

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 quantify 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 water from the riparian
7 zone was a major streamflow source during rainfall events under both wet and dry antecedent
8 conditions. In comparison to other tracer methods, diatoms require taxonomy knowledge and
9 a rather large processing time. However, they can provide unequivocal evidence of
10 hydrological connectivity and potentially be used at larger catchment scales.

11

12 **1 Introduction**

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

26 Despite their usefulness, chemical and isotope tracer-based hydrograph separations do not
27 provide unequivocal evidence of hillslope-riparian-stream (HRS) connectivity. This has been
28 identified as perhaps the key feature for improving our understanding of water origin and the
29 processes that sustain stream flow (Jencso et al., 2010). Consequently, new techniques are
30 desperately needed to gain a process-based understanding of hydrological connectivity
31 (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 km², 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 in a plateau, and GW2, GW3 and GW4 in the transition zone
4 between riparian and hillslope settings (Fig. 1). Wells were around 2 m deep and were
5 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 method. Four probes were installed at 10 cm depth, parallel to the surface and
9 along a 5 m transect perpendicular to the stream (Fig. 1): riparian zone, foot of the hillslope,
10 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 was sampled using 1 and 2
25 m long gutters sealed to the soil surface, which diverted surface runoff to 1 or 2-L plastic,
26 blackened (to prevent light penetration which causes diatom growth) water bottles. Note that
27 what we refer as OF might in fact originate within the forest litter layer (Buttle and Turcotte,
28 1999; Sidle et al., 2007). All gutters were covered to avoid the influence of precipitation.
29 Gutters were regularly cleaned with Milli-Q water to avoid diatoms growth on their surfaces.

30 All water samples were analysed for electrical conductivity (EC), anion and cation
31 concentrations (Cl^- , NO_3^- , SO_4^{2-} , Na^+ , K^+ , Mg^{2+} , Ca^{2+}), silica (SiO_2) and UV-absorbance at
32 254 nm (Abs 254 nm). UV absorbance at 254 nm can be considered as a proxy of DOC

1 (Edzwald et al., 1985). Samples were analysed at the Luxembourg Institute of Science and
2 Technology chemistry laboratory after filtration through WHATMAN GF/C glass fibre filters
3 (<0.45 µm). Prior to analysis, samples were stored at 4° C. Dissolved anions and cations were
4 analysed by ion chromatography (Dionex HPLC), SiO₂ by spectrophotometry (ammonium
5 molybdate method), and UV-absorbance was measured by a Beckmann Coulter
6 spectrophotometer. Isotopic analyses of ¹⁸O/¹⁶O and ²H/H were conducted using a LGR
7 Liquid-Water Isotope Analyser (LWIA) at the Luxembourg Institute of Science and
8 Technology (model DLT-100, version 908-0008) (Penna et al., 2010). The analyser was
9 connected to a LC PAL liquid auto-injector for the automatic and simultaneous measurement
10 of ²H/H and ¹⁸O/¹⁶O ratios in water samples. According to the manufacturer's specifications
11 (Los Gatos Research Inc., 2008), the DLT-100 908-0008 LWIA provides isotopic
12 measurements with a precision below 0.6‰ for ²H/H and 0.2‰ for ¹⁸O/¹⁶O. Data were
13 transformed into δ notion according to Vienna Standard Mean Ocean Water (VSMOW)
14 standards (δ²H and δ¹⁸O in ‰).

15 **3.3 Diatom sampling, sample preparation and analysis**

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

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

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

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

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

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

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

26 **3.4 Hydrograph separation**

27 Two-component hydrograph separation was performed using $\delta^{18}\text{O}$ isotopic composition and
28 the mass balance approach (Pinder and Jones, 1969; Sklash and Farvolden, 1982; Pearce et
29 al., 1986; Sklash et al., 1986). The incremental mean method proposed by McDonnell et al.
30 (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 (SS1₂₀, SS1₆₀, SS2₆₀), 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. 4a). 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. 4b).

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⁻, Na⁺, K⁺, Mg²⁺, Ca²⁺, SiO₂, Abs, δ²H and δ¹⁸O; r²>0.5, p-value<0.01,
2 Fig. 6). These tracers were retained for the PCA analysis. Weaker linear trends were found
3 between NO₃⁻ and the other tracers (r²<0.13) and between SO₄²⁻ and the other tracers
4 (r²<0.43). Neither tracers reached the pre-defined threshold of collinearity (r²>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, *Achnanthidium 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 high (0.21 and 0.73 for the
29 riparian zone and the hillslope samples, respectively) and the null hypothesis was accepted.
30 No diatom valves were found in groundwater or rainfall samples.

31 The riparian zone was characterized by several species that prefer aerial habitats, mainly
32 living on exposed soils or epiphytically on bryophytes. Such species occur mainly on wet

1 and moist or temporarily dry places or live nearly exclusively outside water bodies (Category
2 4 and 5 of Pfister et al. (2009)), such as *Chamaepinnularia evanida* (Hustedt) Lange-Bertalot,
3 *C. parsura* (Hustedt) C.E. Wetzel & Ector, *Eunotia minor* (Kützing) Grunow, *Hantzschia*
4 *abundans* Lange-Bertalot, *Nitzschia harderi* Hustedt, *Orthoseira dendroteres* (Ehrenberg)
5 Round, R.M. Crawford & D.G. Mann, *Pinnularia borealis* Ehrenberg, *P. perirrorata*
6 Krammer, *Stauroneis parathermicola* Lange-Bertalot and *S. thermicola* (J.B. Petersen)
7 J.W.G. Lund.

