<u>Answer to REVIEWER #1 (author's answers are written in bold)</u>

The manuscript presents new information on diatoms as possible hydrological tracers. From that point of view it can be attractive to the readers and I recommend its publications. I have the following comments:

1. The formulations at many places (e.g. page 2393, l. 16-20, l. 20-23; page 2411, l. 23-25; page 2412, l. 6-12) point out that isotopic and chemical tracers have their uncertainties or that they cannot identify the hydrological connectivity. While it is true, it does not mean that isotopes and water chemistry do not provide useful information. I do not understand the reason of stressing the limitations of isotopes and water chemistry (which are well known). Each technique has its advantages and disadvantages and it holds also for isotopes, water chemistry and diatoms. The application of all these techniques in hydrology should be complementary rather than competitive. While isotopes and water chemistry can not provide information on hydrological connectivity, diatoms can not idenfity water sources, quantify hydrograph components or provide the information on transit times the way isotopes and water chemistry do. In fact, both isotopes and water chemistry were used to provide useful information also in this manuscript. Uncertainties with isotopes and water chemistry are not the reasons while we need to use the diatoms. On the contrary, all tracers can help to improve our understanding of hydrological cycle. Therefore, I recommend to skip formulations stressing the fact that isotopes and water chemistry have limitations. The objective of this manuscript is to explore the potential of diatoms as an emerging tracer, not to remind of the uncertainties of other techniques.

We acknowledge anonymous referee #1 for his/her constructive comments on the paper. The effort is highly appreciated. In the following lines we address the main comments outlined in his/her review.

The reviewer #1 stressed that it is not appropriate to justify the use of diatoms as new tracers by listing the limitations of 'standard' tracers in hydrology (i.e. water stable isotopes and chemistry). The reviewer refers to two different parts of the paper: (1) the introduction (page 2393, l. 16-20, l. 20-23) and (2) the discussion (page 2411, l. 23-25; page 2412, l. 6-12). We fully agree with the argument of reviewer #1, and do believe that all tracers –including diatoms- have advantages and disadvantages (as described in our paper). They should be complementary rather than competitive. We have rewritten the introduction of the paper accordingly. We have also deleted the sentence in the

discussion that refers to the limitation of using water stable isotope to trace water flowpahts (page 2411, l. 23-25). Nonetheless, we do not think that the paragraphs referring to large scale tracing (i.e., page 2412, l. 6-12) should be changed. In this paragraph we aimed at pointing out that diatoms might be useful at higher catchment scales, whereas the usefulness of the other tracers have been proved to be limited (e.g. Uhlenbrook et al., 2003; Klaus et al., 2013).

2. Abstract line 20 "(n=11, 2010-2011)" should read "(n-11, 2011-2012)" because the study was conducted in water years 2011 and 2012.

The reviewer also suggested referring to water years by the calendar year in which they end. This has been change in all the manuscript (i.e. abstract, Page 2401, l. 17).

3. Findings summarized in the abstract (which are useful) do not fully answer questions raised at the end of page 2394, especially questions 2 and 3.

Reviewer #1 considered that the findings in the abstract did not fully answer research questions (2) and (3) raised in the manuscript (page 2394, lines 20-25). We have added two new sentences at the end of the abstract, which read like this: "Diatom transport data were compared to two-component hydrograph separation, and end-member mixing analysis (EMMA) using stream water chemistry and stable isotope data. Hillslope overland flow was insignificant during most sampled events. This research suggests that diatoms were likely sourced exclusively from the riparian zone, since it was not only the largest terrestrial and aerophytic diatom reservoir, but also water from the riparian zone was a major streamflow source during rainfall events under both wet and dry antecedent conditions. In comparison with other tracer methods, diatoms require taxonomy knowledge and a rather large processing time. However, they can provide unequivocal evidence of hydrological connectivity and potentially be used at larger catchment scales."

4. Page 2395, l. 10 "… strong seasonality in baseflow exist …" – this is a little confusing, because when talking about baseflow, one would suppose that baseflow was determined using certain technique. Baseflow is a component of hydrograph (a very uncertain one with no universaly accepted definition) rather than a characteristic of flow conditions (high-low flow). Therefore, I would rather speak about "seasonality of low flow" of "streamflow seasonality".

We also agree with Reviewer #1 in that we should avoid referring to 'baseflow' when we have not use any technique for hydrograph separation. Hence, all references to 'baseflow' in the manuscript have been changed to 'low flow'.

5. Page 2396, end it would be useful to characterize the wells a little, e.g. their position on the slope, total depths, depths of screen and aquifer type (I suppose they represent unconfined groundwater… of the same aquifer?). If the soils are not more than 1 m deep, it is interesting to know about the wells. Do they capture also the bedrock groundwater (I suppose the upper bedrock might be weathered, not completely impermeable).

Extra information to characterize the wells was requested (page 2395, lines 12-15). In order to better characterise the subsurface. We have added extra information in the study area to mention that below the soils there are Pleistocene periglacial slope deposits (Juilleret et al., 2011), which exhibits high infiltration rates and high storage capacity (Wrede et al., 2014). Detailed information on the location, position on the slope and screening depths of the piezometers was also added in the manuscript. GW1 was located on the catchment plateau, GW2 near one of the springs, and GW3 and GW4 on the break in slope between riparian and hillslope positions. Wells were around 2 m deep and were screened at least for the lowest 50 cm up to a meter.

6. Page 2397, l.20 – please use 2H/H instead of D/H (to be consistent with notation for oxygen and with line 23)

This was corrected, as well as in page 2397, line 25.

7. Page 2401, l. 17 – Fig. 2 shows water years 2011-2012, not 2010-2012; line 20 instead of "water year 2010-2011" I would write about "water year 2011". While annual precipitation for water year 2011 is mentioned, the same information for water year 2012 is missing. It might be useful to mention it.

The sentence has been corrected and we have added rainfall values for water year 2012.

8. Page 2402, l. 4-5 "…the discharge response represented an ever increasing higher fraction of event rainfall" - How do you know it? If it was a result of some study, please give the reference, otherwise it may not be necessary to mention it in this part of the text.

A major concern of Reviewer #1 was the reference to the estimation of runoff coefficients in the results section (page 2402, lines 4-5). Indeed, we computed event-based runoff coefficients for the sampled events (using the simple "straight line"

separation of baseflow / event flow). However, we avoided giving numbers because it is not obvious (and it is not the scope of this paper) to estimate runoff coefficients for the double peak events that occur in the catchment during wet antecedent conditions. Due to the nature of these events we consider it difficult to determine when the events end. If we consider the end of the events as the return to the pre-event low flow conditions, then recessions might expand over many days resulting in runoff coefficients much higher than 100%. In other cases, rainfall occurs during the falling limb of the hydrograph and a new event starts. As we did not detailed how we estimated the runoff coefficients in the methods section, we have finally opted by removing the text in the results section.

9. Page 2403, l. 4-9. If event water contributions were 50%, 27% and 45%, I would not say that the peaks were formed “mainly” by event water.

The text has been reformulated.

10. Page 2404, l.11-12 “when the catchment was wet, there was a higher contribution of groundwater to streamflow than when the catchment antecedent condition was dry”. It is an interesting finding which may not be incorrect. However, is it not in contradiction with statements on page 2403, l. 6 and 9? Pre-event water contributions were larger in June (dry conditions, event water contributions 27% and 45%) than in November (wet conditions, event water contribution 50% and 16%). While not all pre-event water is formed by groundwater, groundwater is certainly not an event water.

The reviewer also noticed that the statement in page 2404, line 11-12:

“when the catchment was wet, there was a higher contribution of groundwater to streamflow than when the catchment antecedent condition was dry”,

might be in contradiction with what it was stated in page 2403, lines 6-9:

“in winter, when the catchment was wet and flow response was double-peaked, the first peak was formed mainly by event water. This contrasted with the delayed peak that was dominated by pre-event water. For instance, the first peak of the November 2010 event showed a 50% event water contribution, whereas the second delayed peak only 16% (Figure 4b).”

In November, when the catchment was wet, a double peak occurred. The first peak was mainly formed of event-water (50%). We believe that this peak is mainly (but not only) controlled by saturation-excess overland flow in the near-stream areas. On the other

hand, the second peak is mainly formed by pre-event water (event water contribution of 16%). The second peaks represent a much larger volume of water than the first peak (see Fig. 4a), resulting in a much larger volume of pre-event water. Pre-event water contribution in the catchment mainly refers to groundwater.

In contrast, during summer conditions, only the first peak occurred. We estimated maximum event-water contributions of 59.5% and 27% for two consecutive events occurred in June 2010 (see Fig 4b; note that there was a typo here, we apologize for this). These values were larger than the 18% event water contributions of the second peak occurred when the catchment was wet. We agree that the results of the second summer event are not in accordance with the general findings in the catchment. We have thus decided to avoid reporting on this event, and rather sustain our results by citing Wrede et al. (2013). Wrede et al. (2013) first described that pre-event water dominates during the second peak of double peak events, whereas event water dominates when single peaks occur. The manuscript has been edited and we hope that this is clearer.

11. Page 2405, l. 10 I propose using "low flow" instead of "base flow" (see comment 4).

The text has been changed.

12. Page 2405, l. 16-10 – important seasonal changes were not observed. In my opinion Table 3 shows the seasonal differences for the streambed samples. Are they not significant? What is "n" in Table 3? If it is number of samples, is it possible to come to definite conclusion when the numbers of samples for different environments were different?

Concerning the seasonal changes of diatom communities in the catchment, we have now used the non-parametric Mann-Whitney U-test to determine if the samples of the riparian zone and hillslopes collected in summer and winter could come from the same population. However, we could not perform the test for the stream water at low flow and streambed samples due to the small number of samples. As far as we know, this test does not require the same number of samples in the two populations to compare them.

The test was not significant in both cases (two-tailed Mann- Whitney U-test) and thus the null hypothesis was not rejected:

	Z-score	p-value
Riparian samples	**1.252**	**0.21**
Hillslope samples	**0.325**	**0.73**

13. Page 2406, l. 2. “Almost no diatom samples were found in overland flow samples”. This seems to be an interesting finding given that hillslopes had the highest mean % of terrestrial diatoms. It could be assumed that overland flow should mobilize them, although the overland flow occurred rarely. Do you think that the intensity of the overland flow was too small to mobilize the lively diatoms which have certain resistance? Could there be any other reason?

Reviewer #1 wonders if ‘the intensity of overland flow was too small to mobilize the lively diatoms which have a certain resistance’. We found the highest mean % of terrestrial diatoms on the hillslope samples. However, when looking at the absolute numbers the ‘the quantities of terrestrial and aerophytic diatoms found on the hillslopes covered by moss and in the overland flow gutter samples were small and sometimes not sufficient to fully characterize the zone’ (page 2408, lines 20-25). Thus, we believe that we did not find diatoms in the overland flow samples because the diatom reservoir in the hillslope was really small. Moreover, Coles et al. (2015) performed two rainfall simulation experiments over (1) a leaf litter hillslope and (2) a bryophie hillslope of the Weierbach catchment, and overland flow did not occur. The authors simulated 1 in 10 year rainfall events, with high rainfall intensities (40 mm/h).

14. Page 2406, l. 18-20 – systematic increase in terrestrial and aerophytic diatoms. Fig. 8 does not show strong increase for some events, especially the largest ones.

We did not mention that the increase was strong, but systematic.

15. Page 2407, l. 5 “low flow” instead of “base flow”?

The text has been changed.

16. Page 2409, l. 7 – no significant seasonal differences… see comment 11 (streambed diatoms seem to show the difference between summer and winter)

See answer to comment 11.

17. Page 2410, l. 11-12 – conclusion that “hydrological response in spring and summer is largely composed of event water” is not fully in agreement with Fig. 4 (see comment 8), similarly line 20 (the first peak was mainly event water)

See answer to comment 10.

18. Page 2412, l. 6-12. Since this text is not related to exploration of the usefulness of diatoms, I recommend deleting it.

The mentioned paragraph discusses on the concept of hydrological connectivity across catchments scales. As section 5.3 deals with the advantages and limitations of the use of diatoms to infer hydrological connectivity in the HRS system, we do think that the text is relevant as there might be potential to be use at larger catchment scales.

19. Page 2413, l.14-15 “-…riparian zones appear to be the largest diatom reservoir…:” Table 3 seems to indicate that also hillslopes are a large reservoir. Should it not be mentioned as well?

Table 3 shows relative percentages of terrestrial and aerophytic valves, not absolute values. Even though we found the highest relative percentages of terrestrial and aerophytic valves in the hillslope samples, the valves found in absolute numbers were lower.

FINAL NOTE:

We have replaced the term ‘terrestrial and aerophytic diatoms’ for ‘aerial diatoms’ in the manuscript. We considered aerial diatom communities as those communities living exposed to the air outside of lentic and lotic environments, following the definition of Johansen (2010), instead of using other classifications such as those of Petersen (1915, 1935) or Ettl & Gärtner (2014). Therefore, it seems now more appropriate to use the term ‘aerial’ as most species are not strictly terrestrial. We thus considered ‘aerial diatoms’ as those species listed with values 4 and 5 (Van Dam et al., 1994), which includes diatoms “mainly occurring on wet and moist or temporarily dry places” and diatoms “nearly exclusively occurring outside water bodies”, respectively.

References:

Ettl, H. & Gärtner G. (2014) Syllabus der Boden-, Luft- und Flechtenalgen. 2. Auflage. Springer: Berlin Heidelberg.

Johansen JR. (2010). Diatoms of aerial habitats. In The Diatoms: Applications for the Environmental and Earth Sciences, 2nd Edition, Smol JP, Stoermer EF (eds). Cambridge University Press: Cambridge; 465–472.

Klaus, J. and McDonnell, J. J.: Hydrograph separation using stable isotopes: Review and evaluation. J. Hydrol., 505, 47–64, doi:10.1016/j.jhydrol.2013.09.006, 2013.

Petersen J. B. (1915): Studier over danske aerofile alger [Studies on Danish aerophytic algae]. – Det Kongelige Danske Videnskabernes Selskabs Skrifter, Naturvidenskabelig og Mathematisk 12: 272–379.

Petersen JB. (1935). Studies on the biology and taxonomy of soil algae. Dansk Botanisk Arkiv 8(9): 1-183.

Uhlenbrook, S. and Hoeg, S.: Quantifying uncertainties in tracer-based hydrograph separations: a case study for two-, three- and five-component hydrograph separations in a mountainous catchment. Hydrol. Process., 17, 431–453, doi:10.1002/hyp.1134, 2003.

Van Dam, H., Mertens, A., and Sinkeldam, J.: A coded checklist and ecological indicator values of freshwater diatoms from The Netherlands. Neth. J. Aquat. Ecol., 28, 117–133, doi:10.1007/BF02334251, 1994.

Wrede, S., Fenicia, F., Martínez-Carreras, N., Juilleret, J., Hissler, C., Krein, A., Savenije, H. H. G., Uhlenbrook, S., Kavetski, D., and Pfister, L.: Towards more systematic perceptual model development: a case study using 3 Luxembourgish catchments. Hydrol. Process., doi:10.1002/hyp.10393, 2014.

<u>Answer to REVIEWER #2 (author's answers are written in bold)</u>

General comment

This paper focuses on the analysis of stream-riparian-hillslope hydrological connectivity in a humid catchment investigated through the use of the novel tracing technique provided by diatoms. The well-known Luxembourg-based research group was the first, as far as I know, to promote this experimental method a few years ago and this is one of the first applications to investigate hydrological processes at the catchment scale. Overall, the paper is clearly written, with a clear goal, sound analysis and interpretation, and good graphs. However, there are some points that I think the author should address and explain better. First of all, I agree with all comments by the first reviewer, and I'm avoiding to repeat them in my review. I encourage the authors to pay particular attention to the comments reported at the top of page C762 (the comments regarding P2403, L4-9 and P2404, L11-12) and in the central-lower part of page C762 (the comment regarding P2406, L2). In addition, I have also some major concerns and some minor corrections that are reported below and that, in my opinion, should be considered before acceptance of publication to HESS.

We acknowledge anonymous referee #1 for his/her constructive comments on the paper. The effort is highly appreciated. The reviewer agrees with all comments made by Reviewer #1. We kindly ask the reviewer to check how we have answered the comments of reviewer #1.

In the following lines we address the main comments outlined in his/her review.

Specific comments

-The title does not reflect very well the three objectives: indeed, the title suggest a more process-based study, whereas the objectives are more methodologically-oriented. Maybe the title could be changed into "Diatoms as indictor of hydrological connectivity through the riparian-stream system" or something like that.

The first concern of Reviewer #2 is that the title, in his/her opinion, does not reflect well the three objectives of the paper. The Reviewer suggests changing the title to highlight that this is a more methodologically-oriented paper to 'Diatoms as indicator of hydrological connectivity to the riparian-stream-system'.

We have changed the title to 'Hydrological connectivity inferred from diatom transport through the riparian-stream system', as we would like to claim that the paper is both process and methodologically oriented.

-I like the fact that the three objectives reflect well three subsections of the Discussion. However, when one reads the paper for the first time, he/she has a hard time to see the differences between the first objective and the third one. The authors should probably reformulate these two questions in a more univocal way.

Reviewer #2 argued that it is difficult to differentiate between the first and the third objective of the paper. We understand from the comment that the text reads well. It is only the research question that should be reformulated.

Research questions (1) and (3) have been reformulated:

PREVIOUS RESEARCH QUESTIONS:

1. Can terrestrial and aerophytic diatoms be used to reveal hydrological connectivity within the hillslope–riparian–stream system?

