

Diatoms as a tracer of hydrological connectivity: are they supply limited?

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ABSTRACT

Recent work has shown that aerial diatoms are a useful ecological tracer of hydrological connectivity in the hillslope–riparian zone–stream (HRS) system. While such work has improved both our understanding of catchment functioning and aerial diatom taxonomy, assemblages and distribution, further work is hampered by lack of data on diatom population depletion during rainfall events. We still do not know whether or not diatom tracers are supply limited. Here we test the null hypothesis that aerial diatoms exhibit infinite supply in the context of natural rainfall events. Rainfall simulation experiments were conducted in a small forested catchment in northwest Luxembourg. We extracted periodically soil surface samples and overland flow samples for diatom population size and species assemblage analyses. Diatom population size was quantified using a new approach we have developed, which involves extracting diatoms using carbonated water and an isopycnic separation technique. Our results showed that pre-event population size was *c.* 96 100 diatoms per cm² in the riparian zone. During the artificial rainfall event, the diatom population was depleted by 72% to 27 200 diatoms per cm². The diatom assemblage was characteristic of a frequently disturbed environment. Overall, these results suggest that diatoms are supply limited, and are flushed significantly throughout rainfall events. Nevertheless, based on the data from these 1 in 10-year rainstorm simulations, the riparian zone diatom population is unlikely to be exhausted on an event time scale. Further research is now underway to investigate the spatial and temporal variability of aerial diatom communities across a range of storm sizes. Copyright © 2015 John Wiley & Sons, Ltd.

KEY WORDS diatoms; hydrological connectivity; tracers; rainfall disturbance

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INTRODUCTION

Tracing of water flow sources is an important area of study in hydrology. Geochemical and isotopic tracers have revolutionized our understanding of the age, origin and pathways of water at the watershed scale (since the early isotope hydrograph separation work of Sklash and Farvolden, 1979, and end member mixing analysis of Hooper *et al.*, 1990). Current and further progress is now hampered by various assumptions and limitations in the techniques, including unstable end-member solutions (Elsenbeer *et al.*, 1995; Burns, 2002), temporally varying input concentrations (McDonnell *et al.*, 1990) and the need for unrealistic mixing assumptions (Fenicia *et al.*, 2010)—for a recent review see Klaus and McDonnell (2013).

In response to the need for new tracers, Pfister *et al.* (2009) developed a new technique for studying water flowpath connectivity that uses diatoms (unicellular, eukaryotic algae that are ubiquitous in aquatic ecosystems) as natural ecological tracers of surface runoff. Because moisture conditions (and therefore hydrological systems) largely control diatom species distribution, allochthonous diatoms found in stream water samples during rainfall events record the geographical origin of surface runoff at the catchment scale (Pfister *et al.*, 2009; Martínez-Carreras *et al.*, 2015). Through its integration of hydrology and ecology, this new approach to water tracing is thought to provide a holistic view of catchment functionality (as advocated by, for example, Hannah *et al.*, 2007 and Tetzlaff *et al.*, 2007). From a hydrological perspective, the approach complements traditional methods for tracing water sources and flowpaths, by sidestepping some of the current limitations described above. From an ecological and taxonomical perspective, the approach has increased the knowledge of aerial diatom species which previously had been poorly known (Wetzel

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et al., 2013). While proof of concept of the diatom tracer approach has been shown, it is still unclear if diatoms are of infinite supply at the event timescale. To date, work has assumed an unlimited supply (Pfister *et al.*, 2009). However, no studies have yet tested this supply hypothesis.

Early taxonomical work by Petersen (1935) and Lund (1945) described two aerial diatom population depletion mechanisms as a result of rainfall: (i) infiltration into the subsurface; and (ii) overland flow. The stability of the diatom population is likely to be determined by their resilience and their rate of recovery from disturbance (Webster *et al.*, 1975). Their resilience is likely to be controlled by their level of substrate adhesion (resistance) and colony formation (Rimet and Bouchez, 2012) and their size (Stanish *et al.*, 2011), while their recovery is likely to be dependent on their cell division rates. However, the latter might also influence their level of resilience, if the rate is fast enough that population size can be maintained during disturbance. Thus, the impact of the disturbance (by storm rainfall) will be a combination of diatom resilience and recovery, the level of the disturbance (rainfall amount and intensity) and environmental conditions that control overland flow and infiltration processes (such as substrate, soil type and surface gradient). We are not aware of any studies that have quantified the resilience, response or recovery of aerial diatom communities to rainfall disturbance. In comparison, the effect of flood disturbance on in-stream diatom (and other algal) communities has been well-studied (e.g. Power and Stewart, 1987; Yount and Niemi, 1990; Peterson and Stevenson, 1992; Cambra and Gomà, 1997; Soininen and Heino, 2005; Stanish *et al.*, 2011)—comparison to which we refer later in this paper. Here, we consider aerial diatom communities as those communities living exposed to the air outside of lentic and lotic environments, following Johansen (2010).

It is important to determine whether diatoms are of infinite supply at the event timescale so that we can be sure that the absence of aerial diatoms in streamwater samples is a result of missing HRS connectivity and not because of an exhaustion of the diatom supply. Quantifying event-based aerial diatom population depletion is necessary therefore for continued development of aerial diatoms as tracers of connectivity in the HRS system during rainfall events. Such work is also important for further understanding the resilience, response and recovery of aerial diatom communities to hydrological disturbance, given that aerial diatom ecology has not been widely studied. Here we address the following specific questions:

- (i) Is the aerial diatom population mobilized and does it deplete during rainfall events?
- (ii) Is there a preferential depletion of certain diatom species?
- (iii) How does habitat type affect diatom population size?

- (iv) By what mechanism (e.g. overland flow or infiltration) is the diatom population on the soil surface depleted?
- (v) What are the implications of any aerial diatom population depletion for their use as a hydrological tracer?

METHODS

Study site

The research was situated in the Weierbach catchment (0.45 km²; 49°49'42"N, 5°47'49"E) of the Attert experimental basin (290 km² at Bissen) in Luxembourg (also the location of previous diatom research: Rimet *et al.*, 2004; Pfister *et al.*, 2009) (Figure 1). The Weierbach catchment has schistose geology and consists of a plateau cut by deep v-shaped valleys. Cambisols are dominant on the plateau and hillslopes, whereas fluvisols cover the riparian areas (Juilleret *et al.*, 2011). The catchment is covered mainly by *Fagus sylvatica* Linnaeus and *Picea abies* (Linnaeus) H. Karsten woodland. During our study period (June–July 2012), three main habitat types dominated the catchment surface connected to the stream network: (1) hillslopes with little understory where the soil surface is covered with *F. sylvatica* leaf litter; (2) hillslopes where the soil surface is covered with thick bryophyte vegetation (mosses); and (3) a riparian zone (approximately 2–10 m wide) with a bare soil surface (Figure 1). For each habitat, the substrate type(s) are, respectively: (1) *F. sylvatica* leaf litter and bare soil; (2) bryophyte vegetation; and (3) bare soil. We distinguish between ‘habitat’ and ‘substrate’ type because habitat includes the broad physical factors (such as moisture or temperature) and biotic factors (such as food availability), while substrate refers to the surface to which the diatoms are adhered.

Soil plot setup

A circular metal ring (diameter: 0.6 m; area: 0.3 m²) was used to mark the soil plot. It was placed in the three above-mentioned habitat types of the Weierbach catchment (hillslope with leaf litter, hillslope with thick bryophyte cover and bare-soil riparian zone) to account for the effects that different diatom species (assemblages and population sizes differ by habitat and substrate) and habitat surface—leaf litter, moss or bare soil—(through its partitioning of rainfall into different hydrological processes) might have on potential diatom depletion. The ring ensured that the soil plot was isolated from the surrounding area so that there could be no contamination of diatoms from upslope. Each soil plot was located on an area of homogenous vegetation and soil type. Any overland flow from the rainfall simulation was allowed to flow out via an outlet in the downslope side of the metal ring.

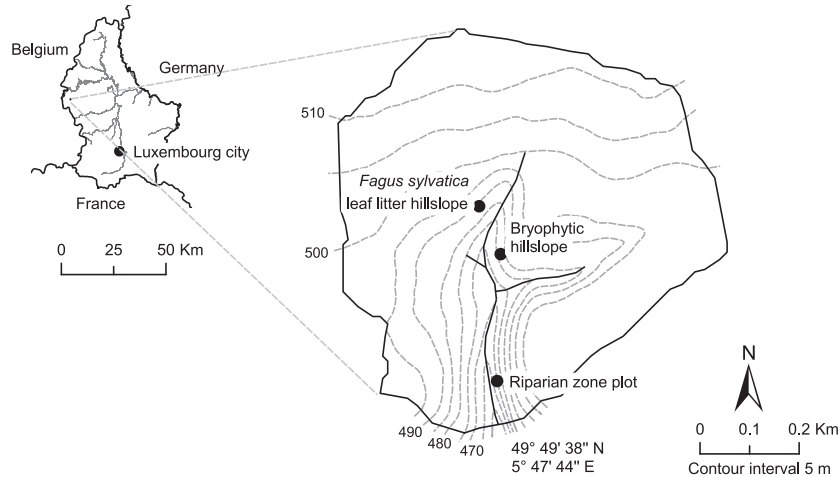


Figure 1. Weierbach catchment location indicating locations of the soil plots.

