

Author response on “Technical note: A time-integrated sediment trap to sample diatoms for hydrological tracing” by Jasper Foets et al.

Jasper Foets on behalf of all co-authors

We are grateful to both referees for their constructive and valuable comments. Below we reply on the suggestions/comments of the reviewers.

Referee1:

Abstract: Half of the abstract are talking about the background and the importance of the study. Limited contents reflect the methods and results of this study

Modified the abstract substantially, focusing more on the results and less on the introduction.

Results: Regarding the goal of treating diatoms as a tracer, I assume that the terrestrial species in the samples are especially important for evaluating the events and the capture rates of the method. I would recommend offering a list of the species list in the Appendix. In addition, regardless of the characteristics of the dominant species in the samples, changes in the terrestrial diatoms would be meaningful as well

We added a full species list with species relative abundances per event in the appendix.

I realize that the auC1 HESSD Interactive comment Printer-friendly version Discussion paper thors provide a figure for the percentage of the terrestrial diatom, while without telling which kind of percentage it is. Is that the species richness changes or abundance or biomass? Whether they have same or different percentages from the two sampling methods?

Table 2 and Figure 7 shows the diatom relative abundances, so we added this to the captions.

Discussion: I really like the introduction part which fully links the sediments and diatoms, and the reason why the diatoms species-specific traits could influence the sampling results. However, its pity that there are no further relevant answers to them either in results or discussion part. I would suggest that it could be extended and supplemented regarding the findings of the present study.

We added a few sentences in the discussion referring to the differences between diatoms and suspended sediment particles. In addition, we referred to the different behaviour of diatoms compared to suspended sediment in the statistics section linking it better with the use of ecological guilds.

Line 336, “JFI” may change to “JF”.

This refers to one of the co-authors: Jean-François Iffly

Referee2:

In each sampling event, the Phillips samples seem to be at the periphery of each cluster (Fig.6), suggesting that there is some systematic bias in assemblages collected, yet the ANOSIM results suggest that in the first two events, samples were representative (i.e., not significantly different). With only two samples to compare with 38 automatic samples per sampling event it is likely that low statistical power increases the chance of a Type II error (although by not pooling the automatic samples actually inflates the replication – akin to pseudoreplication). I think the authors need to be more cautious with the inferences made (e.g., L228, L285, etc.).

We adjusted some sentences in the text, so that we are more careful with our conclusions.

I thought the automatic samplers might be pooled over time so that the comparison between these and the Phillips samples are equivalent, but they are not. Is it not a problem to compare the automatic samples collected every 3 hours with a couple of time-integrated samples using the different sampling method? The weighting using discharge for time-integrated samples in the Mann-Whitney U tests (L153-155, L173) probably helps control for differences across events but it is not clear if the same principle is applied for the cumulative discharge and automatic samples (i.e., temporal auto-correlation of samples collected progressively through the flow event).

We did not agree with pooling the samples, since we would lose too much information on the variability of the different samples. To counteract for potential temporal auto-correlation, we ran a NMDS analysis (figure is added in the text) and compared the position of the centroids in the geometric framework of the NMDS plot using PERMANOVA.

L160 - Removing rare and uncommon taxa is contentious for multivariate community analysis. See Cao et al. (2001) for more regarding the potential issues. Perhaps the authors can better explain why they did this and what (if any) influence it had on their results. Cao, Y., Larsen, D., & Thorne, R. (2001). Rare species in multivariate analysis for bioassessment: Some considerations. Journal of the North American Benthological Society, 20, 144-153. doi:10.2307/1468195

We kept our analysis excluding rare taxa (see authors response to RC2).

L163-167 - The cluster analysis appears to follow practices recommended by Borcard et al. (2011), but Quinn and Keough (2002) highlight one disadvantage of agglomerative cluster analysis relating to the interpretability of the dendrogram. Essentially because the hierarchical approach forms clusters that cannot be later broken, the dendrogram is not a representation of all pairwise dissimilarities in objects like in multidimensional scaling. Thus, it could be useful to visualize a MDS plot of the data to determine their relative dissimilarity – also opening up the potential to use PERMANOVA (“adonis”) to test differences (which has the advantage of being less susceptible to dispersion effects than ANOSIM). That approach has the advantage of using a “strata” term for event and just testing the overall difference between sampling methods. Using a SIMPER analysis could help bolster the observations made at L283-285 about why the sampling methods differ (i.e., there is some systematic bias for certain taxa). If the authors see this as useful, I would also strongly consider pooling the automatic samples (power issues notwithstanding) and using relative abundances. The removal of rare taxa is probably essential here since the much greater effort identifying diatoms for a pooled automatic sample increases the probability of detecting rare taxa. Borcard, D., et al. (2011). Numerical Ecology with R. New York, NY, Springer New York. Quinn, G. and M. Keough (2002). Experimental Design and Data Analysis for Biologists. Cambridge, UK, Cambridge University Press.

We did not pool the data, since we then do not have replicates. As an alternative approach, we specified nestedness in the permanova model. There was a significant effect of treatments (i.e. sampling methods) and thus we tested for dispersion as well. In addition, we did SIMPER tests on the community data. We removed the cluster analysis, since the samples were dependent and temporal auto-correlated making cluster analysis problematic. NMDS and PERMANOVA replaces it. The statistical analysis of the study now involves NMDS, PERMANOVA, ANOSIM, SIMPER and comparison of species relative abundances and derived indices using weighted t-tests or Mann-Withney U test.

Technical note: A time-integrated sediment trap to sample diatoms for hydrological tracing

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Abstract. Diatoms, microscopic, single-celled algae, are present in almost all habitats containing water (e.g. streams, lakes, soil, rocks) ~~and form one of the most common and diverse algal groups in both freshwaters and marine ecosystems.~~ In the terrestrial environment, their diversified species distributions are mainly controlled by physiographical factors and anthropic disturbances. ~~This makes making~~ them useful tracers in catchment hydrology. In their use as a ~~hydrological~~ tracer, diatoms are generally sampled in streams by means of an automated sampling method and as a result, many samples are collected to cover a whole storm run-off event. As diatom analysis is labour intensive, a trade-off has to be made between the number of sites and the amount of samples per site. ~~A potential~~ In a way to reduce this number is by using a time-integrated mass-flux sampler. ~~Here,~~ we explored the potential for the Phillips sampler, a time-integrated mass-flux sampler, to provide a representative sample of the diatom assemblage of a whole storm run-off event. We addressed this by comparing the diatom community composition of the Phillips sampler to the composite community collected by the automatic samplers for three events. ~~Our results indicate that during~~ Non-metric multidimensional scaling showed that, based on the species composition, all three events could be separated from each other, that for two events the Phillips sampler ~~sampled representative samples, whereas was able to sample representative communities and that only for the third event~~ significantly different communities were collected ~~during the third event.~~ These observations were generally confirmed by analysis of similarity, PERMANOVA and comparison of species relative abundances and community-derived indices. However, sediment data of ~~this the third~~ event, which was sampled with automatic samplers, showed much noise meaning that we could not verify if the Phillips sampler sampled representative communities or not. Nevertheless, we believe that this sampler could not only be applied in hydrological tracing using terrestrial diatoms, but ~~may it might~~ also be a useful tool in water quality assessment.

1 Introduction

Tracing of water sources and flow paths is an important field of study in catchment hydrology. Environmental tracers such as geochemical elements and stable isotopes of hydrogen and oxygen in the water molecule have led to new insights in our understanding of the age, origin and pathways of water at the watershed scale during the last few decades. For instance, the

work of Hooper et al. (1990) on end-member mixing analysis showed that stream water could be distributed into unique water signatures that mapped back to measurable water chemical concentrations in the landscape. Likewise, thanks to the use of hydrological tracers, we know that groundwater fulfils a very important role in the storm- and snow-melt run-off generation in streams (Sklash and Farvolden, 1979). However, progress remains stymied by various assumptions and limitations in the techniques, including temporally varying input concentrations (McDonnell et al., 1990), unstable end-member solutions (Elsenbeer et al., 1995; Burns, 2002) and the need for unrealistic mixing assumptions (Fenicia et al., 2010). In response to the need for new tracers, soil diatoms (i.e. diatoms living on the soil surface) have been proposed by Pfister et al. (2009) as a potential tracer for studying hydrological connectivity and surface run-off processes.

Diatoms are microscopic, eukaryotic, unicellular algae and form one of the most common and diverse algal groups in both freshwaters and marine environments (Round et al., 1990). They are pigmented, photosynthetic and because of their high abundances, they play a large role in the exchange of gasses between the atmosphere and biosphere. It has been estimated that they are responsible for 20 % of the total oxygen production on the planet (Scarsini et al., 2019). The characteristic feature of diatoms is their siliceous, highly differentiated cell wall which shows an enormous diversity in shapes and structures. These species-specific cell wall ornamentations enable the identification of diatom taxa and form the basis of diatom taxonomy and systematics. Furthermore, their small cell sizes, commonly varying between 10 and 200 μm in diameter or length, allows them to be easily transported by flowing water within or between elements of the hydrological cycle (Pfister et al., 2009). Besides their high abundances in aquatic environments, they are also present in most terrestrial habitats where their diversified species distributions are mainly controlled by physiographical factors (e.g. pH, moisture... see Lund, 1945; Van de Vijver et al., 2002) and anthropic disturbances (Antonelli et al., 2017; Foets et al., 2020a). In a recent study [Foets et al. \(2020a\)](#) ([Foets et al., 2020a](#)), we showed that indicator species could be assigned to certain land use types (e.g. agricultural field, forest...), whereas in another study we indicated that the absolute abundances of soil diatom were related to available soil moisture content (Foets et al., 2020b). All these aforementioned characteristics make them a useful environmental marker and tracer in catchment hydrology.

In their use as a hydrological tracer, drift diatoms from both aquatic and terrestrial origin are generally sampled by means of an automated sampling method. This allowed researchers to follow the species abundances along the hydrograph and link these changes with the activation of hydrological connectivity. An interesting outcome was that the percentage of terrestrial diatoms in the stream samples increased when a peak in the hydrograph occurred meaning that they are indeed responsive to changes in stream flow conditions (Klaus et al., 2015; Martínez-Carreras et al., 2015; Pfister et al., 2015). As a follow-up to this proof-of-concept work, Pfister et al. (2017) have advocated to better characterize the spatial and temporal dynamics of the terrestrial diatom reservoir - paving the way for new diatom sampling protocols of higher spatial and temporal resolution (Klaus et al., 2015). This should eventually provide new insights into the initiation of hydrological connectivity across the catchment (as inferred from diatom flushing to the stream from various terrestrial reservoirs). However, this is not possible at the moment since diatom analysis is labour intensive (sample processing, cell identification and counting) and new, faster identification techniques (e.g. molecular tools) are not yet "standardized" or widely used (Keck et al., 2018; Rivera et al., 2020). So, when using automatic samplers a trade-off has to be made between the number of sites and the amount of samples per site.