3. What are the advantages and limitations of the use of diatoms to infer hydrological connectivity in the HRS system?

NEW QUESTION:

1. Can aerial diatom transport reveal hydrological connectivity within the hillslope–riparian–stream system?

3. Can aerial diatom be established as a new hydrological tracer?

-P2402, L1-7. These findings are not clearly showed in the manuscript and it's not clear if they come from previous research (in this case insert references). This behaviour should be showed by a new Figure or including a reference to an existing Figure. More importantly, I think that the observation that a second peak (mostly formed by pre-event water, as stated at P2410, L21) does not occur during dry conditions suggests that groundwater (which I assume is the most important component of pre-event water) levels are low and not contribute much to the hydrological response. But this would imply a small contribution of pre-event water, which is not the case (event water dominates during wet conditions). However, later in the manuscript, it's reported that when the catchment was wet there was a higher contribution of groundwater to streamflow. This is quite confusing, we need evidence of these observations, and I think that the author should do a better job to clearly show measurements and observations here and to discuss more in details in section 5 the process interpretation based on them. Finally, we have no clues of how large or small runoff coefficients are: they that should be reported in a Table somewhere (possible Table 4).

As guessed by the reviewer, conclusions about event and pre-event water contributions during runoff events were also drawn from previous studies conducted in the catchment by Wrede et al. (2013). When the catchment was wet, a double peak occurred. The first peak was mainly formed of event-water (50%). We believe that this peak is mainly (but not only) controlled by saturation-excess overland flow in the near-stream areas. On the other hand, the second peak is mainly formed by pre-event water (event water contribution of 16%). The second peaks represent a much larger volume of water than the first peak (see Fig. 4a), resulting in a much larger volume of pre-event water. Pre-event water contribution in the catchment mainly refers to groundwater.

In contrast, during summer conditions, only the first peak occurred. We estimated maximum event-water contributions of 59.5% and 27% for two consecutive events occurred in June 2010 (see Fig 4b; note that there was a typo here, we apologize for this). These values were larger than the 18% event water contributions of the second peak occurred when the catchment was wet. We agree that the results of the second summer event are not in accordance with the general findings in the catchment. We have thus decided to avoid reporting on this event, and rather sustain our results by citing Wrede et al. (2013), which first described this single-double peak event by using silica to discriminate between event and pre-event water during events in the Weierbach catchment. The manuscript has been edited and we hope that this is now clearer. We have also edited Figure 4.

We computed event-based runoff coefficients for the sampled events (using the simple "straight line" separation of baseflow / event flow). However, we avoided giving numbers because it is not obvious (and it is not the scope of this paper) to estimate runoff coefficients for the double peak events that occur in the catchment during wet antecedent conditions. Due to the nature of these events we consider it difficult to determine when the events end. If we consider the end of the events as the return to the pre-event low flow conditions, then recessions might expand over many days resulting in runoff coefficients much higher than 100%. In other cases, rainfall occurs during the

falling limb of the hydrograph and a new event starts. As we did not detailed how we estimated the runoff coefficients in the methods section, we have finally opted by removing the text in the results section.

-P2405, L19. I know very little about diatoms but I guess we can expect no valves in rainfall samples. Is it the same for groundwater? Would it be possible that rainfall infiltration processes during long or intense events facilitate percolation of diatom valves through the vadose zone and down to shallow groundwater? Please, add a few words on this here.

We can expect no terrestrial and aerophytic valves in rainfall samples. We did not find diatoms in groundwater samples. At this state of our research we are not sure if they can infiltrate down to the groundwater. As discussed in page 2409, lines 25-30, we tested if diatoms percolate though different types of soil matrix using fluorescent diatoms (Tauro et al., submitted) and we concluded that it was unlikely.

-P2409, L28. This is a critical point. The authors say that transport of diatoms from the riparian zone to the stream could occur via macropores in the shallow subsurface layers and/or overland in the riparian zone. In principle, I agree with the explanation. However, the authors found very little diatoms in overland flow (P2406, L2) and this seems to be in contrast with their second hypothesis of stream-riparian diatom transport. Moreover, PCA suggests only a minor role of overland flow for streamflow generation (P2404, L8-11). I think that some suggestions should be posed by the authors on this issue.

Indeed, we found very little amounts of diatoms in HILLSLOPE overland flow. We specified in the methods section that we only sampled overland flow on lower hillslope positions (page 2397, line 5-12). End-member mixing analysis was performed only considering hillslope overland flow. To avoid confusions we always refereed to 'hillslope overland flow'. We did not explicitly sample riparian overland flow. But, we sampled litter, moss and vegetation for diatom analysis in the riparian zone. Terrestrial and aerophytic diatoms were much more abundant (in absolute numbers) in the riparian zone than the hillslope. When looking at all the measurements together we hypothesised that the transport of diatoms from the riparian zone to the stream might take place either through (i) a network of macropores in the shallow soils of the riparian zone or (ii) overland flow in the riparian zone.

Minor comments and technical corrections

-P2404, L14-15. Higher contribution of throughfall compared to what? Compared to throughfall when the catchment was dry? Or compared to groundwater? Please, clarify, this is an important part to understand well how the catchment behaves.

We have reformulated the sentence: 'To the contrary, a much higher contribution of throughfall was estimated during summer (events 5-8), when the pre-storm catchment state was dry, than during winter (events 1-2)'.

-P2406, L28. Are the bivariate plots built putting streamflow on the x or y axis? This has to be mentioned to correctly understand the direction of hysteretic loops.

Hysteretic loops have been done with streamflow on the x axis. The sentence in the manuscript has been corrected.

P2392, L11. Here and later in the manuscript: 'assemblages': is this a technical word used to describe biotic communities?

Yes, 'assemblages' is a technical word widely used in ecology. It usually refers to planktonic communities.

P2392, L25-28. These sentences should be modified according to possible changes in the results and discussion about the source of diatoms (role of hillslopes).

As previously explained, we only sampled overland flow on lower hillslope positions (page 2397, line 5-12). We did not explicitly sample riparian overland flow. But, we sampled litter, moss and vegetation for diatom analysis in there. Indeed, our results showed that (i) hillslope overland flow contribution to streamflow during events was minimum; and (ii) presence of terrestrial and aerophytic diatoms on the hillslope samples was really little or zero (in absolute numbers), (iii) the riparian zone was the highest terrestrial and aerophytic diatom reservoir. Our results suggested that diatoms were likely sourced exclusively from the riparian zone.

P2393, L11. Add 'of water' after 'stable isotope'.

It has been changed for 'water stable isotope tracers'.

P2398, L5-7. Skip this, it have already been mentioned.

The sentence has been removed.

P2399, L21. Here, and everywhere in the manuscript, I strongly suggest to avoid using the term ‘concentration’ when referring to the isotopic signature. Technically, it’s not a concentration. I suggest to use ‘isotopic composition’.

We fully agree with the reviewer. We have revised all the manuscript and all references to ‘isotopic concentrations’ have been replaced by ‘isotopic composition’.

P2399, L26. Include ‘isotopic’ between ‘bulk’ and ‘composition’

The sentence has been changed.

P2400, L2. Skip ‘end-member mixing analysis’ and use directly EMMA. The acronym has been already defined at page 2394.

The text has been changed.

P2400, L2. Add ‘that’ after ‘assumes’.

It has been added.

P2400, L10. It’s good that you have included reference but please shortly explain what is the difference between ‘physical mixing’ and ‘equilibrium mixing’ because this is an important concept here.

The EMMA approach is based on the assumption that it is the mixing of the different sources of water (with different geochemical and isotopic signatures) that control stream water geochemical and isotopic signatures. The method assumes linear mixing, and we refer to this as ‘physical mixing’. If equilibrium reactions among solutes of different charge occur (and are dominant) we would not expect linear mixing as equilibrium reactions among solutes of different charge are higher-order polynomials (Hooper, 2003). We refer to this as ‘equilibrium mixing’.

The sentence has been modified in the manuscript.

P2400, L21. Explicit ‘SD’ (I guess standard deviation).

Done.

P2401, L1. ‘was’ should be ‘were’.

This has also been corrected.

P2403, L13. What is ‘riparian water’? Groundwater? Overland flow? Please, clarify.

'Riparian water' refers to soil water in the riparian zone. We have replaced all references to 'riparian water' in the manuscript by 'soil water in the riparian zone'.

We have moved the sentence to the methods section 3.4, and specified which sampling points are included in each end-member type: "Catchment end-members included shallow groundwater (GW1-4), soil water ($SS1_{20}$, $SS1_{60}$, $SS2_{60}$), soil water from the riparian zone (SSr), rainfall (R), throughfall (TH1-2), snow (SN) and overland flow (OF).".

P2403, L16. Since here several solutes were mentioned, it's not clear to which of them the correlation refers to. Please, clarify.

The text has been modified: 'Ten out of the twelve tracers presented linear trends in the solute-solute plots of stream water samples with at least one other tracer (EC, Cl^-, Na^+, K^+, Mg^{2+}, Ca^{2+}, SiO_2, Abs, δ^2H and $\delta^{18}O$; $r^2>0.5$, p-value<0.01, Figure 6). These tracers were retained for further analysis.'

P2403, L17. It's not clear what the authors mean by 'retained for further analysis'. Which? Why? Please, explain.

The sentence has been modified: 'These tracers were retained for the PCA analysis'. Moreover, the steps followed for the hydrograph separation analysis are listed in the methods section 3.3 (see P2400, lines 8 to P 2041, line 14).

P2403, L19. What is the 'pre-defined threshold of collinearity'? Pre-defined by whom? Please, clarify and possible include a reference.

The pre-defined threshold of collinearity is defined in the methods section 3.3. References are also listed there:' stream water concentrations and isotopic compositions (of all samples collected during storm events and low flows at the catchment outlet) were considered conservative when they exhibited at least one linear trend with one other tracer (i.e. r2>0.5, p-value<0.01) (James and Roulet, 2006; Ali et al., 2010; Barthold et al., 2011)'. We do not think that this should be repeated in the results section.

P2403, L21. Skip the definition and use only 'PCA' (acronym already defined earlier in the manuscript).

We have only retained the acronym.

P2404, L24. Change 'wettest' into 'wet'.

This has been changed.

P2405, L7. Is 230 a small or a high or a usual number of taxa? We, as simple hydrologists, have no solid idea.

The number of taxa might be associated to sampling efforts. The number might be higher or lower in different environments. We believe that in order to state if this is a rather high or low value it should be compared to other values.

P2405, L7. I suggest to delete 'catchment-wide'.

We have replaced 'catchment-wide campaigns' by 'seasonal campagins'.

P2405, L12. Replace 'Riparian' with 'riparian'.

This has been changed.

P2406, L4. Replace 'But' with 'However'.

'But' has been replaced by 'However'.

P2407, L15. 'Fig. 10b' should be '9b'.

This has been corrected.

P2407, L25. Although everybody knows what DOC is please explicit the acronym. Moreover, explain and/or give a reference supporting the statement that UV absorbance can be considered a proxy of DOC (it's not immediately intuitive to me).

We have moved the sentence to the 'Methods' to avoid having references in the 'Results' section. We have also added a reference: 'UV absorbance at 254 nm can be considered a proxy of DOC (Edzwald et al., 1985)'.

P2408, L7. Skip 'hillslope-riparian-stream' and use directly HRS, since it was already defined.

'hillslope-riparian-stream' has been replaced by 'HRS'.

P2408, L10-11. Remove (already mentioned).

The sentence has been removed.

P2408, L22. Typo in 'litter'.

The typo has been corrected.

P2410, L9. Use only 'EMMA', without the already mentioned definition.

This has been corrected.

P2411, L14-19. This sentence sounds as already said. Please, try to reformulate.

The sentence has been reformulated.

P2411, L26. Remove 'But'.

It has been removed.

P2412, L4. I think it's more common to use 'ecohydrology' or 'eco-hydroogy' instead of 'hydro-ecology'.

'Hydro-ecology' has been changed by 'eco-hydrology'.

P2413, L10. Replace 'hillslope-riparian-stream' with 'HRS'.

This has been replaced.

Table 2. In the caption, I suggest to remove the sentence in brackets (but keeping the sample size).

This has been done.

Table 3. I suggest to specify in the caption that the valves found on the hillslopes do not include the dry litter zone. Moreover what is the 'baseflow drift'?

The caption of the table has been modified to make it clearer to the reader: "Table 3. Relative percentage of terrestrial and aerophytic valves quantified at distinct zones of the Weierbach catchment. Streambed samples refer to epilithon samples. Riparian zone samples include litter, moss and vegetation. Hillslope samples include litter, moss and surface soil samples. Diatoms were absent on hillslopes covered by dry litter and samples were discarded."

Table 4. Replace 'storm runoff-events' with 'rainfall-runoff events'.

This has been changed.

Fig. 2. In the second panel, use the same label used in Fig. 3, for consistency. I suggest to move the discharge series in the upper panel. I also suggest to change the caption in 'Time series of precipitation, discharge, groundwater depth, volumetric water content…' Also mention what the numbers indicate.

A new figure has been created using the same label for the second panel as in Fig. 3. We tried to move the discharge serie to the upper panel. However, it is then less visual to number the events that were sampled in summer (mainly due to the relative small change on discharge during the dry season compared to the wet season). The caption has been changed following the reviewer recommendation.

Fig. 4. Change the label 'O-18' into 'd18O' or at least '18O'. Change 'winter' response into 'fall-winter' response. Change 'Two components' into 'Two-component'. Delete all that comes after 'using d18O'.

The caption and the label have been corrected. We have changed 'summer and winter response' to 'a) Wet antecedent conditions' and 'b) Dry antecedent conditions'.

Fig. 5. As mentioned above, indicate what 'riparian water' means. Moreover, add if the median or the mean is displayed in the box-plots, as well as percentiles/standard deviation etc.

'Riparian water' refers to soil water in the riparian zone. We have replaced all references to 'riparian water' in the manuscript by 'soil water in the riparian zone'. We understand that, by default, the bottom and top of the box in a boxplot are always the first and third quartiles, and the band inside the box is always the second quartile.

Fig. 6. The part in brackets can be deleted (but keeping the overall sample size). Add 'The' Before 'upper'.

The caption has been corrected.

Fig. 7. Where is the vertical error bar in panel b? Too small to be displayed? SS3R is not in the legend and it's not clear what it indicates. Moreover, how can it be an end-member if some samples (e.g., event 2) fall outside it?

In panel (b) we zoomed in the middle of panel (a) and plotted peakflow stream water samples instead of all the samples. The OF vertical error bars just falls outside the plotted range in panel (b), the reader has to refer to panel (a) to see it displayed. We have better explained this in the new caption.

SS310 refers to soil water in the riparian zone. We have replaced SS310 in all the manuscript for SSr and stated that we refer to 'soil water in the riparian zone'.

Fig. 8. Would it be better to split the Figure in two? Moreover, change '%' into 'percentage'. The last 9 words could be deleted.

We have split the Figure in two and edited the caption.

FINAL NOTE:

We have replaced the term 'terrestrial and aerophytic diatoms' for 'aerial diatoms' in the manuscript. We considered aerial diatom communities as those communities living exposed to the air outside of lentic and lotic environments, following the definition of Johansen (2010), instead of using other classifications such as those of Petersen (1915, 1935) or Ettl & Gärtner (2014). Therefore, it seems now more appropriate to use the term 'aerial' as most species are not strictly terrestrial. We thus considered 'aerial diatoms' as those species listed with values 4 and 5 (Van Dam et al., 1994), which includes diatoms "mainly occurring on wet and moist or temporarily dry places" and diatoms "nearly exclusively occurring outside water bodies", respectively.

References:

Ettl, H. & Gärtner G. (2014) Syllabus der Boden-, Luft- und Flechtenalgen. 2. Auflage. Springer: Berlin Heidelberg.

Johansen JR. (2010). Diatoms of aerial habitats. In The Diatoms: Applications for the Environmental and Earth Sciences, 2nd Edition, Smol JP, Stoermer EF (eds). Cambridge University Press: Cambridge; 465–472.

Petersen J. B. (1915): Studier over danske aerofile alger [Studies on Danish aerophytic algae]. – Det Kongelige Danske Videnskabernes Selskabs Skrifter, Naturvidenskabelig og Mathematisk 12: 272–379.

Petersen JB. (1935). Studies on the biology and taxonomy of soil algae. Dansk Botanisk Arkiv 8(9): 1-183.

Van Dam, H., Mertens, A., and Sinkeldam, J.: A coded checklist and ecological indicator values of freshwater diatoms from The Netherlands. Neth. J. Aquat. Ecol., 28, 117–133, doi:10.1007/BF02334251, 1994.

Wrede, S., Fenicia, F., Martínez-Carreras, N., Juilleret, J., Hissler, C., Krein, A., Savenije, H. H. G., Uhlenbrook, S., Kavetski, D., and Pfister, L.: Towards more

systematic perceptual model development: a case study using 3 Luxembourgish catchments. Hydrol. Process., doi:10.1002/hyp.10393, 2014.

List of all relevant changes made in the manuscript

- The title and research questions (1) and (3) have been edited according to reviewer's comments.
- The abstract has been edited to include some results on research questions (2) and (3).
- Extra information on the characterization of the wells has been added.
- Both reviewers agreed on some confusion regarding event, pre-event water contribution during wet/dry catchment conditions. Figure 4 and text in sections 4.2 have been edited to better explain the results. Moreover, results have been better supported by citing previous work done in the catchment.
- We have reported on the results of a non-parametric test to determine if the seasonal differences in diatom communities were significant.
- Figure 8 was split up in two different figures: new figures 8 and 9.
- We have replaced the term 'terrestrial and aerophytic diatoms' by 'aerial diatoms' as it seems now more appropriated by the ecologists co-authors.
- Captions of Table 2 and 3, and Figures 2, 4, 6-8 have been reformulated.

