

# Examination of aerial diatom flushing across watersheds in Luxembourg, Oregon and Slovakia for tracing episodic hydrological connectivity

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**Abstract:** Hydrological processes research remains a field that is severely measurement limited. While conventional tracers (geochemicals, isotopes) have brought extremely valuable insights into water source and flowpaths, they nonetheless have limitations that clearly constrain their range of application. Integrating hydrology and ecology in catchment science has been repeatedly advocated as offering potential for interdisciplinary studies that are eventually to provide a holistic view of catchment functioning. In this context, aerial diatoms have been shown to have the potential for detecting of the onset/cessation of rapid water flowpaths within the hillslope-riparian zone-stream continuum. However, many open questions prevail as to aerial diatom reservoir size, depletion and recovery, as well as to their mobilisation and transport processes. Moreover, aerial diatoms remain poorly known compared to freshwater species and new species are still being discovered. Here, we ask whether aerial diatom flushing can be observed in three catchments with contrasting physiographic characteristics in Luxembourg, Oregon (USA) and Slovakia. This is a prerequisite for qualifying aerial diatoms as a robust indicator of the onset/cessation of rapid water flowpaths across a wider range of physiographical contexts. One species in particular, (*Hantzschia amphioxys* (Ehr.) Grunow), was found to be common to the three investigated catchments. Aerial diatom species were flushed, in different relative proportions, to the river network during rainfall-runoff events in all three catchments. Our take-away message from this preliminary examination is that aerial diatoms appear to have a potential for tracing episodic hydrological connectivity through a wider range of physiographic contexts and therefore serve as a complementary tool to conventional hydrological tracers.

**Keywords:** Aerial diatoms; Hillslope-riparian zone-stream continuum; Hydrological connectivity.

## INTRODUCTION

Over the past decades, conventional tracers used in experimental hydrology have considerably improved our understanding of time- and geographic sources of streamwater (Hooper et al., 1990; Martinec, 1975; Sklash and Folvolden, 1979). However, these approaches also have well-known limitations (e.g. Burns, 2002; McGuire and McDonnell, 2006) and hydrological processes research remains a field that is severely measurement limited.

In this context, the interface between hydrological and ecological sciences has been recognized as a major research frontier. How hydrological systems control ecological systems and vice-versa is a grand challenge in the emerging field of ecohydrology (Kundzewicz, 2012). This question eventually bears considerable potential for bringing new momentum to our understanding of questions related to water sources and flowpaths (Hannah et al., 2007; Tetzlaff et al., 2007).

In order to overcome limitations inherent to conventional geochemical tracers (e.g. scale constraints, incomplete mixing), recent work by Pfister et al. (2009) has explored the potential for aerial diatoms (i.e. species mainly occurring on wet and moist or temporarily dry places or species occurring nearly exclusively outside water bodies) to serve as a biological tracer of hydrological processes. Diatoms are unicellular eukaryotic algae and they are known to be ubiquitous (Round et al., 1990). Their habitats cover both the aerial and aquatic environment. Aerial diatoms are sensitive to a large variety of environmental

variables, such as light, moisture conditions, temperature, water velocity, salinity, pH, oxygen, inorganic nutrients (carbon, phosphorous, nitrogen, silica), organic carbon and organic nitrogen (Van Dam et al., 1994). Diatoms have been used frequently in geological, archaeological and water quality research (Smol and Stoermer, 2010; Van Dam et al., 1994).

Recent work by Pfister et al. (2009) has shown the potential for aerial diatoms – being flushed to the stream during rainfall-runoff events – to reveal rapid flowpaths connectivity within the hillslope-riparian zone-stream continuum of a small schistous catchment in Luxembourg. In a similar context, diatoms have been used recently as an indicator of tile drainage flow in a lowland catchment in Germany (Wu et al., 2014).

Many questions still exist regarding aerial diatoms and their use as tracers for hydrological processes across different environments (i.e. physiographic settings). Since diatom research has focused mainly on freshwater species (e.g. Bottin et al., 2014; Hlúbiková et al., 2014), new aerial species of diatoms are regularly discovered (Falasco et al., 2015; Furey et al., 2011, 2012; Johansen, 2010; Veselá and Johansen, 2009; Wetzel et al., 2014; Zimmermann et al., 2014). Open questions relate to aerial diatom reservoir size, depletion and recovery rates in case of precipitation events (Coles et al., 2015). Exploratory work in the schistous Weierbach catchment (Luxembourg) has shown that riparian zones hold the largest aerial diatom reservoirs, in comparison to hillslopes (Martínez-Carreras et al., 2015). Mobilisation and (surface or sub-surface)

