

**Answer to Anonymous Referee #2 (author's answers are written in bold)**

The paper was significantly improved compared to the original submission, and valuable changes have been made following the reviewers' indications. Not very clear points have been clarified, further information have been added, a new Figure has been included and some confusing part have been either specified or removed. Moreover, all responses provided by the authors to the reviewers' comments were clear and satisfactory. Overall, I believe that, after fixing some minor issues reported below, the paper can be published on HESS.

**We acknowledge anonymous referee #2 for his/her comments on the paper. In the following lines we address the comments outlined in his/her review.**

Minor comments and technical corrections (the first number refers to the page, the second one to the line)

1,17. '...quantifies' should be 'quantify' ('data' is plural, and has been correctly used as plural later in the manuscript).

**It has been corrected.**

1,21. Delete '(i.e., HRS units)', in my opinion its not needed here.

**We have not deleted it. We believe it clarifies that we link the HRS units to different specific moisture domains of the catchment.**

2,6. Change 'soil riparian zone water' into 'soil water in the riparian zone'.

**It has been changed to 'soil water from the riparian zone'.**

3,30. Change 'climate regime, annual precipitation' into 'climate regime. Annual precipitation'.

**It has been changed.**

4,25. Insert 'electrical' between 'Stream' and 'conductivity'.

**It has been inserted.**

4, 30. 'Data gaps were filled...' Were some data missing due to instrumental failure, or any other specific reasons? They should be shortly mentioned here.

**Yes, some data were missing due to instrument failure: 'Data gaps due to instrument failure were filled with rainfall data from a nearby weather station (49°47'39.2"N 5°49'13.2"E).'**

5,3. Insert 'in' between 'located' and 'a plateau'.

5,3. 'GW2 near one of the springs'. Which spring? And in which catchment zone (riparian, hillslope, transition etc)? Please, specify.

**The sentence has been rewritten: "GW1 was located on in a plateau, and GW2 near one of the springs, and , GW3 and GW4 on in the transition zone between riparian and hillslope settings (Fig.**

1)”. **We have removed the information concerning the location of GW2 near a spring as this can better be seen in Fig. 1.**

5,7. Add ‘reflectometry’ after ‘time-domain’.

**We have added it.**

5,20. Remove ‘positions’.

**We have removed it.**

5,28. I suggest to change ‘direct sampling’ into ‘the influence’.

**This has been changed.**

6,12. Change ‘LWIA’ into ‘Liquid-Water Isotope Analyser

**Instead of changing ‘LWIA’ for ‘Liquid-Water Isotope Analyser’, we have added a couple of lines earlier (line 8) a reference to the acronym used, i.e. ‘Liquid-Water Isotope Analyser (LWIA)’.**

6,11-13. Penna et al. (2010) tested exactly the same version and model of Liquid-Water Isotope Analyser used in this study, and found precisions comparable to those reported here, so this study could be cited. Moreover, the authors could mention if some simple procedures to avoid memory effects (e.g., avoid including in the same run samples whose isotopic composition was expected to be highly different, see Penna et al. (2012)) have been carried out.

Penna D., Stenni B., Šanda M., Wrede S., Bogaard T.A., Gobbi A., Borga M., Fisher B.M.C., Bonazza M., 2010. On the reproducibility and repeatability of laser absorption spectroscopy measurements for  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  isotopic analysis. *Hydrology and Earth System Sciences*, 14, 1551–1566. doi:10.5194/hess-14-1551-2010.

Penna D., Stenni B., Šanda M., Wrede S., Bogaard T.A., Michelini M., Fisher B.M.C., Gobbi A., Mantese N., Zuecco G., Borga M., Bonazza M., Sobotková M., Čejková B., Wassenaar L.I., 2012. Technical Note: Evaluation of between-sample memory effects in the analysis of  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  water samples measured by laser spectrometers. *Hydrology and Earth System Sciences*, 16, 3925–3933, 2012. doi:10.5194/hess-16-3925-2012.

**The instrument that we used was included in the reproducibility and repeatability study by Penna et al. (2010). The reference has been added. Indeed, to avoid memory effect we have tried to analyse samples with similar signatures together. However, we do not think that this information is strictly necessary in the manuscript.**

10,26. Fig. 4b is cited before Fig.4a (at 10,28). So, either swap the two panels in Fig. 4 (so, wet conditions on the left panel and dry conditions on the right panel) or change the sentences at 10,25-28 so that Fig. 4a appears first.

**We have swapped the panels in Fig. 4. All references to Fig. 4a and 4b in the manuscript have been corrected.**

12,28-29. I suggest to change the sentence into 'P values were high (0.21 and 0.73 for the riparian zone and the hillslope samples, respectively), thus the null hypothesis was accepted.

**The sentence has been rewritten.**

Table 3. There was not a large discussion in the text about seasonal differences in sample values that justifies both the inclusion of standard error and standard deviation. So, I suggest to skip SE and keep only SD.

**We agree. We have removed standard error values from the table.**

Fig. 4. Use bars for precipitation instead of lines. Moreover, see my comment for 10,26 above.

**Fig. 4 has been redone using bars.**

Fig. 6, 36,3. Delete 'solute', it's redundant (you already mention 'stream water chemistry').

**We agree. We have removed it.**

Fig. 7. Expand the y-axis scale of the b) panel so that the bars are not cut or touch the plot edge.

**The idea of panel (b) is to zoom into the mixing diagram, if we extend the panel so that the bars are not cut or touch the plot edge then it is difficult to identify the samples. We have not changed the figure, but we have added a sentence in the caption explaining this better: 'Because panel (b) is a zoom into the U1-U2 mixing diagram, the interquartile ranges of some end-members are not fully represented.'**

1 Hydrological connectivity inferred from diatom transport  
2 through the riparian-stream system

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10

11 **Abstract**

12 Diatoms (*Bacillariophyta*) are one of the most common and diverse algal groups (ca. 200 000  
13 species,  $\approx 10\text{-}200\ \mu\text{m}$ , unicellular, eukaryotic). Here we investigate the potential of aerial  
14 diatoms (i.e. diatoms nearly exclusively occurring outside water bodies, on wet, moist or  
15 temporarily dry places) to infer surface hydrological connectivity between hillslope-riparian-  
16 stream (HRS) landscape units during storm runoff events. We present data from the  
17 Weierbach catchment ( $0.45\ \text{km}^2$ , NW Luxembourg) that quantifies the relative abundance of  
18 aerial diatom species on hillslopes and in riparian zones (i.e. surface soils, litter, bryophytes  
19 and vegetation) and within streams (i.e. stream water, epilithon and epipelon). We tested the  
20 hypothesis that different diatom species assemblages inhabit specific moisture domains of the  
21 catchment (i.e. HRS units) and, consequently, the presence of certain species assemblages in  
22 the stream during runoff events offers the potential for recording if there was or not  
23 hydrological connectivity between these domains. We found that a higher percentage of aerial  
24 diatom species was present in samples collected from the riparian and hillslope zones than  
25 inside the stream. However, diatoms were absent on hillslopes covered by dry litter and the  
26 quantities of diatoms (in absolute numbers) were small in the rest of hillslope samples. This  
27 limits their use to infer hillslope-riparian zone connectivity. Our results also showed that  
28 aerial diatom abundance in the stream increased systematically during all sampled events  
29 ( $n=11$ , 2011-2012) in response to incident precipitation and increasing discharge. This

1 transport of aerial diatoms during events suggested a rapid connectivity between the soil  
2 surface and the stream. Diatom transport data were compared to two-component hydrograph  
3 separation, and end-member mixing analysis (EMMA) using stream water chemistry and  
4 stable isotope data. Hillslope overland flow was insignificant during most sampled events.  
5 This research suggests that diatoms were likely sourced exclusively from the riparian zone,  
6 since it was not only the largest aerial diatom reservoir, but also ~~soil-riparian-zone-water~~  
7 soil  
water from the riparian zone was a major streamflow source during rainfall events under both  
8 wet and dry antecedent conditions. In comparison to other tracer methods, diatoms require  
9 taxonomy knowledge and a rather large processing time. However, they can provide  
10 unequivocal evidence of hydrological connectivity and potentially be used at larger catchment  
11 scales.

12

## 13 **1 Introduction**

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

27 Despite their usefulness, chemical and isotope tracer-based hydrograph separations do not  
28 provide unequivocal evidence of hillslope-riparian-stream (HRS) connectivity. This has been  
29 identified as perhaps the key feature for improving our understanding of water origin and the  
30 processes that sustain stream flow (Jencso et al., 2010). Consequently, new techniques are  
31 desperately needed to gain a process-based understanding of hydrological connectivity  
32 (Bracken et al., 2013).

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

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

- 20 1. Can aerial diatom transport reveal hydrological connectivity within the HRS system?
- 21 2. How do diatom results compare to traditional tracer-based and hydrometric methods to  
22 infer hydrological connectivity?
- 23 3. Can aerial diatom be established as a new hydrological tracer?

24

## 25 **2 Study area**

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

30 Weierbach has a temperate, semi-oceanic climate regime. Annual precipitation in the Attert  
31 River basin ranges from 950 mm on the Western border to 750 mm on the Eastern border

1 (average from 1971 to 2000; Pfister et al., 2005). Precipitation is relatively uniform  
2 throughout the year, although strong seasonality in low flow exists due to higher  
3 evapotranspiration from July to September. The annual runoff ratio is high (~55% based on  
4 2005 to 2011 streamflow data) and flow sometimes ceases during summer months.

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

11 Vegetation in the study catchment is mainly mixed Oak-Beech hardwood deciduous forest  
12 (76% of the land cover, *Fagus sylvatica* L. and *Quercus petraea* (Matt.) Liebl.) where the soil  
13 surface is covered with fallen leaves. Conifers cover a smaller part (24% land cover) of the  
14 catchment (*Pseudotsuga menziessii* (Mirb.) Franco and *Picea abies* (L.) H. Karst), and the  
15 soil surface beneath conifers is covered mainly by bryophytes. A well-defined riparian zone  
16 extends up to 3 meters away from the stream channel. Vegetation in the riparian zone includes  
17 *Dryopteris carthusiana* (Vill.) H.P. Fuchs, *Impatiens noli-tangere* L., *Chrysosplenium*  
18 *oppositifolium* L. and *Oxalis acetosella* L.