Rainfall simulation

We used a portable rainfall simulator built, tested and calibrated by Iserloh *et al.* (2012) to produce our artificial events: rainfall intensity of 40 mm h^{-1} , median volume drop diameter of between 1.0 and 1.5 mm and mean momentum of between 0.008 and 0.020 kg ms^{-1} . For each soil plot experiment, the rainfall intensity was held constant at 40 mm h^{-1} for 60 min, a 10-year return period event based upon rainfall data from Holtz (Luxembourg; $49^{\circ}48'17''\text{N}$, $5^{\circ}47'44''\text{E}$) (Pfister *et al.*, 2010). Every 15 min, the rainfall simulator was paused for approximately 5 min to carry out the surface soil and overland flow sampling (described below). The rainfall simulation over the leaf litter hillslope, bryophyte hillslope and riparian bare-soil plots were carried out on 18 June, 22 June and 4 July 2012, respectively, following a minimum of 48 rainless hours to ensure dry antecedent conditions.

Diatom sample preparation and analysis for quantifying diatom population size

As far as we know, there are no methods to determine the absolute abundance of aerial diatoms in soil surface samples. Thus, we describe here a new standardized methodology for this purpose, which is a combination of new protocols that we developed, and also modifications that we made to protocols developed by Ribeiro (2010) and Ribeiro *et al.* (2013) for estuaries. A brief summary of our sample preparation and analysis follows below (and is described fully in Appendix 1).

Surface soil samples (top 4 cm of soil) were taken before initiating the first rainfall event, and then every 15 min throughout the 60-min rainfall event. These surface soil samples were collected from within the experiment's soil plot using small soil sampling rings (5.6-cm diameter). Each sample was composed of three sub-samples (i.e. three 5.6-cm soil sampling rings per sample). Furthermore, any overland

flow that occurred during the rainfall event was collected periodically at the outlet of the soil plot. Overall, analysis of the diatom population sizes in both soil surface and overland flow samples ensured our ability to draw a reasonable picture of any fluctuations in diatom population size.

The diatom populations were fixed in the lab using formaldehyde as soon as possible following sampling (within 6 h) to prevent any population change by cell division. Carbonated water was used to detach the diatoms from the substrate on the surface of the sample (the surface to which the diatoms are adhered), and the diatoms were then separated from the soil mineral particles using an isopycnic separation technique and centrifugation. The resultant diatomaceous solution was left for at least 4 h in 25-ml sedimentation chambers (Utermöhl 1958), after which an inverted light microscope was used to count the number of diatom frustules in a set number of fields of view. Whether the frustules were alive or dead at the time of sampling (determined by the presence or absence of chloroplasts) was recorded. Finally, using Equations (1) and (2), the number of diatoms on the surface (i.e. the substrate) of each soil sub-sample was estimated and expressed in terms of the number of diatoms per cm^2 (Equation (1)), while the number of diatoms in the overland flow sample was estimated and expressed in terms of the number of diatoms contributed from each cm^2 of soil surface (Equation (2), which assumes that diatoms are depleted and overland flow is generated uniformly across the soil plot),

$$T = (N \times V_P \times V_S \times A_C) / (V_C \times V_A \times F \times A_F \times A_R) \quad (1)$$

$$T = (N \times V_{OF} \times A_C) / (V_A \times F \times A_F \times A_P) \quad (2)$$

where:

T = total number of diatoms per cm^2 soil surface
 N = total number of diatoms counted

V_P	= volume of processed sample after centrifuging process (including distilled water to wash in all sediment)
V_C	= volume of processed sample put into chamber (prior to making up to 25 ml with distilled water)
V_S	= volume of initial sample solution (sparkling water, alcohol and sediment mix) (100 ml)
V_A	= volume of aliquot subsample
V_{OF}	= volume of overland flow collected during a 15-min rainfall period
F	= number of fields of view counted
A_F	= area of one field of view
A_C	= area of base of sedimentation chamber (5.31 cm ²)
A_R	= area of soil surface sample (cm ²) (24.6 cm ²)
A_P	= area of soil plot (2.83 × 10 ³ cm ²).

Diatom sample preparation and analysis for determining species assemblages

Ultrastructural details of diatoms were required for species-level identification. To achieve this, we completed a second diatom preparation technique involving mounting the diatoms on slides with Naphrax[®] (a medium with a high refractive index) (Battarbee, 1986). At least 400 valves per slide (i.e. per sub-sample) were identified and the relative abundance of diatoms (‰) was calculated. Ecological information of species was obtained using the software OMNIDIA[®] version 5.3 (Lecointe *et al.*, 1993; database 2012). We used a variety of taxonomic references for species identification, including the atlas of Schmidt (1874–1959), the catalogue of Simonsen (1987), all volumes from series *Diatoms of Europe* (e.g. Lange-Bertalot *et al.*, 2011; Levkov *et al.*, 2013), *Iconographia Diatomologica* (e.g. Lange-Bertalot and Metzeltin, 1996; Lange-Bertalot *et al.*, 1996; Lange-Bertalot *et al.*, 2003; Werum and Lange-Bertalot, 2004), *Bibliotheca Diatomologica* (e.g. Lange-Bertalot, 1993; Kulikovskiy *et al.*, 2010). Recent studies from Germany (Hofmann *et al.*, 2011), Poland (Żelazna-Wieczorek, 2011; Wojtal, 2013) and Czech Republic (Veselá and Johansen, 2009) were also consulted regularly.

Following species-identification, the life form of each diatom was determined using the classification by Rimet and Bouchez (2012). Life forms classify a diatom's likely resistance to disturbance by flowing water or rainfall. Low-profile diatoms tend to have a short stature, including prostrate, adnate (attached to a substrate) and erect diatoms, and are resistant to disturbance. High-profile diatoms tend to be larger and form colonies, and are less able to resist turbulence. Motile diatoms are fast moving species and are well adapted for movement through turbulent systems.

To analyse compositional differences between diatom communities from soil plots and overland flow, we applied non-metric multidimensional scaling (NMDS) ordination techniques, using the software PC-ORD version 6.0 (McCune

and Grace, 2002). NMDS is an iterative search tool for the ranking and placement of n entities (samples) in k dimensions (ordination axes) that minimizes the stress of the k -dimensional configuration. NMDS is therefore used to find a configuration in a given number of dimensions, which preserves rank-order dissimilarities in species composition as closely as possible, such that distance along an NMDS axis corresponds to the relative difference in community composition (Tylianakis *et al.*, 2008). As a distance measure, the Bray–Curtis coefficient (also known as Sørensen coefficient) was used, which is one of the most robust measures for this purpose. We log transformed [$\log_{10}(x+1)$] abundances to lessen the influence of abundant taxa. Additionally, we calculated the degree of entropy in the samples by calculating the evenness (E) of the samples [i.e. measuring the evenness with which individuals are divided among the taxa that are present. For non-zero species values in a sample unit: $E = H' / \ln(S)$, where H' = diversity of species in a sample unit (Shannon Index of information content; Shannon and Weaver, 1949), and S = species richness in a sample unit].

RESULTS

The initial diatom population size (prior to rainfall simulation) on the riparian soil surface was approximately 96 100 diatom cells cm⁻². The diatom population decreased non-linearly with rainfall amount ($r^2=0.99$ for a logarithmic line of best fit; Figure 2). After 10 mm (15 min) of rainfall, the population size had decreased by 46%. After 40 mm (60 min) of rainfall, the diatom population size was depleted by 72% (a total depletion of approximately 68 900 diatoms cm⁻²). However, statistical analysis using the t -test showed that only the diatom population change during the first 10 mm of rainfall application is significant ($p < 0.05$). After 10 mm of rainfall, no significant change in diatom population size was observed.