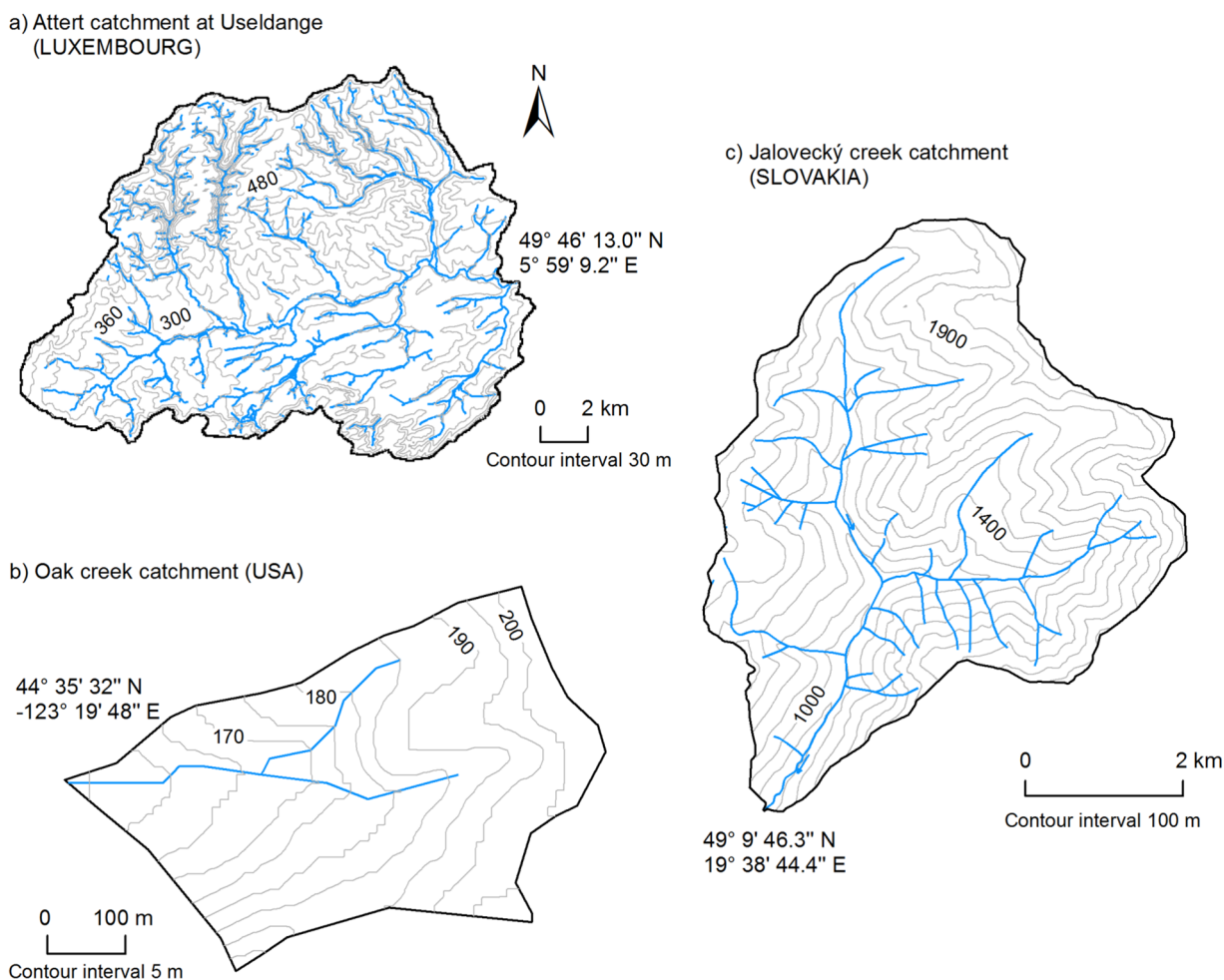
transport processes of aerial diatoms during rainfall-runoff events are currently also investigated (Tauro et al., 2015).

Here, we explore the potential for aerial diatoms to trace rapid water flowpaths in three different environments in Oregon, Slovakia and Luxembourg. Climate conditions are contrasting between the Oregon site (with Mediterranean climate, with wet, mild winters and exceptionally dry, cool summers), the Luxembourg site (with a temperate oceanic climate, characterised by mild and wet winters) and the Slovak site (mountain continental climate with warm summers, cold winters and significant influence of seasonal snow cover). We hypothesize that aerial diatoms are present in all three settings and that they can serve for identifying the onset/cessation of rapid water flowpaths connectivity. More specifically, we ask whether aerial diatom flushing to the stream can be observed in all three catchments. This is a prerequisite for qualifying aerial diatoms as a robust indicator of the onset/cessation of rapid water flowpaths – and their connectedness to the stream – across a wider range of physiographical contexts. Our approach consists in: (1) collecting drift diatoms before, during and after rainfall-runoff events in all three catchments, (2) determining the presence/absence of aerial species in all samples, and (3) relate hydrological descriptors (e.g. precipitation intensity, stormflow coefficients) to aerial diatom contributions to the storm hydrographs.

### STUDY AREAS

The Attert River basin is located in the northwest of Luxembourg (Fig. 1). It covers an area of 249 km<sup>2</sup> and lies on the contact zone of the schistose Ardennes massif and the sedimentary Paris Basin. The Ardennes massif is a high sub-horizontal plateau with average altitudes ranging between 450 and 500 m. It covers the northern part of the catchment and is made of Devonian schists, phyllites and quartzite. The plateau surfaces are mainly under pasture, whereas the steep valley slopes are forested with coniferous and deciduous trees. The sedimentary Paris Basin covers the middle and southern part of the basin and has its topography characterized by gently sloping lowlands. Keuper sandy marls and Jurassic sandstones dominate the lithology. Landuse is characterised by patches of forest and lands dedicated mostly to grassland and agriculture. The hydrological regime of the Attert River is pluvial oceanic (Salvia-Castellv  et al., 2005), with low flows from July to September (largely influenced by evapotranspiration losses), and high flows from December to February (dormant season). The mean annual precipitation for the study area (1954–1996) is estimated 853 mm (Pfister et al., 2000).

