J., Andrades, M. S., & Sánchez-Camazano, M.: Soil properties influencing the adsorption and mobility of penconazole in vineyard soils, Soil science, 165(12), 951-960, 2000.

35 Sannino, F., Filazzola, M. T., Violante, A., & Gianfreda, L.: Fate of herbicides influenced by biotic and abiotic interactions, Chemosphere, 39(2), 333-341, 1999. Schuetz, T., Weiler, M., & Lange, J.: Multitracer assessment of wetland succession: effects on conservative and nonconservative transport processes, Water Resources Research, 48(6), 2012.

Seybold, C. A., Mersie, W., Huang, J., & McNamee, C.: Soil redox, pH, temperature, and water-table patterns of a freshwater tidal wetland, Wetlands, 22(1), 149-158, 2002.

5 Smart, P. L., & Laidlaw, I. M. S.: An evaluation of some fluorescent dyes for water tracing, Water Resources Research, 13(1), 15-33, 1977.

Stang, C., Wieczorek, M. V., Noss, C., Lorke, A., Scherr, F., Goerlitz, G., & Schulz, R.: Role of submerged vegetation in the retention processes of three plant protection products in flow-through stream mesocosms, Chemosphere, 107, 13-22, 2014.

Stehle, S., & Schulz, R.: Agricultural insecticides threaten surface waters at the global scale, Proceedings of the National Academy of Sciences, 112(18), 5750-5755, 2015.

Torrentó, C., Prasuhn, V., Spiess, E., Ponsin, V., Melsbach, A., Lihl, C., ... & Hunkeler, D.: Adsorbing vs. nonadsorbing tracers for assessing pesticide transport in arable soils, Vadose Zone Journal, 2017.

Tournebize, J., Passeport, E., Chaumont, C., Fesneau, C., Guenne, A., & Vincent, B.: Pesticide de-contamination of surface waters as a wetland ecosystem service in agricultural landscapes, Ecological engineering, 56, 51-59, 2013.

15 University of Hertfordshire, PPDB: Pesticide properties database, 2010.

25

USEPA (US Environmental Protection Agency), Pesticide Fact Sheet, Boscalid, 2003.

Vallée, R., Dousset, S., Billet, D., & Benoit, M.: Sorption of selected pesticides on soils, sediment and straw from a constructed agricultural drainage ditch or pond, Environmental Science and Pollution Research, 21(7), 4895-4905, 2014.

Vallée, R., Dousset, S., Billet, D.: Water residence time and pesticide removal in pilot-scale wetlands, Ecol. Eng. 85, 76–84, 20 2015.

Vallée, R., Dousset, S., & Billet, D.: Influence of substrate water saturation on pesticide dissipation in constructed wetlands, Environmental Science and Pollution Research, 23(1), 109-119. 2016

von der Ohe, P. C., Dulio, V., Slobodnik, J., De Deckere, E., Kühne, R., Ebert, R. U., ... & Brack, W.: 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, 2011.

Vorenhout, M., van der Geest, H. G., & Hunting, E. R.: An improved datalogger and novel probes for continuous redox measurements in wetlands, International Journal of Environmental and Analytical Chemistry, 91(7-8), 801-810, 2011.

Vymazal, J.: Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment, Ecological engineering, 25(5), 478-490, 2005.

30 Vymazal, J., & Březinová, T.: The use of constructed wetlands for removal of pesticides from agricultural runoff and drainage: a review, Environment international, 75, 11-20, 2015.

Wernli, H. R.: Einführung in die Tracerhydrologie: hydrologisches Praktikum, Geographisches Institut Univ. Bern, 2009.

Whitmer, S., Baker, L., & Wass, R.: Loss of bromide in a wetland tracer experiment, Journal of Environmental Quality, 29(6), 2043-2045, 2000.

35 Włodarczyk, M.: Influence of formulation on mobility of metazachlor in soil, Environmental monitoring and assessment, 186(6), 3503-3509, 2014.