19

## 20 **3 Methodology**

### 21 **3.1 Hydrometric Monitoring**

22 Table 1 shows a summary of collection methods, sampling resolution and locations in the  
23 Weierbach catchment. Stream water depth at the catchment outlet was measured using a  
24 differential pressure transducer at a 15-minute interval (ISCO 4120 Flow Logger) (Fig. 1).  
25 | Stream electrical conductivity at the outlet was also measured at 15-minute intervals using a  
26 | conductivity meter (WTW). Rainfall was measured with a tipping bucket rain gauge (52203  
27 | model, manufactured by Young, Campbell Scientific Ltd.). One rain gauge was installed  
28 | within a small clearing of the study catchment (see Fig. 1), and another one installed in an  
29 | open area at the Roodt meteorological station, located ≈3.5 km distant from the Weierbach  
30 | (49°48'22.2''N 5°49'52.7''E). Data gaps due to instrument failure were filled with rainfall  
31 | data from a nearby weather station (49°47'39.2''N 5°49'13.2''E).

1 Four groundwater wells were instrumented with real-time TD-Divers data loggers  
2 (Schlumberger Water Services) and WTW conductivity meters – each recording at 15-minute  
3 intervals. GW1 was located ~~on~~in a plateau, ~~and~~ GW2 ~~near one of the springs, and~~ GW3 and  
4 GW4 ~~on~~in the transition zone between riparian and hillslope settings (Fig. 1). Wells were  
5 around 2 m deep and were screened at least for the lowest 50 cm up to a meter.

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

### 11 **3.2 Water sampling and laboratory methods**

12 Fortnightly, cumulative rainfall (R) and throughfall samples under deciduous trees (TH1) and  
13 coniferous trees (TH2) were collected using conical, volumetric rain gauges. A ten bottle  
14 sequential rainfall sampler was installed at the rain gauge located within the Weierbach  
15 (modified from Kennedy et al. (1979)). Three automatic water samplers (ISCO 3700 FS and  
16 6712 FS) were installed immediately upstream of the weir to collect stream water samples  
17 (AS) frequently (0.5 to 4 h) during storm events. Sampling was triggered by flow conditions.  
18 Events were considered separately if they were separated by a period of at least 24h without  
19 rainfall. Stream water at the catchment outlet (SW) and wells (GW1 to GW4) were sampled  
20 fortnightly, as well as prior to, during, and following precipitation events. Soil water was  
21 sampled fortnightly using Teflon suction lysimeters, installed at three locations: deciduous  
22 hillslope (SS1), coniferous hillslope (SS2), and riparian zone (SSr). Three soil depths for each  
23 location: 10 cm for the organic layer (Ah horizon), 20 and 60 cm for the mineral layers (B and  
24 C horizons). Overland flow (OF) that occurred on lower hillslope ~~positions~~ was sampled  
25 using 1 and 2 m long gutters sealed to the soil surface, which diverted surface runoff to 1 or 2-  
26 L plastic, blackened (to prevent light penetration which causes diatom growth) water bottles.  
27 Note that what we refer as OF might in fact originate within the forest litter layer (Buttle and  
28 Turcotte, 1999; Sidle et al., 2007). All gutters were covered to avoid ~~direct sampling the~~  
29 influence of precipitation. Gutters were regularly cleaned with Milli-Q water to avoid diatoms  
30 growth on their surfaces.

31 All water samples were analysed for electrical conductivity (EC), anion and cation  
32 concentrations ( $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ), silica ( $\text{SiO}_2$ ) and UV-absorbance at



1 254 nm (Abs 254 nm). UV absorbance at 254 nm can be considered as a proxy of DOC  
2 (Edzwald et al., 1985). Samples were analysed at the Luxembourg Institute of Science and  
3 Technology chemistry laboratory after filtration through WHATMAN GF/C glass fibre filters  
4 (<0.45 µm). Prior to analysis, samples were stored at 4° C. Dissolved anions and cations were  
5 analysed by ion chromatography (Dionex HPLC), SiO<sub>2</sub> by spectrophotometry (ammonium  
6 molybdate method), and UV-absorbance was measured by a Beckmann Coulter  
7 spectrophotometer. Isotopic analyses of <sup>18</sup>O/<sup>16</sup>O and <sup>2</sup>H/H were conducted using a LGR  
8 Liquid-Water Isotope Analyser ([LWIA](#)) at the Luxembourg Institute of Science and  
9 Technology (model DLT-100, version 908-0008) ([Penna et al., 2010](#)). The analyser was  
10 connected to a LC PAL liquid auto-injector for the automatic and simultaneous measurement  
11 of <sup>2</sup>H/H and <sup>18</sup>O/<sup>16</sup>O ratios in water samples. According to the manufacturer's specifications  
12 (Los Gatos Research Inc., 2008), the DLT-100 908-0008 LWIA provides isotopic  
13 measurements with a precision below 0.6‰ for <sup>2</sup>H/H and 0.2‰ for <sup>18</sup>O/<sup>16</sup>O. Data were  
14 transformed into δ notation according to Vienna Standard Mean Ocean Water (VSMOW)  
15 standards (δ<sup>2</sup>H and δ<sup>18</sup>O in ‰).

### 16 **3.3 Diatom sampling, sample preparation and analysis**

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

20 A small set of stream water and overland flow samples was set aside for geochemical and  
21 isotopic analysis (≈70 mL), the rest of the sample was centrifuged (1250 rpm, 8 minutes) to  
22 concentrate the diatoms.

23 In addition to high-frequency sampling during rainfall events, seasonal sampling campaigns  
24 were carried out throughout the Weierbach catchment to assess the geographic and intra-  
25 annual variability of diatom communities. The following substrates were sampled in the  
26 catchment: (i) litter, bryophytes from the two hillslope classifications (hardwood and  
27 coniferous) and surface soil samples; and (ii) litter, bryophytes, and vegetation in the riparian  
28 zone. Each sample was comprised of five sub-samples collected on a 5-m transect parallel to  
29 the stream (a subsample collected every meter). Only material from the top surface, where  
30 there was greatest incident sunlight, was collected into 1-L plastic bottles. Sample bottles  
31 containing different substrata were filled with carbonated water (1-L), carefully shaken and  
32 left to settle overnight at 0 °C. The next day, the diatom-filled, carbonated water was

1 recovered by passing it through a 1-mm screen. Sample substrate was then rinsed with  
2 additional carbonated water to remove as many diatoms from the sampled substrate as  
3 possible. This procedure was repeated several times until a 2-L sample volume was achieved.  
4 The recovered sample, now with substrate removed, was stored at 0 °C for a minimum of 8  
5 hours to allow diatoms to settle, and the supernatant removed by aspiration.

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

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

19 Diatom valves were identified and counted ( $\approx 400$  valves) on microscopic slides with a light  
20 microscope (Leica DMRX<sup>®</sup>). For the autecological assignment of the diatom species we  
21 relied on: (1) the Denys (1991) diatom ecological classification system refined by Van Dam et  
22 al. (1994), which is, as far as we know, the only formal classification of the occurrence of  
23 freshwater diatoms in relation to moisture; and (2) the associated hydrological units assigned  
24 by Pfister et al. (2009) to the five diatom occurrence classes defined by Van Dam et al.  
25 (1994). We express these results as relative abundance (percentage) of aerial valves, i.e.  
26 categories 4 and 5 of Van Dam's et al. (1994) classification.

### 27 **3.4 Hydrograph separation**

28 Two-component hydrograph separation was performed using  $\delta^{18}\text{O}$  isotopic composition and  
29 the mass balance approach (Pinder and Jones, 1969; Sklash and Farvolden, 1982; Pearce et  
30 al., 1986; Sklash et al., 1986). The incremental mean method proposed by McDonnell et al.  
31 (1990) was used to adjust  $\delta^{18}\text{O}$  rainfall isotopic composition, so that the bulk isotopic

1 composition of rainfall from the beginning of the event to the time of stream sampling was  
2 calculated (i.e. rain that had not yet fallen was excluded from the estimate).

3 Spatial end-member contributions to stream water were explored using EMMA  
4 (Christophersen and Hooper, 1992), which assumes that (i) the stream water is a mixture of  
5 end-member solutions with a fixed composition, (ii) the mixing model is linear and relies on  
6 hydrodynamic mixing, (iii) the solutes used as tracers are conservative, and (iv) the end-  
7 member solutions are distinguishable from one another. Catchment end-members included  
8 shallow groundwater (GW1-4), soil water (SS1<sub>20</sub>, SS1<sub>60</sub>, SS2<sub>60</sub>), soil water from the riparian  
9 zone (SSr), rainfall (R), throughfall (TH1-2), snow (SN) and overland flow (OF). We applied  
10 the diagnostic tools of Hooper (2003), which have been recently applied in the literature  
11 (James and Roulet, 2006; Ali et al., 2010; Barthold et al., 2011; Neill et al., 2011; Inamdar et  
12 al., 2013). Our approach followed three main steps:

13 i. We identified tracers that exhibit conservative linear mixing assuming that stream  
14 water chemistry is controlled by physical mixing of different sources of water and not  
15 by equilibrium mixing (Christophersen and Hooper, 1992; Hooper, 2003; Liu et al.,  
16 2008). The latest would imply equilibrium reactions among solutes of different  
17 charge, which may be approximated by high order polynomials. Hooper (2003)  
18 suggested that conservative and linear mixing of tracers can be evaluated using  
19 bivariate scatter plots. In this study, stream water concentrations and isotopic  
20 compositions (of all samples collected during storm events and low flows at the  
21 catchment outlet) were considered conservative when they exhibited at least one linear  
22 trend with one other tracer (i.e.  $r^2 > 0.5$ ,  $p\text{-value} < 0.01$ ) (James and Roulet, 2006; Ali et  
23 al., 2010; Barthold et al., 2011).

24 ii. We performed a principal component analysis (PCA) on the stream water data. The  
25 PCA was applied on the correlation matrix of the standardized values of tracers  
26 selected in step (i) (i.e. by subtracting the mean concentration or isotopic composition  
27 of each solute and dividing by its standard deviation) (Christophersen and Hooper,  
28 1992). For each water tracer, residuals were defined by subtracting the original value  
29 from its orthogonal projection. A 'good' mixing subspace was indicated by a random  
30 pattern of residuals plotted against the concentration or isotopic composition of the  
31 original values. On the contrary, structure or curvature in the subspace indicates  
32 violation against one of the assumptions of the EMMA approach (i.e. solutes do not

1 mix conservatively) (Hooper, 2003). Eigenvectors were retained until there was no  
2 structure to the residuals. Standardized data were multiplied by the eigenvectors and  
3 projected into the new U space.

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

## 17 **4 Results**

### 18 **4.1 Hydrometric response**

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

29 During wet antecedent conditions, streamflow response of the basin was double peaked, with  
30 a first peak timing coincident with the rainfall input and the second, delayed peak coming a

1 few hours later. On the contrary, when the catchment was dry the hydrological response was  
2 shorter and only a single sharp peak occurred.

