Advanced Review

Terrestrial diatoms as tracers in catchment hydrology: a review

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Diatoms are remarkable organisms. They are present in almost all habitats containing water (e.g., lakes, streams, soils, bark) and rank among the most common algal groups in both freshwaters and marine ecosystems. The ubiquitous character of aquatic diatoms has triggered countless applications as environmental tracers for studies in water quality, paleoclimate reconstruction and sediment tracing. However, diatoms also occur in the terrestrial environment. It is this plethora of diatom life-forms that has recently triggered interest in their use as tracers of hydrological processes. The use of diatoms in catchment hydrology has been very limited. Part of the reason is that until recently, the taxonomy and ecology of terrestrial diatom assemblages were largely unknown. However, in the past decade, much work has been done to quantify terrestrial diatom reservoir size, dynamics, and potential depletion following precipitation events. Therefore, such terrestrial diatoms now hold promise for use in catchment hydrology—for tracing runoff flow sources and pathways across a wide range of spatial scales. Here we review the literature on terrestrial diatoms and describe the various sampling protocols that have been designed and tested for specific applications in hydrological processes research. We review and summarize the work on terrestrial diatom reservoir characterization, transport mechanisms and pathways to show how such diatom-based tracer work might be possible at the catchment scale for rainfall-runoff studies. Finally, we present a vision for future work that might take advantage of terrestrial diatoms in catchment hydrology and discuss the main challenges going forward.

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INTRODUCTION

Diatoms (Bacillariophyta) are present in almost all habitats containing water (e.g., lakes, streams, soils, litter, bark). They count among the most common algal groups in both freshwaters and marine ecosystems.[1] Despite their microscopic size (10–200 microns),[2] diatoms generate an impressive amount of carbon through photosynthesis: almost as much as all rainforests combined.[3] Identification of diatoms date back to the early 1700s as noted by Round et al.[1] Decades later, Otto Friedrich Müller published the first description of a diatom in 1783 (*Vibrio paxillifer* ≡ *Bacillaria paradoxa*). Since then,

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more than 64,000 diatom species have been described—and new species are added to that list almost daily with the number of extant species extrapolated to ca 100,000.[4,5] The identification of diatoms (from the Greek 'diatomos' or 'cut in half') commonly relies on the highly differentiated cell wall (or frustule) that is mostly composed of silica (SiO_2). The diatom frustules have two halves and exhibit a sheer endless diversity in form. It is this species-specific cell wall ornamentation that makes each diatom species unique—a feature that built the foundations of diatom taxonomy and systematics. After removal (e.g., with hydrogen peroxide) of the protoplasm (i.e., general cell content), a diatom is typically identified through a detailed observation of its exoskeleton with a light microscope.

The power of diatom taxonomy and systematics to environmental research and assessment has been long recognized. Since diatom species distributions are largely controlled by environmental variables (light, moisture conditions, temperature, current velocity, salinity, pH, oxygen, inorganic nutrients) and anthropogenic factors (e.g., organic pollution sources, acidification and eutrophication),[13,14] they have become a standard tool in geology, archaeology, and water quality research (e.g., Refs 15 and 16). Diatoms have been used extensively in a wide range of applications as bioindicators of water quality,[17] as biostratigraphical markers in marine deposits,[18,19] as stratigraphic indicators for mineral and petroleum exploration,[20–24] or as indicators of climate change (Box 1).[25–28] But while diatoms have been continued to be used in across the environmental sciences, their use in hydrology has been limited. Seminal work by Pfister et al.[29] has tested the hypothesis that terrestrial diatoms are flushed to the creek during precipitation events—confirming their potential for serving as tracers of the onset/cessation of rapid surface or subsurface flow. The purpose of this paper is to outline and review the current use and the potential for diatoms—specifically terrestrial diatoms and their assemblages—as tracers of water flow paths within catchments. We review the progress made in this area of research in the past decade by describing the various sampling protocols that have been designed and tested for specific applications in hydrological processes research. We then review the work on diatom reservoir characterization, transport mechanisms and pathways. We document diatom-based tracer work in the hillslope-riparian zone-stream continuum and across a set of nested catchments in the mesoscale Attert River basin. Finally, we provide a vision for future diatom work needed to unleash their full potential as a tracer of hydrological processes and possibly even beyond as soil quality indicators.

BOX 1

DIATOM HABITATS

In flowing waters, diatoms typically occupy two main ecological niches: the benthos and the plankton. The so-called benthic diatoms may be qualified as 'attached' (e.g., having one side fixed to the stream bed), 'stalked' (e.g., being fixed to the stream bed by a branched or unbranched mucilaginous pedicel) or 'motile' (i.e., having no attachment mechanism *per se*, but being adapted to gliding locomotion) (see Tapolczai et al.,[6] for a recent review on diatom life-forms and its use in ecological assessments). Planktonic diatoms are free-floating or drifting in flowing water. In lakes and oceans, diatoms (drifting, as well as attached to submerged substrates) are fundamental elements of the food chain providing food and energy to larger organisms, such as fishes. Furthermore, they play a key role in nutrient and heavy metal absorption.[7–10] But outside water bodies, diatom colonies develop in a large variety of substrates, provided that they fulfill certain environmental criteria—among which moisture and light play a prominent role. Soils, rocks, lichen, litter, vegetation, or bark are all offering potential habitats for so-called aerial diatoms—or what we term here as terrestrial diatoms.[11,12]