Hydrological connectivity inferred from diatom transport through the riparian-stream system ~~Hydrological connectivity as indicated by transport of diatoms through the riparian-stream system~~

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Abstract

Diatoms (*Bacillariophyta*) are one of the most common and diverse algal groups (ca. 200 000 species, ≈10-200 µm, unicellular, eukaryotic). Here we investigate the potential of ~~terrestrial and aerophytic~~aerial diatoms (i.e. diatoms nearly exclusively occurring outside water bodies, on wet, moist or temporarily dry places) to infer surface hydrological connectivity between hillslope-riparian-stream (HRS) landscape units during storm runoff events. We present data from the Weierbach catchment (0.45 km^2, NW Luxembourg) that quantifies the relative abundance of ~~terrestrial and aerophytic~~aerial diatom species on hillslopes and in riparian zones (i.e. surface soils, litter, ~~bryophytes~~ bryophytes and vegetation) and within streams (i.e. stream water, epilithon and epipelon). We tested the hypothesis that different diatom species assemblages inhabit specific moisture domains of the catchment (i.e. HRS units) and, consequently, the presence of certain species assemblages in the stream during runoff events offers the potential for recording if there was or not hydrological connectivity between these domains. We found that a higher percentage of ~~terrestrial and aerophytic~~aerial diatom species was present in samples collected from the riparian and hillslope zones than inside the stream.

However, diatoms were absent on hillslopes covered by dry litter and the quantities of diatoms (in absolute numbers) were small in the rest of hillslope samples. This limits~~, limiting~~ their use to infer hillslope-riparian zone connectivity~~ in some parts of the catchment~~. Our results also showed that ~~terrestrial and aerophytic~~aerial diatom abundance in the stream increased systematically during all sampled events (n=11, 2011-2012~~2010-2011~~) in response to incident precipitation and increasing discharge. This transport of ~~terrestrial and aerophytic~~aerial diatoms during events suggested a rapid connectivity between the soil surface ~~–~~and the stream. Diatom transport data ~~was~~ were compared to two-component hydrograph separation, and end-member mixing analysis (EMMA) using stream water chemistry and stable isotope data. Hillslope overland flow was insignificant during most sampled events. This research suggests that diatoms were likely sourced exclusively from the riparian zone, since it was not only the largest ~~terrestrial and aerophytic~~aerial diatom reservoir, but also soil riparian zone water was a major streamflow source during rainfall events under both wet and dry antecedent conditions. In comparison to other tracer methods, diatoms require taxonomy knowledge and a rather large processing time. However, they can provide unequivocal evidence of hydrological connectivity and potentially be used at larger catchment scales.

1 Introduction

The generation of storm runoff is strongly linked to hydrological connectivity—surface and subsurface—that controls threshold changes in flow and concomitant flushing of solutes and labile nutrients (McDonnell, 2013). To date, various approaches to quantify hydrological connectivity have been presented, including hydrometric mapping at hillslope (Tromp-van Meerveld and McDonnell, 2006) and catchment scales (Spence, 2010), connectivity metrics (Ali and Roy, 2010) and high-frequency water table monitoring (Jencso et al., 2009). Perhaps the most popular tool has been the use of environmental tracers for characterising and understanding complex water flow connections within catchments—between soils, channels, overland surfaces, and hillslopes (Buttle, 1998). Chemical tracers and stable isotopes of the water molecule have been widely used for quantifying ~~tracers have enabled quantification of~~ the temporal sources of storm flow (i.e. event and pre-event water) using mass balance equations (see Klaus and McDonnell, 2013 for review). These tracers have also been used together to quantify the geographic sources of runoff using end-member mixing models (EMMA) (see Hooper, 2001 for review).

Despite their usefulness, chemical and isotope tracer-based hydrograph separations do not provide ~~suffer from inherent conceptual limitations (Richey et al., 1998; Burns, 2002). For instance, end-member selection (Hooper et al., 1990), the number of tracers employed (Barthold et al., 2011) and spatial-temporal variation in end-member chemistry (Inamdar et al., 2013) have been shown to influence runoff source apportionment. Perhaps most problematic is that no tracer approach yet allows for~~ unequivocal evidence of hillslope-riparian-stream (HRS) connectivity. This has been identified as perhaps the key feature for improving our understanding of water origin and the processes that sustain stream flow (Jencso et al., 2010). Consequently, new techniques are desperately needed to gain a process-based understanding of hydrological connectivity (Bracken et al., 2013).

Here we build on recent work by Pfister et al. (2009, 2015) and Wetzel et al. (2013) to examine the use of ~~terrestrial and aerophytic~~aerial diatoms (i.e. diatoms nearly exclusively occurring outside water bodies, and on wet, moist or temporarily dry places (Van Dam et al., 1994)), as natural tracers to infer connectivity in the ~~hillslope-riparian-stream (~~HRS~~)~~ system. Diatoms are one of the most common and diverse algal groups (ca. 200 000 species; Round et al., 1990). Due to their small size (~10-200 µm; Mann (2002)), they can be easily transported by flowing water within or between elements of the hydrological cycle (Pfister et al., 2009). Diatoms are present in most terrestrial habitats and their diversified species distributions are largely controlled by physio-geographical factors (e.g. light, temperature, pH and moisture) and anthropogenic pollution (Dixit et al., 2002; Ector and Rimet, 2005).

Our work tests the hypothesis that different diatom species assemblages inhabit specific moisture domains of the HRS system and, consequently, the presence of certain species assemblages in the stream during runoff events has the ability to record periods of hydrological connectivity between these watershed components. We compare diatom results with traditional two-component hydrograph separation, and end-member mixing analysis (EMMA) using stream water chemistry and stable isotope data. We also present soil water content and groundwater level data within the HRS system to facilitate a somewhat holistic understanding of catchment runoff processes (as advocated by Bonell, 1998; Burns, 2002; Lischeid, 2008). Specifically, we addressed the following questions:

1. Can ~~terrestrial and aerophytic~~aerial diatom~~s~~ transport reveal ~~be used to reveal~~ hydrological connectivity within the ~~hillslope-riparian-stream~~HRS system?
2. How do diatom results compare to traditional tracer-based and hydrometric methods to infer hydrological connectivity?
3. ~~What are the advantages and limitations of the use of diatoms to infer hydrological connectivity in the HRS system~~Can aerial diatom be established as a new hydrological tracer?

2 Study area

Our study site is the Weierbach catchment (0.45 km^2, 49°49' N 5°47' E), a sub-catchment of the Attert River and located in the North Western part of the Grand Duchy of Luxembourg (~~Figure~~Fig. 1). The region is known as the Oesling, an elevated sub horizontal plateau cut by deep V-shaped valleys and with averaging altitudes ranging between 450 and 500 m.

Weierbach has a temperate, semi-oceanic climate regime, annual precipitation in the Attert River basin ranges from 950 mm on the Western border to 750 mm on the Eastern border (average from 1971 to 2000; Pfister et al., 2005). Precipitation is relatively uniform throughout the year, although strong seasonality in ~~base~~ low flow exists due to higher evapotranspiration from July to September. The annual runoff ratio is high (~55% based on 2005 to 2011 streamflow data) and flow sometimes ceases during summer months.

The geology of the catchment is dominated by Devonian schists, phyllades and quartzite. The schist bedrock is covered by Pleistocene periglacial slope deposits (Juilleret et al., 2011). Soil depths are shallow (<1 m) and dominated by cambisoils, rankers, lithosoils and colluvisoils. Soil texture is dominated by silt mixed with gravels. The schist bedrock is relatively impermeable, while the soil surface and the Pleistocene periglacial slope deposits ~~while the soil surface~~ exhibit~~s~~ high infiltration rates and high storage capacity (~~van den Bos et al., 2006; Juilleret et al., 2011;~~ Wrede et al., 2014).

Vegetation in the study catchment is mainly mixed Oak-Beech hardwood deciduous forest (76% of the land cover, *Fagus sylvatica* L. and *Quercus petraea* (Matt.) Liebl.) where the soil surface is covered with fallen leaves. Conifers cover a smaller part (24% land cover) of the catchment (*Pseudotsuga menziessii* (Mirb.) Franco and *Picea abies* (L.) H. Karst), and the soil surface beneath conifers is covered mainly by ~~moss~~bryophytes. A well-defined riparian zone extends up to 3 meters away from the stream channel. Vegetation in the riparian zone includes *Dryopteris carthusiana* (Vill.) H.P. Fuchs, *Impatiens noli-tangere* L., *Chrysosplenium oppositifolium* L. and *Oxalis acetosella* L.

3 Methodology

3.1 Hydrometric Monitoring

Table 1 shows a summary of collection methods, sampling resolution and locations in the Weierbach catchment. Stream water depth at the catchment outlet was measured using a differential pressure transducer at a 15-minute interval (ISCO 4120 Flow Logger) (~~Figure~~Fig. 1). Stream conductivity at the outlet was also measured at 15-minute intervals using a conductivity meter (WTW). Rainfall was measured with a tipping bucket rain gauge (52203 model, manufactured by Young, Campbell Scientific Ltd.). One rain gauge was installed within a small clearing of the study catchment (see ~~Figure~~Fig. 1), and another one installed in an open area at the Roodt meteorological station, located ≈3.5 km distant from the Weierbach (49°48’22.2’’~~-~~N 5°4~~7~~9’52.7’’~~-~~E). Data gaps were filled with rainfall data from a ~~A ten bottle sequential rainfall sampler (for later precipitation chemistry and isotope analysis) was installed at the rain gauge located within the Weierbach (modified from Kennedy et al. (1979)).~~ nearby weather station (49°47’39.2’’N 5°49’13.2’’E).

Four groundwater wells ~~(depths ~ 90 mm) w~~were instrumented with real-time TD-Divers data loggers (Schlumberger Water Services) and WTW conductivity meters – each recording at 15-minute intervals. GW1 was located on a plateau, GW2 near one of the springs, and GW3 and GW4 on the transition zone between riparian and hillslope settings. Wells were around 2 m deep and were screened at least for the lowest 50 cm up to a meter.

The volumetric water content (VWC) of soils was measured using water content reflectometers (CS616-L model, Campbell Scientific), which use the time-domain measurement method. Four probes were installed at 10 cm depth, parallel to the surface and along a 5 m transect perpendicular to the stream (~~Figure~~Fig. 1): riparian zone, foot of the hillslope, mid-hillslope and plateau positions.

~~3.1~~3.2 Water sampling and laboratory methods

Fortnightly, cumulative rainfall (R) and throughfall samples under deciduous trees (TH1) and coniferous trees (TH2) were collected using conical, volumetric rain gauges. A ten bottle sequential rainfall sampler was installed at the rain gauge located within the Weierbach (modified from Kennedy et al. (1979)). Three automatic water samplers (ISCO 3700 FS and 6712 FS) were installed immediately upstream of the weir to collect stream water samples (AS) frequently (0.5 to 4 h) during storm events. Sampling was triggered by flow conditions. Events were considered separately if they were separated by a period of at least 24h without rainfall. Stream water at the catchment outlet (SW) and wells (GW1 to GW4) were sampled fortnightly, as well as prior to, during, and following precipitation events. Soil water was sampled fortnightly using Teflon suction lysimeters, installed at three locations:~~- (~~deciduous hillslope (SS1), ~~and~~ coniferous hillslope (SS2), and riparian zone (~~SS3~~SSr)~~)~~,. ~~with t~~Three soil depths for each location: 10 cm for the organic layer (Ah horizon), 20 and 60 cm for the mineral layers (B and C horizons). Overland flow (OF) that occurred on lower hillslope positions was sampled using 1 and 2 m long gutters sealed to the soil surface, which diverted surface runoff to 1 or 2-L plastic, blackened (to prevent light penetration which causes diatom growth) water bottles. Note that what we refer as OF might in fact originate within the forest litter layer (Buttle and Turcotte, 1999; Sidle et al., 2007). All gutters were covered to avoid direct sampling of precipitation. Gutters were regularly cleaned with Milli-Q water to avoid diatoms growth on their surfaces.

All water samples were analysed for electrical conductivity (EC), anion and cation concentrations (Cl^-, NO_3^-, SO_4^{2-}, Na^+, K^+, Mg^{2+}, Ca^{2+}), silica (SiO_2) and UV-absorbance at 254 nm (Abs 254 nm). UV absorbance at 254 nm can be considered as a proxy of DOC (Edzwald et al., 1985). Samples were analysed at the Luxembourg Institute of Science and Technology chemistry laboratory after filtration through WHATMAN GF/C glass fibre filters (<0.45 µm). Prior to analysis, samples were stored at 4° C. Dissolved anions and cations were analysed by ion chromatography (Dionex HPLC), SiO_2 by spectrophotometry (ammonium molybdate method), and UV-absorbance was measured by a Beckmann Coulter spectrophotometer. Isotopic analyses of $^{18}O/^{16}O$ and $^{2}H/H$~~D/H~~ were conducted using a LGR Liquid-Water Isotope Analyser at the Luxembourg Institute of Science and Technology (model DLT-100, version 908-0008). The analyser was connected to a LC PAL liquid auto-injector for the automatic and simultaneous measurement of $^{2}H/H$ and $^{18}O/^{16}O$ ratios in water samples. According to the manufacturer's specifications (Los Gatos Research Inc., 2008), the DLT-100 908-0008 LWIA provides isotopic measurements with a precision below 0.6‰ for $^{2}H/H$ ~~D/H~~ and 0.2‰ for $^{18}O/^{16}O$. Data were transformed into δ notion according to Vienna Standard Mean Ocean Water (VSMOW) standards ($\delta^{2}H$ and $\delta^{18}O$ in ‰).

3.23.3 Diatom sampling, sample preparation and analysis

Diatom analysis was conducted for multiple sample types: stream water, overland flow, epilithon, epipelon, and diatoms attached to different substrates outside the streambed (i.e. litter, bryophytesmosses, vegetation and soils).

Stream water samples were collected using automatic water samples during precipitation events, whereas overland flow samples were collected from gutters that captured overland flow throughout the event. A small amount set of stream water and overland flow samples wasas set aside for geochemical and isotopic analysis (≈70 mL), the rest of the sample was centrifuged (1250 rpm, 8 minutes) to concentrate the diatoms.

In addition to high-frequency sampling during rainfall events, catchment-wideseasonal sampling campaigns were carried out throughout the Weierbach catchment seasonally to assess the geographic and intra-annual variability of diatom communities. The following substrates were sampled in the catchment: (i) litter, moss bryophytes from the two hillslope classifications (hardwood and coniferous) and surface soil samples; and (ii) litter, mossbryophytes, and vegetation in the riparian zone. Each sample was comprised of five sub-samples collected on a 5-m transect parallel to the stream (a subsample collected every meter). Only material from the top surface, where there was greatest incident sunlight, was collected into 1-L plastic bottles. Sample bottles containing different substrata were filled with carbonated water (1-L), carefully shaken and left to settle overnight at 0 °C. The next day, the diatom-filled, carbonated water was recovered by passing it through a 1-mm screen. Sample substrate was then rinsed with additional carbonated water to remove as many diatoms from the sampled substrate as possible. This procedure was repeated several times until a 2-L sample volume was achieved. The recovered sample, now with substrate removed, was stored at 0 °C for a minimum of 8 hours to allow diatoms to settle, and the supernatant removed by aspiration.

During the same catchment-wide campaigns, epilithic (in-stream stone substrata) and epipelic (in-stream sediment or soil substrata) samples were also collected, treated and counted following the European standards CEN 13946 and CEN 14407 (European Committee for Standardization, 2003, 2004). For epilithic samples a minimum of five stones from the main flow and well-lit stream reaches, were brushed to collect the diatom biofilm, while epipelic samples were collected by disturbing small pools with sediment bottoms and then pipetting a superficial layer of 5–10 mm of sediment from reach pools.

All samples were preserved with 4% formaldehyde and treated with hot hydrogen peroxide to obtain clean frustule suspensions. After eliminating the organic matter from the diatom suspensions, diluted HCl was added to remove the calcium carbonate and avoid its precipitation later, which would make diatom frustule observation difficult. Finally, oxidized samples were rinsed with deionized water by decantation of the suspension several times, and permanent slides were mounted with Naphrax®.

Diatom valves were identified and counted (≈400 valves) on microscopic slides with a light microscope (Leica DMRX®). For the autecological assignment of the diatom species we relied on: (1) the Denys (1991) diatom ecological classification system refined by Van Dam et al. (1994), which is, as far as we know, the only formal classification of the occurrence of freshwater diatoms in relation to moisture; and (2) the associated hydrological units assigned by Pfister et al. (2009) to the five diatom occurrence classes defined by Van Dam et al. (1994). We express these results as relative abundance (percentage) of ~~terrestrial and aerophytic~~aerial valves, i.e. categories 4 and 5 of Van Dam's et al. (1994) classification.

~~3.3~~3.4 Hydrograph separation

Two-component hydrograph separation was performed using $\delta^{18}O$ ~~concentrations~~ isotopic composition and the mass balance approach (Pinder and Jones, 1969; Sklash and Farvolden, 1982; Pearce et al., 1986; Sklash et al., 1986). The incremental mean method proposed by McDonnell et al. (1990) was used to adjust $\delta^{18}O$ rainfall ~~concentrations,~~ isotopic composition, so that the bulk isotopic composition of rainfall from the beginning of the event to the time of stream sampling was calculated (i.e. rain that had not yet fallen was excluded from the estimate).

