Ecohydrological characterization of terrestrial diatoms



Ecohydrological characterization of terrestrial diatoms

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Jasper Foets

INVITATION

To attend the public defence of my PhD thesis entitled

Ecohydrological characterization of terrestrial diatoms







Jasper Foets

The defence will be live streamed from 13:25 via weblectures.wur.nl.

PROPOSITIONS

- The potential of diatoms as environmental tracer should be exploited with DNA metabarcoding. (this thesis)
- One should not describe new diatom taxa solely based on morphological characteristics. (this thesis)
- 3. The pressure on scientists to publish in peer-reviewed journals leads to a higher scientific quality.
- 4. Scientific progress is not helped by having predatory journals favouring open access more than high-impact journals.
- 5. In spite of the growing number of PhD students, the quality of PhD research has increased over time.
- 6. The idea that human civilization can be sustainable in the future has no historical precedent.
- 7. From the perspective of the society, Social Media is not 'social' at all.

Propositions belonging to the thesis, entitled

ECOHYDROLOGICAL CHARACTERIZATION OF TERRESTRIAL DIATOMS

Jasper Foets Wageningen, 13 April 2021

ECOHYDROLOGICAL

CHARACTERIZATION OF TERRESTRIAL DIATOMS

Jasper Foets

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This research was conducted under the auspices of the Graduate School for Socio-Economic and Natural Sciences of the Environment (SENSE).

ECOHYDROLOGICAL

CHARACTERIZATION OF TERRESTRIAL

DIATOMS

Jasper Foets

Thesis

submitted in fulfilment of the requirements for the degree of doctor at Wageningen University by the authority of the Rector Magnificus, Prof. Dr A.P.J. Mol, in the presence of the Thesis Committee appointed by the Academic Board to be defended in public on Tuesday 13 April 2021 at 1.30 p.m. in the Aula.

Jasper Foets Ecohydrological characterization of terrestrial diatoms, vi + 153 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2021) With references, with summaries in English and Dutch

ISBN 978-94-6395-676-5 DOI https://doi.org/10.18174/538623 © 2021 J. Foets

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CHAPTER 1

INTRODUCTION

1.1 DIATOMS

Diatoms are single-celled, eukaryotic micro-organisms that form one of the most common, abundant and diverse algal groups in both freshwater and marine environments. They have a typical gold-brown, orange colour thanks to the presence of numerous pigments such as chlorophyll a and c, fucoxanthin, diadinoxanthin and diatoxanthin in their chloroplasts (Figure 1.1; Round et al., 1990). They are also photosynthetic and, despite their small cell sizes (typically ranging between 2 µm and a few millimetres), it is estimated that they are responsible for around 20% of the total oxygen production of the planet (Scarsini et al., 2019). Diatoms are hallmarked by siliceous, highly differentiated cell walls, which exhibit an enormous diversity in shapes and structures. These species-specific cell wall ornamentations are important for the study of diatoms, since they enable the identification of diatom taxa and form the basis of diatom taxonomy and systematics. To date, more than 64,000 diatom taxa have been described, and new taxa are added to that list almost daily with the number of extant taxa extrapolated to 100,000 (Mann and Vanormelingen, 2013). The cell walls of diatoms consist of two overlapping halves, called valves, where the epivalve (upper half) is usually slightly larger than the hypovalve (lower half). Both valves together are termed a frustule and are connected at the side with a girdle or *cingulum*. Besides the presence of silica, the cell wall also contains organic material, which forms a thin coating around the valves and girdle elements and often also a discrete layer beneath them. Diatoms are also known to secrete polysaccharides, some of which may diffuse in the surrounding medium, whereas others may remain as gelled capsules around the cells or as stalks, pads or threads (Round et al., 1990). Since all this organic matter in and around the cell obscures detailed microscopic observations of their exoskeleton, diatom samples are usually treated with an acid (e.g. hydrogen peroxide) prior to microscopic analysis.



FIGURE 1.1: EXAMPLES OF DIATOM CELLS. A, LIGHT (LM) AND SCANNING ELECTRON MICROSCOPE (SEM) IMAGE OF RESPECTIVELY SELLAPHORA HARDERI (SEE FOETS AND WETZEL, 2018 IN APPENDIX 1) AND HUMIDOPHILA IRATA, SHOWING EXTERNAL VALVE STRUCTURES AND ORNAMENTATION, RESPECTIVELY. B, LM IMAGE OF A LIVING DIATOM CELL SHOWING THE PIGMENT DISTRIBUTION. PHOTO CREDIT: CARLOS E. WETZEL (A) AND CARLOS BARRAGÁN (B).

In streams and rivers, diatoms are mainly found in two ecological niches: benthos and plankton in which they can occur either as unicells or colonially. Planktonic diatoms are freefloating in the water, while benthic diatoms can be directly 'attached' to the substratum by one entire surface, 'stalked' by a branched or unbranched mucilaginous pedicel or 'unattached' with no clear method of attachment. These unattached, 'motile' benthic diatoms, especially the so-called biraphid species, are adapted to gliding locomotion on the substrate (Yallop and Kelly, 2006). Furthermore, diatoms are sensitive to numerous environmental variables and anthropogenic factors such as light, current velocity, salinity, oxygen, pH, inorganic nutrients, acidification, organic pollution sources and eutrophication, resulting in strongly diversified species distributions (Lobo et al., 2016). As such, diatoms are commonly used as bioindicators of water quality, in which their quantitative community records are usually converted to reliable and mutually comparable quantitative indices (Foets et al., 2017; Lobo et al., 2016). Several of these indices, such as the Biological Diatom Index (BDI; Lenoir and Coste, 1996), Pollution-Sensitivity Index (IPS; Cemagref, 1982) and the Eutrophication/Pollution Index-Diatom based (EPI-D; Dell'uomo and Torrisi, 2011), are currently included in European directives and used in monitoring programmes. As well as their use in water quality analysis, diatoms have also been used as indicators of climate change, as stratigraphic indicators for mineral and petroleum exploration and biostratigraphic markers in marine deposits (Smol and Stoermer, 2010).

Besides their high abundances in water bodies, numerous diatom taxa are able to colonize a variety of non-aquatic habitats including soils, mosses and rocks. These 'terrestrial' diatoms have been an area of interest for more than 100 years (Fritsch, 1907; Petersen, 1928). But despite this, they are far from being as well-studied as aquatic diatoms, even though they seem to be just as ubiquitous and widespread. One of the reasons may be that most of the research performed on terrestrial habitats has been primarily floristic work with little focus on

the ecology of these communities (see Figure 1.2; Smol and Stoermer, 2010). However, more recently, that focus started to shift towards understanding the ecology of those communities. Moisture and pH have been found to play an important role in structuring diatom communities in terrestrial habitats (Lund, 1945; Van de Vijver et al., 2002; Van Kerckvoorde et al., 2000). For instance, Lund (1945) established tolerance ranges for pH for 24 taxa based on the live material of 66 soil samples, whereas Van de Viiver et al. (2002), who examined 106 soil diatom samples on Île de la Possession (Crozet, sub-Antarctica), was able to define optimum values for soil moisture for the most common taxa in this southern ocean island. More recently, several researchers compared the community composition under different anthropogenic pressures and found strong community responses to disturbances. They observed a large difference in the species' composition between anthropic disturbed and undisturbed areas, while they also noted a higher richness and diversity of species in undisturbed habitats (Heger et al., 2012; Stanek-Tarkowska and Noga, 2012). Additionally, carbon, nitrogen, phosphorus and magnesium have been documented as influencing soil diatom communities (Antonelli et al., 2017; Vacht et al., 2014; Van de Vijver et al., 2002). However, the correlations with carbon, nitrogen and phosphorus may rather be related to anthropic disturbances (e.g. farming practices) as their concentrations are likely affected by them (Antonelli et al., 2017). As a result of their sensitivity, researchers also explored the potential of diatoms to function as a soil bioindicator and even tried to apply the IPS index as an indicator tool to assess the degree of anthropic influence (Antonelli et al., 2017; Barragán et al., 2018; Bérard et al., 2004).



FIGURE 1.2: NUMBER OF PUBLICATIONS ON TERRESTRIAL DIATOMS (A), ALGAE, CYANOBACTERIA AND GREEN ALGAE (B). IN BLACK, THE NUMBER OF PUBLICATIONS ASSIGNED TO THE WEB OF SCIENCE CATEGORY 'ECOLOGY' CATEGORY. THE SEARCH QUERIES USED FOR RETRIEVING THE DATA WERE CONSTRUCTED AS FOLLOWS: TITLE = (ALGAE), (GREEN ALGAE OR CHLOROPHYTA OR CHLOROPHYCEAE), (CYANOBACTERIA OR BLUE-GREEN ALGAE), (DIATOM OR BACILLARIOPHYTA OR BACILLARIOPHYCEAE) FOR RESPECTIVELY ALGAE, GREEN ALGAE, CYANOBACTERIA AND DIATOMS, RESPECTIVELY, EACH TIME IN COMBINATION WITH A TITLE = (SOIL OR TERRESTRIAL OR AERIAL OR SUBAERIAL). AFTERWARDS, THE ARTICLES WERE CHECKED FOR THEIR CORRESPONDENCE WITH OUR SEARCH QUERY.

During all those years of terrestrial diatom research, a number of sampling techniques and protocols were developed and used in order to make the investigation of soil diatoms easier and faster. Generally, two different methods can be distinguished: microscopic examination after cultivation and direct microscopic examination after acid cleaning. The first method can be further divided into liquid cultivation, where culture media are used, and moist plate cultivation, where the soil sample is periodically moistened and incubated in warmer conditions. Both methods are used to further stimulate diatom growth and reproduction and make subsequent microscopic investigations easier and faster. However, using these techniques means that the diatom community is altered and cannot be used to study its ecology. Conversely, direct microscopic observations allow researchers to study diatom communities and their morphology in real-time without the influence of any other artificial effects (Kabirov and Gaisina, 2009). Another advantage of direct microscopic observations is that substantially more diatom taxa are retrieved than with cultivation (see Figure 1.3), giving an actual picture of the living diatom communities (Broady, 1979; Schulz et al., 2016).

However, the main disadvantage of the technique is the presence of high amounts of mineral soil particles, which may obscure diatom cells in the slides (Hayek and Hulbary, 1956). As a result, diatom identification and counting could take several hours per sample until reaching the necessary number of valves per slide is obtained (Barragán et al., 2018).



FIGURE 1.3: COMPARISON OF THE NUMBER OF DIATOM TAXA RETRIEVED BETWEEN DIRECT MICROSCOPIC OBSERVATION (N = 27) AND MICROSCOPIC OBSERVATION AFTER CULTIVATION (LIQUID AND MOIST) (N = 36). A, NUMBER OF DIATOM TAXA ENCOUNTERED FOR EACH STUDY; B, NUMBER OF SAMPLES ANALYSED FOR EACH STUDY. THE LIST OF PUBLICATIONS USED FOR THIS ANALYSIS CAN BE FOUND IN APPENDIX 1.

1.2 TERRESTRIAL DIATOMS IN HYDROLOGY

Hydrological connectivity is often defined as the water-mediated transfer of matter, energy or organisms within or between elements of the hydrologic cycle (Pringle, 2001). The concept was originally developed in ecology and provides a useful framework for understanding rainfall run-off transformations and spatial variations therein (Bracken and Croke, 2007). Various experimental techniques have been developed and used to evaluate this hydrological connectivity for different conceptualizations and environments (Bracken et al., 2013). For example, piezometers are often used to assess and map the hydrological connectivity of shallow water tables over the hillslope-riparian-stream (HRS) continuum (Ali et al., 2011; Jencso and McGlynn, 2011). Also, the use of tracer applications, spatially distributed discharge measurements and soil moisture patterns are common for evaluating hydrological connectivity (Ali and Roy, 2010; Cammeraat, 2002; Glaser et al., 2018; Klaus et al., 2015a). While these techniques work very well in small headwater catchments, detailed hydrometric observations (i.e. soil moisture, piezometer levels) become more difficult to obtain and interpret as the spatial scale increases. With an increasing catchment scale, the use of tracers for isotopic or hydrochemical hydrograph separation to discriminate different time and geographic sources of catchment run-off becomes fuzzy and more ambiguous as the assumptions underlying most conventional tracing techniques are increasingly unrealistic (e.g. complete mixing, stable end-member solutions) (Burns, 2002; Klaus et al., 2015a; Klaus and

McDonnell, 2013). As a response to these limitations, notable efforts have been devoted to the development of new measurement techniques such as fibre optics, thermal imagery and geophysical methods (Antonelli et al., 2020; Pfister et al., 2010; Robinson et al., 2008; Selker et al., 2006), and tracing systems such as fluorescent particles and nanobiotechnologies (Sharma et al., 2012; Tauro et al., 2012a, b). These studies illustrate that innovative multidisciplinary approaches are expected to significantly benefit hydrological sciences and possibly mitigate the limitations of conventional tracers (Burns, 2002; Klaus and McDonnell, 2013).

In response to the need for additional tracers, Pfister et al. (2009) pioneered the possibility of employing terrestrial diatoms to study water flow path connectivity and surface run-off processes. This concept is based on the hypothesis that allochthonous diatoms found in stream water samples during rainfall run-off events could provide information on their geographical origin at the watershed scale thanks to their diversified species' distributions (Figure 1.4). The hypothesis was successfully tested in three contrasted catchments by showing that the proportion of terrestrial diatoms in the drift diatom community increased during precipitation events, indicating the mobilization of diatoms from the terrestrial environment to the stream through surface run-off (Pfister et al., 2015). This proof-of-concept study paved the way for several other studies, which confirmed the potential for terrestrial diatoms to serve as a tracer at the HRS scale (Coles et al., 2016; Klaus et al., 2015b; Martínez-Carreras et al., 2015; Tauro et al., 2016). Tauro et al. (2016) demonstrated in laboratory experiments that diatoms do not percolate through the soil and thus suggested that surface run-off is the most important pathway to the stream. Furthermore, Martínez-Carreras et al. (2015) found that riparian zones are the largest terrestrial diatom reservoirs and the main source for terrestrial diatoms in the streams, whereas Coles et al. (2016) indicated that diatom reservoir exhaustion seems unlikely even during heavy rainfall events. Furthermore, the work by Klaus et al. (2015b) extended the use of terrestrial diatoms to trace hydrological connectivity at large spatial scales. Within a set of seven nested catchments (0.45-249 km²), they showed that different communities of species could be attributed to sub-catchments with different physiographic characteristics. As a result, the occurrence of certain species at the 249 km²-catchment outlet during precipitation events provided information on both the occurrence of riparian-stream connectivity in the different sub-catchments and the source of stream water to the outlet.

Chapter 1: Introduction



FIGURE 1.4: SIMPLIFIED REPRESENTATION OF THE MOBILIZATION OF TERRESTRIAL DIATOMS DURING A RAINFALL RUN-OFF EVENT. THE SCALE BAR (10 µM) REPRESENTS THE SIZE OF THE DIATOM SPECIES. PHOTO CREDIT: ENRICO BONANNO.

Although exploratory work has shown the potential for terrestrial diatoms to serve as complementary tracers in hydrological processes studies, more research is needed to overcome current limitations. For the time being, diatom analysis usually requires expert knowledge, since cell identification is mainly based on morphological characteristics. Therefore, it also requires the manual counting of the different diatom cells in each sample, which is rather time-consuming (i.e. 1-2 hours per sample). In order to reduce this costly and laborious character of diatom analysis, metagenomic and barcoding methods have been proposed as fast and supposedly low-cost identification and quantification tools (Visco et al., 2015; Zimmermann et al., 2015). Although these approaches offer interesting prospects, they are still in their infancy and there are still many hurdles to overcome before they can give the same satisfying results as microscopic analysis (Bailet et al., 2020; Kelly, 2019). Another limitation arises from the lack of ecological knowledge of terrestrial diatoms. Consequently, researchers need to take many samples to characterize the diatom communities in the surrounding terrestrial environment before being able to begin the hydrological tracing experiment (Klaus et al., 2015b). Therefore, there is a prior need for a better understanding of the spatial and temporal dynamics of diatom communities living in terrestrial environments. Concurrently, the main environmental variables driving the species' compositions should be investigated, resulting in more accurately defined ecological optima and ranges for the most common taxa under distinct environmental conditions. Eventually, this new understanding will

enable researchers to model and map species assemblages and their distributions. Besides the lack of ecological knowledge, the collection of stream water samples through automated sampling also generates multiple samples. Moreover, this sampling method restricts research to the more easily reachable stream areas due to the size and weight of the device (ISCO sampler) and its batteries (Klaus et al., 2015b). In order to go beyond these two limitations, new, alternative drift diatom sampling methods should be explored that could replace and/or complement automated sampling, so that the number of samples will decrease, and more sites can be sampled simultaneously.

1.3 OBJECTIVES

In order to address the lack of ecological knowledge on terrestrial diatoms and expand the stream sampling of drift diatoms, the overall aim of this thesis is to improve the applicability of terrestrial diatoms as hydrological tracers, building on exploratory work carried out in recent years in Luxembourg (Antonelli et al., 2017; Klaus et al., 2015b; Pfister et al., 2017b). This is addressed (1) by studying the spatial and temporal dynamics of diatoms living in terrestrial environments and (2) by investigating a new, alternative sampling method to sample drift diatom communities in streams (Figure 1.5). In this way, it should be possible to reduce the number of samples needed for tracing hydrological connectivity using diatoms.

1.4 THESIS OUTLINE

Chapter 2 elaborates on the study area and common sampling and preparation methods used during the research. In the three subsequent chapters, new insights gained from the data gathered through soil sampling are addressed, while in Chapter 6, an alternative sampling method for sampling drift diatom communities is discussed.

Chapter 3: The temporal and spatial variability of soil diatom communities is investigated with a focus on the effect of different anthropic disturbances on the species' composition. Furthermore, the sensitivity of the diatom species to anthropic disturbances is assessed and indicator species for the different disturbances are defined.

Chapter 4: The temporal and spatial variability of the absolute abundances of diatoms on soils are studied and compared with the abundances of green algae and cyanobacteria, two common primary producers on soils. Abundances are derived through pigment analysis using High-Performance Liquid Chromatography (HPLC). Although this approach is frequently used in water quality assessment (Smol et al., 2001), it is the first time it is applied to soil samples.





FIGURE 1.5: OVERVIEW OF THE THESIS STRUCTURE, WITH MAIN ISSUES STUDIED THROUGHOUT THE DIFFERENT CHAPTERS AND RESPECTIVE METHODS APPLIED.

Chapter

Chapter 5: Ecological optima and ranges of common diatom species occurring on soils are defined for pH and soil moisture and compared with previously established autecological values. For this chapter, several datasets are merged including the data gathered in Chapter 2, in order to obtain more robust results.

Chapter 6: The potential for the Phillips sampler, a time-integrated mass-flux sampler, is explored to provide a representative sample of the diatom assemblage of a whole storm run-off event. Its representativeness is evaluated by comparing the diatom community composition of the sampler to the composite community collected by automatic samplers for three events.

CHAPTER 2

STUDY AREA AND METHODOLOGY

2.1 STUDY AREA

Two distinct geomorphological regions dominate the landscape of Luxembourg (Figure 2.1). The southern half of the country, the Gutland region, is characterized by deep valleys cut into the Luxembourg sandstone, alternated with large valleys located in the Keuper marls, while in the northern Oesling (or Eislek) region, Devonian bedrock and deeply incised Vshaped valleys dominate. Altitudes in Luxembourg vary from 140 to 440 m.a.s.l. in the Gutland and from 225 to 559 m.a.s.l. in the Oesling (Cammeraat et al., 2018). Lying in the contact zone between these two contrasted regions, the Attert River basin (249 km²; 49°46'13.0" N. 5°59'9.2" E) is representative of the wider physiographic setting of Luxembourg. The Gutland covers 76% of the total Attert basin area and extends through the middle and southern part of it, whereas the Oesling, situated in the north, represents 24% of the basin (Cammeraat et al., 2018). The basin can be divided into three distinctive physiogeographic units. The first unit, the so-called Luxembourg plateau, is located in the southern part of the Attert River basin and mainly consists of Luxembourg sandstone locally covered by marls, and Luxembourg red sandstone ("Bundsandstein"). Land use in the area is dominated by forests and farmland supported by sandy to clayey soils such as podzols, luvisols, stagnosols, cambisols and regosols. The centre of the basin, called the marly depression, represents 68% of the total basin area. It comprises of very clayey soils (vertisols, planosols, cambisols and stagnosols) that support mainly forests and grassland (Cammeraat et al., 2018). The lithology of the third unit (i.e. the Oesling) mainly consists of schists and phyllades. The regosols and cambisols present in the northern part mainly have a silty soil texture and are mixed with gravels, while forests make up the dominant land use in that area. Farmland in the basin is primarily used for growing corn, wheat and rapeseed, while grasslands are predominantly used for cattle grazing. European beech (Fagus sylvatica L.) and spruce (Picea abies L.) dominate the forested areas. The climate regime of the basin is semi-oceanic.

Due to its complex physiogeographic characteristics, the basin has been the focal point of many (eco)hydrological studies, including studies on hydrological processes, climate variability/change, hydrological modelling and new measurement techniques of hydroclimatological variables (such as thermal IR imagery and ecohydrological tracers) (Antonelli

et al., 2020; Glaser et al., 2018; Pfister et al., 2018). Also, diatom assemblages in streams and on soils in the Attert basin are relatively well-documented thanks to monitoring programmes and scientific research (Antonelli et al., 2017; Barragán et al., 2018; Rimet et al., 2004; Wetzel et al., 2013). Since 2002, a nested set of nine sub-catchments has been implemented in the basin and equipped with a network of several measuring and monitoring stations (Pfister et al., 2018). Given the large amount of previous research already carried out in combination with the physiographic diversity of the area, the Attert River basin provides a perfect setting to investigate the spatio-temporal variability of terrestrial diatoms.

2.2 METHODOLOGY

2.2.1 SAMPLE COLLECTION, PREPARATION AND DIATOM IDENTIFICATION

The soil sampling campaign was performed monthly from October 2017 to November 2018. The summer prior to sampling was relatively wet (on average 88 mm/month), whereas the average monthly precipitation during the sampling period was 56.4 ± 33 mm. The maximum monthly precipitation was observed in December 2017 and January 2018 (111.9 mm and 123.5 mm, respectively). The minimum monthly precipitation was recorded in October 2018 (18 mm). July and August were the warmest months, reaching average temperatures of 20.5°C and 18.7°C, while the most freezing days were encountered during February and March (28 and 12) (data retrieved from Administration des Services Techniques de l'Agriculture, ASTA). Overall, 2018 was characterized by a cold winter and an exceptionally dry and warm summer period (Meteolux, 2019).

2

Chapter



FIGURE 2.1: GEOLOGY AND LAND USE OF THE ATTERT RIVER BASIN IN LUXEMBOURG. THE UPPER MAP SHOWS THE 17 SAMPLING LOCATIONS AND THE BORDERS OF THE COLPACH-HAUT SUB-CATCHMENT, WHILE THE LOWER MAP SHOWS THE SOIL SAMPLING LOCATIONS AND THE 5 × 5 KM GRID. SOURCE: MODIFIED FROM COPERNICUS (2018).

In order to capture the intra-annual and spatial variability of diatom communities and absolute algal abundances, soil samples were taken at the soil surface at 16 sites distributed across the catchment every three to five weeks for a period of 14 months (n = 224 samples). In order to systematically cover the whole area, a grid of 5 × 5 km was mapped on the study area (Figure 2.1). Next, at least one fixed sampling point per cell was chosen considering the differences in geology, soil and land use features as well as previous diatom research in the area (Antonelli et al., 2017; Barragán et al., 2018). During each sampling campaign, a description of the site was made in order to keep track of the environmental changes in between the sampling periods. Characteristics such as type of land use, disturbances, height of the lower vegetation (i.e. grass height was used as an indicator for mowing and grazing) were noted. In addition, soil moisture content and electrical conductivity (EC) were measured 30 times using a FieldScout TDR 300 Soil Moisture Meter (Table 2.1). Of the four soil samples that were taken per site, one was to serve for species' community analysis, one was used for pigment analysis and two were collected additionally for physico-chemical analysis. According to Barragán et al. (2018), one soil sample should be sufficient to have a diatom community representative for a homogeneous area of at least 75 m².

Metal rings (diameter: 5.6 cm, height: 4 cm) were used to collect small soil cores. Upon arrival in the lab, all plant litter was carefully removed from the topsoil layer to keep the sample undisturbed. Diatoms, green algae and cyanobacteria were extracted by rinsing the soil surface with MQ water until a 50 mL falcon tube was completely filled (see Barragán et al., 2018). The samples for community analysis were then fixed with 70% ethanol, while the samples for pigment analysis were kept in the dark at 4°C until pigment extraction. Diatom slides were prepared for microscopic counts following the CEN 13946 procedure (European Committee for Standardization, 2003). A minimum of 200 valves were counted and identified on each slide along random transects using a Leica DMR light microscope with a ×100 oil immersion objective and a magnification of ×1,000 (Bate and Newall, 2002). Diatom identifications were mainly based on following taxonomic references: Ettl and Gärtner (1995); Hustedt (1941); Lange-Bertalot (2001); Lange-Bertalot et al. (2017); Levkov et al. (2013); Lund (1946); Wetzel et al. (2013, 2015).

TABLE 2.1: ENVIRONMENTAL VARIABLES INCLUDED IN THESIS CHAPTERS 3, 4 AND 5. THE METEOROLOGICAL DATA IS CALCULATED AS THE AVERAGE OF THE SEVEN DAYS PRIOR TO SAMPLING AND IS RETRIEVED FROM THE ADMINISTRATION DES SERVICES TECHNIQUES DE L'AGRICULTURE (ASTA). DIATOM BIOVOLUME WAS CALCULATED ACCORDING TO RIMET AND BOUCHEZ (2012).

Variables	Code	Minimum	Maximum	Median
Geographic variables				
Longitude (degrees E)	Lon	49.713	49.835	49.774
Latitude (degrees N)	Lat	5.734	5.961	5.874
Altitude (m)	Alt	261	468	369
Site characteristics				
Habitat type (categorical)	habitat	1	5	3
Shading (ternary)	shading	1	3	2
Disturbance (binary)	disturb	1	2	1
Soil type (categorical)	soil	1	5	3
Vegetation height (cm)	height	0	107	5.7
Soil physico-chemistry				
рН	pН	4.58	8.11	6.53
Soil moisture (%)	VWC	3.64	59.06	19.02
Soil conductivity (µS cm ⁻¹)	EC	2080	3344	2556
Dissolved organic carbon (mg L^{-1})	DOC	1.70	57.07	12.62
Total nitrogen (mg L⁻¹)	TN	0.26	53.70	2.13
Aluminium (mg L⁻¹)	AI	0.04	3.61	0.04
Iron (mg L ⁻¹)	Fe	0.01	0.14	0.01
Phosphorus (mg L ⁻¹)	Р	0.00	0.88	0.03
Potassium (mg L⁻¹)	К	0.33	51.92	8.69
Magnesium (mg L ⁻¹)	Mg	0.00	53.85	11.49
Sodium (mg L ⁻¹)	Na	0.00	6.75	1.14
Silica (mg L⁻¹)	Si	0.00	5.05	0.98
Manganese (mg L⁻¹)	Mn	0.00	7.57	0.46
Meteorology				
Air temperature (°C)	Tair	-3.41	21.52	9.60
Frozen days (d)	Frozen	0	7	0
Precipitation (mm)	Precip	0.0	9.2	0.9
Relative humidity (%)	Humid	58.58	95.88	82.76
Global radiation (W h m ⁻² d ⁻¹)	Radiation	398.46	6226.74	2487.78
Climatological water balance (mm)	CWB	-4.10	6.13	-0.38
Wind speed (m s^{-1})	Wind	0.95	4.13	1.75
Storage deficit (mm)	SD	4.17	378.34	298.47
Diatom variables				
Biovolume (µm ³)	BV	9179.9	85364.8	30804.7

For Scanning Electron Microscope (SEM) analysis, parts of the oxidized suspensions were filtered and rinsed with additional deionized water through a 3 µm IsoporeTM polycarbonate membrane filter (Merck Millipore). Filters were mounted on aluminium stubs and coated with platinum (30 nm) using a BAL-TEC MED 020 Modular High Vacuum Coating System for 30 s at 100 mA. A Hitachi SU-70 ultra-high-resolution analytical field emission SEM (Hitachi High-Technologies Corporation, Tokyo, Japan), operated at 5 kV and with a 10 mm working distance, was used for the analysis. SEM images were taken using the upper (SE-U) detector signal. Photomicrographs were digitally manipulated and plates containing light and scanning electron micrographs were created using CorelDraw X8. For a more detailed and complete description of the soil sampling and algal extraction, we refer to Barragán et al. (2018).

2.3 PHYSICO-CHEMICAL ANALYSIS

After the algal extraction, the first cm of soil of each of the four samples was removed. After having dried the samples for one week at room temperature, the soils were crushed and sieved at 2 mm. The pH was measured according to the ISO 10390 (2005) standard. The bioavailable part of the nutrients (phosphorus (P), total nitrogen (TN), dissolved organic carbon (DOC), silica (Si), magnesium (Mg), manganese (Mn), sodium (Na), potassium (K), iron (Fe) and aluminium (AI)) were all extracted following the method described by Houba et al. (2000). 150 mL of 0.01M CaCl₂ was added to 15 g of soil in a 250 mL glass bottle and this mixture was shaken for two hours. Next, the samples were centrifuged for 15 minutes at 5000 rpm. The supernatant was filtered through 0.7 μ m glass microfibre filters and stored at 4°C prior to the ICP-OES (5110 VDV radial, Agilent) analysis of P, Mg, Mn, K, Si and Na. DOC and TN concentrations were determined directly after the extraction with a Torch Combustion TOC/TN analyser (Teledyne Tekmar). With a view to Fe and Al content analysis, 10 ml of the supernatant was additionally filtered with 0.45 μ m syringe filters (Acrodisc®, Pall) and 100 μ L 1M HCl was added before the ICP-MS (Thermo Elemental X7/Perkin-Elmer© DRC-e) analysis. However, the free metal ion concentrations were not estimated.

2.4 METEOROLOGICAL DATA

Daily meteorological data for the entire study period was retrieved from two weather stations (Useldange and Roodt) situated in the river basin. All data was taken from Useldange, except daily precipitation. For that, Thiessen polygons were calculated to know which sites were closer to which station. Besides this, air temperature was derived for every site using the general equation: -0.65° C × 100 m (Altitude). The station in Useldange is located at 280 m.a.s.l. Additionally, 2°C were deducted for the forested sites. Finally, the 7-day average prior to the sampling day was taken for every meteorological variable (Table 2.1).