A way to increase the number of sampling sites could be by using time-integrated passive samplers. As the name already suggests, they are designed to collect samples that are representative for the whole sampling period. An example is the time-integrated mass-flux sampler designed by Phillips and co-workers (Phillips et al. (2000); Phillips sampler). This sampler, consisting of a PVC-pipe (98 mm x 1 m) with a very small in- and outlet (4 mm), has originally been developed to trap fine-grained (< 62.5 μm) suspended sediment for the assessment of geochemical, physical and magnetic features of transported material (Russell et al., 2000). The functionality of the sampler is based on the large difference in diameter between the inlet of the pipe and the pipe itself. This sudden change in pipe diameter substantially reduces flow velocity and encourages sedimentation of fine particles without significantly disrupting stream flow. This sampler is seen as a simple, inexpensive and easy deployable method particularly for studies requiring a dense sampling network (Phillips et al., 2000). Generally, studies illustrated that the Phillips sampler gives a representative sample of the sediment properties (Russell et al., 2000; Laubel et al., 2002; Walling, 2005) and could therefore for example be used for sediment source fingerprinting (Martínez-Carreras et al., 2010, 2012). However, its use is rather restricted to small streams (Phillips et al., 2000). Although the design could be easily adapted (e.g. increase length and diameter of the pipe) depending on the river flow characteristics, the outcome has not always been positive (McDonald et al., 2010). Furthermore, it is not suited for assessing absolute sediment bed loads and since the sampler grossly underestimate the total fine sediment flux, the samples are also biased towards larger particles (Laubel et al., 2002; Perks et al., 2014). Besides its limitations, this low-cost, easy deployable sampler has reportedly provided satisfying results and as diatoms are a part of suspended sediment (Droppo, 2001) and have mean cell sizes comparable to silt particles (Jones et al., 2012), one would expect that they also behave similarly in stream bodies. Therefore, the sampler could potentially also collect a diatom community representative of the sampling period.

However, diatoms also differ in several aspects of their behaviour from suspended silt particles that could affect the sampling efficiency of the Phillips sampler. First, they have a smaller mass density than silt particles, because their cells do not only consist of silica (generally ranging between 10 and 72 % depending on the environment and species (Round et al. (1990)), but also contain organic material such as protoplasm and polysaccharides. Secondly, their mass density may further decrease due to an increase in thickness of a low-density mucilage sheath or envelope around the cell or colonial unit (Hutchinson, 1967). Additionally, the cell shape and elaborations to the shape will influence their sinking rate. For instance, a unit (colony or individual cell) with a high surface to volume ratio will have a higher viscous drag on the cell as it sinks and hence slow down its descent (Round et al., 1990). On the other hand, moving on or being attached to sediment particles, diatoms produce an extracellular matrix of mucopolysaccharides, which will bind the particles and could eventually form aggregates (Gerbersdorf et al., 2008). Because of that, these attached cells will behave like a larger particle instead. Finally, phyto-benthic diatoms in running waters exhibit a circadian diurnal activity rhythm in which they detach, drift and reattach to a substrate some centimetres or decimetres downstream (Müller-Haeckel, 1973; Müller-Haeckel and Håkansson, 1978). Overall, these different features are often species-specific, meaning that some species could be oversampled while others would be underestimated.

Here, we explore the potential for the Phillips sampler to provide a representative sample of the diatom assemblage of a whole storm run-off event. We addressed this by comparing the diatom community composition of the Phillips sampler to the composite community collected by the automatic samplers. As a control, we also checked if the particle size distribution of

the suspended sediment collected by the Phillips sampler was similar to the composite distribution collected by the automatic sampler. We consider automatic samplers as sampling fully representative samples of a storm run-off event.

95 2 Methodology

2.1 Study site

The sampling location was situated in the south of the Colpach catchment (19.44 km²; 49°46'27" N, 5°48'56" E), a sub-catchment of the Attert experimental basin (249 km²) and located in the western part of the Grand Duchy of Luxembourg. The sub-catchment is mainly underlain by Devonian slate, phyllites and quartzite in the north and marls in the southern part (Fig. 1). Land-use in the area consists of forests (50 %), grass- (24 %) and cropland (23 %) (Juilleret et al., 2011). The altitude in the sub-cathment ranges between 265 m and 530 m, with our sampling site at 313 m a.s.l. Hydrology in a headwater creek of the Colpach catchment, with similar bedrock geology, topography and land use, has been characterised by a fill- and spill system (Wrede et al., 2015) with rapid flow in a highly permeable saprolite layer of weathered schist above the bedrock as the dominant run-off process. Besides the importance of lateral flow, high surface infiltration rates have been observed in the sub-catchment (Van den Bos et al., 2006). For a more detailed description on the hydrological features of the Colpach please see Loritz et al. (2017).

We chose a small, shallow part of the Colpach stream at the outlet of the catchment to conduct our experiment. The stream has a width of around 3 m and had a mean discharge of 0.27 m³ s⁻¹ during 2018. Previously, Martínez-Carreras et al. (2010, 2012) successfully deployed a Phillips sampler at this location. In total, three storm run-off events were sampled between October and December 2018. The first event occurred between 30 October and 5 November, the second between 9 and 17 November and the third between 7 and 12 December. The summer period prior to sampling was exceptionally dry and warm with an average monthly precipitation of 60.8 mm (Meteolux, 2019), whereas the precipitation during October, November and December was 164.8, 138.8 and 144.9 mm respectively (data retrieved from weather station in Useldange, Administration des Services Techniques de l'agriculture (ASTA)). The Colpach has a temperate, semi-oceanic climate regime.

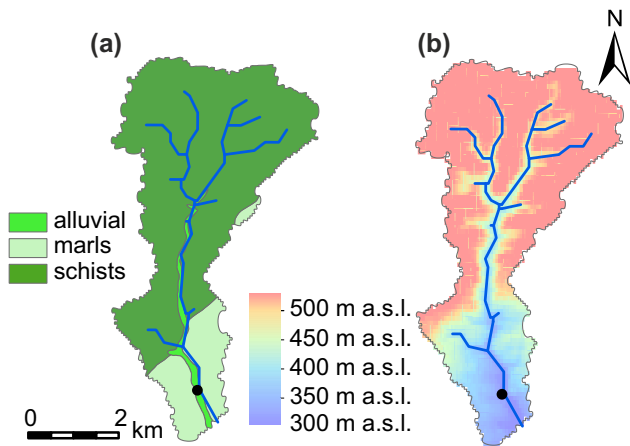


Figure 1. Geology and digital elevation model of the Colpach catchment. (a), Geology. (b), Digital elevation model. The black dot marks the sampling location located at the outlet of the catchment (i.e. Colpach-Haut sub-catchment).

115 2.2 Experimental set-up

In order to sample drift diatoms and suspended sediment, the sampling location (Fig. 2) was equipped with two automatic water samplers (ISCO 6712 FS) to collect instantaneous stream water samples (1 L). Sampling was triggered by flow conditions, set up before a rainfall event occurred. During those events, samples were regularly collected each two or three hours. Furthermore, two Phillips samplers were designed as described by Phillips et al. (2000), except that the diameter of the outlet was 2 mm instead of 4. This was done to increase settling of sediment in the sampler, since the catchment average suspended sediment concentrations are relatively small. The samplers were attached close to the river bank and downstream of the automatic samplers. This was done so it would not interfere with the samples of the automatic samplers (Fig. 2). The Phillips samplers were placed on site just before the event and retrieved when the event was completely finished. Together with the placement/removal of the Phillips samplers, we took a manual grab sample (1 L). The Phillips samplers were emptied in 10 L buckets. In addition, turbidity and conductivity were continuously measured in situ at 5 min. intervals using a YSI 600 OMS optical monitoring system, while water depth was measured with an ISCO 4120 pressure probe logger at a 15 min. time-step. Discharge at 15 min. intervals was estimated using a rating curve between discharge and water level. Upon arrival in the lab, all samples were stored at 4°C prior to analysis.



Figure 2. Pictures of the experimental set-up at the study location. (a), Automatic samplers installed upstream of the bridge. (b), Overview of the installation of the Phillips samplers. They were installed downstream of the bridge. The arrow denotes stream flow. (c), Close-up of the turbidity meter (1) and the Phillips samplers (2).

2.2.1 Diatom preparation and identification

130 After thoroughly shaking the bottles and buckets, a sub-sample (0.5 L) of each sample was taken for diatom analysis. The sub-samples were fixed with 70 % ethanol to prevent possible population change by cell division and left aside for at least one day to let the diatoms settle down. Diatom slides were prepared for microscopic counts following the CEN 13946 procedure (European Committee for Standardization, 2003). Around 70 % of the supernatant was removed by vacuum aspiration, where
 135 tion and 1 mL of HCl (37 %) was added. The mixture was allowed to settle for a day before the supernatant was removed. Afterwards, three repetitions of rinsing with deionized water, decantation and supernatant removal were carried out. The final suspensions were dried on glass cover slips and mounted on permanent slides using Naphrax. A minimum of 400 valves were counted and identified on each slide ($n = 103$) along random transects using a Leica DMR light microscope with a $\times 100$ oil immersion objective and a magnification of $\times 1,000$. Diatom identifications were mainly based on following taxonomic
 140 references: Krammer (2000); Lange-Bertalot (2001); Levkov et al. (2016); Lange-Bertalot et al. (2017).

2.3 Suspended sediment analysis

Suspended sediment concentration (SSC) was determined by filtering a known sub-sample volume between 200 and 500 mL through 1.2 μm Whatman GF/C glass fibre filters using a Millipore vacuum pump. prior to filtering, the filters were dried at 105°C and weighted. Afterwards, the samples were dried (105°C) and weighted again. The concentration of suspended
 145 sediment is the difference between those two weightings divided by the volume of the filtered sample.

Particle size distribution (PSD) was measured in the laboratory using a portable LISST-200X sensor (Sequoia Scientific, Inc., Bellevue, WA), which is a submersible laser-diffraction based particle size analyser. Measurements were done on suspensions inside a test chamber provided by the manufacturer. Before starting them, a background measurement was done with Milli-Q Water. After the chamber was filled, the water was stirred using a magnetic stirrer for a few minutes to ensure all bubbles
 150 disappeared. If still present, bubbles were removed manually from the measuring cell and windows of the chamber. After doing the background measurements, the chamber was filled with a 0.5 L sub-sample and Milli-Q water was added if the

sensor was not fully covered. A magnetic stirrer kept all particles in suspension. Then, a LISST measurement consisting of 120 single measurements was performed in real-time modus using the LISST-SOP200X program. The raw data of each single measurement was then converted to particle size distribution using the 'Random Particle Shape Models' described by Agrawal et al. (2008) and the recorded background scatter. Unfortunately, it was not possible to measure PSD and SSC in all samples, due to limited water volumes collected.