Spatial end-member contributions to stream water were explored using ~~end-member mixing analysis (EMMA)~~EMMA (Christophersen and Hooper, 1992), which assumes that (i) the stream water is a mixture of end-member solutions with a fixed composition, (ii) the mixing model is linear and relies on hydrodynamic mixing, (iii) the solutes used as tracers are conservative, and (iv) the end-member solutions are distinguishable from one another. Catchment end-members included shallow groundwater (GW1-4), soil water ($SS1_{20}$, $SS1_{60}$, $SS2_{60}$), soil water from the riparian zone (SSr), rainfall (R), throughfall (TH1-2), snow (SN) and overland flow (OF). We applied the diagnostic tools of Hooper (2003), which have been recently applied in the literature (James and Roulet, 2006; Ali et al., 2010; Barthold et al., 2011; Neill et al., 2011; Inamdar et al., 2013). Our approach followed three main steps:

i. We identified tracers that exhibit conservative linear mixing assuming that stream water chemistry is controlled by physical mixing of different sources of water and not by equilibrium mixing (Christophersen and Hooper, 1992; Hooper, 2003; Liu et al., 2008). The latest would imply equilibrium reactions among solutes of different charge, which may be approximated by high order polynomials. Hooper (2003) suggested that conservative and linear mixing of tracers can be evaluated using bivariate scatter plots. In this study, stream water concentrations and isotopic compositions (of all samples collected during storm events and ~~baseflow~~ low flows at the catchment outlet) were considered conservative when they exhibited at least one linear trend with one other tracer (i.e. $r^2>0.5$, p-value<0.01) (James and Roulet, 2006; Ali et al., 2010; Barthold et al., 2011).

ii. We performed a principal component analysis (PCA) on the stream water data. The PCA was applied on the correlation matrix of the standardized values of tracers selected in step (i) (i.e. by subtracting the mean concentration or isotopic composition of each solute and dividing by its standard deviation) (Christophersen and Hooper, 1992). For each water tracer, residuals were defined by subtracting the original value from its orthogonal projection. A 'good' mixing subspace was indicated by a random pattern of residuals plotted against the concentration or isotopic composition of the original values. On the contrary, structure or curvature in the subspace indicates violation against one of the assumptions of the EMMA approach (i.e. solutes do not mix conservatively) (Hooper, 2003). Eigenvectors were retained until there was no structure to the residuals. Standardized data ~~was~~ were multiplied by the eigenvectors and projected into the new U space.

iii. Finally, potential end-members were standardized using the mean and standard deviation of the stream water data. Their inter-quartile values (i.e. 25% and 75%) were then multiplied by the eigenvectors and projected into the U space of the stream water samples. Those end-members that best met the constraints of the mixing model theory as described by Christophersen and Hooper (1992) and Hooper (2003) were identified. Similar to previous studies, rather than calculating precise end-member contributions, we investigated the arrangement and relative positioning of all potential end-members with respect to stream flow in the U space (Inamdar et al., 2013). In order to account for end-member temporal variability, end-member concentrations and isotopic compositions for specific storm events were determined by considering ~~solute concentrations measured for~~ the samples collected during the event, as well as the preceding and following months (Inamdar et al., 2013).

4 Results

4.1 Hydrometric response

The hydrometric response for water years ~~2010~~2011-2012 is shown in ~~Figure~~Fig. 2. Diatom sampling commenced in November 2010 when the catchment started to progressively wet up (see groundwater depths and soil volumetric water content in ~~Figure~~Fig. 2). Annual precipitation for the water year ~~2010-~~2011 was 671 mm, a ~20% decrease compared to the average of the preceding four years (873 mm, as measured by the nearby meteorological station, Roodt), and 838 mm for the water year 2012. In January 2011, a 10-year return period rain-on-snow event produced a peak flow of 1.5 mm/h. The high winter discharge levels decreased progressively from February to June 2011 due to reduced precipitation during this period. Afterwards, a dry period extended from July to November 2011. A longer wet period was measured the following year (from December 2011 to July 2012).

During wet antecedent conditions, streamflow response of the basin was double peaked, with a first peak timing coincident with the rainfall input and the second, delayed peak coming a few hours later. ~~Storm-flow runoff coefficients were relatively high and as the catchment wetted up, the discharge response represented an ever-increasing higher fraction of the event rainfall.~~ On the contrary, when the catchment was dry the hydrological response was shorter and only a single sharp peak occurred. ~~Consequently, storm-flow coefficients were much lower.~~

We determined hydrological connectivity along a ~~hillslope-riparian-stream~~HRS transect via hydrometric observations. Water tables in the saprolite and fractured schist bedrock responded significantly to rainfall events. The magnitude of water level change was well-correlated to precipitation amount. Soil volumetric water content (VWC) decreased with distance upslope (VWC hillslope foot > VWC hillslope middle > VWC hillslope plateau (~~Figure~~Fig. 2). The riparian zone showed unchanging values close to saturation during wet periods (≈70%,), which decreased slightly when the catchment was dry (≈65%). For all monitored events, VWC at 10 cm depth responded quickly to incident rainfall at all transect locations (i.e. hillslope foot, middle and plateau), suggesting a vertically infiltrating, wetting front.

During dry antecedent conditions (summer and spring), threshold-like behaviour between soil moisture and discharge was observed at the hillslope foot (~~Figure~~Fig. 3a). Only when the VWC was higher than ≈27-30%~~,~~ did discharge increase significantly (threshold 1 in ~~Figure~~Fig. 3a). A second threshold appeared when the catchment was wet (autumn and winter), stream discharge increased significantly when VWC was above 40% (threshold 2 in ~~Figure~~Fig. 3a). This likely indicated connectivity between the hillslope and riparian compartments and the stream channel. A similar relationship was observed between VWC and depth to groundwater levels (i.e. GW1, GW2 and GW3; ~~Figure~~Fig. 3b).

4.2 Hydrograph separation

Two-component hydrograph separation results using $\delta^{18}O$ ~~concentrations~~ isotopic composition (i.e. pre-event water vs. event water) showed that, in winter, when the catchment was wet and flow response was double-peaked, the first peak had a larger contribution of event water than the delayed peak.~~was formed mainly by event water. This contrasted with the delayed peak that was dominated by pre-event water.~~ For instance, the first peak of the November 2010 event showed a maximum of 50% event water contribution~~,~~. This contrasted with the delayed peak that exhibited only a maximum of 16% event water contribution ~~whereas the second delayed peak only 16%~~ (~~Figure~~Fig. 4b). When the catchment was dry, the response consisted of one sharp peak composed ~~mainly~~ largely of event water ~~(Figure 4a)~~. A maximum ~~E~~event-water contribution~~s~~ of ~~27~~60% ~~and 45% were~~was estimated for a ~~two~~ storm event~~s~~ ~~that~~ occurred in June 2011 (Fig. 4a).

Twelve different tracers measured in the different water compartments of the catchment were used to assess end-member contributions to stream water (~~Figure~~Fig. 5). ~~Catchment end-members included shallow groundwater, soil water, riparian water, rainfall, throughfall, snow and overland flow.~~ Ten out of the twelve tracers presented linear trends in the solute-solute plots of stream water samples with at least one other tracer (EC, Cl^-, Na^+, K^+, Mg^{2+}, Ca^{2+}, SiO_2, Abs, δ^2H and $\delta^{18}O$; $r^2>0.5$, p-value<0.01, ~~Figure~~Fig. 6). These tracers~~, i.e. EC, Cl^-, Na^+, K^+, Mg^{2+}, Ca^{2+}, SiO_2, Abs, δ^2H and $\delta^{18}O$,~~ were retained for ~~further~~ the PCA analysis. Weaker linear trends were found between NO_3^- and the other tracers ($r^2<0.13$) and between SO_4^{2-} and the other tracers (r^2<0.43). Neither tracers reached the pre-defined threshold of collinearity (r^2>0.5), and were therefore not retained.

A ~~Principal Components Analysis (~~PCA~~)~~ analysis was performed on the correlation matrix of stream concentrations and isotopic compositions for the ten selected tracers. The first three principal components explained 91.3% of the variance in stream concentrations and isotopic compositions and were selected to generate a three-dimensional mixing space (U space, Table 2). Plots of residuals of each solute plotted against observed concentrations and isotopic compositions suggested that 3 components were needed to obtain a well-defined mixing subspace. End-member tracer concentrations and isotopic compositions were then projected into the mixing space (~~Figure~~Fig. 7). All stream water samples plotted inside the mixing domain defined by the end-members. Rainfall, throughfall, soil water and ~~riparian water~~soil water from the riparian zone end-members plotted in the upper right quadrant of the U1-U2 mixing space (~~Figure~~Fig. 7a). Shallow groundwater samples were located in the lower left quadrant and snow in the lower right quadrant. Overland flow plotted in the upper left quadrant and was located furthest away from stream water samples and with largest interquartile ranges. Most of the stream water samples were clustered in the immediate vicinity of the ~~riparian water~~soil water from the riparian zone samples, half-way between the throughfall and the groundwater samples. Snow seems to contribute to some stream water samples that placed slightly move toward the lower right quadrant (~~Figure~~Fig. 7a). The large distance between stream water and overland flow samples suggests a minor role of the latter in total runoff generation. Event peakflow samples are highlighted in ~~Figure~~Fig. 7b. In general, results show that when the catchment was wet, there was a higher contribution of groundwater to streamflow (events 1-2 and 10-11) than when the catchment antecedent condition was dry (events 3-9). ~~To the contrary~~However, compared to winter (events 1-2), a much higher contribution of throughfall was estimated during summer (events 5-8), when the pre-storm catchment state was dry.~~.~~

In order to better understand water pathways during each event separately, we plotted stream water samples collected for each event and end-member tracer signatures in the previously determined two-dimensional mixing space (~~Figure~~Fig. 8 and 9). We accounted for end-member temporal variability by plotting not only end-member samples collected the same month as the event occurred, but also the preceding and the following months. Groundwater and rainfall signals remained relatively constant throughout the year, whereas throughfall, riparian and soil water presented higher temporal variability. Results showed that runoff mixing patterns changed between events. During autumn and winter when the catchment was ~~wettest~~ wet (events 1-2, and 10-11), stream water signal composition was most similar to riparian, soil water and groundwater. Only samples collected during the rain-on-snow event (event 2) might have a small contribution of not only overland flow but also snow. Mixing patterns changed during spring and summer when the catchment was drier (i.e. events 3 to 9). As previously seen in ~~Figure~~Fig. 7b, groundwater seems to have a much lower contribution to stream water, since stream water samples now plotted in an intermediate position between throughfall and ~~riparian water~~soil water from the riparian zone (with the exception of event 3, which still has a significant groundwater contribution). Note that overland flow did not occur and the soils were dry during these spring and summer events.

4.3 Seasonal and geographic ~~trends~~ variability in ~~terrestrial and aerophytic~~aerial diatoms communities in the hillslope-riparian-stream system

The qualitative and semi-quantitative analysis of diatom microflora revealed 230 taxa in the Weierbach catchment. Diatom communities from samples collected during the ~~catchment-wide~~seasonal campaigns in the streambed (i.e. epilithon, epipelon and stream water samples) during ~~base~~ low flow were usually composed of species from oligotrophic environments, mainly occurring in water bodies, but also rather regularly on wet and moist surfaces (i.e. r~~R~~iparian zone hydrological functional unit of Pfister et al. (2009), such as *Achnanthes saxonica* Krasske ex Hustedt, *Achnanthidium kranzii* (Lange-Bertalot) Round & Bukthiyarova, *Fragilariforma virescens* (Ralfs) D.M. Williams & Round, *Eunotia botuliformis* F. Wild, Nörpel & Lange-Bertalot, and *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot). Important seasonal changes in relative abundance of ~~terrestrial and aerophytic~~aerial diatoms amongst the sampled habitats were not observed (Table 3). The null hypothesis of equal distributions was tested with the Mann-Whitney U-test for the samples from the riparian zone and the hillslope (too small number of stream water at low flow and streambed samples). P values were too high to reject the null hypothesis ~~)~~(0.21 and 0.73 for the riparian zone and the hillslope samples, respectively). ~~.~~No diatom valves were found in groundwater or rainfall samples.

The riparian zone was characterized by several species that prefer ~~terrestrial and aerophytic~~aerial habitats, mainly living on exposed soils or epiphytically on bryophytes. Such species occurr mainly on wet and moist or temporarily dry places or live nearly exclusively outside water bodies (Category 4 and 5 of Pfister et al. (2009)), such as *Chamaepinnularia evanida* (Hustedt) Lange-Bertalot, *C. parsura* (Hustedt) C.E. Wetzel & Ector, *Eunotia minor* (Kützing) Grunow, *Hantzschia abundans* Lange-Bertalot, *Nitzschia harderi* Hustedt, *Orthoseira dendroteres* (Ehrenberg) Round, R.M. Crawford & D.G. Mann, *Pinnularia borealis* Ehrenberg, *P. perirrorata* Krammer, *Stauroneis parathermicola* Lange-Bertalot and *S. thermicola* (J.B. Petersen) J.W.G. Lund.

Diatoms were completely absent in samples from dry litter on the hillslope and only occurred on bryophytes. Almost no diatoms were found in overland flow samples. The relative abundance of ~~terrestrial and aerophytic~~aerial valves was higher in hillslopes and riparian samples compared to streambed samples (Table 3). ~~But~~ However, we found a higher number of ~~terrestrial and aerophytic~~aerial diatoms (in absolute numbers) in the riparian zone. This emphasizes the importance of the riparian zones as the main terrestrial diatom source during rainfall, when diatoms are mobilized from moist or temporarily dry habitats into the stream channel (Table 3).

4.4 ~~Terrestrial and aerophytic~~Aerial diatom transport during rainfall events

A series of 11 rainfall events were sampled from November 2010 to December 2011 during both wet and dry ~~seasons~~ catchment conditions (Table 4 and ~~Figure~~Fig. 2). ~~Events were considered separately if they were separated by a period of at least 24h without rainfall.~~

Main ~~terrestrial and aerophytic~~aerial species found in stream water during storm events were as follows: *Chamaepinnularia evanida*, *C. obsoleta* (Hustedt) C.E. Wetzel & Ector, *C. parsura*, *Humidophila brekkaensis* (J.B. Petersen) Lowe et al., *H. perpusilla* (Grunow) Lowe et al., *Eolimna tantula* (Hustedt) Lange-Bertalot, *Eunotia minor*, *Pinnularia obscura* Krasske, *P. perirrorata*, *Stauroneis parathermicola*, *S. thermicola*.

Stream water samples taken throughout storm hydrographs showed a systematic increase in ~~terrestrial and aerophytic~~aerial diatoms as a response to incident precipitation and increasing discharge (~~Figure~~Fig. 8 and 9). During events, the minimum increment of aerial valves relative abundance was 8.1% (event 2), whereas the maximum increment was 27% (event 11). The maximum percentage of aerial valves was 43.5% (event 10).

No significant relationship was found between the percentage of ~~terrestrial and aerophytic~~aerial diatoms and instantaneous discharge (r^2=0.13, n=101; discharge on the x axis), most probably due to different diatom abundances on the rising limb of the hydrograph than on the recession limb (i.e. hysteretic effects). Two events showed clockwise hysteretic loops (events 1 and 2); five events showed counter-clockwise hysteretic loops (events 4, 5, 6, 8, and 10) and three showed figure-eight shaped hysteretic loops (events 7, 9 and 11). Although a clear pattern was not observed, results suggest that clockwise hysteretic loops predominated during wet conditions (the greater percentages of ~~terrestrial and aerophytic~~aerial diatoms in streamflow were immediately before peakflow), and counter-clockwise hysteretic loops during dry conditions (the greater percentages were immediately after peakflow).

~~Terrestrial and aerophytic~~Aerial valves comprised less than 15% of the total diatoms in ~~base~~ low flow samples for all events except 6, 9 and 10 (which had 19.2%, 17.1%, and 25.6 %, respectively). Due to technical problems, no ~~base~~ low flow sample was collected for event 3. No relationship was observed between antecedent event rainfall and the percentage of ~~terrestrial and aerophytic~~aerial valves observed during ~~base~~ low flow (n=10, r^2=0.08 and 0.09 for 10 and 20 days of antecedent rainfall, respectively). ~~During events, the minimum increment of terrestrial and aerophytic~~aerial ~~valves relative abundance was 8.1% (event 2), whereas the maximum was 27% (event 11). The maximum percentage of terrestrial and aerophytic~~aerial ~~valves was 43.5% (event 10).~~

At ~~the~~ event scale, there were significant correlations between maximum percentage of ~~terrestrial and aerophytic~~aerial diatoms and event rainfall and maximum event discharge (r^2=0.54, $p < 0.05$, n=10, ~~Figure~~Fig. ~~9~~10a; r^2=0.76, $p < 0.05$, n=10, ~~Figure~~Fig. ~~10~~10b, respectively; the multi-peak event sampled in December 2011 was considered as an outlier). High percentages (>35%) of ~~terrestrial and aerophytic~~aerial diatom relative abundance were measured during dry catchment conditions, compared to when the catchment was wet, where maximum relative abundances were low (<15%). Alternatively, higher maximum percentages of ~~terrestrial and aerophytic~~aerial diatom proportions (>35%) were measured during dry catchment conditions, when events were shorter and more intense.

A significant correlation between percentage of ~~terrestrial and aerophytic~~aerial diatoms with UV absorbance at 254 nm was found (r^2=0.55, $p < 0.05$, n=76, ~~Figure~~Fig. ~~9~~10c). ~~UV absorbance at 254 nm can be considered a proxy of DOC.~~ During rainfall events in the Weierbach catchment, the relative abundance of ~~terrestrial and aerophytic~~aerial diatoms was associated with increased ~~DOC~~ organic matter concentrations in the stream. A similar trend was observed with K^+ (r^2=0.25, $p < 0.05$, n=76), which is also associated with organic matter content. The relative abundance of ~~terrestrial and aerophytic~~aerial diatoms was not correlated with any other tracers.

5 Discussion

5.1 Can ~~terrestrial and aerophytic~~aerial ~~diatoms~~ diatom transport reveal~~detect~~ hydrological connectivity within the hillslope-riparian-stream system?