The Oak creek catchment is located in Benton County, OR, USA (Fig. 1). For this study, we have focused at a small sub-catch-



**Fig. 1.** Maps of a) the Attert River basin – 249 km<sup>2</sup> (Luxembourg), b) the Oak creek catchment – 0.17 km<sup>2</sup> (Oregon, USA) and c) the Jaloveck  creek catchment – 22.2 km<sup>2</sup> (Slovakia). All catchment areas are related to the outlets.

ment of 0.17 km<sup>2</sup>, with altitudes ranging from 155 m to 195 m a.s.l. The geology consists of Tertiary Siletz River Volcanics. Land use is dominated by pasture, with some farm buildings. The catchment has a large riparian zone that extends along the main stretches of the creek. The climate is Mediterranean and annual precipitation is 1109 mm (near Corvallis, Oregon – 1971–2000). For our investigations, we have temporarily installed a recording precipitation gauge and a flow logger (operating at 15 minute time-step in March 2012).

The Jalovecký creek is located in the highest part of the Carpathian Mountains, northern Slovakia (Fig. 1). The catchment covers an area of 22.2 km<sup>2</sup>. Topography is characterised by a mean slope of 30° and elevation ranges from 800 to 2178 m a.s.l. (mean elevation of 1500 m a.s.l.). Crystalline rocks (gneiss, granodiorite) covered with Quaternary moraines and loose slope sediments dominate. Land use is dominated by coniferous forest (mainly spruce), dwarf pine and alpine meadows. Mean annual precipitation in the catchment is 1562 mm and mean annual discharge is 1015 mm (data 1989–2005). Mean annual air temperature at catchment mean elevation is about 3°C. Precipitation is highest in summer (July) and minimum in winter (January). Maximum seasonal discharge occurs in May as a result of the combined effects of snowmelt and spring precipitation. Minimum discharge occurs at the end of winter (February), when precipitation fallen over the last few months (typically since December) has accumulated as seasonal snow cover.

## METHODOLOGY

We monitored precipitation and discharge in all three study catchments (15 to 60 minute time steps). Automatic samplers were installed for drift diatom collection during rainfall-runoff events on the following dates:

- Attert River (Luxembourg): 3–5 December 2011 (n = 16)
- Oak creek (Oregon): 10–14 March 2012 (n = 27)
- Jalovecký creek (Slovakia): 13–15 September 2012 (n = 8)

For all monitored events, we determined the total precipitation and discharge volumes, as well as the corresponding stormflow coefficients (determined through a linear baseflow-stormflow hydrograph separation).

Water samples taken with automatic samplers during the rainfall-runoff events were centrifuged (1250 rpm, 8 minutes) to concentrate the organic material. Subsamples were cleaned (digested) using concentrated hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and heated for 24–48 h using a sand bath. Preparations were then allowed to cool and settle for 24 h, and 80 to 90% of the supernatant was eliminated by vacuum aspiration. A volume of 1 ml of HCl (37%) was then added and the mixture was allowed to stand for 2 h, followed by three repetitions of rinsing and de-

cantation using deionized water, following the procedure CEN 13946 (European Committee for Standardization, 2003). The cleaned suspension was mounted in a permanent slide using Naphrax as mounting medium. For the semi-quantitative analyses all individuals found in random transects under light microscopy across each permanent slide (n = 55) were identified and counted, up to a minimum of 400 valves, using a Leica DMRX microscope at 1000x magnification. The laboratory procedures follow the guidance of the European standard CEN 14407 (European Committee for Standardization, 2014) for identification, enumeration and interpretation of diatom samples from running water.

Identification of diatom assemblages was based mainly on common freshwater floristic works such as Werum and Lange-Bertalot (2004) and Hofmann et al. (2011). Additional information concerning aerial diatom species were found in Petersen (1915, 1928, 1935), Hustedt (1942), Lund (1945), Brendemühl (1949), Simonsen (1987) and Ettl and Gärtner (2014).

Aerial diatom communities, defined as those communities living exposed to the air outside of lentic and lotic environments (Johansen, 2010), were considered as the categories 4 and 5 of the Van Dam et al. (1994) classification. Van Dam et al. (1994) is the only formal classification of the occurrence of freshwater diatoms in relation to moisture and is based on the knowledge of over half a century of diatom research by many authors; we used the associated hydrological units assigned by Pfister et al. (2009) to the five diatom occurrence classes defined by Van Dam et al. (1994). Specifically, we mainly expressed results as percentage (relative abundance) of aerophytic and aerial diatom valves, i.e. categories 4 and 5. The calculations were made using the OMNIDIA software (Lecoite et al., 1993), version 5.3 (updated database 2014).

## RESULTS

### Observed rainfall-runoff events

Results for the three monitored rainfall-runoff events and associated diatom drift assemblages for the three catchments located in Luxembourg, Oregon and Slovakia are shown in Table 1. While precipitation totals were rather similar and moderate in the Luxembourgish and Slovak catchments (ranging from 11.3 to 15.6 mm), the rainfall event recorded in Oregon was quite large (40.1 mm). Maximum precipitation intensity was also significantly higher in the Oak creek (6.6 mm.h<sup>-1</sup>), compared to that measured in the two other catchments (0.9 to 2.4 mm.h<sup>-1</sup>). Runoff response in the catchments was assessed against the antecedent wetness conditions and precipitation characteristics. The precipitation event triggered only marginal runoff response in the Jalovecký creek, with a stormflow coefficient of approximately 0.3%.