2.4 Statistical analysis

We performed weighted t-tests with discharge as weight on the particle size (the 10th, 50th and 90th percentiles) and compared the distributions with a Kolmogorov–Smirnov test. We used discharge as weight, because during periods with higher discharge a larger volume of water is flown through the Phillips sampler and thus contributes more to the time-integrated samples. Furthermore, we plotted flow duration curves using the function *fdc* from the R-package *hydroTSM* (Zambrano–Bigiarini, 2017) to check if the events were fully covered by the automated sampling method. We encountered a problem with the field instrumentation during the third event, as turbidity was exceptionally noisy and measured concentrations too high and unrealistic for the site (see Fig. 3). Therefore, we decided to exclude the event for further analysis on the sediment data.

Before carrying out the statistical analysis on the diatom communities, we reduced the species dataset by only keeping the taxa with a relative abundance of at least 1% in minimum two samples, since the purpose here is to compare the main patterns in the communities between sampling methods. As the species data contains many zeros, we Hellinger transformed the data and took euclidean distances using the *vegdist* function provided by the R-package *vegan* (Oksanen et al., 2019). We then performed ~~several hierarchical agglomerative cluster analyses and evaluated them using Gower's (1983) distances. Eventually, the unweighted arithmetic (UPGMA) cluster analysis showed the best results (i. e. lowest Gower distance). We used the fusion level values~~ non-metric multidimensional scaling (NMDS) analysis. We achieved a stress value below 0.10 indicating an ideal representation of the data (i.e. dissimilarity values) of the obtained dendrogram to identify the "optimal" number of clusters. Furthermore, configuration is close to actual dissimilarities) according to Clarke (1993). Afterwards, we compared the position of the centroids in the geometric framework of the NMDS plot using PERMANOVA. Differences in treatments were additionally analysed with the *betadisper* function to check whether these differences were caused by difference in dispersion (within-group variation) rather than in mean values of the groups. Furthermore, we used analysis of similarity (ANOSIM) ~~was done on the same transformed dataset~~ to test if communities significantly differed between the events and sampling methods (Clarke, 1993). ~~This was whilst accounting for repeated measurements (i.e. pseudo-replication) (Clarke, 1993). Outcomes were~~ assessed with the Monte Carlo permutation test (perm-9999). Besides, species relative abundances were compared using similarity analysis (SIMPER) and unpaired weighted t-test or Mann–Whitney U test (package *sjstats*) with discharge as weight.

Next, we calculated diatom biovolume according to the data provided in Rimet and Bouchez (2012) and Omnidia (Lecointe et al., 1993) as a measure of mean cell size. Also, Shannon–Wiener, Pielou's evenness and specific pollution-sensitivity (IPS; Cemagref (1982)) indices were computed and compared for each method and event using unpaired weighted t-test or Mann–Whitney U test (~~package *sjstats*) with discharge as weight~~. The IPS has been chosen out of many diatom-based in-

dices, because this index takes into account the abundance of each species in the sample, their sensitivity value to organic pollution and their indicator value (i.e., relative probability of each species to occur in one of five saprobity classes (i.e. measure of organic matter present)). ~~In addition, Ecological guilds were assigned~~ As the behaviour of diatoms could differ from suspended sediment particles, we assigned an ecological guild to the diatom species following Passy (2007) and Rimet and Bouchez (2012) to better understand potential differences in sampling methods. Ecological guilds are defined as a group of species which live in the same environment (i.e. tolerant to similar environmental stressors), but may have adapted differently (Rimet and Bouchez, 2012). All aforementioned statistical analyses were performed using the R statistical program (R v. 3.5.0.; <http://www.r-project.org/>).

3 Results

195 The first event occurred after an exceptional dry and warm summer when water levels were very low. Consequently, a maximum catchment discharge of only 0.011 mm h^{-1} was measured (Fig. 3). Turbidity levels also followed the sudden increase in discharge reaching 68.5 NTU. Unfortunately, both the automatic and Phillips samplers were not active during the first peak of the event and this could have influenced our results. The second event happened a few days later and here the discharge, turbidity and SSC increased substantially. A similar pattern in discharge levels as during the first event was seen (i.e. fast responses to precipitation). This is in strong contrast to the third event where the small peak in discharge is now followed by a high, extended peak (i.e. delayed peak). During this event, we measured the highest catchment discharge (0.44 mm h^{-1}) and turbidity levels. However, the latter shows too much noise and therefore we excluded this event for the statistical analysis on the sediment data.

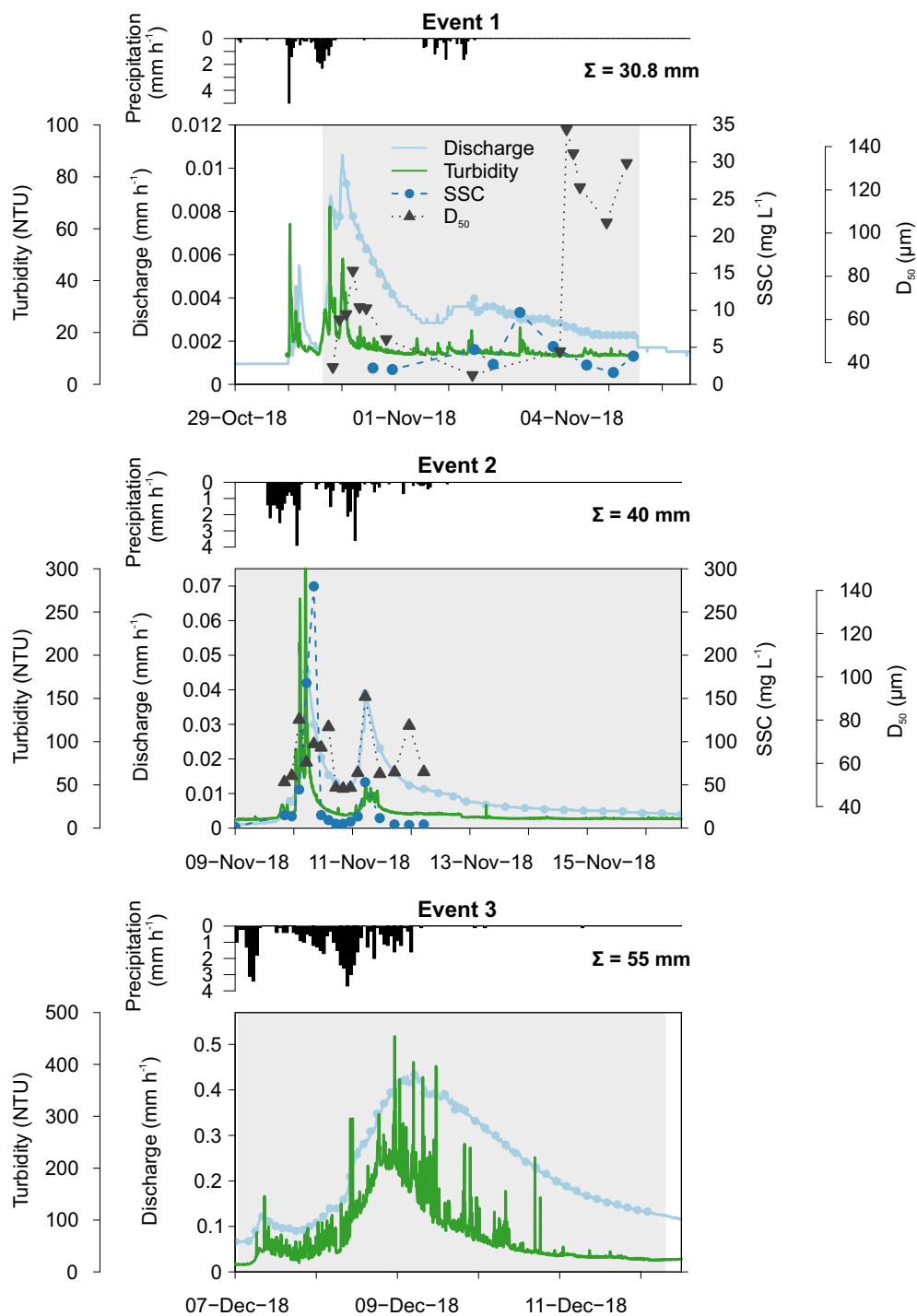


Figure 3. Catchment discharge, suspended sediment concentration (SSC), median particle size (D_{50}), turbidity and precipitation for the three events. Discharge and turbidity were measured at 15 min. intervals, while SSC and D_{50} were analysed from automatic collected water samples. Total precipitation is given per event. Grey background gives the period the Phillips sampler was in the water.

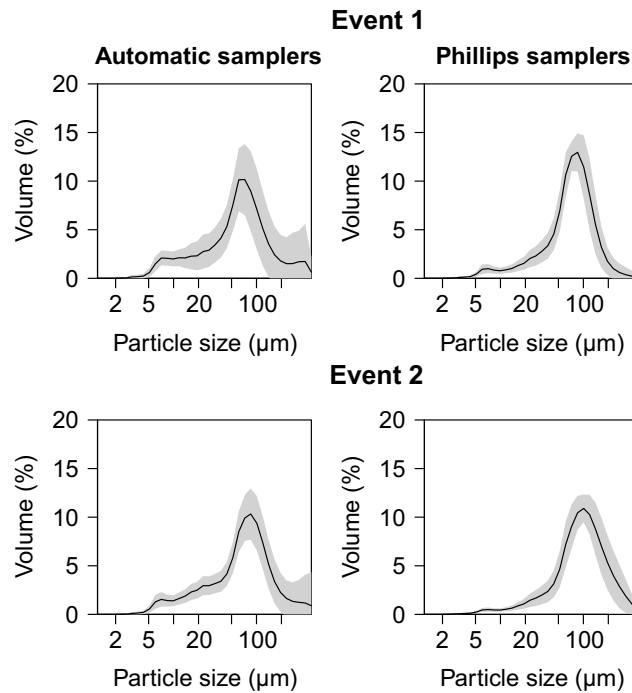


Figure 4. Average particle size distribution for the two sampling methods for the first two events. Grey area indicates the standard variation.

Table 1. Comparison of the particle size (the 10th, 50th and 90th percentiles) between the Phillips sampler and the automatic sampler. Weighted averages and standard deviations are given. *P*-values were derived from weighted t-tests or Mann–Whitney U tests.

	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)
Event 1			
Automatic (n=14)	61.0 ± 23.2	82.0 ± 39.1	124.6 ± 71.5
Phillips trap (n=2)	62.9 ± 19.1	68.6 ± 21.4	78.6 ± 20.4
<i>P</i> value	0.648	0.921	0.607
Event 2			
Automatic (n=16)	53.9 ± 10.6	62.2 ± 13.1	79.5 ± 17.8
Phillips trap (n=2)	85.4 ± 25.1	94.3 ± 27.5	108.0 ± 34.8
<i>P</i> value	0.0297	0.0687	0.175

3.1 Suspended sediment

205 In Figures 3 and 4, median particle sizes (D_{50}) and particle size distributions (PSD) are given for the first two events. In general, median particle sizes follow the changes in turbidity levels. However, this is not the case for the last five samples taken during the first storm run-off event. They do not seem correct as also the SSC shows a different behaviour. We do not know yet what these high levels caused, but this effect is also visible in the PSD, where we observe a high standard variation after $200 \mu\text{m}$.

