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Hydrological connectivity as indicated by transport of diatoms through the riparian–stream system

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Received: 20 January 2015 – Accepted: 29 January 2015 – Published: 24 February 2015

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Published by Copernicus Publications on behalf of the European Geosciences Union.

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Abstract

Diatoms (*Bacillariophyta*) are one of the most common and diverse algal groups (ca. 200 000 species, $\approx 10\text{--}200\ \mu\text{m}$, unicellular, eukaryotic). Here we investigate the potential of terrestrial and aerophytic diatoms (i.e. diatoms nearly exclusively occurring outside water bodies, on wet, moist or temporarily dry places) to infer surface hydrological connectivity between hillslope–riparian–stream (HRS) landscape units during storm runoff events. We present data from the Weierbach catchment ($0.45\ \text{km}^2$, NW Luxembourg) that quantifies the relative abundance of terrestrial and aerophytic diatom species on hillslopes and in riparian zones (i.e. surface soils, litter, bryophytes and vegetation) and within streams (i.e. stream water, epilithon and epipelton). We tested the hypothesis that different diatom species assemblages inhabit specific moisture domains of the catchment (i.e. HRS units) and, consequently, the presence of certain species assemblages in the stream during runoff events offers the potential for recording if there was or not hydrological connectivity between these domains. We found that a higher percentage of terrestrial and aerophytic diatom species was present in samples collected from the riparian and hillslope zones than inside the stream. However, diatoms were absent on hillslopes covered by dry litter, limiting their use to infer hillslope–riparian zone connectivity in some parts of the catchment. Our results also showed that terrestrial and aerophytic diatom abundance in the stream increased systematically during all sampled events ($n = 11$, 2010–2011) in response to incident precipitation and increasing discharge. This transport of terrestrial and aerophytic diatoms during events suggested a rapid connectivity between the soil surface and the stream. Diatom transport data was compared to two-component hydrograph separation, and end-member mixing analysis (EMMA) using stream water chemistry and stable isotope data. This research suggests that diatoms were likely sourced exclusively from the riparian zone, since it was not only the largest terrestrial and aerophytic diatom reservoir, but also riparian zone water was a major streamflow source during rainfall events under both wet and dry antecedent condition.

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1 Introduction

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

Despite their usefulness, chemical and isotope tracer-based hydrograph separations suffer from inherent conceptual limitations (Richey et al., 1998; Burns, 2002). For instance, end-member selection (Hooper et al., 1990), the number of tracers employed (Barthold et al., 2011) and spatial–temporal variation in end-member chemistry (Inamdar et al., 2013) have been shown to influence runoff source apportionment. Perhaps most problematic is that no tracer approach yet allows for unequivocal evidence of hillslope–riparian–stream connectivity. This has been identified as perhaps the key feature for improving our understanding of water origin and the processes that sustain stream flow (Jencso et al., 2010). Consequently, new techniques are desperately needed to gain a process-based understanding of hydrological connectivity (Bracken et al., 2013).

Here we build on recent work by Pfister et al. (2009) and Wetzel et al. (2013) to examine the use of terrestrial and aerophytic diatoms (i.e. diatoms nearly exclusively occur-

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ring outside water bodies, and on wet, moist or temporarily dry places; Van Dam et al., 1994), as natural tracers to infer connectivity in the hillslope–riparian–stream (HRS) system. Diatoms are one of the most common and diverse algal groups (ca. 200 000 species; Round et al., 1990). Due to their small size (~ 10 – $200 \mu\text{m}$; Mann, 2002), they can be easily transported by flowing water within or between elements of the hydrological cycle (Pfister et al., 2009). Diatoms are present in most terrestrial habitats and their diversified species distributions are largely controlled by physio-geographical factors (e.g. light, temperature, pH and moisture) and anthropogenic pollution (Dixit et al., 2002; Ector and Rimet, 2005).

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

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

2 Study area

Our study site is the Weierbach catchment (0.45 km², 49°49' N 5°47' E), a sub-catchment of the Attert River and located in the North Western part of the Grand Duchy of Luxembourg (Fig. 1). The region is known as the Oesling, an elevated sub horizontal plateau cut by deep V-shaped valleys and with averaging altitudes ranging between 450 and 500 m.

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

The geology of the catchment is dominated by Devonian schists, phyllades and quartzite. Soil depths are shallow (< 1 m) and dominated by Dystric Cambisols. Soil texture is dominated by silt mixed with gravels. The schist bedrock is relatively impermeable while the soil surface exhibits high infiltration rates and high storage capacity (van den Bos et al., 2006; Juilleret et al., 2011; Wrede et al., 2014).

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

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3 Methodology

Table 1 shows a summary of collection methods, sampling resolution and locations in the Weierbach catchment. Stream water depth at the catchment outlet was measured using a differential pressure transducer at a 15 min interval (ISCO 4120 Flow Logger) (Fig. 1). Stream conductivity at the outlet was also measured at 15 min intervals using a conductivity meter (WTW). Rainfall was measured with a tipping bucket rain gauge (52 203 model, manufactured by Young, Campbell Scientific Ltd.). One rain gauge was installed within a small clearing of the study catchment (see Fig. 1), and another one installed in an open area at the Roodt meteorological station, located ≈ 3.5 km distant from the Weierbach ($49^{\circ}48' N 5^{\circ}47' E$). A ten bottle sequential rainfall sampler (for later precipitation chemistry and isotope analysis) was installed at the rain gauge located within the Weierbach (modified from Kennedy et al., 1979). Four groundwater wells (depths ~ 90 mm) were instrumented with real-time TD-Divers data loggers (Schlumberger Water Services) and WTW conductivity meters – each recording at 15 min intervals.