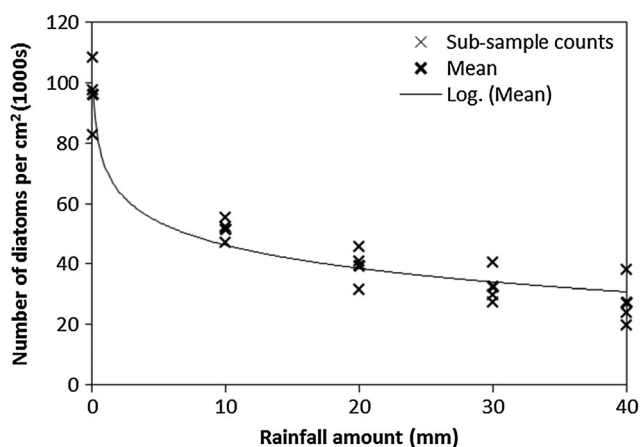


Figure 2. Sub-sample and mean (of sub-samples for each time step) diatom population sizes per cm² of the riparian zone plot's soil surface. A logarithmic line of best fit was fitted to the mean data.

Pre-storm proportions of alive and dead cells in the soil samples were approximately equal (Figure 3). In terms of absolute abundance, the number of alive diatoms then decreased by *c.* 84% by the end of the 1-h rainfall simulation. In contrast, the number of dead diatoms initially decreased by *c.* 42% after the first 10 mm of rainfall, but then stabilized for the remainder of the simulation.

Overland flow did not occur on either the leaf litter or the bryophyte hillslope soil plots, while it did on the riparian zone soil plot. The total volume of overland flow collected at the outlet of the riparian soil plot was 11.21 (99% of the 11.31 of rainfall inputted to the soil plot over 60 min). The overland flow contained an estimated 27 800 diatoms per cm^2 of soil surface (Figure 4). Thus, the overland flow contained just 40% of the total number of diatoms depleted from the soil surface ($68\,900\text{ diatoms cm}^{-2}$).

We observed that the number of living diatoms in overland flow decreased—however without depleting—over the course of the experiment. In the first 15 min, *c.* 11 100 aerial diatoms

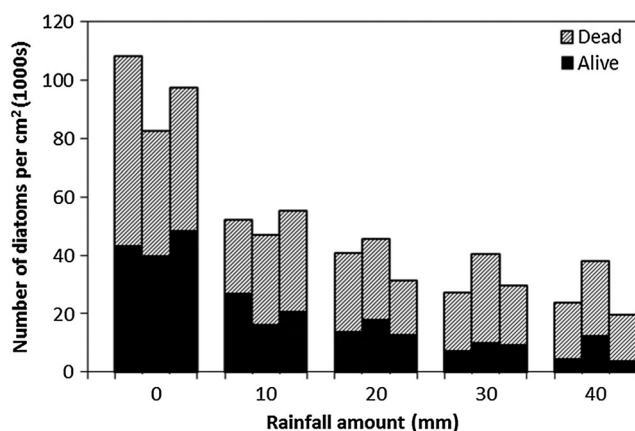


Figure 3. Numbers of alive and dead diatoms in each soil surface subsample from the riparian zone plot.

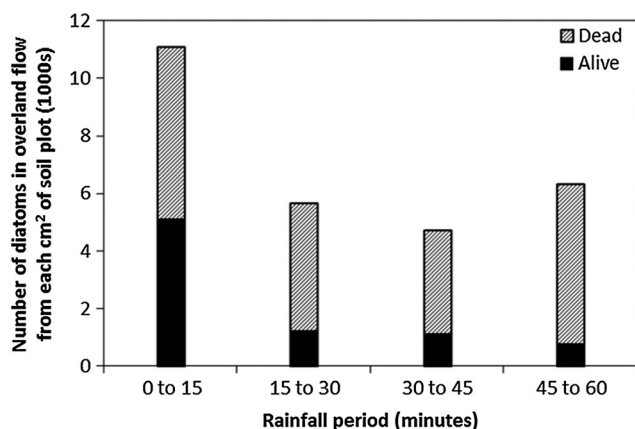


Figure 4. Number of dead and alive diatoms in overland flow from the riparian zone plot for each 15-min rainfall period, calculated as the number of diatoms contributed from each cm^2 of soil plot.

per cm^2 of soil surface were exported (46% of which were alive). The number of diatoms in overland flow remained fairly constant throughout the remainder of the experiment (*c.* 5500 diatoms per cm^2 of soil surface, 19% of which were alive) (Figure 4).

Diatoms identified from the 400-valve samples ($n=27$) represented 76 taxa (see Table I in Appendix 2). Thirteen species occurred in all samples: *Achnantheidium minutissimum* (Kützing) Czarnecki, *Cavinula intractata* (Hustedt) Lange-Bertalot sensu Werum and Lange-Bertalot (2004), *Diploneis fontanella* Lange-Bertalot, *Eolimna tantula* (Hustedt) Lange-Bertalot, *Eunotia minor* (Kützing) Grunow, *Fragilariforma virescens* (Ralfs) D.M. Williams & Round, *Gomphonema productum* (Grunow) Lange-Bertalot & E. Reichardt, *G. varioeduncum* Jüttner *et al.*, *Placoneis ignorata* (Schimanski) Lange-Bertalot in Rumrich *et al.*, *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot, *P. lanceolatum* (Brébisson) Lange-Bertalot, *Stauroneis parathermicola* Lange-Bertalot in Hofmann *et al.* and *Sellaphora seminulum* (Grunow) D.G. Mann. The number of taxa observed during the experiment was *c.* 39 species per sample ($n=27 \pm 3$; min. = 33; max. = 44). The two most common species in the samples, *P. frequentissimum* (Lange-Bertalot) Lange-Bertalot and *P. lanceolatum* (Brébisson) Lange-Bertalot, had a mean abundance of 29.3% and 19.2%, respectively. The total abundances of both species (which have similar habitat and life form) in the overland flow samples tended to decrease because their abundance ranged from up to 50% in the first event to less than 32% in the last event (Figure 5). The dominant species present are known typically as having preferences for aerial habitats, mainly living epiphytically on bryophytes or on exposed soil, such as *Chamaepinnularia evanida* (Hustedt) Lange-Bertalot, *C. parsura* (Hustedt) C.E. Wetzel & Ector, *Nitzschia harderi* Hustedt in Brendemühl, *Stauroneis parathermicola* Lange-Bertalot in Hofmann *et al.*, and *S. thermicola* (J.B. Petersen) J.W.G. Lund. Diatom communities were also composed of species from oligotrophic environments (e.g. *Achnantheidium kranzii* (Lange-Bertalot) Round & Bukhtiyarova, *Fragilariforma virescens*, *Eunotia botuliformis* Wild, Nörpel & Lange-Bertalot, *E. minor* (Kützing) Grunow, and *Planothidium lanceolatum* (Brébisson) Lange-Bertalot). An elevated relative abundance of the colonial species *F. virescens* was also observed. This colony forming species is typical of terrestrial environments (Van Dam *et al.*, 1994). Results from the spatial ordination analysis (NMDS) clearly separated soil surface samples from overland flow samples (Figure 6), highlighting distinct community structures in both sets of samples and indicating a potential preferential depletion of certain diatom species. Although the overall floristic composition was similar in terms of species composition, samples from overland flow showed less evenness compared to samples from soil plots (Figure 7).