210 However, we do not observe this second peak in the PSD of the Phillips samples. This difference in the distribution between the
two methods was confirmed by the Kolmogorov–smirnov test ($D = 0.31$, $P = 0.036$), while there was no significant difference
for the second event ($D = 0.25$, $P = 0.21$). In addition, we observe that particle sizes of the Phillips samples are similar to the
automatic samples ($P > 0.05$), except for the 10th percentile of the second event ($t = -2.39$, $P = 0.03$) (Table 1). Besides, we
notice that particle sizes of the time-integrated method substantially increased for the second event, whereas the mean sizes
decreased for the automated sampling method. Although, the Phillips sampler has a tendency to undersample smaller particles,
215 it integrates the sediment particle sizes and distributions well on both occasions.

3.2 Diatom communities

Generally, the flow duration curves show that the three events were well covered with the automatic samplers, because the
discharge at the time the automatic sampler collected the samples used for diatom analysis (blue line) follows a similar pattern
than the discharge estimated at 15 min. intervals (black line) (Fig. 5). Also, the samples are well distributed over the different
220 sampling periods. Both results show us that our sampling was not biased towards certain periods of the events.

We identified a total of 233 different taxa, including varieties, subspecies and forms, belonging to 65 genera. After using
the cut-off criteria, 71 species (94 % of the total valves counted) were kept in the statistical analyses (see Table A1). Most
species rich genera were then *Nitzschia*, *Navicula*, *Fragilaria* and *Planothidium* comprising respectively 15, 10, 5 and 4 taxa,
whereas *Navicula gregaria* (15 % of the total valves counted), *Navicula germainii* (9 %), *Nitzschia palea* (7.2 %), *Nitzschia*
225 *tenuis* (6.5 %) and *Planothidium lanceolatum* (4.2 %) were the most abundant species. An average species richness/sample of
 43 ± 6.8 was observed with 37.9 ± 6.3 , 43.7 ± 4.1 and 49 ± 3.9 during the first, second and third event respectively. Terrestrial
diatoms were consistently found to react to the precipitation pulses with the average proportion of terrestrial diatoms in the
water samples increasing to maximum of 6.6 % (event 1), 8.4 % (event 2) and 9.7 % (event 3) during periods of high discharge
(see Fig. A1 in Appendix).

230 Based on the ~~cluster analysis and its respective fusion level values, five different groups could be established (Fig. 6). Samples
of event 3 and 2 are each divided over two groups, whereas almost all samples of event 1 belong to one group. There is a clear
separation between~~ NMDS analysis, we are able to separate the three different events from each other, especially the diatom
communities taken during the third event ~~from the ones of the~~ are very different from the other two events, ~~since the branches
of the dendrogram are very long and splitting occurred early in the process~~ (Fig. 6). Our PERMANOVA analysis confirmed
this observation ($F = 70.5$, $P = 0.001$). Concerning the ~~samples taken with the Phillips sampler~~ different sampling methods,
235 we observe that the ~~ones~~ Phillips samples of the third event are ~~very significantly~~ different than most of the ~~other~~ automatic
samples of that event (~~both located in a different cluster~~ $F = 4.1$, $P = 0.001$). Likewise, the time-integrated samples of the second
event are quite distinct from each other, ~~since the samples are also assigned to a different cluster. On the contrary,~~ ($F = 2.3$, P
 $= 0.02$), while the Phillips samples of event 1 are not really distinguishable from each other or from the automatic samples. As
240 the sampling methods showed differences for event 2 and 3, we checked whether these differences may be caused by a different
within-group variation (dispersion) instead of different mean values of the groups. The analysis eventually gave no significant
difference ($P = 0.114$) indicating no effect of dispersion. Overall, the ~~cluster~~ NMDS and PERMANOVA analysis groups the

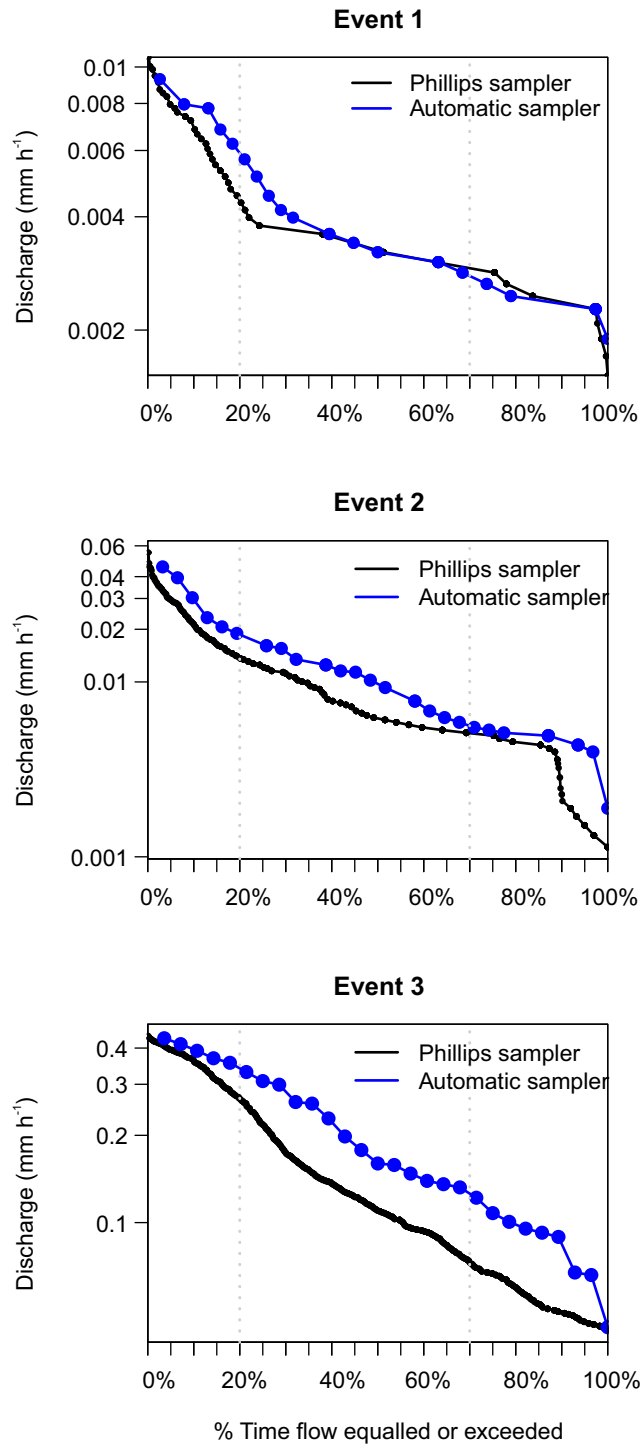


Figure 5. Flow duration curves covering the sampling period of the Phillips samplers. Blue line, samples taken with an automatic sampler for diatom analysis. Black line, continuous measurements of discharge at 15 min. intervals.

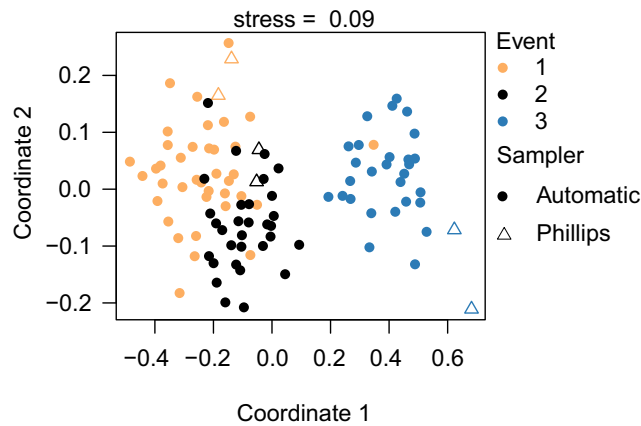


Figure 6. Dendrogram of the diatom community data retrieved from unweighted arithmetic Non-metric dimensional scaling (UPGMA/NMDS) cluster analysis. The y-axis gives the value of the distance metric (idiatom communities indicating the different events and sampling methods, e. euclidean) between clusters. Species data was Hellinger transformed and indicates the similarity between the two groups euclidean distance was used. The five different clusters are given in the bar. A stress value below 0.10 represents an ideal representation of the data according to Clarke (1993).

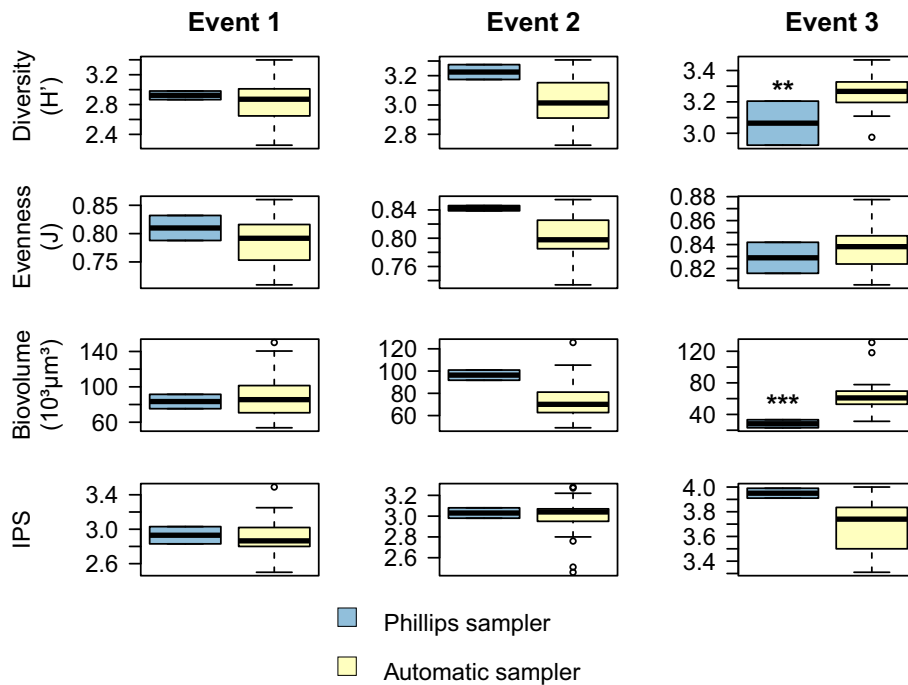


Figure 7. Comparison of the diatom community data between the two sampling methods for each event. Shannon–Wiener index (H') generally ranges between 1.5 (low diversity) and 3.5 (high diversity). Pielou's evenness (J) ranges between 0 (low evenness) and 1 (high evenness). The specific pollution-sensitivity index (IPS) ranges between 1 (poor water quality) and 5 (high quality). **, $P < 0.01$; ***, $P < 0.001$.