TERRESTRIAL DIATOM SAMPLING AND IDENTIFICATION

While aquatic diatom communities have been studied for more than a century, terrestrial diatom communities (i.e., diatom assemblages which can be found on the soil surface, rocks, lichen, litter, vegetation, or bark) have received much less attention. The lack of knowledge on aerial diatom assemblages—both taxonomic and ecological—stands as a major limitation to their use as tracers in catchment hydrology and beyond. Until very recently, we have not known terrestrial diatom reservoir size, dynamics, and potential depletion following precipitation events. Terrestrial diatom species distribution and abundance are largely controlled by soil pH and land use.[12,30–33]

Collection of terrestrial diatoms living on plants or rocks (phytobenthic diatoms) can be removed with a toothbrush and mixed with water in a glass bottle.

Drift diatoms (i.e., free-floating terrestrial diatoms in water) can be collected via grab sampling or an automatic sampler (e.g., connected to a recording stream gauge and programmed to pump water from a creek during the rising and falling limbs of a storm hydrograph) (Figure 1(a)).

Soil samples are collected using 'soil sampling rings' (Figure 1(d)–(f)). Each sample is composed of three subsamples (i.e., three soil sampling rings per sample). Sparkling water is used typically to extract diatoms from the soil samples. Carbon dioxide gas bubbles (from the sparkling water) act to detach the diatoms from the substrate. This procedure can also be used in a quantitative way (e.g., the so-called Utermöhl method), taking into account all dilutions made during the procedure (see Coles et al.[34] for a detailed protocol). The base of the soil sample is covered with parafilm to prevent water from leaking (Figure 1(g) and (h)). Water is then poured on the top of the soil sampling ring, so to entirely cover the surface of the soil sample (Figure 1(g)). A pestle is used to gently tap the soil surface and dislodge the diatoms. Next, water is carefully poured into a clean bottle. The same extraction procedure is repeated three times for each sampling ring in order to collect sufficient volumes of diatom material. Finally, the diatoms obtained from the three rings are merged into one single sample bottle—representative of a specific sampling site.

The next stage of the sample preparation involves separating the living diatoms from the soil mineral particles via an isopycnic separation technique using the silica solution Ludox (Sigma-Aldrich, St. Louis, MO, USA). Density gradient centrifugation is used to separate the very different densities of mineral and organic soil fractions. The main potential diatom substrates (e.g., leaves, litter, moss, and herbaceous vegetation) must be first identified and then sampled using 1-L sampling bottles. Only material well exposed to direct sunlight and from the top surface is sampled.

Upon arrival in the laboratory, the bottles containing the different diatom-substrate samples are filled with sparkling water, carefully shaken and left in the fridge overnight (essentially to keep diatoms in a normal diel 12:12-hour cycle and to avoid asexual reproduction of the cells). The next day, water is poured through a 1 mm sieve. The sample is then rinsed with sparkling water to extract the diatoms. This procedure is repeated multiple times—until a 2-L bottle is filled. The recovered 2-L sample is then stored in a fridge for a minimum of 8 h to allow the diatoms to settle. After this storage period, the samples are brought back to the laboratory, where the supernatant is carefully removed by aspiration using a vacuum pump. The remaining water contains the diatoms that have settled and concentrated in a smaller volume (approximately 200 mL).

All collected diatom samples are treated and prepared following standard procedures.[35,36] Microscopic slides for light microscopy observations are prepared using a refractive resin (Naphrax, Brunel Microscopes Ltd, Chippenham, UK) as mounting medium (as in Figure 2(a) and (d)). For scanning electron microscopy (SEM), subsamples of treated diatom suspensions are concentrated on a polycarbonate membrane filter with a 3-μm mesh, attached to aluminum stubs and sputtered with a 30-