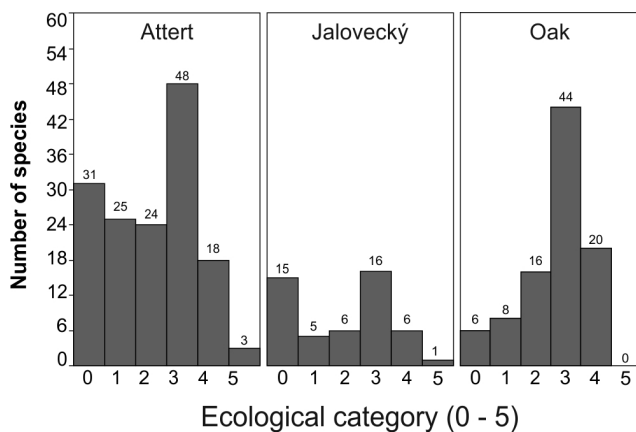
**Table 1.** Precipitation totals, maximum intensities, total runoff, stormflow and stormflow coefficients for the Attert basin, Oak creek and Jalovecký creek.

Catchment	Date event	Total precip. (mm)	Max. precip. (mm.h <sup>-1</sup> )	Total runoff volume (mm)	Stormflow volume (mm)	Stormflow coeff. (%)
Attert basin	03–05.12.2011	11.3	0.9	1.76	1.01	9.0
Jalovecký creek	13–14.09.2012	15.6	2.4	0.73	0.05	0.3
Oak creek	12–13.03.2012	40.1	6.6	9.9	6.92	17.3

Summer 2012 was extremely dry in this catchment. No runoff event occurred since mid-June, which is very unusual. Therefore, the single rainfall event on 13<sup>th</sup> September (Fig. 4) caused just a small runoff response. For the Attert River, the response was significantly higher with a stormflow coefficient of 9%. Temporal distribution of rainfall in the Attert basin (Fig. 3) was different from that in the Jaloveck  creek catchment. Several rainfall events which had occurred prior to the studied event had significantly increased catchment wetness. Thus, the runoff response to rainfall on 4<sup>th</sup> December 2011 was more pronounced. The large total rainfall and the high maximum precipitation intensity monitored in the Oak creek (Fig. 5) generated a stormflow coefficient of approximately 17%. Unlike in the other two catchments, a second flood hydrograph was triggered in the Oak creek catchment, although incident precipitation was relatively small during the second event. It could thus be assumed that the second event was dominated by delayed subsurface flow.

**Diatom assemblages**

Diatom assemblages and composition were very distinct between the three catchments in terms of species composition and functional species structure (Fig. 2). In the Attert basin in Luxembourg we found a higher species richness – i.e. species per sample – (S[number of species] = 149; n[number of samples] = 16), compared to the Oak creek in Oregon (S = 94; n = 27) and the Jaloveck  creek in Slovakia (S = 49; n = 8). Most species were classified as ‘mainly occurring in water bodies’, but also ‘regularly on wet and moist places’ (Category 3 of Van Dam et al., 1994; Fig. 2). The Attert River and the Oak creek exhibited the highest number of aerial species (Categories 4 & 5 of Van Dam et al., 1994), with 21 and 20 species respectively. In the Jaloveck  creek we only found 7 aerial species.



**Fig. 2.** Species distribution histogram according to the categories of Van Dam et al. (1994) in the Attert basin, Oak and Jaloveck  creeks. Ecological categories for moisture conditions: 0 = unknown; 1 = never, or only very rarely, occurring outside water bodies; 2 = mainly occurring in water bodies, sometimes on wet places; 3 = mainly occurring in water bodies, also rather regularly on wet and moist places; 4 = mainly occurring on wet and moist or temporarily dry places; 5 = nearly exclusively occurring outside water bodies.

This can be explained by the fact that runoff response was very small in the Jaloveck  creek. Isotopic data confirmed that runoff was dominated by the water which was stored in the

catchment before the rainfall (isotopic data not shown here). Thus, the diatom data in the Jaloveck  creek catchment represent low flow conditions, i.e. between the runoff events rather than a runoff event situation.

The maximum relative abundance of an individual species was lowest in the Attert River and in the Jaloveck  creek, in comparison to the Oak creek. In the Attert River *Hantzschia amhyoxis* (Ehr.) Grunow (2.10%) and *Navicula tenelloides* Hust. (1.57%) were the most frequent aerial diatom species, while in the Jaloveck  creek *Nupela lapidosa* (Krasske) Lange-Bert. (1.68%) was most represented (Table 2). In relative numbers, the Oak creek had more aerial species than the other two catchments, with *Nitzschia hantzschiana* Lange-Bert. (12.44%), *Mayamaea atomus* (K tz.) Lange-Bertalot (5.94%), *Caloneis tenuis* (W. Greg.) Krammer (3.17%), *Adlafia minuscula* (Grunow) Lange-Bert. (2.00%), *Hantzschia abundans* Lange-Bert. (1.49%), *Hantzschia amphioxys* (Ehr.) Grunow (1.49%), *Hantzschia vivax* (W. Smith) Grunow (1.21%), *Navicula subseminulum* Hust. (1.07%), and *Pinnularia palatina* Lange-Bert. & W. Kr ger (1.02%) as the main aerial species. Only *Hantzschia amphioxys* was common to the three catchments. A larger number of species could not be classified in the categories 1 to 5 as their ecology still remains unknown (in that case a default value of zero was attributed). The highest number of ‘unknown’ ecology (category 0) species was determined for the Attert River, where 31 species could not be classified in any of the categories (Fig. 2).