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

15 **4.4 Aerial diatom transport during rainfall events**

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

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

27 No significant relationship was found between the percentage of aerial diatoms and
28 instantaneous discharge ($r^2=0.13$, $n=101$; discharge on the x axis), most probably due to
29 different diatom abundances on the rising limb of the hydrograph than on the recession limb
30 (i.e. hysteretic effects). Two events showed clockwise hysteretic loops (events 1 and 2); five
31 events showed counter-clockwise hysteretic loops (events 4, 5, 6, 8, and 10) and three showed

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

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

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

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

25

26 **5 Discussion**

27 **5.1 Can aerial diatoms transport reveal hydrological connectivity within the** 28 **hillslope-riparian-stream system?**

29 Our central hypothesis for this study was that aerial diatoms could indicate connectivity
30 within the HRS system. In order to test this hypothesis, we sampled from potential upland

1 catchment sources (i.e. hillslope and riparian zones), and within the streambed (i.e. epilithon,
2 epipelon and stream water samples).

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

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

23 We examined the aerial diatoms transported in the stream water during runoff events. We
24 observed an increase in the relative abundance of aerial diatoms with discharge for all
25 sampled events regardless of antecedent wetness conditions. Hence, during storm events there
26 was an increase in the relative proportion of diatoms in categories 4 and 5 of Van Dam's et al.
27 (1994) classification. Similar results were reported by Pfister et al. (2009). These observations
28 imply hydrological connectivity between the riparian soil surface and the stream for all
29 events. The use of aerial diatoms to infer hydrological connectivity in the Weierbach
30 catchment thus remains limited to the riparian-stream system as no diatoms were found on the
31 hillslopes covered by dry litter.

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

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

17 **5.2 How do diatom results compare to the other methods to infer hydrological** 18 **connectivity?**

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

1 largest storm on record –overland flow was a significant contributor to stream discharge,
2 likely due to rapid snowmelt onto surface-saturated area (Fig. 8 and 9).

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

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

22 **5.3 Can aerial diatom be established as a new hydrological tracer?**

23 Storm hydrograph separation using stable isotope tracers has resulted in major advances in
24 catchment hydrology. However, despite their usefulness, these methods do not provide
25 unequivocal evidence of hydrological connectivity in the HRS system. In comparison,
26 diatoms can provide evidence of riparian-stream connectivity. Further research is needed to
27 better understand diatom transport processes (and associated water flowpaths) in headwater
28 catchments. Future studies should focus on expanding our understanding of terrestrial diatom
29 taxonomy and ecology, which are scarce or lacking for a large number of taxa (Wetzel et al.,
30 2013, 2014). Even though this new data source will have its own individual measurement
31 uncertainty (McMillan et al., 2012), diatoms offer the possibility to tackle open questions in
32 hydrology and eco-hydrology.

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

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

29

30 **6 Conclusions**

31 We investigated the potential for aerial diatoms, i.e. diatoms nearly exclusively occurring
32 outside water bodies and on wet and moist or temporarily dry places (Van Dam et al., 1994),

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

14

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24

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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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4

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

2

3

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.

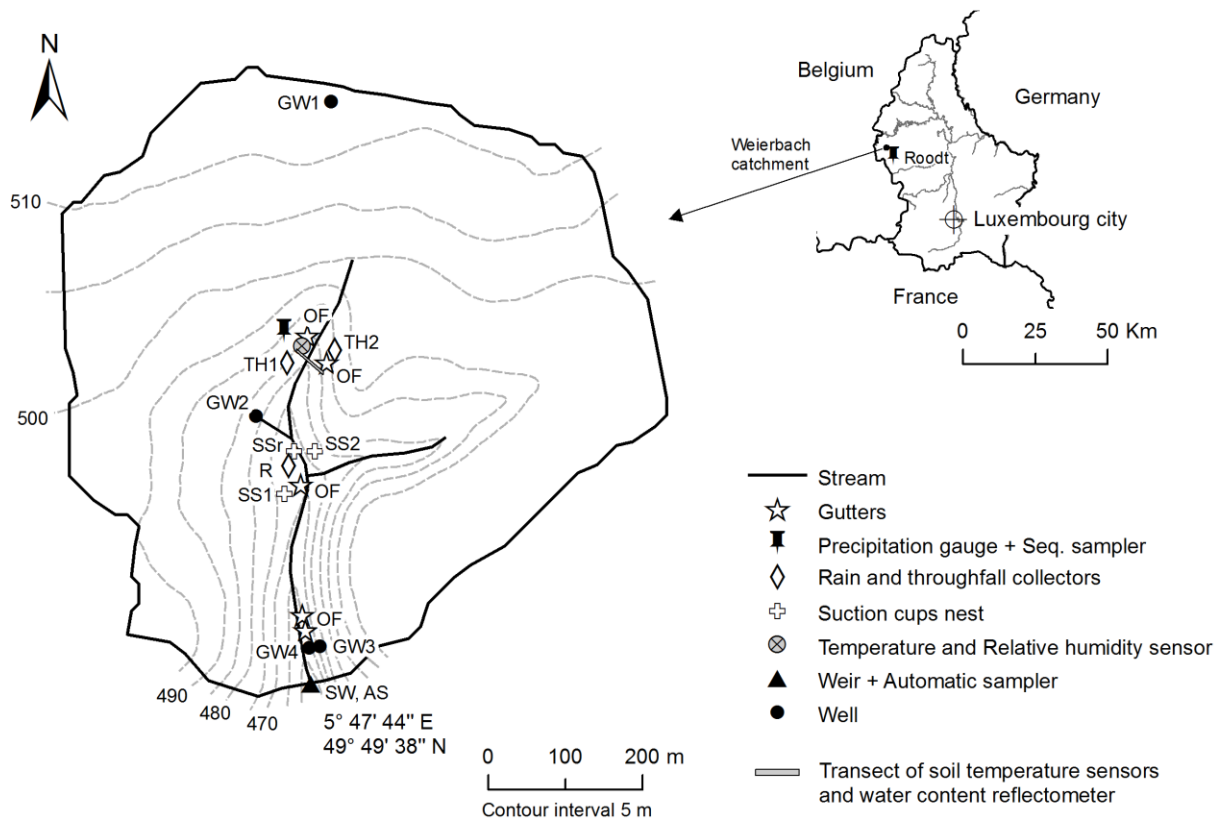
	Sample	n	Min [%]	Max [%]	Mean [%]	S.D. [%]
Summer 2010	Stream water at low flow	3	10.1	19.4	14.9	4.6
	Streambed	6	14.8	21.7	19.0	2.7
	Riparian zone	25	8.5	61.5	22.9	16.9
	Hillslope	12	11.6	96.6	36.5	27.0
Winter 2011	Streamwater at low flow	8	5.9	16.1	9.8	3.3
	Streambed	2	5.0	8.8	6.9	2.7
	Riparian zone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