Our central hypothesis for this study was that ~~terrestrial and aerophytic~~aerial diatoms could indicate connectivity within the ~~hillslope-riparian-stream (~~HRS~~)~~ system. In order to test this hypothesis, we sampled from potential upland catchment sources (i.e. hillslope and riparian zones), and within the streambed (i.e. epilithon, epipelon and stream water samples). ~~We also collected hillslope overland flow samples using gutters, installed as closely as possible to the soil surface.~~

Before testing our central hypothesis, we tested for the existence of distinguishable diatom species assemblages on the hillslope, the riparian zone and the stream. Only if diatom assemblages are distinguishable between these zones can their presence in the channel during rainfall events serve as a proxy for HRS connectivity. Results showed clear differences in diatom species assemblages between the hillslopes, riparian zone and streams, with higher relative abundance of ~~terrestrial and aerophytic~~aerial diatoms in the hillslopes and riparian zones compared to the stream (Table 3). Diatoms are usually abundant in moist environments (Van de Vijver and Beyens, 1999; Nováková and Poulíčková, 2004; Chen et al., 2012; Vacht et al., 2014) but in spite the presence of diatoms in ~~moss~~bryophytes-covered areas of the hillslopes, we did not find any diatom valves in hillslopes covered by dry litteer. Moreover, the quantities of ~~terrestrial and aerophytic~~aerial diatoms found on the hillslopes covered by ~~moss~~ bryophytes and in the overland flow gutter samples were small and sometimes not sufficient to fully characterize the zone (due the rarity of some species but also linked to sampling difficulties). This constrained the use of ~~terrestrial and aerophytic~~aerial diatoms to infer hillslope-riparian zone connectivity in some parts of the Weierbach catchment because of a limited diatom reservoir on hillslopes~~ covered by dry litter~~.

Despite the highest relative abundance of ~~terrestrial and aerophytic~~aerial valves on the hillslope compared to the riparian zone, the riparian zone was still the largest ~~terrestrial and aerophytic~~aerial diatom reservoir (in absolute numbers) with the highest probability of connecting to the stream (Table 3). We did not observe significant seasonal differences in diatom species assemblages among the different sampled habitats.

We examined the ~~terrestrial and aerophytic~~aerial diatoms transported in the stream water during runoff events. We observed an increase in the relative abundance of ~~terrestrial and aerophytic~~aerial diatoms with discharge for all sampled events regardless of antecedent wetness conditions. Hence, during storm events there was an increase in the relative proportion of diatoms in categories 4 and 5 of Van Dam's et al. (1994) classification. Similar results were reported by Pfister et al. (2009). These observations imply hydrological connectivity between the riparian soil surface and the stream for all events. The use of ~~terrestrial and aerophytic~~aerial diatoms to infer hydrological connectivity in the Weierbach catchment thus remains limited to the riparian-stream system as no diatoms were found on the hillslopes covered by dry litter.

Even though ~~terrestrial and aerophytic~~aerial diatoms do not live in microhabitats with flowing water, they were found in stream water samples during ~~baseflow~~ low flow conditions preceding storm events (Table 3). This indicated that the 'stock' of ~~terrestrial and aerophytic~~aerial diatoms in the catchment before the sampled events was not completely exhausted during previous events. Similar conclusions were drawn by Coles et al. (*under review*), who examined diatom population depletion effects during rainfall and found that while ~~terrestrial and aerophytic~~aerial diatom populations in the riparian zone were depleted in response to rainfall disturbance, rainfall was unlikely to completely exhaust the diatom reservoir.

We hypothesize that the transport of diatoms from the riparian zone to the stream might take place either through (i) a network of macropores in the shallow soils of the riparian zone or (ii) overland flow in the riparian zone. The potential for diatoms to be transported through the subsurface matrix was investigated using fluorescent diatoms and soil columns by Tauro et al. (*under review*). Results demonstrated that sub-surface transport of diatoms through the sub-surface matrix was unlikely. However, the potential for transport of diatoms through heterogeneous macropore networks remains unexplored. The increased relative abundance of ~~terrestrial and aerophytic~~aerial diatoms in the stream event water could also be explained by yet undocumented, surface or near-surface pathways.

5.2 How do diatom results compare to the other methods to infer hydrological connectivity?

Two-component hydrograph separation and ~~end-member mixing analysis (~~EMMA~~)~~ provide valuable information on water sources and flowpaths. Using these methods we learned that in the Weierbach catchment, during spring and summer, the hydrological response was largely composed of event water (see an example of dry antecedent catchment conditions in ~~Figure~~Fig. 4a). Similar conclusions were drawn by Wrede et al. (2014) using dissolved silica. Accordingly, EMMA results suggest canopy throughfall, rainfall and riparian soil water were the main water sources (~~Figure~~Fig. 8 and 9). As observed in other headwater catchments (e.g. Penna et al., 2011), discharge likely increased due to channel interception and riparian runoff leading to clear and singular hydrograph peaks (~~Figure~~Fig. 4a). During fall and winter, when the catchment was at its wettest state, double peaked hydrographs characterized the event hydrological response. Hydrograph separation indicated that the first peak was mainly event water and the delayed, second peak was mostly pre-event water (~~Figure~~Fig. 4b; Wrede et al., 2014). During these events, soil water, groundwater, and throughfall contributed substantially to total discharge (~~Figure~~Fig. 8 and 9). Hillslope overland flow was insignificant during most sampled events. Only for event 2 – the largest storm on record –overland flow was a significant contributor to stream discharge, likely due to rapid snowmelt onto surface-saturated area (~~Figure~~Fig. 8 and 9).

During all sampled events the relative abundance of ~~terrestrial and aerophytic~~aerial diatoms increased with discharge indicating hydrological connectivity between the riparian zone and the stream. These findings are consistent with the hydrograph separation results. ~~Terrestrial and aerophytic~~Aerial diatoms could reach the stream as saturated areas expand during rainfall events. Accordingly, we found a significant correlation between percentage of ~~terrestrial and aerophytic~~aerial diatoms with UV absorbance (proxy of DOC). DOC concentrations associated with runoff storm often come mainly from the near-stream riparian zones (Boyer et al., 1997). Controls on surface saturated and subsurface mixing processes are currently being investigated in the Weierbach riparian zone using infrared imagery and groundwater metrics (Pfister et al., 2010).

Hydrological connectivity between hillslopes and the stream has also been previously defined by water table connections between the hillslope and the riparian zone (Vidon and Hill, 2004; Ocampo et al., 2006; Jencso et al., 2010; McGuire and McDonnell, 2010). While our results showed that overland flow did not occur on hillslopes during most sampled events, the VWC measurements and timing of the hydrograph response suggest that subsurface hydrological connectivity along the ~~hillslope-riparian-stream~~HRS system occurs during wet catchment conditions (~~Figure~~Fig. 3). Hence, if ~~terrestrial and aerophytic~~aerial diatoms found on the ~~moss-covered~~ hillslopes ~~(we did not find any diatom valves on hillslopes covered by dry litter)~~, might reach the stream through ~~deeper~~ sub-surface ~~macropore~~ flowpaths remains unknown. ~~-~~Others have demonstrated that tracer transport can occur at larger time scales that extend beyond individual events (McGuire and McDonnell, 2010). Whether this may also be true for diatoms remains to be explored.

5.3 Can aerial diatom be established as a new hydrological tracer?

~~5.3 On the use of diatoms to infer hydrological connectivity in the HRS system~~

Storm hydrograph separation using stable isotope tracers has resulted in major advances in catchment hydrology. However, despite their usefulness, these methods do not provide unequivocal evidence of hydrological connectivity in the HRS system. ~~This is mainly due to inherent conceptual limitations (Richey et al., 1998; Burns, 2002).~~ In comparison, diatoms can provide evidence of riparian-stream connectivity. ~~But f~~Further research is needed to better understand diatom transport processes (and associated water flowpaths) in headwater catchments. Future studies should focus on expanding our understanding of terrestrial diatom taxonomy and ecology, ~~that~~ which are scarce or lacking for a large number of taxa (Wetzel et al., 2013, 2014). Even though this new data source will have its own individual measurement uncertainty (McMillan et al., 2012), diatoms offer the possibility to tackle open questions in hydrology and ~~hydro-ecology~~eco-hydrology.

A key issue with the concept of hydrological connectivity is how it can be applied across and between environments. Uncertainties increase when applying two-component hydrograph separation at large scales. For instance Klaus and McDonnell (2013) note that quantifying the spatial variability in the isotope signal of rainfall and snowmelt can be difficult in large catchments and in catchments with complex topography. Similarly, some studies showed that for meso-scale catchments, only qualitative results of the contribution of a runoff component can be obtained by the hydrograph separation techniques (Uhlenbrook and Hoeg, 2003). For ~~terrestrial and aerophytic~~aerial diatoms to be useful and a way forward to increase our understanding of hydrological pathways at a range of scales, they must be also relevant across environments and scales (Bracken et al., 2013). The current concepts related to ~~hillslope-riparian-stream~~HRS connectivity are best-suited to humid, temperate settings (Beven, 1997; Bracken and Croke, 2007) and represent only very specific settings (Bracken et al., 2013). Previous investigations in Luxembourg have shown that freshwater diatom assemblages in headwater streams have regional distributions strongly affected by geology, as well as anthropogenic factors (e.g. organic pollution sources and eutrophication) (Rimet et al., 2004). Hence, we speculated that diatoms have potential in headwater systems, and at larger catchment scales to determine connectivity between contrasting geological zones.

The need to account for the temporal variability in end-member chemistry and to collect high-frequency data on both—stream water as well as potential runoff end-members – has been well-recognized (Inamdar et al., 2013). As noted by Tetzlaff et al. (2010), seasonality should also be considered when using living organisms to trace water flowpaths. Diatom end-members must be sampled seasonally in order to ensure that populations have not undergone demographic changes. Indeed, this increases the sampling needs and the overall laboratory procedures of an already time-consuming approach (i.e. sampling, pre-treating the samples, mounting permanent slides and diatom identification). A potential alternative to reduce processing time is to develop new techniques such as to dye diatom valves and use them to trace water flowpaths (see Tauro et al., *under review*). The use of dyed diatoms under field conditions for experimental hydrology remains unexplored.

6 Conclusions

We investigated the potential for ~~terrestrial and aerophytic~~aerial diatoms, i.e. diatoms nearly exclusively occurring outside water bodies and on wet and moist or temporarily dry places (Van Dam et al., 1994), to serve as natural tracers capable of detecting connectivity within the ~~hillslope-riparian-stream~~HRS system. We found that the relative abundance of ~~terrestrial and aerophytic~~aerial diatoms in stream water samples collected during storm events increased with runoff during all seasons. Sampling of the potential catchment sources of diatoms in the HRS system and inside the stream channel (i.e. epilithon, epipelon and stream water samples) indicated that riparian zones appear to be the largest ~~terrestrial and aerophytic~~aerial diatom reservoir. Few diatom valves were found in overland flow samples and diatoms were completely absent on leaf-covered hillslopes, occurring only in hillslope samples with bryophytes ~~mosses~~ and limiting the use of ~~terrestrial and aerophytic~~aerial diatoms do infer hillslope-riparian zone connectivity. ~~Nontheless~~Nonetheless, we have shown the use of diatoms to quantify riparian-stream connectivity as the relative abundance of ~~terrestrial and aerophytic~~aerial diatoms increased with discharge during all sampled events. Although further research is needed to determine the exact pathways that ~~terrestrial and aerophytic~~aerial diatoms use to reach the stream, diatoms offer the possibility of address open questions in hydrology at small and large catchment scales.

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Table 1. Summary of collection methods, sampling resolution and locations in the Weierbach catchment.

	Component	Resolution	Method	N° locations
Hydrology	Discharge	15 min	Stage-discharge rating curve	1 (outlet)
	Precipitation	15 min	Tipping bucket	2
	Water table depth	15 min	TD-driver	4
	Soil moisture	30 min	Water content reflectometer	4
	Stream conductivity	15 min	Conductivity meter	1 (outlet)
	Groundwater conductivity	30 min	Conductivity meter	2
Geochemistry and isotopes	Groundwater	Fortnightly	Manual	4
	Overland flow (hillslope)	Accum. events	Gutters	5
	Precipitation	Accum. fortnightly	Rain gauge	1
	Precipitation	~2.5 mm increments	Sequential rainfall sampler	1
	Snow	Sporadic	Manual	Spots
	Soil water	Accum. fortnightly	Suction cups	3
	Stream water	1-6 h (events)	ISCO automatic sampler	1 (outlet)
	Stream water	Fortnightly	Manual	3
	Throughfall	Accum. fortnightly	Rain gauge	2
Diatoms	Epilithon	Once per season	Manual	3
	Epipelon	Once per season	Manual	3
	Overland flow (hillslope)	Accum. events	Gutters	5
	Stream water	1-6 h (events)	ISCO automatic sampler	1 (outlet)
	Stream water	Monthly	Manual	1 (outlet)
	Substrates	Once per season	Manual	16

Table 2. Variance explained by each eigenvector (~~data collected in the Weierbach catchment,~~ n=210).

Eigenvectors	Proportion of variance explained, %	Accumulated variance explained, %
1	57.6	57.6
2	20.5	78.1
3	13.2	91.3
4	2.8	94.0
5	2.3	96.4
6	1.4	97.8
7	0.8	98.6
8	0.6	99.2
9	0.5	99.7
10	0.3	100

Table 3. ~~P~~Relative percentage of ~~terrestrial and aerophytic~~aerial valves quantified at distinct zones of the Weierbach catchment. Streambed samples refer to epilithon samples. Riparian zone samples include litter, bryophytes and vegetation. Hillslope samples include litter, bryophytes and surface soil samples. Diatoms were absent on hillslopes covered by dry litter and samples were discarded.

	Sample	n	Min [%]	Max [%]	Mean [%]	S.E. [%]	S.D. [%]
Summer 2010	~~Baseflow~~ Stream water at low flow ~~drift~~	3	10.1	19.4	14.9	2.7	4.6
	Streambed	6	14.8	21.7	19.0	1.1	2.7
	Riparian zone	25	8.5	61.5	22.9	3.4	16.9
	Hillslope	12	11.6	96.6	36.5	7.8	27.0
Winter 2011	~~Baseflow~~ Streamwater at low flow ~~drift~~	8	5.9	16.1	9.8	1.2	3.3
	Streambed	2	5.0	8.8	6.9	1.9	2.7
	Riparian zone	39	12.4	67.2	21.9	1.9	12.0
	Hillslope	16	11.3	100.0	40.4	6.6	26.4

Table 4. General hydrological characteristics of the sampled rainfall-runoff ~~storm runoff~~ events occurred from October 2010 to December 2011 in the Weierbach catchment.

	Beginning of precipitation	Duration	Total P	Maximum intensity	Antecedent P, 10 days	Antecedent P, 20 days	~~Baseflow~~Pre-event discharge	Maximum discharge
		[h]	[mm]	[mm·15min^{-1}]	[mm]	[mm]	[L·s^{-1}]	[L·s^{-1}]
Event 1	11 Nov 2010	154	65	1.2	42	49	5.4	60.4
Event 2	6 Jan 2011	142	45	0.9	-	-	6.1	187.5
Event 3	31 May 2011	14	26	5.4	1	4	0.1	12.2
Event 4	18 Jun 2011	10	10	3.2	8	71	0.1	3.0
Event 5	20 Jun 2011	14	26	6.4	25	62	0.3	9.2
Event 6	22 Jun 2011	13	10	2.6	51	89	0.4	3.4
Event 7	16 Jul 2011	29	31	2.2	6	8	0	5.2
Event 8	6 Aug 2011	12	20	8.1	7	21	0	3.6
Event 9	17 Sep 2011	49	15	1.4	12	22	0	2.1
Event 10	1 Dec 2011	46	10	0.8	2	3	0.1	1.5
Event 11	3 Dec 2011	124	57	2.7	13	14	0.2	13.1