Table 2. Most abundant diatom species for the whole study campaign. The weighted averages (in percent) and standard error (between brackets) of the relative abundances are given. Species marked with an "*" indicates that the species relative abundance is significantly different between the two sampling methods for that event.

Species	Event 1		Event 2		Event 3	
	Automatic (n = 38)	Phillips (n=2)	Automatic (n = 31)	Phillips (n = 2)	Automatic (n = 28)	Phillips (n = 2)
<i>Navicula germainii</i> ³	14.60 ± 0.77	11.42 ± 6.24	9.28 ± 0.61	9.41 ± 0.92	1.21 ± 0.18	0.00 ± 0.00
<i>Navicula gregaria</i> ³	13.89 ± 0.54	13.66 ± 0.82	18.71 ± 0.51	16.01 ± 0.03	14.05 ± 0.60*	8.21 ± 0.10*
<i>Nitzschia tenuis</i> ³	10.87 ± 0.30	8.13 ± 0.06	3.54 ± 0.15	5.50 ± 0.27	0.22 ± 0.07	0.00 ± 0.00
<i>Nitzschia palea</i> ³	8.99 ± 0.35	5.71 ± 0.14	9.32 ± 0.15	4.04 ± 0.17	2.90 ± 0.04	1.47 ± 0.00
<i>Navicula cryptocephala</i> ³	4.50 ± 0.29	2.96 ± 1.74	6.09 ± 0.39	3.79 ± 0.42	2.50 ± 0.18	1.72 ± 0.35
<i>Nitzschia dissipata</i> ³	3.54 ± 0.38	5.77 ± 1.08	4.91 ± 0.51	4.15 ± 0.50	1.35 ± 0.22	0.37 ± 0.09
<i>Nitzschia recta</i> ³	3.53 ± 0.02	1.69 ± 0.00	1.12 ± 0.04	1.22 ± 0.09	0.22 ± 0.09	0.00 ± 0.09
<i>Nitzschia linearis</i> ³	3.36 ± 0.54	1.33 ± 0.38	1.81 ± 0.60	2.57 ± 1.31	0.29 ± 0.28	0.00 ± 0.17
<i>Cyclotella meneghiniana</i> ⁴	3.03 ± 0.46*	10.92 ± 2.59*	1.76 ± 0.22*	3.54 ± 0.42*	0.28 ± 0.05	0.12 ± 0.09
<i>Nitzschia acicularis</i> ⁴	2.37 ± 0.29	0.25 ± 0.18	2.08 ± 0.33	0.49 ± 0.35	0.12 ± 0.04	0.12 ± 0.09
<i>Planothidium lanceolatum</i> ²	2.09 ± 0.08	2.32 ± 0.00	3.02 ± 0.12	2.08 ± 0.00	9.11 ± 0.13	9.20 ± 0.35
<i>Melosira varians</i> ¹	1.79 ± 0.22	2.04 ± 0.38	1.86 ± 0.31	3.68 ± 1.40	1.23 ± 0.25	0.00 ± 0.00
<i>Achnanthyidium minutissimum</i> ²	1.59 ± 0.25	3.54 ± 0.68	2.59 ± 0.29	1.71 ± 0.01	8.46 ± 0.46*	16.16 ± 3.44*
<i>Achnanthyidium rivulare</i> ²	1.46 ± 0.20	1.44 ± 0.31	2.45 ± 0.26	3.79 ± 0.07	2.75 ± 0.29*	7.35 ± 0.68*
<i>Reimeria sinuata</i> ²	1.41 ± 0.05	0.85 ± 0.08	1.95 ± 0.17	2.81 ± 0.60	5.19 ± 0.20*	1.96 ± 0.62*
<i>Cocconeis euglypta</i> ²	1.28 ± 0.16	1.57 ± 0.22	1.26 ± 0.13	2.44 ± 0.34	3.32 ± 0.24*	1.10 ± 0.26*
<i>Gomphonema parvulum</i> ¹	1.04 ± 0.13	2.21 ± 0.73	1.03 ± 0.11	1.46 ± 0.51	2.84 ± 0.30	1.47 ± 0.52
<i>Fragilaria gracilis</i> ⁴	0.88 ± 0.11	0.00 ± 0.00	2.88 ± 0.18	2.94 ± 0.87	1.52 ± 0.15	0.86 ± 0.26
<i>Planothidium frequentissimum</i> ²	0.66 ± 0.24	0.72 ± 0.31	1.01 ± 0.29	1.10 ± 0.43	2.51 ± 0.39	3.07 ± 1.66
<i>Fragilaria vaucheriae</i> ¹	0.33 ± 0.10*	1.57 ± 0.22*	0.23 ± 0.07	0.00 ± 0.00	3.35 ± 0.20*	1.72 ± 0.00*
<i>Sellaphora atomoides</i> ³	0.19 ± 0.06	0.12 ± 0.00	1.36 ± 0.06	0.85 ± 0.09	1.42 ± 0.14*	5.76 ± 0.60*
<i>Sellaphora subseminulum</i> ³	0.16 ± 0.35	0.00 ± 0.00	0.23 ± 0.36	0.12 ± 0.12	1.54 ± 0.73*	5.76 ± 0.84*
<i>Achnanthyidium catenatum</i> ²	0.13 ± 0.05	0.00 ± 0.00	0.51 ± 0.11	0.98 ± 0.34	0.81 ± 0.14*	4.41 ± 0.17*
<i>Rossethidium petersenii</i> ²	0.08 ± 0.21	0.00 ± 0.10	0.20 ± 0.21	0.00 ± 0.08	1.16 ± 0.31*	2.21 ± 0.35*
<i>Planothidium daonense</i> ²	0.07 ± 0.09	0.00 ± 0.16	0.06 ± 0.07	0.12 ± 0.08	0.74 ± 0.16*	2.08 ± 0.44*

Ecological guilds: ¹ High profile, ² Low profile, ³ Motile, ⁴ Planktonic

diatom communities relatively well per event with ~~only a few mismatches occurring during the process~~some time-integrated samples differentiating from the automatic ones.

245 Overall, analysis of similarity (ANOSIM), species relative abundances (Table 2) and their derived indices (Fig. 7) all ~~confirm~~
~~the outcome of the cluster analysis. The~~seem to indicate a similar result as the NMDS. ANOSIM enabled the detection and
split of the communities based on the different events (Global $R = 0.6433$, $P = 0.0001$). Moreover, there was also a significant
difference between the community composition of the automatic sampler and the Phillips sampler for the third event ($R = 0.804$,
 $P = 0.002$), whereas the communities taken during the other two events could not be separated from each other (~~event 1: $R =$~~
250 ~~0.361~~ 0.1713 , $P = 0.07$; ~~event 2: $R = 0.129$, $P = 0.249$~~ 0.08). A similar outcome to previous analyses is seen when we compare the
abundances of the most abundant diatom species with each other. Aside from *Navicula gregaria* and *Navicula cryptocephala*
which are dominant throughout the entire study, the abundance of the other dominant taxa differ substantially between the first
two and the third event. For instance, *Navicula germainii*, *Nitzschia tenuis* and *Cyclotella meneghiniana* only sparingly occur
in the third event, while we observe the reverse for *Planothidium lanceolatum*, *Sellaphora subseminulum* and *Achnanthydium*
255 *minutissimum*. Interestingly, this follows a shift in ecological guilds going from an assemblage containing more motile species
(i.e. unattached free living species immersed on the sediment matrix surrounded by exopolysaccharides) to more colonial
and strongly attached taxa. Regarding the sampling methods, dominant taxa such as *N. germainii*, *N. gregaria*, *N. tenuis* and
Nitzschia palea vary little in the first two events, except for *C. meneghiniana* and to a lesser extent *Fragilaria vaucheriae* which
both occur significantly more in the samples of the Phillips sampler. In contrast to those events, the abundances of *N. gregaria*,
260 *Achnanthydium rivulare*, *A. minutissimum*, *Reimeria sinuata*, *Sellaphora atomoides*, *F. vaucheriae* and *S. subseminulum* are all
very different between the methods for the last event. Of those two communities, the one collected with the automatic samplers
resembles more the communities of event 1 and 2. Concerning the indices, Shannon–Wiener, species evenness and IPS do not
show any significant difference between the methods for the first two events ($P > 0.05$), while only a higher diversity in the
automatic samplers is noted for the third event ($t = 2.76$, $df = 28$, $P = 0.01$). Also, the overall diatom size was higher in the
265 automatic samples of the third event compared to the time-integrated samples (Chisq = 7.76, $df = 28$, $P < 0.0001$), whereas
it was not significantly different for the first two events. Despite that indices do not differ for the second event, we notice that
diversity, evenness and biovolume tend to have higher values for the Phillips samplers. The results of our analyses indicate that
the Phillips sampler could possibly be a valid tool to collect a time-integrated diatom community representative for the entire
sampling period.

270 4 Discussion

4.1 Evaluation of the events and samplings

The purpose of this study was to test if the Phillips sampler could be used to collect a time-integrated diatom assemblage as an
alternative to automated sampling methods. To evaluate the sampler, three storm run-off events were simultaneously sampled
with automatic samplers and analysed. The events were well and thoroughly covered as shown in the flow duration curves.
275 Of those three, two different types of hydrographs were generated and ~~cluster~~NMDS analysis showed that the events could

be separated based on the diatom species composition. Even though we only sampled at one location during a relatively short period (30 October–12 December), we were able to observe quite some variation among events and communities.

Furthermore, previous results of Klaus et al. (2015) and Pfister et al. (2017) confirm that we did sample representative communities of the stream. Like us, they did an event-based sampling in the Colpach catchment during the same period of year
280 (both in December). The latter is also important, since diatoms exhibit seasonal succession and thus species composition could change significantly during the year (Wetzel, 2001; Wu et al., 2016). Klaus et al. (2015) analysed 28 samples in which they found 221 different species, while we also observed 231 taxa. Likewise, during the first precipitation pulse the percentage of terrestrial diatoms in the samples increased to 8.9 % at a discharge of around 0.18 mm h^{-1} , which is also the proportion that we found. Of the 15 most abundant species in their study, 12 were also present in our study of which eight were abundant including
285 *N. gregaria*, *P. lanceolatum*, *N. cryptocephala* and *N. linearis*. Similarly, of the 15 dominating species in Pfister et al. (2017), 11 were present in this study of which six were dominant (e.g. *N. gregaria*, *N. palea*, *A. minutissimum*, *P. lanceolatum*). Our research also confirms the observation of Martínez-Carreras et al. (2015), who found that the relative abundance of terrestrial diatoms increased with higher discharge. Unfortunately, since we encountered too much noise in the turbidity and sediment analysis of the third event, it is difficult to draw robust conclusions from that event. The reasons for this noise are still unknown,
290 and may be linked to a local accumulation of sediment and fine material around and on the sampling tubing of the automatic samplers and a miss-cleaning of the turbidity sensor. But aside from that, results indicate that overall sampling was done properly, that there was some variation between and during the events and that samples were representative for the site.