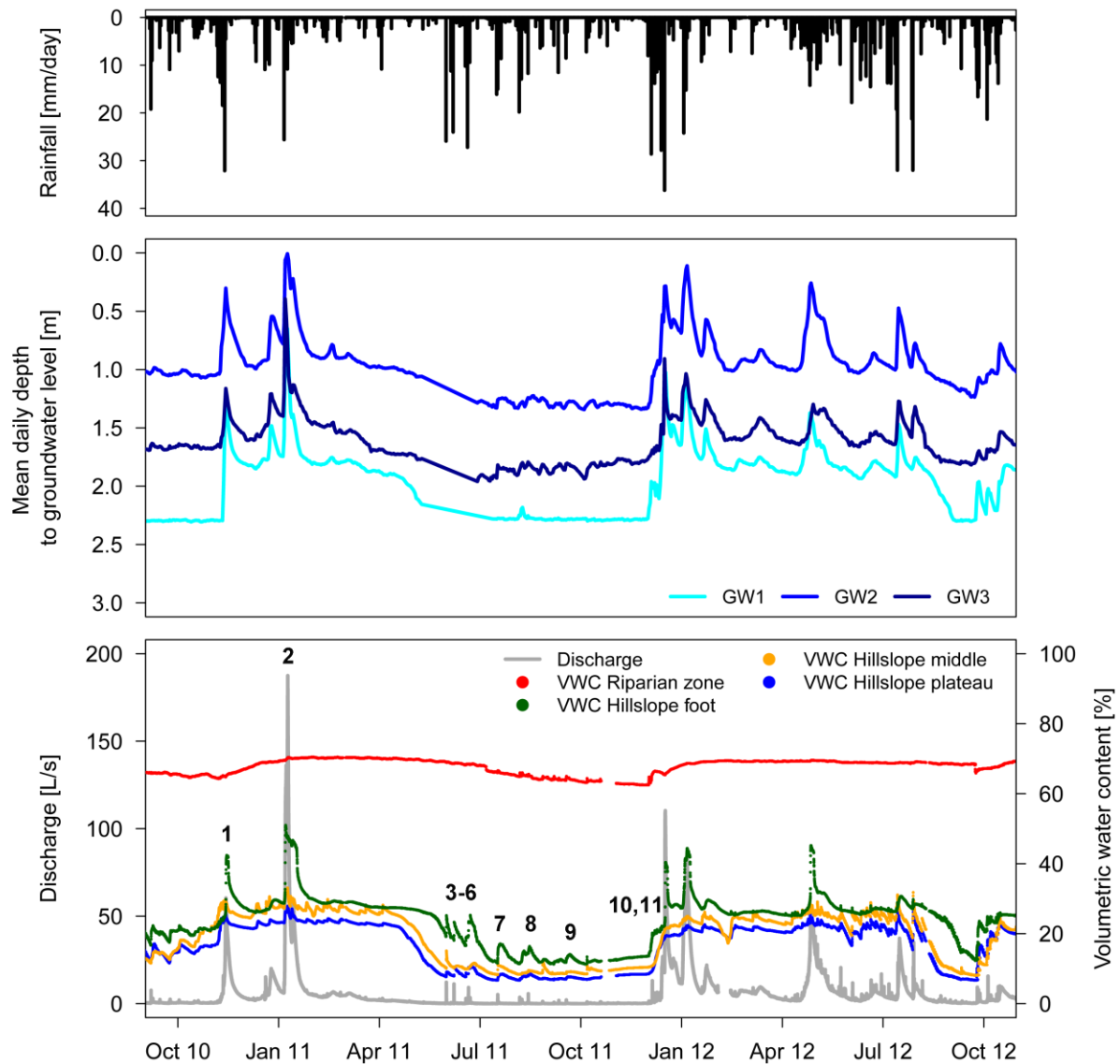
1 Table 4. General hydrological characteristics of the sampled rainfall-runoff events occurred
 2 from October 2010 to December 2011 in the Weierbach catchment.