CHAPTER 3

TEMPORAL AND SPATIAL VARIABILITY OF TERRESTRIAL DIATOMS AT THE CATCHMENT SCALE: CONTROLS ON COMMUNITIES

ABSTRACT

Diatoms are generally regarded as inhabitants of water bodies. However, numerous taxa are able to survive and reproduce in a variety of non-aquatic ecosystems. Although terrestrial diatoms are discussed extensively in the literature, most of those studies covered floristic aspects and few information exists on their ecology. This lack of knowledge thwarts their potential use as environmental markers in various applications. As a way forward, we investigated the seasonal patterns and the role of different disturbances on the community composition. We collected soil diatom samples in 16 sites across the Attert River basin (Luxembourg) every four weeks for a period of 14 months. Our results indicate that forests create a stable microhabitat for diatoms and that temporal variation of the diatom communities is mainly controlled by farming practices rather than seasonal changes in environmental variables. We also found out that communities need one to two months to re-establish a new, stable community after a significant change in the environment. We were able to confirm the applicability of the Pollution-Sensitivity Index (IPS) to identify anthropic disturbances.

This chapter is based on: Foets, J., Wetzel, C.E., Teuling, A.J., Pfister, L., 2020. Temporal and spatial variability of terrestrial diatoms at the catchment scale: controls on communities. PeerJ 1–19. doi:10.7717/peerj.8296

3.1 INTRODUCTION

Diatoms are generally regarded as inhabitants of water bodies. However, numerous taxa are able to survive and reproduce in a variety of terrestrial ecosystems such as soils, mosses, wet walls and rocks (Smol and Stoermer, 2001). Generally, those environments are much harsher for diatoms than aquatic habitats (Ress, 2012). Variables such as moisture and temperature could vary significantly over the course of a day or between two consecutive days and hence, diatoms could experience frequent and prolonged periods of desiccation in certain moments. Therefore, terrestrial diatom species have developed several methods (both morphologically and physiologically) to cope with the variable and moisture limited conditions. For instance, as the characteristic siliceous cell wall consists of many pores, they often decrease its number or create structures to enclose them on the out- or inside to prevent water loss (Lowe et al., 2007; Ress, 2012). This adaptability suggests that diatoms not only survive on soils, but also could thrive and even be the dominant algal group.

Freshwater diatoms are very well studied and generally regarded as a good bioindicator for water quality assessment due to their high diversity, rapid turn-over and sensitivity to numerous environmental conditions (e.g. pH, organic and inorganic pollution) (Prygiel and Coste, 1993; van Dam et al., 1994). In order to estimate degradation levels of water bodies several diatom-based indices such as BDI (Biological Diatom Index; Lenoir and Coste, 1996), IPS (Pollution-Sensitivity Index; Cemagref, 1982) and the EPI-D (Eutrophication/Pollution Index-Diatom based; Dell'uomo and Torrisi, 2011) have been developed and incorporated in legislatives. While the ecology of aquatic species is well understood, little ecological information exists on terrestrial diatoms, despite being discussed in an extensive literature. This is because most of the publications consist solely of floristic lists (i.e. qualitative data) and researchers often used culturing methods prior to microscope examination. Due to the latter, many diatom species were overlooked, and relative abundances were not reflecting the prevailing environmental conditions (Broady, 1979). Nevertheless, some notable ecological studies exist. Van de Vijver et al. (2002) found that moisture content and nutrient concentrations (particularly SO_4^{-2} and PO_4^{-3}) were the main environmental variables influencing the diatom communities, whereas in the study of Antonelli et al. (2017), pH and anthropic disturbances caused by farming practices were seen as the principal explanatory variables. Similarly, studies comparing different disturbance factors showed that soil diatoms are quite responsive to agricultural practices (Heger et al., 2012; Stanek-Tarkowska et al., 2018; Stanek-Tarkowska and Noga, 2012). It was even possible to separate those different land use types based on IPS values (Antonelli et al., 2017). Although, knowing that anthropic disturbances play a key role in shaping the species' composition of soil diatom communities, many details on species occurrences and sensitivities are still unknown.

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The variability of physical, chemical and biological conditions over the course of a year cause significant seasonal changes in the biomass and composition of dominant freshwater diatom species in temperate regions (Wetzel, 2001). For example, different diatom species reach peak populations at different times during the annual cycle, thereby growing under different environmental conditions (Köster and Pienitz, 2006). This is, however, not always the case in (verv) stable ecosystem types such as springs where seasonality is often totally absent (Cantonati et al., 2006). While this behaviour is very well documented for aquatic diatoms, it is still an open question in soil diatom ecology. To the best of our knowledge, only two studies briefly discussed this topic. Both, however, had contrasting results with Lund (1945), who observed a garden soil for several years, reporting no seasonality in the community composition, whereas Antonelli et al. (2017) found signs of seasonality reflected by the different species' compositions in the communities over three seasons. It should be noted that in both cases the temporal resolution was relatively coarse. Lund (1945) took his samples when environmental conditions were 'optimal' leading to sampling 26 of the possible 58 months. Antonelli et al. (2017), on the other hand, sampled once every four months. In order to have a better idea of the behaviour of terrestrial diatoms and subsequently to use them in future applications (e.g. soil bioindicator, hydrological tracer), it is essential to understand the seasonal patterns of the communities and their primary causes.

Natural catchments are very heterogeneous areas and exhibit spatio-temporal variability of soil moisture, land surface temperature and vegetation at a range of scales. (He et al., 2018; Troch et al., 2009; Western et al., 1999). Within a catchment those variables are linked through the distribution of energy and temperature (varying with exposure and altitude), and with varying wetness conditions (varying from infiltration and deep groundwater tables in the upslope parts to exfiltration and shallow groundwater tables in valleys and convergence zones) (Burt and Butcher, 1985). Nevertheless, since most of the variability in hydrological conditions and radiation/temperature will by definition occur at the catchment scale, this has long been a central scale in (eco)hydrological research. One such heterogeneous catchment is the Attert River basin in Luxembourg, which is characterized by a wide variety in physiographic settings (Pfister et al., 2018). As a result, it has been the centrepiece for recent studies on terrestrial diatoms and particularly their use as hydrological tracers (Pfister et al., 2017b). In the light of those studies, many different areas of the basin were investigated and soil diatom communities characterized. Klaus et al. (2015b) examined 302 samples (including stream samples) in six nested catchments and found 85 different terrestrial species. Also, Antonelli et al. (2017) took soil diatom samples at 34 locations across the whole basin, while Barragán et al. (2018) sampled around four of those to test a new sampling strategy. As a result of those studies, several new species were discovered and described (Barragán et al.,

2017; Wetzel et al., 2013, 2014). In spite of previous efforts, a full, detailed characterization of terrestrial diatoms over the catchment and across seasons is still lacking.

The purpose of our study is to provide more detailed information on the temporal and spatial variability of soil diatoms. This paper mainly focuses on the temporal variability of terrestrial diatom communities and has special attention for seasonal patterns and the role of land uses (e.g. forest, agricultural fields and (un)disturbed grasslands), as anthropic disturbances, on the community composition.

3.2 MATERIALS & METHODS

3.2.1 STUDY AREA AND WEATHER CONDITIONS

See Chapter 2.

3.2.2 STATISTICAL ANALYSIS

Before carrying out the statistical analysis, the species dataset was reduced by only keeping the taxa with a relative abundance of minimum 2% in at least two samples or 4% in one sample. Samples which contained less than 100 valves were removed from the analysis (18 of the 224 samples). Of the 18 samples, 12 were taken during the dry summer period and in the aftermath of it (July, 1; August, 3; September, 5; October, 3). A Detrended Correspondence Analysis (DCA) was performed to see how communities relate to each other and to estimate the length of the gradient (Hill and Gauch, 1980). The analysis revealed a gradient longer than 5.0 S.D. (Standard deviation of species turnover). Also, the nonparametric statistical test Analysis of Similarities (ANOSIM) using Bray-Curtis as a dissimilarity metric, was used to test if communities significantly differ between land use types (Clarke, 1993). Temporal variation was assessed using the same analysis performed for each type of land use. Significance of both analyses were tested with the default Monte Carlo permutation test (perm = 999). The effect of geographical distance on the species' composition of the communities was assessed with a Mantel test. Species data were transformed (Bray-Curtis) to a dissimilarity matrix and tested if there was a significant correlation with the geographical distance matrix. The latter was obtained using the *rdist.earth* function from the R-package fields (Douglas Nychka et al., 2017).

The characteristic species and their indicator value for the different land uses (i.e. agricultural field, grazed grassland, agricultural grassland, undisturbed grassland and forest) were assessed using the option *IndVal.g*, which is implemented in the *multipatt* function included in the *indicspecies* package. This function takes into account species presences/absences and relative abundances per type of land use (De Cáceres et al., 2010).

Also, the Shannon-Wiener index was computed for each sample with the function *diversity*. Additionally, IPS values were calculated (Cemagref, 1982) for the five different land uses to check if differences in communities could be expressed by a single value. This index takes into account the abundance of each species in the sample, their sensitivity value (i.e. sensitivity to pollution) and their indicator value (i.e. relative probability of each species to occur in one of five saprobity classes). Calculations for both, Shannon-Wiener and IPS index, were done on the whole dataset of soil diatom species. The IPS indices were computed with the software Omnidia (v. 6.0.8, 2018) (Lecointe et al., 1993) and compared with ANOVA (Analysis of Variance). IPS was chosen for this study, since it is the index with the highest number of taxa included and the most updated. Furthermore, a CONISS cluster analysis was done for sites 9, 10 and 13 to see when diatom community composition changed following land management practices. This was conducted with the function *strat.plot* in the *Rioja* package (Juggings, 2017). All aforementioned statistical analyses were performed using the R statistical program (R v. 3.5.0.; http://www.r-project.org/) and additional functions from the R-package *vegan* v. 2.5-4 (Oksanen et al., 2019) (if not mentioned differently).

3.3 RESULTS

We identified a total of 302 different taxa, including varieties, subspecies and forms, belonging to 44 genera. After using the cut-off criteria, 111 species (90% of the total valves counted) were kept in the statistical analyses. Most species-rich genera were *Pinnularia*, *Nitzschia*, *Mayamaea*, *Stauroneis* and *Sellaphora* comprising respectively 17, 14, 12, 6 and 5 taxa, whereas *Hantzschia amphioxys* (Ehrenberg) (19% of the total valves counted), *Mayamaea permitis* (Hustedt) Bruder & Medlin (8%), *Nitzschia pusilla* (Kützing) Grunow (6%), *Pinnularia obscura* Krasske (5%) and *Hantzschia abundans* Lange-Bertalot (5%) were the most abundant species. An average species richness per sample of 22 ± 8.3 was observed with the lowest values in December (18.3 ± 6.3) and October 2018 (17.8 ± 7.1). Maxima were noted in November 2017 (49), February (45) and July (45). The Shannon-Wiener index was similar between the 14 months (average 2.01 ± 0.17).

The first two axes of the DCA explained respectively 9.0 and 6.6% (both P < 0.05) of the total variance. The DCA clustered sites regarded as agricultural field, grazed grassland and agricultural grassland together, while (undisturbed) grassland is set apart from that cluster (Figure 3.1). Samples taken in forested areas are spread along the second axis of the plot. Additionally, we were able to distinguish four different sites (12, 7, 4, 2) within those samples, suggesting that species' composition of the communities is quite different from each other and that the composition generally does not change much over time (Figure 3.2). Indeed, site 12, a deciduous forest located on marls, had repeatedly very high abundances of *Humidophila*

irata (Krasske) R.L.Lowe et al. and to a lesser extent *Sellaphora harderi* (Hustedt) J.Foets & C.E.Wetzel, while species of the genus *Eunotia* together with *Pinnularia perirrorata* Krammer and *Pinnularia schoenfelderii* Krammer (not on Figure 3.2) were often very abundant on site 7. It is also one of the few sites where *H. amphioxys* was totally absent during the whole sampling period. Site 7 is situated in the schists area and the soils are typically very acidic, since the area is dominated by pine trees. On site 4, located in the schists area and covered by mixed forest, communities are primarily influenced by the riparian zone of the stream nearby. Hence, communities are generally very diverse, in particular with many different *Nitzschia* and *Navicula* species present in low abundances. Finally, site 2, characterized by sandy soils and a mixed forest, is generally dominated by *Chamaepinnularia obsoleta* (Hustedt) C.E.Wetzel & Ector, *Stauroneis thermicola* (J.B.Petersen) J.W.G.Lund and *Humidophila biceps* (Grunow) P.Furey, K.Manoylov & R.L.Lowe.



FIGURE 3.1: RESULTS OF THE DETRENDED CORRESPONDENCE ANALYSIS (DCA). IN DCA, SAMPLES ARE ORDERED BASED ON COMMUNITY (DIS)SIMILARITIES. SAMPLES CLOSE TO EACH OTHER SUGGEST SIMILAR COMMUNITIES. SAMPLES ARE COLOURED ACCORDING TO THEIR LAND USE TYPE.

An overview of the different land use types and their characteristic diatom species, selected by the indicator species analysis, is shown in Figure 3.3. Indicative species for agricultural fields are *Luticola ventricosa* (Kützing) D.G.Mann, *Stauroneis* (cf.) *borichii* (J.B.Petersen) J.W.G.Lund and *Navicula cincta* (Ehrenberg) Ralfs *var. heufleri* Grunow f. *curta*, while *Pinnularia microstauron* (Ehrenberg) Cleve var. *angusta* Krammer, *Navicula veneta* Kützing and *Sellaphora lundii* C.E.Wetzel, Barragán & Ector are characteristic for grazed grasslands. Moreover, *S. lundii* together with *Mayamaea agrestis* (Hustedt) Lange-Bertalot and *Pinnularia subrupestris* Krammer could typically be found on agricultural grasslands, whereas *Mayamaea fossalis* (Krasske) Lange-Bertalot and *Sellaphora nana* (Hustedt) Lange-Bertalot et al. are indicative for undisturbed grassland soils. Finally, *H. irata*,

C. obsoleta and *S. harderi* were only present on forested soils. A complete list with all the characteristic taxa per habitat type is given in the supplemental material (Table A3.1).



FIGURE 3.2: RELATIVE ABUNDANCES OF TERRESTRIAL DIATOM SPECIES OVER THE SAMPLING PERIOD. ONLY DIATOM TAXA WITH THE MOST SIGNIFICANT INDICATOR VALUE ARE LISTED. DIATOM TAXA ARE ABBREVIATED FOLLOWING OMNIDIA. ALL CODES AND RESPECTIVE FULL SPECIES NAMES COULD BE FOUND IN TABLE A3.1. SITES (1–16) ARE ORDERED ACCORDING TO THEIR LAND USE TYPE (RED, AGRICULTURAL FIELD; YELLOW, GRAZED GRASSLAND; ORANGE, AGRICULTURAL GRASSLAND; GREY, GRASSLAND; PURPLE, FOREST) AND SAMPLES ARE ORDERED CHRONOLOGICALLY. THE COLOUR INDICATES THE TAXON RELATIVE ABUNDANCE (WHITE, ABSENT; BLACK, > 50%).

Additionally, we examined which factors were responsible for the (dis)similarities between the different communities over the sampling period. We analysed whether those differences are rather related to the geographical distances between the sites (communities closer to each other are more similar), the seasonal variation in environmental factors or to external forces (e.g. farming practices), often related to the type of habitat. The Mantel test revealed no significant relations between the geographical distance and the community similarities (r = -0.09, P = 0.758). Regarding the temporal variation, only communities related to agricultural fields differed significantly between some months (Figure 3.4). Nevertheless, we were able to split the samples into three main groups based on the overlap of the notches.

The first group contains the period from October 2017 till March 2018 with *Mayamaea atomus* (Kützing) Lange-Bertalot and *N. pusilla* dominant (see Figure 3.2). The second group includes

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the samples taken from June to October 2018 when *H. amphioxys* became more dominant. April and November 2018 form the third 'transition' group with intermediate communities. However, the same analysis was more successful in separating the different land uses (Figure 3.5). Only agricultural grassland and undisturbed grassland could not be separated based on the community compositions, while forested soils have significantly different communities. The latter is clearly visible in Figure 3.2, where species in the lower part of the heatmap such as *Sellaphora atomoides* C.E.Wetzel & Van de Vijver, *N. veneta*, *S. thermicola*, *Stauroneis parathermicola* Lange-Bertalot and *Humidophila* spp. are more abundant on undisturbed sites. On the contrary, disturbed areas are especially dominated by *H. amphioxys*, *H. abundans*, *N. pusilla* and *M. atomus*. The Shannon-Wiener index also revealed a significant lower diversity for agricultural fields compared to the other types (F= 8.8, df = 4, P < 0.0001). Thus, the type of habitat has stronger effects on the diatom community than the seasonal and geographical factors.



FIGURE 3.3: HABITAT IMPRESSION AND SCANNING ELECTRON IMAGES OF SOME OF THE DOMINANT DIATOM SPECIES. HABITATS: A, AGRICULTURAL FIELD (SITE 9); B, GRAZED GRASSLAND (SITE 15); C, AGRICULTURAL GRASSLAND (SITE 9); D, UNDISTURBED GRASSLAND (SITE 13); E, FOREST (SITE 12). DIATOM TAXA: F, *LUTICOLA VENTRICOSA* (KÜTZING) D.G.MANN; K, *STAURONEIS* (CF.) BORICHII (J.B.PETERSEN) J.W.G.LUND; G, *HUMIDOPHILA CONTENTA* (GRUNOW) R.L.LOWE ET AL.; L, *NAVICULA VENETA* KÜTZING; H, *SELLAPHORA LUNDI*I C.E.WETZEL, BARRAGÁN & ECTOR; M, *HANTZSCHIA CALCIFUGA* E.REICHARDT & LANGE-BERTALOT; I, *MAYAMAEA FOSSALIS* (BOCK) LANGE-BERTALOT; N, *SELLAPHORA NANA* (HUSTEDT) LANGE-BERTALOT ET AL.; J, *HUMIDOPHILA IRATA* (KRASSKE) R.L.LOWE ET AL.; C, *SELLAPHORA HARDERI* (HUSTEDT) J.FOETS & C.E.WETZEL. THE SCALE BAR IS 5 µM. PHOTO CREDIT: JASPER FOETS AND CARLOS E. WETZEL.

Also, large shifts in the community composition were observed following land management practices. An example is site 9. From October to March the site was regarded as an agricultural grassland and except for mowing once or twice, no other disturbances took place (see Figure 3.2). During that period, the community consisted mainly of *S. thermicola*, *N. pusilla*, *M. atomus* and *M. permitis*. Between the sampling in March and April, the site was ploughed and manured transforming the area to an agricultural field. The field remained in this

condition for the rest of the study period in which crops were grown and harvested twice. Following those farming practices, the species' composition changed after a lag-phase of one to two months to a community dominated by *H. amphioxys*, *H. abundans* and *S. borichii*. A more detailed figure of the last example is shown in the supplemental material (Figure A3.1). Furthermore, similar community shifts occurred in sites 13 (from undisturbed to grazed grassland between June and July; Figure A3.2) and 10 (from agricultural grassland to agricultural field between May and June; Figure A3.3). Also, there, *H. amphioxys* and *H. abundans* replaced the more sensitive *N. pusilla*, *M. atomus* and *M. permitis*.



FIGURE 3.4: RESULTS OF THE ANALYSIS OF SIMILARITIES. THE PLOT SHOWS THE DISSIMILARITY BETWEEN AND WITHIN DIFFERENT HABITAT TYPES. NOT OVERLAPPING NOTCHES INDICATE THAT THE MEDIANS SIGNIFICANTLY DIFFER (CHAMBERS ET AL., 1983). BTW, BETWEEN-GROUP DISSIMILARITY; AF, AGRICULTURAL FIELD; GG, GRAZED GRASSLAND; AG, AGRICULTURAL GRASSLAND; UG, UNDISTURBED GRASSLAND; F, FOREST.



FIGURE 3.5: RESULTS OF THE ANALYSIS OF SIMILARITIES. THE PLOT SHOWS THE DISSIMILARITY BETWEEN THE DIFFERENT MONTHS. ONLY SAMPLES TAKEN ON AGRICULTURAL FIELDS ARE INCLUDED IN THE GRAPH. NOT OVERLAPPING NOTCHES INDICATE THAT THE MEDIANS SIGNIFICANTLY DIFFER (CHAMBERS ET AL., 1983). BTW, BETWEEN-GROUP DISSIMILARITY.

The IPS values show to be a good parameter in separating the different land uses (F = 59.6, df = 4, P < 0.0001) (Figure 3.6). Agricultural fields, undisturbed grassland and forest could be separated from all the other land use types. Only the samples from the semidisturbed types, grazed grassland and agricultural grassland, could not be separated from each other (P = 0.89). Also, agricultural fields comprise the species which are least sensitive to pollution (= lowest values), while undisturbed sites consist mainly of sensitive species (= highest values). Unfortunately, not all of the species have attributed IPS values yet (on average $85.6 \pm 5.8\%$) and thus the final calculations included only $90.7\% (\pm 11.9\%)$ of the total abundance.



FIGURE 3.6: COMPARISON OF POLLUTION-SENSITIVITY INDEX (IPS) VALUES BETWEEN HABITAT TYPES. AF, AGRICULTURAL FIELD; GG, GRAZED GRASSLAND; AG, AGRICULTURAL GRASSLAND; UG, UNDISTURBED GRASSLAND; F, FOREST. NOT OVERLAPPING NOTCHES INDICATE THAT THE MEDIANS DIFFER (CHAMBERS ET AL., 1983). ***, HABITAT IS SIGNIFICANTLY DIFFERENT FROM ALL THE OTHER HABITATS.

3.4 DISCUSSION

3.4.1 SEASONAL VARIATION

Our research showed that seasonal variation in soil diatom communities was rather limited or almost absent when looking at the communities in the forested sites. A reason behind this stationarity could be that forests create a relatively stable microhabitat for diatoms. They exhibit generally a higher and more constant moisture than grasslands (James et al., 2003). The temperature fluctuations are usually smaller and maximum temperatures are lower. Also, the forest canopy provides a good protection against direct UV-radiation, which is considered to be harmful to algae (Ress, 2012), and ensures that the topsoil layer dries out less quickly. Although, forest soils are receiving high amounts of leaf litter and seasonal changes resulting from that (i.e. decomposition and mineralization processes), but this does not seem to have any visible effect on soil diatoms. Similar to forests, several spring types have shown to create a stable environment for aquatic diatoms (i.e. fairly constant pH, temperature and trophy level), leading to limited seasonal changes in the communities (Cantonati, 1998). Cantonati et al. (2006) therefore concluded that this facilitates the use of diatoms as ecological indicators since fewer numbers of surveys are required. The same principle could be applied to forest soils, meaning that sampling once a year should be sufficient.

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In addition to the minor seasonal influences in forests, we did not observe any significant temporal variation in the other habitat types, except for communities living on agricultural fields. There, we observed a general change from *Mayamaea* to *Hantzschia* dominated communities. However, this variation could be explained by farming practices such as ploughing and manuring, since the compositional changes in the communities coincides with the beginning (April–May) and ending (October–November) of the 'farming season'. Nevertheless, we noticed some small signs of seasonality, especially by some species who are strongly dependent on soil moisture content (e.g. *Nitzschia solgensis* Cleve-Euler; Foets et al., 2020d). Also, the fact that we experienced an exceptionally dry and warm summer period during the sampling campaign did not help to observe potential seasonality, since sufficient moisture availability is essential for diatom survival and reproduction (Van de Vijver et al., 2002). So, seasonality in diatom community composition could occur in (disturbed) grassland soils, but it is rather unlikely that it will play a significant role in the composition of soil diatom communities.

3.4.2 HABITAT VARIATION

We found that the type of land use was a key factor in defining the species' composition. This result is consistent with previous works that showed different communities with different levels of disturbance (Antonelli et al., 2017; Stanek-Tarkowska et al., 2018; Stanek-Tarkowska and Noga, 2012; Vacht et al., 2014). In contrast to these works, we compared two to three habitat types more at the same time. Although our analysis could not split the communities sampled on agricultural grasslands from the ones taken on undisturbed grasslands, our results still show that species are perhaps more sensitive and indicative for anthropic disturbances than we previously thought. Indeed, we were able to define several indicator species for each type of land use and when comparing those species with earlier works, we noticed many similarities. For instance, Barragán et al. (2018) also reported *S. lundii* from a meadow and *P. perrirorata* and *Eunotia* spp. from a forest site, whereas Stanek-Tarkowska et al. (2013) and Stanek-Tarkowska and Noga (2012) observed *S. borichii* in high abundances on farmland. This habitat specificity of soil diatoms is an interesting outcome and should be explored more often in the future, certainly in the light of using soil diatoms as environmental markers.

Another interesting outcome of this study was the follow-up of the communities after a drastic change in the landscape following land management practices. Two of our 16 sites (9 and 10) went from an agricultural grassland to an agricultural field and one site (13) was transformed from an undisturbed to a grazed grassland. Generally, species colonizing the early stages of a secondary succession have fast immigration rates rather than high

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reproduction and growth rates (Stevenson and Peterson, 1991; Stevenson, 1986). This is also what we observed in the three cases with *Pinnularia borealis* Ehrenberg, *H. amphioxys* and *H. abundans* often replacing *N. pusilla* and *Mayamaea* spp. The former three taxa are very common, large terrestrial diatoms (\pm 75 µm) and are known to be well-adapted to living in extreme environments (Dodd and Stoermer, 1962; Pipe and Cullimore, 1984; Stock et al., 2018; van Dam et al., 1994). These diatoms are considered to be slow growers, since they spend a large part of their energy in developing complex structures to withstand (very) harsh conditions (Pinseel et al., 2019; Ress, 2012). For example, *Hantzschia* spp. create internal valves to better retain moisture and decrease water loss (Dodd and Stoermer, 1962; Round et al., 1990). On the other hand, *N. pusilla* and *Mayamaea* spp. are rather small (10–15 µm) and more fragile (i.e. less silicified cells) species. We therefore expect them to grow and reproduce faster, but they need preferably a more stabilized habitat. Consequently, changes in land use could have large, similar effects on the community, even in (semi-)disturbed areas.

Besides the identification of important pioneer species, we also noticed that soil diatom communities need between one and two months to colonize a new, disturbed habitat depending on the prevailing environmental conditions. This recolonization period is longer compared to freshwater communities. They generally need 14 days to one month to establish a new, stable community (Blinn et al., 1980; Oemke and Burton, 1986). We stipulate that this difference is mainly due to three reasons: 1) the absolute abundance of soil diatoms is much lower compared to freshwater diatoms (Loescher, 1972), 2) water is a more efficient dispersal mechanism than wind (Kristiansen, 1996) and 3) nutrient exchange via osmosis and reproduction is easier in aquatic conditions. However, it is to be noted that some shifts happened during exceptionally warm, dry weather conditions and therefore recolonization could be shorter in more optimal (moist) conditions. Unfortunately, we were not able to monitor a second succession stage to see if communities go back to their previous composition or to a different one. So, the re-establishment of a stable community on disturbed terrestrial habitats generally happens after one–two months with the more tolerant species replacing the rather sensitive ones.

3.4.3 **IPS** AND FUTURE MODIFICATIONS

Despite that the IPS index was developed for water quality assessment, it works very well in separating the different land use types as it is shown here and previously by Antonelli et al. (2017) and Barragán et al. (2018). Nevertheless, this index should be adapted to terrestrial environments if it is to be used further. In the first place, we should be able now (i.e. with the recent results) to fill in the missing indicator and pollution values for, at least, the most abundant and common terrestrial diatom taxa. The IPS index was chosen, because it is
species-specific and is supported by a large and updated database. However, some important terrestrial diatoms are not incorporated in it. For instance, Antonelli et al. (2017) could not include certain abundant species in their analysis, while some of our IPS values were calculated using only 60% of the total valves counted. Secondly, there is a need to update and re-evaluate the indicator and sensitivity values, since changes in diatom taxonomy happen very fast leading to new names and/or species descriptions. This in turn leads then to different results in quality indices calculations (Kahlert et al., 2016). Also, both values are inferred from aquatic surveys and many of them are probably not reflecting the behaviour of diatoms in terrestrial habitats (Antonelli et al., 2017). Finally, we should look at incorporating an additional factor to make the index more specific for terrestrial habitats and eventually decrease the intravariability and increase the inter-variability between the land use types. For instance, by multiplying that factor it will give more weight to the very specific indicator taxa. Hopefully, with those modifications we will be able to predict anthropic disturbances more precisely in the future.

3.5 CONCLUSIONS

In this study, we investigated the temporal and spatial variability of soil diatom communities at the catchment scale. Our results indicate that forests create a stable microhabitat for diatoms and that temporal variation is mainly related to farming practices rather than seasonal changes in environmental variables. This was highlighted by the changes in the species' composition of the communities after drastic anthropogenic interventions in the habitat. Our study showed that communities need one to two months to re-establish a new, stable community after a significant change in the environment. However, we were not able to observe whether communities would go back to their original composition when the habitat would change to its previous state. We eventually confirmed the earlier results of Antonelli et al. (2017) regarding the applicability of the IPS index to predict different anthropic disturbances and stressed that, with the recent results, we should be able to adapt and update the indicator and sensitivity values for terrestrial diatom species so that this will improve the accuracy and predictability of the index on soils.

CHAPTER 4

TEMPORAL AND SPATIAL VARIABILITY OF TERRESTRIAL DIATOMS AT THE CATCHMENT SCALE: CONTROLS ON PRODUCTIVITY AND COMPARISON WITH OTHER SOIL ALGAE

ABSTRACT

Terrestrial diatoms are an integral component of the soil microbial community. However, their productivity and how it compares to other algal groups remains poorly known. This lack of knowledge hampers their potential use as environmental markers in various applications. As a way forward, we investigated the seasonal and spatial patterns of diatom assemblages and the role of environmental factors on the soil diatom productivity. We collected soil algal samples in 16 sites across the Attert River basin (Luxembourg) every four weeks for a period of 12 months. The algal abundances were then derived from pigment analysis using High-Performance Liquid Chromatography (HPLC). Our results indicate that diatom productivity is mainly controlled by factors related to soil moisture availability leading to seasonal patterns, whereas the concentration of green algae remained stable over the course of the study period. Generally, anthropic disturbed habitats contained less living diatom cells than undisturbed habitats. Also, we learned that diatoms can be the dominant algal group at periods of the year with high soil moisture.