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

3.1 Water sampling and laboratory methods

Fortnightly, cumulative rainfall (R) and throughfall samples under deciduous trees (TH1) and coniferous trees (TH2) were collected using conical, volumetric rain gauges. Three automatic water samplers (ISCO 3700 FS and 6712 FS) were installed immediately upstream of the weir to collect stream water samples (AS) frequently (0.5 to 4 h) during storm events. Sampling was triggered by flow conditions. Stream water at the catchment outlet (SW) and wells (GW1 to GW4) were sampled fortnightly, as well as

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prior to, during, and following precipitation events. Soil water was sampled fortnightly using Teflon suction lysimeters, installed at three locations (deciduous hillslope – SS1 – and coniferous hillslope – SS2 – and riparian zone – SS3), with three soil depths for each location: 10 cm for the organic layer (Ah horizon), 20 and 60 cm for the mineral layers (B and C horizons). Overland flow (OF) that occurred on lower hillslope positions was sampled using 1 and 2 m long gutters sealed to the soil surface, which diverted surface runoff to 1 or 2 L plastic, blackened (to prevent light penetration which causes diatom growth) water bottles. Note that what we refer as OF might in fact originate within the forest litter layer (Buttle and Turcotte, 1999; Sidle et al., 2007). All gutters were covered to avoid direct sampling of precipitation. Gutters were regularly cleaned with Milli-Q water to avoid diatoms growth on their surfaces.

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

3.2 Diatom sampling, sample preparation and analysis

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

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

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

During the same catchment-wide campaigns, epilithic (in-stream stone substrata) and epipelic (in-stream sediment or soil substrata) samples were also collected, treated and counted following the European standards CEN 13946 and CEN 14407 (European

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Committee for Standardization, 2003, 2004). For epilithic samples a minimum of five stones from the main flow and well-lit stream reaches, were brushed to collect the diatom biofilm, while epipelic samples were collected by disturbing small pools with sediment bottoms and then pipetting a superficial layer of 5–10 mm of sediment from reach pools.

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

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

3.3 Hydrograph separation

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

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Spatial end-member contributions to stream water were explored using end-member mixing analysis (EMMA) (Christophersen and Hooper, 1992), which assumes (i) the stream water is a mixture of end-member solutions with a fixed composition, (ii) the mixing model is linear and relies on hydrodynamic mixing, (iii) the solutes used as tracers are conservative, and (iv) the end-member solutions are distinguishable from one another. We applied the diagnostic tools of Hooper (2003), which have been recently applied in the literature (James and Roulet, 2006; Ali et al., 2010; Barthold et al., 2011; Neill et al., 2011; Inamdar et al., 2013). Our approach followed three main steps:

- i. We identified tracers that exhibit conservative linear mixing assuming that stream water chemistry is controlled by physical mixing and not by equilibrium mixing (Christophersen and Hooper, 1992; Hooper, 2003; Liu et al., 2008). Hooper (2003) suggested that conservative and linear mixing of tracers can be evaluated using bivariate scatter plots. In this study, stream water concentrations (of all samples collected during storm events and baseflow at the catchment outlet) were considered conservative when they exhibited at least one linear trend with one other tracer (i.e. $r^2 > 0.5$, p value < 0.01) (James and Roulet, 2006; Ali et al., 2010; Barthold et al., 2011).
- ii. We performed a principal component analysis (PCA) on the stream water data. The PCA was applied on the correlation matrix of the standardized values of tracers selected in step (i) (i.e. by subtracting the mean concentration of each solute and dividing by its SD) (Christophersen and Hooper, 1992). For each water tracer, residuals were defined by subtracting the original value from its orthogonal projection. A “good” mixing subspace was indicated by a random pattern of residuals plotted against the concentration of the original values. On the contrary, structure or curvature in the subspace indicates violation against one of the assumptions of the EMMA approach (i.e. solutes do not mix conservatively) (Hooper, 2003). Eigenvectors were retained until there was no structure to the residuals. Stan-

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

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

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

4.2 Hydrograph separation

Two-component hydrograph separation results using $\delta^{18}\text{O}$ concentrations (i.e. pre-event water vs. event water) showed that, in winter, when the catchment was wet and flow response was double-peaked, the first peak was formed mainly by event water.

This contrasted with the delayed peak that was dominated by pre-event water. For instance, the first peak of the November 2010 event showed a 50% event water contribution, whereas the second delayed peak only 16% (Fig. 4b). When the catchment was dry, the response consisted of one sharp peak composed mainly of event water (Fig. 4a). Event-water contributions of 27 and 45% were estimated for two storm events that occurred in June 2011.

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

A Principal Components Analysis (PCA) analysis was performed on the correlation matrix of stream concentrations for the ten selected tracers. The first three principal components explained 91.3% of the variance in stream concentrations and were selected to generate a three-dimensional mixing space (U space, Table 2). Plots of residuals of each solute plotted against observed concentrations suggested that 3 components were needed to obtain a well-defined mixing subspace. End-member tracer concentrations were then projected into the mixing space (Fig. 7). All stream water samples plotted inside the mixing domain defined by the end-members. Rainfall, throughfall, soil

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water and riparian water end-members plotted in the upper right quadrant of the U1-U2 mixing space (Fig. 7a). Shallow groundwater samples were located in the lower left quadrant and snow in the lower right quadrant. Overland flow plotted in the upper left quadrant and was located furthest away from stream water samples and with largest interquartile ranges. Most of the stream water samples were clustered in the immediate vicinity of the riparian water samples, half-way between the throughfall and the groundwater samples. Snow seems to contribute to some stream water samples that placed slightly move toward the lower right quadrant (Fig. 7a). The large distance between stream water and overland flow samples suggests a minor role of the latter in total runoff generation. Event peakflow samples are highlighted in Fig. 7b. In general, results show that when the catchment was wet, there was a higher contribution of groundwater to streamflow (events 1–2 and 10–11) than when the catchment antecedent condition was dry (events 3–9). To the contrary, a much higher contribution of throughfall was estimated during summer (events 5–8), when the pre-storm catchment state was dry.

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

water samples now plotted in an intermediate position between throughfall and riparian water (with the exception of event 3, which still has a significant groundwater contribution). Note that overland flow did not occur and the soils were dry during these spring and summer events.

4.3 Seasonal and geographic trends in terrestrial and aerophytic diatoms in the hillslope–riparian–stream system

The qualitative and semi-quantitative analysis of diatom microflora revealed 230 taxa in the Weierbach catchment. Diatom communities from samples collected during the catchment-wide campaigns in the streambed (i.e. epilithon, epipelon and stream water samples) during base flow were usually composed of species from oligotrophic environments, mainly occurring in water bodies, but also rather regularly on wet and moist surfaces (i.e. Riparian zone hydrological functional unit of Pfister et al. (2009), such as *Achnanthes saxonica* Krasske ex Hustedt, *Achnantheidium kranzii* (Lange-Bertalot) Round and Bukthiyarova, *Fragilariforma virescens* (Ralfs) D.M. Williams and Round, *Eunotia botuliformis* F. Wild, Nörpel and Lange-Bertalot, and *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot). Important seasonal changes in relative abundance of terrestrial and aerophytic diatoms amongst the sampled habitats were not observed (Table 3). No diatom valves were found in groundwater or rainfall samples.