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

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

## 20 **4.2 Hydrograph separation**

21 Two-component hydrograph separation results using  $\delta^{18}\text{O}$  isotopic composition (i.e. pre-event  
22 water vs. event water) showed that, in winter, when the catchment was wet and flow response  
23 was double-peaked, the first peak had a larger contribution of event water than the delayed  
24 peak. For instance, the first peak of the November 2010 event showed a maximum of 50%  
25 event water contribution. This contrasted with the delayed peak that exhibited only a  
26 maximum of 16% event water contribution (Fig. 4ba). When the catchment was dry, the  
27 response consisted of one sharp peak composed largely of event water. A maximum event-  
28 water contribution of 60% was estimated for a storm event occurred in June 2011 (Fig. 4ab).

29 Twelve different tracers measured in the different water compartments of the catchment were  
30 used to assess end-member contributions to stream water (Fig. 5). Ten out of the twelve  
31 tracers presented linear trends in the solute-solute plots of stream water samples with at least

1 one other tracer (EC, Cl<sup>-</sup>, Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, SiO<sub>2</sub>, Abs, δ<sup>2</sup>H and δ<sup>18</sup>O; r<sup>2</sup>>0.5, p-value<0.01,  
2 Fig. 6). These tracers were retained for the PCA analysis. Weaker linear trends were found  
3 between NO<sub>3</sub><sup>-</sup> and the other tracers (r<sup>2</sup><0.13) and between SO<sub>4</sub><sup>2-</sup> and the other tracers  
4 (r<sup>2</sup><0.43). Neither tracers reached the pre-defined threshold of collinearity (r<sup>2</sup>>0.5), and were  
5 therefore not retained.

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

29 In order to better understand water pathways during each event separately, we plotted stream  
30 water samples collected for each event and end-member tracer signatures in the previously  
31 determined two-dimensional mixing space (Fig. 8 and 9). We accounted for end-member  
32 temporal variability by plotting not only end-member samples collected the same month as

1 the event occurred, but also the preceding and the following months. Groundwater and  
2 rainfall signals remained relatively constant throughout the year, whereas throughfall, riparian  
3 and soil water presented higher temporal variability. Results showed that runoff mixing  
4 patterns changed between events. During autumn and winter when the catchment was wet  
5 (events 1-2, and 10-11), stream water signal composition was most similar to riparian, soil  
6 water and groundwater. Only samples collected during the rain-on-snow event (event 2)  
7 might have a small contribution of not only overland flow but also snow. Mixing patterns  
8 changed during spring and summer when the catchment was drier (i.e. events 3 to 9). As  
9 previously seen in Fig. 7b, groundwater seems to have a much lower contribution to stream  
10 water, since stream water samples now plotted in an intermediate position between  
11 throughfall and soil water from the riparian zone (with the exception of event 3, which still  
12 has a significant groundwater contribution). Note that overland flow did not occur and the  
13 soils were dry during these spring and summer events.

#### 14 **4.3 Seasonal and geographic variability in aerial diatom communities in the** 15 **hillslope-riparian-stream system**

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

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

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

#### 17 **4.4 Aerial diatom transport during rainfall events**

18 A series of 11 rainfall events were sampled from November 2010 to December 2011 during  
19 both wet and dry catchment conditions (Table 4 and Fig. 2). Main aerial species found in  
20 stream water during storm events were as follows: *Chamaepinnularia evanida*, *C. obsoleta*  
21 (Hustedt) C.E. Wetzel & Ector, *C. parsura*, *Humidophila brekkaensis* (J.B. Petersen) Lowe et  
22 al., *H. perpusilla* (Grunow) Lowe et al., *Eolimna tantula* (Hustedt) Lange-Bertalot, *Eunotia*  
23 *minor*, *Pinnularia obscura* Krasske, *P. perirrorata*, *Stauroneis parathermicola*, *S. thermicola*.

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

29 No significant relationship was found between the percentage of aerial diatoms and  
30 instantaneous discharge ( $r^2=0.13$ ,  $n=101$ ; discharge on the x axis), most probably due to  
31 different diatom abundances on the rising limb of the hydrograph than on the recession limb



1 (i.e. hysteretic effects). Two events showed clockwise hysteretic loops (events 1 and 2); five  
2 events showed counter-clockwise hysteretic loops (events 4, 5, 6, 8, and 10) and three showed  
3 figure-eight shaped hysteretic loops (events 7, 9 and 11). Although a clear pattern was not  
4 observed, results suggest that clockwise hysteretic loops predominated during wet conditions  
5 (the greater percentages of aerial diatoms in streamflow were immediately before peakflow),  
6 and counter-clockwise hysteretic loops during dry conditions (the greater percentages were  
7 immediately after peakflow).

8 Aerial valves comprised less than 15% of the total diatoms in low flow samples for all events  
9 except 6, 9 and 10 (which had 19.2%, 17.1%, and 25.6 %, respectively). Due to technical  
10 problems, no low flow sample was collected for event 3. No relationship was observed  
11 between antecedent event rainfall and the percentage of aerial valves observed during low  
12 flow ( $n=10$ ,  $r^2=0.08$  and  $0.09$  for 10 and 20 days of antecedent rainfall, respectively).

13 At event scale, there were significant correlations between maximum percentage of aerial  
14 diatoms and event rainfall and maximum event discharge ( $r^2=0.54$ ,  $p < 0.05$ ,  $n=10$ , Fig. 10a;  
15  $r^2=0.76$ ,  $p < 0.05$ ,  $n=10$ , Fig. 10b, respectively; the multi-peak event sampled in December  
16 2011 was considered as an outlier). High percentages ( $>35\%$ ) of aerial diatom relative  
17 abundance were measured during dry catchment conditions, compared to when the catchment  
18 was wet, where maximum relative abundances were low ( $<15\%$ ). Alternatively, higher  
19 maximum percentages of aerial diatom proportions ( $>35\%$ ) were measured during dry  
20 catchment conditions, when events were shorter and more intense.

21 A significant correlation between percentage of aerial diatoms with UV absorbance at 254 nm  
22 was found ( $r^2=0.55$ ,  $p < 0.05$ ,  $n=76$ , Fig. 10c). During rainfall events in the Weierbach  
23 catchment, the relative abundance of aerial diatoms was associated with increased organic  
24 matter concentrations in the stream. A similar trend was observed with  $K^+$  ( $r^2=0.25$ ,  $p < 0.05$ ,  
25  $n=76$ ), which is also associated with organic matter content. The relative abundance of aerial  
26 diatoms was not correlated with any other tracers.

27

## 1 **5 Discussion**

### 2 **5.1 Can aerial diatoms transport reveal hydrological connectivity within the** 3 **hillslope-riparian-stream system?**

4 Our central hypothesis for this study was that aerial diatoms could indicate connectivity  
5 within the HRS system. In order to test this hypothesis, we sampled from potential upland  
6 catchment sources (i.e. hillslope and riparian zones), and within the streambed (i.e. epilithon,  
7 epipelon and stream water samples).

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

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

28 We examined the aerial diatoms transported in the stream water during runoff events. We  
29 observed an increase in the relative abundance of aerial diatoms with discharge for all  
30 sampled events regardless of antecedent wetness conditions. Hence, during storm events there  
31 was an increase in the relative proportion of diatoms in categories 4 and 5 of Van Dam's et al.  
32 (1994) classification. Similar results were reported by Pfister et al. (2009). These observations

1 imply hydrological connectivity between the riparian soil surface and the stream for all  
2 events. The use of aerial diatoms to infer hydrological connectivity in the Weierbach  
3 catchment thus remains limited to the riparian-stream system as no diatoms were found on the  
4 hillslopes covered by dry litter.

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

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

## 21 **5.2 How do diatom results compare to the other methods to infer hydrological** 22 **connectivity?**

23 Two-component hydrograph separation and EMMA provide valuable information on water  
24 sources and flowpaths. Using these methods we learned that in the Weierbach catchment,  
25 during spring and summer, the hydrological response was largely composed of event water  
26 (see an example of dry antecedent catchment conditions in Fig. [4a4b](#)). Similar conclusions  
27 were drawn by Wrede et al. (2014) using dissolved silica. Accordingly, EMMA results  
28 suggest canopy throughfall, rainfall and riparian soil water were the main water sources (Fig.  
29 8 and 9). As observed in other headwater catchments (e.g. Penna et al., 2011), discharge  
30 likely increased due to channel interception and riparian runoff leading to clear and singular  
31 hydrograph peaks (Fig. [4a4b](#)). During fall and winter, when the catchment was at its wettest  
32 state, double peaked hydrographs characterized the event hydrological response. Hydrograph

1 separation indicated that the first peak was mainly event water and the delayed, second peak  
2 was mostly pre-event water (Fig. 4b4a; Wrede et al., 2014). During these events, soil water,  
3 groundwater, and throughfall contributed substantially to total discharge (Fig. 8 and 9).  
4 Hillslope overland flow was insignificant during most sampled events. Only for event 2 – the  
5 largest storm on record –overland flow was a significant contributor to stream discharge,  
6 likely due to rapid snowmelt onto surface-saturated area (Fig. 8 and 9).

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

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

### 26 **5.3 Can aerial diatom be established as a new hydrological tracer?**

27 Storm hydrograph separation using stable isotope tracers has resulted in major advances in  
28 catchment hydrology. However, despite their usefulness, these methods do not provide  
29 unequivocal evidence of hydrological connectivity in the HRS system. In comparison,  
30 diatoms can provide evidence of riparian-stream connectivity. Further research is needed to  
31 better understand diatom transport processes (and associated water flowpaths) in headwater  
32 catchments. Future studies should focus on expanding our understanding of terrestrial diatom

1 taxonomy and ecology, which are scarce or lacking for a large number of taxa (Wetzel et al.,  
2 2013, 2014). Even though this new data source will have its own individual measurement  
3 uncertainty (McMillan et al., 2012), diatoms offer the possibility to tackle open questions in  
4 hydrology and eco-hydrology.