## **Tables and Figures**

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

Property		Unit	UR	SRB	Br-	Boscalid	Penconazole	Metazachlor
Chemical formula			$C_{20}H_{10}O_5Na_2$	$C_{27}H_{29}N_2NaO_7S_2$	NaBr	$C_{18}H_{12}Cl_2N_2O$	$C_{13}H_{15}Cl_2N_3$	C14H16ClN3O
Chemical family			Xanthene dye	Xanthene dye	Inorganic salt	Carboxamide	Triazole	Chloroacetamide
Molecular mass <sup>a</sup>		g mol <sup>-1</sup>	376.3	580.7	102.89	342.033	283.064	277.098
Aqueous solubility		g L <sup>-1</sup>	25.0 <sup>b</sup>	70.0 <sup>b</sup>	850 °	0.0046 <sup>d</sup>	0.073 <sup>d</sup>	0.450 <sup>d</sup>
Aqueous diffusion coefficient		cm <sup>2</sup> s	3.5 x 10 <sup>-6 c</sup>	4.7 x 10 <sup>-6 c</sup>	-	4.4 x 10 <sup>-12 g</sup>	-	-
Soil degradation	DT50	days	-	-	stable <sup>c</sup>	200 <sup>d</sup> (persistent)	117 <sup>d</sup> (persistent)	8.6 <sup>d</sup> (non-persistent)
Dissipation rate on plant matrix	RL <sub>50</sub>	days	-	-	-	6.9 <sup>d</sup>	65.6 <sup>d</sup>	-
Photolytic stability	DT <sub>50</sub>	days	0.5 °	34 °	stable <sup>c</sup>	30 <sup>d</sup> (stable)	4 <sup>d</sup> (moderately fast)	stable <sup>d</sup>
Hydrolytic stability	DT <sub>50</sub>	days	stable <sup>c</sup>	stable <sup>c</sup>	stable <sup>c</sup>	stable <sup>d</sup>	stable <sup>d</sup>	stable <sup>d</sup>
Water-sediment	DT <sub>50</sub>	days	stable <sup>i</sup>	stable <sup> i</sup>	-	-	853 <sup>d</sup> (stable)	20.6 d (fast)
Organic carbon - water partitioning	Koc	L kg <sup>-1</sup>	0-62 <sup>b</sup>	147-498 <sup>b</sup>	-	772.0 <sup>f</sup>	2205 <sup>f</sup>	134.0 <sup>f</sup>
Octanol - water	Log Kow		1.26-3.56 <sup>h</sup>	0.21-4.77 <sup>h</sup>	-	2.96 <sup>d</sup>	3.72 <sup>d</sup>	2.49 <sup> d</sup>

partitioning (at pH 7) (-)Information not available

<sup>*a*</sup> From ChemID database (2017)

<sup>b</sup> From Sabatini (2000)

10 <sup>c</sup> From Leibundgut et al. (2009)

<sup>d</sup> From Pesticide Properties DataBase, University of Hertfordshire.

<sup>e</sup> From Merck Millipore (http://www.merckmillipore.de)

<sup>f</sup> From PAN Pesticides Database (.pesticideinfo.org/Search\_Chemicals.jsp)

<sup>g</sup> From Martin et al. (2017)

15 <sup>h</sup> From EPA Chemistry Dashboard

<sup>i</sup> 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	$0.2 \pm 0.0 \underline{2}$
	Mean final organic carbon content **	%	Sand	$0.8 \pm 1.4$
	Mean initial dithionite-extractable Fe (Fed)*	g Kg <sup>-1</sup>	Sand	$1.0\pm0.0\underline{1}$
	Mean final dithionite-extractable Fe (Fed)**	g Kg <sup>-1</sup>	Sand	$1.1\pm0.2$
	pH (H <sub>2</sub> O)	-	Sand	9.1
	pH (CaCl <sub>2</sub> )	-	Sand	8.1
	Diameter*	mm	Sand	0-2
			Gravel	4-8
	Bulk density*	Kg L <sup>-1</sup>	Sand	1.5
			Gravel	1.6
	Porosity	%	Sand	42
			Gravel	45
	Height	cm	Sand	32
			Gravel	10

	Surface area	m <sup>2</sup>	-	0.7
	Mass	Kg	Sand	430.8
			Gravel	124.0
	Redox potential***	mV	Sand and gravel	$-328 \pm 10.7$ to
				$+740\pm25.6$
	Conductivity***	dS/m	Sand and gravel	$0 \text{ to } 0.4 \pm 0.1$
Plants	Density	N°	Typha latifolia	4
			Phragmites australis	7
		Plants m <sup>-2</sup>	Typha latifolia	10.8
			Phragmites australis	18.9
	Mean initial height	cm	Typha latifolia	$79.8 \pm 18.6$
			Phragmites australis	$76.9 \pm 10.1$

\* 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 <sup>-1</sup>	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	$34.1\pm3.1$
Volume of total clean water injected in the flushings	L	$355.1\pm20.5$
Hydraulic retention time	Days	$62.5\pm2.12$

*Values represent means*  $\pm$  *standard deviation.* 

.