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

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diatoms in streamflow were immediately before peakflow), and counter-clockwise hysteretic loops during dry conditions (the greater percentages were immediately after peakflow).

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

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

A significant correlation between percentage of terrestrial and aerophytic diatoms with UV absorbance at 254 nm was found ($r^2 = 0.55$, $p < 0.05$, $n = 76$, Fig. 9c). UV absorbance at 254 nm can be considered a proxy of DOC. During rainfall events in the Weierbach catchment, the relative abundance of terrestrial and aerophytic diatoms was associated with increased DOC concentrations in the stream. A similar trend was observed with K^+ ($r^2 = 0.25$, $p < 0.05$, $n = 76$), which is also associated with organic

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matter content. The relative abundance of terrestrial and aerophytic diatoms was not correlated with any other tracers.

5 Discussion

5.1 Can terrestrial and aerophytic diatoms detect hydrological connectivity within the hillslope–riparian–stream system?

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

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

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connectivity in some parts of the Weierbach catchment because of a limited diatom reservoir on hillslopes covered by dry litter.

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

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

Even though terrestrial and aerophytic diatoms do not live in microhabitats with flowing water, they were found in stream water samples during baseflow conditions preceding storm events (Table 3). This indicated that the “stock” of terrestrial and aerophytic diatoms in the catchment before the sampled events was not completely exhausted during previous events. Similar conclusions were drawn by Coles et al. (2015), who examined diatom population depletion effects during rainfall and found that while terrestrial and aerophytic diatom populations in the riparian zone were depleted in response to rainfall disturbance, rainfall was unlikely to completely exhaust the diatom reservoir.

We hypothesize that the transport of diatoms from the riparian zone to the stream might take place either through (i) a network of macropores in the shallow soils of the riparian zone or (ii) overland flow in the riparian zone. The potential for diatoms to be transported through the subsurface matrix was investigated using fluorescent diatoms

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and soil columns by Tauro et al. (2015). Results demonstrated that sub-surface transport of diatoms through the sub-surface matrix was unlikely. However, the potential for transport of diatoms through heterogeneous macropore networks remains unexplored. The increased relative abundance of terrestrial and aerophytic diatoms in the stream event water could also be explained by yet undocumented, surface or near-surface pathways.

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

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

During all sampled events the relative abundance of terrestrial and aerophytic diatoms increased with discharge indicating hydrological connectivity between the riparian zone and the stream. These findings are consistent with the hydrograph separation

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5 results. Terrestrial and aerophytic diatoms could reach the stream as saturated areas expand during rainfall events. Accordingly, we found a significant correlation between percentage of terrestrial and aerophytic diatoms with UV absorbance (proxy of DOC). DOC concentrations associated with runoff storm often come mainly from the near-

10 stream riparian zones (Boyer et al., 1997). Controls on surface saturated and subsurface mixing processes are currently being investigated in the Weierbach riparian zone using infrared imagery and groundwater metrics (Pfister et al., 2010). Hydrological connectivity between hillslopes and the stream has also been previously defined by water table connections between the hillslope and the riparian zone (Vidon and Hill, 2004; Ocampo et al., 2006; Jencso et al., 2010; McGuire and McDonnell, 2010). While our results showed that overland flow did not occur on hillslopes during most sampled events, the VWC measurements and timing of the hydrograph response suggest that subsurface hydrological connectivity along the hillslope–riparian–stream system occurs during wet catchment conditions (Fig. 3). Hence, if terrestrial and aerophytic diatoms found on the moss-covered hillslopes (we did not find any diatom valves on hillslopes covered by dry litter), might reach the stream through deeper sub-surface macropore flowpaths remains unknown. Others have demonstrated that tracer transport can occur at larger time scales that extend beyond individual events (McGuire and McDonnell, 2010). Whether this may also be true for diatoms remains to be explored.

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

25 Storm hydrograph separation using stable isotope tracers has resulted in major advances in catchment hydrology. However, despite their usefulness, these methods do not provide unequivocal evidence of hydrological connectivity in the HRS system. This is mainly due to inherent conceptual limitations (Richey et al., 1998; Burns, 2002). In comparison, diatoms can provide evidence of riparian–stream connectivity. But further research is needed to better understand diatom transport processes (and associated water flowpaths) in headwater catchments. Future studies should focus on expanding

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

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

The need to account for the temporal variability in end-member chemistry and to collect high-frequency data on both – stream water as well as potential runoff end-
25 members – has been well-recognized (Inamdar et al., 2013). As noted by Tetzlaff et al. (2010), seasonality should also be considered when using living organisms to trace water flowpaths. Diatom end-members must be sampled seasonally in order to ensure that populations have not undergone demographic changes. Indeed, this increases the sampling needs and the overall laboratory procedures of an already

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time-consuming approach (i.e. sampling, pre-treating the samples, mounting permanent slides and diatom identification). A potential alternative to reduce processing time is to develop new techniques such as to dye diatom valves and use them to trace water flowpaths (see Tauro et al., 2015). The use of dyed diatoms under field conditions for experimental hydrology remains unexplored.

6 Conclusions

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

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Acknowledgements. Funding for this research was provided by the Luxembourg National Research Fund (FNR) in the framework of the BIGSTREAM (C09/SR/14), ECSTREAM (C12/SR/40/8854) and CAOS (INTER/DFG/11/01) projects. We gratefully acknowledge Delphine Collard for technical assistance in diatom samples treatment and preparation, François Barnich for the water chemistry analyses, and Jean-François Iffly, Christophe Hissler, Jérôme Juilleret and Julian Klaus for their constructive comments on the project and technical assistance in the field.