	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 ⁻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

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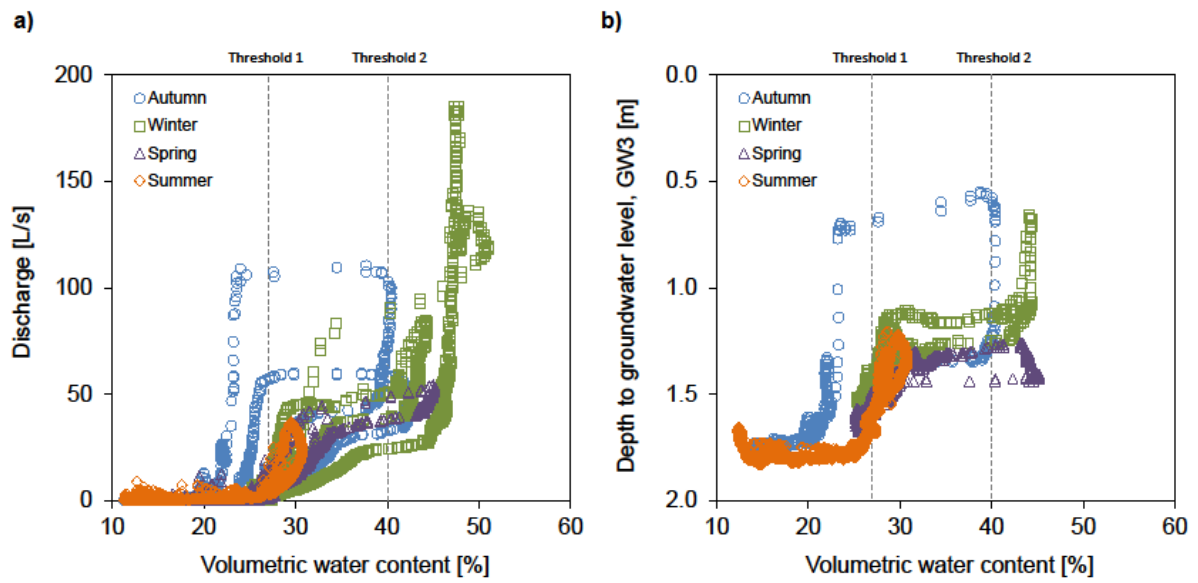
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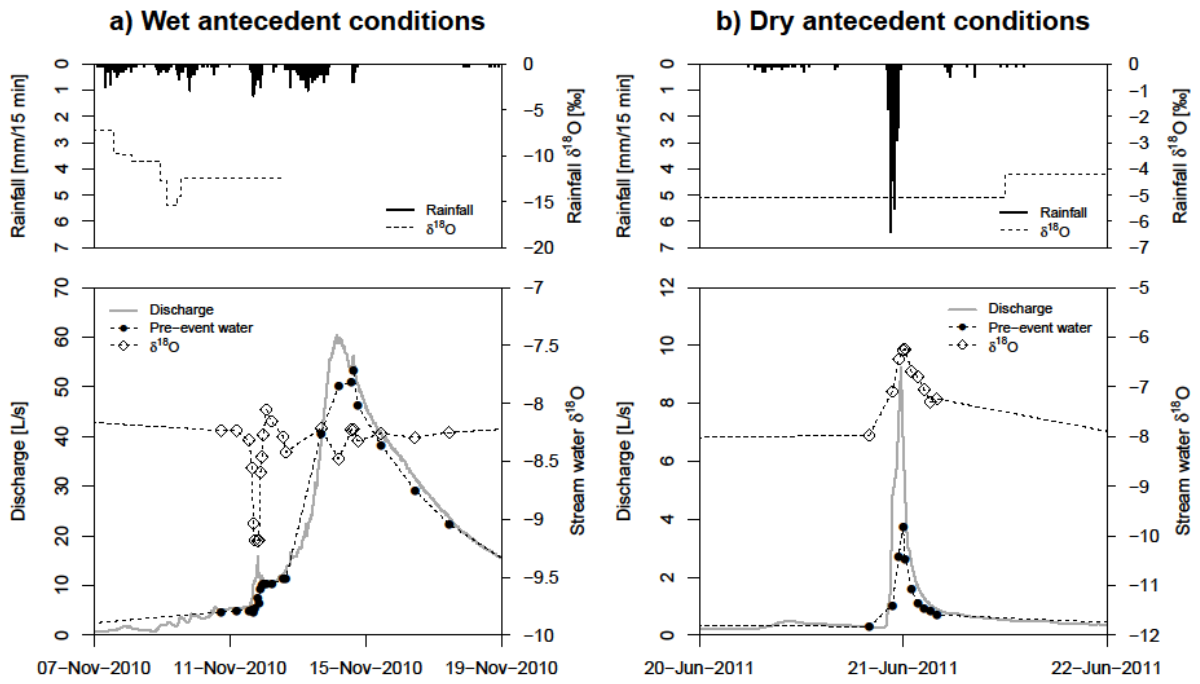
Figure 2. Time series of daily rainfall measured at the Roodt meteorological station (≈ 3.5 km distant from the Weierbach) (upper plot), mean daily groundwater depth at three different locations (GW1: plateau, GW2: close to a spring, and GW3: hillslope foot) (middle plot) and soil volumetric water content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



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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).