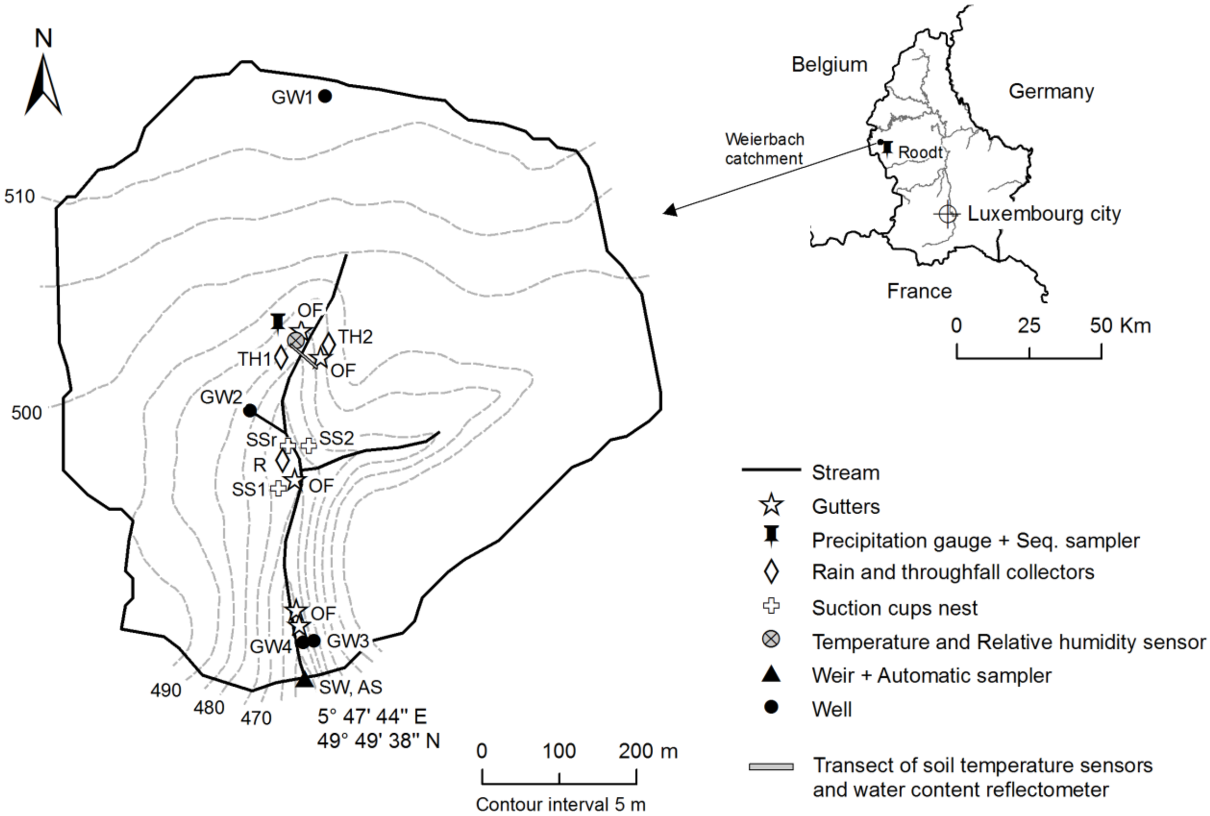
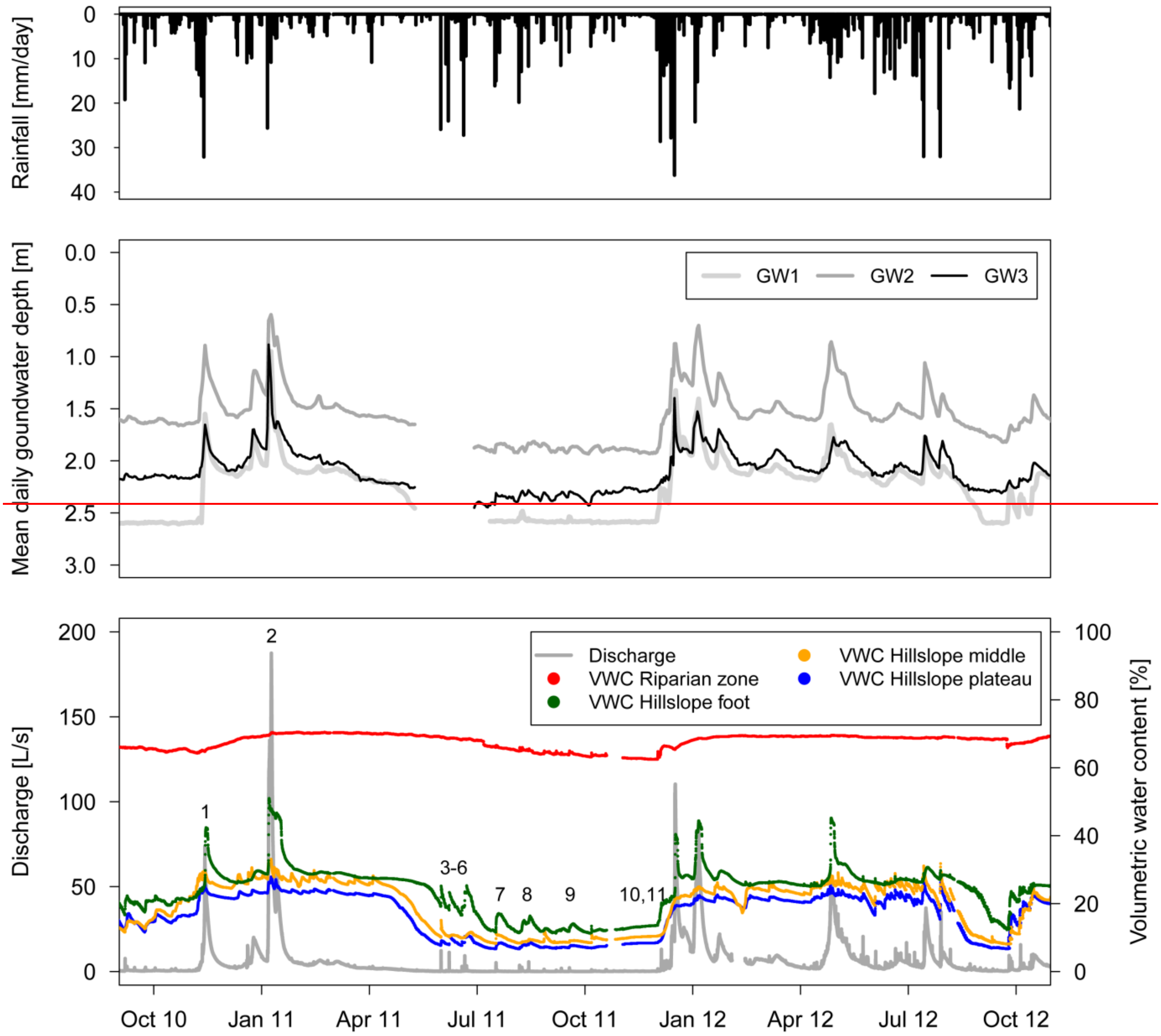


Figure 1. Detailed map of topography and instrumentation locations in the Weierbach catchment (Northwest of Luxembourg City).

1



Rainfall [mm/day]
0
10
20
30
40
Mean daily goundwater depth [m]
0.0
0.5
1.0
1.5
2.0
2.5
3.0
GW1
GW2
GW3
Discharge [L/s]
200
150
100
50
0
Volumetric water content [%]
100
80
60
40
20
0
Discharge
VWC Riparian zone
VWC Hillslope foot
VWC Hillslope middle
VWC Hillslope plateau
1
2
3-6
7
8
9
10,11
Oct 10
Jan 11
Apr 11
Jul 11
Oct 11
Jan 12
Apr 12
Jul 12
Oct 12

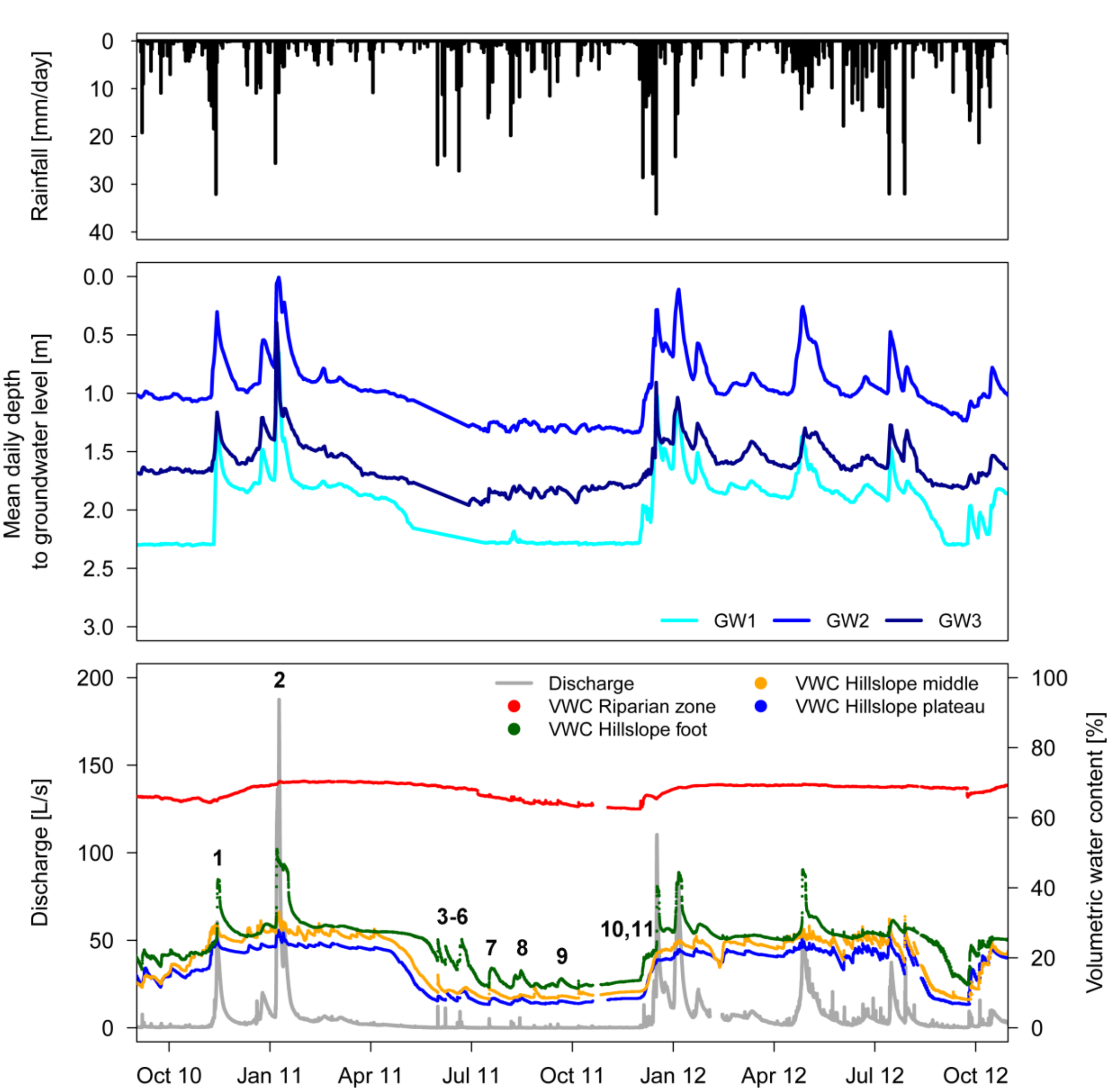


Figure 2. Time series of daily rainfall measured at the Roodt meteorological station (≈3.5 km distant from the Weierbach) (upper plot), mean daily groundwater depth at three different locations (GW1: plateau, GW2: close to a spring, and GW3: hillslope foot) (middle plot) and soil volumetric water content measured in a transect from the hillslope plateau to the riparian zone along with corresponding water discharge (lower plot). Numbers in the lower plot identify sampled storm events.~~Soil volumetric water content measured in a transect from the hillslope plateau to the riparian zone along with corresponding water discharge (blue line) (lower plot), numbers identify sampled storm events; mean daily groundwater depth at three different locations (GW1: plateau, GW2: close to a spring, and GW3: hillslope foot) (middle~~

plot) and daily rainfall measured at the Roodt meteorological station (≈3.5 km distant from the Weierbach) (upper plot).

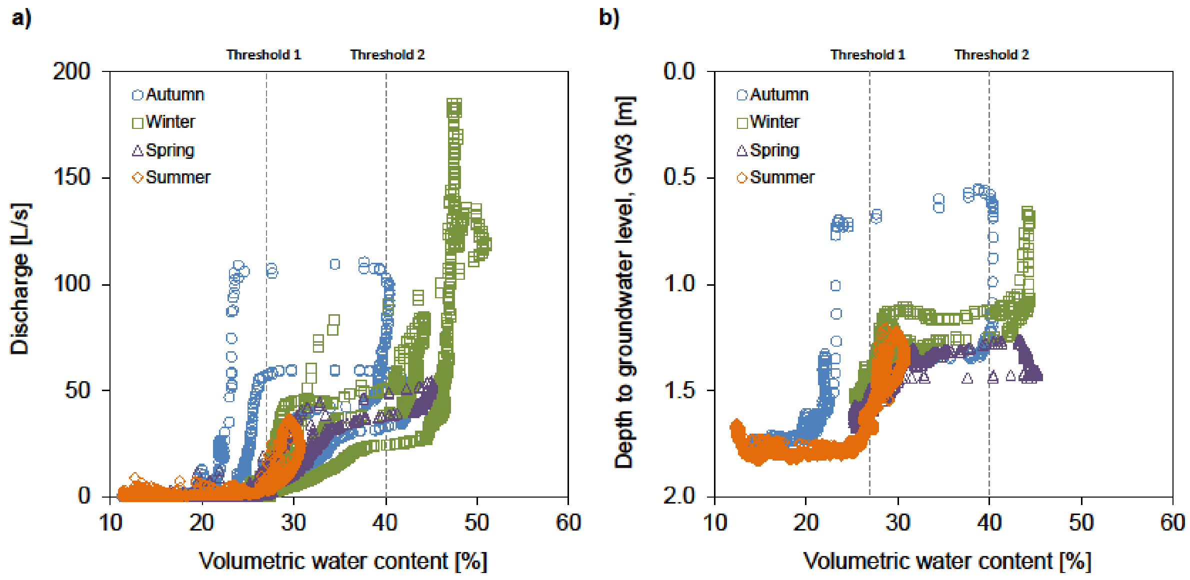
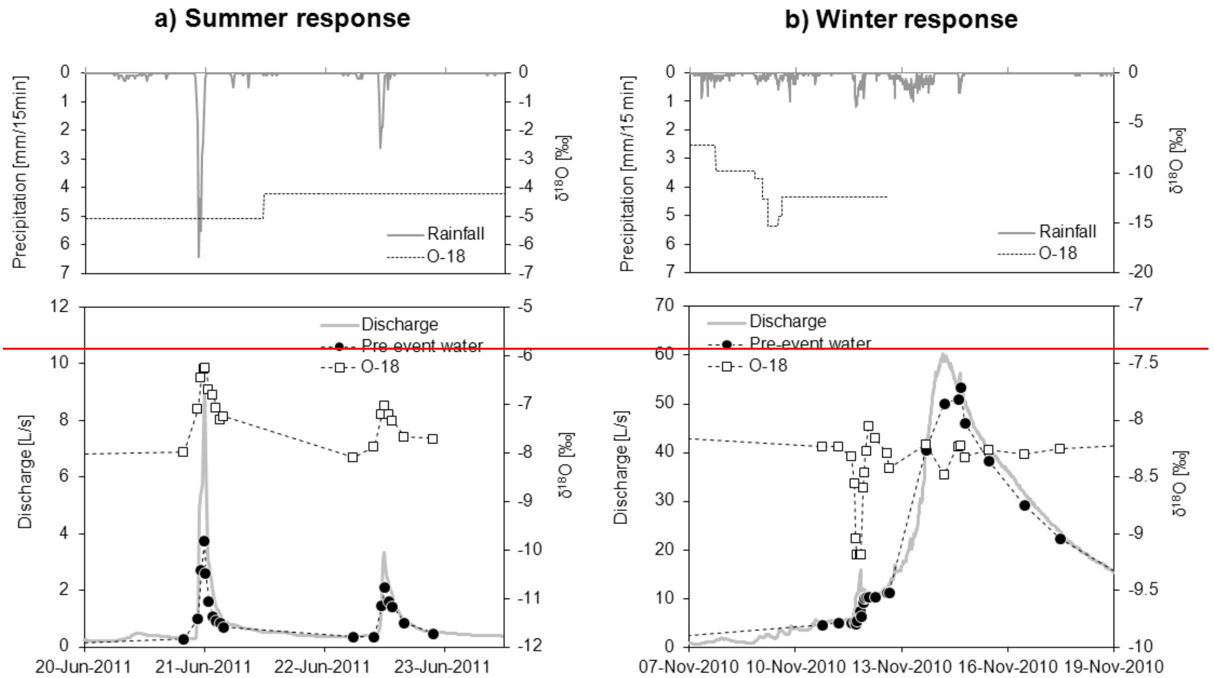


Figure 3. Relationship between (a) volumetric water content (hillslope foot) and discharge, and (b) between volumetric water content and depth to groundwater level for the period plotted in Figure 2. Vertical dashed lines represent two threshold values (see details in the text).