References

- Ali, G. A. and Roy, A. G.: Shopping for hydrologically representative connectivity metrics in a humid temperate forested catchment, *Water Resour. Res.*, 46, W12544, doi:10.1029/2010WR009442, 2010.
- Ali, G. A., Roy, A. G., Turmel, M. C., and Courchesne, F.: Source-to-stream connectivity assessment through end-member mixing analysis, *J. Hydrol.*, 392, 119–135, doi:10.1016/j.jhydrol.2010.07.049, 2010.
- Barthold, F. K., Tyralla, C., Schneider, K., Vaché, K. B., Frede, H. G., and Breuer, L.: How many tracers do we need for end member mixing analysis (EMMA)? A sensitivity analysis, *Water Resour. Res.*, 47, W08519, doi:10.1029/2011WR010604, 2011.
- Beven, K.: TOPMODEL: a critique, *Hydrol. Process.*, 11, 1069–1085, doi:10.1002/(SICI)1099-1085(199707)11:9<1069::AID-HYP545>3.0.CO;2-O, 1997.
- Bonell, M.: Selected challenges in runoff generation research in forests from the hillslope to headwater drainage basin scale, *J. Am. Water Resour. As.*, 34, 765–785, doi:10.1111/j.1752-1688.1998.tb01514.x, 1998.
- Boyer, E. W., Hornberger, G. M., Bencala, K. E., and Mcknight, D. M.: Response characteristics of DOC flushing in an alpine catchment, *Hydrol. Process.*, 11, 1635–1647, doi:10.1002/(SICI)1099-1085(19971015)11:12<1635::AID-HYP494>3.0.CO;2-H, 1997.
- Bracken, L. J. and Croke, J.: The concept of hydrological connectivity and its contribution to understanding runoff-dominated geomorphic systems, *Hydrol. Process.*, 21, 1749–1763, doi:10.1002/hyp.6313, 2007.

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- Bracken, L. J., Wainwright, J., Ali, G. A., Tetzlaff, D., Smith, M. W., Reaney, S. M., and Roy, A. G.: Concepts of hydrological connectivity: research approaches, pathways and future agendas, *Earth-Sci. Rev.*, 119, 17–34, doi:10.1016/j.earscirev.2013.02.001, 2013.
- Burns, D. A.: Stormflow-hydrograph separation based on isotopes: the thrill is gone – what's next?, *Hydrol. Process.*, 16, 1515–1517, doi:10.1002/hyp.5008, 2002.
- Buttle, J. M.: Fundamentals of small catchment hydrology, in: *Isotope Tracers in Catchment Hydrology*, edited by: Kendall, C. and McDonnell, J. J., Elsevier, Amsterdam, 1–49, 1998.
- Buttle, J. M. and Turcotte, D. S.: Runoff processes on a forested slope on the Canadian Shield, *Nord. Hydrol.*, 30, 1–20, doi:10.2166/nh.1999.001, 1999.
- Chen, X., Bu, Z., Yang, X., and Wang, S.: Epiphytic diatoms and their relation to moisture and moss composition in two montane mires, Northeast China, *Fund. Appl. Limnol.*, 181, 197–206, doi:10.1127/1863-9135/2012/0369, 2012.
- Christophersen, N. and Hooper, R. P.: Multivariate analysis of stream water chemical data: the use of principal components analysis for the end-member mixing problem, *Water Resour. Res.*, 28, 99–107, doi:10.1029/91WR02518, 1992.
- Coles, A. E., Wetzel, C. E., Martínez-Carreras, N., Ector, L., McDonnell, J. J., Frentress, J., Klaus, J., Hoffmann, L., and Pfister, L.: Diatoms as a tracer of hydrological connectivity: are they supply limited?, *Ecology*, in review, 2015.
- Denys, L.: A check-list of the diatoms in the Holocene deposits of the Western Belgian coastal plain with a survey of their apparent ecological requirements, I. Introduction, ecological code and complete list, Professional Paper 1991/2-246, Service Géologique de Belgique-Belgische Geologische Dienst, Brussels, 41 pp., 1991.
- Dixit, S. S., Dixit, A. S., and Smol, J. P.: Diatom and chrysophyte transfer functions and inferences of post-industrial acidification and recent recovery trends in Killarney lakes (Ontario, Canada), *J. Paleolimnol.*, 27, 79–96, doi:10.1023/A:1013571821476, 2002.
- Ector, L. and Rimet, F.: Using bioindicators to assess rivers in Europe: an overview, in: *Modelling Community Structure in Freshwater Ecosystems*, edited by: Lek, S., Scardi, M., Verdonschot, P. F. M., Descy, J. P., and Park, Y. S., Springer-Verlag, Berlin, Heidelberg, 7–19, 2005.
- European Committee for Standardization: Water quality – guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers, [EN 13946:2003], European Committee for Standardization, Brussels, 2003.

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

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

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

Hooper, R. P., Christophersen, N., and Peters, N. E.: Modelling streamwater chemistry as a mixture of soilwater end-members – an application to the Panola Mountain catchment, Georgia, U.S.A., *J. Hydrol.*, 116, 321–343, doi:10.1016/0022-1694(90)90131-G, 1990.