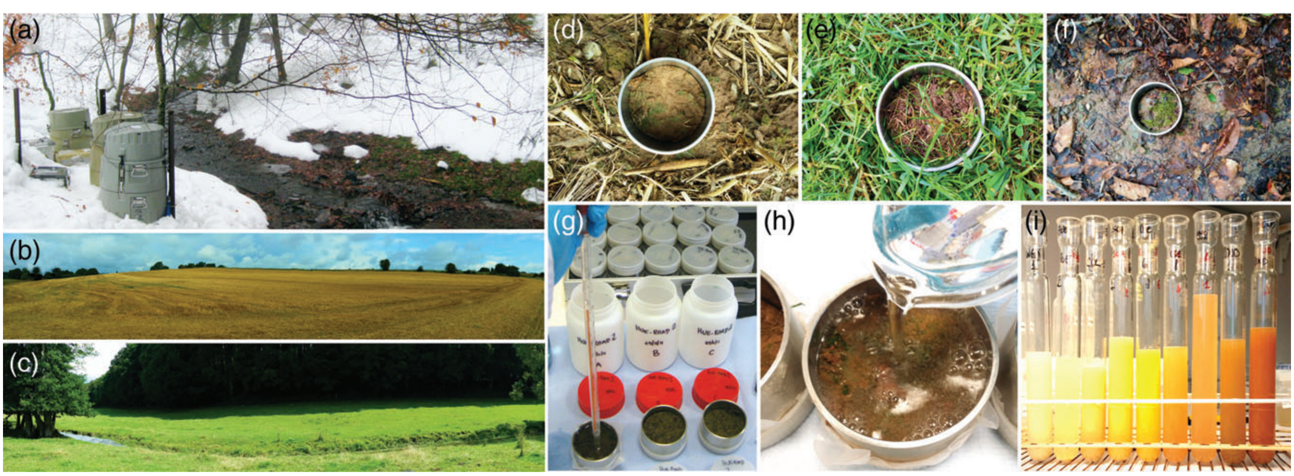

FIGURE 1 | (a) Automatic sampler (ISCO Automatic sampler (Teledyne Isco, Inc., US)) installed in the Weierbach catchment (Attert River basin, Luxembourg) used to collect drifting diatoms; (b, c) common landscape surrounding the streams in the Attert River basin (Luxembourg); (d–f) soil diatom sampling rings in (d) agricultural, (e) grassland/pasture, and (f) forested areas; (g, h) soil sampling ring filled with sparkling water (view from top) and (i) digestion of organic matter with hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl).

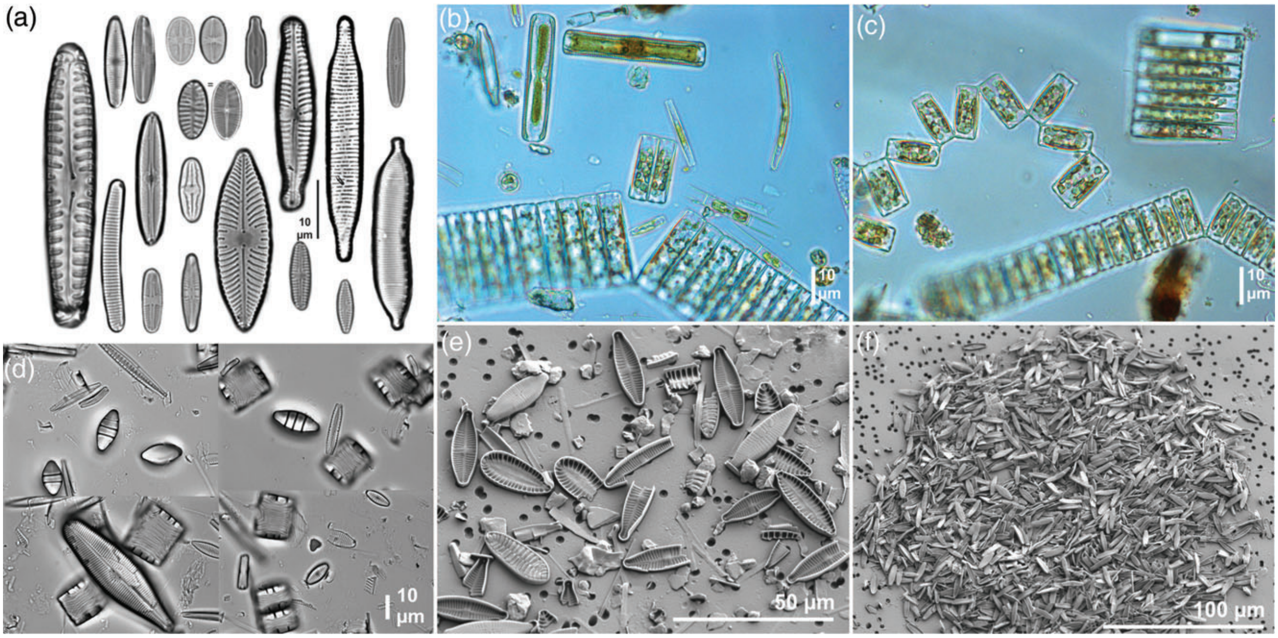

FIGURE 2 | Examples of diatom cells observed with different instruments: (a) light microscope images of species belonging to distinct genera and classes, collected in the Weierbach catchment (Attert River basin, Luxembourg); (b, c) show raw live material collected with ISCO samplers ISCO automatic samplers (Teledyne Isco, Inc., US) following a rainfall event. Alive specimens (with chloroplasts) showing solitary and colonial habits; (d) water sample after digestion of organic matter showing 'cleaned' frustules in a permanent slide ready for observation and counting with a light microscope; (e, f) scanning electron microscope (SEM) images showing valves under high magnification for accurate identification of species.

nm platinum layer. Accurate taxonomy techniques using SEM on subsamples of treated diatom suspensions are carried out with a Hitachi SU-70 field emission scanning electron microscope (or any other SEM instrument with similar performances) using an accelerated voltage of 5 kV (Figure 2(e) and (f)).

Diatom valves are counted (~400 valves are required to adequately represent species assemblages) and identified on each slide along random transects with a Leica DMRX light microscope (Leica Microsystems GmbH, Wetzlar, Germany) (or any other microscope with similar performances and equipped with a 100× oil immersion objective) at a magnification of 1000× using the most updated taxonomic books and monographs (e.g., Refs 37–39). The ecological classification is typically based on the Van Dam et al.[15] system using the software (OMNIDIA 6.0.2)[40], refined and updated with the inclusion of information concerning poorly known aerial species (e.g., Refs 41–44).