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

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

1

## 2 **6 Conclusions**

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

18

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28

## 1 **References**

- 2 Ali, G. A. and Roy, A. G.: Shopping for hydrologically representative connectivity metrics in  
3 a humid temperate forested catchment. *Water Resour. Res.*, 46, W12544,  
4 doi:10.1029/2010WR009442, 2010.
- 5 Ali, G. A., Roy, A. G., Turmel, M. C., and Courchesne, F.: Source-to-stream connectivity  
6 assessment through end-member mixing analysis. *J. Hydrol.*, 392, 119–135,  
7 doi:10.1016/j.jhydrol.2010.07.049, 2010.
- 8 Barthold, F. K., Tyralla, C., Schneider, K., Vaché, K. B., Frede, H. G., and Breuer, L.: How  
9 many tracers do we need for end member mixing analysis (EMMA)? A sensitivity analysis.  
10 *Water Resour. Res.*, 47, W08519, doi:10.1029/2011WR010604, 2011.
- 11 Beven, K.: TOPMODEL: a critique. *Hydrol. Process.*, 11, 1069–1085, 1997.
- 12 Bonell, M.: Selected challenges in runoff generation research in forests from the hillslope to  
13 headwater drainage basin scale. *J. Am. Water Resour. As.*, 34, 765–785, doi:10.1111/j.1752-  
14 1688.1998.tb01514.x, 1998.
- 15 Boyer, E. W., Hornberger, G. M., Bencala, K. E., and Mcknight, D. M.: Response  
16 characteristics of DOC flushing in an alpine catchment. *Hydrol. Process.*, 11, 1635–1647,  
17 doi:10.1002/(SICI)1099-1085(19971015)11:12<1635::AID-HYP494>3.0.CO;2-H, 1997.
- 18 Bracken, L. J. and Croke, J.: The concept of hydrological connectivity and its contribution to  
19 understanding runoff-dominated geomorphic systems. *Hydrol. Process.*, 21, 1749–1763,  
20 doi:10.1002/hyp.6313, 2007.
- 21 Bracken, L. J., Wainwright, J., Ali, G. A., Tetzlaff, D., Smith, M. W., Reaney, S. M., and  
22 Roy, A. G.: Concepts of hydrological connectivity: Research approaches, pathways and future  
23 agendas. *Earth-Sci. Rev.*, 119, 17–34, doi:10.1016/j.earscirev.2013.02.001, 2013.
- 24 Burns, D. A.: Stormflow-hydrograph separation based on isotopes: the thrill is gone – what's  
25 next? *Hydrol. Process.*, 16, 1515–1517, doi:10.1002/hyp.5008, 2002.
- 26 Buttle, J. M.: Fundamentals of small catchment hydrology, in: Kendall, C. and McDonnell, J.  
27 J. (Eds.): *Isotope tracers in catchment hydrology*, Elsevier, Amsterdam, 1–49, 1998.
- 28 Buttle, J. M. and Turcotte, D. S.: Runoff processes on a forested slope on the Canadian  
29 Shield. *Nord. Hydrol.*, 30, 1–20, doi:10.2166/nh.1999.001, 1999.

1 Chen, X., Bu, Z., Yang, X., and Wang, S.: Epiphytic diatoms and their relation to moisture  
2 and moss composition in two montane mires, Northeast China. *Fund. Appl. Limnol.*, 181,  
3 197–206, doi:10.1127/1863-9135/2012/0369, 2012.

4 Christophersen, N. and Hooper, R. P.: Multivariate analysis of stream water chemical data:  
5 The use of principal components analysis for the end-member mixing problem. *Water Resour.*  
6 *Res.*, 28, 99–107, doi:10.1029/91WR02518, 1992.

7 Coles, A. E., Wetzel, C. E., Martínez-Carreras, N., Ector, L., McDonnell, J. J., Frentress, J.,  
8 Klaus, J., Hoffmann, L., and Pfister, L.: Diatoms as a tracer of hydrological connectivity: are  
9 they supply limited? *Ecohydrology* (under review).

10 Denys, L.: A check-list of the diatoms in the Holocene deposits of the Western Belgian  
11 coastal plain with a survey of their apparent ecological requirements. I. Introduction,  
12 ecological code and complete list. Service Géologique de Belgique-Belgische Geologische  
13 Dienst, Professional Paper 1991/2-246, 41 pp., 1991.

14 Dixit, S. S., Dixit, A. S., and Smol, J. P.: Diatom and chrysophyte transfer functions and  
15 inferences of post-industrial acidification and recent recovery trends in Killarney lakes  
16 (Ontario, Canada). *J. Paleolimnol.*, 27, 79–96, doi:10.1023/A:1013571821476, 2002.

17 Ector, L. and Rimet, F.: Using bioindicators to assess rivers in Europe: an overview, in: Lek,  
18 S., Scardi, M., Verdonshot, P. F. M., Descy, J. P., and Park, Y. S. (Eds.): *Modelling*  
19 *Community Structure in Freshwater Ecosystems*, Springer-Verlag, Berlin, Heidelberg, 7–19,  
20 2005.

21 Edzwald, J.K., Becker, W.C., Wattier, K.L.: Surrogate parameters for monitoring organic  
22 matter and THM precursors. *J. Am. Water Works Ass.*, 77, 122-727, 1985.

23 European Committee for Standardization: Water quality - guidance standard for the routine  
24 sampling and pretreatment of benthic diatoms from rivers. [EN 13946:2003]. European  
25 Committee for Standardization, Brussels, 2003.

26 European Committee for Standardization: Water quality – guidance standard for the  
27 identification, enumeration and interpretation of benthic diatom samples from running waters.  
28 [EN 14407:2004]. European Committee for Standardization, Brussels, 2004.

29 Hooper, R. P.: Applying the scientific method to small catchment studies: a review of the  
30 Panola Mountain experience. *Hydrol. Process.*, 15, 2039–2050, doi:10.1002/hyp.255, 2001.



1 Hooper, R. P.: Diagnostic tools for mixing models of stream water chemistry. *Water Resour.*  
2 *Res.*, 39, 1055, doi:10.1029/2002WR001528, 2003.

3 Inamdar, S., Dhillon, G., Singh, S., Dutta, S., Levia, D., Scott, D., Mitchell, M., Van Stan, J.,  
4 and McHale, P.: Temporal variation in end-member chemistry and its influence on runoff  
5 mixing patterns in a forested, Piedmont catchment. *Water Resour. Res.*, 49, 1828–1844,  
6 doi:10.1002/wrcr.20158, 2013.

7 James, A. L. and Roulet, N. T.: Investigating the applicability of end-member mixing analysis  
8 (EMMA) across scale: A study of eight small, nested catchments in a temperate forested  
9 watershed. *Water Resour. Res.*, 42, W08434, doi:10.1029/2005WR004419, 2006.

10 Jencso, K. G., McGlynn, B. L., Gooseff, M. N., Bencala, K. E., and Wondzell, S. M.:  
11 Hillslope hydrologic connectivity controls riparian groundwater turnover: Implications of  
12 catchment structure for riparian buffering and stream water sources. *Water Resour. Res.*, 46,  
13 W10524, doi:10.1029/2009WR008818, 2010.

14 Jencso, K. G., McGlynn, B. L., Gooseff, M. N., Wondzell, S. M., Bencala, K. E., and  
15 Marshall, L. A.: Hydrologic connectivity between landscapes and streams: Transferring  
16 reach- and plot-scale understanding to the catchment scale. *Water Resour. Res.*, 45, W04428,  
17 doi:10.1029/2008WR007225, 2009.

18 Juilleret, J., Iffly, J. F., Pfister, L., and Hissler, C.: Remarkable Pleistocene periglacial slope  
19 deposits in Luxembourg (Oesling): pedological implication and geosite potential. *B. Soc.*  
20 *Naturalistes Luxemb.*, 112, 125–130, 2011.

21 Kennedy, V. C., Zellweger, G. W., and Avanzino, R. J.: Variation of rain chemistry during  
22 storms at two sites in northern California. *Water Resour. Res.*, 15, 687–702,  
23 doi:10.1029/WR015i003p00687, 1979.

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

26 Lischeid, G.: Combining hydrometric and hydrochemical data sets for investigating runoff  
27 generation processes: Tautologies, inconsistencies, and possible explanations. *Geogr.*  
28 *Compass*, 2, 255–280, doi:10.1111/j.1749-8198.2007.00082.x, 2008.

1 Liu, F., Bales, R. C., Conklin, M. H., and Conrad, M. E.: Streamflow generation from  
2 snowmelt in semi-arid, seasonally snow-covered, forested catchments, Valles Caldera, New  
3 Mexico. *Water Resour. Res.*, 44, W12443, doi:10.1029/2007WR006728, 2008.

4 Mann, D. G.: Diatoms: organism and image, in: du Buf, H. and Bayer, N. M. (Eds.):  
5 Automatic Diatom Identification, World Scientific Publishing, Singapore, 9–40, 2002.

6 McDonnell, J. J.: Are all runoff processes the same? *Hydrol. Process.*, 27, 4103–4111,  
7 doi:10.1002/hyp.10076, 2013.

8 McDonnell, J. J., Bonell, M., Stewart, M. K., and Pearce, A. J.: Deuterium variations in storm  
9 rainfall: Implications for stream hydrograph separation. *Water Resour. Res.*, 26, 455–458,  
10 doi:10.1029/WR026i003p00455, 1990.

11 McGuire, K. J. and McDonnell, J. J.: Hydrological connectivity of hillslopes and streams:  
12 Characteristic time scales and nonlinearities. *Water Resour. Res.*, 46, W10543,  
13 doi:10.1029/2010WR009341, 2010.

14 McMillan, H., Krueger, T., and Freer, J.: Benchmarking observational uncertainties for  
15 hydrology: rainfall, river discharge and water quality. *Hydrol. Process.*, 26, 4078–4111,  
16 doi:10.1002/hyp.9384, 2012.

17 Neill, C., Chaves, J. E., Biggs, T., Deegan, L. A., Elsenbeer, H., Figueiredo, R. O., Germer,  
18 S., Johnson, M. S., Lehmann, J., Markewitz, D., and Piccolo, M. C.: Runoff sources and land  
19 cover change in the Amazon: an end-member mixing analysis from small watersheds.  
20 *Biogeochemistry*, 105, 7–18, doi:10.1007/s10533-011-9597-8, 2011.

21 Nováková, J. and Pouličková, A.: Moss diatom (Bacillariophyceae) flora of the Nature  
22 Reserve Adršpašsko-Teplické Rocks (Czech Republic). *Czech Phycol.*, 4, 75–86, 2004.

23 Ocampo, C. J., Sivapalan, M., and Oldham, C.: Hydrological connectivity of upland-riparian  
24 zones in agricultural catchments: Implications for runoff generation and nitrate transport. *J.*  
25 *Hydrol.*, 331, 643–658, doi:10.1016/j.jhydrol.2006.06.010, 2006.

26 Pearce, A. J., Stewart, M. K., and Sklash, M. G.: Storm runoff generation in humid headwater  
27 catchments: 1. Where does the water come from? *Water Resour. Res.*, 22, 1263–1272,  
28 doi:10.1029/WR022i008p01263, 1986.