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

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

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

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

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

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

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

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12, 2391–2434, 2015

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- Penna, D., Tromp-van Meerveld, H. J., Gobbi, A., Borga, M., and Dalla Fontana, G.: The influence of soil moisture on threshold runoff generation processes in an alpine headwater catchment, *Hydrol. Earth Syst. Sci.*, 15, 689–702, doi:10.5194/hess-15-689-2011, 2011.
- Pfister, L., Wagner, C., Vansuypeene, E., Drogue, G., and Hoffmann, L.: Atlas climatique du Grand-Duché de Luxembourg, Musée National d'Histoire Naturelle, Société des Naturalistes Luxembourgeois, Centre de Recherche Public – Gabriel Lippmann, Administration des Services Techniques de l'Agriculture, Luxembourg, 80 pp., 2005.
- Pfister, L., McDonnell, J. J., Wrede, S., Hlúbiková, D., Matgen, P., Fenicia, F., Ector, L., and Hoffmann, L.: The rivers are alive: on the potential for diatoms as a tracer of water source and hydrological connectivity, *Hydrol. Process.*, 23, 2841–2845, doi:10.1002/hyp.7426, 2009.
- Pfister, L., McDonnell, J. J., Hissler, C., and Hoffmann, L.: Ground-based thermal imagery as a simple, practical tool for mapping saturated area connectivity and dynamics, *Hydrol. Process.*, 24, 3123–3132, doi:10.1002/hyp.7840, 2010.
- Pinder, G. F. and Jones, J. F.: Determination of the ground-water component of peak discharge from the chemistry of total runoff, *Water Resour. Res.*, 5, 438–445, doi:10.1029/WR005i002p00438, 1969.
- Richey, D. G., McDonnell, J. J., Erbe, M. W., and Hurd, T. M.: Hydrograph separations based on chemical and isotopic concentrations: a critical appraisal of published studies from New Zealand, North America and Europe, *J. Hydrol. (New Zealand)*, 37, 95–111, 1998.
- Rimet, F., Ector, L., Cauchie, H. M., and Hoffmann, L.: Regional distribution of diatom assemblages in the headwater streams of Luxembourg, *Hydrobiologia*, 520, 105–117, doi:10.1023/B:HYDR.0000027730.12964.8c, 2004.
- Round, F. E., Crawford, R. M., and Mann, D. G.: *The Diatoms. Biology and Morphology of the Genera*, Cambridge University Press, Cambridge, 1990.
- Sidle, R. C., Hirano, T., Gomi, T., and Terajima, T.: Hortonian overland flow from Japanese forest plantations – an aberration, the real thing, or something in between?, *Hydrol. Process.*, 21, 3237–3247, doi:10.1002/hyp.6876, 2007.
- Sklash, M. G. and Farvolden, R. N.: The use of environmental isotopes in the study of high-runoff episodes in streams, in: *Isotope Studies of Hydrologic Processes*, edited by: Perry, E. C. J. and Montgomery, C. W., Northern Illinois University Press, Illinois, 65–73, 1982.

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Sklash, M. G., Stewart, M. K., and Pearce, A. J.: Storm runoff generation in humid headwater catchments: 2. A case study of hillslope and low-order stream response, *Water Resour. Res.*, 22, 1273–1282, doi:10.1029/WR022i008p01273, 1986.

Spence, C.: A paradigm shift in hydrology: storage thresholds across scales influence catchment runoff generation, *Geogr. Compass*, 4, 819–833, doi:10.1111/j.1749-8198.2010.00341.x, 2010.

Tauro, F., Martínez-Carreras, N., Barnich, F., Juilleret, J., Wetzel, C. E., Ector, L., Hissler, C., Frentress, J., Hoffmann, L., McDonnell, J. J., and Pfister, L.: Diatom percolation through soils, *Ecohydrology*, in review, 2015.

Tetzlaff, D., Soulsby, C., and Birkel, C.: Hydrological connectivity and microbiological fluxes in montane catchments: the role of seasonality and climatic variability, *Hydrol. Process.*, 24, 1231–1235, doi:10.1002/hyp.7680, 2010.

Tromp-van Meerveld, H. J. and McDonnell, J. J.: Threshold relations in subsurface stormflow: 1. A 147-storm analysis of the Panola hillslope, *Water Resour. Res.*, 42, W02410, doi:10.1029/2004WR003778, 2006.

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

Vacht, P., Puusepp, L., Koff, T., and Reitalu, T.: Variability of riparian soil diatom communities and their potential as indicators of anthropogenic disturbances, *Est. J. Ecol.*, 63, 168–184, doi:10.3176/eco.2014.3.04, 2014.

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

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

Van de Vijver, B. and Beyens, L.: Moss diatom communities from Ile de la Possession (Crozet, Subantarctica) and their relationship with moisture, *Polar Biol.*, 22, 219–231, doi:10.1007/s0030000050414, 1999.

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

Wetzel, C. E., Martínez-Carreras, N., Hlúbiková, D., Hoffmann, L., Pfister, L., and Ector, L.: New combinations and type analysis of Chamaepinnularia species (Bacillariophyceae) from aerial habitats, *Cryptogamie Algol.*, 34, 149–168, doi:10.7872/crya.v34.iss2.2013.149, 2013.

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

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

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

	Component	Resolution	Method	N° locations
Hydrology	Discharge	15 min	Stage-discharge rating curve	1 (outlet)
	Precipitation	15 min	Tipping bucket	2
	Water table depth	15 min	TD-driver	4
	Soil moisture	30 min	Water content reflectometer	4
	Stream conductivity	15 min	Conductivity meter	1 (outlet)
	Groundwater conductivity	30 min	Conductivity meter	2
Geochemistry and isotopes	Groundwater	Fortnightly	Manual	4
	Overland flow (hillslope)	Accum. events	Gutters	5
	Precipitation	Accum. fortnightly	Rain gauge	1
	Precipitation	~ 2.5 mm increments	Sequential rainfall sampler	1
	Snow	Sporadic	Manual	Spots
	Soil water	Accum. fortnightly	Suction cups	3
	Stream water	0.5–4 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	0.5–4 h (events)	ISCO automatic sampler	1 (outlet)
	Stream water	Monthly	Manual	1 (outlet)
	Substrates	Once per season	Manual	16

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

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

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Table 3. Percentage of terrestrial and aerophytic valves quantified at distinct zones of the Weierbach catchment.

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

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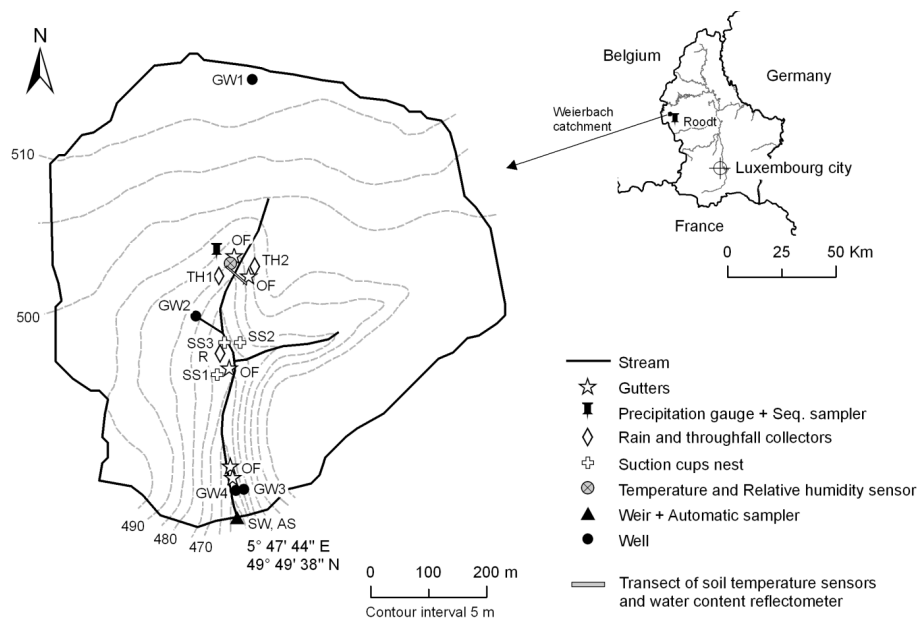


Figure 1. Detailed map of topography and instrumentation locations in the Weierbach catchment (Northwest of Luxembourg City).