This Chapter is based on: Foets, J., Wetzel, C.E., Teuling, A.J., Pfister, L., 2020. Temporal and spatial variability of terrestrial diatoms at the catchment scale: controls on productivity and comparison with other soil algae. PeerJ 8, 1–21. doi:10.7717/peerj.9198

4.1 INTRODUCTION

Diatoms are microscopic, unicellular algae and form one of the most common and diverse algal groups in both freshwaters and marine environments (Round et al., 1990). They are pigmented, photosynthetic and because of their high abundances, they play a large role in the exchange of gasses between the atmosphere and biosphere. It has been estimated that they are responsible for 20% of the total oxygen production of the planet (Scarsini et al., 2019). While diatoms are generally regarded as inhabitants of water bodies, numerous taxa are able to survive and reproduce in a variety of terrestrial ecosystems such as soils, mosses, wet walls and rocks (Smol and Stoermer, 2001). Generally, those environments are much harsher for diatoms than aquatic habitats (Ress, 2012). Variables such as moisture and temperature can vary considerably not only seasonally, but also over the course of a day or between two consecutive days. As a consequence, diatoms are typically exposed to frequent and prolonged periods of desiccation. Terrestrial diatom species have developed several features (both morphologically and physiologically) to cope with the temperature variability and limited moisture conditions. For instance, as the characteristic siliceous cell wall consists of many pores, they often decrease its number or create structures to enclose them on the out- or inside to prevent water loss (Lowe et al., 2007; Ress, 2012). This adaptability suggests that diatoms do not only survive on soils, but that they eventually also could thrive and even be the dominant algal group in a terrestrial environment.

As an integral component of the soil microbial community, diatoms play an important role in the functioning of the soil ecosystem. By moving on or being attached to the soil surface, they produce an extracellular matrix of mucopolysaccharides, which will bind soil particles and eventually stabilize the soil (Booth, 1941; Jewson et al., 2006; Paterson, 1986; Tolhurst et al., 2002). This aggregation will subsequently reduce water loss by evaporation, limit soil erosion and improve water infiltration, thereby providing a favourable habitat for seed germination (Booth, 1941; Hoffmann, 1989). Moreover, they promote the release of nutrients from insoluble compounds and the weathering of silicates by creating a slightly acidic environment (Hoffmann, 1989; Wu et al., 2013). Schmidt et al. (2016) found that they even might be an important carbon input source for decomposing soil animals. Their death and decay also provide organic matter, which other microorganisms and plants can potentially utilize (Fritsch, 1907; Shubert and Starks, 1979; Starks et al., 1981). As climate change is highly likely to increase the frequency and persistence of droughts in the near future (Berg and Sheffield, 2018), there is growing concern about potential negative impacts that this change could have on the viability and functions of terrestrial diatoms and subsequently also on higher plants.

In temperate regions, the seasonal variability in meteorological (e.g. humidity), chemical (e.g. nutrients) or biological conditions (e.g. vegetation height) is not causing any significant changes in the composition of soil diatom communities according to Foets et al. (2020) and Lund (1945). However, seasonal variations might prevail in the diatom biomass with possible maxima observed in spring and autumn in response to changes in temperature and precipitation (Lund, 1945). A similar observation for soil algae was reported by Stokes (1940), who noticed sharp increases in algal numbers after periods of rain and found that the optimum moisture range for algal growth lies between 40 and 60% of the moisture-holding capacity of the soil. This amount would probably be ideal to produce a soil solution with enough available nutrients to support algal growth (Pringsheim, 1950; Starks and Shubert, 1982). Furthermore, Davey (1991) and Grondin and Johansen (1995) reported an increased algal biomass (in algal numbers and in chlorophyll a concentration) after snow cover. Although Lund (1945) is the only study (of all aforementioned studies) to date to have analysed specifically the terrestrial diatom production, the temporal and spatial scale of that study was relatively limited (i.e. one garden soil in optimal conditions). There is a pressing need for a better understanding of the seasonal patterns in diatom production across contrasted environmental settings. This stands as a prerequisite for their subsequent use in future applications (e.g. as soil bioindicator or as hydrological tracer).

Algae and cyanobacteria use pigments and other compounds to regulate the spectral composition and the intensity of incoming light (photon flux density). This allows them to maximize photosynthetic efficiency whilst preventing photochemical degradation of cellular components and indirect loss of function mediated by reactive oxygen species (Vincent, 2000). Two types of pigments are involved in those processes. Chlorophylls, particularly a, b and c, are the central components in light-harvesting, while carotenoids, including xanthophylls, are mainly engaged in photoprotection (Demmig-Adams and Adams, 2000). In contrast to higher plants, which all contain the same major carotenoids, algae have different characteristic carotenoids. So far, around 1100 different natural carotenoids have been characterized and reported in the literature (Fernandes et al., 2018). This diversity and specificity allow us to use certain carotenoids as so-called biomarkers or chemotaxonomical markers to infer the production of different algal groups. Furthermore, recent developments in High-Performance Liquid Chromatography (HPLC) allow for rapid and accurate determinations of pigments and their derivatives in aquatic and sediment samples. Pigment concentrations have now become a common tool in paleolimnological research and water quality assessments (Duong et al., 2019; Reavie et al., 2017; Verleyen et al., 2004). However, except for a few studies on soil primary production (Davey, 1991; Davey and Rothery, 1992; Shubert and Starks, 1979; Starks

and Shubert, 1982), no other attempts have been reported to date to assess soil diatom production and its environmental controls.

Previously, we have investigated the temporal and spatial variability in the composition of soil diatom communities (Foets et al., 2020a). There, we found that forests create a stable microhabitat for diatoms, that the temporal variation of the communities is mainly controlled by farming practices, and that they need one to two months to re-establish a new, stable community after a significant change in the environment. Here, we leverage these findings and investigate how diatom production changes over space and time in a mesoscale catchment compared to green algae and cyanobacteria. The catchment scale was selected, since this is the natural unit in which environmental variables such as radiation, temperature and soil moisture show the largest variability. The aim of this study is mainly exploratory, but while analysing the data for Foets et al. (2020a), we also developed two hypotheses. Our first hypothesis states that soil diatom productivity (i.e. concentration of fucoxanthin) is controlled by meteorological conditions. Our second hypothesis stipulates that the biomass changes depending on the type of habitat, since communities in (anthropic) disturbed areas contain generally larger species (i.e. higher pigment concentration) compared to undisturbed areas (Foets et al., 2020a).

4.2 MATERIAL AND METHODS

4.2.1 STUDY AREA AND WEATHER CONDITIONS

See Chapter 2.

4.2.2 METEOROLOGICAL DATA

See Chapter 2.

4.2.3 SOIL SAMPLING AND PIGMENT ANALYSIS

Of the total 224 samples collected during the sampling campaign (see Chapter 2), 192 samples (i.e. from December 2017 to November 2018) were analysed for pigment analysis. The day after algal extraction, the samples were centrifuged for 10 minutes at 4000 rpm. The supernatants were discarded and the residues freeze-dried for approximately 24 hours. Next, the pigments were extracted two times with 5 mL methanol by shaking for 15 minutes at 25 Hz. with a mixer mill (MM 400 Retsch). After centrifugation (4000 rpm., 10 min), organic phases were pooled and extracts were concentrated by vacuum evaporation.

The pigments were separated and quantified by HPLC with Diode-Array Detection (HPLC-DAD, 1260 Infinity, Agilent). An Acquity UPLC HSS T3 column at 30°C was used

(2.1 × 100 mm, 1.7 µm) with a mobile phase consisting of 50mM ammonium acetate solution, acetonitrile/dichloromethane/methanol (75/10/15 v/v/v), and ethyl acetate. Pigments were eluted at a flow rate of 300 µL min⁻¹ with a ternary gradient. The compounds of interest were identified by their retention time and the spectral data was compared with certified standards provided by Sigma-Aldrich and Carotenature. The detection limit for the standards was 30 ng per sample. Quantification was done at 450, 464, 620 and 655 nm using external calibration. Chlorophyll and carotenoid concentrations were expressed in ng per sample (50 mL) and transformed to μ g L⁻¹ to make the concentrations comparable with other studies. The different pigments used in the analysis, their affinity and interpretation are listed in Table 4.1.

Pigment	Code	Affinity	Interpretation	
Chlorophyll a (incl. derivatives)	TChla	All photosynthetic algae	Primary production/Total algal production	
Fucoxanthin	Fx	B*	Diatom production	
Diatoxanthin	Da	B, X*, E	Diatoms, measure for irradiance	
Diadinoxanthin	Dd	B, X*, E*	Diatoms, measure for irradiance	
Zeaxanthin Lutein	Ze Lu	Cy*, R*, B, E, Cl Cl*	Cyanobacteria production Green algae production	
(Dd + Da) Fx ⁻¹			Mean irradiance per diatom	

TABLE 4.1: SUMMARY OF PROXIES EMPLOYED IN THIS STUDY.

*, MAJOR CAROTENOID IN MOST SPECIES OF THE CLASS. CY, CYANOPHYTA (CYANOBACTERIA); E, EUGLENOPHYTA; CL, CHLOROPHYTA (~GREEN ALGAE); B, BACILLARIOPHYCEAE (~DIATOMS); R, RHODOPHYTA; X, XANTHOPHYCEAE. TABLE DERIVED FROM TAKAICHI (2011) AND VERLEYEN ET AL. (2004).

4.2.4 PHYSICO-CHEMICAL ANALYSIS

See Chapter 2.

4.2.5 SCANNING ELECTRON MICROSCOPE ANALYSIS

A scanning electron analysis was carried out to get information regarding diatoms within the soil matrix (e.g. their abundance, their way of living). Additional soil samples were taken in November 2017 at an agricultural grassland site (site 9, see Foets et al., 2020a). A part of the soil samples were carefully placed on aluminium stubs and analysed with a Quanta 200 Field Emission Gun Scanning Electron Microscope (FEG SEM) (Phillips-FEI). In addition, the microscope was equipped with a Genesis XM 4i Energy Dispersive Spectrometer (EDS) system for chemical analysis. For complementary EDS analysis, a large field detector was used, as our examination was done in a low-vacuum environment (150 Pa).

4.2.6 STATISTICAL ANALYSES

Based on meteorological and discharge datasets, two soil moisture availability proxies were calculated for the Attert River basin. First, daily water storage deficits (SD) were inferred from water balance calculations as per Pfister et al. (2017a). Second, daily differences

between precipitation input and potential evapotranspiration loss (PET) were calculated. The FAO Penman-Monteith equation was used for calculating PET (Allen et al., 1998). For these calculations, it was assumed that the soil heat flux was zero and the psychrometric constant equal to 0.065.

Correlations between continuous environmental variables and between pigment concentrations were assessed using Spearman rank correlation. One of each correlated (i.e. $R^2 > 0.5$ or < -0.5) environmental variable was retained for further discriminant analyses (see section 4.3.2). Also, possible spatial autocorrelation of the pigment measurements was assessed with a Mantel test. For doing that, pigment data were transformed to a dissimilarity matrix (Bray-Curtis) and tested if there was significant correlation (perm = 999) with the geographical distance matrix. The latter was obtained using the *rdist.earth* function from the R-package *fields* (Douglas Nychka et al., 2017). Before carrying out the indirect and direct ordination analysis, pigment data revealed a gradient length smaller than 2.0 S.D. (standard deviation) (Hill and Gauch, 1980). Therefore, we relied on a Redundancy Analysis (RDA) as an exploration method.

The variation partitioning technique (Legendre et al., 2005) was used to quantify the relative contributions of three matrices of variables (soil chemistry, meteorology, and site characteristics) to the explained variance and to test whether and to what extent their contributions to the composition of the pigment concentrations could be separated. Testable fractions were analysed with RDA. For testing our first hypothesis, a multiple regression analysis was carried out on the fucoxanthin data to identify the variables that are significantly related to diatom abundances. A generalized mixed model (Imer function) was formed with 'site' as a random variable to account for repeated measurements. Prior to model building, fucoxanthin concentrations were log-transformed (x + 0.0001) to get normal distributed data and environmental variables were rescaled. Variable selection with backward elimination was done using the step function from the *ImerTest* package (Kuznetsova et al., 2017). Afterwards, the residuals of the final model were checked. For testing our second hypothesis, diatom biovolume was calculated by multiplying the raw diatom community data given in Foets (2019) with the species size measurements given in Rimet and Bouchez (2012) and Omnidia (v. 6.0.8, 2018) (Lecointe et al., 1993). Next, the variability of biovolume and pigment concentrations were tested over qualitative variables (e.g. month, soil and habitat type) with Analysis of Variance (ANOVA), whilst accounting for repeated measurements, or the nonparametric Kruskal-Wallis test. Homoscedasticity was checked with the Breusch-Pagan test. Thereafter, the significant results were analysed in more detail by relying on Tukey's range or the pairwise Wilcoxon test, respectively. All aforementioned statistical analyses were

performed using the R statistical program (R v. 3.5.3.; http://www.r-project.org/) and additional functions from the R-package *vegan* v. 2.5-5 (Oksanen et al., 2019).

4.3 RESULTS

4.3.1 SOIL MATRIX

Diatoms live at the top of the soil surface and with 15 cells per approximately 0.2 mm² (= 7500 cells cm⁻²) diatoms are relatively abundant even on a small area such as shown in Figure 4.1B. The occurring species are mainly *Nitzschia pusilla* (Kützing) Grunow, *Sellaphora nana* (Hustedt) Lange-Bertalot et al. and *Hantzschia amphioxys* (Ehrenberg) Grunow. Bacilli-shaped bacteria are also present at the soil surface (Figure 4.1C). However, because they are so small, it is impossible to see whether the bacteria are cyanobacteria or not. Green algae are absent on the picture. This general observation is supported by the average pigment concentrations measured on that particular site (site 9), which indicate that diatoms on average (9.0 µg L⁻¹) are more abundant than green algae (2.6 µg L⁻¹) and cyanobacteria (0.7 µg L⁻¹).

Besides the topography, the SEM picture also gives an idea of the chemical composition of the top layer (Table 4.2). The contrast of the picture is based on the convention that "heavier" elemental areas (= higher atomic number) are shown in light grey and low atomic number elements in darker grey. Our subsequent analysis showed that the percentage of carbon and oxygen in *H. amphioxys* is much higher than at the soil surface (difference of respectively \pm 100% and 50%), while the silica concentration is approximately the same. Interestingly, the aluminium concentration at the soil is high (11.0%) compared to the other elements and around a third of that percentage is found in the diatom (3.4%). In addition to the composition mode, chemical maps could be made displaying the chemical contrast in a coloured pixel map (Figure 4.1G – I). This means that the higher the elemental concentration is, the higher the coloured pixel density will be. Since oxygen and silica had the highest concentrations, they gave the best pixel maps of all present chemical elements. While the map of silica is only able to show us roughly the contours of *H. amphioxys*, some differences in the external valve structure are visible on the oxygen map.



Figure 4.1: Illustration of diatoms living on the soil surface. Images were taken with a Scanning Electron Microscope (SEM). The soil sample is from an agricultural grassland site (site 9; see Figure 3.2 in Chapter 3) sampled in November 2017. A, soil sample on aluminium stub; B, SEM image; C, close-up of the dashed rectangle showing baciteria; D, life material showing pigment distribution in *Hantzschia Amphioxys* (Ehrenberg) Grunow; E, SEM picture showing external valve structures; F, SEM picture showing the EDS 1 and EDS 2 analysed zones; G, SEM picture; H, chemical map showing oxygen concentration at the surface; I, chemical map showing silica concentration at the surface; 1, *Sellaphora Nana* (Husted) Lange-Bertalot et al.; 2, *Nitzschia Pusilla* (Kützing) Grunow; 3, *H. Amphioxys*; 4, *Mayamaea* sp.; 5, bacteria. The scale bar is 10 µm (if not indicated otherwise). Photo credit: Carlos E. Wetzel (A, D and E), Jean-Luc Biagi (B and F–I).

Element	EDS 1 (%)	EDS 2 (%)
С	22.7	12.5
N	3.0	
0	36.2	24.5
Mg	0.4	0.8
A	3.4	11.0
Si	24.9	26.3
Р	0.4	0.3
S	0.4	0.3
К	3.2	6.1
Ca	1.4	2.9
Fe	4.0	5.5
Ti		0.8

 TABLE 4.2: ELEMENT DISTRIBUTION FOR TWO EXAMPLE POINTS.
 WEIGHT PERCENTAGES ARE DERIVED FROM THE SCANNING

 ELECTRON ANALYSIS INDICATED IN FIGURE 4.1C.
 THE WEIGHT PERCENTAGE OF AN ELEMENT IS THE WEIGHT OF THAT ELEMENT

 MEASURED IN THE SAMPLE DIVIDED BY THE WEIGHT OF ALL ELEMENTS IN THE SAMPLE MULTIPLIED BY 100.
 100.

4.3.2 CONTROLLING ENVIRONMENTAL VARIABLES

Chlorophyll a (cf. primary production, see Table 4.1) had the highest average concentration over the sampling campaign ($12.7 \pm 13.5 \mu g L^{-1}$), followed by lutein (cf. green algae) and fucoxanthin (cf. diatoms), with concentrations of $5.7 \pm 10.0 \mu g L^{-1}$ and $5.0 \pm 8.2 \mu g L^{-1}$, respectively. We determined the lowest average concentrations in zeaxanthin (cf. cyanobacteria; $1.1 \pm 1.5 \mu g L^{-1}$) and diatoxanthin ($0.6 \pm 0.3 \mu g L^{-1}$). However, the differences between the concentrations of fucoxanthin and lutein were not significant when considering the whole dataset (Wilcoxon test, P = 0.089), whereas the concentrations of zeaxanthin were significantly different for both fucoxanthin and lutein (P < 0.001). This finding is confirmed when comparing the different habitat types with each other (Figure 4.2A). In addition, the figure shows that the type of habitat does not have an influence on those three algal groups (Kruskal-Wallis, df = 4, P > 0.05). However, diatom biovolume significantly differs between the different habitats with disturbed areas having generally smaller communities than undisturbed habitats (ANOVA, df = 4, P < 0.001) (Figure 4.2B).

Results of the correlation analyses showed that the environmental descriptors, physico-chemical and meteorological variables were not correlated between each other (R^2 not higher than 0.5 for all the variables). DOC and TN ($R^2 = 0.58$) and EC and moisture ($R^2 = 0.91$) were correlated. Furthermore, most of the meteorological variables were correlated with each other ($R^2 > 0.5$ or < -0.5) and as a result, only radiation and SD of the meteorological variables were included in the RDA analysis, while K and EC were removed. Concerning the different pigments, fucoxanthin was correlated with diadinoxanthin ($R^2 = 0.64$), while lutein was positively correlated with zeaxanthin ($R^2 = 0.59$) and with total chlorophyll a ($R^2 = 0.58$).



FIGURE 4.2: IMPACT OF HABITAT TYPE ON ALGAL CONCENTRATIONS AND DIATOM BIOVOLUME. A, CONCENTRATION OF DIATOMS, CYANOBACTERIA AND GREEN ALGAE. B, COMPARISON OF DIATOM BIOVOLUMES BETWEEN HABITAT TYPES. BIOVOLUME IS CALCULATED ACCORDING TO RIMET AND BOUCHEZ (2012). AF, AGRICULTURAL FIELD; GG, GRAZED GRASSLAND; AG, AGRICULTURAL GRASSLAND; UG, UNDISTURBED GRASSLAND; F, FOREST. NOT OVERLAPPING NOTCHES INDICATE THAT THE MEDIANS DIFFER (CHAMBERS ET AL., 1983).

There was no indication of spatial autocorrelation in the pigment data according to the Mantel tests (P > 0.05). The total variation explained by the RDA was 40%. The first two RDA axes covered respectively 40 and 5% of the total explained variation. However, only the first axis resulted as significant after permutation test (P = 0.001 and P = 0.158 respectively). In the 2D ordination, fucoxanthin and lutein are mainly related to the first axis, which is determined by radiation, TN and SD, while the other carotenoids follow more the second axis, which is correlated with soil moisture content (Figure 4.3). Of those mentioned variables, most exhibit a strong seasonal gradient (e.g. radiation, soil moisture and SD). The Venn diagram (Figure 4.4) shows that meteorological variables explain in total (including joint effects) 23.7% of the total variation (F = 3.80, df = 7, P = 0.02), whereas the site characteristic variables and soil chemical factors explain respectively 13.3 (F = 2.88, df = 5, P = 0.01) and 9.1% (F = 2.57, df = 11, P = 0.027). However, only 37.7% of the variation could be explained. Furthermore, multiple regression analysis revealed that diatom productivity (log-transformed) has a strong seasonal gradient with radiation, SD, and vegetation height all significant (ANOVA, F > 8.73, P < 0.05), whereas pH is an important factor (F = 31.49, P < 0.001) in creating a spatial gradient. Besides, diatom productivity is positively correlated with pH and vegetation height, whereas a negative relation with radiation and SD exist.

Chapter 4: Controls on productivity



FIGURE 4.3: RESULTS OF REDUNDANCY ANALYSIS (RDA) ON THE RELATION BETWEEN ENVIRONMENTAL VARIABLES AND PIGMENT DATA. PIGMENT DATA IS HELLINGER TRANSFORMED. PIGMENTS: FX, FUCOXANTHIN; LU, LUTEIN; ZE, ZEAXANTHIN; DD, DIADINOXANTHIN; DA, DIATOXANTHIN. VARIABLES: DOC, DISSOLVED ORGANIC CARBON; SD, STORAGE DEFICIT; TN, TOTAL NITROGEN.

4.3.3 SPATIAL AND TEMPORAL VARIABILITY PIGMENT CONCENTRATIONS AND DIATOM BIOVOLUME

Diatom abundance shows a strong seasonal variability with a peak in February and March (Figure 4.5B), which occurred after the two wettest months and coincides with the coldest period of the sampling campaign. In the subsequent spring and summer period (April–September), abundances are low with an average of 3.9 μ g L⁻¹ (± 3.8 μ g L⁻¹) per month. A slight increase is again visible in November. In contrast to the diatoms, green algae show less seasonality and their concentrations remain relatively high throughout the whole campaign. Furthermore, chlorophyll a reaches its highest concentrations in February and March following the pattern of fucoxanthin (Figure 4.5C). Also, a peak occurred in June reaching on average 24.0 μ g L⁻¹ while experiencing a precipitation of 90.8 mm that month. The lowest average chlorophyll a concentrations were measured at the beginning of the campaign in December and January (2.7 μ g L⁻¹ and 4.1 μ g L⁻¹ respectively).



FIGURE 4.4: VARIATION PARTITIONING OF THE PIGMENT COMPOSITION DATA. GRAPH IS REPRESENTED AS A VENN DIAGRAM, INDICATING THREE GROUPS OF VARIABLES (SOIL CHEMISTRY, SITE CHARACTERISTICS AND METEOROLOGY) WITH THEIR PERCENTAGES OF EXPLAINED VARIANCE. THE DIAGRAM SHOWS THE PARTITIONING OF THE EXPLAINED VARIANCE INTO SEVEN COMPONENTS. A, PARTIAL EFFECTS OF THE SOIL CHEMISTRY; B, PARTIAL EFFECTS OF THE SITE CHARACTERISTICS; C, PARTIAL EFFECTS OF METEOROLOGY; D, PARTIAL JOINT EFFECTS OF SOIL CHEMISTRY AND SITE CHARACTERISTICS; E, PARTIAL JOINT EFFECTS OF SOIL CHEMISTRY AND METEOROLOGY; F, PARTIAL JOINT EFFECTS OF SITE CHARACTERISTICS AND METEOROLOGY; G, PARTIAL JOINT EFFECTS OF THE THREE GROUPS. NUMBERS OUTSIDE THE CIRCLES STAND FOR THE TOTAL VARIANCE (IN PERCENT) EXPLAINED BY EACH VARIABLE (INCLUDING ALL JOINT EFFECTS). METEOROLOGY INCLUDES AIR TEMPERATURE, NUMBER OF FREEZING DAYS, PRECIPITATION, RADIATION, RELATIVE HUMIDITY, WIND SPEED AND PET. SOIL CHEMISTRY INCLUDES NA, MN, FE, PH, SI, P, DOC, TN, K, AL, FE AND NA. SITE CHARACTERISTICS INCLUDES VEGETATION HEIGHT, SOIL MOISTURE CONTENT, SITE, TYPE OF HABITAT AND LEVEL OF DISTURBANCE.

Since larger diatoms generally contain more pigments, the diatom biovolume was calculated to check if the temporal variability of the fucoxanthin concentration was influenced by their cell size. Opposed to the fucoxanthin concentration, the diatom biovolume did not change significantly during the year (ANOVA, F = 2.29, df = 11, P > 0.05) (Figure 4.5C), meaning that the temporal variability in the fucoxanthin concentration is not related to the absolute size of the diatom community. Finally, in Figure 4.5D the ratio between the photoprotective carotenoids in diatoms (i.e. diadinoxanthin and diatoxanthin) and the light harvesting pigment fucoxanthin is given. This ratio generally follows the pattern in radiation/temperature levels during the year. In summer, diatoms sometimes had twice the amount of photoprotective pigments than fucoxanthin during summertime, whereas in winter the average ratio was less than or equal to one in the first three months of study campaign (Kruskal-Wallis, df = 11, P < 0.001).



FIGURE 4.5: TEMPORAL DYNAMICS OF ATMOSPHERIC CONDITIONS AND DIATOM CHARACTERISTICS DURING THE SAMPLING CAMPAIGN. A, DAILY PRECIPITATION (BLUE BARS) AND AIR TEMPERATURE (RED LINE) FLUCTUATIONS DURING THE SAMPLING CAMPAIGN (1 DECEMBER 2017–30 NOVEMBER 2018) RETRIEVED FROM USELDANGE WEATHER STATION (LUXEMBOURG); B, TEMPORAL VARIATION OF GREEN ALGAE AND DIATOM BUOVALUME. DIATOM BIOVOLUME HAS BEEN CALCULATED ACCORDING TO RIMET AND BOUCHEZ (2012). D, TEMPORAL VARIABILITY OF THE RATIO BETWEEN THE PHOTOPROTECTIVE (DA + DD) AND LIGHT-HARVESTING (FX) PIGMENTS OF DIATOMS.

4.4 DISCUSSION

4.4.1 SOIL MICROBIAL COMMUNITY

The SEM image of the soil surface showed a diverse microbial community. Both diatoms and (cyano)bacteria were present. Surprisingly, green algae seemed absent. Generally, green algae are an important part of the soil algal community (Rindi, 2011; Starks et al., 1981), which our pigment data also confirms. However, for that agricultural grassland site our data indicated that usually diatoms are more abundant than green algae during the period the sample was taken (i.e. November). Besides, species of the algal classes Xanthophyta, Rodophyta and Euglenophyta could also be present on soils. However, they generally occur in low abundances. Therefore, we did not expect to see them on the picture. Despite that pigment concentrations could be a good estimate for algal abundances, the amount of each pigment per algal cell is also dependent on its size (i.e. larger cells have a higher amount of each pigment). Overall, terrestrial diatoms and green algae have similar cell sizes (Ress, 2012; Rindi, 2011), whereas cyanobacteria have on average smaller dimensions. Therefore, the pigment signal is lower for the same number of living cells. This could explain why there are (cyano)bacteria present whilst having a lower pigment concentration than green algae. Thus, for pigment analysis, size does matter and this makes it difficult to compare pigment data with algal counts.

4.4.2 SPATIAL AND TEMPORAL VARIABILITY IN RELATION TO ENVIRONMENTAL FACTORS

In this study, we analysed 25 environmental variables and related them to the different concentrations of fucoxanthin over the entire 12-month study period. Of those 25, we found that pH, radiation, SD and height of the lower vegetation were significant in explaining 45% of the variability in diatom productivity. The relation with pH was positive, suggesting that diatoms prefer higher pH values. This is in agreement with an earlier work by Hoffmann (1989), indicating that most diatoms reach their highest abundances on neutral to alkaline soils. However, our maximum soil pH extended only to 8.11, meaning that we were unable to conclude on an optimum occurring or not. Regarding radiation and SD, both variables can serve as a proxy for soil moisture availability. The negative relation between those factors and fucoxanthin concentration was therefore expected, since water, which is often a limiting factor, is essential for diatom survival and reproduction (Camburn, 1982; Van de Vijver et al., 2002). We also noticed that SD was a better factor for explaining the temporal and spatial variability in the concentration of fucoxanthin than precipitation and soil moisture content. This is probably because it takes both variables into account. In this case, soil moisture was a point measurement and does not give any information of the antecedent conditions, while precipitation (average of seven days prior to sampling day) does not inform on how much

water is still prevailing in the upper soil layers. Finally, we found a positive relation between vegetation height and diatom abundances. This could indicate that the capacity of a higher vegetation to better keep moisture and protect against UV-radiation is important for diatom growth — particularly in warm and dry conditions (Ress, 2012; Zhang et al., 2013). Despite having analysed many different environmental factors, we were still not able to explain a large part of the variation.

We observed maxima in the chlorophyll a concentration in winter and in June, following month(s) of high precipitation. Stokes (1940) reported the same observation and considered the antecedent moisture conditions as responsible for the high abundances in summer. Regarding the peak during winter, he considered snow cover as being important for algal growth and conjectured that a blanket of snow keeps the soil moist and warmer than the surrounding atmosphere — creating favourable conditions for algal development. However, the results of Davey (1991) and Grondin and Johansen (1995) refute the latter, since the disappearance of snow led instead to a steep increase in the algal concentration. Although we encountered snowfall during February and March, the snow layer did not prevail for long. Thus, our winter peak could be rather the result of changes between the presence and absence of snow cover, so that soil algae at one point in time are protected against harsh winter conditions and at another point, they are able to maintain photosynthesis. An additional reason for the winter peak could be that under those low light conditions soil algae maximizes their chlorophyll a content to optimize their photosynthetic activity and decrease their chlorophyll a content under high-irradiance conditions in summer (Bohne and Linden, 2002; Neidhardt et al., 1998; Quesada and Vincent, 1993). Indeed, according to Kuczynska et al. (2015) those changes are usually fast since they are engaged in basic processes (e.g. photosynthesis and photoprotection), which are essential for cell life. Thus, seasonality in chlorophyll a concentration is probably the result of the algal growth in combination with their physiological state. In addition, chlorophyll a comprises different algal groups which could respond in a different way to varying environments.

Indeed, diatoms and green algae reacted differently to seasonal conditions. While diatoms exhibited very high abundances in winter and lower abundances during summer, the abundances of green algae remained high throughout the sampling period. This indicates that green algae are more tolerant to dry and warm environmental conditions than diatoms. The reasons for this could lie in the mucilage production and the cell aggregation ability (e.g. forming filaments) of soil green algae. It is known that for green algae the extracellular matrix is well resistant against periods of desiccation and could contain a considerable amount of moisture (up to 97% of the total weight) (Boney, 1980; Rindi, 2011; Shephard, 1987). Furthermore, by forming filaments, they support a high self-protection against water loss

(Karsten and Holzinger, 2014). Although many soil diatom species have developed adaptations to fluctuating moisture availabilities in terrestrial habitats (including the production of a mucopolysaccharide matrix), the adaptations made by terrestrial green algae seem to be more efficient (Ress, 2012; Shephard, 1987).