- 1 Penna, D., Tromp-Van Meerveld, H. J., Gobbi, A., Borga, M., and Dalla Fontana, G.: The  
2 influence of soil moisture on threshold runoff generation processes in an Alpine headwater  
3 catchment. *Hydrol. Earth Syst. Sc.*, 15, 689–702, doi:10.5194/hess-15-689-2011, 2011.
- 4 [Penna, D., Stenni, B., Šanda, M., Wrede, S., Bogaard, T.A., Gobbi, A., Borga, M., Fisher,  
5 B.M.C., Bonazza, M., On the reproducibility and repeatability of laser absorption  
6 spectroscopy measurements for  \$\delta^2\text{H}\$  and  \$\delta^{18}\text{O}\$  isotopic analysis. \*Hydrology and Earth System  
7 Sciences\*, 14, 1551–1566. doi:10.5194/hess-14-1551-2010, 2010.](#)
- 8 Pfister, L., McDonnell, J. J., Hissler, C., and Hoffmann, L.: Ground-based thermal imagery as  
9 a simple, practical tool for mapping saturated area connectivity and dynamics. *Hydrol.  
10 Process.*, 24, 3123–3132, doi:10.1002/hyp.7840, 2010.
- 11 Pfister, L., McDonnell, J. J., Wrede, S., Hlúbíková, D., Matgen, P., Fenicia, F., Ector, L., and  
12 Hoffmann, L.: The rivers are alive: on the potential for diatoms as a tracer of water source and  
13 hydrological connectivity. *Hydrol. Process.*, 23, 2841–2845, doi:10.1002/hyp.7426, 2009.
- 14 Pfister, L., Wagner, C., Vansuypeene, E., Drogue, G., and Hoffmann, L.: Atlas climatique du  
15 Grand-Duché de Luxembourg, Musée National d'Histoire Naturelle, Société des Naturalistes  
16 Luxembourgeois, Centre de Recherche Public - Gabriel Lippmann, Administration des  
17 Services Techniques de l'Agriculture, Luxembourg, 80 p., 2005.
- 18 Pfister, L., Wetzel, C.E., Martínez-Carreras, N., Iffly, J.F., Klaus, J., Holko, L., McDonnell,  
19 J.J.: Examination of aerial diatom flushing across watersheds in Luxembourg, Oregon and  
20 Slovakia for tracing episodic hydrological connectivity. *J. Hydrol. Hydromech.*, 63, XX–XX,  
21 doi:10.1515/johh-2015-0031, 2015.
- 22 Pinder, G. F. and Jones, J. F.: Determination of the ground-water component of peak  
23 discharge from the chemistry of total runoff. *Water Resour. Res.*, 5, 438–445,  
24 doi:10.1029/WR005i002p00438, 1969.
- 25 Rimet, F., Ector, L., Cauchie, H. M., and Hoffmann, L.: Regional distribution of diatom  
26 assemblages in the headwater streams of Luxembourg. *Hydrobiologia*, 520, 105–117,  
27 doi:10.1023/B:HYDR.0000027730.12964.8c, 2004.
- 28 Round, F. E., Crawford, R. M., and Mann, D. G.: The diatoms. Biology and morphology of  
29 the genera, Cambridge University Press, Cambridge, 1990.

- 1 Sidle, R. C., Hirano, T., Gomi, T., and Terajima, T.: Hortonian overland flow from Japanese  
2 forest plantations – an aberration, the real thing, or something in between? *Hydrol. Process.*,  
3 21, 3237–3247, doi:10.1002/hyp.6876, 2007.
- 4 Sklash, M. G. and Farvolden, R. N.: The use of environmental isotopes in the study of high-  
5 runoff episodes in streams, in: Perry, E.C.J. and Montgomery, C.W. (Eds.): *Isotope Studies of*  
6 *Hydrologic Processes*, Northern Illinois University Press, Illinois, 65–73, 1982.
- 7 Sklash, M. G., Stewart, M. K., and Pearce, A. J.: Storm runoff generation in humid headwater  
8 catchments: 2. A case study of hillslope and low-order stream response. *Water Resour. Res.*,  
9 22, 1273–1282, doi:10.1029/WR022i008p01273, 1986.
- 10 Spence, C.: A paradigm shift in hydrology: Storage thresholds across scales influence  
11 catchment runoff generation. *Geogr. Compass*, 4, 819–833, doi:10.1111/j.1749-  
12 8198.2010.00341.x, 2010.
- 13 Tauro, F., Martínez-Carreras, N., Barnich, F., Juilleret, J., Wetzel, C. E., Ector, L., Hissler, C.,  
14 Frenress, J., Hoffmann, L., McDonnell, J. J., and Pfister, L.: Diatom percolation through  
15 soils. *Ecohydrology* (under review).
- 16 Tetzlaff, D., Soulsby, C., and Birkel, C.: Hydrological connectivity and microbiological  
17 fluxes in montane catchments: the role of seasonality and climatic variability. *Hydrol.*  
18 *Process.*, 24, 1231–1235, doi:10.1002/hyp.7680, 2010.
- 19 Tromp-van Meerveld, H. J. and McDonnell, J. J.: Threshold relations in subsurface  
20 stormflow: 1. A 147-storm analysis of the Panola hillslope. *Water Resour. Res.*, 42, W02410,  
21 doi:10.1029/2004WR003778, 2006.
- 22 Uhlenbrook, S. and Hoeg, S.: Quantifying uncertainties in tracer-based hydrograph  
23 separations: a case study for two-, three- and five-component hydrograph separations in a  
24 mountainous catchment. *Hydrol. Process.*, 17, 431–453, doi:10.1002/hyp.1134, 2003.
- 25 Vacht, P., Puusepp, L., Koff, T., and Reitalu, T.: Variability of riparian soil diatom  
26 communities and their potential as indicators of anthropogenic disturbances. *Est. J. Ecol.*, 63,  
27 168–184, doi:10.3176/eco.2014.3.04, 2014.
- 28 Van Dam, H., Mertens, A., and Sinkeldam, J.: A coded checklist and ecological indicator  
29 values of freshwater diatoms from The Netherlands. *Neth. J. Aquat. Ecol.*, 28, 117–133,  
30 doi:10.1007/BF02334251, 1994.

1 van den Bos, R., Hoffmann, L., Juilleret, J., Matgen, P., and Pfister, L.: Regional runoff  
2 prediction through aggregation of first-order hydrological process knowledge: a case study.  
3 *Hydrolog. Sci. J.*, 51, 1021–1038, doi:10.1623/hysj.51.6.1021, 2006.

4 Van de Vijver, B. and Beyens, L.: Moss diatom communities from Ile de la Possession  
5 (Crozet, Subantarctica) and their relationship with moisture. *Polar Biol.*, 22, 219–231,  
6 doi:10.1007/s003000050414, 1999.

7 Vidon, P. G. F. and Hill, A. R.: Landscape controls on the hydrology of stream riparian zones.  
8 *J. Hydrol.*, 292, 210–228, doi:10.1623/hysj.51.6.1021; 2004.

9 Wetzel, C. E., Martínez-Carreras, N., Hlúbiková, D., Hoffmann, L., Pfister, L., and Ector, L.:  
10 New combinations and type analysis of *Chamaepinnularia* species (Bacillariophyceae) from  
11 aerial habitats. *Cryptogamie Algol.*, 34, 149–168, doi:10.782/crya.v34.iss2.2013.149, 2013.

12 Wetzel, C. E., Van de Vijver, B., Kopalová, K., Hoffmann, L., Pfister, L., and Ector, L.: Type  
13 analysis of the South American diatom *Achnanthes haynaldii* (Bacillariophyta) and  
14 description of *Planothidium amphibium* sp. nov., from aerial and aquatic environments in  
15 Oregon (USA). *Plant Ecol. Evol.*, 147, 439–454, doi:10.5091/plecevo.2014.1058, 2014.

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

20

1 Table 1. Summary of collection methods, sampling resolution and locations in the Weierbach  
 2 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

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1 Table 2. Variance explained by each eigenvector (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