**Diatom drift**

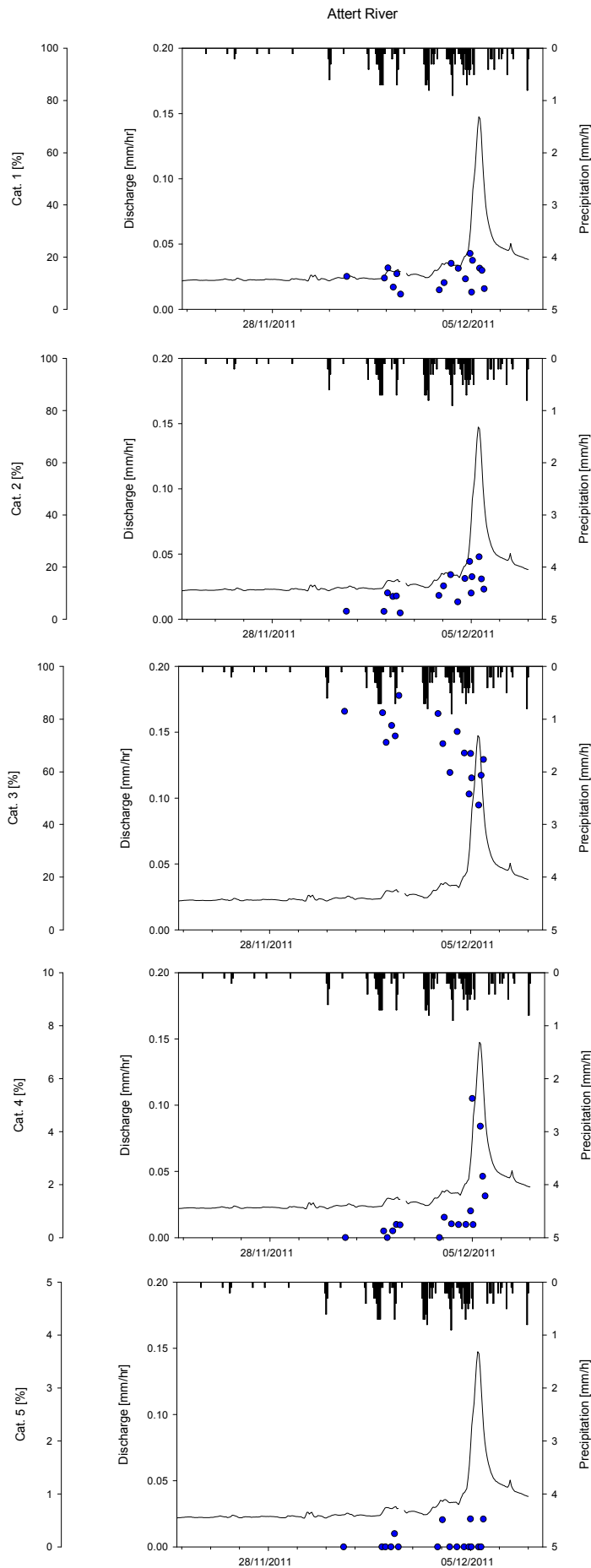
The diatom drift observed in the Attert River (Fig. 3) was largely dominated by category 3 species (aquatic to subaerial). The contribution from this category decreased from 85% at low flow conditions to approximately 55% at peak flow. Category 2 species (occasional aerophytic) increased from 5 to 20% during the runoff event. Throughout the event, category 1 species (aquatic strict) did not exhibit any specific trend (varying between 5 and 20%). Categories 4 (aerophytic strict) and 5 (terrestrial) increased from zero to 5 and 0.5% respectively during the flood event.

In the Jaloveck  creek (Fig. 4), the category 3 species (aquatic to subaerial) have dominated the diatom drift throughout the runoff event (approximately 75%). No clear trend could be observed for categories 1 (aquatic strict, ranging between 0 and 5%) and 2 (occasional aerophytic, ranging between 7 and 20%). Category 4 (aerophytic strict) gradually decreased from approximately 4% prior to the rainfall event to almost zero percent during the runoff event. Category 5 (terrestrial) did not exhibit any trend during the runoff event, with values remaining close to zero percent during most of the observation period.

In the Oak creek (Fig. 5), contributions to diatom drift were highest from category 3 (aquatic to subaerial), decreasing from 88% prior to the precipitation event to 70% during the flood peak. Category 1 (aquatic strict) exhibited a high variability, with contributions ranging from 0.5 to 7% throughout the event. Contributions from category 2 (occasional aerophytic) slightly decreased from approximately 10% prior to precipitation to almost zero during the flood event. In the Oak creek, contributions from category 4 (aerophytic strict) increased from 0.5 at low flow to 25% at peak flow. Simultaneously, contributions from category 5 (terrestrial) increased from zero to 2%.

**Table 2.** List of aerial species (categories 4 and 5 of Van Dam et al., 1994) from the three catchments in Luxembourg (Attert River), Oregon (Oak creek) and Slovakia (Jalovecký creek). Maximal relative abundance (%) values are shown and species with >1% are marked in bold.