4.2 On the potential for the Phillips sampler to collect drift diatoms

Several statistical analyses were executed on sediment and diatom data to verify if the Phillips sampler could be a useful
295 tool in regards to sampling drift diatoms. As shown here and previously by several other studies, the Phillips sampler gave reasonably good results concerning particle sizes and particle size distributions compared to automated sampling methods (Phillips et al., 2000; Walling, 2005; Perks et al., 2014; Smith and Owens, 2014). Oversampling of bigger sediment particles, as often mentioned in those studies, did occur, but this effect was rather limited in this research. ~~There was also no difference in the diatom~~ Despite differences in behaviour between diatoms and suspended sediment particles (e.g. smaller mass density, formation of aggregates and circadian diurnal activity rhythm), there was only one time-integrated sample that deviated from the other time-integrated and automatic samples regarding the first two events. besides that, no significant differences in the
300 communities and its derivatives such as biovolume and diversity between the two sampling methods for the first two events were observed, though communities tend to be bigger and more diverse for the Phillips samples for the second event. However, time-integrated communities sampled during the third event ~~deviated~~ differed from the assemblages collected with the automatic
305 sampler. This is probably because of the same reason which caused so much noise on the sediment data. Since those samples were collected by means of automated sampling, one could argue that the Phillips sampler should give the better results of the two methods. This is, however, not reflected in the diatom communities, because they actually differed more from the communities sampled in the previous events. On the other hand, the stream did have a different behaviour, higher discharge and water level compared to the other events and this likely led to the activation, detachment and transportation of species with

310 another behaviour or belonging to a different ecological guild (e.g. more strongly attached species) (see examples in Round
et al. (1990); Jewson et al. (2006); Rimet and Bouchez (2012)). Indeed, high motile and planktonic species such as *Navicula* sp.
and *Nitzschia* sp. were replaced by colonial and/or attached species (e.g. *Achanthidium* sp. and *Planothidium* sp.) and this shift
was more pronounced in the Phillips sampler. Thus, the Phillips sampler ~~is~~ seems to be able to sample a representative diatom
community of a storm run-off event, though we were not able to check whether underestimation of certain species occurred
315 during the third event.

4.3 Potential way forward

Today, different research avenues can be followed in the exploration of drift diatom sampling methods for hydrological tracing.
Here, the Phillips sampler was successfully applied, though our work was restricted to a single location. Therefore, future
studies should test the sampler also in other settings that have a different hydrological behaviour and diatom communities. It
320 would be interesting to investigate if studies would then be able to confirm our results and especially give information on its
efficiency at higher discharges, which is still not ~~completely~~ clear from our study. Furthermore, future research should not only
be limited to the Phillips sampler. Also, other passive samplers such as the pumped active suspended sediment (PASS) sampler
designed by Doriean et al. (2019) and the bidirectional time-integrated mass-flux sampler (TIMS) developed by Elliott et al.
(2017) should be able to collect ~~a representative diatom community~~ representative diatom communities. The operating principle
325 of both samplers is the same as the Phillips sampler with the difference that the PASS works at a constant, pre-defined flow rate
enabling the measurements of time-weighted average SSC and PSD, while the Bidirectional TIMS is developed for estuaries
and has a L-shaped outlet preventing inflow from the other direction. Another possible avenue would be to investigate what
the minimum number of point samples is that we need and at which periods we need to take them (e.g. one at peak discharge,
during rising and falling limb) to get a community representing the entire event. Importantly, the diurnal circadian rhythm of
330 benthic diatoms should be taken into account (Müller-Haeckel, 1973; Müller-Haeckel and Håkansson, 1978). Eventually, this
information could then significantly reduce the number of samples to be analysed. Besides, molecular techniques (i.e. DNA
metabarcoding and High-Throughput Sequencing) in diatom research are developing fast and since these techniques are faster
and less expensive than microscopical examinations, they could also open the path to higher frequency sampling (Vasselon
et al., 2017; Rivera et al., 2020). So, in general, this research has unlocked ~~many~~ new possibilities for collecting drift diatoms
335 which could pave the way for better using diatoms in hydrological tracing.

Increased amounts of suspended sediments due to anthropogenic factors can have significant negative impacts on water
quality and aquatic biota. Besides that it reduces primary production through a reduction in light penetration, it also acts as an
important vector for the transfer of nutrients and metals in fluvial systems (Bowes et al., 2003; Ballantine et al., 2008; Bilotta
and Brazier, 2008). Therefore, it is important to identify the sediment sources, what their relative contribution is to the overall
340 sediment load and to know the effect on the ecological functioning of the system so that remedial measures could be taken.
A way to investigate this is with sediment fingerprinting. There, several tracers (e.g. mineralogy, nitrogen and carbon stable
isotopes) are used to serve as sediment fingerprints (Haddadchi et al., 2013). As (drift) diatoms are often associated with those
particles, they could provide additional information on sediment quality, its sources and transport (Jewson et al., 2006; Jones

et al., 2012). More interestingly, they could even be sampled and analysed together with the suspended sediment to determine potential (sediment) sources and their relative contribution in a catchment (Pfister et al., 2017). Besides, the use of a time-integrated sampler enables us also to collect a phytoplankton community, even at different depths (McDonald et al., 2010). Generally, in water quality assessment, phytoplankton is sampled with grab samples (Soylu and Gönülol, 2003; Abonyi et al., 2012). However, the composition of planktonic diatoms is quite variable in time and changes occur relatively fast in comparison to benthic communities (Round et al., 1990). Therefore, it would be interesting to test if a time-integrated suspended sediment sampler would also give a representative phytoplankton community for a certain period of time. So, the use of a time-integrated mass-flux sampler could not only assist sediment fingerprinting, but potentially also improve the analysis of water quality.

5 Conclusions

Here, we investigated if the Phillips sampler, a time-integrated mass-flux sampler, was able to sample representative diatom communities during a storm run-off event. This was done by comparing the suspended sediment concentrations, particle size distributions and drift diatom assemblages with point samples collected by automatic samplers. ~~Our~~ Most of our results indicate that during two storm run-off events the Phillips sampler sampled representative samples, whereas significantly different communities were collected during the third event. However, sediment data of this event, which was sampled with automatic samplers, showed much noise meaning that we could not verify if the Phillips sampler sampled representative communities or not. Nevertheless, we believe that this sampler could not only be applied in hydrological tracing using terrestrial diatoms, but may also be a useful tool in water quality assessment.

Data availability. Foets, J., Martínez-Carreras, N., Iffly, J., Pfister, L.: A time-integrated sediment trap to sample diatoms for hydrological tracing, Mendeley Data, V1, doi: 10.17632/hgbf5bpwkh.1 <https://data.mendeley.com/datasets/hgbf5bpwkh/draft?a=961d56b1-f410-4201-b0fe-c1d80dca84c4>, 2020

Author contributions. JF, CEW, AJT, NMC and LP designed and directed the study. JFI planned and carried out the field work. NMC Carried out the analysis of the sediment data. JF prepared the diatom slides and carried out diatom identifications. JF, CEW, NMC, LP, and AJT discussed and interpreted the results. JF prepared the paper with contributions from all co-authors

Competing interests. The authors declare that they have no conflict of interest.

Financial support. This work was supported by the Luxembourg National Research Fund (FNR) (PRIDE15/10623093/HYDRO-CSI).

370 *Acknowledgements.* The authors thank one anonymous reviewer and Dr. Francis Burdon for their constructive comments that improved our manuscript.

Appendix A

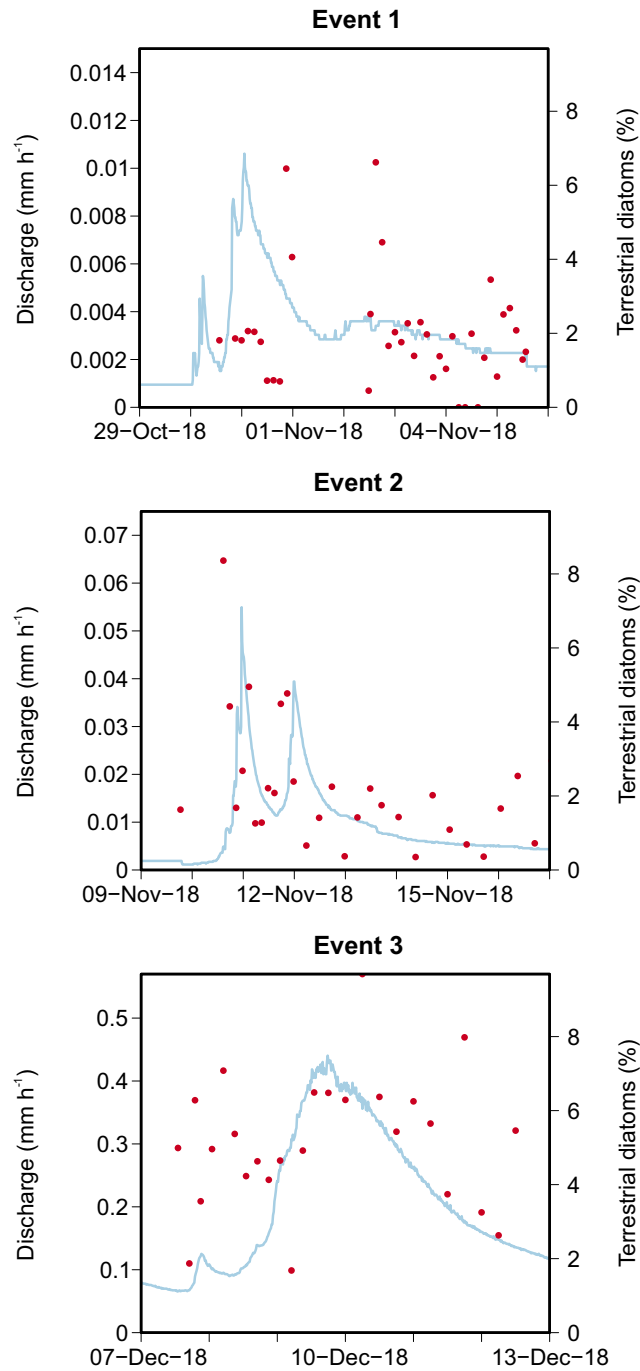


Figure A1. Discharge and percentage relative abundances of terrestrial diatoms in the community for the three storm run-off events.

Table A1. Species list with species relative abundances (%) per event. The list only includes taxa with a relative abundance of at least 1% in minimum two samples.