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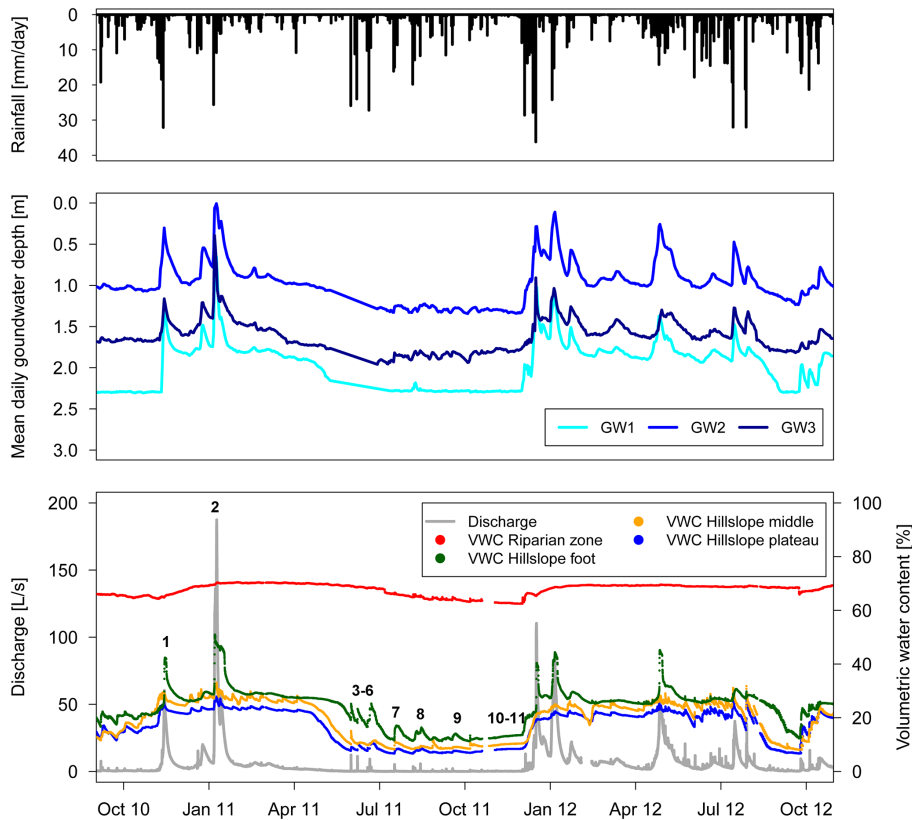


Figure 2. Soil volumetric water content measured in a transect from the hillslope plateau to the riparian zone along with corresponding water discharge (grey line) (lower plot), numbers identify sampled storm events; mean daily groundwater depth at three different locations (GW1: plateau, GW2: close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

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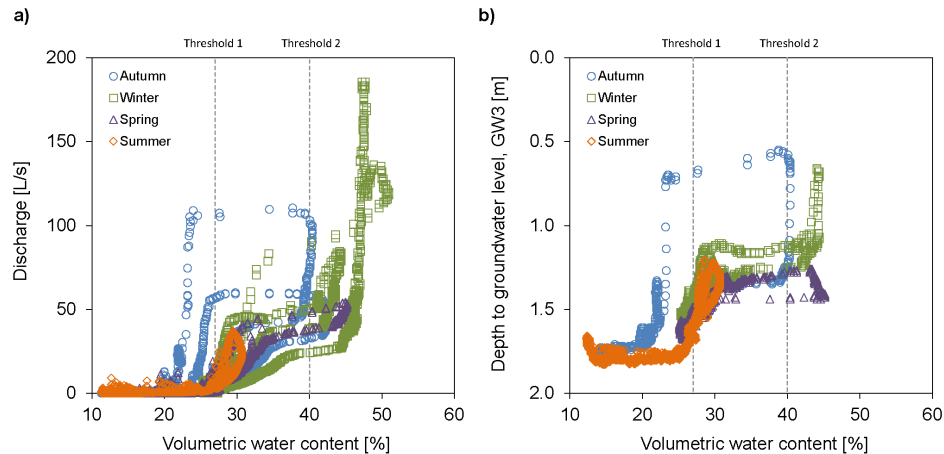


Figure 3. Relationship between (a) volumetric water content (hillslope foot) and discharge, and (b) between volumetric water content and depth to groundwater level for the period plotted in Fig. 2. Vertical dashed lines represent two threshold values (see details in the text).

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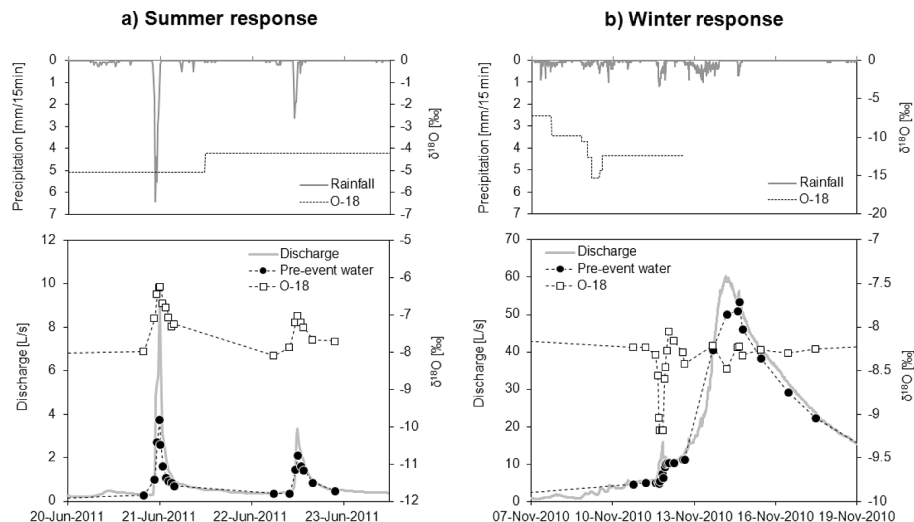


Figure 4. Two components hydrograph separation for **(a)** the 20 and 22 June 2011 events (summer response) and **(b)** the 7 November 2010 event (winter response) using $\delta^{18}O$ concentrations to identify event and pre-event water contribution to streamflow.