Species	Attert basin	Jalovecký creek	Oak creek
<i>Achnanthes coarctata</i> (Bréb. ex W. Smith) Grunow	0.52		
<i>Adlafia bryophila</i> (J.B. Petersen) Gerd Moser et al.	0.52		
<b><i>Adlafia minuscula</i> (Grunow) Lange-Bert.</b>			<b>2.00</b>
<i>Caloneis molaris</i> (Grunow) Krammer			0.66
<b><i>Caloneis tenuis</i> (W. Greg.) Krammer</b>			<b>3.17</b>
<i>Chamaepinnularia obsoleta</i> (Hust.) C.E. Wetzel & Ector			0.25
<i>Diploneis</i> aff. <i>krammeri</i> Lange-Bert. & E. Reichardt			0.48
<b><i>Eolimna tantula</i> (Hust.) Lange-Bert.</b>	0.52		<b>5.49</b>
<b><i>Eunotia minor</i> (Kütz.) Grunow</b>	0.52		<b>2.79</b>
<i>Eunotia paludosa</i> Grunow			0.66
<i>Eunotia trinacria</i> Krasske	0.52		
<i>Fallacia insociabilis</i> (Krasske) D.G. Mann	0.51		
<i>Geissleria acceptata</i> (Hust.) Lange-Bert. & Metzeltin	0.52	0.98	
<b><i>Gomphonema</i> aff. <i>angustatum</i> (Kütz.) Rabenh.</b>			<b>5.17</b>
<b><i>Hantzschia abundans</i> Lange-Bert.</b>	0.52		<b>1.49</b>
<b><i>Hantzschia amphioxys</i> (Ehr.) Grunow</b>	<b>2.10</b>	0.48	<b>1.49</b>
<b><i>Hantzschia vivax</i> (W. Smith) Grunow</b>			<b>1.21</b>
<i>Humidophila perpusilla</i> (Grunow) Lowe et al.		0.65	
<i>Luticola mutica</i> (Kütz.) D.G. Mann	0.52		
<i>Luticola nivalis</i> (Ehr.) D.G. Mann	0.52		
<i>Luticola ventricosa</i> (Kütz.) D.G. Mann	0.52		
<b><i>Mayamaea atomus</i> (Kütz.) Lange-Bertalot</b>	0.51		<b>5.94</b>
<i>Microcostatus krasskei</i> (Hust.) J.R. Johansen & J.C. Sray		0.60	
<i>Navicula cincta</i> (Ehr.) Ralfs	0.51		
<b><i>Navicula subseminulum</i> Hust.</b>	0.26		<b>1.07</b>
<b><i>Navicula tenelloides</i> Hust.</b>	<b>1.57</b>		0.48
<b><i>Nitzschia hantzschiana</i> Lange-Bert.</b>			<b>12.44</b>
<i>Nitzschia harderi</i> Hust.	0.51		
<i>Nitzschia solgensis</i> Cleve-Euler	0.50		
<i>Nitzschia terrestris</i> (J.B. Petersen) Hust.			0.49
<b><i>Nupela lapidosa</i> (Krasske) Lange-Bert.</b>		<b>1.68</b>	
<i>Pinnularia borealis</i> Ehr.		0.65	
<i>Pinnularia divergentissima</i> (Grunow) P.T. Cleve			0.50
<b><i>Pinnularia palatina</i> Lange-Bert. &amp; W. Krüger</b>			<b>1.02</b>
<i>Placoneis hambergii</i> (Hust.) Bruder & Medlin			0.66
<i>Psammothidium bioretii</i> (H. Germ.) Bukht. & Round	0.52		
<i>Psammothidium</i> cf. <i>marginulatum</i> (Grunow) Bukht. & Round		0.96	
<i>Stauroneis thermicola</i> (J.B. Petersen) J.W.G. Lund	0.26		
<i>Tryblionella debilis</i> Arn. ex O'Meara	0.52		
<b>Maximum of aerial species per sample =</b>	2.1%	1.68%	12.44%
<b>Number of aerial species =</b>	21	7	20



**Fig. 3.** Precipitation, discharge and percentages of diatom categories in streamwater (Attert River basin).

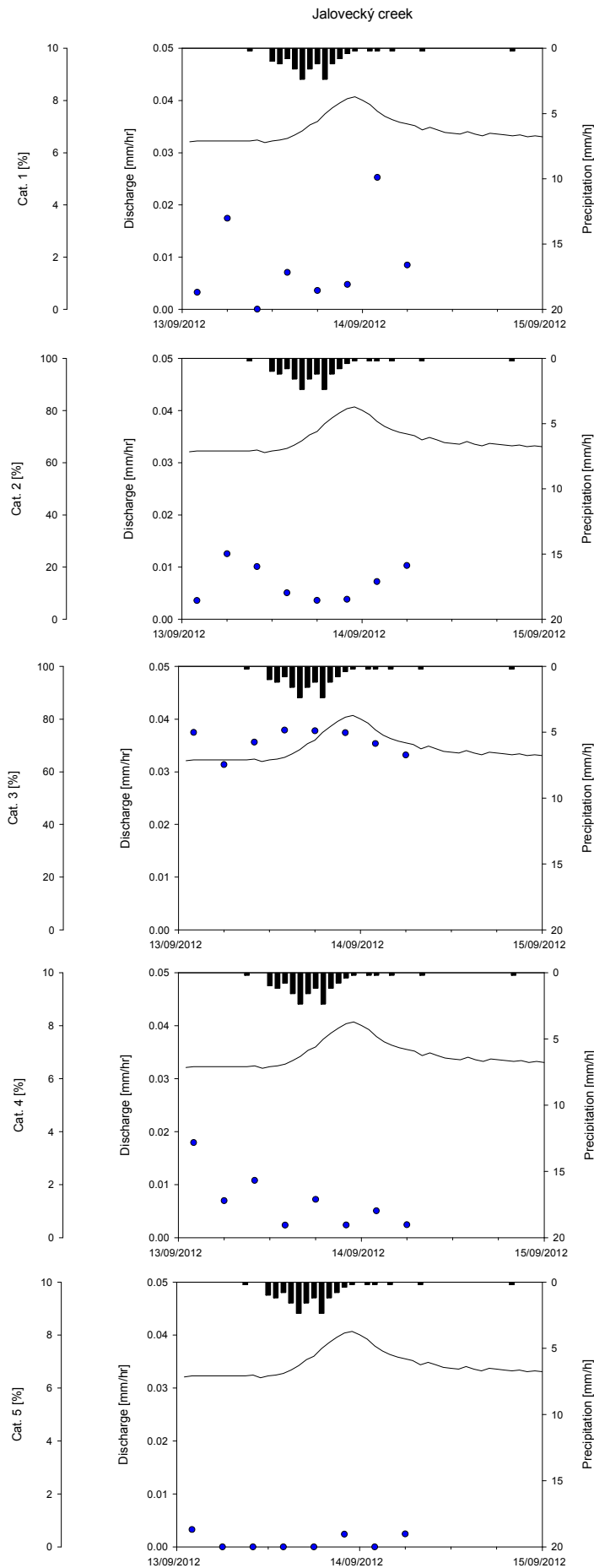


Fig. 4. Precipitation, discharge and percentages of diatom categories in streamwater (Jalovecký creek).