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

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

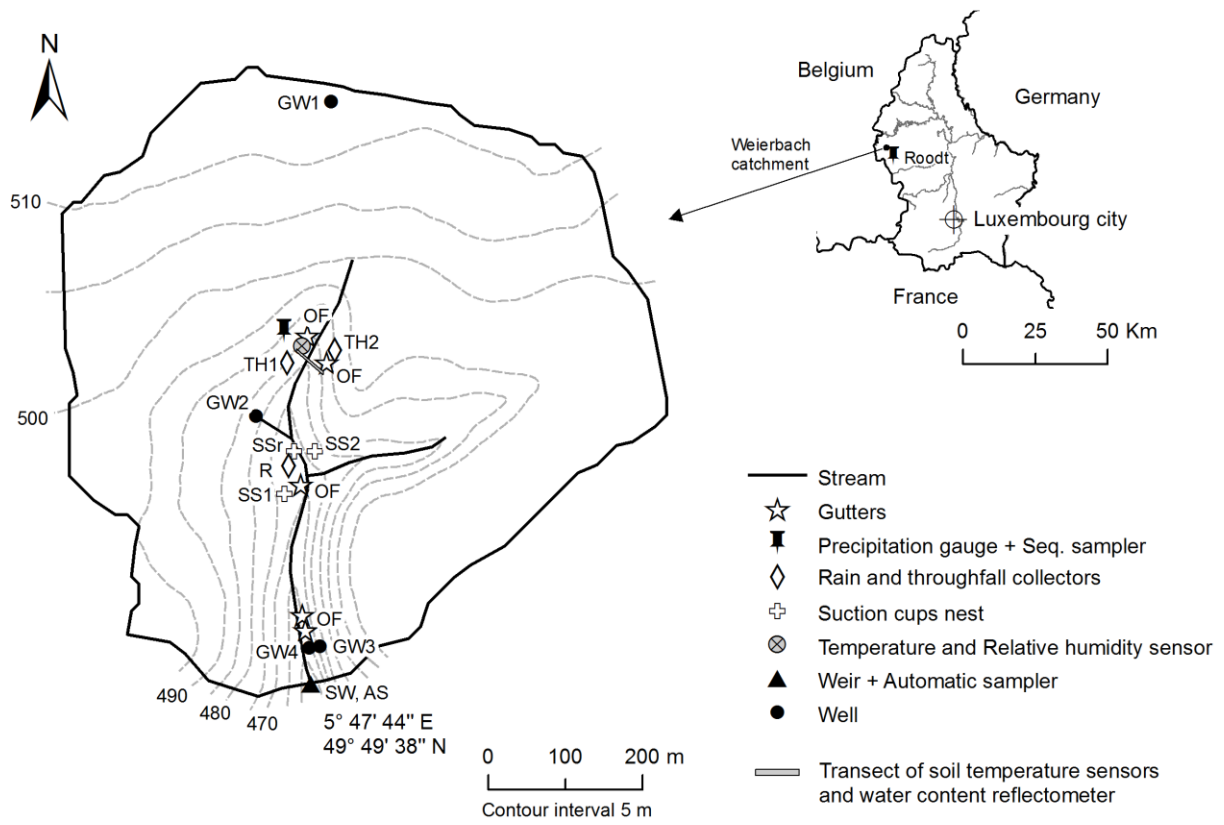


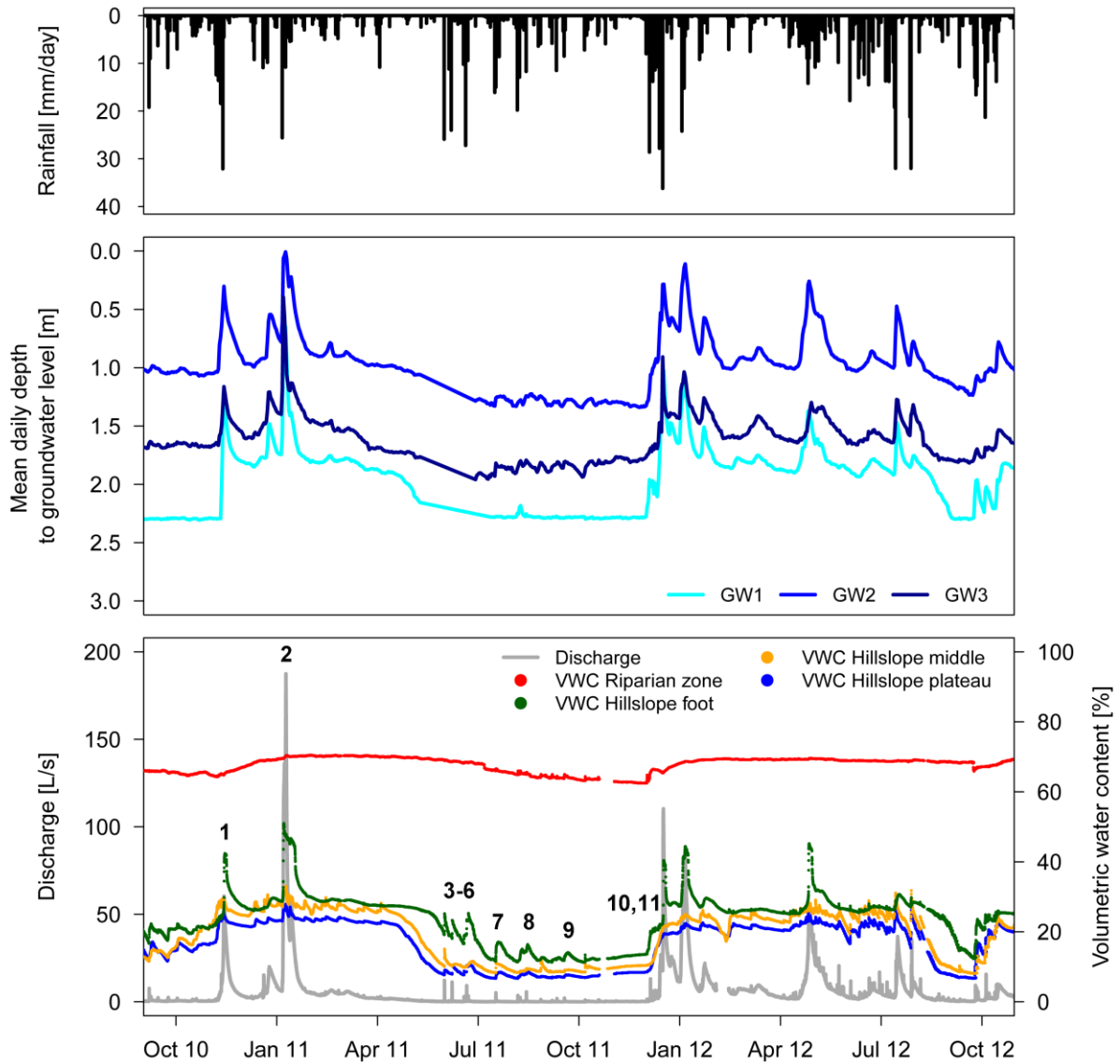
1 Table 4. General hydrological characteristics of the sampled rainfall-runoff events occurred  
 2 from October 2010 to December 2011 in the Weierbach catchment.

	Beginning of precipitation	Duration	Total P	Maximum intensity	Antecedent P, 10 days	Antecedent P, 20 days	Pre-event discharge	Maximum discharge
		[h]	[mm]	[mm·15min <sup>-1</sup> ]	[mm]	[mm]	[L·s <sup>-1</sup> ]	[L·s <sup>-1</sup> ]
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

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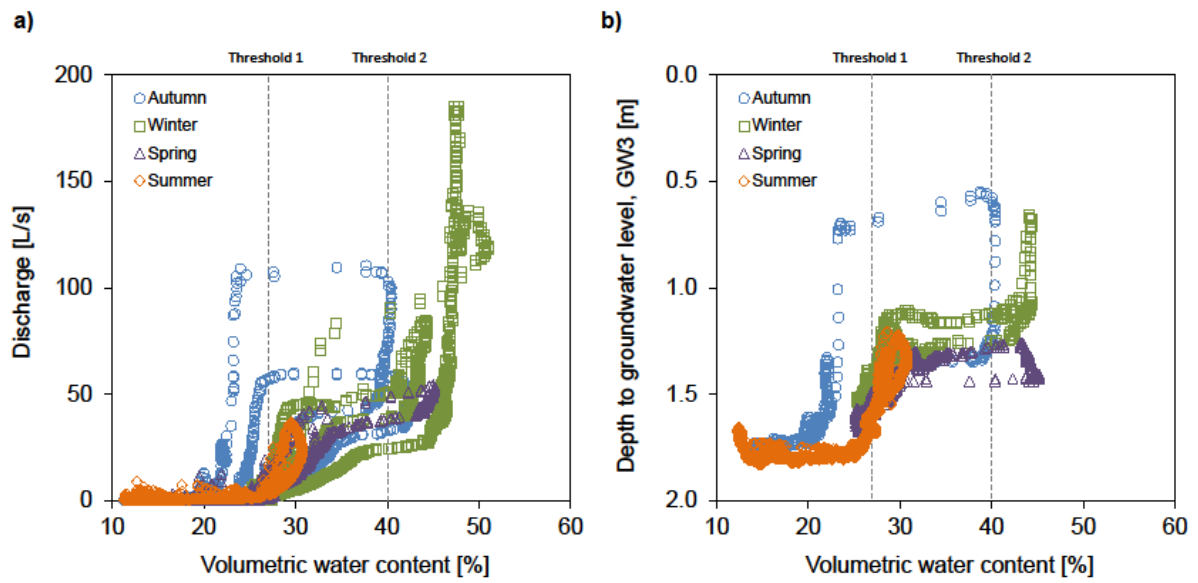
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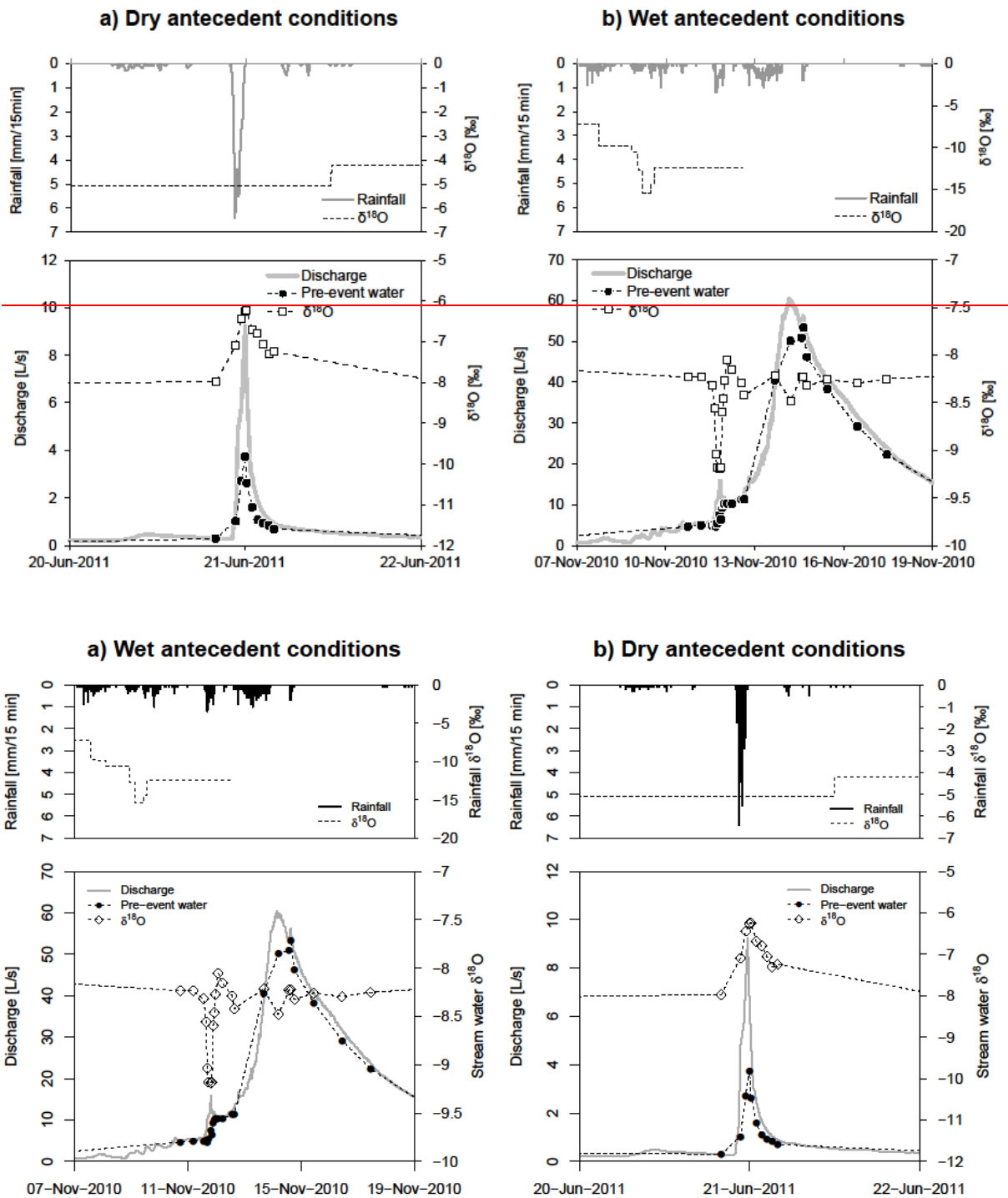
Figure 2. Time series of daily rainfall measured at the Roodt meteorological station ( $\approx 3.5$  km distant from the Weierbach) (upper plot), mean daily groundwater depth at three different locations (GW1: plateau, GW2: close to a spring, and GW3: hillslope foot) (middle plot) and soil volumetric 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.