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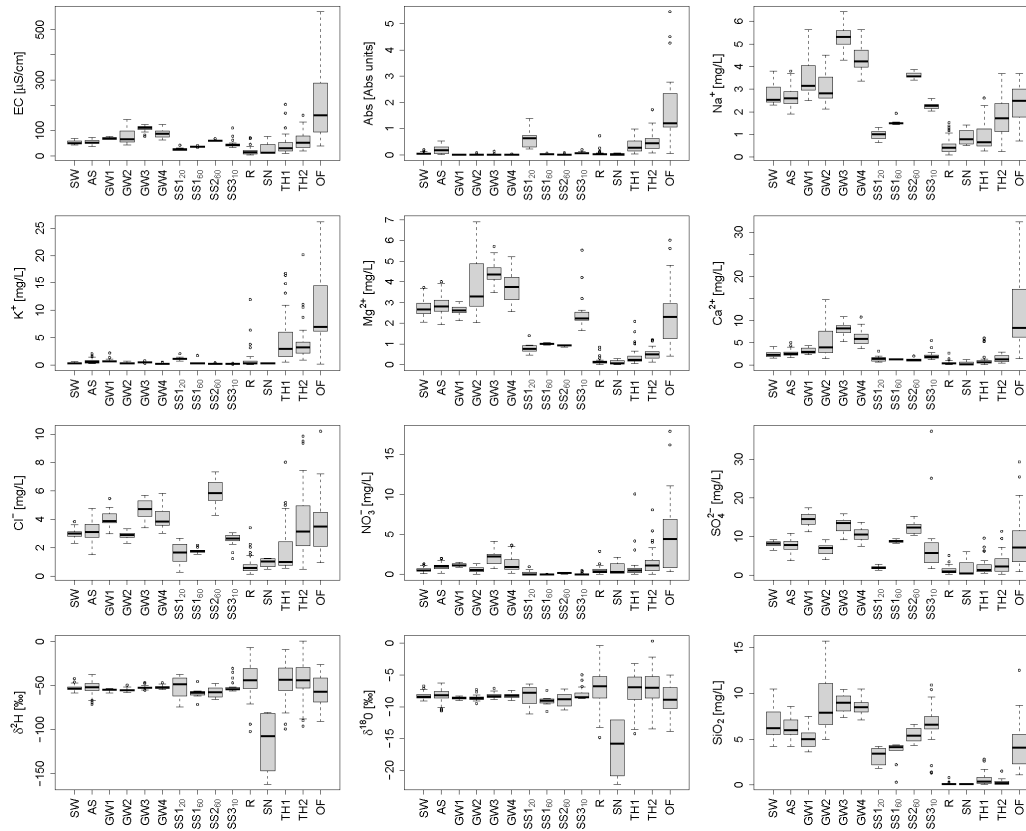


Figure 5. Boxplots of tracers measured for stream water sampled fortnightly (SW, $n = 47$) and using automatic samplers (AS, $n = 179$), groundwater (GW1, $n = 24$; GW2, $n = 49$; GW3, $n = 49$; GW4, $n = 47$), soil water (SS1₂₀, $n = 22$; SS1₆₀, $n = 10$; SS2₆₀, $n = 9$), riparian water (SS3₁₀, $n = 21$), 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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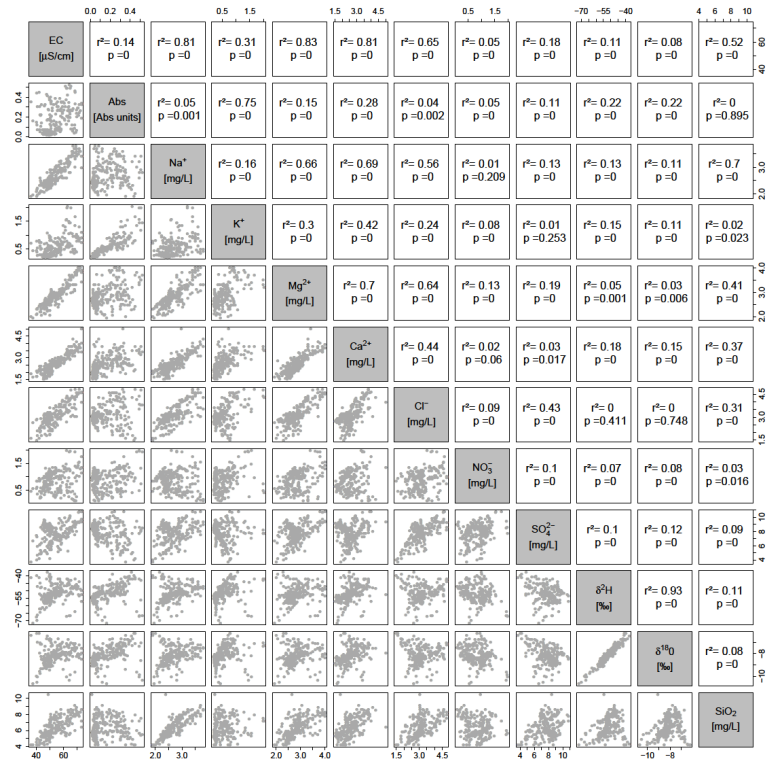


Figure 6. Bivariate solute plots of stream water chemistry and stable isotope data collected at the outlet of the Weierbach catchment ($n = 226$, i.e. 47 grab stream water samples collected fortnightly from October 2010 to September 2012 – SW – and 179 stream water samples collected during runoff events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