Despite the fact that diatom abundances are strongly related to moisture availability, the latter obviously does not affect the diversity and composition of their assemblages. Johansen (1993) observed high diatom diversity in arid environments, whereas, in a related study by Foets et al. (2020a), diatom composition and diversity did not change significantly during the year. In addition, the latter also showed that diatom communities have a strong spatial component and are controlled by the amount of anthropic disturbance with larger species often being more tolerant (cf. H. amphioxys, Pinnularia borealis Ehrenberg). Such observations were also visible in the results of our biovolume calculations. However, there were no spatial differences in fucoxanthin concentrations, meaning that overall less living diatom cells are present in disturbed compared to undisturbed areas. Conversely, the temporal variation in the measured fucoxanthin concentration is not influenced by the diatom biovolume, since the results of the biovolume did not show any temporal variation. Additionally, like chlorophyll, fucoxanthin has an important function in light-harvesting and its concentration is therefore affected in the same way as chlorophyll by different irradiance levels (Kuczynska et al., 2015; Xia et al., 2013). Because of that, we would expect the differences in the number of living diatom cells between the summer and winter period to be smaller than what we would expect from the results derived from the pigment analysis. Besides the fact that the size and physiological state of diatoms are important for pigment concentration, we could conclude that the distribution of diatom communities is mainly controlled by spatial factors related to the amount of disturbance and that diatom abundances are generally linked to temporal factors related to (soil) moisture availability.

4.4.3 RECOMMENDATIONS AND PERSPECTIVES

Pigment analyses proved to be a useful tool in identifying microbial communities and deriving their abundances (Leavitt and Hodgson, 2001). Here, we applied the same algal extraction method as described in Barragán et al. (2018) for diatom community analysis. However, it is recommended by the authors that only samples of bare soil or with only low presence of grass or other vegetation are taken. This is because dense vegetation does not allow a proper rinsing of the soil substrate and subsequently hinders the detachment of algal communities. As we followed this recommendation, we believe that we equally extracted the different algal groups. Furthermore, we used average diatom biovolume measurements taken from aquatic specimens (including terrestrial species occurring in those habitats). However,

previous studies of Ress (2012), Stanek-Tarkowska et al. (2013) and Van de Vijver and Beyens (1997) all reported that cell sizes could be significantly different (both larger and smaller) between terrestrial and aquatic habitats. We did not incorporate this, but as those changes are not unidirectional, we believe that there would only be a minimal effect on some of the results. Besides, we were not able to compare our pigment concentrations with other studies, since many different preparation, extraction and analytical methods are used and subsequently pigment concentrations are expressed in different units (e.g. μ g L⁻¹, mg cm⁻², mg g⁻¹ soil) (Cartaxana and Brotas, 2003; Kuczynska et al., 2015; Leavitt and Hodgson, 2001; Schagerl and Künzl, 2007).

Moreover, only few studies exist on the ecology of terrestrial diatoms compared to aquatic diatoms. This is rather surprising, since terrestrial diatoms and other algae are arguably affected more directly than aquatic algae by climatic changes and can be expected to respond in an immediate way (Ress, 2012; Rindi, 2011; Souffreau et al., 2013a). Therefore, future research should give more attention to terrestrial microbial communities, certainly in the light of climate change.

4.5 CONCLUSIONS

In this study, we investigated the temporal and spatial variability of soil diatom abundances and compared them with the abundances of green algae and cyanobacteria. The abundances were derived from pigment analysis using HPLC. Our results supported our first hypothesis that diatom abundances show seasonal succession and that their abundances are mainly controlled by factors influencing the (soil) moisture availability (e.g. radiation and storage deficit). However, our second hypothesis was rejected, since the effect of habitat type, which played a key role in shaping the diatom communities (Foets et al., 2020a) and subsequently their biovolume, was not seen in the pigment concentrations. This led to the conclusion that overall less diatom cells are present in disturbed than in undisturbed habitats. Contrary to diatoms, green algal productivity remained stable over the course of the study period. Also, we observed that diatoms could have higher abundances than green algae and cyanobacteria at periods of the year with high soil moisture. Concerning future studies, more focus should be on the ecology of terrestrial diatoms and other algae, since they are affected more directly than aquatic algae by climatological conditions (Hinder et al., 2012; Rindi, 2011).

CHAPTER 5

AUTECOLOGY OF TERRESTRIAL DIATOMS UNDER ANTHROPIC DISTURBANCE AND ACROSS CLIMATE ZONES

ABSTRACT

Like aquatic diatoms, terrestrial diatoms are sensitive to multiple environmental factors such as pH, anthropic disturbances, soil moisture and nitrogen. While most aquatic diatom species have well-known autecological values for several important variables, qualifying them as biological indicators, this potential has not yet been untapped for terrestrial diatoms. Here, we aim to establish autecological values for pH and soil moisture content using weighted averaging for common, widespread terrestrial diatoms and compare them with previous calculated indicator values and tolerance ranges. In order to provide robust autecological values, we combined the data of ecological studies carried out across a range of climate conditions. We found that our validation metrics improved considerably after removing samples collected in anthropic disturbed habitats, suggesting that anthropic disturbance is the principal factor defining taxon occurrences. Besides our results showing similarities with previous studies, they also showed a significant improvement on the existing indicator values. Moreover, we expanded the list of terrestrial diatoms to 249 taxa that may serve as environmental markers in different research fields.

The chapter is based on: Foets, J., Stanek-Tarkowska, J., Teuling, A.J., Van de Vijver, B., Wetzel, C.E., Pfister, L., 2020. Autecology of terrestrial diatoms under anthropic disturbance and across climate zones. Ecological Indicators 122. doi:10.1016/j.ecolind.2020.107248

5

5.1 INTRODUCTION

Diatoms are a group of microscopic, single-celled algae living in almost all moist and aquatic environments with sufficient light (Dixit et al., 1992). Most species show very specific preferences for a broad range of environmental factors such as pH, nutrients and salinity, making them one of the most commonly used bioindicators for water quality assessment and paleolimnological analysis (Smol et al., 2001; Smol and Stoermer, 2010). In order to infer degradation levels of water bodies or reconstruct past environmental conditions, autecological indicator values are regularly established (Carayon et al., 2019; Van de Vijver et al., 2002). These values are generally derived from weighted averaging; a simple, reliable and extensively used technique for estimating taxa indicator values, which assumes that taxa abundance follows a unimodal relationship with a given environmental variable. Indicator values are the basis of some widely used diatom indices such as the IPS (specific Pollution-Sensitivity Index; Cemagref, 1982) and BDI (Biological Diatom Index; Lenoir and Coste, 1996). Also, they have been gathered in synthetic trait matrices to be used for other types of ecological diagnosis (Carayon et al., 2020; Taylor et al., 2007; van Dam et al., 1994). Overall, the calculation of autecological values for diatoms has proven to be a very useful tool in many aspects of water quality research (Poikane et al., 2020).

While aquatic diatoms are commonly studied and ecologically well characterized, mainly due to their general use in water quality monitoring programmes, studies on limnoterrestrial diatom communities (i.e. here used as diatom assemblages that can be found on soils) are rather scarce. However, ecological studies showed that diatom communities on soils are also quite responsive to several environmental variables such as soil moisture and pH (Antonelli et al., 2017; Lund, 1945; Van de Vijver et al., 2002; Van de Vijver and Beyens, 1998; Van Kerckvoorde et al., 2000). As a result, Van de Vijver et al. (2002), who investigated 106 soil diatom samples on Île de la Possession (Crozet, sub-Antarctica), could establish optimum values of soil moisture for the most common taxa in their dataset occurring there. Also, Lund (1945), who sampled 66 different soils in the UK, was able to determine tolerance ranges of pH for 24 taxa. In addition to soil moisture and pH, disturbances caused by farming practices also play a key role in structuring terrestrial diatom assemblages (Antonelli et al., 2017; Foets et al., 2020a; Heger et al., 2012; Stanek-Tarkowska and Noga, 2012; Vacht et al., 2014). Foets et al. (2020a) found that disturbed areas were less diverse, and that land uses with different disturbance levels could be differentiated solely based on the community composition. They also noticed that the species' composition remains stable throughout the year meaning that variation in soil moisture availability, irradiance and temperature does not play a significant role. Besides the direct influence of these disturbances on diatoms, they indirectly affect other variables as well such as organic matter, nitrogen and carbon content in the soil, which in turn

also impact diatoms (Stanek-Tarkowska et al., 2018). Both nitrogen and carbon are often found as significant explanatory variables for the diatom species' composition (Antonelli et al., 2017; Vacht et al., 2014), while organic matter increases the moisture holding capacity of the soil and acts as a buffer against dryness (Stanek-Tarkowska et al., 2018; Stanek-Tarkowska and Noga, 2012). Thus, terrestrial diatoms are sensitive to several environmental variables, but so far, most studies focused on the communities' structure and not on the species preferences.

Pending more ecological knowledge on terrestrial diatoms, several studies explored their potential as environmental markers. Many of them tried to explain the occurrence of diatoms on soils based on the ordinal classifications created by van Dam et al. (1994) (see for instance Antonelli et al., 2017; Stanek-Tarkowska et al., 2013; Stanek-Tarkowska and Noga, 2012). Despite that these classifications are based on data acquired from aquatic samples in the Netherlands (and ecological values reported in the published literature), they are nevertheless frequently used in many European countries as a reference for autecological studies. However, for numerous terrestrial taxa, similar values are lacking, as they only occur sporadically in aquatic environments, and assigned values may not really reflect their behaviour in true terrestrial conditions (Barragán et al., 2018). There is a pressing need for assigning autecological values to terrestrial diatoms, reflecting their preferences in terrestrial environments and which would enable us to unlock their potential as environmental markers.

Despite the worldwide application of some diatom-based indices (e.g. IPS), there is nowadays a tendency to develop indices and indicator values specific to certain regions, since these values would better reflect environmental conditions in that area (Carayon et al., 2020; Lavoie et al., 2009). Unfortunately, data on terrestrial diatom ecology remains scarce and developing an index or defining indicator values at the local scale is at present still not possible. Therefore, we aim to provide robust autecological values for common diatom taxa living on soils based on data of several ecological studies carried out across a range of climate and geographical conditions. We will analyse several environmental variables, which previously showed to influence diatom communities living on soils. This shall enable us not only to use them later as environmental indicators, but also to expand the existing toolbox of environmental markers applied in for example hydrology and soil science.

5.2 MATERIAL AND METHODS

5.2.1 DESCRIPTION OF THE DATA

In this study, the terrestrial diatom community and environmental data were gathered from the studies of Antonelli et al. (2017), Barragán et al. (2018), Foets et al. (2020a, b),

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Stanek-Tarkowska et al. (2015, 2018), Stanek-Tarkowska and Noga (2012), Van de Vijver et al. (2002) and Van Kerckvoorde et al. (2000). The data covers four distinct regions and three different climate types (Figure 5.1).



FIGURE 5.1: LOCATIONS OF THE SAMPLING SITES WITH INDICATION OF THEIR CLIMATIC REGION ACCORDING TO THE KÖPPEN-GEIGER CLASSIFICATION (BECK ET AL., 2018). A, LUXEMBOURG; B, POLAND; C, ÎLE DE LA POSSESSION (ÎLES CROZET, SUB-ANTARCTICA); D, ZACKENBERG (GREENLAND). SOURCE: ESRI, GLOBAL MAPPING INTERNATIONAL, U.S. CENTRAL INTELLIGENCE AGENCY (THE WORLD FACTBOOK).

The studies of Foets et al. (2020a, b) were conducted in the Attert River basin in Luxembourg. Terrestrial diatom samples were taken at the soil surface according to the method described by Barragán et al. (2018) in 16 sites around the catchment every 3–5 weeks for a period of 14 months (October 2017 to November 2018) totalizing 206 samples that were included in the present study. Subsequently, the same soil samples were pooled and used for pH and nutrient analysis. Of the several environmental variables included in that study, only pH measured in 1:5 H₂O, volumetric soil moisture content (VWC, expressed in percent) measured *in situ* (30 times around the sampling area), and the bioavailable fraction of total nitrogen (TN), phosphorus (P) and dissolved organic carbon (DOC) (both in mg L⁻¹ soil extraction) were used. The latter two were analysed with ICP-OES after a 0.01M CaCl₂ extraction following Houba et al. (2000). Furthermore, they incorporated five different types of

land use (forest, undisturbed grassland, grassland disturbed by cattle grazing, grassland disturbed by agriculture and agricultural fields).

Similar to Foets et al. (2020a, b), the study of Antonelli et al. (2017) was also carried out in the Attert River basin from August 2014 to March 2015. Diatoms were sampled as in Foets et al. (2020a) at 34 locations during three sampling campaigns totalling 92 samples. Topsoil samples for physico-chemical analysis were collected simultaneously with a shovel. Contrary to Foets et al. (2020a), only three land use types were distinguished. However, based on photographs and additional information on the sampling areas, it was possible to adjust the different land uses according to their classification. Besides, soil moisture was measured gravimetrically (in percent) rather than volumetrically, pH in H₂O, KCl and CaCl and C, H and N (all expressed in percent) using a CHN analyser. Of those variables, pH_{H2O} , N and C were included. While pH is merged with the results of Foets et al. (2020a, b), N and C are regarded as different variables.

Barragán et al. (2018) sampled four locations in the Attert basin comprising four different anthropogenic pressures. At each location, they collected 10 soil samples for diatom analysis following the method described in Coles et al. (2016) and determined soil moisture volumetrically. Since soil moisture measurements were done as in Foets et al. (2020b), both variables were aggregated. Also, for this study, photographs and information on the locations were provided and enabled us to classify the different land uses.

In Stanek-Tarkowska and Noga (2012), two agricultural fields under different tillage systems, located in the Subcarpathian region (SE Poland), were sampled once. The samples were collected at a depth of 0–3 cm and put into petri dishes. In that study, some samples were also cultured, but these were excluded here. Besides, they also collected soil samples from the surface layer (0–10 cm) of which they measured pH in 1:2.5 KCl. These values were converted to pH 1:5 H_2O following the Equation (5.1) provided in Kabała et al. (2016) before aggregating the data.

$pH_{H201:5} = -1.95 + 11.58 * log_{10}(pH_{KCl1:2.5})(5.1)$

The study of Stanek-Tarkowska et al. (2015) was also conducted in Poland. There, one site located in Pogórska Wola was sampled for nine consecutive months in 2011. Soil samples for diatom analyses were collected from a 0–3 cm deep layer and placed in petri dishes (three replicates), whereas samples for physico-chemical analysis were taken from the topsoil layer (0–5 cm) at the same time. pH was measured in a 1:2.5 KCl solution and was converted following Equation 5.1 before data merging.

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Stanek-Tarkowska et al. (2018) collected monthly samples from April to November for four consecutive years (2013–2016) at two agricultural fields near Rzeszów (Poland) (n = 60). There, two replicates for diatom analyses were taken from the 0–5 cm soil layer and placed in petri dishes, while undisturbed soil samples were taken with 100 cm³ cylinders for physico-chemical analysis. pH was determined as in Foets et al. (2020a, b), while gravimetric soil moisture content was converted to volumetric moisture content using soil bulk density. Both variables were used in our study without further adjustments.

The dataset of Van Kerckvoorde et al. (2000) included 30 diatom samples originating from 30 different sites in the Zackenberg area (NE Greenland) covering an area of approximately 4 km². For those samples, the upper 3 cm of the soils was collected. In addition to diatom community data, soil moisture and pH were measured similar to Foets et al. (2020b), while SOM was derived from LOI (Loss-On-Ignition). All three variables were included without modification in the final dataset.

Finally, Van de Vijver et al. (2002) analysed 106 samples collected on the sub-Antarctic Île de la Possession (Îles Crozet). Of those, 29 originated from permanently wet areas or moistened rocks (i.e. moisture content > 75 %) and were excluded from the final dataset, as they did not reflect the true terrestrial soil conditions as is the case in the other studies. In the sub-Antarctic study, several environmental variables were assessed, but only pH 1:5 H₂O and VWC were incorporated, because the other variables were measured in a different way and could therefore not be converted.

After merging the community datasets, the entire dataset was made taxonomically consistent by updating species names to the most recent diatom taxonomy, putting synonyms together and assigning four-letter codes to each taxon according to the latest Omnidia version (March 2019, Lecointe et al. 1993). Since identification of terrestrial diatoms is often sparse in the literature, several publications were used, including Ettl and Gärtner (1995), Krammer (2000), Lange-Bertalot (2001), Lange-Bertalot et al. (2011, 2017), Levkov et al. (2013) and Lund (1946), along with some essential studies on terrestrial algae (Brendemühl, 1949; Hustedt, 1941; Petersen, 1915, 1928, 1935) and some recent studies on terrestrial diatoms (Reichardt, 2008, 2012; Wetzel et al., 2015). Available diatom pictures from the different studies were used to make the datasets taxonomic consistent as much as possible. Taxon names were kept in the broad sense (i.e. *sensu lato*) as our aim was to give general autecological values. This resulted in a final dataset including 516 soil diatom samples across 166 different sites, covering 710 taxa (including varieties, subspecies and forms), and the following variables: pH, VWC, TN, DOC, N, C and SOM. Table 5.1 provides an overview of all data.

TABLE 5.1: OVERVIEW OF THE DIFFERENT DATASETS AND VARIABLES INCLUDED IN THIS STUDY. THE NUMBER OF CELLS PER SAMPLE IS THE MINIMUM NUMBER OF DIATOM CELLS THAT WAS TARGETED IN THE CORRESPONDING STUDY. HOWEVER, SOMETIMES THIS NUMBER COULD NOT BE REACHED DUE TO A LOW PRESENCE OF DIATOMS IN THE SAMPLES. CONVERTED OR MODIFIED FACTORS ARE INDICATED BY ^(**).

Dataset	Location	No. of samples	No. of diatom cells/sample	Variables used in this study
Van de Vijver et al. (2002)	Crozet, sub- Antarctica	77 (106×1)	500	pH, VWC
Van Kerckvoorde et al. (2000)	Zackenberg, Greenland	30 (30×1)	500	pH, VWC, SOM
Foets et al. (2020a, b)	Attert basin, Luxembourg	206 (16×14)	200	pH, VWC, TN, P, DOC, land use
Antonelli et al. (2017)	Attert basin, Luxembourg	92 (34×3)	200	pH, C, N, land use*
Barragán et al. (2018)	Attert basin, Luxembourg	40 (4×10)	400	VWC, land use*
Stanek-Tarkowska and Noga (2012)	Dabrowa, Poland	2 (2×1)	400	pH*
Stanek-Tarkowska et al. (2015)	Pogórska Wola, Poland	9 (1×9)	400	pH*
Stanek-Tarkowska et al. (2018) ¹	Rzeszów, Poland	60 (2×30)	400	pH, soil moisture

¹, STUDY ONLY INCLUDED THE MOST ABUNDANT TAXA. THEREFORE, IT WAS ONLY USED FOR THE CALCULATION OF THE AUTECOLOGICAL VALUES.

5.2.2 STATISTICS

The Shannon-Wiener diversity and species richness were calculated for each sample. However, the communities given in Stanek-Tarkowska et al. (2018) were excluded from further analysis, as the data only contained the 14 most abundant taxa and not the entire communities. Furthermore, the significance of soil moisture, pH and type of land use in explaining the variation in those two variables was investigated. For this, a generalized mixed model with 'site' as random variable to account for repeated measurements was set up. The Shannon-Wiener index between four different regions (i.e. Luxembourg, Poland, sub-Antarctica and Greenland) and five land use types (i.e. forest, undisturbed grassland, grazed grassland, agricultural grassland and agricultural field) was analysed with Analysis of Variance (ANOVA) and the differences were assessed further with parametric *post hoc* tests. Species rarefaction curves for each region and land use type were established using the function *specaccum* incorporated in the *vegan* R-package (Oksanen et al., 2019).

Next, the community dataset was reduced by removing rare taxa. A taxon was considered 'rare' when it did not occur with a minimum relative abundance of 2.5% in at least five samples (i.e. 0.01% of the samples). The identification of most of those taxa was also uncertain (i.e. sp., cf. or aff.). Then, species autecological values were computed using weighted averaging regression (ter Braak and Looman, 1986). After the calculation of the

ecological indicator values, the inverse algorithm (i.e. WA calibration) was used to build a model predicting value based on floristic data. These two values were then compared and assessed after bootstrap cross-validations (n = 1000) using Root Mean Square Error (RMSE; Wallach and Goffinet, 1989) and correlation coefficients as model validation metrics. The best results (i.e. lowest RMSE and highest r²) were obtained with data that was tolerance downweighted and transformed according to inverse deshrinking models.

Additionally, a training-set containing half of the samples (i.e. all odd samples) for pH and soil moisture was created. Then the goodness-of-fit was assessed by passively fitting the passive samples (i.e. all even samples) into a constrained ordination of the training-set with pH or soil moisture as the sole constraint. The passive samples were positioned as supplementary samples within the ordination space by means of transition equations given in ter Braak and Šmilauer (2002). This determines subsequently a score for each passive sample by taking the weighted average (Canonical Correspondence Analysis, CCA) of the species scores extracted from the ordination of the training-set samples. Hence, the even samples are positioned within the ordination without influencing the underlying ordination solely based on the training set (Birks et al., 2012). CCA as ordination method was chosen, since DCA on the reduced species data revealed a unimodal distribution for the first four axes (S.D. > 4). Next, the distribution of the squared residual distances between each sampling point and its fitted position on the first constrained axis for the training-set was calculated. Any passive sample that has a squared residual fit greater than the 90th percentile distance for the training-set samples is poorly fitted within the calibration function model (Birks et al., 1990). Afterwards, the two sample sets were switched, and the analysis was done again to verify the previous outcome. All previous calculations were done with the residLen function from the analogue Rpackage (Simpson and Oksanen, 2020).

After estimating autecological values (optimum and tolerance) for each taxon, the optimum values of pH and soil moisture were compared with the updated indicator values of the same variables assigned by van Dam et al. (1994). Differences between the two sets of optimum values were tested using ANOVA. In case of significant differences were found, the Tukey HSD test was used to reveal which categories deviated from the others. The normality and homoscedasticity of the model residuals were checked prior to statistical analysis. All aforementioned statistical analyses were performed using the R statistical program (R v. 3.6.3.; http://www.r-project.org/) and additional functions from the R-packages *vegan* (v. 2.5- 6; Oksanen et al., 2019), *analogue* (v. 0.17-4; Simpson and Oksanen, 2020) and *rioja* (v. 0.9-21; Juggings, 2017).

5.3 RESULTS

An overall mean species richness per sample of 21.7 ± 11.1 with a maximum of 81 and a minimum of 2 was observed in the entire dataset (Figure 5.2). From the species rarefaction curves, we observe that communities sampled in undisturbed areas have generally a higher species richness per sample than disturbed sites. A closer look reveals that after approximately 80 samples counted, we should find between 300 and 350 different species in forested sites, which is twice the number found for agricultural fields. Also, the rarefaction curves for the samples collected in Greenland and sub-Antarctica reach a clear asymptote at approximately 250 taxa.

The Shannon diversity values are significantly lower in areas that are highly disturbed by agriculture (AF) (F = 13.74, df = 443, R² = 0.028, P < 0.01), while pastures, undisturbed grassland and forest did not present a significantly different diversity (P > 0.05). Furthermore. we notice that the communities collected in Greenland and Poland have a lower diversity than the ones taken in Luxembourg and sub-Antarctica (F = 11.22, df = 452, R² = 0.063, P < 0.05). It must be noted however, that all samples from the Polish sampling sites used here are coming from anthropic disturbed areas and are therefore comparable to samples from disturbed fields (AF, AG) elsewhere. However, the sites were not assigned to a land use type. since verification material (i.e. pictures and additional notes on the sampling areas) was not available. In addition, we ran a generalized mixed model with 'site' as random variable to check whether differences in species richness and diversity could be explained by land use, soil moisture and/or pH. The first model revealed that the type of land use (P < 0.01) and soil pH (P < 0.05), which was positively correlated, explained 84.5% of species richness, whereas the second one showed that they both explained 62.7% of the species diversity $(P_{\text{landuse}} < 0.05, P_{\text{pH}} < 0.01)$. Soil moisture was not significant in both models (P > 0.05). Although samples are coming from contrasted environments, the results indicate that the species richness and diversity are similar between the samples and are likely driven by the same environmental factors.

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FIGURE 5.2: SHANNON-WIENER AND SPECIES RICHNESS OF THE SAMPLES AND COMPARISON BETWEEN LAND USES AND REGIONS. A, FREQUENCY BAR PLOT OF THE TAXON RICHNESS PER SAMPLE; B, SAMPLE-BASED SPECIES RAREFACTION CURVES PER LAND USE TYPE; C, SHANNON-DIVERSITY PER REGION AND LAND USE TYPE. F, FOREST; UG, UNDISTURBED GRASSLAND; GG, GRAZED GRASSLAND; AG, AGRICULTURAL GRASSLAND; AF, AGRICULTURAL FIELD; PR, PRISTINE (I.E. SUB-ANTARCTICA AND GREENLAND). LUX, LUXEMBOURG; POL, POLAND; ANT, SUB-ANTARCTICA; GREEN, GREENLAND. THESE ANALYSES INCLUDE 456 SAMPLES AND RARE SPECIES.

After using the cut-off criteria of a 2.5% relative abundance in five samples, 248 of the 710 taxa were kept in the statistical analyses, covering 48 genera of which *Pinnularia* (45 taxa), *Nitzschia* (18), *Navicula* (13), *Mayamaea* (13), *Humidophila* (13) and *Eunotia* (12) were the most species-rich genera. The most abundant taxa in the soil dataset are *Hantzschia amphioxys* (Ehrenberg) Grunow (12.0 % of total relative abundance), *Mayamaea atomus* (Kützing) Lange-Bertalot (5.9 %), *M. permitis* (Hustedt) Bruder & Medlin (5.6 %), *Pinnularia obscura* Krasske (5.1 %) and *Nitzschia pusilla* (Kützing) Grunow (4.6 %). Of all the taxa, only *Luticola mutica* s.l. (Kützing) D.G.Mann, species complex *Pinnularia borealis* Ehrenberg (see Pinseel et al., 2019), *P. obscura*, *M. atomus* and *Encyonema silesiacum* (Bleisch) D.G.Mann occurred in all four regions, while *Caloneis lancettula* (Schulz) Lange-Bertalot & Witkowski, *Mayamaea excelsa* (Krasske) Lange-Bertalot, *Stauroneis parathermicola* Lange-Bertalot, *Craticula molestiformis* (Hustedt) Lange-Bertalot and *Humidophila irata* (Krasske) R.L.Lowe et al. were some of the taxa only observed in the samples from Luxembourg. *Achnanthidium*

pyrenaicum Hustedt was only noted in samples from Poland, whereas *Stauroneis thermicola* (J.B.Petersen) J.W.G.Lund and *Sellaphora nana* (Hustedt) Lange-Bertalot et al. occurred only in samples from both Luxembourg and Poland. Typical taxa exclusively found in sub-Antarctica include for instance *Psammothidium aretasii* (Manguin) Lange-Bertalot, *Frankophila maillardii* (Le Cohu) Lange-Bertalot, *Humidophila comperei* (Le Cohu & Van de Vijver) R.L.Lowe et al., *Pinnularia subantarctica* (Manguin) Van de Vijver & Le Cohu, *Achnanthidium sieminskae* Witkowski, Kulikovskiy & Riaux-Gobin and *Humidophila ingeae* (Van de Vijver) R.L.Lowe et al., whereas *Caloneis aerophila* Bock, *Eunotia fallax* Cleve and *Pinnularia lagerstedtii* (Cleve) Cleve-Euler were only present in samples from Greenland.

TABLE 5.2: VALIDATION OF THE WEIGHTED AVERAGE OF DIFFERENT ENVIRONMENTAL VARIABLES. RESULTS OF THE VALIDATION (R² AND RMSE) ARE GIVEN FOR THE DATA THAT WAS TOLERANCE DOWN-WEIGHTED AND INVERSE DESHRINKED. THE REFERENCE BETWEEN BRACKETS GIVES THE STUDY WHERE THE VARIABLE WAS ANALYSED. ALSO, THE NUMBER OF SAMPLES INCLUDED IN THE ANALYSIS AND THEIR RANGE ARE PROVIDED. SOM, SOIL ORGANIC MATTER; VWC, VOLUMETRIC MOISTURE CONTENT; RMSE, ROOT MEAN SQUARE ERROR.

Variable (Study)	n	Range (units)	۲²	RMSE
TN (Foets et al., 2020a, b)	206	0.25 – 53.70 (mg L ⁻¹ soil extraction)	0.16	9.32
DOC (Foets et al., 2020a, b)	206	1.70 – 57.07 (mg L ⁻¹ soil extraction)	0.18	9.20
P (Foets et al., 2020a, b)	206	0 – 0.88 ́ (mg L ^{−1} soil extraction)	0.31	0.16
C/N (Antonelli et al., 2017)	89	4.61 – 29.83	0.07	4.63
SOM (Van Kerckvoorde et al., 2000)	30	9.93 – 79.85 (%)	0.29	17.34
pH (see Table 5.1)	388	4 - 8.45	0.57	0.52
VWC (see Table 5.1)	411	2.27 – 75.5 (%)	0.62	9.61

We calculated weighted averages for TN, P, DOC, C/N, SOM, pH and VWC and validated the calculated with the observed values. Bar the latter two, all other variables are coming from one single study (Table 5.1) and therefore those analyses are restricted to smaller sample sizes. The outcome of the validations show generally (very) low correlation coefficients and relative high RMSE values for TN, C/N, P, DOC and SOM (Table 5.2), while pH and soil moisture reach an overall r² of respectively 0.57 and 0.62 (Figure 5.3). Furthermore, we divided the samples based on the type of land use (anthropic disturbed vs. undisturbed) and ran the same analyses again. For both pH and soil moisture content, we now observed increased correlation coefficients. pH reached 0.68 and had a RMSE of 0.47 compared to 0.56 and 0.44 when only disturbed habitats were considered, whereas for soil moisture a substantial difference of r² was seen between disturbed (0.34) and undisturbed areas (0.69). The RMSE also increased with 0.5 compared to the overall value.

In addition to evaluating the regression between the observed and estimated environmental variables, we also calculated 'goodness-of-fit' statistics from a CCA ordination for pH and soil moisture. Therefore, we subdivided the samples to create two equally large datasets: a training set and a passive sample set. For pH, both sets contained 194 samples, whereas for soil moisture the sets contained respectively 206 and 205 samples. The distribution of the squared residuals for all four sets of samples is shown in Figure 5.4. In the training sets, we observe that for both pH and VWC 10% of the samples were poorly fitted (i.e. outside 90th percentile), whereas those values increased to 16.5 (pH) and 14.6% (VWC) for the passive samples. Similar percentages were found when we switched the sample sets. So, the analysis of the squared residual distances for the passive samples to pH and soil moisture indicates that 82–85% of the samples are well fitted within the unimodal response-model framework and that we can therefore be confident of the estimated autecological values.



FIGURE 5.3: RELATIONSHIPS BETWEEN OBSERVED AND ESTIMATED VALUES USING WEIGHTED AVERAGING. A SEPARATE ANALYSIS HAS BEEN DONE FOR THE DISTURBED (AG, GG, AF) AND UNDISTURBED HABITATS (UG, F, PR). HERE, ONLY TAXA WERE INCLUDED WITH A MINIMUM ABUNDANCE OF 2.5% IN AT LEAST FIVE SAMPLES. REGARDING PH, 202 AND 173 DATA POINTS WERE INCLUDED IN THE VALIDATION OF THE UNDISTURBED AND DISTURBED HABITATS RESPECTIVELY, WHILE THERE WERE 209 AND 142 SAMPLES FOR SOIL MOISTURE.