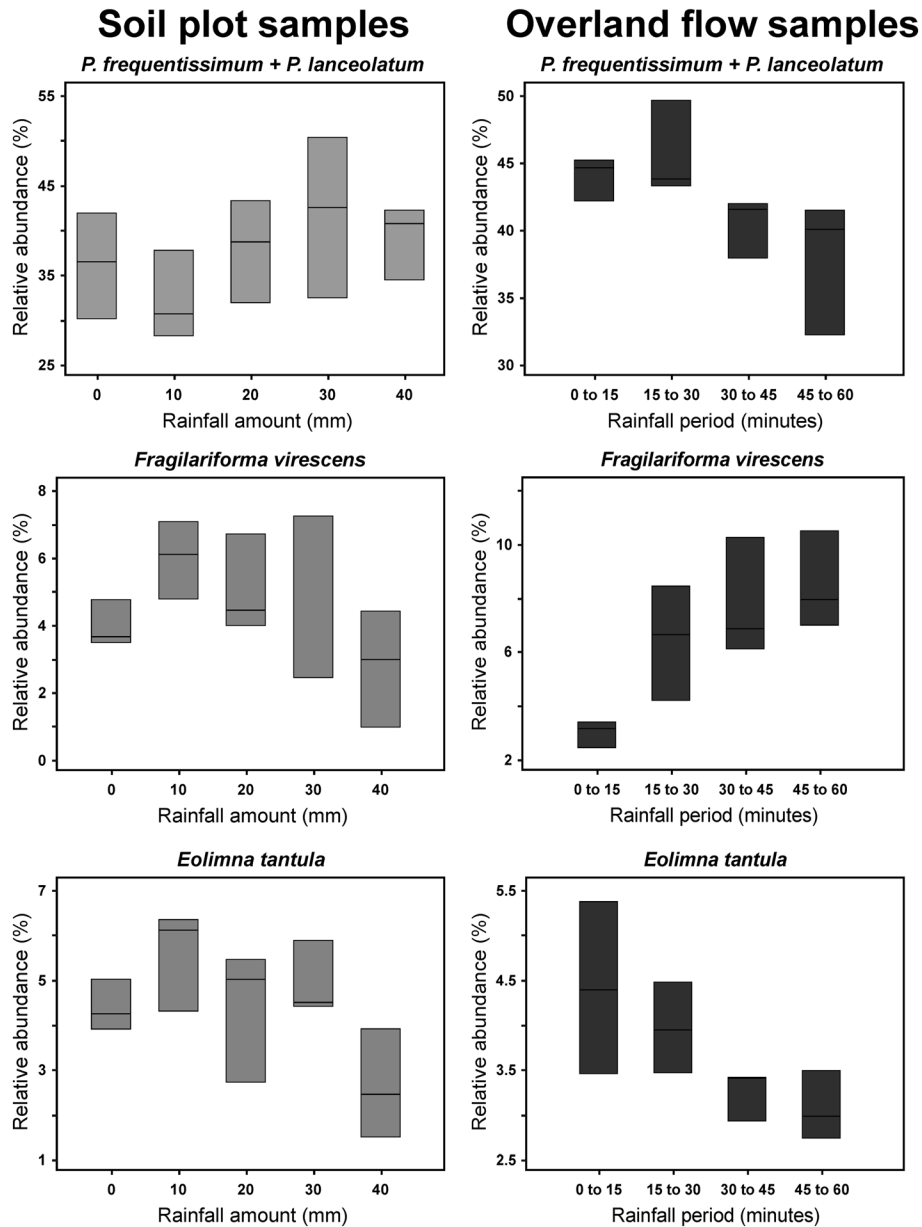


Figure 5. Relative abundances of diatom species from soil samples and overland flow samples from the riparian zone. The species shown are representative of the three life forms (low-profile, high-profile and motile) and the different responses to rain events. The life forms are: low-profile (high resistance): *Planothidium frequentissimum* and *P. lanceolatum*; high-profile (medium resistance): *Fragilariforma virescens* and motile (low resistance): *Eolimna tantula*.

Of the three habitats on which we based our rainfall simulations, only the riparian zone bare-soil plot yielded sufficient diatom data for quantitative investigations. Therefore, only the results generated from the riparian zone experiment are presented here. No diatoms were found in samples from the leaf litter hillslope soil plot. Some diatoms were extracted from samples from the bryophyte hillslope soil plot, but in too small abundances to provide reliable results. Overland flow did not occur on either the leaf litter or the bryophyte hillslope soil plots, while it did on the riparian zone soil plot. We focus here on the results generated from the riparian zone experiment.

DISCUSSION

Does the aerial diatom population deplete during rainfall events?

With regards to the use of diatoms as tracers of hydrological connectivity, our results indicate that diatoms are supply limited. We found that, during a 1 in 10-year storm, aerial diatom population size depleted with increasing rainfall amounts. Within the riparian zone, diatom population size depleted by 72% from *c.* 96 100 to *c.* 27 200 diatoms per square centimetre following 40 mm (60 min) of simulated rainfall. Nevertheless, the fact that we did not see an

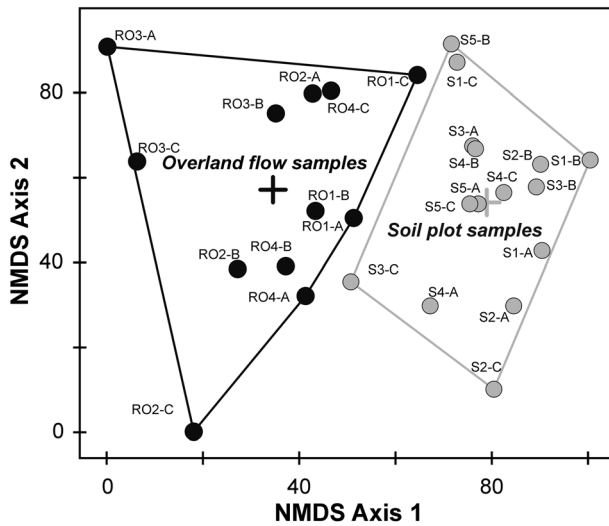


Figure 6. Nonmetric multidimensional scaling (NMDS) of species communities from soil plot samples (grey dots) and from overland flow samples (runoff, black dots) from the riparian zone. Centroids for the respective groups are shown by crosses.

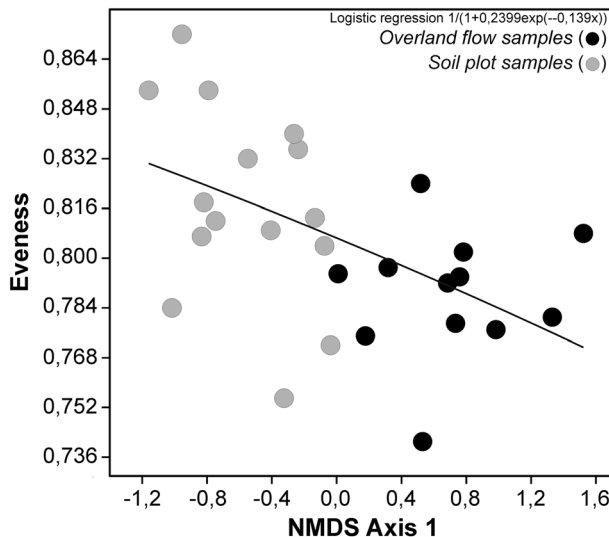


Figure 7. Relationships between scores on the first axis of the nonmetric multidimensional scaling (NMDS) and the evenness (E) of species assemblage from soil plot samples (grey dots) and from overland flow samples (runoff, black dots) from the riparian zone.

exhaustion of diatoms on the soil surface in the simulated 1 in 10-year rainfall event suggest substantial potential for supply on an event timescale.

Is there a preferential depletion of certain diatom species?

We found that the species assemblage in the riparian zone was dominated by low-profile diatoms. This is consistent with Power and Stewart (1987) who found that frequent disturbance truncates algal successional sequences by favouring very adhesive or resistant diatoms, or diatoms with high recolonization and growth rates. Our results

showed no preferential removal of motile species. Furthermore, there was no indication that low-profile diatoms dominated in the latter stages of the rainfall simulation (Figure 5). Although the results of the NMDS analysis showed two separate diatom groupings (Figure 6), the differences between were due mainly to the degree of entropy (a measure of unpredictability or uncertainty in a random variable) of both datasets (Figure 7). This suggests that although, at the community-level, diatoms are adapted to living in a frequently disturbed site, this does not necessarily translate to species-level resistance to a single-event disturbance.

We were not able to identify, for an individual diatom frustule, both its species and whether it was alive or dead at the time of sampling. New techniques are required to achieve this. This would enable the species types and life forms of those diatoms that were alive to be analysed separately from the equivalent data for the dead (and thus inert) diatoms. This is particularly important for studies of aerial diatom ecology and for further understanding the resilience, response and recovery of aerial diatoms to hydrological disturbance. It might be that the species and life form assemblages of the live diatoms in fact do vary in response to disturbance, and that the dead diatom data have clouded any trend here. For indicators of hydrological processes, we suggest that it is important to differentiate between alive and dead diatoms. This is because soil profiles can contain very high numbers of dead diatoms, which are easily preserved because of their siliceous cell walls (Mackay *et al.*, 2003). Therefore, for example, any surface return of groundwater, mixing of surface water with soil water, or soil surface erosion by rainfall might cause dead and buried diatoms to resurface.

How does habitat type affect diatom population size?

Our measured, pre-disturbance diatom population size on the bare-soil substrate of the riparian zone (96 100 per cm²) is similar to diatom population sizes reported by Baume and Straub (1993) for a moist terrestrial zone (54 000–153 000 diatoms per cm²). Of the different terrestrial environments that Baume and Straub (1993) studied (the only other study we know of that has estimated diatom population sizes in a similar environment), this zone yielded by far the greatest diatom population. Like that study, the riparian zone in our study also yielded the greatest diatom population. The high soil moisture content of the riparian zone is likely to be the dominant factor behind the high diatom populations. Soininen and Heino (2005) showed a significant positive relationship between local abundance and local population persistence. Therefore, the large abundance of diatoms may be a causative factor in preventing population extinction in the samples because of efficient cell division. However, like us, Soininen and Heino (2005) were unable to judge whether

it was the high abundance that increased local population persistence, or whether a high persistence enabled a high abundance.

We are unaware of any other work that has investigated the existence of a diatom population living on a leaf litter hillslope. We found no diatoms in samples from this habitat type of our study site. High moisture contents and light levels are typically important ecological factors in the colonization of an environment by diatoms (Van de Vijver and Beyens, 1999). Therefore, we hypothesize that leaf surfaces of *F. sylvatica* were too dry to support a diatom population on the leaf litter substrate. Further, light conditions at the soil surface were likely too low to support a diatom population on the soil substrate beneath.