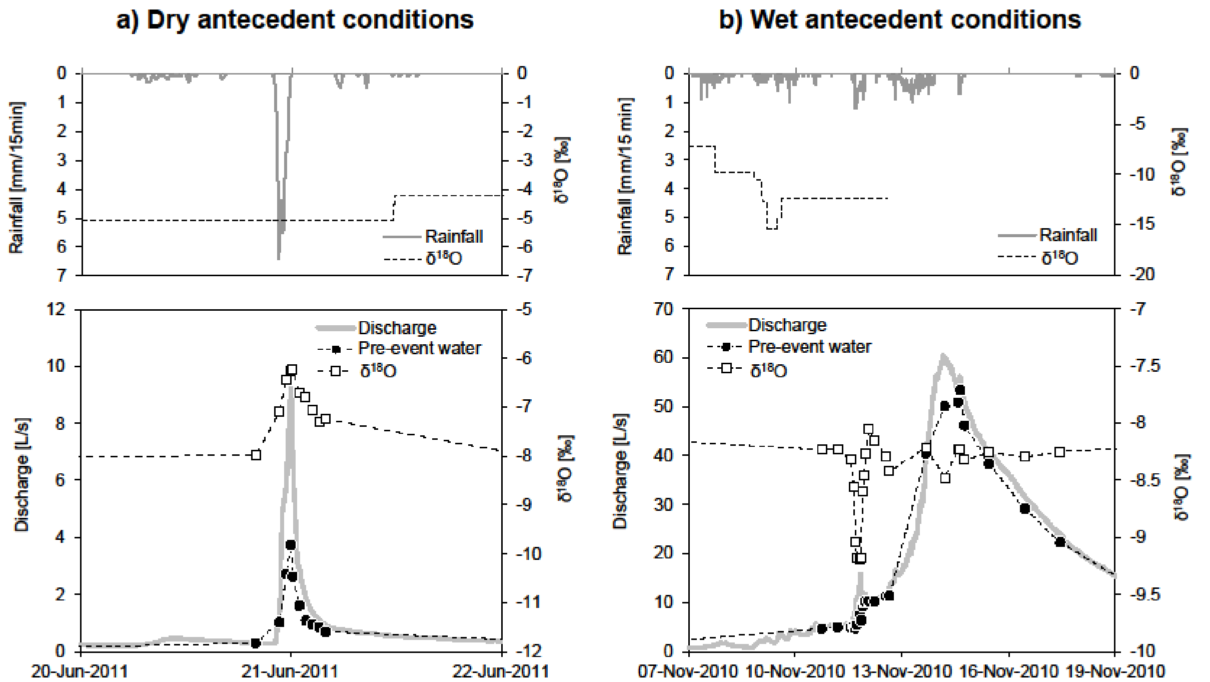
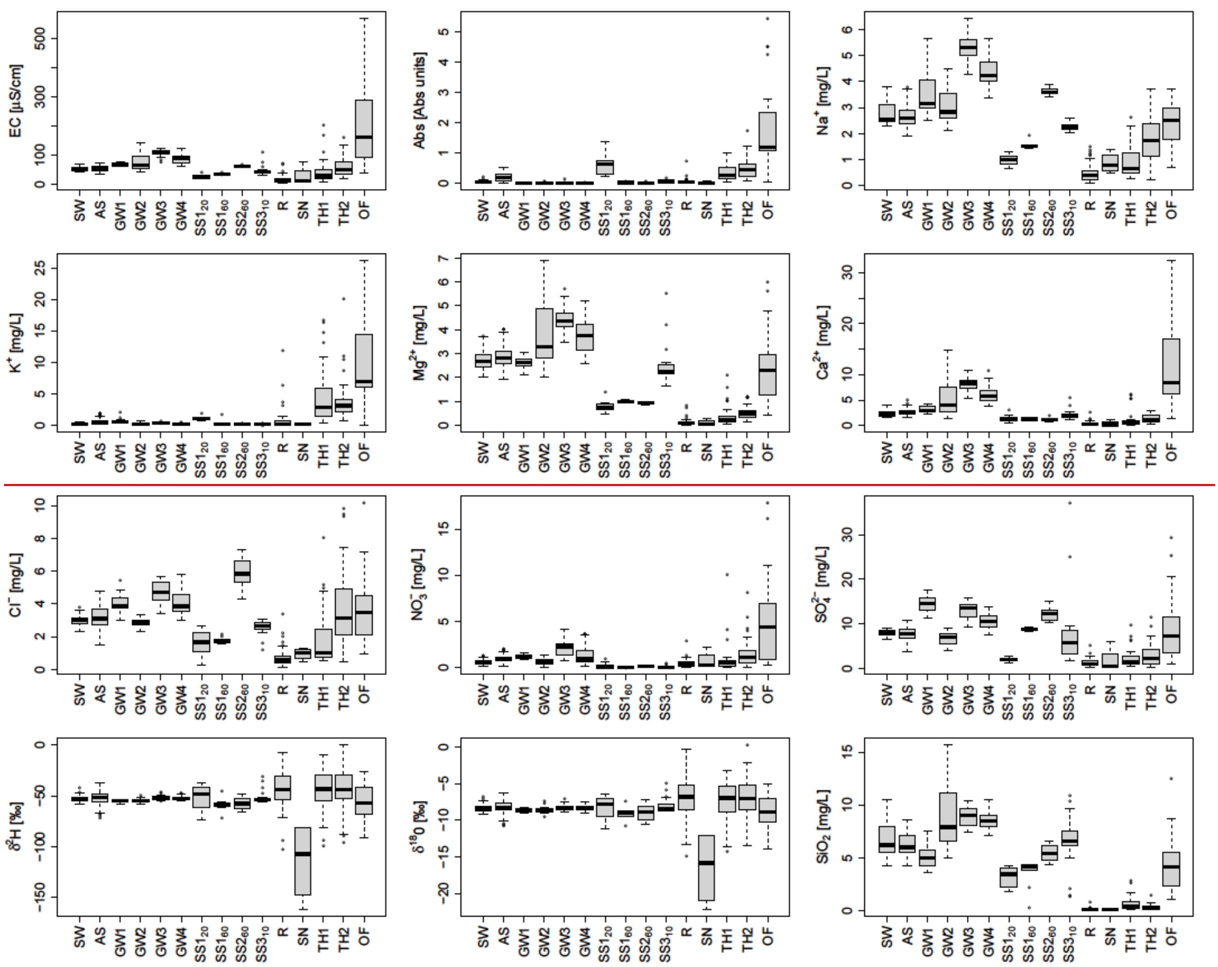


Figure 4. Two~~-~~-component~~s~~ hydrograph separation for (a) the 20th and 22nd June 2011 events (summer response) and (b) the 7th November 2010 event (winter response) using $\delta^{18}O$~~$\delta^{18}O$ concentrations~~ isotopic composition.~~to identify event and pre-event water contribution to streamflow.~~



EC [µS/cm]
Abs [Abs units]
Na+ [mg/L]
K+ [mg/L]
Mg2+ [mg/L]
Ca2+ [mg/L]
Cl− [mg/L]
NO3− [mg/L]
SO42− [mg/L]
δ2H [‰]
δ18O [‰]
SiO2 [mg/L]
SW
AS
GW1
GW2
GW3
GW4
SS1₂₀
SS1₆₀
SS2₆₀
SS3₁₀
R
SN
TH1
TH2
OF

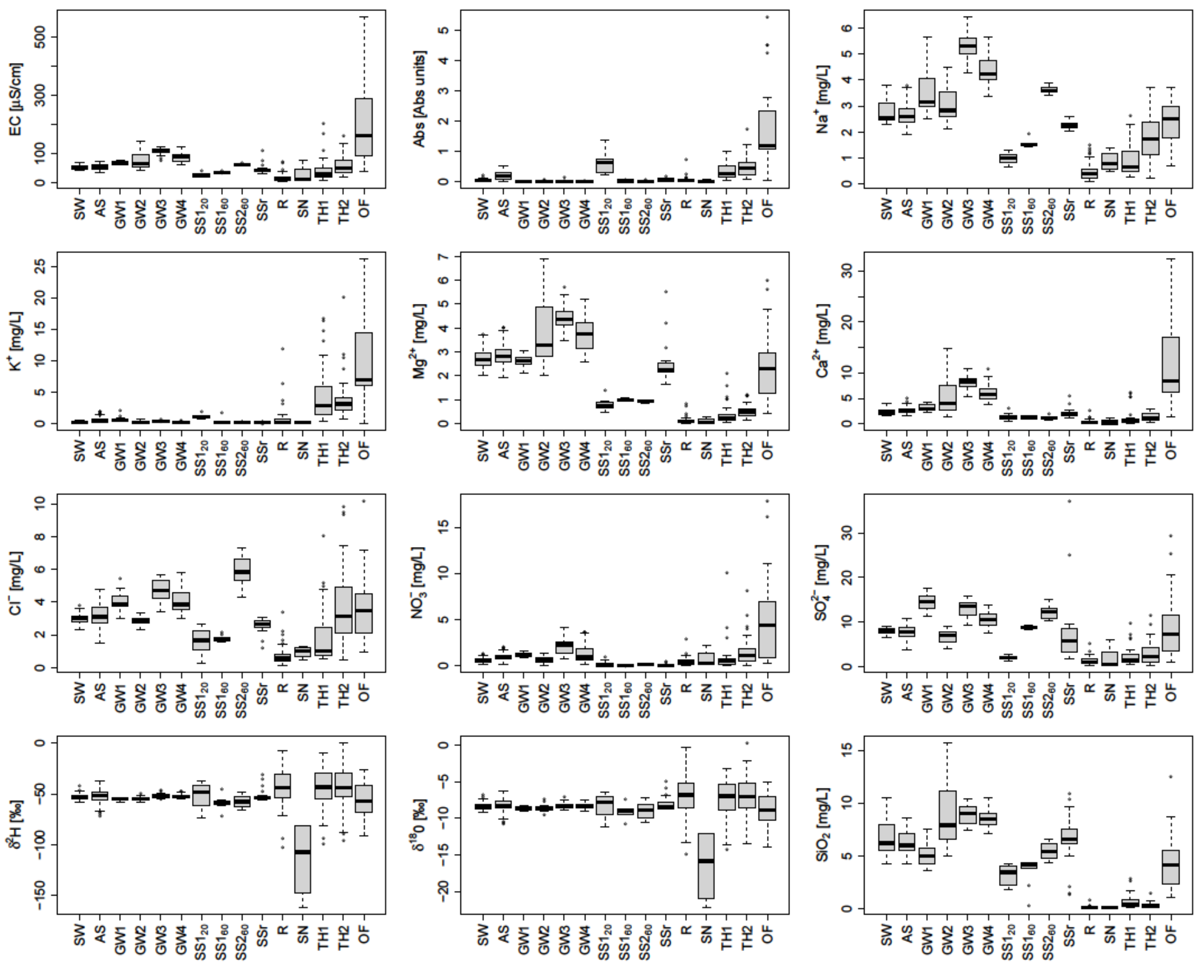


Figure 5. Boxplots of tracers measured for stream water sampled fortnightly (SW, n=47) and using automatic samplers (AS, n=179), groundwater (GW1, n=24; GW2, n= 49; GW3, n=49; GW4, n=47), soil water ($SS1_{20}$, n=22; $SS1_{60}$, n=10; $SS2_{60}$, n=9), soil ~~riparian~~ water from the riparian zone (SSr~~S3$_{10}$~~, n=21), rainfall (R, n=44), snow (SN, n=4), throughfall (TH1, n=35; TH2, n=38) and overland flow (OF, n=21). Outliers were discarded.

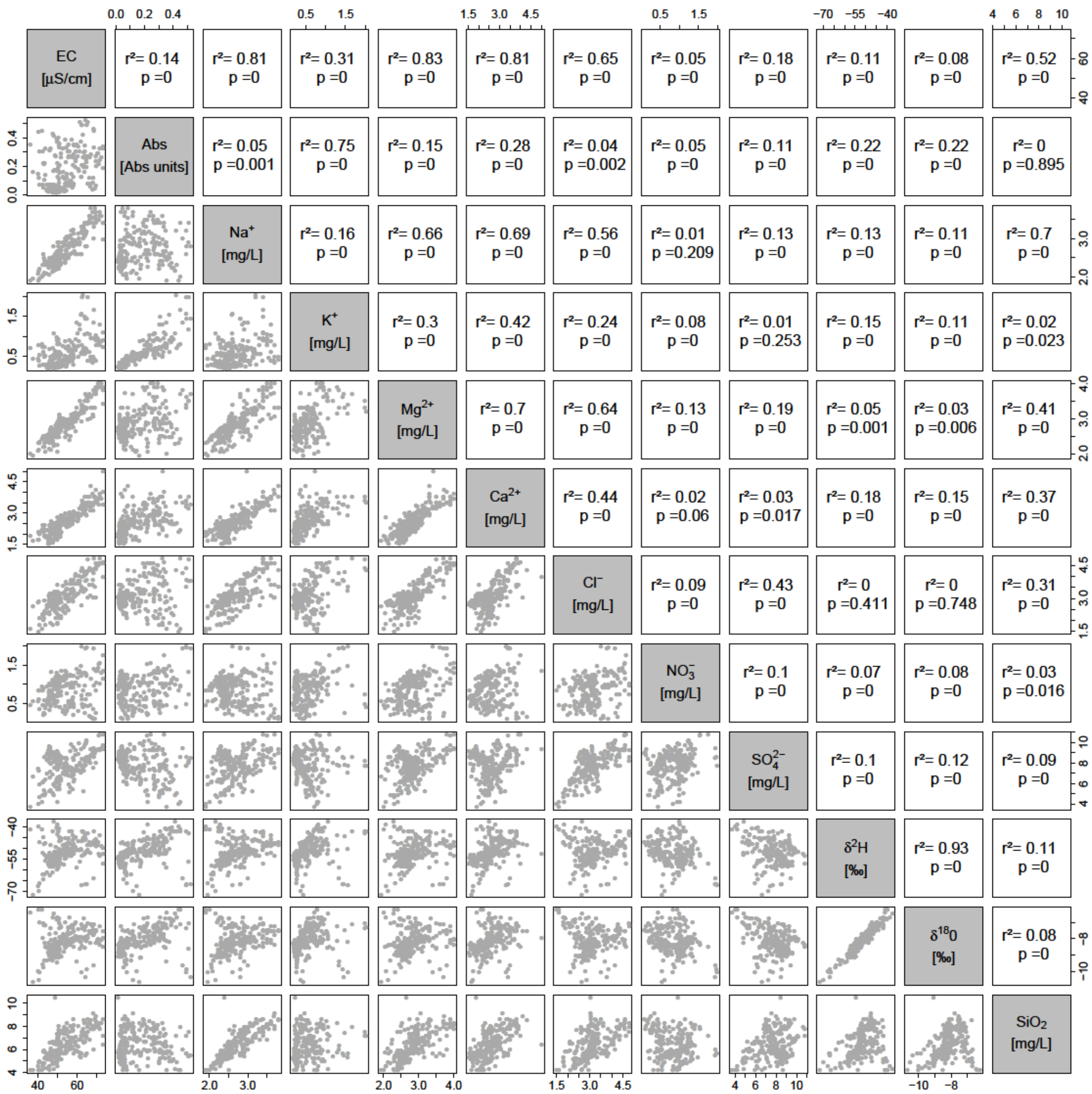


Figure 6. Bivariate solute plots of stream water chemistry and water stable isotope data collected at the outlet of the Weierbach catchment (n=226; SW and AS displayed in Fig. 5~~, i.e. 47 grab stream water samples collected fortnightly from October 2010 to September 2012 (SW) and 179 stream water samples collected during runoff events using automatic samplers (AS)~~). ~~Upper~~ The upper part of the diagonal shows the Pearson's correlation coefficient and its significance at the 0.95 confidence level.

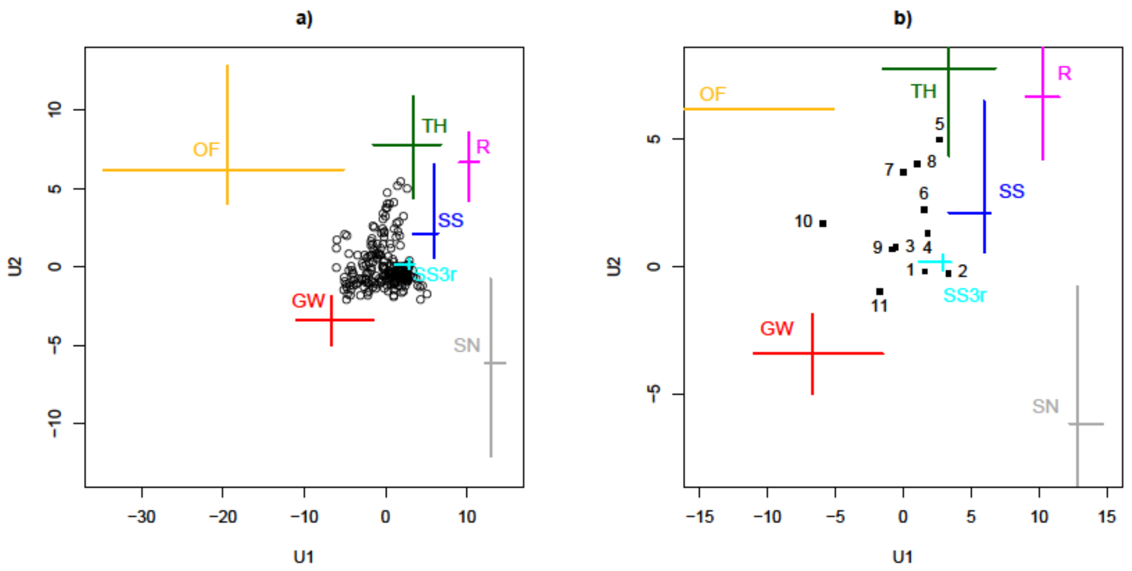
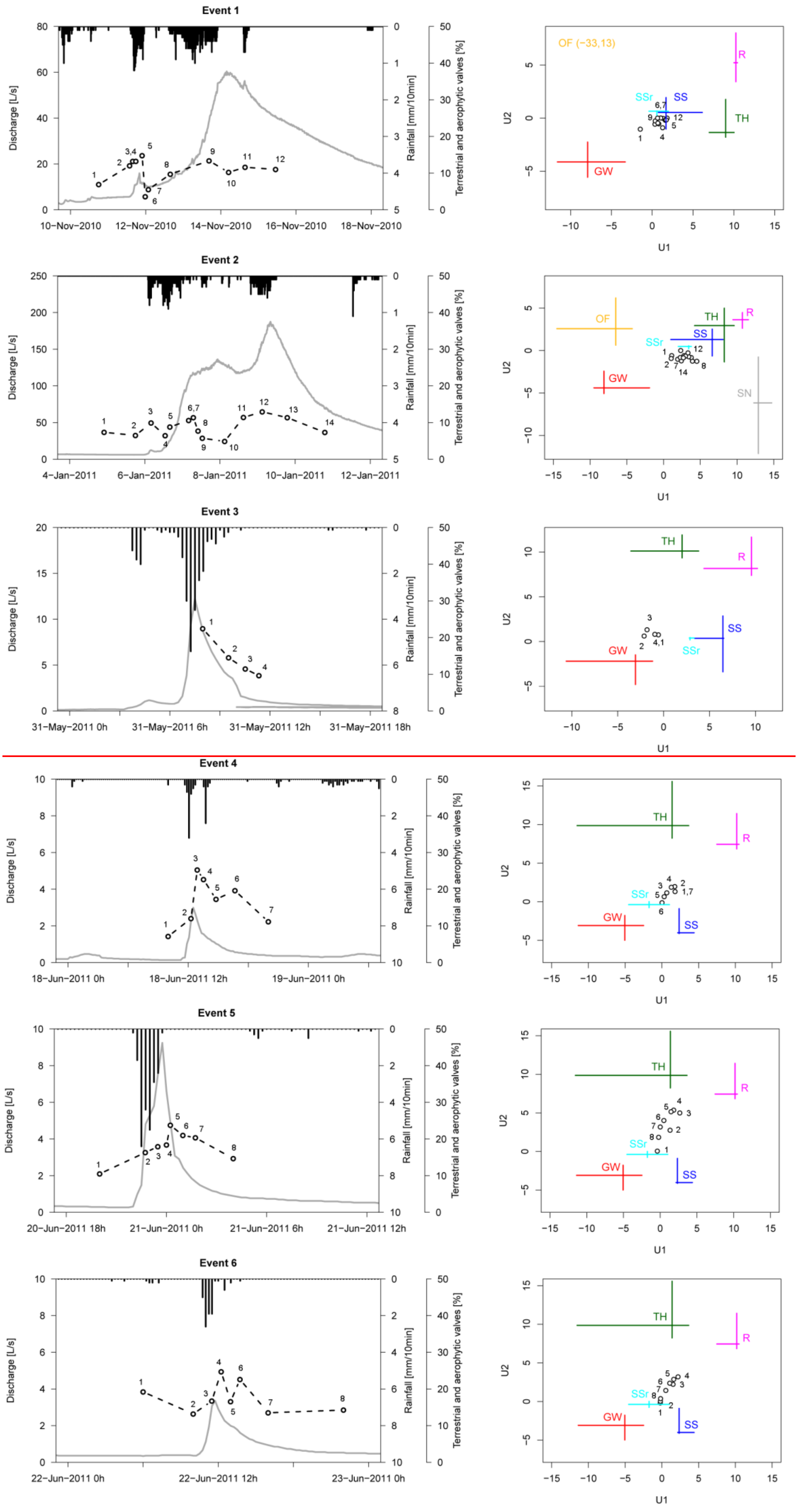


Figure 7. (a) U1-U2 mixing diagram of stream water tracers (black circles; AS + SW in Figure 5) and (b) zoom into the U1-U2 mixing diagram showing event peakflow stream water samples (black squares; numbers identify storm events in Figure 2)~~ in the U1-U2 mixing diagram~~. Sampling points data plotted in Figure 5 were grouped in 7 end-members ~~(GW: groundwater, SN: snow, SS: soil solution, SSr: riparian water, OF: overland flow, R: rainfall, TH: throughfall)~~ and the interquartile ranges of each end-member were projected into the new mixing space (U-space~~)~~; GW: groundwater, SN: snow, SS: soil water, SSr: soil water from the riparian zone, OF: overland flow, R: rainfall, TH: throughfall).