FIGURE 5.4: DENSITY PLOTS OF THE SQUARED RESIDUAL FIT TO PH AND SOIL MOISTURE. THE RESULTS ARE GIVEN FOR THE TRAINING DATA SET (ODD SAMPLES; UPPER PANELS) AND THE PASSIVE SAMPLES (EVEN SAMPLES; LOWER PANELS) DERIVED FROM PASSIVELY OVERLAYING THE ODD SAMPLES ON TO A CANONICAL CORRESPONDENCE ANALYSIS ORDINATION (CCA) OF THE TRAINING-SET SAMPLES. THE PH TRAINING SET INCLUDES 194 SAMPLES, WHILE THE SET FOR SOIL MOISTURE YIELDS 206 SAMPLES. THE LABELLED DASHED LINES ARE FOR THE 90TH, 95TH, AND 99TH PERCENTILES OF THE DISTRIBUTIONS OF THE TWO SETS OF SQUARED RESIDUAL LENGTHS. SAMPLES LYING BEYOND THE 99TH PERCENTILE ARE EXTREMELY POORLY FITTED TO PH AND SOIL MOISTURE, RESPECTIVELY, THOSE BETWEEN THE 95TH AND 99TH PERCENTILES ARE VERY POORLY FITTED, AND THOSE SAMPLES BETWEEN THE 90TH AND 95TH PERCENTILES ARE VERY POORLY FITTED.

The obtained autecological values were then compared with previous studies of Van de Vijver et al. (2002) and Lund (1945) for respectively volumetric soil moisture content and pH (Table 5.3). Prior to comparing the values, the diatom identifications made by Lund (1946) were checked if his identifications were the same as ours. Lund (1945) unfortunately determined pH using a colorimeter, whereas in our study, an electrometric method was used. Although the outcomes of the two methods should not deviate much more than 0.2 units from each other according to Haines et al. (1983), comparisons should be interpreted with care. Nevertheless, it seems that all ranges for pH overlap well with the values of Lund (1945). However, Tryblionella debilis Arnott has a very small tolerance range (7-7.2) specified by Lund (1945), whereas we calculated a rather broad pH tolerance ranging from 6.0 to 7.8. Also, Humidophila contenta s.I. (Grunow) R.L.Lowe et al. (including Humidophila biceps (Grunow) P.Furey, K.Manoylov & R.L.Lowe) occurs on very acidic soils (pH = 3.9) according to Lund (1945), while this study indicates that both taxa are normally present on soils with a pH between 5.7 and 7 and 5.18 and 6.28, respectively. Furthermore, we see some larger differences between the ranges for soil moisture content. The ranges assigned by Van de Vijver et al. (2002) tend to go more towards higher moisture contents and are generally broader, compared to ours. This is not only the case for common, widespread taxa, but also for taxa such as Humidophila crozetikerguelensis (Le Cohu & Van de Vijver) R.L.Lowe et al.,

H. comperei and *Planothidium aueri* (Krasske) Lange-Bertalot which were only found in the samples of Van de Vijver et al. (2002). However, this did not result in non-overlapping ranges, except for the very common *Hantzschia abundans* Lange-Bertalot. For that species, we found a range between 7.5 and 31%, while the tolerance calculated by Van de Vijver et al. (2002) ranged between 31 and 71%. Bar this exception, the tolerance ranges for both soil moisture and pH overlap well with previous research.

TABLE 5.3: PREVIOUS AND CALCULATED TOLERANCE RANGES. PREVIOUS RANGES ARE BASED ON STUDIES OF VAN DE VIJVER ET AL. (2002) FOR SOIL MOISTURE AND LUND (1945) CONCERNING PH. LUND DETERMINED PH COLORIMETRICALLY, WHILE HERE IT WAS DETERMINED ELECTROMETRICALLY IN A 1:5 WATER SOLUTION. THEREFORE, VALUES MAY DEVIATE A BIT. *, SPECIES ONLY PRESENT IN THE SAMPLES OF VAN DE VIJVER ET AL. (2002).

Taxon	Van de Vijver et al. (2002)	This study	Taxon	Lund (1945)	This study
PBOR	0–75	12.9–43.5	PBOR	4.4-6.0	5.4–6.6
POBS	18–76	11.7–40	NCIH	6.9–7.4	7.1–7.9
ABRY	21–80	13.5–54	SEAT	6.2-7.2	6.0–7.3
HAMP	0–50	10.3–30.7	TDEB	7–7.2	6.0–7.8
MAAT	5–46	17.2–36.6	HMON	6.9-8.2	7.0–8.1
IMUT	19–43	12.3–40.4	IMUT	6.0-8.3	5.6-6.9
HUCO	13–62	8.7–33.4	HUCO (incl.	3 9-8 3	5.7–7, 5.18–6.28
	17–69	13.5–30.3	NPAI	5 9–7 4	6.1–7.6
HABU	31–71	7.5–31.3	PMIC	4.6-6.6	5.1–6.8
DCOP*	0–43	2.2-34.4		1.0 0.0	
HUC7*	20–58	23.9–51.1			
	5–40	10.2–41.2			
PAUE*	20–79	18.8–56.8			

Optima and tolerance values for common on soils occurring diatom taxa with respect to pH and soil moisture are shown in Figure 5.5 and Figure 5.6. There, we observe that some taxa such as *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst, *S. nana* and *Meridion circulare* (Greville) C.A.Agardh are very sensitive to pH. For example, *E. exigua* only occurs in very acidic environments, while *S. nana* is rather restricted to soils with a neutral pH. On the contrary, several species including *H. ingeae*, *Luticola robusta* Van de Vijver, Ledeganck & Beyens, *Frustulia vulgaris* (Thwaites) De Toni, *H. crozetikerguelensis* and *Stauroneis pseudoagrestis* Lange-Bertalot & Werum are rather tolerant to pH and could be present on a wide range of soils. We observe similar patterns for VWC. Certain species such as *Adlafia minuscula* (Grunow) Lange-Bertalot var. *minuscula*, *Adlafia minuscula* var. *muralis* (Grunow) Lange-Bertalot, *E. exigua* and *Chamaepinnularia obsoleta* (Hustedt) C.E.Wetzel & Ector only require a low moisture content to grow and reproduce (i.e. low optimum values), whereas *Pinnularia isselana* Krammer, *Halamphora montana* (Krasske) Levkov,

Nitzschia solgensis Cleve-Euler and *Navicula cincta* var. *heufleri* Grunow need a soil that has a VWC of at least 40 to 50%. Furthermore, for *Humidophila brekkaensis* (J.B.Petersen) R.L.Lowe et al., Mayamaea alcimonica (E.Reichardt) C.E.Wetzel, Barragán & Ector, *Pinnularia perirrorata* Krammer and *Pinnularia angliciformis* Van de Vijver & Beyens it seems that other environmental factors are more important than soil moisture, since they have large tolerance values. On the other hand, taxa such as *Fragilaria pectinalis* Lyngbye, *Nitzschia adamata* Hustedt, *Mayamaea agrestis* (Hustedt) Lange-Bertalot and *P. lagerstedtii* are very sensitive to soil moisture. Interestingly, *H. amphioxys*, the most generally known terrestrial diatom, has also a rather limited moisture range and is generally not present on soils anymore when the moisture content reaches 35 to 40%. Overall, these autecological values will improve our knowledge of the ecology of terrestrial diatoms and enable us to better use these organisms as environmental markers.



FIGURE 5.5: OPTIMUM AND TOLERANCE VALUES OF PH FOR THE DOMINANT SOIL DIATOM TAXA (10% IN 10 SAMPLES). DIATOM TAXA ARE ABBREVIATED WITH A FOUR-LETTER CODE FOLLOWING OMNIDIA. THE DIATOM TAXA WITH THEIR RESPECTIVE OMNIDIA CODES ARE GIVEN IN TABLE A5.1.

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FIGURE 5.6: OPTIMUM AND TOLERANCE VALUES OF VOLUMETRIC SOIL MOISTURE CONTENT FOR THE DOMINANT SOIL DIATOM TAXA (10% IN 10 SAMPLES). DIATOM TAXA ARE ABBREVIATED WITH A FOUR-LETTER CODE FOLLOWING OMNIDIIA. THE DIATOM TAXA WITH THEIR RESPECTIVE OMNIDIA CODES ARE GIVEN IN TABLE A5.1.


FIGURE 5.7: DERIVED AUTECOLOGICAL VALUES FOR PH (A) AND SOIL MOISTURE (B) IN COMPARISON WITH INDICATOR VALUES ASSIGNED BY VAN DAM ET AL. (1994) (UPDATED VERSION). THE WIDTH OF THE BOXES INDICATES THE NUMBER OF SPECIES WITH SMALLER BOXES YIELDING LESS SPECIES. A: 1, PH < 5.5; 2, PH < 7; 3, PH = 7; 4, MAINLY OCCURRING ON PH > 7; 5, EXCLUSIVELY OCCURRING ON PH > 7. B: 1, RARELY OCCURRING OUTSIDE WATER BODIES; 2, SOMETIMES OCCURRING ON WET PLACES; 3, REGULARLY OCCURRING ON WET AND MOIST PLACES; 4, MAINLY OCCURRING ON TERRESTRIAL PLACES; 5, ALMOST EXCLUSIVELY OCCURRING ON TERRESTRIAL PLACES.

Finally, we analysed if our inferred optimum values correspond to the categories for pH and moisture assigned by van Dam et al. (1994) (Figure 5.7). The latter indicates how likely it is that a species will occur in a terrestrial environment and at which prevailing moisture conditions. Concerning pH, we see that our optimum values correspond well with the different van Dam et al. (1994) categories (F = 9.87, df = 115, R² = 0.23, P < 0.001). However, our autecological values indicate that it would be better to take a pH of 6.5 instead of 7 to differentiate the categories 2, 3 and 4 from each other when evaluating soils. Contrary to pH, there was no significant difference of our optimum values for VWC between the different categories (F = 0.35, df = 81, R² = -0.03, P = 0.84). This is interesting, since we expected that taxa categorized as aquatic require a higher threshold for soil moisture than terrestrial taxa to be present on terrestrial habitats. Now it seems that, independent of their assigned category, diatoms generally need a VWC of around 20% to grow and reproduce on soils.

5.4 DISCUSSION

For this research, we combined eight different datasets on terrestrial diatoms coming from four distinct regions, encompassing 516 soil samples from 166 different sites and covering 710 taxa. A first analysis of this data revealed significant relationships of species diversity and richness with pH and anthropic disturbance, while soil moisture was not important. These observations are in line with previous research. Hoffmann (1989) and Lund (1945) noted that diatoms generally prefer neutral to alkaline soils. However, both pointed out that pH is often correlated with other variables (e.g. CaCO₃) and that it will change the nutrient availability in soils, which could also affect diatoms. Regarding different land uses, Barragán et al. (2018) and Foets et al. (2020a) observed that less disturbed areas were more species rich and diversified. The difference between the two extremes (agricultural field and forest) observed

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here was 150 taxa, doubling the number of species occurring on agricultural fields. Even though soil moisture is crucial for diatoms to grow and reproduce, recent studies indicated that soil moisture does not influence the community composition as such (Foets et al., 2020a; Zhang et al., 2020). However, it does play a key role in the absolute diatom abundances (i.e. primary production) (Foets et al., 2020b). Overall, these analyses confirm the main patterns in the variability of diatom communities and reveal a huge difference in species richness between undisturbed and disturbed areas.

In a next step of our analysis, we checked the sensitivity of diatom taxa for pH, VWC, TN, P, DOC and SOM. Despite that these variables all have been documented in having an effect on the community composition (Antonelli et al., 2017; Lund, 1945; Vacht et al., 2014; Van de Vijver et al., 2002; Van Kerckvoorde et al., 2000), we only found that the optimum values assigned for pH and soil moisture gave decent results with r²-values of respectively 0.57 and 0.62. A side note here is that considerably less data has been included in the calculation for TN, P, DOC, SOM and C/N. Thus, adding more samples to the dataset may improve the model metrics for those variables. However, research by Gremmen et al. (2007) and Van de Vijver et al. (2002) on terrestrial diatoms revealed high r²-values of 0.85 and 0.68 for respectively altitude and soil moisture, including less data points than we did. However, these studies only used data coming from anthropogenic undisturbed environments situated on sub-Antarctic islands. This latter factor is also very important, as our validation metrics improved considerably after removing the samples collected in anthropogenic disturbed habitats, whilst reaching values similar to Van de Vijver et al. (2002). This observation indicates that anthropogenic disturbance is perhaps the principal factor defining taxon occurrences. In order to improve the validation metrics for nitrogen, carbon and organic matter, it will be important that, apart from adding data, a significant part of the data should come from anthropogenic undisturbed sites. Only then, we will know if we could get acceptable autecological values for those variables and eventually apply them in future research and management.

After validating the calculated autecological values for pH and soil moisture, we compared them with previous research of Lund (1945) and Van de Vijver et al. (2002). Although pH was measured differently, the ranges seem to overlap well and there is no real indication that the ones established by Lund (1945) are constantly more acidic or alkali than ours suggesting that the outcome of the two methods does indeed not seem to diverge much from each other. We noticed that the tolerance ranges for soil moisture calculated by Van de Vijver et al. (2002) tend to go more towards higher values and are generally broader. This is probably due to the selection and removal of the data, since the same observation is done for taxa exclusively occurring in sub-Antarctic samples. While Van de Vijver et al. (2002) included

diatoms found on soils ranging between 0 and 100% saturation, we only selected sites with a maximum saturation of 75.5%. In addition, our samples generally had a lower volumetric moisture content averaging 28%, whereas Van de Vijver et al. (2002) had an average content of around 45%. Considering those differences, the ranges of moisture (and pH) overlap very well and show that data selection plays an important role in calculating indicator values.

Apart from comparing the tolerance ranges of pH and soil moisture, we also checked whether the updated van Dam et al. (1994) classification is in accordance with those optimum values. We found that diatom taxa occurring in both aquatic and terrestrial environments have similar preferences in both environments meaning that the categories for pH are still useful in terrestrial settings. However, if used for soils, the threshold value between categories 2, 3 and 4 should be set around 6.5 instead of 7 to have a better interpretation. Contrary to pH, the categories for soil moisture did not work for terrestrial environments and results indicate that taxa, either assigned as rather aquatic or terrestrial by van Dam et al. (1994), can (frequently) occur on soils if the moisture concentration is minimum 20%. A similar observation was reported by Stokes (1940), who found that algae function and grow best when 40 to 60% of moisture-holding capacity of the soil is reached. Besides, this outcome also affects the use of diatoms as hydrological tracers, since in those studies van Dam et al. (1994) categories 4 and 5 were used to classify diatoms as being 'terrestrial' (Pfister et al., 2009, 2017b). Knowing that diatoms from other categories also regularly occur on soils, we should revise the classification, which eventually would lead to the inclusion of more diatoms that can be used for tracing hydrological connectivity. Although the classification of van Dam et al. (1994) has proven to be very useful, it does not always provide the correct ecological answers for diatoms present on soils.

As mentioned before, adding data, independent of the location, to the existing dataset might still improve the validation and optimum values for the investigated variables provided that the community data is taxonomically consistent. This is, however, not easy since taxonomy in diatom research changes constantly and verification is rather time-consuming and not always possible. In this study, we could not verify everything and due to this inconsistency, it is possible that (much) 'noise' ended up in the results. However, since terrestrial diatoms are far less studied than strictly aquatic diatoms, taxonomical changes occur in a slower pace. Very often, old diatom publications such as Ettl and Gärtner (1995), which is a compilation of terrestrial diatom studies in Europe, and Lund (1945, 1946), among other references mentioned earlier are still consulted for identification making taxonomical harmonization easier between the different datasets. Noise will also result from differences in soil moisture between the soil moisture observation and the diatom sample, since soil moisture can be highly variable even at small distances (Teuling and Troch, 2005). Also, we did not

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account for (pseudo-)cryptic species (i.e. genetically different species, but morphologically (almost) undistinguishable) such as *P. borealis* and *H. amphioxys* (Maltsev and Kulikovskiy, 2017; Pinseel et al., 2020, 2019; Souffreau et al., 2013b). Both are known to have developed different adaptations and/or tolerances to certain climatic conditions (Souffreau, 2010; Souffreau et al., 2011). As they are widespread, common diatoms, we can imagine that many more cosmopolitan species such as *H. contenta*, *H. abundans*, *P. obscura* are also (pseudo-)cryptic. A way to solve this is to switch to molecular techniques (i.e. DNA metabarcoding and high-throughput sequencing) for community analysis. These techniques are seen as a fast, efficient and low-cost solution to the rather time-consuming microscopic diatom identifications (Rivera et al., 2020; Vasselon et al., 2017). It will also enable us to increase sampling frequency and to combine and link different soil organisms with each other, paving the way for monitoring programmes and other related applications (Fløjgaard et al., 2019; Orgiazzi et al., 2015). Moreover, it will be also a solution for cryptic diversity and thus it will still be possible to combine datasets from different climatic regions.

Another point that we should be aware of when collecting data for intercomparing purposes is to align and standardize the soil sampling and analysis of environmental variables. Due to this, we were not able to fully utilize our combined dataset. Of the different variables included here, we believe more focus should be on soil organic matter, since an encouraging r² of 0.29 was obtained based on a limited number of only 30 samples. Besides, it is strongly related to anthropic disturbances and the moisture-holding capacity of the soil (Hudson, 1994; Stanek-Tarkowska et al., 2018), both of which diatoms are responsive to and likely sensitive as well. Furthermore, in regards of their potential usage as a measure for soil quality, SOM is often seen as an important indicator (Bünemann et al., 2018) and it will therefore be interesting to explore this variable more in relation to diatoms.

5.5 CONCLUSIONS

In this study, we defined autecological values for pH and soil moisture content for the most common, widespread soil diatoms and compared them with previous research of Lund (1945), van Dam et al. (1994) and Van de Vijver et al. (2002). Besides our results showing similarities with those studies, they also indicated a significant improvement on the existing indicator values. Moreover, we expanded the list of terrestrial diatoms to 249 taxa to be used as environmental markers in different research fields. We believe that future studies should focus on molecular techniques as it will be possible to speed up the identification process and to better discriminate cryptic species. Additionally, soil sample collection and nutrient analysis should be standardized in order to enable a better pooling of data concerning diatom ecology in the future.

CHAPTER 6

TECHNICAL NOTE: A TIME-INTEGRATED SEDIMENT TRAP TO SAMPLE DIATOMS FOR HYDROLOGICAL TRACING

Diatoms, microscopic single-celled algae, are present in almost all habitats containing water (e.g. streams, lakes, soil and rocks). In the terrestrial environment, their diversified species distributions are mainly controlled by physiographical factors and anthropic disturbances which makes them useful tracers in catchment hydrology. In their use as a tracer, diatoms are generally sampled in streams by means of an automated sampling method; as a result, many samples must be collected to cover a whole storm run-off event. As diatom analysis is labourintensive, a trade-off must be made between the number of sites and the number of samples per site. To reduce this sampling effort, we explored the potential for the Phillips sampler, a time-integrated mass-flux sampler, to provide a representative sample of the diatom assemblage of a whole storm run-off event. We addressed this by comparing the diatom community composition of the Phillips sampler to the composite community collected by automatic samplers for three events. Non-metric Multidimensional Scaling (NMDS) showed that, based on the species' composition, (1) all three events could be separated from each other, (2) the Phillips sampler was able to sample representative communities for two events and (3) significantly different communities were only collected for the third event. These observations were generally confirmed by Analysis of Similarity (ANOSIM), Permutational Multivariate Analysis of Variance (PERMANOVA), and the comparison of species relative abundances and community-derived indices. However, sediment data from the third event, which was sampled with automatic samplers, showed a large amount of noise; therefore, we could not verify if the Phillips sampler sampled representative communities or not. Nevertheless, we believe that this sampler could not only be applied in hydrological tracing using terrestrial diatoms, but it might also be a useful tool in water quality assessment.

The chapter is based on: Foets, J., Wetzel, C.E., Martínez-Carreras, N., Teuling, A.J., Iffly, J.-F., Pfister, L., 2020. Technical note: A time-integrated sediment trap to sample diatoms for hydrological tracing. Hydrology and Earth System Sciences 24. doi:10.5194/hess-24-4709-2020

6.1 INTRODUCTION

Tracing of water sources and flow paths is an important field of study in catchment hydrology. Environmental tracers such as geochemical elements and stable isotopes of hydrogen and oxygen in the water molecule have led to new insights in our understanding of the age, origin and pathways of water at the watershed scale over the last few decades. For instance, the work of Hooper et al. (1990) on end-member mixing analysis showed that stream water could be distributed into unique water signatures that mapped back to measurable water chemical concentrations in the landscape. Likewise, thanks to the use of hydrological tracers, we know that groundwater fulfils a very important role in the storm and snowmelt run-off generation in streams (Sklash and Farvolden, 1979). However, progress remains stymied by various assumptions and limitations in the techniques, including temporally varying input concentrations (McDonnell et al., 1990), unstable end-member solutions (Burns, 2002; Elsenbeer et al., 1995) and the need for unrealistic mixing assumptions (Fenicia et al., 2010). In response to the need for new tracers, soil diatoms (i.e. diatoms living on the soil surface) have been proposed by Pfister et al. (2009) as a potential tracer for studying hydrological connectivity and surface run-off processes.

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

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

A way to increase the number of sampling sites could be with the use of time-integrated passive samplers. As the name already suggests, these devices are designed to collect samples that are representative of the whole sampling period. An example of such a device is the time-integrated mass-flux sampler designed by Phillips et al. (2000) - the Phillips sampler. This sampler, which consists of a PVC pipe (98 mm × 1000 mm) with a very small inlet and outlet (4 mm), was originally developed to trap fine-grained (< 62.5 µm) suspended sediment for the assessment of geochemical, physical and magnetic features of transported material (Russell et al., 2000). The functionality of the sampler is based on the large difference in the diameter between the inlet of the pipe and the pipe itself. This sudden change in pipe diameter substantially reduces the flow velocity and encourages the sedimentation of fine particles without significantly disrupting streamflow. This sampler is seen as a simple, inexpensive and easily deployable method, particularly for studies requiring a dense sampling network (Phillips et al., 2000). Generally, studies have illustrated that the Phillips sampler gives a representative sample of the sediment properties (Laubel et al., 2002; Phillips et al., 2000; Walling, 2005) and could, therefore, be employed for use in techniques such as sediment source fingerprinting (Martínez-Carreras et al., 2010, 2012). However, its use is rather restricted to small streams (Phillips et al., 2000). Although the design could be easily adapted (e.g. increase the length and diameter of the pipe) depending on the river flow characteristics, the outcome has not always been positive (McDonald et al., 2010). Furthermore, it is not suited

for the assessment of absolute sediment bed loads, and as the sampler grossly underestimates the total fine sediment flux, the samples are also biased towards larger particles (Laubel et al., 2002; Perks et al., 2014). Aside from its above-mentioned limitations, this low-cost, easily deployable sampler has reportedly provided satisfying results. Moreover, as diatoms are a part of suspended sediment (Droppo, 2001) and have mean cell sizes comparable to silt particles (Jones et al., 2012), one would expect that they also behave similarly in stream bodies; therefore, the sampler could potentially also collect a diatom community representative of the sampling period.

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

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

6.2 MATERIAL AND METHODS

6.2.1 STUDY SITE

The sampling location was situated in the south of the Colpach catchment (19.44 km²; 49°46′27″ N, 5°48′56′ E), a sub-catchment of the Attert experimental basin (288 km²) which is located in the western part of the Grand Duchy of Luxembourg (Figure 2.1). The sub-catchment is mainly underlain by Devonian slate, phyllites and quartzite in the north and marls in the south (Figure 6.1). Land use in the area consists of forests (50%), grasslands (24%) and croplands (23%) (Juilleret et al., 2011). The altitude in the sub-catchment ranges between 265 and 530 m, and our sampling site was located at 313 m.a.s.l. Hydrology in a headwater creek of the Colpach catchment, with similar bedrock geology, topography and land use, has been characterized by a fill and spill system (Wrede et al., 2015) with rapid flow in a highly permeable saprolite layer of weathered schist above the bedrock as the dominant run-off process. Besides the importance of lateral flow, high surface infiltration rates have been observed in the sub-catchment (Van den Bos et al., 2006). For a more detailed description on the hydrological features of the Colpach catchment, please see Loritz et al. (2017).

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



FIGURE 6.1: GEOLOGY (A) AND DIGITAL ELEVATION MODEL (B) OF THE COLPACH CATCHMENT. THE BLACK DOT MARKS THE SAMPLING LOCATION, WHICH IS LOCATED AT THE OUTLET OF THE CATCHMENT (I.E. THE COLPACH-HAUT SUB-CATCHMENT).

6.2.2 EXPERIMENTAL SET-UP

In order to sample drift diatoms and suspended sediment, the sampling location (Figure 6.2) was equipped with two automatic water samplers (ISCO 6712 FS) to collect instantaneous stream water samples (1 L). Sampling was triggered by flow conditions and was set up before a rainfall event occurred. During these events, samples were regularly collected every 2 or 3 hours. Furthermore, two Phillips samplers were designed as described by Phillips et al. (2000) — except that the diameter of the outlet was 2 mm instead of 4 mm. This was done to increase the settling of sediment in the sampler, as the catchment average suspended sediment concentrations are relatively small. The samplers were attached close to the riverbank and downstream of the automatic samplers so that they would not interfere with the samples of the automatic samplers (Figure 6.2). The Phillips samplers were placed on site just before the event and were retrieved when the event was completely finished. Concurrently with the placement and removal of the Phillips samplers, a manual grab sample (1 L) was taken. The Phillips samplers were emptied into 10 L buckets. In addition, turbidity and conductivity were continuously measured in situ at 5 min intervals using a YSI 600OMS optical monitoring system, while water depth was measured with an ISCO 4120 pressure probe logger at a 15 min time step. Discharge at 15 min intervals was estimated using a rating curve between discharge and water level. Upon arrival in the laboratory, all samples were stored at 4°C prior to analysis.



FIGURE 6.2: PICTURES OF THE EXPERIMENTAL SET-UP AT THE STUDY LOCATION. A, AUTOMATIC SAMPLERS INSTALLED UPSTREAM OF THE BRIDGE. B, AN OVERVIEW OF THE INSTALLATION OF THE PHILLIPS SAMPLERS DOWNSTREAM OF THE BRIDGE. THE ARROW DENOTES STREAMFLOW. C, A CLOSE-UP OF THE TURBIDITY METER (1) AND THE PHILLIPS SAMPLERS (2).

6.2.3 DIATOM PREPARATION AND IDENTIFICATION

After thoroughly shaking the bottles and buckets, a subsample (0.5 L) of each sample was taken for diatom analysis. The subsamples were fixed with 70% ethanol to prevent a possible population change by cell division and were left aside for at least one day to let the diatoms settle down. Diatom slides were prepared for microscopic counts following the European Committee for Standardization (2003). Around 70% of the supernatant was removed by vacuum aspiration, whereafter samples were cleaned using H_2O_2 and heated (100°C) for 24 hours in a sand bath. The supernatant was removed by aspiration and 1 mL of HCI (37%) was added. The mixture was allowed to settle for one day before the supernatant was removed. Afterwards, three repetitions of rinsing with deionized water, decantation and supernatant removal were carried out. The final suspensions were dried on glass cover slips and mounted on permanent slides using Naphrax. A minimum of 400 valves were counted and identified on each slide (n = 103) along random transects using a Leica DMR light microscope with a ×100 oil immersion objective and a magnification of ×1000. Diatom identifications were mainly based on following taxonomic references: Krammer (2000), Lange-Bertalot (2001), Lange-Bertalot et al. (2017) and Levkov et al. (2016).

6.2.4 SUSPENDED SEDIMENT ANALYSIS

The suspended sediment concentration (SSC) was determined by filtering a known subsample volume between 200 and 500 mL through 1.2 μ m Whatman GF/C glass fibre filters using a Millipore vacuum pump. Prior to filtering, the filters were dried at 105°C and weighed. Afterwards, the samples were dried (105°C) and weighed again. The concentration of suspended sediment was calculated as the difference between these two weights divided by the volume of the filtered sample.

The particle size distribution (PSD) was measured in the laboratory using a portable LISST-200X sensor (Sequoia Scientific, Inc., Bellevue, WA), which is a submersible laserdiffraction-based particle size analyser. Measurements were carried out on suspensions

inside a test chamber provided by the manufacturer. Before undertaking the measurements, a background measurement was carried out with Milli-Q water. After the chamber was filled, the water was stirred for a few minutes using a magnetic stirrer to ensure that all bubbles disappeared. If bubbles were still present, they were removed manually from the measuring cell and the windows of the chamber. After carrying the background measurements out, the chamber was filled with a 0.5 L subsample and Milli-Q water was added if the sensor was not fully covered. The magnetic stirrer kept all particles in suspension. Next, a LISST measurement consisting of 120 single measurements was performed in real-time mode using the LISST-SOP200X program. The raw data from each single measurement were then converted to the particle size distribution using the 'Random Particle Shape Models' described by Agrawal et al. (2008) and the recorded background scatter. Unfortunately, it was not possible to measure the PSD and SSC in all samples due to the limited water volumes collected.

6.2.5 STATISTICAL ANALYSIS

Weighted *t* tests were performed using the discharge as the weight on the particle size (the 10th, 50th and 90th percentiles), and the distributions were compared using a Kolmogorov-Smirnov test. Discharge was used as the weight because a larger volume of water flows through the Phillips sampler during periods of higher discharge and, thus, contributes more to the time-integrated samples. Furthermore, flow duration curves were plotted using the *fdc* function from the *hydroTSM* R package (Zambrano-Bigiarini, 2017) in order to check if the events were fully covered by the automated sampling method. A problem was encountered with the field instrumentation during the third event, as the turbidity was exceptionally noisy and the measured concentrations were too high and unrealistic for the site (see Figure 6.3). Therefore, it was decided to exclude the event from further analysis of the sediment data.