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

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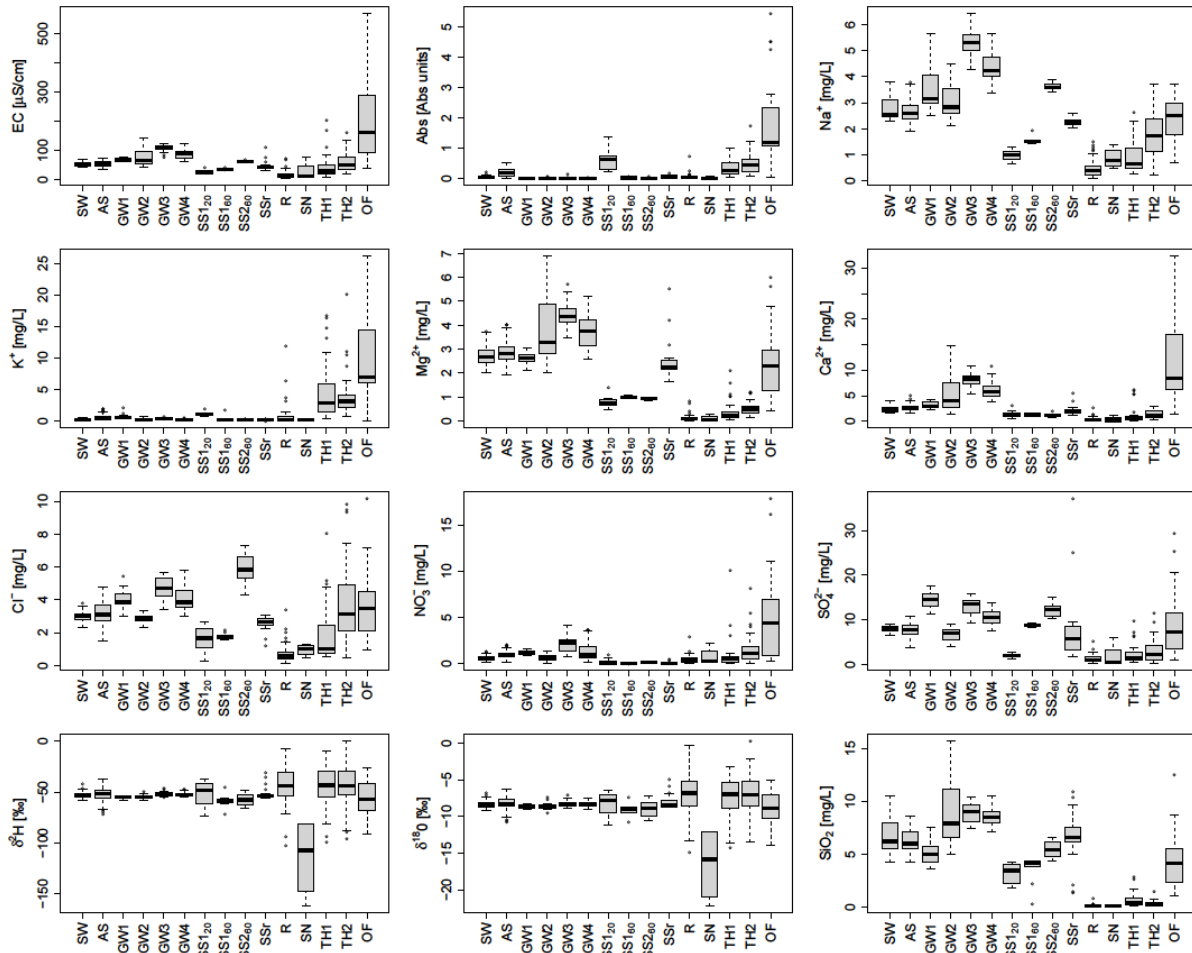


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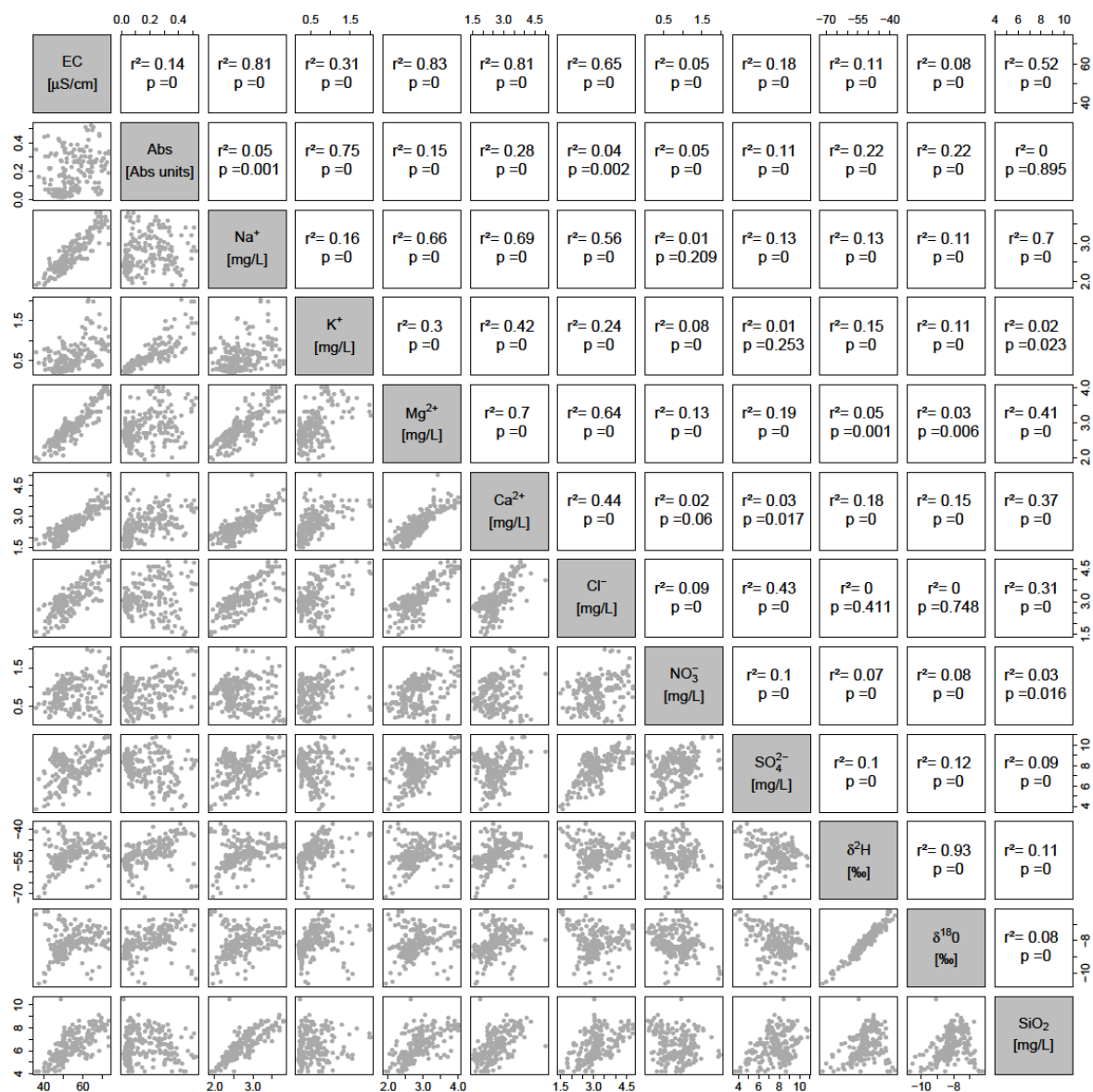
3 Figure 4. Two-component hydrograph separation for (a) the 7th November 2010 event (wet  
 4 antecedent conditions) and (ab) the 20th and 22nd June 2011 events (dry antecedent  
 5 conditionssummer response) and (b) the 7th November 2010 event (winter response) using  
 6  $\delta^{18}\text{O}$  isotopic composition.

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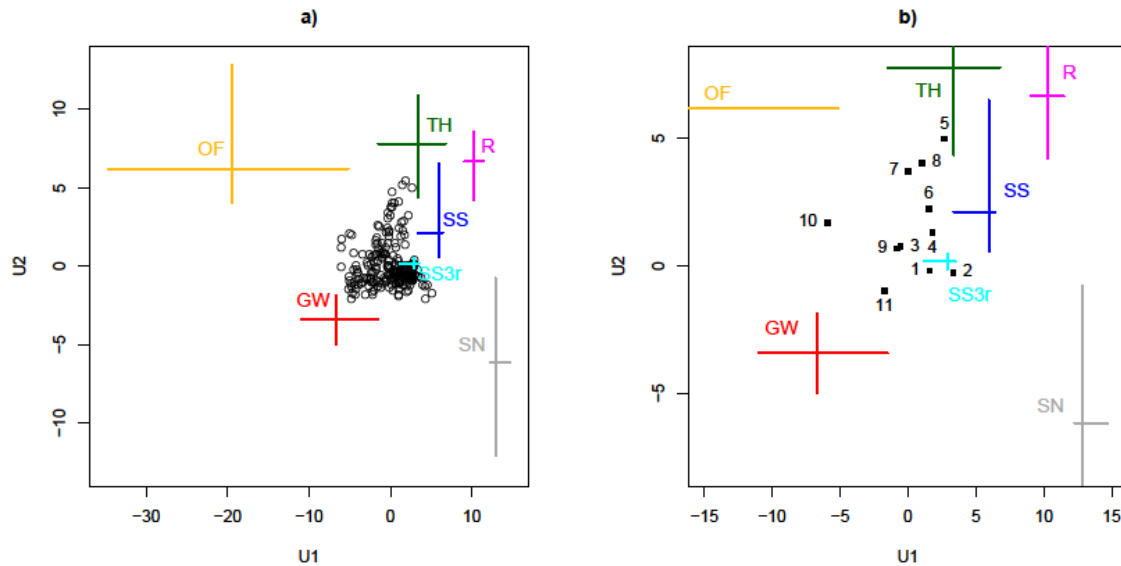
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Figure 5. Boxplots of tracers measured for stream water sampled fortnightly (SW, n=47) and using automatic samplers (AS, n=179), groundwater (GW1, n=24; GW2, n= 49; GW3, n=49; GW4, n=47), soil water (SS1<sub>20</sub>, n=22; SS1<sub>60</sub>, n=10; SS2<sub>60</sub>, n=9), soil water from the riparian zone (SSr, 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.



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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). The upper part of the diagonal shows the Pearson's correlation coefficient and its significance at the 0.95 confidence level.