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

	I	
Substance	LOD [ng L <sup>-1</sup> ]	LOQ [ng L <sup>-1</sup> ]
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<sup>-</sup> measured during the: 1) first and 2) second run for the different zones, phases (saturation and drying) and depths.

1)	Donth	Saturatio	on		Drying					Saturatio	on			
1)	(cm)	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
	3	0.08	0.17	0.19	0.34	0.41	-	-	-	0.33	0.35	0.21	0.17	0.03
Non- vegetated	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
	3	0.20	0.70	0.79	0.76	0.79	-	-	-	0.48	0.55	0.45	0.39	0.08
Vagatatad	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
vegetated	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)	Donth	Saturation		Drying				Saturation						
2)	(cm)	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
	3	0.07	0.20	0.46	-	-	-	-	-	0.43	0.53	0.62	0.35	0.03
Non-	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
vegetated	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
	3	0.18	0.59	0.53	-	-	-	-	-	0.09	0.16	0.18	0.08	0.01
Manada d	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
vegetated	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 of the breakthrough curves between the vegetated and non-vegetrated zones for the different solutes and the first and second run.

D d		<b>T</b> .		a 1	
<u>Depth</u>		<u>First run</u>	_	Second run	
<u>(cm)</u>		<u>rho</u>	<u>p-value</u>	<u>rho</u>	<u>p-value</u>
<u>3</u>	$\underline{\mathbf{Br}}_{\mathbf{nv}}$ : $\underline{\mathbf{Br}}_{\mathbf{v}}$	<u>0.43</u>	<u>0.09</u>	<u>0.53</u>	0.05*
	<u>UR<sub>nv</sub>: UR<sub>v</sub></u>	<u>0.30</u>	<u>0.26</u>	<u>0.33</u>	<u>0.25</u>
	$\underline{SRB_{nv}}: \underline{SRB_{v}}$	<u>0.79</u>	0.26	0.32	<u>0.26</u>
	Bos <sub>nv</sub> : Bos <sub>v</sub>	<u>0.79</u>	<u>0.06</u>	<u>0.76</u>	<u>0.03*</u>
	Pen <sub>nv</sub> : Pen <sub>v</sub>	<u>0.46</u>	<u>0.35</u>	<u> </u>	<u> </u>
	<u>Met<sub>nv</sub>: Met<sub>v</sub></u>	<u>0.59</u>	0.22	<u>0.58</u>	<u>0.13</u>
	Met-ESAnv: Met-ESAv	<u>0.40</u>	<u>0.43</u>	<u> </u>	<u>_</u>
	<u>Met-OA<sub>nv</sub>: Met-OA<sub>v</sub></u>	<u>-</u>	<u>_</u>	<u>-</u>	<u> </u>
<u>15</u>	<u>Br<sub>nv</sub>: Br<sub>v</sub></u>	<u>0.49</u>	<u>0.06</u>	<u>0.63</u>	<u>0.02*</u>
	<u>UR<sub>nv</sub>: UR<sub>v</sub></u>	<u>0.53</u>	0.04*	0.05	<u>0.86</u>
	$\underline{SRB_{nv}}: \underline{SRB_{v}}$	<u>0.51</u>	0.04*	<u>-0.01</u>	<u>0.98</u>
	Bos <sub>nv</sub> : Bos <sub>v</sub>	<b>_</b>	± 1	<u> </u>	±
	Pen <sub>nv</sub> : Pen <sub>v</sub>	<u>_</u>	<u>-</u>	<u> </u>	<u>-</u>
	<u>Met<sub>nv</sub>: Met<sub>v</sub></u>	<u>_</u>	<u>-</u>	<u> </u>	<u>-</u>
	<u>Met-ESA<sub>nv</sub>: Met-ESA<sub>v</sub></u>	<u> </u>	<u>_</u>	<u> </u>	<u> </u>
	<u>Met-OA<sub>nv</sub>: Met-OA<sub>v</sub></u>	<u>_</u>	_	<u>-</u>	<u>-</u>
<u>27</u>	$\underline{\mathbf{Br}}_{\mathbf{nv}}$ : $\underline{\mathbf{Br}}_{\mathbf{v}}$	<u>0.58</u>	0.02*	<u>0.97</u>	<u>&lt;0.001***</u>
	<u>UR<sub>nv</sub>: UR<sub>v</sub></u>	<u>0.85</u>	<0.001***	<u>0.78</u>	<0.001***
	SRB <sub>nv</sub> : SRB <sub>v</sub>	<u>0.67</u>	0.004**	<u>0.64</u>	0.01**
	Bos <sub>nv</sub> : Bos <sub>v</sub>	<u> </u>	<u>_</u>	<u> </u>	<u> </u>
	Pen <sub>nv</sub> : Pen <sub>v</sub>	<u>_</u>	± 1	<u> </u>	± 1
	<u>Met<sub>nv</sub>: Met<sub>v</sub></u>	<u>_</u>	± 1	<u>0.76</u>	<u>0.03*</u>
	Met-ESA <sub>nv</sub> : Met-ESA <sub>v</sub>	<u>-</u>		-	
	Met-OA <sub>nv</sub> : Met-OA <sub>v</sub>	<u>-</u>		-	
<u>39</u>	Br <sup>-</sup> <sub>nv</sub> : Br <sup>-</sup> <sub>v</sub>	0.53	0.03*	0.27	0.35
	$\overline{\mathbf{UR}_{nv}:\mathbf{UR}_{v}}$	<u>0.84</u>	<0.001***	<u>0.95</u>	< 0.001***
	$\underline{\mathbf{SRB}}_{\mathbf{nv}}$ : $\underline{\mathbf{SRB}}_{\mathbf{v}}$	<u>-0.06</u>	0.83	<u>0.73</u>	0.003**