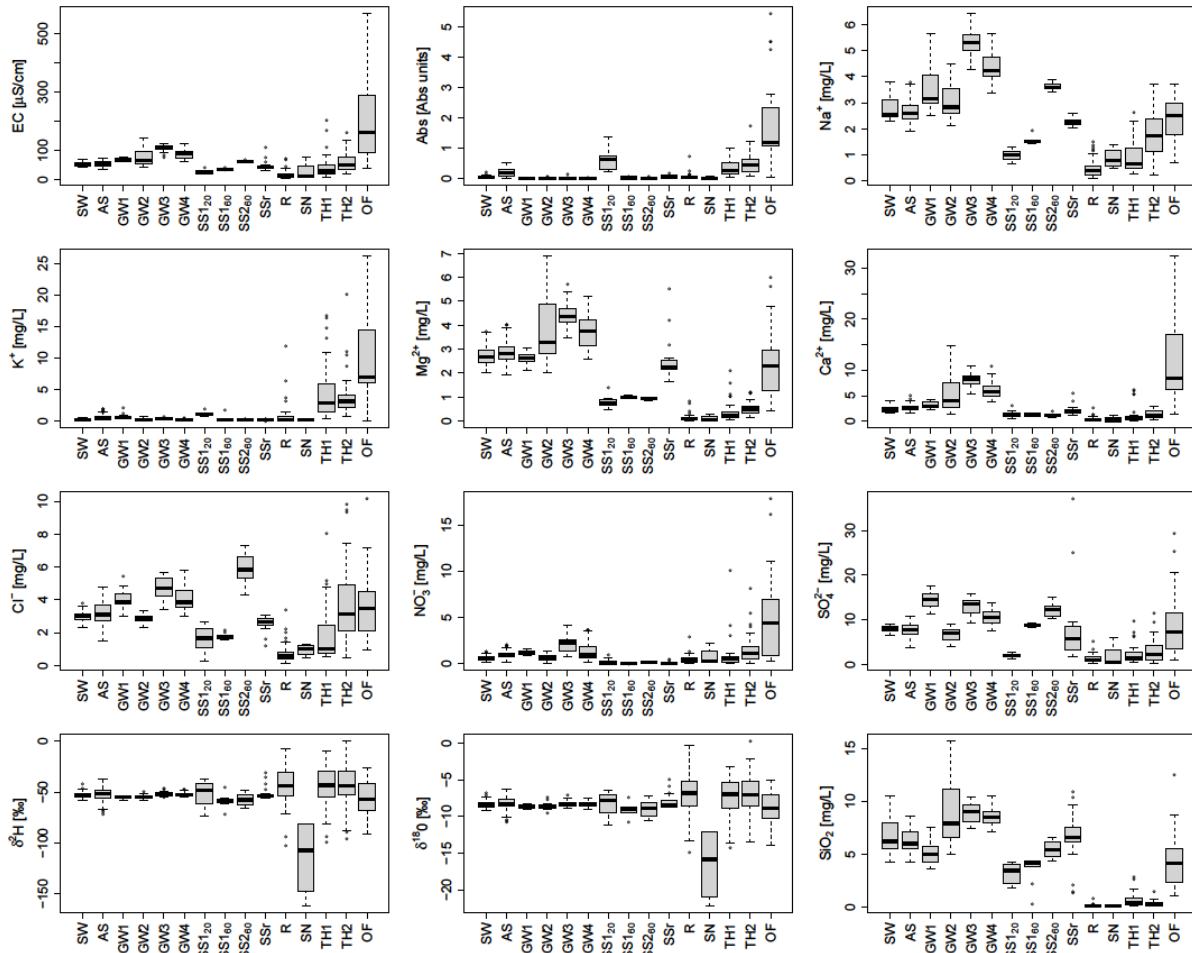
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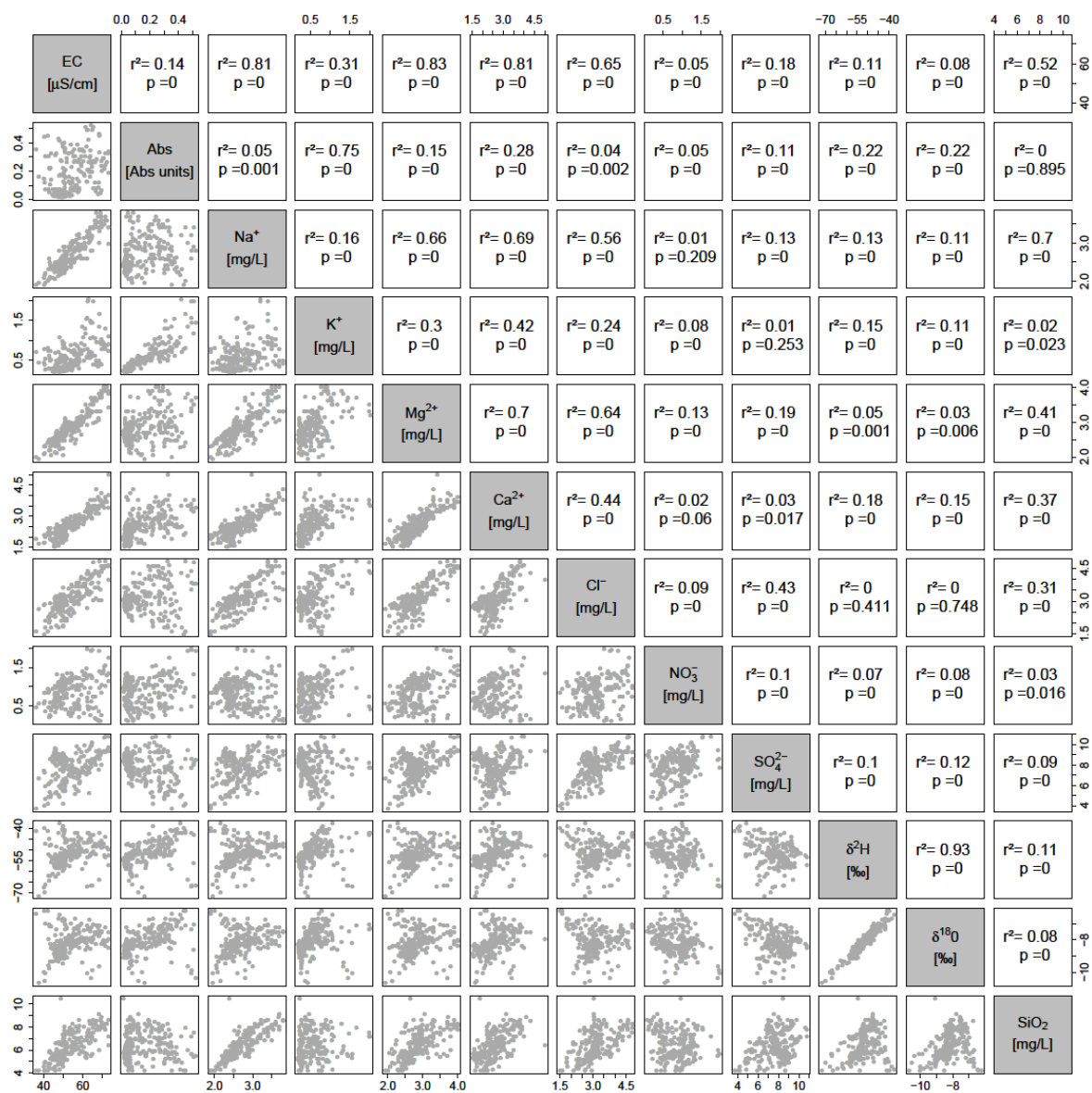
2 Figure 4. Two-component hydrograph separation for (a) the 7th November 2010 event (wet
 3 antecedent conditions) and (b) the 20th and 22nd June 2011 events (dry antecedent
 4 conditions) using $\delta^{18}\text{O}$ isotopic composition.