Very few diatoms were present in our bryophyte soil plot samples, and there were too few to obtain a reliable estimation of the relative species composition. This again mirrors the results obtained by Baume and Straub (1993), where they estimated *c.* 100 diatoms per cm² in their moss vegetation environment. Nevertheless, previous studies have documented rich bryophyte diatom communities, even in relatively dry terrestrial zones (e.g. Johansen, 2004; Cantonati *et al.*, 2009). Van de Vijver and Beyens (1999) showed that moisture content, rather than light, was the most important ecological factor in the distribution of diatoms in terrestrial habitats. Thus, we hypothesize that diatoms would reside within the bryophyte layer, as opposed to on its surface. If this is the case, our method of diatom extraction using carbonated water is not suitable because this targets the surface of the sample. Further research is needed to develop alternative techniques for quantifying bryophyte diatom population size (such as total digestion of a vegetation section).

In this study, we have not explored how the chemical nature of the different substrates might affect diatom population size (for example, the influence of soil cations on the level surface adhesion of diatoms; Geesey *et al.*, 2000) because this was beyond the scope of the research.

What have we learned about the nature of the observed diatoms?

For the diatom communities studied here, we classified the species by ecological requirements according to Van Dam *et al.* (1994) (provided in Appendix 2). We consider as aerial those diatoms listed as category 4 or 5, which includes diatoms 'mainly occurring on wet and moist or temporarily dry places' and diatoms 'nearly exclusively occurring outside water bodies', respectively (Van Dam *et al.*, 1994). Of the species observed in this study, 43% are classified as category 3 ('mainly occurring in water bodies, also rather regularly on wet and moist places'), while 26% are considered aerial (category 4 and 5) and 5% are aquatic (category 1 and 2). We modified some of the categorical values. For example, species

such as *Chamaepinnularia obsoleta* (Hustedt) C.E. Wetzel & Ector and *Chamaepinnularia parsura* (Hustedt) C.E. Wetzel & Ector are common in the Weierbach catchment, with some of them present in 70% of the analysed samples. In this case we decided that these are 'aerial species', based on the type material collected in bryophytes from forests in Germany (Hustedt, 1942; Wetzel *et al.*, 2013).

Among the aerial species, we highlight the presence of *Eunotia minor* (Kützing) Grunow, *Stauroneis parathermicola* Lange-Bertalot in Hofmann *et al.* and *Cavinula intractata* (Hustedt) Lange-Bertalot sensu Werum and Lange-Bertalot (2004). These three aerial species were present in all analysed samples (100% frequency), despite their low relative abundances. Similarly, other truly aerial species were present in the majority of samples (up to 63%) albeit in low relative abundances. Indeed, many diatom assemblages we have observed (both in this study and in soils from nearby areas) show a high diversity. The assemblage described here closely mirrors the *Fragilarietum virescentis* association of Kolbe (1932) for European aerial-aquatic sites. The dominant species found in this study are widely reported from aerial environments (e.g. Van de Vijver *et al.*, 2013; Ettl and Gärtner, 2014).

Overall, the true diversity of aerial diatoms is thought to be widely underrepresented, and as far as we know, there is no work synthesizing this information. We suggest that we still only poorly understand the ecology of many species. Further, we know even less about the dynamics in the riparian zone, where the species communities in this study are living. Most importantly, we believe that generic preferences cannot be attributed equally to species, which is why we can consider different species of *Pinnularia* to be aquatic or aerial.

By what mechanism (e.g. overland flow or infiltration) is the diatom population on the soil surface depleted?

The number of diatoms counted in the overland flow (27 800 diatoms per cm² of soil surface) draining the riparian soil plot was only 40% of the total number of diatoms depleted from the soil surface (68 900 diatoms cm⁻²). This suggests that there is another route by which diatoms are lost from the soil surface. Infiltration of diatoms into the subsurface may be an additional depletion mechanism. Vertical movement of diatoms was hypothesized by Lund (1945). Since then, Hart and Humphreys (1997) observed vertical movement of diatoms through the soil matrix, and Fishkis *et al.* (2010) observed the same for phytoliths (siliceous microscopic structures with a similar size to diatoms). However, both studies observed diatom infiltration to depths of only *c.* 40–80 cm over one year of rainfall simulation. Furthermore, both studies argued that the relatively large size of diatoms should preclude their downward movement through the soil matrix. Therefore, movement into the subsurface would be

restricted to macropores. Given that diatoms are typically 10–200 μm in their largest dimension (Mann, 2002; Pfister *et al.*, 2009) (equivalent to the silt and fine sand fraction), the nature of the soil type in the Weierbach riparian zone (a silty loamy matrix; Juilleret *et al.*, 2011) means that their infiltration into the subsurface is unlikely. Other research at our study site has also suggested that diatom infiltration through the soil matrix is not possible because of their relatively large size (Tauro *et al.*, 2013).

There are two alternative explanations for the fact that the loss of diatoms on the soil surface is not entirely accounted for by overland flow. The first is a possible reflection of the uncertainties of scaling undertaken using our calculations (Equations (1) and (2)). The second explanation is that some diatoms mobilized by overland flow might be deposited at the lowest point of the soil plot, where the overland flow water was occasionally observed to artificially pond prior to flowing through the outlet of the metal ring.

What are the implications of any aerial diatom population depletion for their use as a hydrological tracer?

The fact that diatom reservoir exhaustion appears to be unlikely even in heavy rainfall events—as shown by our experiment—supports their use as an indicator of connectivity. We suggest that the value of diatoms as hydrological tracers remains in their indication of the onset and cessation of connectivity and, therefore, the period during which overland flow occurs within the HRS system. For this purpose, it is not necessary to know the absolute diatom population size in the upslope contributing area, just that the diatom reservoir persists and therefore that the presence or absence of aerial diatom species in streamflow indicates connectivity onset or cessation. The value of this tracer is only lost if the available diatom pool is exhausted, which our results show to be unlikely. As further support of this, although the surface soil diatom population showed a rapid initial depletion (by 42%) in response to the first 10 mm of rainfall, the diatom population size in the subsequent samples did not deplete significantly. This might indicate that the population size shows a threshold response to heavy rainfall. Further research undertaking repeat experiments would be useful to further constrain the depletion rate and rainfall amount required for complete exhaustion.

CONCLUSIONS

We found that aerial diatom populations do decrease in response to rainfall disturbance. However, at the event timescale (here, we simulated a 1 in 10-year rainfall event), the diatom reservoir is not exhausted. The riparian zone

diatom population was depleted by overland flow. Given other research has suggested that diatom infiltration through the soil matrix is impossible because of their relatively large size, we argue that diatom population depletion would therefore be unlikely without the occurrence of overland flow. Therefore, at the event timescale, we suggest that diatoms remain useful indicators of the onset and cessation of HRS connectivity by overland flow.

We have discussed a number of mechanisms that we believe affect the diatom population size in response to rainfall disturbance. Several relate to the resistance of the diatoms themselves to disturbance. Their large population size no doubt also plays a role in avoiding population exhaustion, especially because this would allow high levels of vegetative multiplication by cell division. This latter aspect of resilience and response to disturbance was indistinguishable from other likely resistive measures in our results. Furthermore, we are unable to say whether large population size is a cause or an effect of population persistence.

Further research we intend to undertake includes investigations of the spatial and temporal variability of aerial diatom communities—an essential research avenue to pursue if we are to demonstrate the full potential for tracing the onset and cessation of connectivity within the HRS system. Additionally, we will further investigate appropriate sampling methodologies for bryophytic substrate. This will extend the new approach we have presented here for the quantification of aerial diatom population size.

APPENDIX 1

Full description of the standardized methodologies developed for diatom sample preparation and analysis for quantifying diatom population size

Step 1: *Surface soil and overland flow sampling*

In order to capture the change in diatom population size as a result of rainfall, surface soil samples were taken before initiating the first rainfall event, and then every 15 min throughout the 60-min rainfall event. Surface soil samples were collected using soil sampling rings (5.6-cm diameter). Each sample was composed of three sub-samples (i.e. three soil sampling rings per sample). Each surface soil sample was taken adjacent to previous samples (rather than upslope or downslope). This minimized the effect that diatom population extractions could have on the population sizes of later samples. Once pressed into the ground and sealed with plastic caps, the rings were left in position until the end of the rainfall simulation. Leaving the capped rings fixed in the ground until after the last rainfall event

meant that the area of the soil plot decreased by a known amount ($7.38 \times 10^{-3} \text{ cm}^2$; the surface area of three soil sampling rings, which is equivalent to 2.6% of the plot area and was accounted for in calculations of diatom population size) at each time step and we avoided introducing new surfaces during the rainfall event to the soil plot (an area of the subsurface soil that would be exposed after each soil sample extraction) where the initial diatom population size was unknown. Rainfall in and runoff out were not possible within the soil sampling ring. After the last rainfall event, all soil samples were removed simultaneously by cutting around the ring and extracting the top 4 cm of soil. The base was sealed using parafilm and a second plastic cap.