Before carrying out the statistical analysis on the diatom communities, the species dataset was reduced by only retaining taxa with a relative abundance of at least 1% in a minimum of two samples; the purpose of this was to compare the main patterns in the communities between sampling methods. As the species data contained many zeros, a Hellinger transformation was used on the data and Euclidean distances were taken using the *vegdist* function provided by the *vegan* R package (Oksanen et al., 2019). Non-metric Multidimensional Scaling (NMDS) analysis was then performed. A stress value below 0.10 was achieved, indicating an ideal representation of the data (i.e. the configuration is close to actual dissimilarities) according to Clarke (1993). Afterwards, the position of the centroids in the geometric framework of the NMDS plot was compared using Permutational Multivariate Analysis of Variance (PERMANOVA). Differences in treatments were additionally analysed

with the *betadisper* function to check whether these differences were caused by a difference in dispersion (within-group variation) rather than a difference in the mean values of the groups. Furthermore, Analysis of Similarity (ANOSIM) was used to test if communities significantly differed between the events and sampling methods whilst accounting for repeated measurements, i.e. pseudo-replication (Clarke, 1993). Outcomes were assessed with the Monte Carlo permutation test (perm = 9999). Moreover, species relative abundances were compared using Similarity Percentages analysis (SIMPER) and an unpaired weighted *t* test or Mann-Whitney U test (*sjstats* package) with the discharge as weight.

Next, the diatom biovolume was calculated according to the data provided in Rimet and Bouchez (2012) and Omnidia (Lecointe et al., 1993) as a measure of the mean cell size. Also, the Shannon-Wiener, Pielou's evenness and specific Pollution-Sensitivity (IPS; Cemagref, 1982) indices were computed and compared for each method and event using an unpaired weighted *t* test or Mann-Whitney U test. The IPS was chosen from many different diatom-based indices, as this index considers the abundance of each species in the sample, their sensitivity value to organic pollution and their indicator value (i.e. the relative probability of each species to occur in one of five saprobity classes and, in turn, the measure of organic matter present). As the behaviour of diatoms could differ from suspended sediment particles, an ecological guild was assigned to the diatom species following Passy (2007) and Rimet and Bouchez (2012) in order to better understand potential differences in sampling methods. Ecological guilds are defined as a group of species that live in the same environment (i.e. tolerant to similar environmental stressors) but may have adapted differently (Rimet and Bouchez, 2012). All the aforementioned statistical analyses were performed using the R statistical program (R v. 3.5.0.; http://www.r-project.org/, last access: 21 July 2020).

6.3 RESULTS

The first event occurred after an exceptionally dry and warm summer when water levels were very low. Consequently, a maximum catchment discharge of only 0.011 mm h⁻¹ was measured (Figure 6.3). Turbidity levels also followed the sudden increase in discharge, reaching 68.5 NTU. Unfortunately, both the automatic and Phillips samplers were not active during the first peak of the event, and this may have influenced our results. The second event occurred a few days later, and the discharge, turbidity and SSC increased substantially during this period. A similar pattern in discharge levels was seen as during the first event (i.e. fast responses to precipitation). This is in strong contrast to the third event, where the small peak in discharge was instead followed by a high, extended peak (i.e. delayed peak). During the third event, we measured the highest catchment discharge (0.44 mm h⁻¹) and turbidity levels.



However, the latter showed too much noise; therefore, this event was excluded from the statistical analysis of the sediment data.

FIGURE 6.3: CATCHMENT DISCHARGE, SUSPENDED SEDIMENT CONCENTRATION (SSC), MEDIAN PARTICLE SIZE (D_{50}), TURBIDITY AND PRECIPITATION FOR THE THREE EVENTS. DISCHARGE AND TURBIDITY WERE MEASURED AT 15 MIN INTERVALS, WHEREAS SSC AND D_{50} were analysed from automatically collected water samples. Total precipitation is given per event. The grey shading represents the period during which the Phillips sampler was in the water.

6.3.1 SUSPENDED SEDIMENT

In Figure 6.3 and Figure 6.4, median particle sizes (D₅₀) and PSDs are given for the first two events. In general, the median particle sizes follow the changes in turbidity levels. However, this is not the case for the last five samples taken during the first storm run-off event. These values do not seem correct, as the SSC also shows different behaviour. We do not currently know what caused these high levels, but this effect is also visible in the PSD where a high standard variation is observed after 200 µm. However, we do not observe this second peak in the PSD of the Phillips samples. This difference in the distribution between the two methods was confirmed by the Kolmogorov-Smirnov test (D = 0.31, *P* = 0.036), although there was no significant difference for the second event (D = 0.25, *P* = 0.21). In addition, we observe that the particle sizes of the Phillips samples are similar to those of the automatic samples (*P* > 0.05), except for the 10th percentile of the second event (t = -2.39, P = 0.03) (Table 6.1). Moreover, we notice that the particle sizes of the mean sizes decreased for the automated sampling method. Although, the Phillips sampler tends to undersample smaller particles, it integrates the sediment particle sizes and distributions well on both occasions.

TABLE 6.1: COMPARISON OF THE PARTICLE SIZE, THE 10TH (D10), 50TH (D50) AND 90TH (D90) PERCENTILES, BETWEEN THE PHILLIPS SAMPLER AND THE AUTOMATIC SAMPLER. WEIGHTED AVERAGES AND STANDARD DEVIATIONS ARE GIVEN. P VALUES WERE DERIVED FROM WEIGHTED *T* TESTS OR MANN-WHITNEY U TESTS.

		D ₁₀	D ₅₀	D90
			(µm)	
	Automatic (n = 14)	61.0 ± 23.2	82.0 ± 39.1	124.6 ± 71.5
Event 1	Philips (n = 2)	62.9 ± 19.1	68.6 ± 21.4	78.6 ± 20.4
	P value	0.648	0.921	0.607
	Automatic (n = 16)	53.9 ± 10.6	62.2 ± 13.1	79.5 ± 17.8
Event 2	Philips (n = 2)	85.4 ± 25.1	94.3 ± 27.5	108.0 ± 34.8
	P value	0.0297	0.0687	0.175

6.3.2 DIATOM COMMUNITIES

Generally, the flow duration curves show that the three events were well covered by the automatic samplers, because the discharge at the time the automatic sampler collected the samples used for diatom analysis (blue line; Figure 6.5) follows a similar pattern to the discharge estimated at 15 min intervals (black line; Figure 6.5). Furthermore, the samples are well distributed over the different sampling periods. Both results show us that our sampling was not biased towards certain periods of the events.



FIGURE 6.4: AVERAGE PARTICLE SIZE DISTRIBUTION FOR THE TWO SAMPLING METHODS FOR THE FIRST TWO EVENTS. THE GREY SHADED AREA INDICATES THE STANDARD VARIATION.

We identified a total of 233 different taxa, including varieties, subspecies and forms, belonging to 65 genera. After using the cut-off criteria, 71 species (94% of the total valves counted) were retained in the statistical analyses (see Table A6.1). The most species-rich genera were then *Nitzschia*, *Navicula*, *Fragilaria* and *Planothidium*, comprising 15, 10, 5 and 4 taxa respectively, whereas *Navicula gregaria* (15% of the total valves counted), *Navicula germainii* (9%), *Nitzschia palea* (7.2%), *Nitzschia tenuis* (6.5%) and *Planothidium lanceolatum* (4.2%) were the most abundant species. An average species richness per sample of 43 ± 6.8 was observed, with values of 37.9 ± 6.3, 43.7 ± 4.1 and 49 ± 3.9 during the first, second and third events respectively. Terrestrial diatoms were consistently found to react to the precipitation pulses, with the average proportion of terrestrial diatoms in the water samples increasing to a maximum of 6.6% (event 1), 8.4% (event 2) and 9.7% (event 3) during periods of high discharge (see Figure A6.1).





FIGURE 6.5: FLOW DURATION CURVES COVERING THE SAMPLING PERIOD OF THE PHILLIPS SAMPLERS. THE BLUE LINE SHOWS THE SAMPLES TAKEN WITH AN AUTOMATIC SAMPLER FOR DIATOM ANALYSIS AND THE BLACK LINE SHOWS THE CONTINUOUS MEASUREMENTS OF DISCHARGE AT 15 MIN INTERVALS.

Chapter 6

Chapter 6: Phillips sampler



FIGURE 6.6: NON-METRIC MULTIDIMENSIONAL SCALING (NMDS) ANALYSIS OF THE DIATOM COMMUNITIES, INDICATING THE DIFFERENT EVENTS AND SAMPLING METHODS. SPECIES DATA UNDERWENT A HELLINGER TRANSFORMATION AND THE EUCLIDEAN DISTANCE WAS USED. A STRESS VALUE BELOW 0.10 CONSTITUTES AN IDEAL REPRESENTATION OF THE DATA ACCORDING TO CLARKE (1993).



FIGURE 6.7: COMPARISON OF THE DIATOM COMMUNITY DATA BETWEEN THE TWO SAMPLING METHODS FOR EACH EVENT. THE SHANNON-WIENER INDEX (H') GENERALLY RANGES BETWEEN 1.5 (LOW DIVERSITY) AND 3.5 (HIGH DIVERSITY). PIELOU'S EVENNESS (J) RANGES BETWEEN 0 (LOW EVENNESS) AND 1 (HIGH EVENNESS). THE SPECIFIC POLLUTION-SENSITIVITY INDEX (IPS) RANGES BETWEEN 1 (POOR WATER QUALITY) AND 5 (HIGH QUALITY). CALCULATIONS WERE BASED ON RELATIVE ABUNDANCE DATA. **, P < 0.01; ***, P < 0.001.

Based on the NMDS analysis, we can separate the three different events from each other; in particular, the diatom communities taken during the third event are very different from the other two events (Figure 6.6). Our PERMANOVA analysis confirmed this observation (F = 70.5, P = 0.001). Concerning the different sampling methods, we observe that the Phillips samples from the third event are significantly different from most of the automatic samples from that event (F = 4.1, P = 0.001). Likewise, the time-integrated samples of the second event are quite distinct from each other (F = 2.3, P = 0.02), whereas the Phillips samples of event 1 are not distinguishable from each other or from the automatic samples. As

the sampling methods showed differences for events 2 and 3, we checked whether these differences may have been caused by a different within-group variation (dispersion) instead of different mean values of the groups. The analysis eventually gave no significant difference (P = 0.114), indicating that there was no significant dispersion effect. Overall, the NMDS and PERMANOVA analysis grouped the diatom communities relatively well per event with some time-integrated samples being differentiated from the automatic samples.

TABLE 6.2: MOST ABUNDANT DIATOM SPECIES FOR THE WHOLE STUDY CAMPAIGN. THE WEIGHTED AVERAGES	3 (IN PERCENT)
AND STANDARD ERROR OF THE RELATIVE ABUNDANCES ARE GIVEN. AN '*' INDICATES THAT THE SPECIES RELATI'	/E ABUNDANCE
IS SIGNIFICANTLY DIFFERENT BETWEEN THE TWO SAMPLING METHODS FOR THAT EVENT.	

	Event 1		Event 2		Event 3	
Species	Automatic	Phillips	Automatic	Phillips	Automatic	Phillips
Navicula germainii ^c	(11 - 30) 14.60 ± 0.77	(11 - 2) 11.42 ± 6.24	(11 - 31) 9.28 ± 0.61	(11 - 2) 9.41 ± 0.92	(11 - 20) 1.21 ± 0.18	(1 - 2) 0.00 ± 0.00
Navicula gregaria ^c	13.89 ± 0.54	13.66 ± 0.82	18.71 ± 0.51	16.01 ± 0.03	14.05 ±	8.21 ± 0.10*
Nitzschia tenuis ^c	10.87 ± 0.30	8.13 ± 0.06	3.54 ± 0.15	5.50 ± 0.27	0.22 ± 0.07	0.00 ± 0.00
Nitzschia palea ^c	8.99 ± 0.35	5.71 ± 0.14	9.32 ± 0.15	4.04 ± 0.17	2.90 ± 0.04	1.47 ± 0.00
Navicula cryptocephala ^c	4.50 ± 0.29	2.96 ± 1.74	6.09 ± 0.39	3.79 ± 0.42	2.50 ± 0.18	1.72 ± 0.35
Nitzschia dissipata ^c	3.54 ± 0.38	5.77 ± 1.08	4.91 ± 0.51	4.15 ± 0.50	1.35 ± 0.22	0.37 ± 0.09
Nitzschia recta ^c	3.53 ± 0.02	1.69 ± 0.00	1.12 ± 0.04	1.22 ± 0.09	0.22 ± 0.09	0.00 ± 0.09
Nitzschia linearis ^c	3.36 ± 0.54	1.33 ± 0.38	1.81 ± 0.60	2.57 ± 1.31	0.29 ± 0.28	0.00 ± 0.17
Cyclotella meneghiniana ^d	$3.03 \pm 0.46^{*}$	10.92 ± 2.59*	1.76 ± 0.22*	3.54 ± 0.42*	0.28 ± 0.05	0.12 ± 0.09
Nitzschia acicularis ^d	2.37 ± 0.29	0.25 ± 0.18	2.08 ± 0.33	0.49 ± 0.35	0.12 ± 0.04	0.12 ± 0.09
Planothidium lanceolatum ^b	2.09 ± 0.08	2.32 ± 0.00	3.02 ± 0.12	2.08 ± 0.00	9.11 ± 0.13	9.20 ± 0.35
Melosira varians ^a	1.79 ± 0.22	2.04 ± 0.38	1.86 ± 0.31	3.68 ± 1.40	1.23 ± 0.25	0.00 ± 0.00
Achnanthidium minutissimum ^b	1.59 ± 0.25	3.54 ± 0.68	2.59 ± 0.29	1.71 ± 0.01	8.46 ± 0.46*	16.16 ± 3.44*
Achnanthidium rivulare ^b	1.46 ± 0.20	1.44 ± 0.31	2.45 ± 0.26	3.79 ± 0.07	2.75 ± 0.29*	$7.35 \pm 0.68^{*}$
Reimeria sinuata ^b	1.41 ± 0.05	0.85 ± 0.08	1.95 ± 0.17	2.81 ± 0.60	5.19 ± 0.20*	1.96 ± 0.62*
Cocconeis euglypta ^b	1.28 ± 0.16	1.57 ± 0.22	1.26 ± 0.13	2.44 ± 0.34	3.32 ± 0.24*	1.10 ± 0.26*
Gomphonema parvulum ^a	1.04 ± 0.13	2.21 ± 0.73	1.03 ± 0.11	1.46 ± 0.51	2.84 ± 0.30	1.47 ± 0.52
Fragilaria gracilis ^d	0.88 ± 0.11	0.00 ± 0.00	2.88 ± 0.18	2.94 ± 0.87	1.52 ± 0.15	0.86 ± 0.26
Planothidium frequentissimum ^b	0.66 ± 0.24	0.72 ± 0.31	1.01 ± 0.29	1.10 ± 0.43	2.51 ± 0.39	3.07 ± 1.66
Fragilaria vaucheriaeª	0.33 ± 0.10*	1.57 ± 0.22*	0.23 ± 0.07	0.00 ± 0.00	$3.35 \pm 0.20^{*}$	1.72 ± 0.00*
Sellaphora atomoides ^c	0.19 ± 0.06	0.12 ± 0.00	1.36 ± 0.06	0.85 ± 0.09	1.42 ± 0.14*	$5.76 \pm 0.60^{*}$
Sellaphora subseminulum ^c	0.16 ± 0.35	0.00 ± 0.00	0.23 ± 0.36	0.12 ± 0.12	1.54 ± 0.73*	5.76 ± 0.84*
Achnanthidium catenatum ^b	0.13 ± 0.05	0.00 ± 0.00	0.51 ± 0.11	0.98 ± 0.34	0.81 ± 0.14*	4.41 ± 0.17*
Rossithidium petersenii ^b	0.08 ± 0.21	0.00 ± 0.10	0.20 ± 0.21	0.00 ± 0.08	1.16 ± 0.31*	2.21 ± 0.35*
Planothidium daonense ^b	0.07 ± 0.09	0.00 ± 0.16	0.06 ± 0.07	0.12 ± 0.08	0.74 ± 0.16*	2.08 ± 0.44*

THE ECOLOGICAL GUILDS ARE AS FOLLOWS: ^a, HIGH PROFILE; ^b, LOW PROFILE; ^c, MOTILE; ^d, PLANKTONIC.

Overall, the ANOSIM, species relative abundances (Table 6.2) and their derived indices (Figure 6.7) all seemed to indicate similar results to the NMDS. ANOSIM enabled the detection and split of the communities based on the different events (global R = 0.6433, P = 0.0001). Moreover, there was also a significant difference between the community composition of the automatic sampler and the Phillips sampler for the third event (R = 0.804,

P = 0.002), whereas the communities taken during the other two events could not be separated from each other (R = 0.1713, P = 0.08). A similar outcome to previous analyses is seen when we compare the abundances of the most abundant diatom species with each other. Aside from *N. gregaria* and *Navicula cryptocephala*, which are dominant throughout the entire study. the abundance of the other dominant taxa differ substantially between the first, second and the third events. For instance, N. germainii, N. tenuis and Cvclotella meneghiniana only occur sparingly in the third event, whereas we observe the inverse for P. lanceolatum, Sellaphora subseminulum and Achnanthidium minutissimum. Interestingly, this follows a shift in ecological guilds: from an assemblage containing more motile species (i.e. unattached freeliving species immersed on the sediment matrix surrounded by exopolysaccharides) to more colonial and strongly attached taxa. Regarding the sampling methods, dominant taxa such as N. germainii, N. gregaria, N. tenuis and N. palea vary little during the first two events, except for C. meneghiniana and to a lesser extent Fragilaria vaucheriae which both occur significantly more in the samples from the Phillips sampler. In contrast to those events, the abundances of N. gregaria, Achnanthidium rivulare, A. minutissimum, Reimeria sinuata, Sellaphora atomoides, F. vaucheriae and S. subseminulum are all very different between the methods for the last event. Of these two communities, the one collected with the automatic samplers more appropriately resembles the communities of events 1 and 2. Concerning the indices, the Shannon-Wiener, species evenness and IPS do not show any significant difference between the methods for the first two events (P > 0.05), and only a higher diversity in the automatic samplers is noted for the third event (t = 2.76, df = 28, P = 0.01). Moreover, the overall diatom size was higher in the automatic samples of the third event compared with the time-integrated samples (χ^2 = 7.76, df = 28, P < 0.0001), whereas it was not significantly different for the first two events. Despite the fact that indices do not differ for the second event, we notice that the diversity, evenness and biovolume tend to have higher values for the Phillips samplers. The results of our analyses indicate that the Phillips sampler could possibly be a valid tool for collecting a time-integrated diatom community representative of the entire sampling period.

6.4 **DISCUSSION**

6.4.1 EVALUATION OF THE EVENTS AND SAMPLING METHODS

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

composition. Even though we only sampled at one location over a relatively short period (30 October–12 December), we were able to observe a noteworthy amount of variation among events and communities.

Furthermore, previous results from Klaus et al. (2015b) and Pfister et al. (2015) confirm that we did sample representative communities of the stream. Like us, they carried out eventbased sampling in the Colpach catchment at the same time of year (December). The time of vear is important, as diatoms exhibit seasonal succession; thus, the species' composition could change significantly during the year (Wetzel, 2001; Wu et al., 2016). Klaus et al. (2015b) analysed 28 samples in which they found 221 different species, while we also observed 231 taxa. Likewise, Klaus et al. (2015b) found that the percentage of terrestrial diatoms in the samples increased to 8.9% during the first precipitation pulse at a discharge of around 0.18 mm h^{-1} , which is also the proportion that we found. Of the 15 most abundant species in their study, 12 were also present in our study and 8 of these 12 were abundant, including N. gregaria, P. lanceolatum, N. cryptocephala and N. linearis. Similarly, of the 15 dominant species in Pfister et al. (2015), 11 were present in this study and 6 of these 11 were dominant, including N. gregaria, N. palea, A. minutissimum and P. lanceolatum. Our research also confirms the observation of Martínez-Carreras et al. (2015), who found that the relative abundance of terrestrial diatoms increased with higher discharge. Unfortunately, as we encountered too much noise in the turbidity and sediment analysis of the third event, is it difficult to draw robust conclusions from that event. The reasons for this noise are still unknown and may be linked to a local accumulation of sediment and fine material on and around the sampling tubing of the automatic samplers and inadequate cleaning of the turbidity sensor. However, aside from this noise, the results indicate that (1) sampling was generally carried out properly, (2) there was some variation between and during the events, and (3) samples were representative of the site.

6.4.2 THE POTENTIAL FOR THE PHILLIPS SAMPLER TO COLLECT DRIFT DIATOMS

Several statistical analyses were executed on sediment and diatom data to verify if the Phillips sampler could be a useful tool for sampling drift diatoms. As shown here (and previously by several other studies), the Phillips sampler gives reasonably good results concerning particle sizes and particle size distributions when compared to automated sampling methods (Perks et al., 2014; Phillips et al., 2000; Smith and Owens, 2014; Walling, 2005). The oversampling of bigger sediment particles, as often mentioned in previous studies, did occur, but this effect was rather limited in this research. Despite differences in the behaviour of diatoms and suspended sediment particles (e.g. smaller mass density, formation of aggregates and circadian diurnal activity rhythm), there was only one time-integrated

sample that deviated from the other time-integrated and automatic samples for the first two events. Besides this one sample, no significant differences in the communities and their derivatives, such as biovolume and diversity, were observed between the two sampling methods for the first two events, although communities tend to be bigger and more diverse for the Phillips samples for the second event. However, time-integrated communities sampled during the third event differed from the assemblages collected with the automatic sampler. This is probably due to the same reason as the excessive noise in the sediment data. As these samples were collected using automated sampling, one could argue that the Phillips sampler should give the better results of the two methods. This is, however, not reflected in the diatom communities, because they differed more from the communities sampled in the previous events. Conversely, the stream did show different behaviour, higher discharge and water level, compared with the other events, and this likely led to the activation, detachment and transportation of species that show different behaviour or species that belong to a different ecological guild, e.g. more strongly attached species (see examples in Jewson et al., 2006; Rimet and Bouchez, 2012; Round et al., 1990). Indeed, highly motile and planktonic species such as Navicula spp. and Nitzschia spp. were replaced by colonial and/or attached species (e.g. Achnanthidium spp. and Planothidium spp.), and this shift was more pronounced in the Phillips sampler. Thus, the Phillips sampler seems to be able to sample a representative diatom community for a storm run-off event, although we were not able to check whether the underestimation of certain species occurred during the third event.

6.4.3 POTENTIAL WAY FORWARD

Today, different research avenues can be followed in the exploration of drift diatom sampling methods for hydrological tracing. Here, the Phillips sampler was successfully applied, although our work was restricted to a single location. Therefore, future studies should aim to test the sampler in other settings that have a different hydrological behaviour and diatom communities. It would be interesting to investigate if studies would then be able to confirm our results and, specifically, provide information on the efficiency of the Phillips sampler at higher discharges, which is still not clear from our study. Furthermore, future research should not be limited to the Phillips sampler alone; other passive samplers such as the Pumped Active Suspended Sediment (PASS) sampler designed by Doriean et al. (2019) and the bidirectional Time-Integrated Mass-flux Sampler (TIMS) developed by Elliott et al. (2017) should also be able to collect representative diatom communities. The operating principle of both of these samplers is the same as that of the Phillips sampler with the difference being that the PASS works at a constant, predefined flow rate, enabling the measurements of time-weighted average SSC and PSD, whereas the bidirectional TIMS was developed for estuaries and has a L-shaped outlet preventing inflow from the other direction.

Another possible avenue would be the investigation of the minimum number of point samples that is needed and the periods during which the samples should be taken (e.g. one at peak discharge, during rising and falling limb) to get a community representing the entire event. Importantly, the diurnal circadian rhythm of benthic diatoms should also be taken into account (Müller-Haeckel, 1973; Müller-Haeckel and Håkansson, 1978). Eventually, this information could then significantly reduce the number of samples analysed. Furthermore, molecular techniques (i.e. DNA metabarcoding and high-throughput sequencing) are developing quickly in diatom research, and as these techniques are faster and less expensive than microscopical examinations, they could also open the door to higher-frequency sampling (Rivera et al., 2020; Vasselon et al., 2017). Thus, this research has unlocked new possibilities for collecting drift diatoms which could pave the way for the better use of diatoms in hydrological tracing.

Increased amounts of suspended sediments due to anthropogenic factors can have significant negative impacts on water quality and aquatic biota. It can also reduce primary production through a reduction in light penetration and can act as an important vector for the transfer of nutrients and metals in fluvial systems (Ballantine et al., 2008; Bilotta and Brazier, 2008; Bowes et al., 2003). Therefore, it is important to identify sediment sources and their relative contribution to the overall sediment load in order to establish their effect on the ecological functioning of the system so that remedial measures can be taken. A way to investigate this is with sediment fingerprinting. Regarding this technique, several tracers (e.g. mineralogy, nitrogen and carbon stable isotopes) are used to serve as sediment fingerprints (Haddadchi et al., 2013). As (drift) diatoms are often associated with those particles, they could provide additional information on sediment quality, its sources and its transport (Jewson et al., 2006; Jones et al., 2012). More interestingly, they could even be sampled and analysed with the suspended sediment in order to determine potential (sediment) sources and their relative contribution in a catchment (Pfister et al., 2017b). Furthermore, the use of a timeintegrated sampler also enables us to collect a phytoplankton community, even at different depths (McDonald et al., 2010). Generally, in water quality assessment, phytoplankton is sampled with grab samples (Abonyi et al., 2012; Soylu and Gönülol, 2003). However, the composition of planktonic diatoms is quite variable in time and changes occur relatively quickly in comparison to benthic communities (Round et al., 1990). Therefore, it would be interesting to test if a time-integrated suspended sediment sampler would also give a representative phytoplankton community for a certain period. Hence, the use of a time-integrated mass-flux sampler could not only assist sediment fingerprinting but could potentially also improve the analysis of water quality.

6.5 CONCLUSIONS

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

CHAPTER 7

SYNTHESIS

7.1 MAIN FINDINGS

This thesis focused on improving the understanding of terrestrial diatoms in relation to hydrological processes and their use as hydrological markers. The objectives were to investigate how the communities and absolute abundances of terrestrial diatoms change spatially and temporally; to know what the main environmental variables causing these changes are; and to explore a new sampling method for drift diatoms to replace and/or complement conventional stream sampling methods. Furthermore, from the ecological values of significant environmental variables for the most common taxa. To achieve these objectives, data was gathered through extensive fieldwork in the Attert River basin (Luxembourg). Soil sampling was conducted monthly at 16 sites for a period of 14 months (Chapter 3 and Chapter 4), while three storm run-off events were also sampled (Chapter 6). Besides field data collection, we also took advantage of previous ecological research in the area and elsewhere in the world to establish more robust autecological values (Chapter 5). Our main findings are addressed in four research questions (RQs) presented below, while a summary of the thesis synthesis is given in Figure 7.1.

RQ 1: How do diatom communities and absolute abundances vary temporally and what are the main drivers (Chapter 3 and Chapter 4)?

Diatom communities did not vary seasonally during our 14-month sampling campaign, meaning that seasonal variability in meteorological (e.g. humidity), chemical (e.g. nutrients) or biological conditions (e.g. vegetation height) did not significantly influence diatom communities. However, substantial variations did occur, but they were primarily related to farming practices such as grazing by cattle or ploughing. After such a drastic change (i.e. from anthropic undisturbed to disturbed), diatoms needed between one and two months to reestablish a stable community. Forests seemed to create stable micro-habitats for diatoms, since no changes in the species' compositions were noted. Contrary to the communities, the absolute abundances did change seasonally following the soil moisture availability. In optimal circumstances (i.e. when moisture availability was high), they reached abundances even

higher than green algae and cyanobacteria, two common groups of photosynthetic microorganisms occurring on soils. These abundances were not related to the type of habitat or the level of disturbance as seen with diatom communities.

RQ 2: How do diatom communities and absolute abundances vary spatially and what are the main drivers (Chapter 5)?

Our results showed that anthropic disturbance is the principal factor defining species occurrences. Forests and other non-anthropic disturbed habitats were generally very rich in species and could sometimes harbour more than 40 species per sample, whereas disturbed areas often contained only 15 species or less. Typically for those disturbed soils were the species *Hantzschia amphioxys*, *Stauroneis borichii*, *Mayamaea atomus* and *Luticola ventricosa*, while *Sellaphora harderi*, *humidophila irata*, *Sellaphora nana*, *Mayamaea fossalis* were some of our defined indicator taxa for undisturbed areas. Concurrently, the applicability of the pollution-sensitivity index (IPS), a diatom index developed for analysing organic pollution in waterbodies, was checked regarding soil disturbance. Our analysis revealed a good distinction between different levels of anthropic disturbance based on the IPS values. Besides diatom communities, absolute diatom abundances did not vary significantly between habitat or soil types. Only pH was found to influence their abundances.

RQ 3: Can autecological values be defined for environmental variables influencing diatom communities and, if so, how do they differ from previous diatom research (Chapter 3, Chapter 4 and Chapter 5)?

We were able to define optimum values and tolerance ranges for pH and soil moisture for the most common diatom taxa occurring on soils. We compared them with previous values assigned by van Dam et al. (1994) and found that for pH, they generally follow these categories. For soil moisture, however, we noticed that all diatom taxa, independent of their moisture category assigned by van Dam et al. (1994), have their soil moisture optimum around or above 25%. Furthermore, we also checked wether we could establish optimum values for soil organic matter, phosphorus, total nitrogen and carbon content, but this was not possible due to low numbers in the validation metrics.



Building upon the work of Antonelli et al. (2017)

FIGURE 7.1: SUMMARY OF THE THESIS SYNTHESIS. MAIN FINDINGS OF THE RESEARCH AND FUTURE STEPS REGARDING THE USE OF TERRESTRIAL DIATOMS AS HYDROLOGICAL TRACERS.

RQ 4: Can the Phillips sampler be used to sample representative drift diatom communities for a whole storm run-off event (Chapter 6)?

To address this question, samples from Phillips samplers were compared with samples collected with automatic samplers. In addition to diatom community analysis, suspended sediment concentration and particle size distribution were analysed to verify the correct functioning of the different samplers. For storm run-off events with relatively low discharges (max. 0.07 mm h^{-1}), we found that both the suspended sediment and diatom communities

were comparable between automatic sampling and sampling with Phillips samplers, while for the event with high discharge (max. 0.4 mm h⁻¹), communities were significantly different between the two methods. The latter, however, could not be verified, since there was too much noise in the sediment analysis possibly due to local accumulation of sediment and fine material on and around the tubing of the automatic samplers. Additionally, the stream also showed a different hydrological behaviour leading to a shift in the diatom species' composition. Species with a colonial and/or attached lifeform replaced highly motile and planktonic species. Overall, our results showed that the Phillips sampler could replace and/or complement automated sampling, especially under low discharge conditions.

Our findings showed that the ecology of terrestrial diatoms, both in drift and on soils, is a very complex issue. There are several environmental variables that play an important role in different aspects of the diatom assemblages and occurrences. Their influence also differs according to the level (i.e. species, community or ecosystem) at which they act and the level of prevailing anthropic disturbances. Despite this complexity, the behaviour of diatoms in drift is comparable with suspended sediment particles under certain circumstances. On the other hand, their sensitivity to environmental variables could open up possibilities for terrestrial diatoms to function as environmental markers in various scientific fields.