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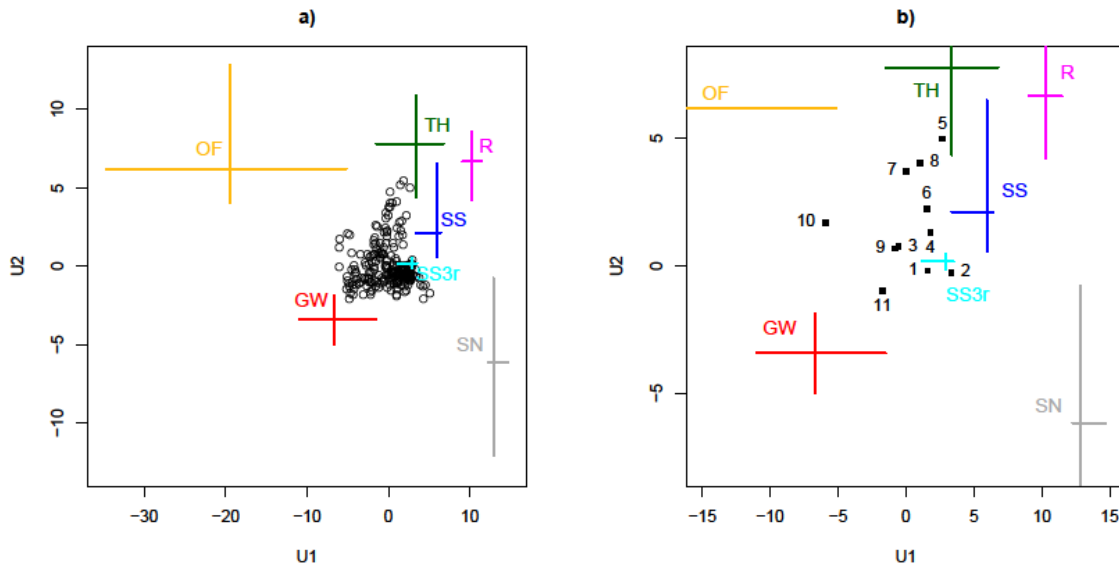
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Figure 5. Boxplots of tracers measured for stream water sampled fortnightly (SW, n=47) and using automatic samplers (AS, n=179), groundwater (GW1, n=24; GW2, n= 49; GW3, n=49; GW4, n=47), soil water (SS1₂₀, n=22; SS1₆₀, n=10; SS2₆₀, n=9), soil water 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.