Any overland flow was collected at the outlet of the soil plot in one-litre bottles (a separate bottle was used for each 15-min period), and the volumes recorded (V_{OF}). Analysis of the diatom population sizes in both soil surface and overland flow samples ensured that we are able to draw a reasonable picture of any fluctuations in diatom population size.

Step 2: Fixing the diatom population size

It was crucial to fix the diatom populations as soon as possible following sampling to prevent any population change by vegetative multiplication. To begin with, 10 ml of formaldehyde (40%) was added to a measuring cylinder. Sparkling water was then poured into the top of a soil sampling ring onto the soil surface. It is thought that the carbon dioxide gas bubbles in the sparkling water detach the diatoms from the substrate. A pestle was also used to lightly tap the soil surface to aid dislodging. After five seconds, the sparkling water was poured into the measuring cylinder with the formaldehyde. This was repeated until the solution in the measuring cylinder was made up to exactly 100 ml (V_{S}). The mixed solution was then transferred to labelled storage bottles.

To fix the diatom population in the overland flow samples, 10 ml of formaldehyde was added directly to the overland flow sampling bottles and shaken well to ensure mixing.

Step 3: Extracting the diatoms from soil samples

The next stage of the sample preparation involved separating the diatoms from the soil mineral particles via an isopycnic separation technique using the silica solution Ludox® (adapted from Ribeiro, 2010; Ribeiro *et al.*, 2013). Density gradient centrifugation is suitable because of the very different densities of mineral and organic soil fractions. This step was only carried out for the soil surface samples, and not for the overland flow samples. The fixed 100-ml solution from Step 2 was homogenized in a vortex mixer. A 5-ml aliquot subsample (V_{A}) was

transferred to a polypropylene centrifuge tube, to which was added 30 ml of fully concentrated Ludox® HS 40, with a density of 1.31 g cm^{-3} .

The sample was then centrifuged at 4700 rpm for 15 min to accelerate the sedimentation of the mineral fraction of the sediment (including fine silts and clays) to the bottom of the tube in a pellet. Organic material and the silica sol (including the diatoms) did not deposit at this g force. Microscopic analysis confirmed the absence of diatoms in the pellet, which was discarded. The supernate was poured evenly into four new centrifuge tubes and each solution was made up to 50 ml using pure distilled water. This was to rinse the excess Ludox® and separate the organic material from the silica material by centrifuging at 3000 rpm for 10 min. The supernate was discarded, and the new pellet retained. The tubes were then filled again with distilled water, and the process was repeated twice to ensure complete elimination of the Ludox® vestiges. The final four rinsed pellets were then combined in a single tube for that sample. The final volume was recorded (V_{P}).

Step 4: Setting up the sedimentation chambers

The sedimentation procedure followed Utermöhl (1958) for quantifying diatom population size. A sub-sample (V_{C}) of the sample prepared in Step 3 was transferred to a 25-ml sedimentation chamber. The chambers were then completely filled using pure distilled water, covered and left for at least four hours to allow sedimentation. The volume of the subsample (V_{C}) for the chambers was varied by trial and error, depending on the concentration of diatoms and sediment present in the sample, as determined in Step 5 from microscopic analysis.

For the overland flow samples, an aliquot sub-sample (V_{A}) of the formaldehyde fixed solution was transferred to the sedimentation chamber, which was then filled using pure distilled water and left to settle.

Step 5: Analysing the diatom populations

An inverted light microscope, with a known area of field of view (A_{F}), was then used to analyse the sedimented diatoms. All diatom frustules in at least 50 fields of view (F) were counted. Whether frustules were alive or dead at the time of sampling (determined by the presence or absence of chloroplasts) was recorded. Following this, the number of diatoms in each soil sub-sample, was estimated and expressed in terms of the number of diatoms per cm^2 , as determined by Equation (1). The diatoms in the overland flow chamber were counted in the same way. The number of diatoms in the overland flow sample was then estimated (and expressed in terms of the number of diatoms contributed from each cm^2 of soil surface), as determined by Equation (2).

DIATOMS AS A TRACER OF HYDROLOGICAL CONNECTIVITY

Appendix 2. List of diatom species observed during the experiment, including samples from soil plots ($n=15$) and overland flow ($n=12$). Maximum relative abundance (Max. (%)), mean relative abundance (Mean (%)) and frequency of occurrence (Freq. (%)) of the 76 taxa found in the riparian zone of the Weierbach catchment (Luxembourg) are provided. Categories (Cat.) of Van Dam *et al.* (1994) are also indicated: (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. Bold values are modified values (i.e. not following Van Dam *et al.*, 1994), according to the most similar species or previous synonyms of recently described species.

Species	Cat.	Freq. (%)	Mean (%)	Max. (%)	S.D.	Figure
<i>Achnantheidium kranzii</i> (Lange-Bertalot) Round & Bukhtiyarova	0	92.6	1.8	6.0	1.3	19–22
<i>Achnantheidium lineare</i> W. Smith	0	7.4	0.1	1.0	0.3	13–15
<i>Achnantheidium minutissimum</i> (Kützing) Czarnecki	3	100.0	3.6	8.5	2.2	16–18
<i>Achnantheidium subatomoides</i> (Hustedt) O. Monnier, Lange-Bertalot & Ector	1	29.6	0.2	1.0	0.3	11–12
<i>Adlafia brockmanni</i> (Hustedt) Bruder & F. Hinz	4	37.0	0.3	1.5	0.4	60
<i>Adlafia bryophila</i> (J.B. Petersen) Gerd Moser, Lange-Bertalot & Metzeltin	5	63.0	0.6	3.9	0.8	29
<i>Adlafia lange-bertalotii</i> O. Monnier & Ector	5	7.4	0.0	0.5	0.1	30
<i>Caloneis leptosoma</i> (Grunow) Krammer	4	48.1	0.6	3.9	0.9	69
<i>Caloneis cf. macei</i> Fusey sensu Werum and Lange-Bertalot (2004)	4	33.3	0.3	1.0	0.4	83
<i>Cavinula cocconeiformis</i> (Gregory ex Greville) D.G. Mann & Stickle	3	14.8	0.1	0.5	0.2	64
<i>Cavinula intractata</i> (Hustedt) Lange-Bertalot sensu Werum and Lange-Bertalot (2004)	4	100.0	1.5	4.4	0.9	63
<i>Cavinula variostrata</i> (Krasske) D.G. Mann & Stickle	4	11.1	0.1	1.2	0.3	65
<i>Chamaepinnularia evanida</i> (Hustedt) Lange-Bertalot	4	81.5	1.4	5.3	1.3	38
<i>Chamaepinnularia obsoleta</i> (Hustedt) C.E. Wetzel & Ector in Wetzel <i>et al.</i>	4	70.4	0.7	2.0	0.7	39
<i>Chamaepinnularia parsura</i> (Hustedt) C.E. Wetzel & Ector in Wetzel <i>et al.</i>	4	33.3	0.2	1.0	0.3	37
<i>Cymbopleura amphicephala</i> (Nägeli) Krammer	3	18.5	0.1	1.0	0.2	55
<i>Diploneis fontium</i> E. Reichardt & Lange-Bertalot in Reichardt	0	25.9	0.2	1.7	0.5	58
<i>Diploneis fontanella</i> Lange-Bertalot	0	100.0	2.3	3.9	0.9	59
<i>Eolimna tantula</i> (Hustedt) Lange-Bertalot	3	100.0	4.1	6.3	1.2	36
<i>Eolimna vekhovii</i> (Lange-Bertalot & Genkal) Lange-Bertalot & Kulikovskiy	0	40.7	0.3	1.0	0.4	33–34
<i>Eunotia bilunaris</i> (Ehrenberg) M.G.M. Souza	3	77.8	0.7	2.9	0.7	4
<i>Eunotia botuliformis</i> Wild, Nörpel & Lange-Bertalot in Lange-Bertalot	0	85.2	1.0	2.5	0.8	7
<i>Eunotia exigua</i> (Brébisson ex Kützing) Rabenhorst	3	3.7	0.0	0.5	0.1	8
<i>Eunotia glacialifalsa</i> Lange-Bertalot	3	18.5	0.1	0.5	0.2	1
<i>Eunotia incisadistans</i> Lange-Bertalot	2	70.4	0.7	2.5	0.7	6
<i>Eunotia minor</i> (Kützing) Grunow	4	100.0	3.1	5.7	1.2	5
<i>Eunotia nymanniana</i> Grunow	3	29.6	0.2	1.7	0.4	3
<i>Eunotia paratridentula</i> Lange-Bertalot & Kulikovskiy	0	18.5	0.1	0.5	0.2	9
<i>Eunotia soleirolii</i> (Kützing) Rabenhorst	3	3.7	0.1	1.5	0.3	2
<i>Eunotia tenella</i> (Grunow) Hustedt	3	44.4	0.3	1.5	0.4	10
<i>Fragilaria nevadensis</i> Linares-Cuesta & Sánchez-Castillo	0	92.6	1.6	5.4	1.2	40
<i>Fragilariforma virescens</i> (Ralfs) D.M. Williams & Round	3	100.0	5.3	10.5	2.4	43–49
<i>Frustulia vulgaris</i> (Thwaites) De Toni	3	55.6	0.4	1.5	0.4	76
<i>Gomphonema acidoclinatum</i> Lange-Bertalot & E. Reichardt	3	66.7	0.4	2.0	0.4	51
<i>Gomphonema productum</i> (Grunow) Lange-Bertalot & E. Reichardt	3	100.0	4.2	8.2	1.7	53
<i>Gomphonema subclavatum</i> Grunow	3	85.2	1.1	2.5	0.7	50
<i>Gomphonema varioeduncum</i> Jüttner <i>et al.</i>	3	100.0	2.7	6.1	1.4	54
<i>Gomphonema variscohercynicum</i> Lange-Bertalot & E. Reichardt	0	3.7	0.0	0.5	0.1	52
<i>Humidophila brekkaensis</i> (J.B. Petersen) Lowe <i>et al.</i>	4	22.2	0.1	0.5	0.2	31
<i>Humidophila perpusilla</i> (Grunow) R.L. Lowe <i>et al.</i>	5	37.0	0.3	2.0	0.6	32
<i>Karayevia oblongella</i> (Östrup) Aboal (including <i>Achnanthes saxonica</i>)	3	18.5	0.1	1.0	0.3	23–24
<i>Mayamaea permissis</i> (Hustedt) Bruder & Medlin	3	25.9	0.1	1.0	0.1	28
<i>Meridion constrictum</i> Ralfs	2	92.6	1.5	3.7	1.0	41
<i>Navicula exilis</i> Kützing	0	96.3	1.8	4.5	1.1	61
<i>Neidium alpinum</i> Hustedt sensu Hofmann <i>et al.</i> (2011)	3	14.8	0.1	0.5	0.2	73
<i>Neidium bisulcatum</i> (Lagerstedt) Cleve	3	25.9	0.2	1.5	0.4	74
<i>Neidium carteri</i> Krammer	0	3.7	0.0	0.5	0.1	75
<i>Nitzschia amphibia</i> Grunow	3	3.7	0.0	0.5	0.1	68
<i>Nitzschia harderi</i> Hustedt in Brendemühl	5	51.9	0.3	1.0	0.4	67