TERRESTRIAL DIATOMS AS TRACERS IN HYDROLOGY

As suggested by several studies, the analysis of water samples taken in a creek or river provide a detailed footprint of a catchment's set of physiographic characteristics on diatom communities. The Attert River basin in Luxembourg has been a focal point for studies of terrestrial diatoms and their use as tracers in hydrology. There, diatoms have been sampled in stream water and across the landscape from a set of seven-nested catchments covering a wide range of soil, geological bedrock and land-use conditions. Early work by Rimet et al.[31] showed how these environmental variables influenced the structure of benthic diatoms. The Attert River basin is characterized by two distinct geological regions[45]: its left-bank tributaries are characterized by Devonian bedrock (belonging to the Ardennes massif) while the right-bank tributaries are characterized by alternating layers of Mesozoic bedrock (with sandstone and marls dominating). Stream water has low carbonate hardness in the schistous catchments, whereas further south high carbonate hardness is linked to the presence of sandstone and marls. Rimet et al.[31] found that on the basis of geology and stream water chemistry, Luxembourg could be separated in two regions: schistose streams with low carbonate hardness (median 3.5°F) in the northern part of the country while the southern part presented high carbonate hardness streams (median 21.8°F), notably connected to the presence of sandstone and limestone substrata. A TWINSPAN classification (or 'Two-Way-

INdicator-SPecies-ANalysis'—a hierarchical sample classification procedure) carried out by the authors on diatom assemblages led to the definition of two groups of samples for these two regions.

As an example, stream water pH appears to have a strong control on the abundance of the diatom species *Fragilariforma virescens* (Ralfs) D.-M. Williams and Round—catchments with low water pH values also tending to exhibit the highest relative abundance of that species (Figure 3). Consequently, *F. virescens* has emerged as an indicator species for catchments in the Attert River basin with low pH of water and dominated by schist bedrock.

A detrended correspondence analysis (DCA—an ordination technique commonly used in ecology) based on 629 samples collected during four major rain events (one in 2011 and three in 2014) yielded up to 753 species that can be separated in two groups of samples corresponding to these two geological regions. The carbonate hardness that is related to the nature of the geological substratum appeared to be the major structuring variable for the assemblage composition. The multiple nested catchment analysis reported by Klaus et al.[46] also indicates a higher similarity reflected by the connectivity of the streams draining from distinct locations within the Attert River basin. Figure 4 shows physiographic and water chemistry data from the Attert basin revealing distinct spatial patterns in diatom assemblages along the uniform and mixed bedrock geologies characteristic of the seven-nested catchments.

It is this documented sensitivity of diatom species to a wide range of environmental parameters prevailing at local and catchment scales that makes them a promising candidate for characterizing hydrological conditions and source locations for the runoff mixture in the stream. In other words, either single diatom species or combinations thereof, may constitute unique or representative markers of specific physiographic and ecohydrological settings. Since landscape configuration reportedly has a significant impact on water quality, high landscape diversity improves water quality.[32,47–49] This in turn might also reflect on diatom assemblages (planktonic or benthic) sampled in the Attert River basin.

Diatom literature[50,51] stresses the importance of ion concentration and trophic status as major environmental drivers of diatom distributions in lakes and streams,[52] while physical factors typically have a lesser effect on community structure. Moreover, studies where spatial configuration accounted for a significant proportion of the community variance clearly outnumbered the studies suggesting strict local environmental control. However, freshwater diatom communities are also strongly spatially structured[31,50] and pure spatial factors account sometimes for a high percentage in the total explainable community variation[53] and thereby suggest that diatoms lack strict ubiquitous dispersal. Wu et al.[54] have recently advocated new (time-variant) sampling frequencies accounting for the strong seasonality in diatom population dynamics.

In the Attert River basin, this is illustrated by the distinct spatial structure of diatom communities found in three experimental catchments with uniform bedrock geologies: schists in the Weierbach catchment, sandstone in the (upper) Huewelerbach catchment, and marls in the Wollefsbach catchment. These spatially structured diatom communities can be visualized along the DCA axis plotted against pH and conductivity (the two main measured parameters structuring diatom communities) (Figure 5).

Percolation of diatoms through soils was investigated by Tauro et al.[55] in a series of laboratory experiments. They labeled frustules of purified diatomite and *Conticribra weissflogii* (Grunow) K. Stachura-Suchoples and D.M. Williams cultures with rhodamine 123 and 2-(4-pyridyl)-5[4-

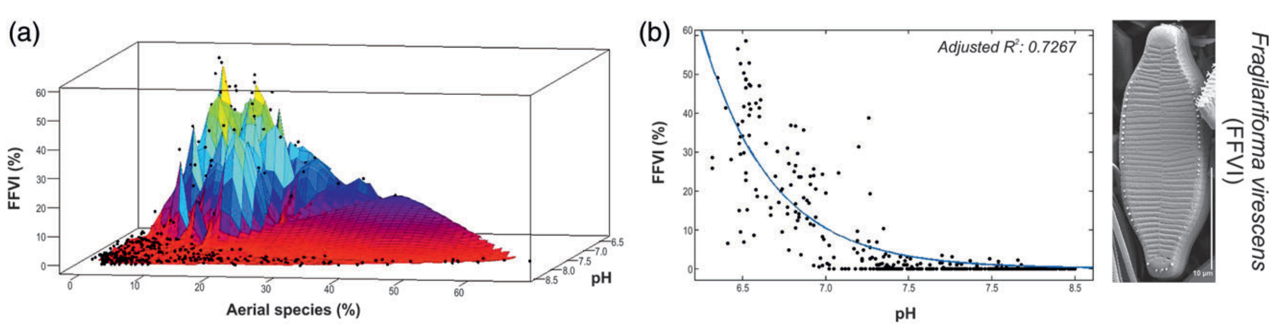

FIGURE 3 | (a) Relative abundance (in %) of *F. virescens* (FFVI) as related to the percentage of aerial species and (b) pH of water (data from all seven-nested catchments in the Attert River basin).