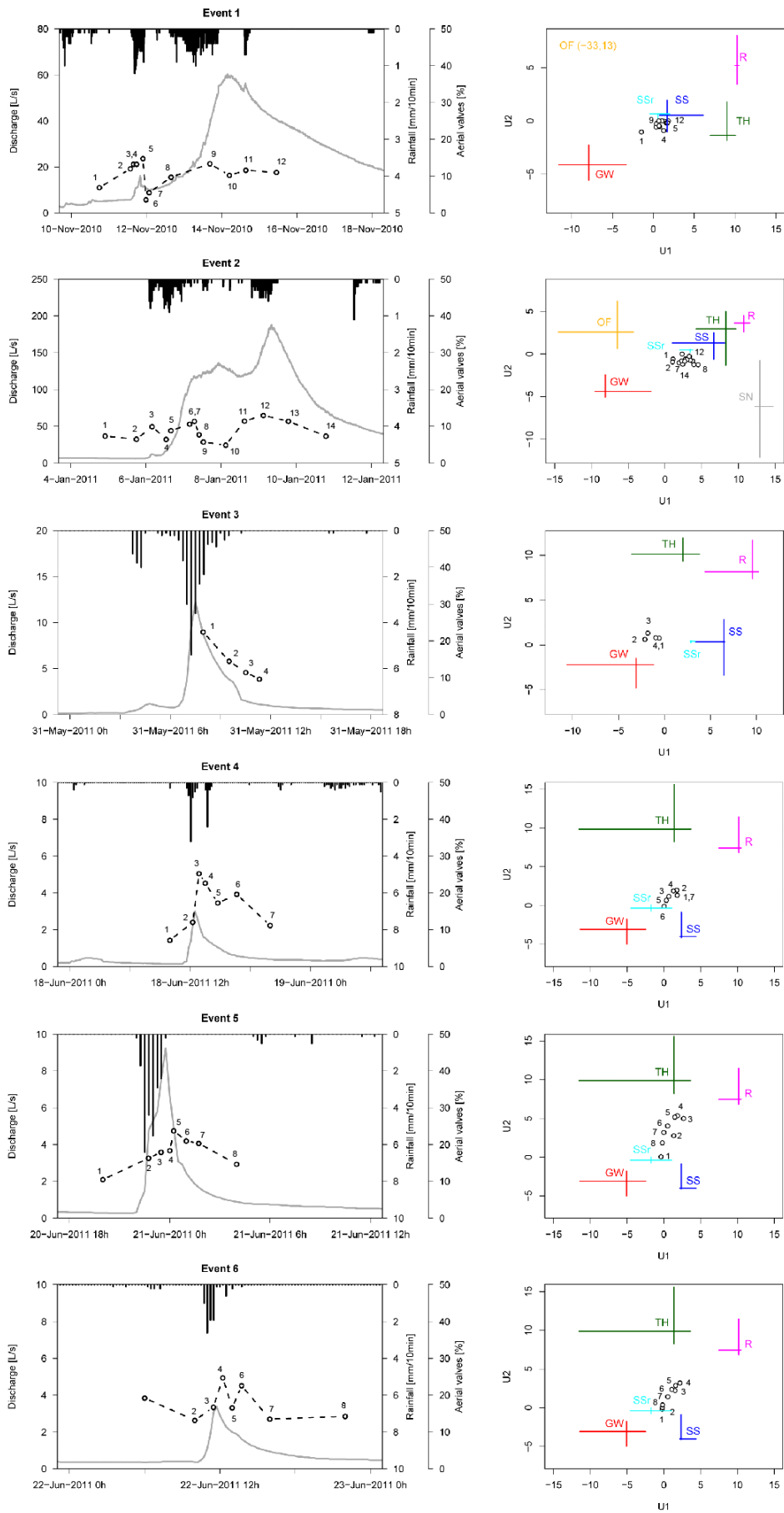
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Figure 6. Bivariate plots of stream water chemistry and water stable isotope data collected at the outlet of the Weierbach catchment (n=226; SW and AS displayed in Fig. 5). The upper part of the diagonal shows the Pearson's correlation coefficient and its significance at the 0.95 confidence level.