(Continues)

Appendix 2. (Continued)

Species	Cat.	Freq. (%)	Mean (%)	Max. (%)	S.D.	Figure
<i>Pinnularia acrosphaeria</i> W. Smith	3	37.0	0.2	1.0	0.3	78
<i>Pinnularia dornii</i> Metzeltin in Lange-Bertalot & Metzeltin	0	33.3	0.4	2.0	0.6	72
<i>Pinnularia isselana</i> Krammer	0	59.3	0.7	2.5	0.8	71
<i>Pinnularia nodosa</i> (Ehrenberg) W. Smith	3	70.4	0.6	2.9	0.7	82
<i>Pinnularia obscura</i> Krasske	4	85.2	1.0	2.5	0.7	85
<i>Pinnularia peracuminata</i> Krammer	4	48.1	0.4	1.5	0.6	81
<i>Pinnularia perirrorata</i> Krammer	4	81.5	1.1	3.9	0.9	84
<i>Pinnularia sinistra</i> Krammer	0	59.3	0.4	1.5	0.5	86
<i>Pinnularia stomatophora</i> (Grunow) Cleve	4	7.4	0.0	0.5	0.1	80
<i>Pinnularia subcommutata</i> var. <i>nonfasciata</i> Krammer	0	40.7	0.3	2.0	0.5	70
<i>Pinnularia viridiformis</i> Krammer	0	14.8	0.1	0.7	0.2	79
<i>Placoneis ignorata</i> (Schimanski) Lange-Bertalot in Rumrich <i>et al.</i>	3	100.0	2.5	6.6	1.6	66
<i>Planothidium frequentissimum</i> (Lange-Bertalot) Lange-Bertalot	3	100.0	20.3	31.7	4.7	27
<i>Planothidium lanceolatum</i> (Brébisson) Lange-Bertalot	3	100.0	19.2	26.8	4.1	25–26
<i>Reimeria sinuata</i> (W. Gregory) Kociolek & Stoermer	3	3.7	0.0	0.5	0.1	57
<i>Reimeria uniseriata</i> S.E. Sala, J.M. Guerreiro & Ferrario	3	3.7	0.0	0.5	0.1	56
<i>Sellaphora pseudopupula</i> (Krasske) Lange-Bertalot sensu Werum and Lange-Bertalot (2004)	0	96.3	1.6	4.0	0.9	62
<i>Sellaphora seminulum</i> (Grunow) D.G. Mann	3	100.0	3.1	5.7	1.4	35
<i>Stauroneis acidoclinata</i> Lange-Bertalot & Werum	0	48.1	0.3	2.0	0.4	92
<i>Stauroneis acuta</i> W. Smith	3	18.5	0.1	1.0	0.2	93
<i>Stauroneis kriegeri</i> R.M. Patrick	3	11.1	0.1	0.5	0.2	89
<i>Stauroneis parathermicola</i> Lange-Bertalot in Hofmann <i>et al.</i>	4	100.0	1.7	4.5	0.8	87
<i>Stauroneis silvahassiacae</i> Lange-Bertalot & Werum	0	7.4	0.0	0.7	0.2	91
<i>Stauroneis smithii</i> Grunow	3	3.7	0.0	0.5	0.1	90
<i>Stauroneis thermicola</i> (J.B. Petersen) J.W.G. Lund	4	25.9	0.2	1.0	0.3	88
<i>Staurosira venter</i> (Ehrenberg) Cleve & J.D. Möller	1	7.4	0.0	0.5	0.1	42
<i>Surirella angusta</i> Kützing	3	33.3	0.2	2.0	0.4	77

APPENDIX 3

Light micrographs of diatom taxa identified during the experiment.

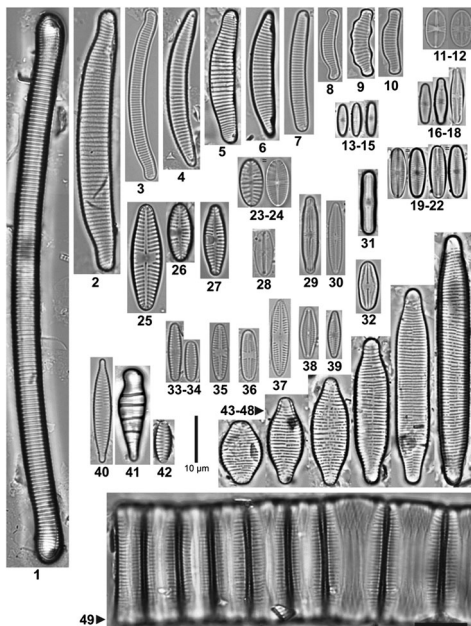


Fig. 1: *Eunotia glacialisfalsa* Lange-Bertalot; Fig. 2: *Eunotia soleirolii* (Kützing) Rabenhorst; Fig. 3: *Eunotia nymanniana* Grunow; Fig. 4: *Eunotia bilunaris* (Ehrenberg) M.G.M. Souza; Fig. 5: *Eunotia minor* (Kützing) Grunow; Fig. 6: *Eunotia incisadistans* Lange-Bertalot; Fig. 7: *Eunotia botuliformis* Wild, Nörpel & Lange-Bertalot in Lange-Bertalot; Fig. 8: *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst; Fig. 9: *Eunotia paratridentula* Lange-Bertalot & Kulikovskiy; Fig. 10: *Eunotia tenella* (Grunow) Hustedt; Figs. 11–12: *Achnanthis subatomoides* (Hustedt) O. Monnier, Lange-Bertalot & Ector; Figs. 13–15: *Achnanthis lineare* W. Smith; Figs. 16–18: *Achnanthis minutissimum* (Kützing) Czarniecki; Figs. 19–22: *Achnanthis kranzii* (Lange-Bertalot) Round & Bukhtiyarova; Figs. 23–24: *Achnanthis saxonica* Krasske in Hustedt; Figs. 25–26: *Planothidium lanceolatum* (Brébisson) Lange-Bertalot; Fig. 27: *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot; Fig. 28: *Mayamaea atomus* (Kützing) Lange-Bertalot; Fig. 29: *Adlafia bryophila* (J.B. Petersen) Gerd Moser, Lange-Bertalot & Metzeltin; Fig. 30: *Adlafia langebertalotii* O. Monnier & Ector; Fig. 31: *Humidophila brekkaensis* (J.B. Petersen) R.L. Lowe *et al.*; Fig. 32: *Humidophila perpusilla* (Grunow) R.L. Lowe *et al.*; Figs. 33–34: *Eolimna vekhovii* (Lange-Bertalot & Genkal)