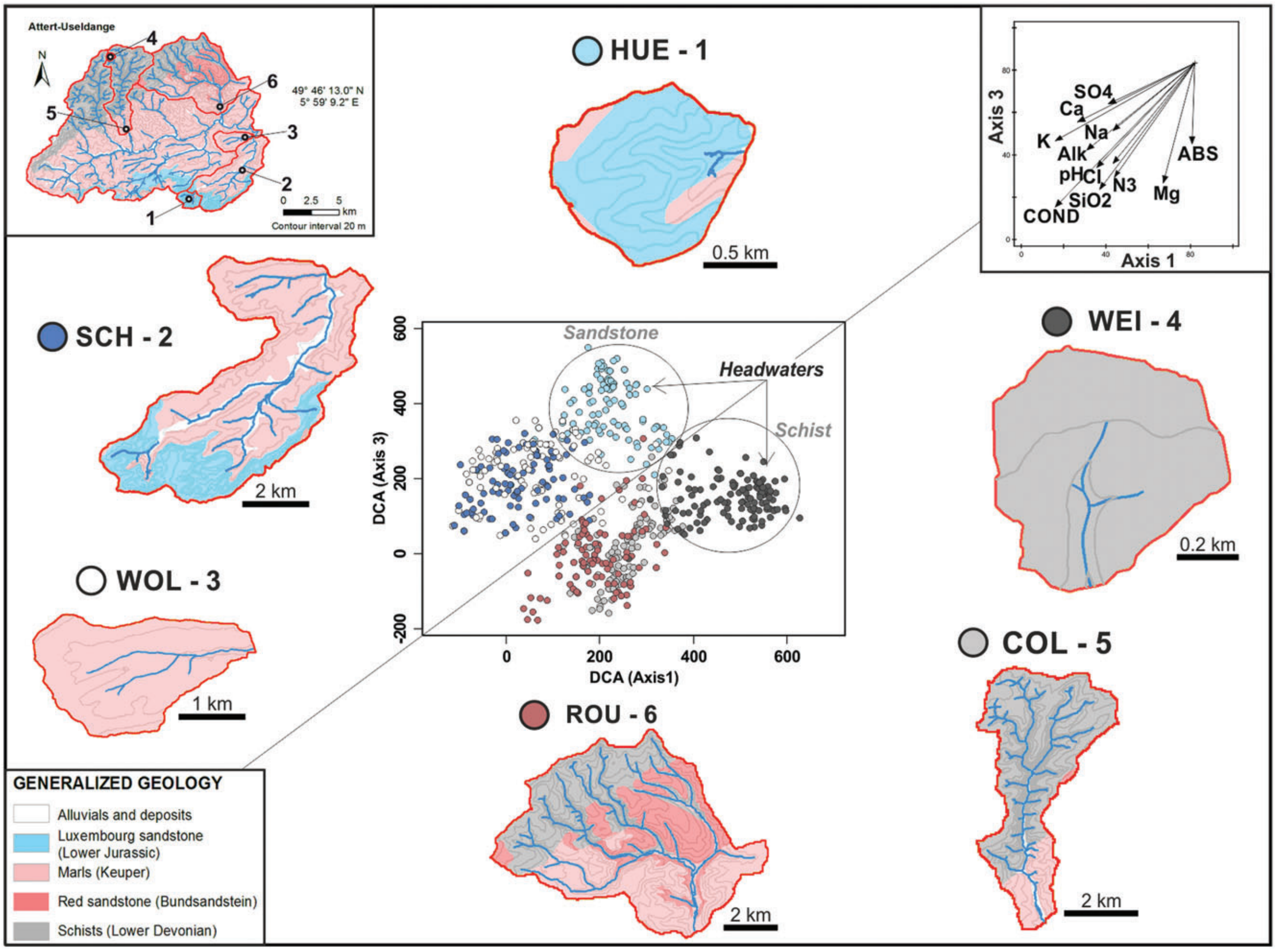

FIGURE 4 | Detrended correspondence analysis (DCA) ordination using diatom species data from six distinct geological units collected with ISCO samplers ISCO automatic samplers (Teledyne Isco, Inc., US) during rainfall events in the Attert River basin, Luxembourg. Each point represents a diatom species. Groups were defined using an indicator species analysis based on the subcatchments. Catchment abbreviations: HUE, Upper Huewelerbach; SCH, Schwebich; WOL, Wollefsbach; WEI, Weierbach; COL, Colpach; and ROU, Roudbach.

dimethylaminoethyl-aminocarbamoyl)-methoxy]phenyloxazole (PDMPO). The soil column experiments consisted in the application of 3600 mL of water for a maximum duration of 10 h over soils covered with labeled diatoms. Microscopy and spectro-fluorometry analysis did not reveal any fluorescent diatoms in the