Taxon	Event 1	Event 2	Event 3
<i>Achnanthydium catenatum</i> (Billy et Marvan) Lange-Bertalot	0.16	0.51	0.97
<i>Achnanthydium kranzii</i> (Lange-Bertalot) Round et Bukhtiyarova	0.00	0.01	0.61
<i>Achnanthydium minutissimum</i> (Kützing) Czarnecki	1.78	1.93	8.71
<i>Achnanthydium rivulare</i> Potapova et Ponader	1.46	2.08	3.29
<i>Amphora pediculus</i> (Kützing) Grunow	1.03	0.84	0.67
<i>Cavinula cocconeiformis</i> (Gregory) D.G. Mann et Stickle	0.07	0.10	0.56
<i>Cocconeis euglypta</i> Ehrenberg	1.12	1.29	3.09
<i>Cocconeis pseudolineata</i> (Geitler) Lange-Bertalot	0.11	0.02	0.52
<i>Conticribra weisfloggi</i> (Grunow) Stachura-Suchoples et Williams	0.77	0.82	0.51
<i>Cyclotella meneghiniana</i> Kützing	3.50	2.22	0.35
<i>Encyonema silesiacum</i> (Bleisch) D.G. Mann	0.40	0.26	0.91
<i>Eunotia botuliformis</i> Wild, Nörpel et Lange-Bertalot	0.02	0.00	0.56
<i>Eunotia implicata</i> Nörpel-Schempp, Alles et Lange-Bertalot	0.02	0.05	0.29
<i>Eunotia minor</i> (Kützing) Grunow	0.15	0.21	0.69
<i>Fragilaria gracilis</i> Østrup	0.78	2.81	1.62
<i>Fragilaria pectinalis</i> (O.F. Muller) Lyngbye	0.30	0.58	0.84
<i>Fragilaria rumpens</i> (Kützing) Carlson	0.21	0.78	1.11
<i>Fragilaria tenera</i> (W. Smith) Lange-Bertalot var. <i>tenera</i>	0.11	0.74	0.00
<i>Fragilaria vaucheriae</i> (Kützing) J.B. Petersen	0.42	0.22	3.03
<i>Fragilariforma virescens</i> (Ralfs) D.M. Williams et Round	0.15	0.18	1.07
<i>Gomphonema micropus</i> Kützing	0.00	0.01	0.48
<i>Gomphonema minutum</i> (C. Agardh) C. Agardh	0.23	0.31	0.63
<i>Gomphonema parvulum</i> (Kützing) Kützing	1.08	1.16	2.36
<i>Halamphora montana</i> (Krasske) Levkov	0.25	0.36	0.25
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	0.08	0.15	0.22
<i>Hippodonta capitata</i> (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowski	1.35	0.94	0.31
<i>Mayamaea permitis</i> (Hustedt) Bruder et Medlin	0.06	0.24	0.24
<i>Melosira varians</i> C. Agardh	1.71	1.78	1.36
<i>Meridion circulare</i> (Gréville) C. Agardh var. <i>circulare</i>	0.07	0.05	0.47
<i>Meridion circulare</i> var. <i>constrictum</i> (Ralfs) Van Heurck	0.35	0.39	0.78
<i>Navicula cryptocephala</i> Kützing	4.19	5.25	2.55
<i>Navicula exilis</i> Kützing	0.52	0.44	0.11
<i>Navicula germainii</i> Wallace	14.30	10.40	1.28
<i>Navicula gregaria</i> Donkin	13.41	18.52	14.49
<i>Navicula hofmanniae</i> Lange-Bertalot	0.05	0.00	0.35
<i>Navicula lanceolata</i> (C. agardh) Ehrenberg	0.74	0.78	1.06
<i>Navicula rhynchocephala</i> Kützing	0.70	0.63	0.27
<i>Navicula tennelloides</i> Hustedt	0.16	0.27	0.31
<i>Navicula trivialis</i> (Lange-Bertalot)	0.36	0.31	0.03
<i>Navicula veneta</i> Kützing	0.21	0.54	0.13
<i>Nitzschia acicularis</i> (Kützing) W. Smith	2.17	2.20	0.16
<i>Nitzschia acidoclinata</i> Lange-Bertalot	0.07	0.01	0.71
<i>Nitzschia amphibia</i> Grunow	0.45	0.31	0.26
<i>Nitzschia dissipata</i> (Kützing) Grunow ssp. <i>dissipata</i>	3.48	5.02	1.65
<i>Nitzschia heufferiana</i> Grunow	0.40	0.44	0.00

References

- Abonyi, A., Leitão, M., Lançon, A. M., and Padisák, J.: Phytoplankton functional groups as indicators of human impacts along the River Loire (France), *Hydrobiologia*, 698, 233–249, <https://doi.org/10.1007/s10750-012-1130-0>, 2012.
- 375 Agrawal, Y. C., Whitmire, A., Mikkelsen, O. A., and Pottsmith, H. C.: Light scattering by random shaped particles and consequences on measuring suspended sediments by laser diffraction, *J. Geophys. Res.*, 113, 1–11, <https://doi.org/10.1029/2007JC004403>, 2008.
- Antonelli, M., Wetzel, C. E., Ector, L., Teuling, A. J., and Pfister, L.: On the potential for terrestrial diatom communities and diatom indices to identify anthropic disturbance in soils, *Ecol. Indic.*, 75, 73–81, <https://doi.org/10.1016/j.ecolind.2016.12.003>, 2017.
- Ballantine, D. J., Walling, D. E., Collins, A. L., and Leeks, G. J.: The phosphorus content of fluvial suspended sediment in three lowland groundwater-dominated catchments, *J. Hydrol.*, 357, 140–151, <https://doi.org/10.1016/j.jhydrol.2008.05.011>, 2008.
- 380 Bilotta, G. S. and Brazier, R. E.: Understanding the influence of suspended solids on water quality and aquatic biota, *Water Res.*, 42, 2849–2861, <https://doi.org/10.1016/j.watres.2008.03.018>, 2008.
- Bowes, M. J., House, W. A., and Hodgkinson, R. A.: Phosphorus dynamics along a river continuum, *Sci. Total Environ.*, 313, 199–212, [https://doi.org/10.1016/S0048-9697\(03\)00260-2](https://doi.org/10.1016/S0048-9697(03)00260-2), 2003.
- 385 Burns, D. A.: Stormflow-hydrograph separation based on isotopes: the thrill is gone - what's next?, *Hydrol. Process.*, 16, 1515–1517, <https://doi.org/10.1002/hyp.5008>, 2002.
- Cemagref: Etude des méthodes biologiques d'appréciation quantitative de la qualité des eaux., Tech. rep., Cemagref, Division Qualité des Eaux, Lyon, 1982.
- Clarke, K. R.: Non-parametric multivariate analyses of changes in community structure, *Aust. J. Ecol.*, 18, 117–143, <https://doi.org/10.1111/j.1442-9993.1993.tb00438.x>, 1993.
- 390 Doriean, N. J., Teasdale, P. R., Welsh, D. T., Brooks, A. P., and Bennett, W. W.: Evaluation of a simple, inexpensive, in situ sampler for measuring time-weighted average concentrations of suspended sediment in rivers and streams, *Hydrol. Process.*, 33, 678–686, <https://doi.org/10.1002/hyp.13353>, 2019.
- Droppo, I. G.: Rethinking what constitutes suspended sediment, *Hydrol. Process.*, 15, 1551–1564, <https://doi.org/10.1002/hyp.228>, 2001.
- 395 Elliott, E. A., Monbureau, E., Walters, G. W., Elliott, M. A., McKee, B. A., and Rodriguez, A. B.: A novel method for sampling the suspended sediment load in the tidal environment using bi-directional time-integrated mass-flux sediment (TIMS) samplers, *Estuar. Coast. Shelf Sci.*, 199, 14–24, <https://doi.org/10.1016/j.ecss.2017.08.029>, 2017.
- Elsenbeer, H., Lorieri, D., and Bonell, M.: Mixing model approaches to estimate storm flow sources in an overland flow-dominated tropical rain forest catchment, *Water Resour. Res.*, 31, 2267–2278, <https://doi.org/10.1029/95WR01651>, 1995.
- 400 Fenicia, F., Wrede, S., Kavetski, D., Pfister, L., Hoffmann, L., Savenije, H. H. G., and McDonnell, J. J.: Assessing the impact of mixing assumptions on the estimation of streamwater mean residence time, *Hydrol. Process.*, 24, 1730–1741, <https://doi.org/10.1002/hyp.7595>, 2010.
- Foets, J., Wetzel, C. E., Teuling, A. J., and Pfister, L.: Temporal and spatial variability of terrestrial diatoms at the catchment scale : controls on communities, *PeerJ*, pp. 1–19, <https://doi.org/10.7717/peerj.8296>, 2020a.
- 405 Foets, J., Wetzel, C. E., Teuling, A. J., and Pfister, L.: Temporal and spatial variability of terrestrial diatoms at the catchment scale: controls on productivity and comparison with other soil algae, *PeerJ*, pp. 1–21, <https://doi.org/10.7717/peerj.9198>, 2020b.
- Gerbersdorf, S. U., Jancke, T., Westrich, B., and Paterson, D. M.: Microbial stabilization of riverine sediments by extracellular polymeric substances, *Geobiology*, 6, 57–69, <https://doi.org/10.1111/j.1472-4669.2007.00120.x>, 2008.