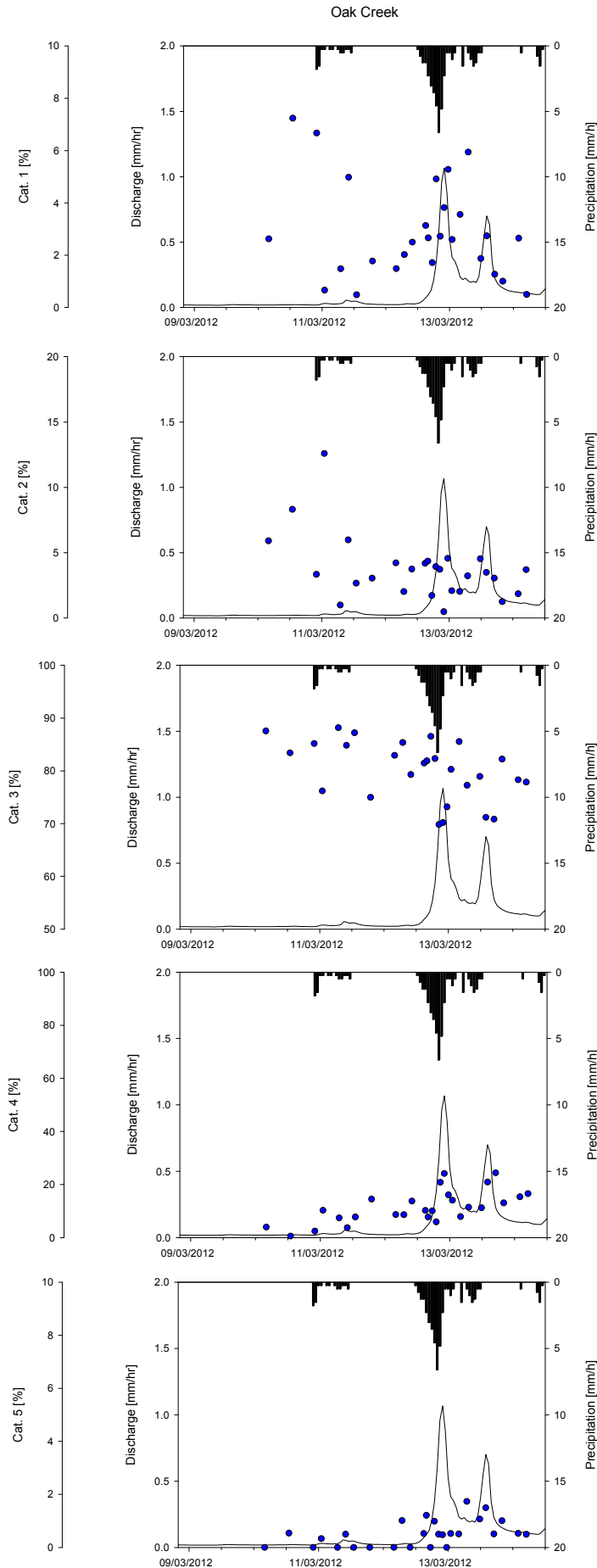


Fig. 5. Precipitation, discharge and percentages of diatom categories in streamwater (Oak creek).



## DISCUSSION

### Diatom assemblages across sites: what did we find?

Our investigations showed very different diatom assemblages in the three studied catchments. We found the highest species richness in the Attert River basin (149 sp.), followed by the Oak creek (94 sp.) and the Jalovecký creek (49 sp.). While the number of collected samples was also smallest in the Jalovecký creek (the sampling protocols in the three catchments eventually being largely influenced by the size and shape of the observed hydrographs), we may nonetheless consider that this finding is likely to reflect the physiographic characteristics of the three studied catchments. The Oak creek and the Jalovecký creek catchments were much smaller than the Attert basin that covers a wide range of geological and land use settings. The size and physiographic diversity of the Attert basin appears to offer a larger variety of diatom habitats. The Oak creek and Jalovecký creek catchments exhibited less heterogeneous physiographical characteristics and consequently a smaller range of potential diatom habitats.

We found aerial diatom species in streamwater in all three catchments during the sampled rainfall-runoff events. While we identified 21 and 20 aerial species in the Attert River and Oak creek respectively, we only found 7 aerial species in the Jalovecký creek. The Oak creek exhibited the highest relative aerial species richness, with 21% of the total species population of the catchment. The presence of a very large riparian zone in the Oak creek, offering favourable conditions and habitats to aerial species, might explain this large difference in aerial species richness.