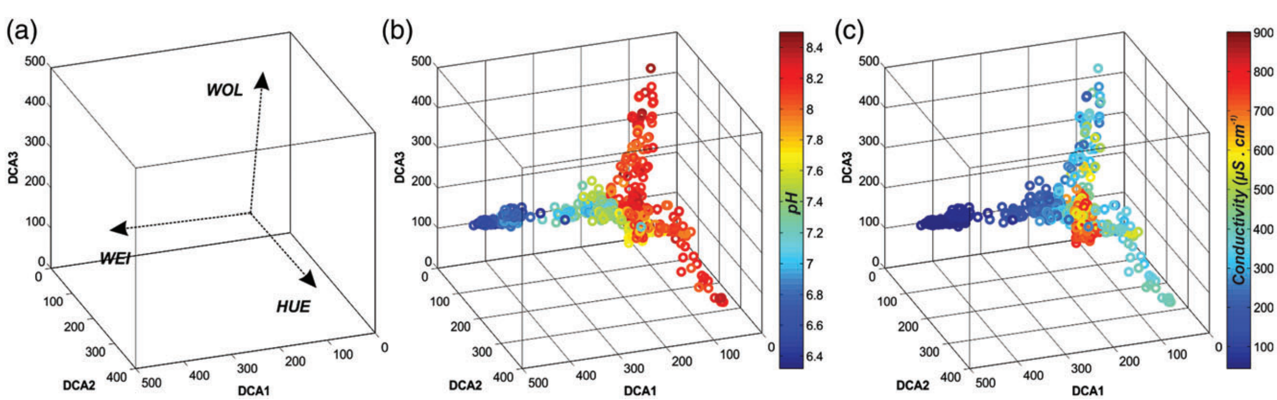

FIGURE 5 | 3D scatter plot of detrended correspondence analysis (DCA) based on diatom data from drifting samples collected during rain events. The three main geologies are structuring the three axis (i.e., Weierbach (WEI): schists, low pH; Upper Huewelerbach (HUE): calcareous sandstone, high pH; Wollefsbach (WOL): marls, moderate conductivity and pH).

percolated material collected at the bottom of the soil columns.

In another series of laboratory experiments by De Graaf et al.,[56] diatom percolation (speed of percolation, speed of percolation over time, and species distribution) was studied through undisturbed soil columns sampled in three catchments of the Attert River basin with distinct geological bedrock settings (marls, sandstone, and schists). After artificial rainfall sprinkling, *Pseudostaurosira* sp. and *Melosira* sp. (used as percolation proxies) were rapidly transported through all three soil columns—following breakthrough curves previously obtained for rhodamine dye experiments. Macroporosity is likely to have largely controlled the observed percolation of diatoms through the soil columns.

Recent research into diatom communities in stream water samples has shown that biovolumetric content (μm^3) tends to decrease with larger relative proportions of aerial species (Figure 6). This finding suggests that small-sized species dominate in aerial communities—and subsequently their predisposition to mobilization and transport through either overland flow or percolation through macropores (near or in riparian areas) during precipitation events. This hypothesis requires further investigations in the near future.

The artificial rainfall and labeled diatom percolation experiments, as well as recent work on biovolumetric content in water samples, supports the idea that terrestrial diatoms can be used as biological indicators of the onset/cessation of overland flow across the hillslope-riparian-stream (HRS) continuum.

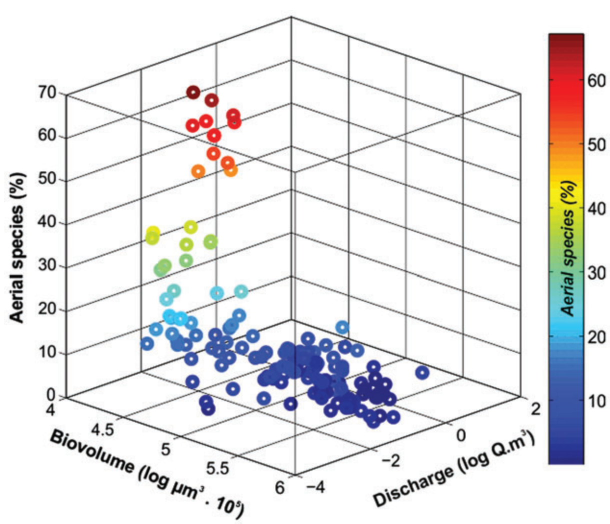

FIGURE 6 | Samples with high content of aerial species are in general dominated by smaller species, which lead to a decrease in the relative biovolumetric content (μm^3).

A VISION FOR TERRESTRIAL DIATOMS IN CATCHMENT HYDROLOGY RESEARCH

Future applications of terrestrial diatoms as tracers in catchment hydrology hold great potential. Much of this relates to quantifying hydrological connectivity. Connectivity as conceptual framework for understanding catchment systems is focused on structural (spatial patterns in the landscape) and functional, and process-based (interaction of spatial patterns and hydrological processes) connectivity.[57]

Terrestrial Diatoms For Quantifying Surface Hydrological Connectivity In The HRS Continuum

The extraordinary variety of diatom species (approximately 100,000 species; ≈10–200 μm) and their strong dependence on environmental conditions makes them potentially highly useful for connectivity quantification. Pfister et al.[29] showed that terrestrial diatoms can be transported by overland flow to the stream network during precipitation events. The ability to trace overland flow and connectivity within the HRS continuum was shown whereby terrestrial diatom frustules were found in stream water samples collected during the rising and falling limbs of the storm hydrograph. Pfister et al.[29] carried out proof-of-concept work in the Attert River basin, where three locations had been selected for their distinct physiogeographic characteristics: (1) the forested Weierbach catchment (0.45 km^2), where bedrock geology is dominated by Devonian schist and phyllades and quartzite; (2) the mainly forested Huewelerbach catchment (2.7 km^2), dominated by alternating layers of sandstone and marls; (3) the Attert River basin in Useldange (250 km^2), where bedrock is composed by schists, marls, and sandstone. The experimental protocol consisted of three steps: terrestrial diatom sampling, sample processing (essentially preparation of microscopic slides), and distributing identified diatom frustules among five classes of occurrence (according to the Van Dam et al.[15] diatom ecological classification system—each class corresponding to distinct habitat wetness conditions).