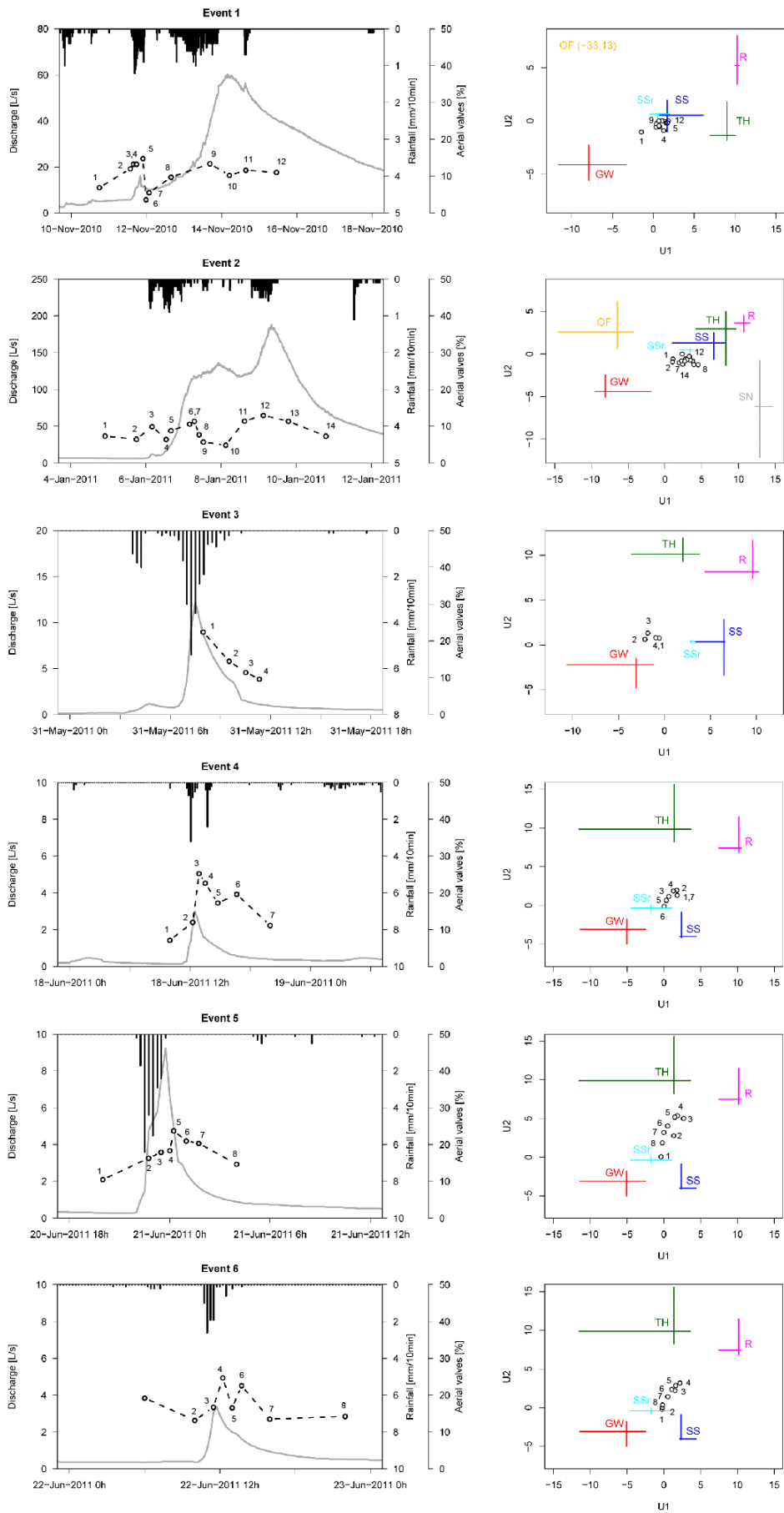


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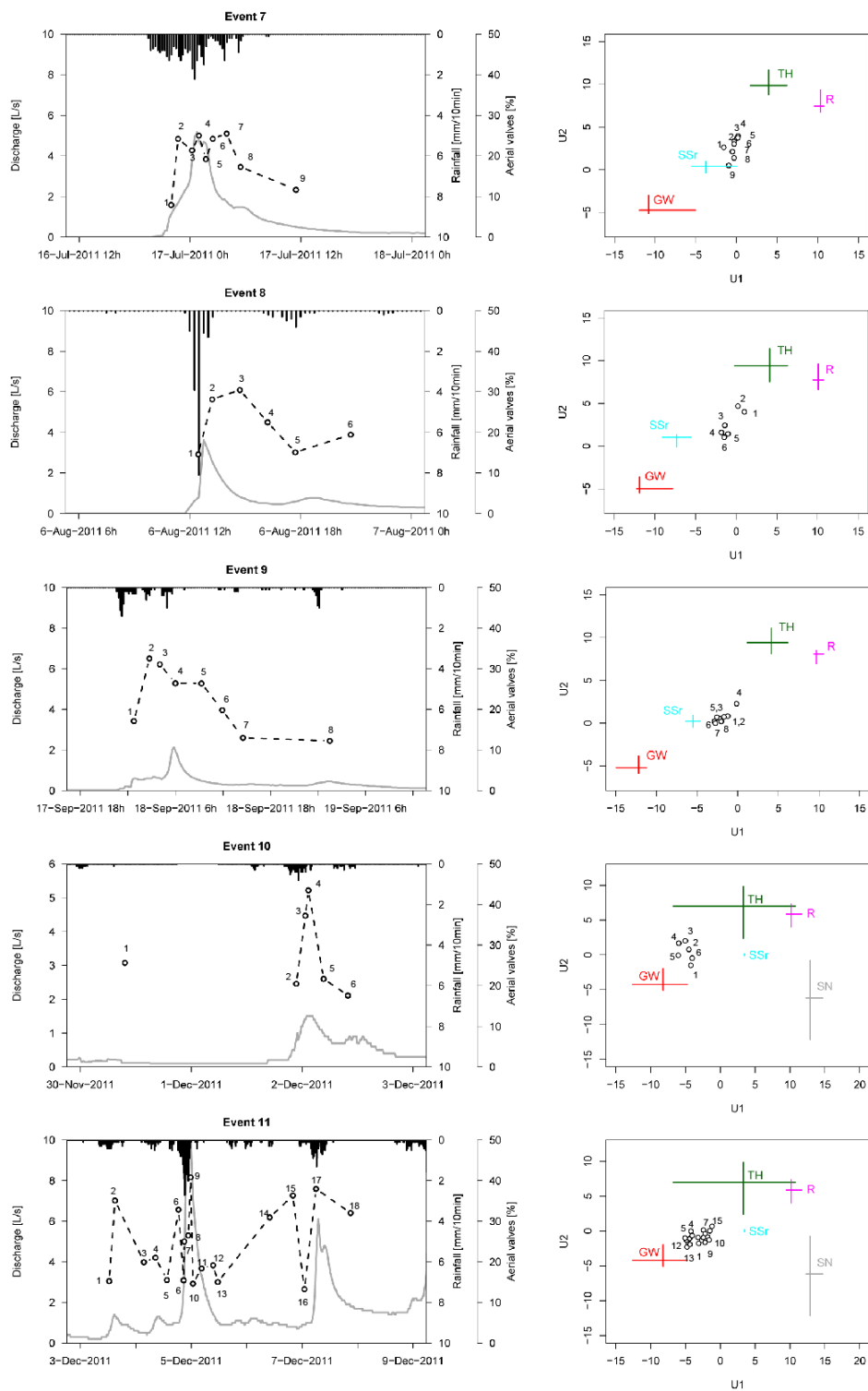
2 Figure 7. (a) U1-U2 mixing diagram of stream water tracers (black circles; AS + SW in  
 3 Figure 5) and (b) zoom into the U1-U2 mixing diagram showing event peakflow stream water  
 4 samples (black squares; numbers identify storm events in Figure 2). Sampling points data  
 5 plotted in Figure 5 were grouped in 7 end-members and the interquartile ranges of each end-  
 6 member were projected into the new mixing space (U-space; GW: groundwater, SN: snow,  
 7 SS: soil water, SSr: soil water from the riparian zone, OF: overland flow, R: rainfall, TH:  
 8 throughfall). Because panel (b) is a zoom into the U1-U2 mixing diagram, the interquartile  
 9 ranges of some end-members are not fully represented.

10





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



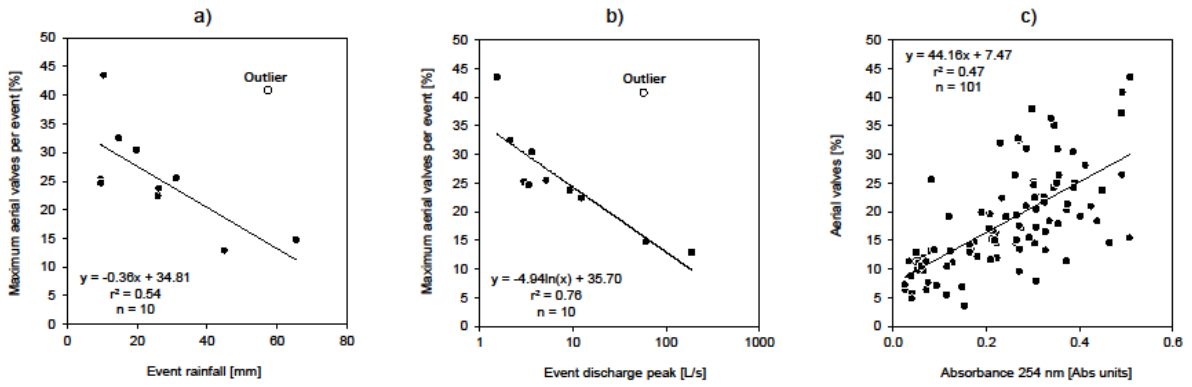
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2 Figure 9. Hydrograph, hyetograph and percentage of aerial valves in the stream water for the  
 3 events 7-11 in the Weierbach catchment (left), and U1-U2 mixing diagrams for each event.  
 4 End-members are rainfall (R), throughfall (TH), snow (SN), soil water (SS), soil water from  
 5 the riparian zone (SSr) and groundwater (GW). Bars represent end-member values

1 interquartile ranges of samples collected during the month when the event occurred, as well as  
2 the previous and following month.

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3 Figure 10. Correlations between (a) maximum percentage of aerial valves in the stream water  
 4 per event and event rainfall, (b) maximum percentage of aerial valves in the stream water per  
 5 event and maximum event discharge, and (c) percentage of aerial valves in the stream water  
 6 and UV-absorbance at 254 nm.