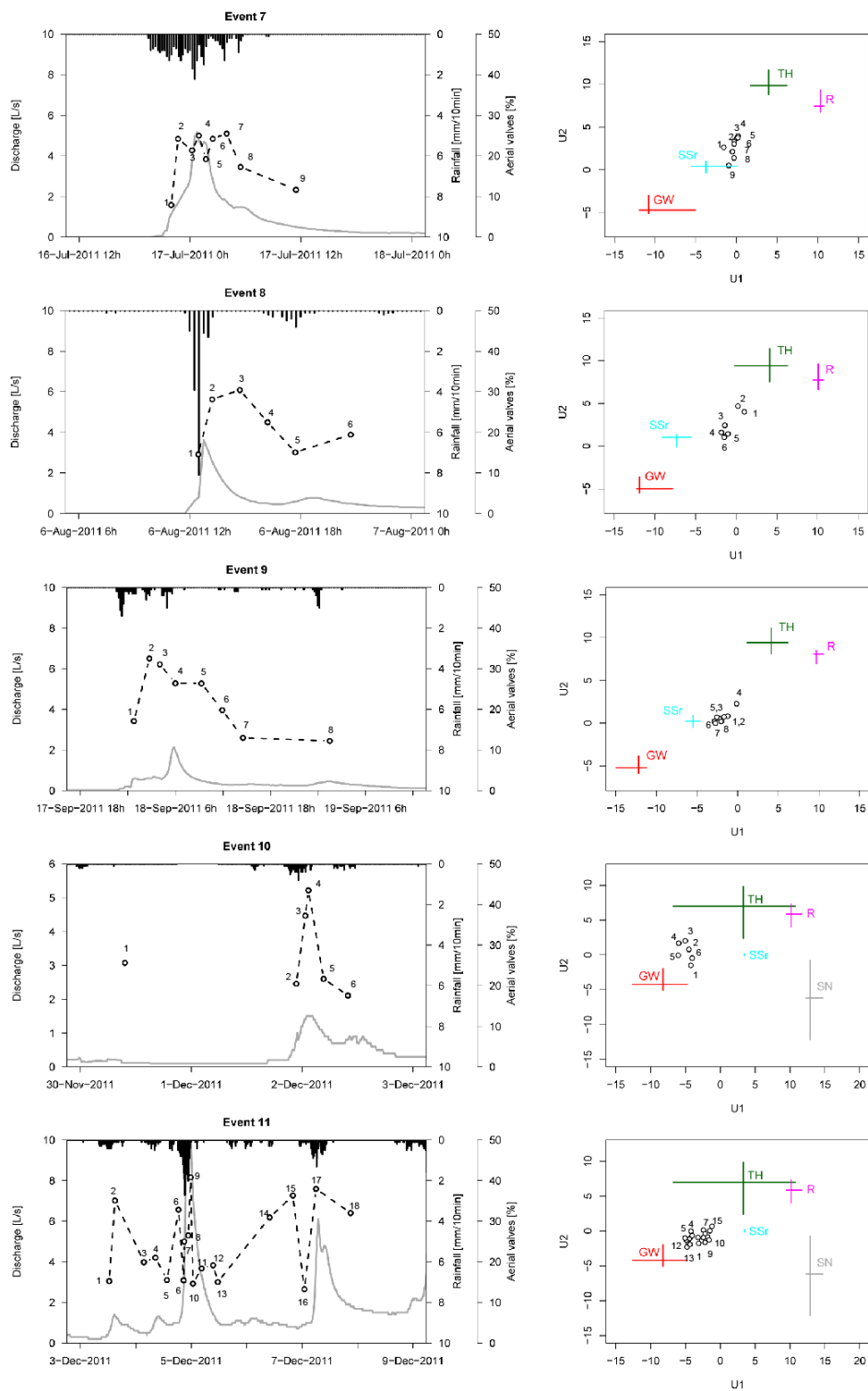


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 2 Figure 7. (a) U1-U2 mixing diagram of stream water tracers (black circles; AS + SW in
 3 Figure 5) and (b) zoom into the U1-U2 mixing diagram showing event peakflow stream water
 4 samples (black squares; numbers identify storm events in Figure 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.



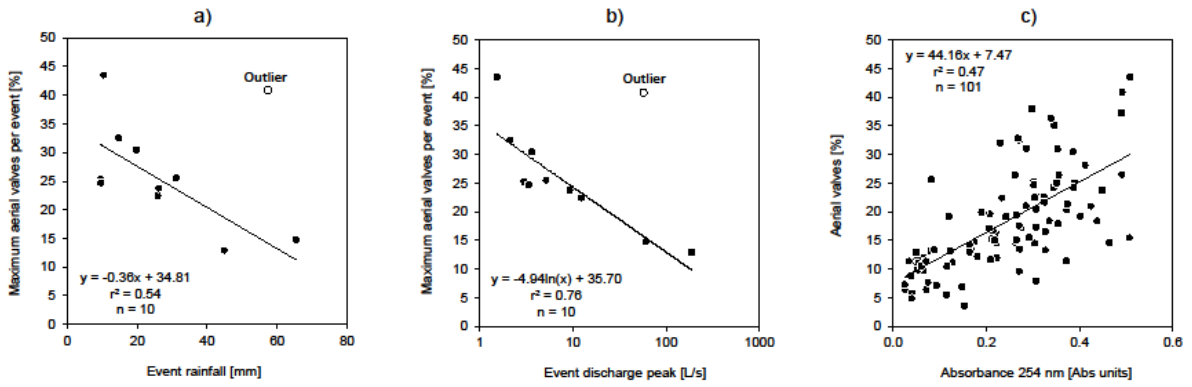
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2 Figure 9. Hydrograph, hyetograph and percentage of aerial valves in the stream water for the
 3 events 7-11 in the Weierbach catchment (left), and U1-U2 mixing diagrams for each event.
 4 End-members are rainfall (R), throughfall (TH), snow (SN), 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.