When used in the Attert River basin by Pfister et al.,[29] these sampling and analytical protocols revealed, for the first time, substantial increase in terrestrial diatom frustules in stream water samples along the rising limb of two storm hydrographs. This exploratory work confirmed that terrestrial diatoms could be flushed from their terrestrial habitats to the stream—showing that diatoms can be used as tracers of the onset/cessation of surface hydrological connectivity. That initial work was followed up and confirmed by further research and sampling campaigns in experimental catchments located in Oregon and Slovakia—with contrasted physiographic and climatic conditions.[58]

With the diatom sampling protocol having been restricted to stream water, early research did not provide any information on terrestrial diatom habitats and the spatial variability of terrestrial diatom assemblages in diverse environmental settings. Exploring the potential for terrestrial diatoms to trace water flow paths requires additional research into diatom reservoirs. Recent work by Martínez-Carreras et al.[59] has investigated terrestrial diatom communities in the Weierbach catchment. Their sampling campaign covered a wide range of habitats in the HRS continuum (including epilithon, epipelon, and stream water samples). Sample analysis revealed 230 taxa in the Weierbach catchment. Eventually, the number of diatom valves found in overland flow, or on hillslope litter, was rather limited compared to the sites covered by bryophytes. Riparian zones were found to be the largest aerial diatom reservoir by far. No significant seasonal changes were noticed in the relative abundances of aerial diatoms across the various habitats. A total of 11 rainfall events were sampled from November 2010 to December 2011, covering both dry and wet antecedent conditions. The substantial flushing of terrestrial diatoms during precipitation events confirmed saturation excess flow (as inferred from conventional tracer work) in riparian wetlands as the dominating runoff generating process during single-peaked events.[59] They concluded from their research that terrestrial diatoms were best-suited for determining rapid surface and subsurface hydrological connectivity between the riparian zone and the stream.

Klaus et al.[46] further documented that in the sandstone-dominated Huewelerbach, most runoff is fed by groundwater contributions. In the absence of riparian areas, rapid surface or subsurface flows play a minor role in runoff generation. Only very small amounts of terrestrial diatom frustules are flushed to the stream during precipitation events (with occasional onset of forest road connectedness triggering surface runoff contributions, and thereby also inflows of terrestrial diatoms). Previous tracer work in the marls-dominated Wollefsbach catchment has revealed a predominance of rapid preferential and subsurface contributions to runoff generation during precipitation events[45]—in addition to occasional tile drain contributions.

Terrestrial Diatom Tracing of Hydrological Connectivity at Larger Catchment Scales

With proof-of-concept work having confirmed the potential for terrestrial diatoms to trace hydrological connectivity in the HRS continuum,[59] the next major step will be to investigate their potential for tracing hydrological connectivity across a wide range of spatial scales. However, disentangling the origin and subsequent pathways of terrestrial diatoms is a more challenging exercise at larger scales. The diversity and complexity of a catchment's physiographic characteristics, water sources, and flow paths increase with catchment size; leading to multiple solutions in terms of terrestrial diatom sources and transport pathways from headwater catchments to the main river branch.

Recent work by Klaus et al.[46] has tested the potential for aerial diatoms to trace hydrological connectivity between their habitat and the stream network within a set of seven-nested catchments (0.45–247 km^2) in the Attert River basin. While the species richness was highly variable among catchments, it did not appear to be related to catchment size. However, the proportion of terrestrial diatoms was clearly decreasing with larger catchment areas. Within their set of seven-nested catchments, Klaus

BOX 2

TERRESTRIAL DIATOM RESERVOIR DYNAMICS

While the work in the Attert basin showed the potential for terrestrial diatoms as tracers of hydrological connectivity, the question has remained about their conservativeness as a tracer and whether or not their supply is flushed or exhausted through a rain event. Coles et al.[34] tested the hypothesis that the supply of aerial diatoms is infinite during rainfall events. They carried out artificial rainfall experiments in selected riparian soil plots located in the Weierbach experimental catchment, a tributary of the Attert River basin, simulating a 1 in 10-year rainfall event. At regular intervals, they collected soil and overland flow samples in order to determine diatom population size and assemblages. As a result of the sprinkling experiment, the population size decreased from 96,100 to 27,200 diatoms/cm^2 (i.e., a decrease of 72%). The main finding of their study was that while terrestrial diatoms were not of infinite supply, they nonetheless continued to be flushed throughout the entire simulated rainfall event. This suggests that the terrestrial diatom reservoir is not likely to be exhausted for a 1 in 10-year precipitation event.

et al.[46] did not find any evidence for terrestrial diatom reservoir depletion—a finding confirmed by artificial sprinkling experiments carried out by Coles et al.[34] in the Weierbach catchment (Box 2).