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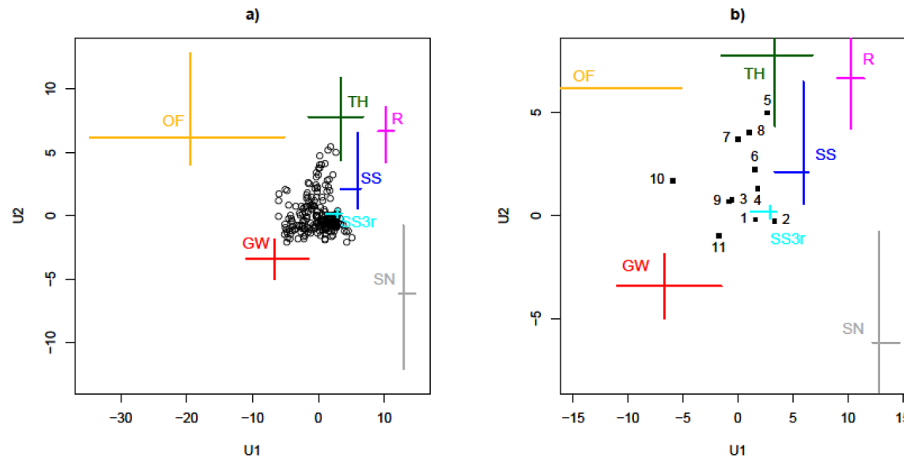


Figure 7. (a) U1–U2 mixing diagram of stream water tracers (AS + SW in Fig. 5) and (b) event peakflow stream water samples (numbers identify storm events in Fig. 2) in the U1–U2 mixing diagram. Sampling points data plotted in Fig. 5 were grouped in 7 end-members (GW: groundwater, SN: snow, SS: soil solution,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

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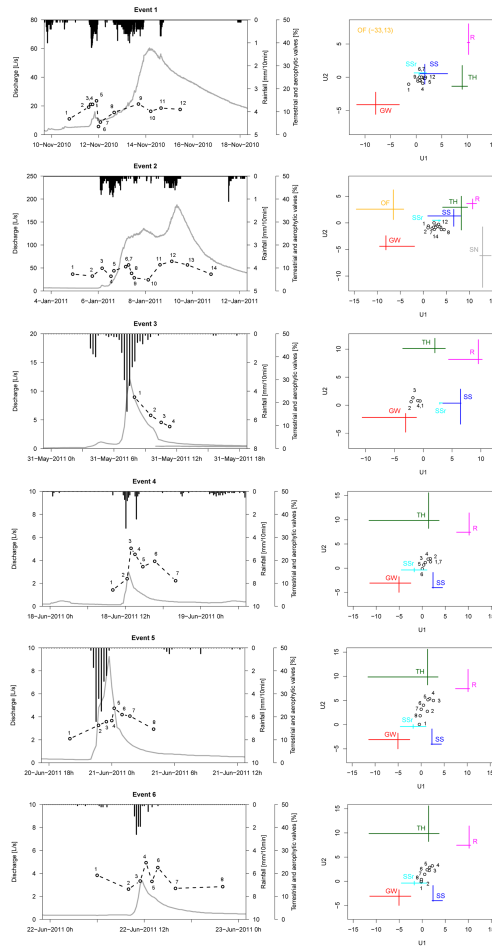


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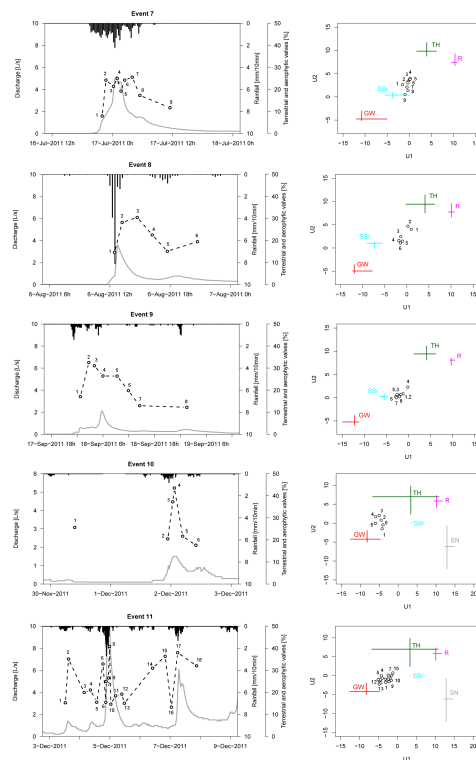


Figure 8. Hydrograph, hietograph and % of terrestrial and aerophytic valves in the stream water for the 11 sampled events in the Weierbach catchment (left panels), and U1-U2 mixing diagrams for each event. End-members are rainfall (R), throughfall (TH), snow (SN), soil water (SS), riparian water (SSr) and groundwater (GW). Bars represent end-member values interquartile ranges of samples collected during the month when the event occurred, as well as the previous and following month, with the objective of accounting for end-member temporal variability.

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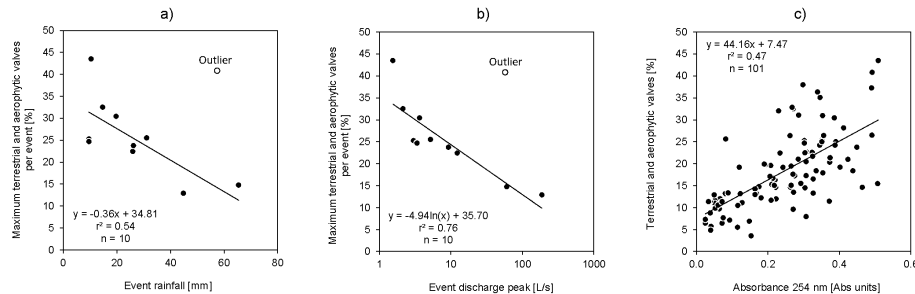


Figure 9. Correlations between **(a)** maximum percentage of terrestrial and aerophytic valves in the stream water per event and event rainfall, **(b)** maximum percentage of terrestrial and aerophytic valves in the stream water per event and maximum event discharge, and **(c)** percentage of terrestrial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

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