Lange-Bertalot & Kulikovskiy; Fig. 35: *Sellaphora seminulum* (Grunow) D.G. Mann; Fig. 36: *Eolimna tantula* (Hustedt) Lange-Bertalot; Fig. 37: *Chamaepinnularia parsura* (Hustedt) C.E. Wetzel & Ector in Wetzel *et al.*; Fig. 38: *Chamaepinnularia evanida* (Hustedt) Lange-Bertalot; Fig. 39: *Chamaepinnularia obsoleta* (Hustedt) C.E. Wetzel & Ector in Wetzel *et al.*; Fig. 40: *Fragilaria nevadensis* Linares-Cuesta & Sánchez-Castillo; Fig. 41: *Meridion constrictum* Ralfs; Fig. 42: *Staurosira venter* (Ehrenberg) Cleve & J.D. Möller; Figs. 43–49: *Fragilariforma virescens* (Ralfs) D.M. Williams & Round.

APPENDIX 4

Light micrographs of diatom taxa identified during the experiment.

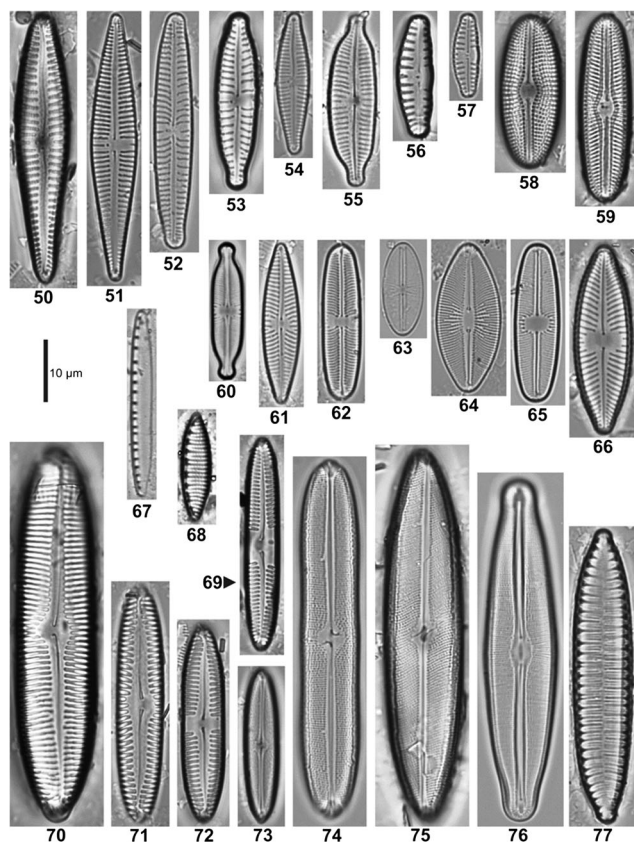


Fig. 50: *Gomphonema subclavatum* Grunow; Fig. 51: *Gomphonema acidoclinatum* Lange-Bertalot & E. Reichardt; Fig. 52: *Gomphonema variscohercynicum* Lange-Bertalot & E. Reichardt; Fig. 53: *Gomphonema productum* (Grunow) Lange-Bertalot & E. Reichardt; Fig. 54: *Gomphonema varioreduncum* Jüttner *et al.*; Fig. 55: *Cymbopleura amphicephala* (Nägeli) Krammer; Fig. 56: *Reimeria uniseriata* S.E. Sala, J.M. Guerreiro & Ferrario; Fig. 57: *Reimeria sinuata* (W. Gregory) Kociolek & Stoermer; Fig. 58: *Diploneis fontium* E. Reichardt & Lange-Bertalot in

Reichardt; Fig. 59: *Diploneis fontanella* Lange-Bertalot; Fig. 60: *Adlafia brockmanii* (Hustedt) Bruder & F. Hinz; Fig. 61: *Navicula exilis* Kützing; Fig. 62: *Sellaphora pseudopupula* (Krasske) Lange-Bertalot sensu Werum and Lange-Bertalot; Fig. 63: *Cavinula intractata* (Hustedt) Lange-Bertalot sensu Werum and Lange-Bertalot; Fig. 64: *Cavinula cocconeiformis* (Gregory ex Greville) D.G. Mann & Stickle; Fig. 65: *Cavinula variostrata* (Krasske) D.G. Mann & Stickle; Fig. 66: *Placoneis ignorata* (Schimanski) Lange-Bertalot in Rumrich *et al.*; Fig. 67: *Nitzschia harderi* Hustedt in Brendemühl; Fig. 68: *Nitzschia amphibia* Grunow; Fig. 69: *Caloneis leptosoma* (Grunow) Krammer; Fig. 70: *Pinnularia subcommutata* var. *nonfasciata* Krammer; Fig. 71: *Pinnularia domestica* E. Reichardt; Fig. 72: *Pinnularia dornii* Metzeltin in Lange-Bertalot & Metzeltin; Fig. 73: *Neidium alpinum* Hustedt sensu Hofmann *et al.*; Fig. 74: *Neidium bisulcatum* (Lagerstedt) Cleve; Fig. 75: *Neidium carteri* Krammer; Fig. 76: *Frustulia vulgaris* (Thwaites) De Toni; Fig. 77: *Surirella angusta* Kützing.

APPENDIX 5

Light micrographs of diatom taxa identified during the experiment.

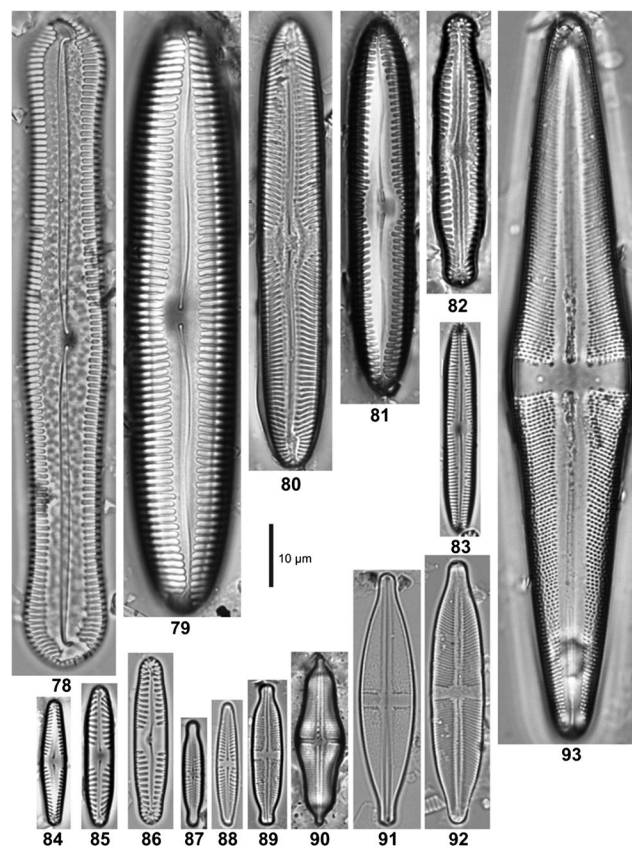


Fig. 78: *Pinnularia acrosphaeria* W. Smith; Fig. 79: *Pinnularia viridiformis* Krammer; Fig. 80: *Pinnularia stomatophora* (Grunow) Cleve; Fig. 81: *Pinnularia peracuminata* Krammer; Fig. 82: *Pinnularia nodosa* (Ehrenberg) W. Smith; Fig. 83: *Caloneis* cf. *macei* Fusey sensu Werum and Lange-Bertalot; Fig. 84: *Pinnularia perirrorata* Krammer; Fig. 85: *Pinnularia obscura* Krasske; Fig. 86: *Pinnularia sinistra* Krammer; Fig. 87: *Stauroneis parathermicola* Lange-Bertalot in Hofmann *et al.*; Fig. 88: *Stauroneis thermicola* (J.B. Petersen) J.W.G. Lund; Fig. 89: *Stauroneis kriegei* R.M. Patrick; Fig. 90: *Stauroneis smithii* Grunow; Fig. 91: *Stauroneis silvahassiaca* Lange-Bertalot & Werum; Fig. 92: *Stauroneis acidoclinata* Lange-Bertalot & Werum; Fig. 93: *Stauroneis acuta* W. Smith.

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