Event 1
Event 2
Event 3
Event 4
Event 5
Event 6
Discharge [L/s]
Rainfall [mm/10min]
Terrestrial and aerophytic valves [%]
10-Nov-2010
12-Nov-2010
14-Nov-2010
16-Nov-2010
18-Nov-2010
4-Jan-2011
6-Jan-2011
8-Jan-2011
10-Jan-2011
12-Jan-2011
31-May-2011 0h
31-May-2011 6h
31-May-2011 12h
31-May-2011 18h
18-Jun-2011 0h
18-Jun-2011 12h
19-Jun-2011 0h
20-Jun-2011 18h
21-Jun-2011 0h
21-Jun-2011 6h
21-Jun-2011 12h
22-Jun-2011 0h
22-Jun-2011 12h
23-Jun-2011 0h
U1
U2
OF (−33,13)
OF
R
TH
SS
SSr
GW
SN

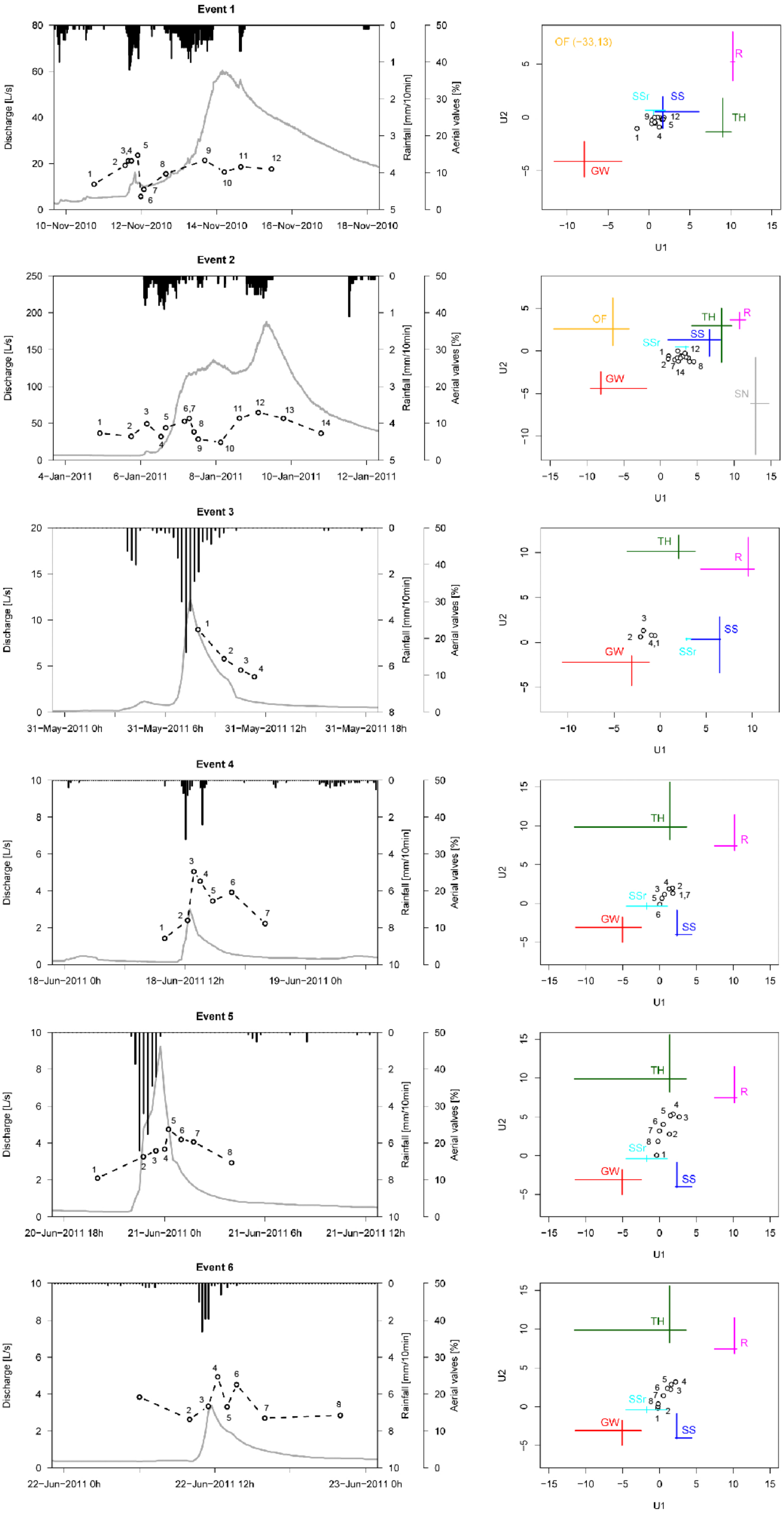
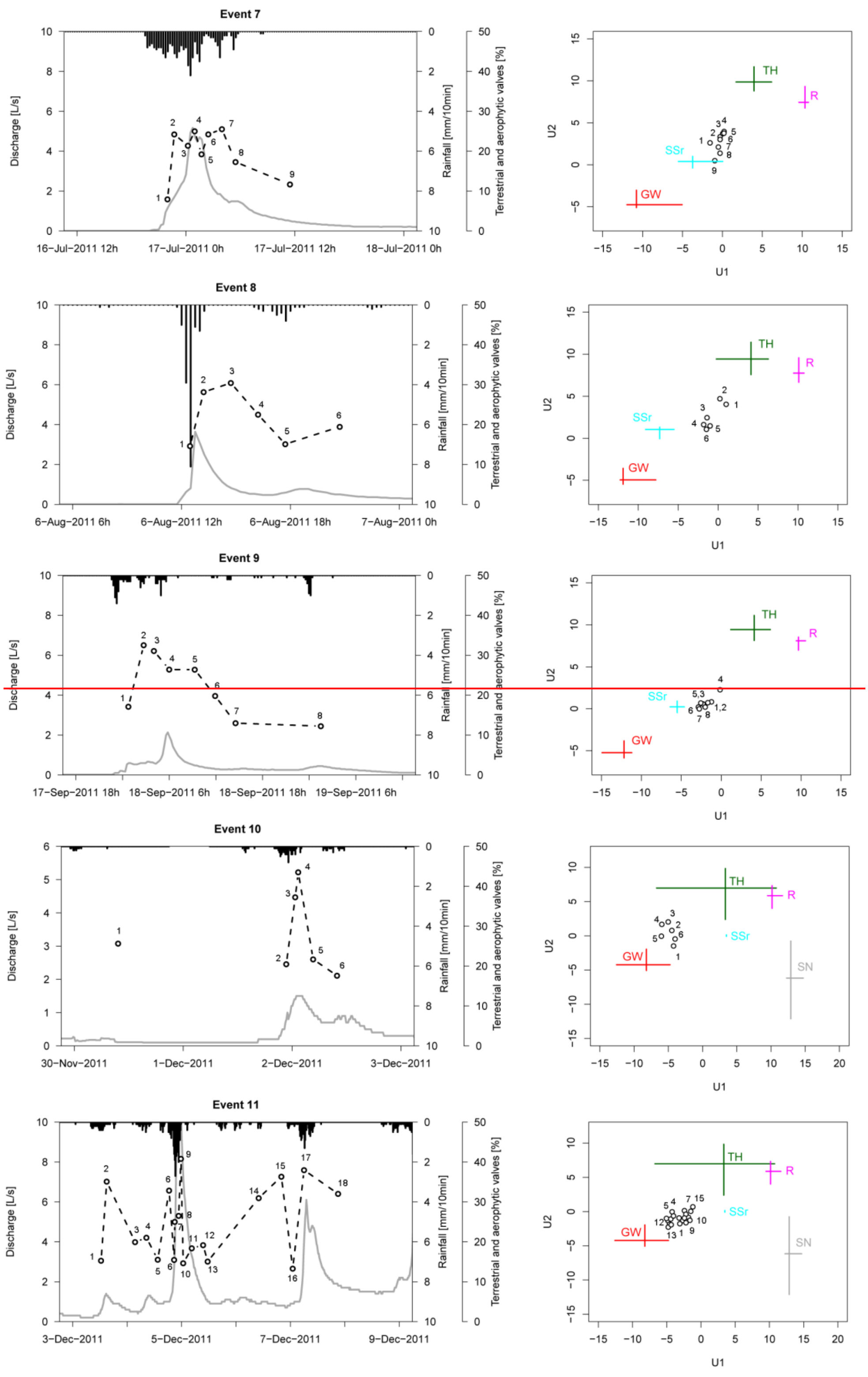
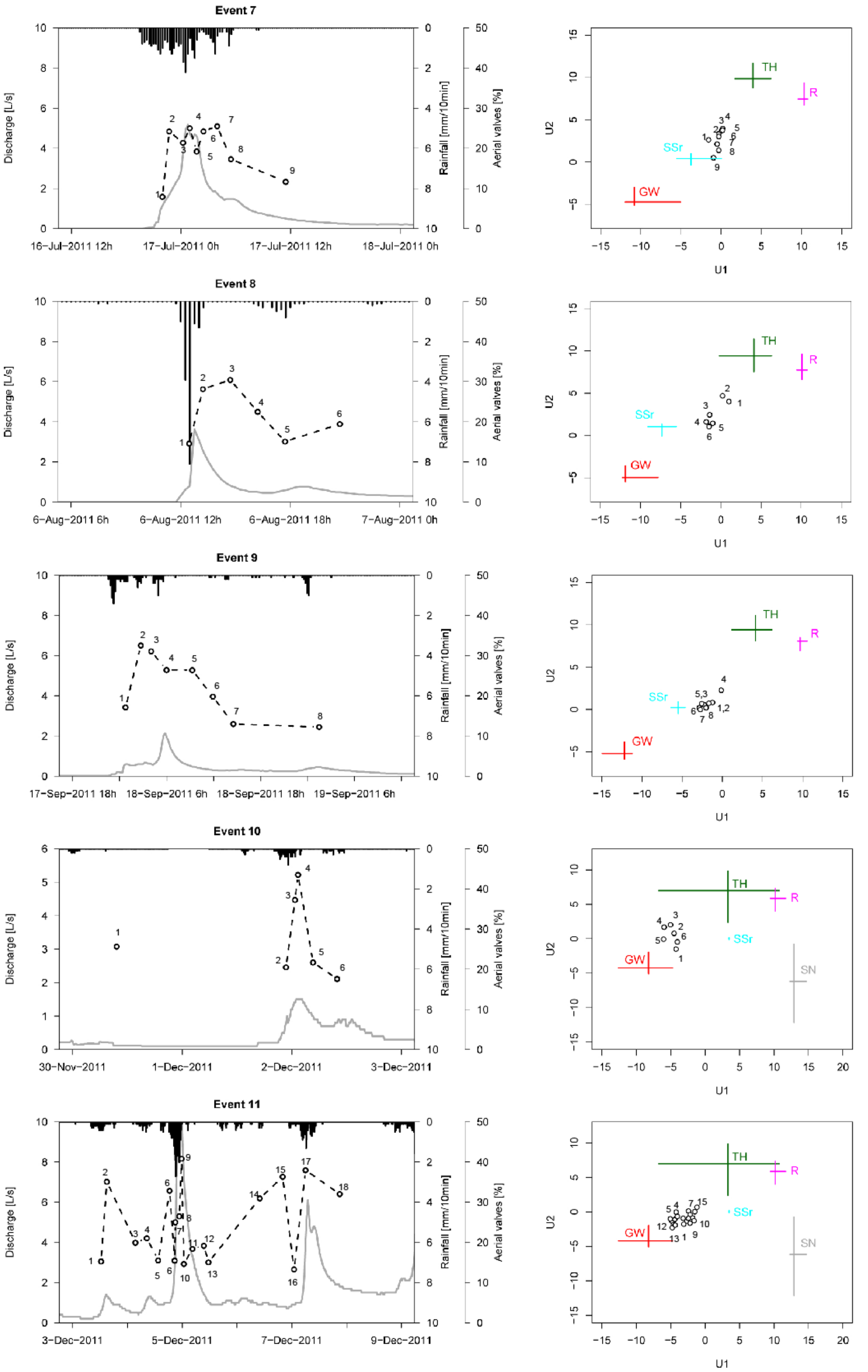

Event 1
Discharge [L/s]
Rainfall [mm/10min]
Aerial valves [%]
10−Nov−2010
12−Nov−2010
14−Nov−2010
16−Nov−2010
18−Nov−2010
OF (−33,13)
R
SSr
SS
TH
GW
U1
U2
Event 2
4−Jan−2011
6−Jan−2011
8−Jan−2011
10−Jan−2011
12−Jan−2011
OF
SN
Event 3
31−May−2011 0h
31−May−2011 6h
31−May−2011 12h
31−May−2011 18h
Event 4
18−Jun−2011 0h
18−Jun−2011 12h
19−Jun−2011 0h
Event 5
20−Jun−2011 18h
21−Jun−2011 0h
21−Jun−2011 6h
21−Jun−2011 12h
Event 6
22−Jun−2011 0h
22−Jun−2011 12h
23−Jun−2011 0h

Figure 8. Hydrograph, hyetograph and percentage of aerial valves in the stream water for the events 1-6 in the Weierbach catchment (left), and U1-U2 mixing diagrams for each event. End-members are rainfall (R), throughfall (TH), snow (SN), soil water (SS), soil water from the riparian zone (SSr) and groundwater (GW). Bars represent end-member values interquartile ranges of samples collected during the month when the event occurred, as well as the previous and following month.


Event 7
Event 8
Event 9
Event 10
Event 11
Discharge [L/s]
Rainfall [mm/10min]
Terrestrial and aerophytic valves [%]
16−Jul−2011 12h
17−Jul−2011 0h
17−Jul−2011 12h
18−Jul−2011 0h
6−Aug−2011 6h
6−Aug−2011 12h
6−Aug−2011 18h
7−Aug−2011 0h
17−Sep−2011 18h
18−Sep−2011 6h
18−Sep−2011 18h
19−Sep−2011 6h
30−Nov−2011
1−Dec−2011
2−Dec−2011
3−Dec−2011
5−Dec−2011
7−Dec−2011
9−Dec−2011
U1
U2
TH
R
SSr
GW
SN



~~8~~9. Hydrograph, hyetograph and percentage of ~~terrestrial and aerophytic~~aerial valves in the stream water for the events 7-11 in the Weierbach catchment (left), and U1-U2 mixing diagrams for each event. End-members are rainfall (R), throughfall (TH), snow (SN), soil water (SS), soil water from the riparian zone (SSr) and groundwater (GW). Bars represent end-member values interquartile ranges of samples collected during the month when the event occurred, as well as the previous and following month.

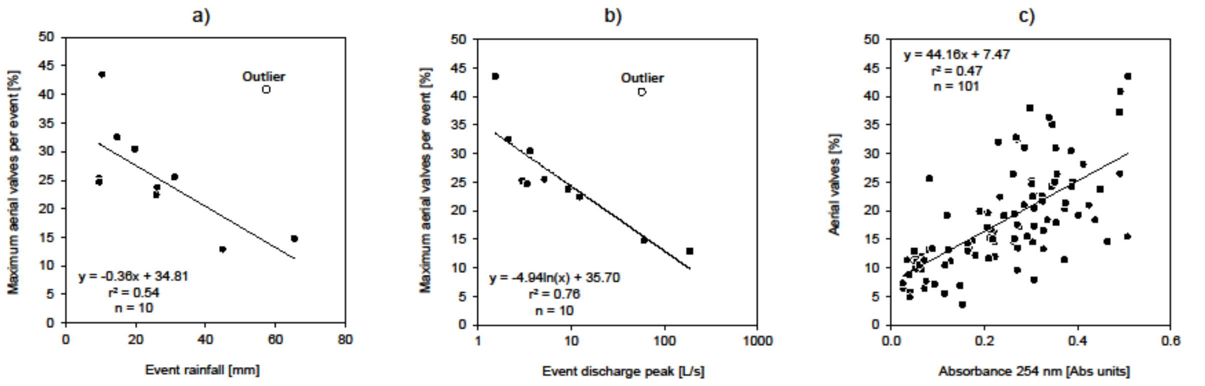


Figure ~~9~~10. Correlations between (a) maximum percentage of ~~terrestrial and aerophytic~~aerial valves in the stream water per event and event rainfall, (b) maximum percentage of ~~terrestrial and aerophytic~~aerial valves in the stream water per event and maximum event discharge, and (c) percentage of ~~terrestrial and aerophytic~~aerial valves in the stream water and UV-absorbance at 254 nm.