Bos <sub>nv</sub> : Bos <sub>v</sub>	<u>-</u>	<u>-</u>	<u>-</u>	<u> </u>
Pen <sub>nv</sub> : Pen <sub>v</sub>	<u>-</u>	<u>-</u>	<u>-</u>	<u> </u>
<u>Met<sub>nv</sub>: Met<sub>v</sub></u>	<u>0.40</u>	<u>0.44</u>	0.76	0.03*
Met-ESA <sub>nv</sub> : Met-ESA <sub>v</sub>	<u> </u>	<u> </u>	<u> </u>	<u>-</u>
Met-OA <sub>nv</sub> : Met-OA <sub>v</sub>	<u>-</u>	<u> </u>	<u>-</u>	<u>-</u>
Signif. Codes: 0.001 '***'; 0.01 '**';	0.05 '*'			
nv = non-vegetated; v = vegetated				

Table 65: Spearman rank correlation between the breakthrough curves of Br<sup>-</sup> of the first and the second experimental run and the different depths and zones.

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

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

5

Table 76: Percentage of tracers, pesticides and their TPs recovered from the outlet water after the flushing of the system and the removal10for the first and second run.

	Recovery water outlet (%)		Mean recovery	Removal (%)		Mean removal
	<u>First</u> 1º run	<u>Second</u> 2º run	(%)	<u>First</u> 1º run	<u>Second</u> 2º run	(%)
Br⁻	76.5	76.7	$76.6 \pm 0.1$	23.5	23.3	$23.4\pm0.1$
UR	30.3	28.8	$29.6 \pm 1.1$	69.7	71.2	$70.5 \pm 1.1$
SRB	36.4	38.0	$37.2 \pm 1.1$	63.6	62.0	$62.8 \pm 1.1$
Boscalid	27.9	24.9	$26.4 \pm 2.1$	72.1	75.1	$73.6 \pm 2.1$
Penconazole	17.3	20.7	$19.0 \pm 2.4$	82.7	79.3	$81.0 \pm 2.4$
Metazachlor	7.5	7.4	$7.5 \pm 0.1$	92.5	92.6	$92.6\pm0.1$
Met-ESA	6.1	5.4	$5.8 \pm 0.5$	-	-	-
Met-OA	0	0.8	$0.4 \pm 0.6$	-	-	-

Mean recovery and removal represent means  $\pm$  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

10 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 10 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.





**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 (A) corresponds to the upward vertical filling and B) to the flow on the surface while the ponding was forming), (2) injection of clean water (tap water) from the bottom, (3) flushing of the system with clean water (tap

5 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.



**Figure 5:** Breakthrough curves of the different tracers, pesticides and their TPs in terms of relative concentrations (C/C<sub>0</sub>) (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

5 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<sup>-</sup> 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. Lag units are given in days (corresponding to the dates when the observations were made). The blue lines represent the approximate 95% confidence intervals.



**Figure 7:** 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.

5



Figure 8: 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: 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.

5 Recoveries of tracers, pesticides and their TPs at the outlet



10

Figure 10: 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).

15



5 Figure 11: 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.