While a major finding of our study is that aerial diatoms are present in all three catchments and flushed to the streams during rainfall-runoff events, only one aerial species (*Hantzschia amphioxys*) was actually common to the three investigated catchments during the sampled events. To date, we have a very limited understanding on how aerial diatoms map across landscapes (Sherwood et al., 2014; Van Eaton et al., 2013), geological settings, or topographic and climatic gradients. The vast majority of investigations on the variability of diatom assemblages across regions have been limited to freshwater diatoms (e.g. Antoniadou et al., 2014; Bottin et al., 2014; Hlúbíková et al., 2014). There have been virtually no investigations on aerial diatom communities along soil and/or altitude gradients. The vast majority of investigations on the relationships between (moss-inhabiting) diatoms and environmental conditions (moisture) has been focusing on the Antarctic region (e.g. Beyens, 1989; Gremmen et al., 2007; Kopalová et al., 2014; Van de Vijver and Beyens, 1998; Van de Vijver et al., 2008) with a few examples from the northern hemisphere in Europe (e.g. Nováková and Pouličková, 2004; Veselá and Johansen, 2009) and North America (e.g. Johansen et al., 1981, 2004; Lowe and Collins, 1973; Lowe et al., 2007).

The presence of aerial diatoms in our three study catchments is an important prerequisite for their general use as a tracer of hydrological processes. Moreover, the obvious relationship between aerial diatom assemblages and prevailing physiographic characteristics opens new possibilities for the use of diatoms as a tracer of water source across multiple scales and physiographic contexts. Prior to that, though, systematic investigations are still required for characterizing aerial diatom assemblages across landscapes, as well as determination of diatom reservoir sizes and depletion during rainfall flushing (Coles et al., 2015).

### Drift diatom mobilization during events

Precipitation events monitored in the Attert basin and Oak creek catchment triggered significant increases in discharge. Due to a 3 months dry spell, the precipitation event recorded in the Jalovecký creek catchment only generated a very small response. These differences are likely related to the fact that sampling campaigns in the Attert River basin and the Oak creek catchment took place during the winter period, when the catchments had already been through a significant wetting-up phase. In the Jalovecký creek, sampling was conducted in late summer, shortly after unusually dry conditions.

The diatom drift analysis showed differences between catchments in the relative contributions from the five diatom categories. Contributions from category 1 (aquatic strict) were minimal and not related to changes in discharge in all three catchments. While contributions through category 2 (occasional aerophytic) roughly ranged between 10 and 20% in the Jalovecký creek (with an apparent decrease during the rainfall event), they were less significant and showed opposite trends in the Attert River (increasing) and the Oak creek (a slight decrease). Contributions from category 3 (aquatic to subaerial) largely dominated in all three catchments. While in the Jalovecký creek they remained rather stable throughout the rainfall-runoff event, contributions decreased with rising discharge in the Oak creek and the Attert River. In the Oak creek, this decrease was largely compensated by a strong increase in contributions from category 4 (aerophytic strict). In the Attert River, this compensation effect was essentially driven by rising contributions from category 2, and to a much lesser extent by those from category 4. Relative contributions through category 5 (terrestrial) were close to zero in the Jalovecký creek. While being most of the time close to zero in the Jalovecký creek and the Attert River, they temporarily reached values close to 2% in the Oak creek during the rainfall-runoff event.

The very small changes observed in contributions from categories 4 and 5 (aerial diatoms) in the mountainous Jalovecký creek tend to suggest a limited contribution of rapid flowpaths to discharge generation. This is consistent with the low stormflow coefficient and with our knowledge of the catchment's behaviour, i.e. rare overland flow and high infiltration capacities of the soils (Holko and Kostka, 2006) and with isotopic data collected during the event (data not shown here). In the Attert River we observed a noticeable increase in combined contributions through categories 4 and 5, suggesting a more significant role of near-stream aerial diatom reservoirs (and thus eventually temporarily connected riparian zones). This is also implied by the substantial stormflow coefficient of 9%. This effect was even more exacerbated in the Oak creek, where we observed the strongest increase in combined contributions by categories 4 and 5, alongside a substantial stormflow coefficient of 17%. From these findings we can infer the existence of aerial diatom reservoirs and their temporary connectedness – through rapid surface or subsurface flowpathways – to the stream network in all three catchments. However, the differences in the flushing responses might be related to differing diatom reservoir sizes and/or contrasted rapid water flow pathways, as well as the noticeable differences in precipitation intensities observed in the three catchments.

## CONCLUSION

Our investigations in three contrasted catchments were a follow-up to the work of Pfister et al. (2009). We have been able to confirm that aerial diatoms can be found in all three catchments and that they are flushed to the streams during rainfall-runoff events. The existence of aerial diatom reservoirs and their connectedness to the stream are fundamental prerequisites for their application as hydrological tracers.

We compared the abundances of diatom communities in three catchments with contrasting physiographic characteristics. Categories 4 and 5 (aerial diatoms) were mobilized in all three investigated catchments during a rainfall-runoff event. While these results document the existence of diatom reservoirs in each study catchment, their respective size and connectedness to the stream network exhibited significant differences. Strictly aerophytic communities showed a clear increase during the events in wetter conditions in the Attert and Oak creek catchments. They did not vary during the small event that occurred after a long dry period in the Jalovecky creek catchment. Further investigations are needed for assessing the spatial and temporal variability of aerial diatom reservoirs in individual catchments – as a function of prevailing environmental conditions.

Beyond tracking their existence in very contrasted environments, further research on aerial diatoms is needed. Diatom reservoir size and the probability of diatom supply being exhausted during prolonged rainfall events is of crucial interest in the context of hydrological studies. Furthermore, we need to explore the (species-specific) diatom concentration-discharge relationships during flood hydrographs. If clockwise or counter-clockwise hysteretic, these relationships could indicate hydrological processes such as ‘wash off’ or shallow subsurface transport routes. With aerial diatoms being occasionally observed during low flow conditions, we need to investigate how conservatively diatoms are transported downstream, or whether they are deposited temporarily.

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