- Gower, J.: Comparing classifications, in: Numer. Taxon., edited by Felsenstein, J., pp. 137–155, Springer, Berlin, Heidelberg, 410 https://doi.org/10.1007/978-3-642-69024-2_21, 1983.
- Haddadchi, A., Ryder, D. S., Evrard, O., and Olley, J.: Sediment fingerprinting in fluvial systems: Review of tracers, sediment sources and mixing models, Int. J. Sediment Res., 28, 560–578, [https://doi.org/10.1016/S1001-6279\(14\)60013-5](https://doi.org/10.1016/S1001-6279(14)60013-5), 2013.
- 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, [https://doi.org/https://doi.org/10.1016/0022-1694\(90\)90131-G](https://doi.org/https://doi.org/10.1016/0022-1694(90)90131-G), 1990. 415
- Hutchinson, G.: Introduction to lake biology and the limnoplankton, in: A treatise Limnol., p. 1115, Wiley, New York, 2 edn., 1967.
- Jewson, D. H., Lowry, S. F., and Bowen, R.: Co-existence and survival of diatoms on sand grains, Eur. J. Phycol., 41, 131–146, <https://doi.org/10.1080/09670260600652903>, 2006.
- Jones, J. I., Duerdoth, C. P., Collins, A. L., Naden, P. S., and Sear, D. A.: Interactions between diatoms and fine sediment, Hydrol. Process., 420 pp. 2267–2274, <https://doi.org/10.1002/hyp>, 2012.
- Juilletet, J., Iffly, J. F., Pfister, L., and Hissler, C.: Remarkable Pleistocene periglacial slope deposits in Luxembourg (Oesling): pedological implication and geosite potential, Bull. la Société des Nat. Luxemb., 112, 125–130, 2011.
- Keck, F., Vasselon, V., Rimet, F., Bouchez, A., and Kahlert, M.: Boosting DNA metabarcoding for biomonitoring with phylogenetic estimation of operational taxonomic units’ ecological profiles, Molecular Ecology Resources, 18, 1299–1309, <https://doi.org/10.1111/1755-0998.12919>, 2018. 425
- Klaus, J., Wetzel, C. E., Martínez-Carreras, N., Ector, L., and Pfister, L.: A tracer to bridge the scales: on the value of diatoms for tracing fast flow path connectivity from headwaters to meso-scale catchments, Hydrol. Process., 29, 5275–5289, <https://doi.org/10.1002/hyp.10628>, 2015.
- Krammer, K.: The genus *Pinnularia*, in: Diatoms of Europe: Diatoms of the European inland waters and comparable habitats, edited by Lange-Bertalot, H., vol. 1, p. 703, ARG Gantner Verlag KG, Ruggell, 2000. 430
- Lange-Bertalot, H.: *Navicula* sensu stricto; 10 genera separated from *Navicula* sensu lato *Frustulia*, in: Diatoms of Europe: Diatoms of the European inland waters and comparable habitats, edited by Lange-Bertalot, H., vol. 2, p. 526, Gantner Verlag KG, Ruggell, 2001.
- Lange-Bertalot, H., Hofmann, G., Werum, M., Cantonati, M., and Kelly, M. G.: Freshwater benthic diatoms of Central Europe: Over 800 common species used in ecological assessment, Koeltz Botanical Books Schmittens-Oberreifenberg, Germany, 2017.
- 435 Laubel, A., Kronvang, B., Fjorback, C., and Larsen, S. E.: Time-integrated suspended sediment sampling from a small lowland stream, SIL Proceedings, 1922-2010, 28, 1420–1424, <https://doi.org/10.1080/03680770.2001.11902689>, 2002.
- Lecointe, C., Coste, M., and Prygiel, J.: “Omnidia”: software for taxonomy, calculation of diatom indices and inventories management, Hydrobiologia, 269, 509–513, <https://doi.org/10.1007/BF00028048>, 1993.
- Levkov, Z., Mitic-Kopanja, D., and Reichardt, E.: The diatom genus *Gomphonema* from the Republic of Macedonia, in: Diatoms of Europe: Diatoms of the European inland waters and comparable habitats, edited by Lange-Bertalot, H., vol. 8, p. 552, Koeltz Scientific Books, Königstein, 2016. 440
- Loritz, R., Hassler, S. K., Jackisch, C., Allroggen, N., Van Schaik, L., Wienhöfer, J., and Zehe, E.: Picturing and modeling catchments by representative hillslopes, Hydrol. Earth Syst. Sci., 21, 1225–1249, <https://doi.org/10.5194/hess-21-1225-2017>, 2017.
- Lund, J. W. G.: Observations on soil algae. I. The ecology, size and taxonomy of British soil diatoms, New Phytol., 44, 196–219, 1945.

- 445 Martínez-Carreras, N., Krein, A., Gallart, F., Iffly, J. F., Pfister, L., Hoffmann, L., and Owens, P. N.: Assessment of different colour parameters for discriminating potential suspended sediment sources and provenance: A multi-scale study in Luxembourg, *Geomorphology*, 118, 118–129, <https://doi.org/10.1016/j.geomorph.2009.12.013>, 2010.
- Martínez-Carreras, N., Krein, A., Gallart, F., Iffly, J. F., Hissler, C., Pfister, L., Hoffmann, L., and Owens, P. N.: The influence of sediment sources and hydrologic events on the nutrient and metal content of fine-grained sediments (Attert River basin, Luxembourg), *Water, Air, Soil Pollut.*, 223, 5685–5705, <https://doi.org/10.1007/s11270-012-1307-1>, 2012.
- 450 Martínez-Carreras, N., Wetzel, C. E., Frentress, J., Ector, L., McDonnell, J. J., Hoffmann, L., and Pfister, L.: Hydrological connectivity inferred from diatom transport through the riparian-stream system, *Hydrol. Earth Syst. Sci.*, 19, 3133–3151, <https://doi.org/10.5194/hess-19-3133-2015>, 2015.
- McDonald, D. M., Lamoureux, S. F., and Warburton, J.: Assessment of a time-integrated fluvial suspended sediment sampler in a high arctic setting, *Geogr. Ann. Ser. A Phys. Geogr.*, 92, 225–235, <https://doi.org/10.1111/j.1468-0459.2010.00391.x>, 2010.
- 455 McDonnell, J. J., Bonell, M., Stewart, M. K., and Pearce, A. J.: Deuterium variations in storm rainfall: Implications for stream hydrograph separation, *Water Resour. Res.*, 26, 455–458, <https://doi.org/10.1029/WR026i003p00455>, 1990.
- Müller-Haeckel, A. and Håkansson, H.: The diatom flora of a small stream near Abisko (Swedish Lapland) and its annual periodicity, judged by drift and colonisation, *Arch. für Hydrobiol.*, 84, 199–217, 1978.
- 460 Müller-Haeckel, A.: Different patterns of synchronization in diurnal and nocturnal drifting algae in the subarctic summer, *Aquil. Ser Zool*, 14, 19–22, 1973.
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGinn, D., Minchin, P. R., O’Hara, R., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., and Wagner, H.: *Vegan: community ecology package*. R package version 2.5-5, <https://cran.r-project.org/package=vegan>, 2019.
- 465 Passy, S. I.: Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters, *Aquat. Bot.*, 86, 171–178, <https://doi.org/10.1016/j.aquabot.2006.09.018>, 2007.
- Perks, M. T., Warburton, J., and Bracken, L.: Critical assessment and validation of a time-integrating fluvial suspended sediment sampler, *Hydrol. Process.*, 28, 4795–4807, <https://doi.org/10.1002/hyp.9985>, 2014.
- 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, <https://doi.org/10.1002/hyp.7426>, 2009.
- 470 Pfister, L., Wetzel, C. E., Martínez-Carreras, N., Iffly, J. F., Klaus, J., Holko, L., and McDonnell, J. J.: Examination of aerial diatom flushing across watersheds in Luxembourg, Oregon and Slovakia for tracing episodic hydrological connectivity, *J. Hydrol. Hydromechanics*, 63, <https://doi.org/10.1515/johh-2015-0031>, 2015.
- 475 Pfister, L., Wetzel, C. E., Klaus, J., Martínez-Carreras, N., Antonelli, M., Teuling, A. J., and McDonnell, J. J.: Terrestrial diatoms as tracers in catchment hydrology: a review, *Wiley Interdiscip. Rev. Water*, 2017.
- Phillips, J. M., Russell, M. A., and Walling, D. E.: Time-integrated sampling of fluvial suspended sediment: A simple methodology for small catchments, *Hydrol. Process.*, 14, 2589–2602, [https://doi.org/10.1002/1099-1085\(20001015\)14:14<2589::AID-HYP94>3.0.CO;2-D](https://doi.org/10.1002/1099-1085(20001015)14:14<2589::AID-HYP94>3.0.CO;2-D), 2000.
- 480 Rimet, F. and Bouchez, A.: Life-forms, cell-sizes and ecological guilds of diatoms in European rivers, *Knowl. Manag. Aquat. Ecosyst.*, 406, 1–12, <https://doi.org/10.1051/kmae/2012018>, 2012.

- Rivera, S. F., Vasselon, V., Bouchez, A., and Rimet, F.: Diatom metabarcoding applied to large scale monitoring networks: Optimization of bioinformatics strategies using Mothur software, *Ecol. Indic.*, 109, 1–13, <https://doi.org/10.1016/j.ecolind.2019.105775>, 2020.
- Round, F., Crawford, R., and Mann, D.: *The Diatoms. Biology and morphology of the genera*, Cambridge University Press, Cambridge, 1990.
- 485 Russell, M. A., Walling, D. E., and Hodgkinson, R. A.: Appraisal of a simple sampling device for collecting time-integrated fluvial suspended sediment samples, *IAHS-AISH Publ.*, pp. 119–127, 2000.
- Scarsini, M., Marchand, J., Manoylov, K. M., and Schoefs, B.: Photosynthesis in diatoms, in: *Diatoms Fundam. Appl.*, edited by Seckbach, J. and Gordon, R., chap. 8, pp. 191–211, Scrivener Publishing LLC, New Jersey, <https://doi.org/10.1002/9781119370741>, 2019.
- 490 Sklash, M. G. and Farvolden, R. N.: The role of groundwater in storm runoff, *J. Hydrol.*, 43, 45–65, [https://doi.org/10.1016/S0167-5648\(09\)70009-7](https://doi.org/10.1016/S0167-5648(09)70009-7), 1979.
- Smith, T. B. and Owens, P. N.: Flume- and field-based evaluation of a time-integrated suspended sediment sampler for the analysis of sediment properties, *Earth Surf. Process. Landforms*, 39, 1197–1207, <https://doi.org/10.1002/esp.3528>, 2014.
- Soylu, E. N. and Gönülol, A.: Phytoplankton and seasonal variations of the River Ye ú ılırmak, Amasya, *Turkish J. Fish. Aquat. Sci.*, 3, 17–24, 2003.
- 495 Van de Vijver, B., Ledeganck, P., and Beyens, L.: Soil diatom communities from Ile de la Possession, *Polar Biol.*, 25, 721–729, <https://doi.org/10.1007/s00300-002-0392-9>, 2002.
- 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, *Hydrol. Sci. J.*, 51, 1021–1038, <https://doi.org/10.1623/hysj.51.6.1021>, 2006.
- 500 Vasselon, V., Rimet, F., Tapolczai, K., and Bouchez, A.: Assessing ecological status with diatoms DNA metabarcoding: Scaling-up on a WFD monitoring network (Mayotte island, France), *Ecol. Indic.*, 82, 1–12, <https://doi.org/10.1016/j.ecolind.2017.06.024>, 2017.
- Walling, D. E.: Tracing suspended sediment sources in catchments and river systems, *Sci. Total Environ.*, 344, 159–184, <https://doi.org/10.1016/j.scitotenv.2005.02.011>, 2005.
- Wetzel, R. G.: *Limnology: lake and river ecosystems*, gulf professional publishing, 2001.
- 505 Wu, N., Faber, C., Sun, X., Qu, Y., Wang, C., Ivetic, S., Riis, T., Ulrich, U., and Fohrer, N.: Importance of sampling frequency when collecting diatoms, *Sci. Rep.*, 6, 1–9, <https://doi.org/10.1038/srep36950>, 2016.
- Zambrano-Bigiarini, M.: *hydroTSM: Time Series Management, analysis and interpolation for hydrological modelling*, <https://doi.org/10.5281/zenodo.839864>, 2017.