Eventually, Klaus et al.[46] found terrestrial diatom species bound to physiographic source areas to map across the complete set of nested catchments during a rainfall-runoff event—showing their potential for tracing hydrological connectivity (from multiple source areas) over a wide range of scales (up to 250 km^2).

Regardless of how promising terrestrial diatom work may appear from exploratory work described above, it is important to note that many other interesting new artificial (particle) tracers have been tested in recent years. Sharma et al.[60] have proposed polylactic acid microspheres with incorporated strands of synthetic DNA and paramagnetic iron nanoparticles as an almost unlimited source of labeled hydrological tracers—all exhibiting identical transport properties that remain perfectly distinguishable from each other. Along the similar lines, Tauro et al.[61] introduced nontoxic fluorescent nanoparticles made of beeswax for tracing surface water flow paths. This, nonexhaustive, list of recent efforts for designing new polyvalent natural and artificial tracers eventually is a perfect illustration of the need for an extended tracer toolbox, complementing the set of existing conventional geochemical and isotope tracers.

Diatoms as Hydrological Tracers: Current Limitations, Knowledge Gaps and Research Needs

Albeit exploratory work in multiple contrasted environments has shown the potential for terrestrial diatoms to serve as complementary tracers in hydrological processes studies, more work is needed to overcome current limitations.

For the time being, any diatom-based tracer experiment requires expert knowledge from an experienced taxonomist, needed throughout the entire sequence of diatom sampling, sample preparation and sample analysis. In order to reduce the cumbersome and costly character of diatom analysis, alternative avenues for the identification and quantification of diatoms have been explored. Automated or computer-aided diatom identification tools have been developed and tested, such as SHERPA (image segmentation and outline feature extraction tool for diatoms and other objects, Ref 62), or PlanktoVision (automated analysis system for the identification of phytoplankton, Ref 63). Recently, metagenomic analysis or barcoding methods have been proposed as complementary tools for identifying and quantifying diatoms from environmental DNA and RNA samples[64]. Albeit offering interesting prospects, these approaches will not lead to the abandonment of taxonomic skills and expert knowledge.

Many Earth Science disciplines have long recognized the potential that lies in the use of diatoms. Their widespread use is documented through more than 17,300 papers (source: ISI Web of Science database) that have referred to the keyword ‘diatoms’ over the past decade. Published in a wide variety of journals (e.g., Journal of Paleolimnology, Palaeogeography, Palaeoclimatology, Palaeoecology, Quaternary Science Reviews, Holocene), many of these manuscripts have used diatoms as a tool for understanding environmental impacts related to global change. Moreover, many identification guides and accessible material are currently being produced and are freely available for a wide community of users. Many publications are directly related to the implementation of the European Water Framework Directive, which includes diatom analysis as a routine tool for water quality assessment in all EU member states.

Consequently, using diatoms as an environmental tracer is not accessible *per se*, but rather an approach that is still occupying a niche in disciplines such as hydrology or ecohydrology. The recent call by Stubbington et al.[65] for a better understanding of the connectivity of aquatic–terrestrial environments of temporary streams in temperate zones is a telling example of a potential application field for terrestrial diatom tracing. However, before the establishment of diatoms as a tracer in hydrological or ecohydrological studies, there is a prior need for a better understanding of spatial and temporal dynamics of diatom communities in terrestrial environments. Since the diatom communities living on terrestrial habitats are way less known than the aquatic ones, more detailed studies on these communities are urgently needed. Further results will help to more accurately define the ecological optima and range of many species (including population dynamics) under distinct environmental conditions.

Species assemblage and distribution modeling will be an important next step for testing the potential for individual terrestrial diatom species as source area indicators. Prior to this, a better knowledge of terrestrial diatom habitats will be required. While the Van Dam classification system has been useful for ecological water quality status assessments, it has limitations inherent to its original design. New schemes are sorely needed for terrestrial diatom species classification and for testing the potential for terrestrial diatoms to serve as soil quality indicators. Such information could form the basis of a terrestrial diatom-based soil quality index, paving the way for new hydrological tracer work at increasingly larger spatial scales.

CONCLUSION

Terrestrial diatom research to date has shown that diatom assemblages are largely controlled by physiographic characteristics of their habitats. Despite encouraging research results in what remains a set of preliminary studies—and within a rather restricted range of physiographic settings—multiple questions remain unanswered: How are terrestrial diatom assemblages affected by seasonal variations in meteorological conditions? What are the terrestrial diatom reservoir dynamics in contrasted climates? How do terrestrial diatom reservoir depletion dynamics vary across different habitats through precipitation events of varying intensity, duration, and magnitude? Better understanding of terrestrial diatom reservoir dynamics and controls is clearly needed for a better use of such tracers through the storm hydrograph.

As terrestrial diatom sampling and analytical methods will get perfected, more fundamental knowledge on diatom populations will be gained, eventually paving the way for further research into hydrological connectivity over a large range of spatial scales and physiographic settings. Furthermore, as more knowledge on terrestrial diatom assemblages and dynamics will become available, we see further opportunities for research into diatom-based assessment of soil properties and quality (as is already the case for water quality assessment with diatom-based indexes in the EU Water Framework Directive).

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