7.2 HOW DO OUR FINDINGS COMPARE TO PREVIOUS RESEARCH?

While aquatic diatoms are commonly studied and ecologically well-characterized, ecological studies on terrestrial diatoms are rather scarce (Smol and Stoermer, 2010). This knowledge gap seriously hampers their potential use as environmental markers in, among others, hydrological, forensic and soil sciences. It is very well-documented that diatoms in aquatic environments show seasonal fluctuations in their community compositions (Köster and Pienitz, 2006; Wetzel, 2001), whereas this was only discussed in two studies for communities under terrestrial conditions. However, both studies reported contrasting observations. Lund (1945) did not document seasonality, whereas Antonelli et al. (2017) did find indications of seasonal changes. Our results confirmed the observations made by Lund (1945), as there were no seasonal changes in the species' composition. For diatom absolute abundances, on the other hand, Lund (1945) reported seasonality, with the highest abundances occurring in spring and autumn and the lowest values in February, whereas we noticed the highest abundances in winter (i.e. February) and the lowest values during the summer period following the soil moisture availability. Apart from our work and the work of Lund (1945), no other study has examined diatom abundances separately. Also, studies examining algal communities in their entirety (mainly comprising of Chlorophyta, diatoms and cyanobacteria) found that soil

moisture availability plays a crucial role in the algal absolute abundances (Davey, 1991; Grondin and Johansen, 1995; Stokes, 1940).

In addition to soil moisture availability, pH was also positively related to diatom abundances suggesting that diatoms prefer higher pH values. This result agrees with the work of Hoffmann (1989), who stated that most diatoms reach their highest abundances on neutral to alkaline soils. However, our maximum measured soil pH extended only to 8.11, meaning that we were unable to conclude wether an optimum occurred or not. We could extend this value further to 8.45 when we combined different datasets for the calculation of autecological values. Contrary to the abundances, these values suggest that diatoms living on soils prefer lower pH values than under aquatic conditions. We found that a pH of 6.5, instead of 7 as documented by van Dam et al. (1994), should be used to differentiate the categories 2, 3 and 4 from each other for evaluating soils. The similar behaviour of diatom species between aquatic and terrestrial environments is however not reflected in the optimum values for soil moisture. We also expected that taxa categorized by van Dam et al. (1994) as aquatic would have higher moisture optimum values than for taxa assigned as typically terrestrial, but our analysis showed that all categories had average values of around 25%. A similar observation was reported by Stokes (1940), who found that algae function and grow best when the moisture-holding capacity of soils reach 40 to 60%. Aside from pH and soil moisture, we could not calculate optimum values for soil organic matter, total nitrogen, carbon and phosphorus, despite the fact that they were also reported as important variables influencing diatom community compositions (Antonelli et al., 2017; Vacht et al., 2014; Van de Vijver et al., 2002; Van Kerckvoorde et al., 2000). This was possibly due to the limited data availability of these variables compared to pH and soil moisture. Generally, these variables are less investigated than the latter two and often different methodologies are used to prepare and analyse them, making it impossible to merge and compare the data.

While soil moisture is essential for diatom survival and reproduction (Camburn, 1982; Van de Vijver et al., 2002), it had no significant effect on the species' composition. Instead, we found that the type of land use was the key factor. This result is consistent with previous works that explored communities under different levels of disturbance (Antonelli et al., 2017; Stanek-Tarkowska et al., 2018; Stanek-Tarkowska and Noga, 2012; Vacht et al., 2014). These studies also observed that communities often comprised the same species under similar disturbance levels. For instance, *S. borichii* prefers farmlands, while *Pinnularia perirrorata* and *Eunotia* spp. are typically found on forested soils (Barragán et al., 2018; Stanek-Tarkowska et al., 2013; Stanek-Tarkowska and Noga, 2012). Furthermore, we observed that diatoms in terrestrial environments needed 14 to 30 days more to recolonize a recently disturbed site compared to freshwater communities (i.e. 14 days–1 month; Blinn et al., 1980; Mark P Oemke

and Burton, 1986). This is logical, considering the importance of water for diatoms and its limited availability in terrestrial environments. Therefore, we expect the recolonization period to be shorter in optimal (very moist) conditions and longer when it is dryer. During this period, species such as *H. amphioxys* and *P. borealis*, which are known to be well-adapted to living in extreme environments (Bérard et al., 2004; Dodd and Stoermer, 1962; Pinseel et al., 2019; Pipe and Cullimore, 1984; Stock et al., 2018) dominated diatom assemblages. They often replaced small, more fragile (i.e., less silicified) species such as *Nitzschia* spp. and *Mayamaea* spp.

7.3 IMPLICATIONS FOR THE APPLICABILITY OF TERRESTRIAL DIATOMS AS HYDROLOGICAL TRACERS

The use of terrestrial diatoms as hydrological markers suffer from the collection and diatom analysis of a high number of soil and stream samples, a number that can now be significantly reduced thanks to several of our research findings. First and foremost, we know that diatom communities do not vary seasonally, meaning that sampling once a year or every two to three years should be enough to characterize the whole area (i.e. 75 m²; Barragán et al., 2018). Only when drastic changes occur in the landscape should additional samples be taken. However, when considering farmlands and other very disturbed anthropic areas, this is not really required since they generally harbour few species and often only the most common ones, providing little distinctive, ecological information on the site. Furthermore, we found that diatom absolute abundances are positively related to soil moisture availability. This information will save time, because soil samples containing a high number of diatoms are easier and faster to count. Therefore, we recommend sampling during the wettest periods of the year. In temperate climate regions, this period generally ranges from October to March. Additionally, with our defined autecological values for pH and indicator taxa for different levels of disturbance, we can now try to map or model species distributions based on those variables (see Figure 7.2 for an example). Although the results are preliminary, they could already form a template when time is limited. Further to mapping diatoms, we also found that our assigned optimum values of soil moisture did not correspond at all with the van Dam et al. (1994) classification, which played an important role in previous hydrological tracer studies (see Coles et al., 2016; Martínez-Carreras et al., 2015; Pfister et al., 2009). There, the different categories were used to indicate whether a diatom was terrestrial (categories 4 and 5) or aquatic (categories 1 to 3). Since we found that there were as many 'aquatic taxa' as 'terrestrial taxa' on soils, we recommend using our provided composite list of diatom taxa regularly occurring on soils (see Table A5.1). As a result, more taxa can be used as environmental markers in tracing hydrological connectivity.



FIGURE 7.2: EXAMPLES OF SPECIES DISTRIBUTION MAPPING IN LUXEMBOURG BASED ON THE TYPE OF LAND USE AND PH VALUES. HERE, THE SPECIES DISTRIBUTIONS ARE THE CENTROIDS OF THE OVERLAPPING POLYGONS OF LAND USE AND PH.

Finally, as the Phillips sampler is able to sample representative drift diatom communities, we can reduce the number of stream samples needed for characterizing their assemblages and sample at other, less reachable sites. Although the sampler opens new possibilities for future hydrological studies, there are still some limitations associated with it that should be kept in mind. First, the suitability of the Phillips sampler is very much restricted to small streams. Although the design could be easily adapted (e.g. increase the length and diameter of the pipe) to the river flow characteristics, the outcome was not always positive (McDonald et al., 2010). Secondly, the sampler cannot completely replace automated sampling, since it will not provide any indications of the onset or cessation of run-off processes. So, for research questions related to that specific topic, automatic samplers should still be the preferred option. Finally, there is currently no scientific evidence that the sampler works well under high discharge conditions. Considering that the Phillips sampler generally oversamples larger sediment particles under those circumstances (Laubel et al., 2002; Perks et al., 2014), we would also expect larger diatoms to be sampled. However, this was not the case as the biovolume decreased. So, while this approach may not work for sediment particles, it might work better for diatom sampling. Overall, the Phillips sampler extends the current toolbox in both hydrological and diatom research, but further research is needed to go beyond its limitations.

So, are we now able to really roll out diatoms as hydrological tracers? Unfortunately, the answer is no. Despite reducing the sampling effort, tracing hydrological connectivity using diatoms remains costly and labour intensive. Moreover, terrestrial diatom communities provide less information on site-specific ecological conditions than aquatic diatoms, since they are less sensitive to environmental variables and there are significantly less taxa that occur on soils than in freshwater environments (Smol and Stoermer, 2010). Nevertheless, research on terrestrial diatoms remains scarce and considering the vast amount of research that has been conducted on diatoms in aquatic environments, we could state that the research on terrestrial diatoms is only just beginning.

7.4 NEW RESEARCH AVENUES

Different research avenues can be followed in the light of terrestrial diatom ecology and its subsequent use as environmental tracers in, amongst others, catchment hydrology. So far, most studies on terrestrial diatoms have been conducted in Europe, mainly in Luxembourg and Poland, as well as in (sub-)Antarctica and some areas in North America, whereas other places were barely investigated, suggesting that many species are still undiscovered. As forests and other undisturbed habitats are a source of interesting, rare and, likely, new diatom taxa (Barragán et al., 2017; Foets and Wetzel, 2018; Van de Vijver et al., 2004; Wetzel et al., 2013, 2017), future research should particularly focus on those undisturbed habitats when investigating unexamined areas. It would also be interesting to investigate how different diatom communities are between nearby micro-habitats in a forest. Another avenue that could be explored, relates to the fact that diatoms (and other algae) in terrestrial environments are affected more directly by climatic changes than in aguatic habitats (Ress, 2012; Rindi, 2011; Souffreau et al., 2013a). As the frequency and persistence of droughts increases as a result of climate change (Berg and Sheffield, 2018), investigating the differences in the morphology and physiology of diatoms between those two environments and between different climatic zones may reveal how diatoms adapt to harsher, moisturelimited conditions. Eventually, these differences could then serve as a proxy of possible adaptions to climatic changes and give an indication of how resilient diatoms are.

Future avenues may also arise from our work with the Phillips sampler. A possible step would be to try to use other suspended sediment samplers such as the pumped active suspended sediment sampler (Doriean et al., 2019) and the bidirectional time-integrated mass-flux sampler (Elliott et al., 2017) to sample drift diatoms. Both samplers have the same operating principle as the Phillips sampler and thus should be able to function equally well for diatoms. Also, future research should try to use diatoms in combination with suspended sediment analysis and source tracing. Since (drift) diatoms are often associated with those particles, they could provide additional information on sediment quality, its sources and its transport (Jewson et al., 2006; Jones et al., 2012). Conversely, suspended sediment samplers may be applied to collect phytoplankton for water quality assessment. Currently, phytoplankton is sampled in rivers and streams with grab samples and hence only provides information over a small period (Abonyi et al., 2012; Soylu and Gönülol, 2003). Using a time-integrated sampler will give a better representation of the plankton community, provided that it is used under stable stream conditions, and thus will give a more representative assessment of the water quality.

Furthermore, the introduction of molecular techniques (i.e. diatom metabarcoding and high-throughput sequencing) in terrestrial diatom research will be an essential next step for using terrestrial diatoms in ecological applications and as hydrological tracers. These techniques are generally regarded as a fast, efficient and low-cost solution to the rather timeconsuming microscopic diatom identifications (Rivera et al., 2020; Vasselon et al., 2017). They will also enable researchers to increase the sampling frequency on soils and to combine and link different soil organisms (fungi, bacteria, plants...) with each other using environmental DNA (eDNA; Orgiazzi et al., 2015). Moreover, these approaches will improve diatom identification, as they may reveal (pseudo-)cryptic (i.e. genetically different species, but morphologically (almost) undistinguishable) species, such as P. borealis and H. amphioxys (Maltsev and Kulikovskiy, 2017; Pinseel et al., 2019, 2020; Souffreau et al., 2013b). However, as mentioned earlier in Chapter 1, these techniques are still very much under development with many hurdles still to overcome such as standardizing the methodology (Bailet et al., 2020; Kelly, 2019). So, at a more advanced stage, molecular techniques will play a key role in future terrestrial diatom research and the quantitative use of terrestrial diatoms in ecological applications.

APPENDIX

APPENDIX TO CHAPTER 1

PUBLICATION OF THE ARTICLE: FOETS, J., WETZEL, C.E., 2018. SELLAPHORA HARDERI (HUSTEDT) COMB. NOV. (BACILLARIOPHYTA, SELLAPHORACEAE), AN OVERLOOKED TERRESTRIAL DIATOM. NOTULAE ALGARUM 1–5.

Navicula harderi Hustedt (in Brendemühl 1949: 439) was originally described as a terrestrial diatom from deciduous forest soils in northern Germany. Its presence has been noted and illustrated in some recent studies of soils (Noga *et al.* 2014; Barragan *et al.* 2018), semi-terrestrial habitats such as springs and wetlands (Denys and Oosterlynck 2015; Isheva and Ivanov 2016; Szigyártó *et al.* 2017), caves (Klemenčič and Rhovšek 2005), and in rivers and streams (Juggins *et al.* 2016). *Navicula harderi* frequently co-occurs with *Sellaphora subseminulum* (Hustedt) C.E.Wetzel; indeed, both were closely compared in the same publication using material from the same sample. Due their high morphological similarity using light microscopy these species were considered conspecific by some authors (e.g. Ettl and Gärtner 1995; Krammer and Lange-Bertalot 1985, 1986). However, Reichardt (2006) and Wetzel (2015) argued that they should not be considered synonyms based on striae density and the distinct large central area.

While investigating samples from a sandy soil (deciduous forest) in Luxembourg, we found two populations identified as *N. harderi* (> 5%) and *S. subseminulum* (< 1%). Since unprocessed material for both species is unavailable at the Hustedt collection in Bremerhaven (**BRM**), Light (LM) and Scanning Electron Microscopy (SEM) were performed in samples from Luxembourg to resolve and illustrate the ultrastructural characteristics of both species. These specimens agreed in morphology with the light microscopy of the types provided by Simonsen (1987, pl. 540: Figs. 1–7; 10–12).

Light microscopy analysis of *N. harderi* (Figs. 2–31) revealed rhombic–lanceolate valves with moderately pointed apices (as highlighted in Hustedt's illustrations, here reproduced as Fig. 1). Straight and filiform raphe, and a variable axial area going from narrow linear to wide broad in the middle portion. A butterfly-like central area limited on the borders by the very short (usually five or six) median striae. The striae continue shortly on the valve mantle with the presence of mostly one areola per stria on the valve mantle. External shallow grooves enclosing the raphe system were observed constantly in all specimens (Figs. 32–40); beyond the helictoglossa, a larger foramen-like (pit) can be seen (Fig. 41) at the poles. Based on our measurements from material, the species is $6-12 \mu m \log and has a width of 3–4 \mu m$. It has radiant striae with a number of 30–35 in 10 μm .

Appendix

Navicula harderi fits into the group of small-celled Sellaphora Mereschkowsky (1902: 186) species such as S. seminulum (Grunow) D.G.Mann (1989: 2), and the diatom currently referred to as Sellaphora atomoides (Grunow) C.E.Wetzel & Van de Vijver in Wetzel et al. (2005: 219) which are characterized by small dimensions with barely resolvable features under the light microscope. Although a key feature of the genus Sellaphora is the shape of the chloroplast ('endochrome'): i.e. a single H-shaped plate and gives the genus its name (Sellaphora = 'saddle-bearer') (Mann et al., 2008), the chloroplast morphology has not been documented in many naviculoid diatoms, including N. harderi. However, other morphological characteristics of Sellaphora, according to Round et al. (1990: 552), include naviculoid and solitary cells, with uninterrupted uniseriate (rarely biseriate), radiate or parallel striae. The striae contain small round to irregular squared poroids, which are occluded near their internal apertures by hymenes. Transapically orientated bar-like thickenings occur at the poles in some species (see Mann et al., 2008). Adjacent to the axial area there may be a non-porous conopeum. The valve face is flat, except that it is often grooved near the raphe externally, and curves fairly gently into shallow or moderately deep mantles. The raphe system is central and straight. Terminal fissures are usually present and may be deflected or hooked. The central external raphe endings are expanded and slightly deflected towards the primary side, and the central internal endings are also turned or deflected towards the primary side. We should also add that many, if not all, species possess an internal apical pit at both poles.

Based on the valve ultrastructure analysis and similarities with other small-celled *Sellaphora* species (*Sellaphoraceae*), we propose the following combination:

Sellaphora harderi (Hustedt) J.Foets & C.E.Wetzel (Figs. 1-42)

Basionym: *Navicula harderi* Hustedt in Brendemühl 1949, *Archiv für Mikrobiologie*, vol. 14, p. 439, Fig. 4.

Holotype: slide N12/80. Wenzen bei Göttingen. Buchenwaldrand, Bremerhaven (**BRM**), Germany, as indicated in Simonsen (1987: 354).

Additional illustrations: Krammer and Lange-Bertalot (1986: 229, pl. 76, figs 1–6; *pro parte*, includes *Navicula subseminulum* on Fig. 7).

Appendix

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FIGURES 1–42: SELLAPHORA HARDERI. FIG. 1. ORIGINAL DRAWINGS OF NAVICULA HARDERI HUSTEDT (IN BRENDEMÜHL 1949, FIG. 4). FIGS. 2–42: LM AND SEM IMAGES OF A POPULATION COLLECTED FROM SOIL (DECIDUOUS FOREST) IN LUXEMBOURG (ATTERT RIVER BASIN) SHOWING RHOMBIC–LANCEOLATE VALVES WITH MODERATELY POINTED APICES (FIGS 2–31). FIGS 32–42: SEM IMAGES. NOTE THE LARGE CENTRAL AREA AND REDUCED STRIAE. SHALLOW GROOVES ENCLOSING THE RAPHE SYSTEM MAINLY NEAR THE CENTRAL AREA.



FIGURES 43–63: SELLAPHORA SUBSEMINULUM (HUSTEDT) C.E.WETZEL. FIG. 43. ORIGINAL DRAWINGS OF NAVICULA SUBSEMINULUM HUSTEDT (IN BRENDEMÜHL 1949, FIG. 6). FIGS 44–63: LM AND SEM IMAGES OF A POPULATION COLLECTED FROM SOIL (DECIDUOUS FOREST) IN LUXEMBOURG (ATTERT RIVER BASIN). NOTE THE LARGE CENTRAL AREA AND SHORT STRIAE AS WELL AS THE SHALLOW GROOVES ENCLOSING THE RAPHE SYSTEM MAINLY NEAR THE CENTRAL AREA. INTERNAL PROXIMAL RAPHE ENDINGS DEPRESSED AND HOOKED.

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APPENDIX TO CHAPTER 3

TABLE A3.1: LIST OF THE SIGNIFICANT INDICATOR TAXA FOR THE FIVE LAND USE TYPES. A BLACK RECTANGLE INDICATES THAT THE SPECIES IS AN INDICATOR FOR THAT LAND USE TYPE. AF, AGRICULTURAL FIELD; GG, GRAZED GRASSLAND; AG, AGRICULTURAL GRASSLAND; UG, UNDISTURBED GRASSLAND; F, FOREST.

Taxon name	CODE	AF	GG	AG	UG	F	P value
Achnanthidium minutissimum (Kützing) Czarnecki	ADMI						**
Cavinula intractata (Hustedt) Lange-Bertalot	CITT						**
<i>Chamaepinnularia obsoleta</i> (Hustedt) C.E.Wetzel & Ector	СНОВ						**
Eunotia botuliformis Wild, Nörpel-Schempp & Lange-Bertalot	EBOT						**
Eunotia exigua (Brebisson) Rabenhorst	EEXI						**
Eunotia minor (Kützing) Grunow	EMIN						*
Eunotia palatina Lange-Bertalot & W.Krüger	EPLT						*
Fragilariforma virescens (Ralfs) D.M.Williams & Round	FFVI						*
<i>Geissleria paludosa</i> (Hustedt) Lange-Bertalot & Metzeltin	GPAL						*
Halamphora montana (Krasske) Levkov	HLMO						**
<i>Humidophila biceps</i> (Grunow) P.Furey, K.Manoylov & R.L.Lowe	DBIC						*
<i>Humidophila irata</i> (Krasske) R.L.Lowe et al.	HUIR						**
Meridion circulare (Greville) C.Agardh	MCIR						*
Nitzschia cf. palea (Kützing) W.Smith	NPALc						*
Nitzschia harderi Hustedt	NIHD						**
Nitzschia solgensis Cleve-Euler	NSOL						**
Odonthidium mesodon (Kützing) Kützing	OMES						**
Pinnularia perrirorata Krammer	PPRI						**
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	PLFR						**
<i>Sellaphora harderi</i> (Hustedt) J.Foets & C.E.Wetzel	SHRD						**
Tryblionella debilis Arnott	TDEB						*
Mayamaea fossalis (Bock) Lange-Bertalot	MAFO						**
Sellaphora nana (Hustedt) Lange-Bertalot et al.	SENA						**
Adlafia minuscula var. minuscula (Grunow) Lange-Bertalot	ASDMS						*
<i>Humidophila brekkaensis</i> (J.B.Petersen) R.L.Lowe et al.	HBRE						**
<i>Microcostatus aerophilus</i> Stanek-Tarkowska, Noga, C.E.Wetzel & Ector	MAER						**
Nitzschia acidoclinata Lange-Bertalot	NACD						**
Mayamaea aff. fossalis (Krasske) Lange-Bertalot	MAFOa						**
<i>Pinnularia microstauron</i> (Ehrenberg) Cleve var. <i>angusta</i> Krammer	PMIA						**
Navicula veneta Kützing	NVEN						*
Humidophila contenta (Grunow) R.L.Lowe et al.	нсот						**

Taxon name	CODE	AF	GG	AG	UG	F	P value
Pinnularia molaris (Grunow) Cleve	PMOL						*
Nitzschia palea var. palea (Kützing) W.Smith	NPAL						**
Nitzschia perminuta (Grunow) M.Peragallo	NIPM						**
Pinnularia subcapitata f. typica J.B.Petersen	PSCT						**
Sellaphora atomoides C.E.Wetzel & Van de Vijver	SEAT						**
Stauroneis parathermicola Lange-Bertalot	SPTH						**
Mayamaea agrestis (Hustedt) Lange-Bertalot	MAGR						**
Pinnularia subrupestris Krammer	PSRU						*
<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg var. <i>sudetica</i> (Hilse) Hustedt	PVSU						**
Sellaphora lundii C.E.Wetzel, Barragán & Ector	SLUN						**
Hantzschia calcifuga E.Reichardt & Lange- Bertalot	HCAL						**
Sellaphora subseminulum (Hustedt) C.E.Wetzel	SSSE						**
Stauroneis thermicola (J.B.Petersen) J.W.G.Lund	STHE						**
Luticola ventricosa (Kützing) D.G.Mann	LVEN						**
Stauroneis cf. borichii (J.B.Petersen) J.W.G.Lund	SBORv						**
<i>Navicula cincta</i> (Ehrenberg) Ralfs var. <i>heufleri</i> Grunow f. <i>curta</i>	NCIH						*
Stauroneis borichii (J.B.Petersen) J.W.G.Lund	SBOR						**
Craticula minuscoloides (Hustedt) Lange-Bertalot	CMNO						**
<i>Mayamaea excelsa</i> (Krasske) Lange-Bertalot	MAEX						**
Surirella terricola Lange-Bertalot & Alles	STER						**
Hantzschia abundans Lange-Bertalot	HABU						**
Hantzschia amphioxys (Ehrenberg) Grunow	HAMP						**
<i>Luticola frequentissima</i> Levkov, Metzeltin & Pavlov	LFRQ						**
Mayamaea alcimonica (E.Reichardt) Monnier & Ector	MALC						**
Mayamaea atomus (Kützing) Lange-Bertalot	MAAT						**
Nitzschia pusilla (Kützing) Grunow	NIPU						**
Pinnularia borealis Ehrenberg	PBOR						**



FIGURE A3.1: TEMPORAL SUCCESSION OF THE MOST ABUNDANT DIATOM SPECIES OF SITE 9. THE SITE CHANGED FROM AN AGRICULTURAL GRASSLAND TO AN AGRICULTURAL FIELD BETWEEN THE SAMPLING IN MARCH AND APRIL 2018. CONISS CLUSTER ANALYSIS INDICATES TWO DIFFERENT DIATOM COMMUNITIES.



FIGURE A3.2: TEMPORAL SUCCESSION OF THE MOST ABUNDANT DIATOM SPECIES OF SITE 13. THE SITE CHANGED FROM AN UNDISTURBED GRASSLAND TO A GRAZED GRASSLAND BETWEEN THE SAMPLING IN JUNE AND JULY 2018. CONISS CLUSTER ANALYSIS INDICATES TWO DIFFERENT DIATOM COMMUNITIES.



FIGURE A3.3: TEMPORAL SUCCESSION OF THE MOST ABUNDANT DIATOM SPECIES OF SITE 10. THE SITE CHANGED FROM AN AGRICULTURAL GRASSLAND TO AN AGRICULTURAL FIELD BETWEEN THE SAMPLING IN MAY AND JUNE 2018. CONISS CLUSTER ANALYSIS INDICATES TWO DIFFERENT DIATOM COMMUNITIES.

FIGURE A3.4: LIGHT MICROSCOPE IMAGES OF SOME DIATOMS FREQUENTLY FOUND ON SOILS. 1, MAYAMAEA EXCELSA; 2, MAYAMAEA PERMITIS; 3, MAYAMAEA FOSSALIS; 4, MAYAMAEA ATOMUS; 5, MAYAMAEA AGRESTIS; 6, MAYAMAEA LACUNALACINIATA; 7, MAYAMAEA FOSSALIS; 4, MAYAMAEA ATOMUS; 5, MAYAMAEA AGRESTIS; 6, MAYAMAEA LACUNALACINIATA; 7, MAYAMAEA FF. ARIDA; 8, SELLAPHORA NANA; 9, SELLAPHORA LUNDII; 10, SELLAPHORA ATOMOIDES; 11, SELLAPHORA SUBSEMINULUM; 12, MICROCOSTATUS SP.1; 13, MICROCOSTATUS SP.2; 14, MICROCOSTATUS AEROPHILUS; 15, HUMIDOPHILA IRATA; 16, HUMIDOPHILA PERPUSSILA; 17, HUMIDOPHILA CONTENTA; 18, ADLAFIA BRYOPHILA; 19, ADLAFIA MINISCULA VAR. MURALIS; 20, CRATICULA MINUSCOLOIDES; 21, LUTICOLA VENTRICOSA; 22, LUTICOLA IMBRICATA; 23, LUTICOLA ACIDOCLINATA; 24, CHAMAEPINNULARIA KROOKIFORMIS; 25, CHAMAEPINNULARIA OBSOLETA; 26, NAVICULA MICROCARI; 27, NAVICULA CINCTA VAR. HEUFLERIANA F. CURTA; 28, NAVICULA RECENS; 29, NAVICULA VAUCHERIAE VAR. DENSISTRIATA; 30, NAVICULA FRITSCHII; 31, GOMPHONEMA CF. ANGUSTATUM; 32, GOMPHONEMA MICROPUS; 33, GOMPHONEMA MINUTUM; 34, PINNULARIA MICROSTAURON; 35, PINNULARIA SUBRUPESTRIS; 36, PINNULARIA SUBCAPITATA F. TYPICA; 38, PINNULARIA MICROSTAURON VAR. ANGUSTA; 39, PINNULARIA OBSOLRA; 40, PINNULARIA SUBCAPITATA F. TYPICA; 38, PINNULARIA MICROSTAURON VAR. ANGUSTA; 39, PINNULARIA GOBSOLRA; 40, PINNULARIA PERRIRORATA; 41, GEISSLERIA PALUSTRIS; 42, MUELLERIA GIBBULA; 43, MUELLERIA TERRESTRIS. THE SCALE BAR IS 10 µM.



FIGURE A3.5: LIGHT MICROSCOPE IMAGES OF SOME DIATOMS FREQUENTLY FOUND ON SOILS.1, FRAGILARIA RUMPENS; 2, FRAGILARIA PECTINALIS; 3, ACHNANTHIDIUM MINUTISSIMUM; 4, PSAMMOTHIDIUM SUBATOMOIDES; 5, PLANOTHIDIUM LANCEOLATUM; 6, PLANOTHIDIUM FREQUENTISSIMUM; 7, EUNOTIA BOTULIFORMIS; 8, EUNOTIA EXIGUA; 9, PLACONEIS HAMBERGII; 10, NITZSCHIA PALEA VAR. TENUIROSTRIS; 11, NITZSCHIA AMPHIBIA; 12, NITZSCHIA HARDER; 13, NITZSCHIA PALEA VAR. TENUIROSTRIS; 11, NITZSCHIA PALEA VAR. DEBILIS; 14, NITZSCHIA HANTZSCHIANA; 15, NITZSCHIA PALEA VAR. PALEA; 16, NITZSCHIA HARDER; 17, CAVINULA INTRACTATA; 18, CAVINULA JAERNEFELTII; 19, STAURONEIS PSEUDOAGRESTIS; 20, STAURONEIS BORICHI; 21, STAURONEIS PARATHERMICOLA; 23, SURIRELLA ANGUSTA; 24, SURIRELLA ROBA; 25, SURIRELLA TERRICOLA; 26, HANTZSCHIA AMPHIOXYS; 27, HANTZSCHIA CALCIFUGA; 28, HANTZSCHIA ABUNDANS. THE SCALE BAR IS 10 μM.

APPENDIX TO CHAPTER 5

TABLE A5.1: OPTIMUM AND TOLERANCE VALUES OF PH AND VOLUMETRIC SOIL MOISTURE CONTENT (VWC) FOR THE MOST ABUNDANT DIATOMS OCCURRING ON SOILS. THE DIATOM TAXA ARE GIVEN WHICH OCCURRED IN AT LEAST FIVE SAMPLES WITH AN ABUNDANCE OF MINIMUM 2.5%. THE FOUR-LETTER CODES ARE RETRIEVED FROM OMNIDIA.

Diatom taxa	Code	pH optima	pH tolerance	VWC optima (%)	VWC tolerance (%)
Adlafia bryophila	ABRY	6.40	0.63	33.71	20.26
Adlafia bryophiloides	ABYD	NA	NA	40.66	13.77
Achnanthidium eutrophilum	ADEU	6.44	0.60	14.83	10.29
Adlafia linearis	ADFL	NA	NA	38.95	12.74
Adlafia frenotii	ADFR	NA	NA	14.86	13.67
Achnanthidium kranzii	ADKR	6.15	0.45	35.33	28.75
Achnanthidium minutissimum	ADMI	6.85	0.92	29.36	15.55
Adlafia minuscula var. muralis	ADMM	5.40	0.96	12.95	8.72
Adlafia minuscula	ADMS	6.00	0.81	15.51	10.32
Achnanthidium pyrenaicum	ADPY	NA	NA	24.69	6.82
Achnanthidium hoffmannii	AHOF	6.90	0.55	NA	NA
Achnanthidium modestiforme	AMDF	5.68	0.59	53.47	15.14
Achnanthidium sieminskae	ASIE	5.89	0.71	45.74	19.13
Achnanthidium strictum	ASTU	7.47	0.64	NA	NA
Caloneis aerophila	CAER	5.66	0.47	36.86	8.14
Caloneis leptosoma	CALE	6.39	0.82	NA	NA
Chamaepinnularia aliena	CALN	NA	NA	59.42	11.01
Caloneis sp.3	CALS3	6.79	1.00	NA	NA
Chamaepinnularia australomediocris	CAUM	NA	NA	46.80	21.72
Caloneis cf. bacillum	CBAC	6.47	0.72	34.31	13.54
Caloneis fontinalis	CFON	7.21	1.04	NA	NA
Chamaepinnularia evanida	CHEV	5.97	0.60	50.58	22.09
Chamaepinnularia muscicola	CHMC	6.60	0.95	44.19	19.18
Chamaepinnularia obsoleta	CHOB	5.97	0.59	15.41	12.94
Chamaepinnularia parsura	CHPP	NA	NA	53.38	20.24
Cavinula intractata	CITT	6.03	0.52	36.93	22.42
Caloneis lancettula	CLCT	6.99	0.65	NA	NA
Craticula molestiformis	CMLF	7.05	0.40	NA	NA
Craticula minusculoides	CMNO	7.01	0.64	27.29	8.55
Caloneis molaris	CMOL	7.12	0.75	NA	NA
Cocconeis costata var. costata	COCC	NA	NA	12.57	18.57
Cocconeis sp.	COCS	NA	NA	28.24	34.35
Cocconeis pediculus	CPED	7.19	0.62	NA	NA
Cocconeis placentula var. lineata	CPLI	6.04	0.60	NA	NA
Diatomella balfouriana	DBAL	6.15	0.60	34.67	18.81

Diatom taxa	Code	pH optima	pH tolerance	VWC optima (%)	VWC tolerance (%)
Humidophila biceps	DBIC	5.73	0.55	33.15	16.82
<i>Denticula</i> sp.	DENS	NA	NA	30.62	0.00
Distrionella germainii	DIGM	6.20	0.69	41.73	15.24
Diploneis subovalis	DSBO	5.86	0.57	42.12	12.32
Eunotia botuliformis	EBOT	5.66	0.63	23.50	21.87
Eunotia clotii	ECLO	5.61	0.59	44.61	14.29
Eunotia exigua	EEXI	5.20	0.22	13.51	5.40
Eunotia fallax	EFAL	5.74	0.19	45.74	14.27
Eunotia incisa	EINC	NA	NA	59.51	14.65
Eunotia minor	EMIN	5.90	0.58	49.76	22.75
Eunotia muscicola	EMUS	NA	NA	46.75	16.54
Eunotia palatina	EPLT	5.23	0.45	12.19	5.79
Eunotia praerupta	EPRA	5.79	0.39	41.45	12.58
Encyonema silesiacum	ESLE	6.13	0.74	NA	NA
Eunotia tenella	ETEN	5.47	0.45	31.94	31.13
Eunotia bidens	EUBI	5.67	0.17	32.61	6.54
Eunotia paludosa	EUPA	NA	NA	43.27	22.18
Frustulia cirisiae	FCIR	6 60	0.38	24 54	11.17
Fragilaria capucina var	FCTW	6.22	0.78	53.13	16.85
capucina morphotype 2		0.22	0110	00110	
Fragilariforma virescens	FFVI	5.86	0.53	60.39	11.12
Fragilaria gracilis	FGRA	6.72	0.73	27.09	12.98
Frankophila maillardii	FKMA	6.05	0.67	41.86	17.17
Fragilaria nevadensis	FNEV	NA	NA	62.93	0.00
Fragilaria pectinalis	FPEC	6.51	0.53	21.46	3.97
Fistulifera pelliculosa	FPEL	6.45	0.88	NA	NA
Fragilariopsis kerguelensis	FPKE	6.40	0.69	NA	NA
Fragilaria rumpens	FRUM	6.74	0.72	24.88	8.63
Fragilaria vaucheriae	FVAU	6.89	0.70	NA	NA
Frustulia vulgaris	FVUL	7.09	1.07	40.93	19.84
Gomphonema affine	GAFF	5.96	0.52	44.82	13.61
Gomphonema angustatum	GANG	6.49	0.65	23.47	7.99
Gomphonema micropus	GMIC	6.62	0.93	27.26	17.52
Gomphonema minutum f.	GMIN	6.71	0.78	26.83	9.84
minutum					
Geissleria paludosa	GPAL	6.20	0.73	30.94	12.47
Gomphonema parvulum var. parvulum	GPAR	6.38	0.79	NA	NA
Gomphonema productum	GPRO	5.90	0.61	62.10	6.52
Gomphonema varioreduncum	GVRD	6.61	0.76	NA	NA
Hantzschia abundans	HABU	6.83	0.73	19.38	11.87
Hantzschia amphioxys	HAMP	6.47	0.66	20.54	10.28
Humidophila brekkaensis	HBRE	5.92	0.79	22.96	22.55
Hantzschia calcifuga	HCAL	6.83	0.66	35.22	19.18
Humidophila comperei	HCOP	6.66	0.38	17.14	16.21
Hantzschia hyperborea	HHYP	6.15	0.34	46.65	12.17

Diatom taxa	Code	pH optima	pH tolerance	VWC optima (%)	VWC tolerance (%)
Halamphora montana	HLMO	7.52	0.52	24.53	9.13
Humidophila nienta	HNIE	6.25	0.61	40.93	14.65
Humidophila perpusilla	HPEP	5.86	0.36	43.10	11.89
Humidophila arcuata	HUAC	5.89	0.42	42.59	16.84
Humidophila contenta s.l.	HUCO	6.35	0.69	20.88	12.41
Humidophila	HUCZ	5.59	0.84	37.52	13.62
crozetikerguelensis		5 76	0.91	10 15	14 55
Humidophila ingeae		5.70	0.61	42.45	0.20
Humidophila liata Humidophila latestriata		7.03 NA	0.01	20.49	0.30 9.17
		6.22	0.40	29.13	0.17
Humidophila Subantarctica		0.23	0.49	20.71	21.75
		NA 6.24	0.64	40.90	9.24
		0.34	0.04	10.29	0.24
		6.02	0.59	19.50	11.04
		0.03	0.70	41.04	7 11
				20.73	2.09
		0.47	0.57	21.72	2.90
		INA 6.00		20.74	14.04
		6.00	0.60	20.24	14.00
Luticola nivalis		5.90	0.52		NA 0.40
Luticola paramutica		6.00	0.56	24.66	9.10
Luticola robusta	LROB	6.29	0.98	31.64	12.25
Luticola ventriconfusa	LVCF	7.44	0.53	NA	NA
Luticola ventricosa	LVEN	7.44	0.65	16.25	5.06
Mayamaea atomus		6.65	0.59	26.91	9.68
Mayamaea ct. atomus 2	MAAT2	6.84	0.24	26.12	14.45
Microcostatus aerophilus	MAER	6.08	0.59	18.00	12.25
Mayamaea excelsa	MAEX	6.69	0.56	20.18	9.77
Mayamaea fossalis	MAFO	6.41	0.40	31.49	14.07
Mayamaea agrestis	MAGR	6.83	0.45	22.78	4.78
Mayamaea alcimonica	MALC	6.61	0.65	24.64	9.58
Mayamaea asellus	MASE	6.57	0.84	NA	NA
Mayamaea terrestris	MAYI	7.22	0.64	NA	NA
Meridion circulare var.	MCIR	6.81	0.30	18.20	9.08
Meridion constrictum	MCON	6.21	0.58	NA	NA
Melosira sp.2	MELS2	NA	NA	34.47	20.41
Mayamaea fossalis var.	MFOB	6.46	0.96	17.22	12.03
obsidialis					
Mayamaea fossaloides	MFSL	7.18	0.59	31.19	8.46
Melosira guillaumini	MGUI	NA	NA	24.99	32.65
Microcostatus sp.1	MISP1	6.56	0.84	19.73	8.04
Microcostatus krasskei	MKRA	5.35	0.78	17.35	17.25
Mayamaea permitis	MPMI	6.81	0.70	24.69	8.43
Mayamaea recondita	MRCD	6.40	0.65	28.02	20.40
Muelleria gibbula	MUGI	6.80	0.81	16.04	9.91

Diatom taxa	Code	pH optima	pH tolerance	VWC optima (%)	VWC tolerance (%)
Muelleria terrestris	MUTE	7.04	1.34	19.25	8.91
Muelleria varipunctata	MVPU	6.72	0.30	12.22	10.03
Nitzschia acidoclinata	NACD	6.65	0.70	25.36	13.45
Neidium alpinum	NALP	5.57	0.49	NA	NA
Nitzschia amphibia	NAMP	6.87	0.81	21.15	7.10
Navicula bicephala	NBIC	6.34	0.47	33.59	19.53
Navicula cincta var. heufleri	NCIH	7.47	0.46	21.52	9.60
Navicula cryptocephala	NCRY	6.22	0.49	NA	NA
Navicula cryptotenella	NCTE	7.42	0.60	NA	NA
Nitzschia dissipata var. media	NDME	6.50	0.63	16.84	9.15
Navicula exilis	NEXI	6.63	0.84	NA	NA
Navicula fritschii	NFRH	7.01	0.62	25.56	15.44
Navicula frugalis	NFRU	7.28	0.76	NA	NA
Navicula gregaria	NGRE	6.78	0.83	30.94	19.28
Nitzschia hantzschiana	NHAN	6.54	0.63	21.50	6.35
Nitzschia frustulum	NIFR	7.26	0.74	NA	NA
Nitzschia harderi	NIHD	7.22	0.70	26.12	12.00
Nitzschia perminuta	NIPM	6.15	0.63	28.00	10.78
Nitzschia pusilla	NIPU	6.79	0.53	25.10	9.36
Nitzschia solita	NISO	NA	NA	30.62	0.00
Navicula lanceolata	NLAN	7.49	0.68	NA	NA
Navicula moenofranconica	NMFC	7.72	0.54	NA	NA
Nitzschia obtusangula	NOTA	7.83	0.41	NA	NA
Nitzschia palea var. debilis	NPAD	6.50	0.67	22.19	12.32
Nitzschia paleacea	NPAE	6.28	0.58	21.69	7.79
<i>Nitzschia palea</i> var. <i>palea</i>	NPAL	6.72	0.63	21.83	8.40
Nitzschia cf. palea	NPALc	6.54	0.38	12.02	7.45
<i>Nitzschia palea</i> var.	NPAT	6.68	0.53	22.29	6.65
tenuirostris Nitzschia solgensis	NSOL	7 47	0 70	24 10	11 11
Navicula tenelloides	NTEN	7.40	0.79	NA	NA
Navicula trivialis	NTRV	7.57	0.51	NA	NA
Navicula vaucheriae	N\/ALL	7.20	0.47	24.27	8 17
Navicula veneta		7.20	0.51	25.47	6.99
Nitzschia adamata		NA NA	NA	20.47	2.24
Nitzschia supralitorea	NZSU	7 20	0.69	20.79	5 53
Odonthidium mesodon	OMES	6.41	0.05	16.86	6.45
Orthoseira roeseana		5.23	1 10	20.36	11 30
Psammothidium abundans		6.13	0.50	29.50	16.90
Pinpularia acidicala		6.12	0.50	40.30 51.36	10.90
Pinnularia aciulcula		6.07	0.04	56 12	21 //
Finnularia anylicilorinis Pinnularia annondiaulata		5.07	0.04	50.15 NIA	∠1.44 NA
var. irrorata		0.74	0.39	INA 04.40	
Psammotniaium aretasii	PAIS	b.34	0.42	31.46	15.28
Planothidium aueri	PAUE	5.92	0.67	37.64	18.92

Diatom taxa	Code	pH optima	pH tolerance	VWC optima (%)	VWC tolerance (%)
Pinnularia brebissonii var.	PBBB	6.03	0.97	17.30	6.52
Pinnularia bullacostae	PBLC	6.29	0.64	19.67	7.09
Pinnularia borealis	PBOR	5.98	0.59	28.20	15.30
Pinnularia bottnica	PBOT	5.99	0.84	31.80	18.66
Pinnularia brebissonii	PBRE	6.09	1.00	NA	NA
Pinnularia carteri	PCAT	6.07	0.48	46.80	14.00
Psammothidium confusum	PCFU	5.88	0.61	49.16	20.64
Pinnularia cuneorostrata	PCNR	6.44	0.52	20.65	13.37
Pinnularia crozetii	PCRO	NA	NA	36.47	16.54
Planothidium cyclophorum	PCYC	6.38	0.66	41.64	19.57
Parlibellus delognei	PDEL	7.02	0.53	31.20	28.42
Pinnularia domestica	PDMS	6.41	0.90	NA	NA
Pinnularia divergentissima	PDVG	6.06	0.63	48.33	20.55
Placoneis elginensis	PELG	7.72	1.14	NA	NA
Pinnularia frauenbergiana	PFCA	7.02	1.13	57.91	0.00
Pinnularia frequentis	PFQT	NA	NA	57.91	0.00
Psammothidium germainii	PGER	6.76	0.44	25.13	17.20
Psammothidium	PICO	5.95	0.55	35.42	14.70
incognitum Psammothidium investians	PINV	NA	NA	52.18	18.02
Pinnularia isselana	PISS	7.66	0.99	NA	NA
Pinnularia kolbei	PKOL	6.42	0.89	24.73	15.50
Pinnularia lagerstedtii	PLAG	5.97	0.65	36.50	5.64
Planothidium	PLFR	7.46	0.87	26.09	12.06
frequentissimum					
Placoneis hambergii	PLHA	6.44	0.79	NA	NA
Placoneis ignorata	PLIG	7.02	0.65	NA	NA
Psammothidium manguinii	PMAN	6.17	0.54	28.56	15.72
Pinnularia microstauron var. angusta	PMIA	6.38	0.56	18.24	7.91
Pinnularia microstauron var. microstauron	PMIC	6.00	0.84	21.22	12.15
Pinnularia microstauron var. nonfasciata	PMNF	5.48	0.39	58.21	12.61
Pinnularia molaris	PMOL	6.19	0.60	17.20	7.50
Pinnularia obscuriformis	POBF	6.46	0.27	39.95	26.16
Pinnularia obscura	POBS	6.34	0.66	25.82	14.12
Pinnularia petersenii	PPET	6.67	0.44	27.66	20.60
Pinnularia perirrorata	PPRI	5.46	0.75	43.38	26.61
Pinnularia aff. perirrorata	PPRIa	6.58	0.92	41.91	18.21
Planothidium quadripunctatum	PQDP	NA	NA	42.44	20.38
Pinnularia rabenhorstii	PRAB	NA	NA	34.56	9.69
Pinnularia rhombarea var. serrata	PRHS	6.35	0.82	37.41	20.04
Pinnularia rabenhorstii var. subantarctica	PRSA	NA	NA	13.82	15.38

Diatom taxa	Code	pH optima	pH tolerance	VWC optima (%)	VWC tolerance (%)
Pinnularia rupestris	PRUP	5.46	0.15	43.71	14.39
Psammothidium subatomoides	PSAT	6.59	0.54	27.98	13.35
Pinnularia subcapitata var.	PSCA	6.26	0.51	NA	NA
Pinnularia subcapitata f.	PSCTx	6.04	0.45	20.53	12.51
Pseudostaurosira elliptica	PSDE	6.48	0.32	36.98	8.92
Pinnularia schimanskii	PSHK	6.26	0.52	18.53	13.41
Pinnularia schoenfelderi	PSHO	5.88	0.80	18.37	13.14
Pinnularia silvatica	PSIL	NA	NA	57.25	17.59
Pinnularia sinistra	PSIN	6.00	0.55	59.92	2.57
Pseudostaurosira naveana	PSNA	6.51	0.51	31.84	17.94
Pinnularia subrostrata	PSRO	5.54	0.19	34.37	6.15
Pinnularia subrupestris	PSRU	6.97	0.61	40.18	19.58
Psammothidium stauroneioides	PSTD	6.36	0.49	25.77	14.01
Pinnularia stricta	PSTI	6.29	0.60	15.11	10.66
Pinnularia subantarctica var. elongata	PSUE	6.01	0.57	54.62	15.08
Planothidium lanceolatum	PTLA	6.66	0.79	39.56	21.15
Pinnularia viridis var. sudetica	PVSU	6.78	0.72	28.61	12.63
Rhoicosphenia abbreviata	RABB	7.35	0.59	NA	NA
Rhopalodia rupestris	RRUP	NA	NA	37.02	15.72
Reimeria sinuata	RSIN	6.20	0.66	NA	NA
Surirella angusta var. constricta	SACO	6.75	0.59	38.06	11.08
Surirella angusta	SANG	7.09	0.89	NA	NA
Stauroneis borrichii	SBOR	6.20	0.89	25.28	11.36
Stauroneis aff. Borichii	SBORa	7.39	0.70	17.16	8.59
Sellaphora atomoides	SEAT	6.65	0.66	28.01	15.95
Sellaphora elorantana	SELO	6.39	0.95	43.55	18.95
Sellaphora lundii	SELU	7.40	0.55	NA	NA
Sellaphora nana	SENA	6.56	0.30	26.99	8.58
Stauroforma exiguiformis	SEXG	6.31	0.51	22.40	13.17
Sellaphora hustedtii	SHUS	NA	NA	57.91	0.00
Simonsenia delognei	SIDE	7.11	0.72	21.60	7.58
Stauroneis lecohui	SLEC	NA	NA	41.60	18.27
Staurosirella leptostauron	SLEP	6.16	0.53	36.15	16.02
Stauroneis minutula	SMNT	6.56	0.62	NA	NA
Stauroneis muriella	SMUR	7.31	0.89	NA	NA
Surirella ovata	SOVA	7.07	0.46	20.94	6.48
Stauroneis cf.	SPAGx	7.10	0.61	26.80	12.13
pseudoagrestis	000.	0.50	4.00		
Stauroneis pseudoagrestis	SPDA	6.59	1.02	NA	NA
Stauroneis parathermicola	SPTH	6.47	0.87	29.24	20.87
Sellaphora saugerresii	SSGE	6.31	0.76	50.34	17.40
Sellaphora subseminulum	SSSE	6.72	0.59	30.07	17.83

Diatom taxa	Code	pH optima	pH tolerance	VWC optima (%)	VWC tolerance (%)
Surirella terricola	STER	7.36	0.46	25.50	8.97
Stauroneis thermicola	STHE	6.38	0.83	26.72	16.17
Stauroneis kriegeri	STKR	6.62	0.88	NA	NA
Surirella minuta	SUMI	7.19	0.73	NA	NA
Tryblionella debilis	TDEB	6.88	0.91	15.68	10.59

APPENDIX TO CHAPTER 6



FIGURE A6.1: DISCHARGE AND RELATIVE ABUNDANCES OF TERRESTRIAL DIATOMS IN THE COMMUNITY FOR THE THREE STORM RUN-OFF EVENTS. THE RELATIVE ABUNDANCE OF TERRESTRIAL DIATOMS HAS BEEN CALCULATED USING THE VAN DAM ET AL. (1994) CATEGORIES (I.E. CATEGORIES 4 AND 5).

 TABLE A6.1: LIST OF SPECIES SHOWING SPECIES RELATIVE ABUNDANCES (%) PER EVENT. THE LIST ONLY INCLUDES TAXA

 WITH A RELATIVE ABUNDANCE OF AT LEAST 1% IN A MINIMUM OF TWO SAMPLES.*, SPECIES ASSIGNED AS TERRESTRIAL.

Taxon	Event 1	Event 2	Event 3
Achnanthidium catenatum (Billy & Marvan) Lange-Bertalot	0.16	0.51	0.97
Achnanthidium kranzii (Lange-Bertalot) Round & Bukhtiyarova	0	0.01	0.61
Achnanthidium minutissimum (Kützing) Czarnecki	1.78	1.93	8.71
Achnanthidium rivulare Potapova & Ponader	1.46	2.08	3.29
Amphora pediculus (Kützing) Grunow	1.03	0.84	0.67
Cavinula cocconeiformis (Gregory) D.G.Mann & Stickle	0.07	0.1	0.56
Cocconeis euglypta Ehrenberg	1.12	1.29	3.09
Cocconeis pseudolineata (Geitler) Lange-Bertalot	0.11	0.02	0.52
Conticribra weisfloggi (Grunow) Stachura-Suchoples & D.M.Williams	0.77	0.82	0.51
Cyclotella meneghiniana Kützing	3.5	2.22	0.35
Encyonema silesiacum (Bleisch) D.G.Mann	0.4	0.26	0.91
Eunotia botuliformis Wild, Nörpel & Lange-Bertalot*	0.02	0	0.56
Eunotia implicata Nörpel-Schempp, Alles & Lange-Bertalot	0.02	0.05	0.29
Eunotia minor (Kützing) Grunow*	0.15	0.21	0.69
Fragilaria gracilis Østrup	0.78	2.81	1.62
Fragilaria pectinalis (O.F.Muller) Lyngbye	0.3	0.58	0.84
Fragilaria rumpens (Kützing) Carlson	0.21	0.78	1.11
Fragilaria tenera (W.Smith) Lange-Bertalot var. tenera	0.11	0.74	0
Fragilaria vaucheriae (Kützing) J.B.Petersen	0.42	0.22	3.03
Fragilariforma virescens (Ralfs) D.M.Williams & Round	0.15	0.18	1.07
Gomphonema micropus Kützing	0	0.01	0.48
Gomphonema minutum (C.Agardh) C.Agardh	0.23	0.31	0.63
Gomphonema parvulum (Kützing) Kützing	1.08	1.16	2.36
Halamphora montana (Krasske) Levkov*	0.25	0.36	0.25
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow*	0.08	0.15	0.22
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski	1.35	0.94	0.31
Mayamaea permitis (Hustedt) Bruder & Medlin^	0.06	0.24	0.24
Melosira varians C.Agardh	1./1	1.78	1.36
Meridion circulare (Gréville) C.Agardh var. circulare	0.07	0.05	0.47
Meridion circulare var. constrictum (Ralfs) Van Heurck	0.35	0.39	0.78
Navicula cryptocephala Kützing	4.19	5.25	2.55
Navicula exilis Kützing	0.52	0.44	0.11
Navicula germainii Wallace	14.3	10.4	1.28
Navicula gregaria Donkin	13.41	18.52	14.49
Navicula hotmanniae Lange-Bertalot	0.05	0	0.35
Navicula lanceolata (C.Agardh) Ehrenberg	0.74	0.78	1.06
Navicula rhynchocephala Kützing	0.7	0.63	0.27
	0.16	0.27	0.31
Navicula trivialis (Lange-Bertalot)	0.36	0.31	0.03
<i>Navicula veneta</i> Kutzing	0.21	0.54	0.13
Nitzschia acicularis (Kutzing) W.Smith	2.17	2.2	0.16
	0.07	0.01	0.71
Nitzschia amphibia Grunow	0.45	0.31	0.26
Nitzschia dissipata (Kützing) Grunow ssp. dissipata	3.48	5.02	1.65

Taxon	Event 1	Event 2	Event 3
Nitzschia heufleriana Grunow	0.4	0.44	0
Nitzschia intermedia Hantzsch	0.02	0.01	0.23
Nitzschia linearis (C.Agardh) W.Smith	3.48	2.07	0.38
Nitzschia palea (Kützing) W.Smith var. palea	8.59	8.83	3.42
Nitzschia palea var. debilis (Kützing) Grunow*	0.42	0.31	0
Nitzschia palea var. tenuirostris sensu Lange-Bertalot*	0.6	0.63	0.22
Nitzschia pura Hustedt	0.01	0.18	0
Nitzschia pusilla Grunow*	0.4	0.52	0.42
Nitzschia recta Hantzsch	3.1	1.34	0.2
Nitzschia soratensis E.Morales & Vis	0.24	0.19	0.69
Nitzschia tenuis W.Smith	12.79	4.5	0.28
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	0.65	0.99	2.51
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	2.15	2.85	8.49
Geissleria acceptata (Hustedt) Lange-Bertalot & Metzeltin*	0.18	0.2	0.17
Psammothidium daonense (Lange-Bertalot) Lange-Bertalot	0.06	0.11	0.86
Reimeria sinuata (Gregory) Kociolek & Stoermer	1.47	1.76	4.45
Rossithidium petersenii (Hustedt) Round & Bukhtiyarova	0.09	0.08	1.12
Sellaphora atomoides (Grunow) C.E.Wetzel & Van de Vijver*	0.22	1.02	1.9
Sellaphora pupula (Kützing) Mereschkowsky	0.73	0.85	0.4
Sellaphora subseminulum (Hustedt) C.E.Wetzel*	0.16	0.22	1.72
Stauroneis parathermicola Lange-Bertalot*	0.02	0.14	0.67
Stauroneis thermicola (J.B.Petersen) J.W.G.Lund*	0.07	0.11	0.47
Staurosirella pinnata (Ehrenberg) D.M.Williams & Round	0.04	0.06	0.89
Surirella angusta Kützing*	0.24	0.36	0.08
Tabellaria flocculosa (Roth) Kützing	0.16	0.13	0.66
Ulnaria acus (Kützing) Aboal	0.22	0.69	0.07
Ulnaria ulna (Nitzsch) Compère	0.46	1.1	1.03



FIGURE A6.2: LIGHT MICROSCOPE IMAGES OF SOME DIATOMS FOUND IN THE DRIFT. 1, FRAGILARIA VAUCHERIAE; 2, PLANOTHIDIUM ROSTRATUM; 3, REIMERIA SINUATA; 4, ACHNANTHIDIUM RIVULARE; 5, COCCONEIS PLACENTULA VAR. EUGLYPTA; 6, COCCONEIS PLACENTULA VAR. PLACENTULA; 7, COCCONEIS PEDICULUS; 8, SELLAPHORA PUPULA; 9, NAVICULA LANCEOLATA; 10, NAVICULA GERMAINII; 11, NAVICULA GREGARIA; 12, GOMPHONEMA PARVULUM; 13, ENCYONEMA VENTRICOSA; 14, AMPHORA PEDICULUS; 15, CYCLOTELLA MENEGHINIANA; 16, MELOSIRA VARIANS; 17, HIPPODONTA CAPITATA; 18, NITZSCHIA SORATENSIS; 19, NITZSCHIA DISSIPATA VAR. MEDIA; 20, NITZSCHIA ACICULARIS; 21, NITZSCHIA RECTA; 22, NITZSCHIA TENUIS. THE SCALE BAR IS 10 µM.

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DATA AVAILABILITY

The data used in this research are publicly available online at following data repositories and websites.

Chapter 3: Foets, J., 2019. Temporal and spatial variability of terrestrial diatoms at the catchment scale: data. Mendeley Data, v2. doi:10.17632/9s28gvnr53.2

Chapter 4: Foets, J., Wetzel, C.E., Teuling, A.J., Pfister, L., 2020. Temporal and spatial variability of terrestrial diatoms at the catchment scale: controls on productivity and comparison with other soil algae. PeerJ 8. doi:10.7717/peerj.9198 (*Data in supplemental material*)

Chapter 5: Foets, J., Stanek-Tarkowska, J., Teuling, A.J., Van de Vijver, B., Wetzel, C.E., Pfister, L., 2021. Autecology of terrestrial diatoms under anthropic disturbance and across climate zones. Ecological Indicators 122. doi:10.1016/j.ecolind.2020.107248. (*R script in supplemental material*)

Chapter 6: Foets, J., Martínez-Carreras, N., Iffly, J.-F., Pfister, L., 2020 A time-integrated sediment trap to sample diatoms for hydrological tracing. Mendeley Data, v1. doi:10.17632/hgbf5bpwkh.1

SUMMARY

In the past decade, terrestrial diatoms, a group of microscopic algae occurring on soils, have been studied for tracing water sources and pathways across a wide range of spatial scales. Despite encouraging results, the innovative tracer concept has reached its limits due to the lack of ecological knowledge on terrestrial diatoms and restrictions proper to the sampling locations. In order to improve the applicability of diatoms as hydrological tracers, the temporal and spatial dynamics of terrestrial diatoms were investigated and a new, alternative sampling method to sample drift diatom communities was explored.

Chapter 2 elaborates on the study area and common sampling and preparation methods used in Chapters 3 and 4. The research was conducted in the Attert River basin (Luxembourg and Belgium), where every four weeks soil diatom samples were collected at 16 sites for a period of 14 months. Concurrently, several biological, physico-chemical and meteorological variables were measured, analysed or retrieved from databases. Overall, the fieldwork resulted in a dataset comprising 224 soil samples.

In Chapter 3, the spatial and temporal variability of terrestrial diatom communities and their sensitivity to anthropic disturbances were studied. The results indicate that forests create a stable microhabitat for diatoms. On agricultural land, the temporal variation of the diatom communities is mainly controlled by farming practices, rather than seasonal changes in environmental variables. We also found that communities needed one to two months to re-establish a new, stable community after a significant change in the environment. Furthermore, indicator species were assigned for the different levels of disturbance, paving the way for species assembly dynamics modelling.

In Chapter 4, the temporal and spatial dynamics of diatom abundances on soils were studied and related to the abundances of cyanobacteria and green algae. The abundances of the different algal groups were derived from pigment analysis using High-Performance Liquid Chromatography (HPLC). Our results indicate that diatom productivity is mainly controlled by factors related to soil moisture availability leading to seasonal patterns, whereas the concentration of green algae remained stable over the course of the study period. Generally, anthropic disturbed habitats contained less living diatom cells than undisturbed habitats. Also, it was observed that diatoms can be the dominant algal group at periods of the year with high soil moisture. These results show that diatoms can play a significant role in functioning of the soil ecosystem and that more attention should be paid, certainly in the light of climatic changes, to this algal group.

Summary

The autecology of diatom taxa living on soils was investigated in Chapter 5. There, the data of several ecological studies carried out across a range of climate conditions was combined in order to provide more robust autecological values. Of several environmental variables analysed, autecological values of pH and soil moisture for common terrestrial diatoms could be established. Also, the validation metrics improved considerably after removing the samples collected in anthropic disturbed habitats, which suggest that anthropic disturbance is the principal factor defining taxon occurrences. Besides our autecological values showing similarities with previous studies, they also indicated a significant improvement on the existing optimum values and tolerance ranges for both pH and soil moisture content.

In Chapter 6, the potential for the Phillips sampler, a time-integrated mass-flux sampler, was assessed to provide a representative sample of the diatom assemblage of a whole storm runoff event. The representativeness of the sampler was tested by comparing the diatom community composition of the sampler to the composite community collected by automatic samplers for three events. As a control, the particle size distribution of the suspended sediment was compared between the two methods. Results showed that the species' composition was different between the three events and that for the two events with the lowest discharge the Phillips sampler collected representative communities. Communities for the third event were significantly different between the methods, but this could not be verified due to noise in the sediment data collected with automatic samplers. Overall, it is concluded that the Phillips sampler could be a useful sampler to replace and/or complement automatic sampling.

The final Chapter (7) summarizes and discusses the outcomes of Chapters 3 to 6 and looks at future research avenues that can be followed. In general, the results of the thesis allow to reduce the sampling effort and speed up the analysis of diatom-based hydrological tracer studies. They also indicate that many more diatoms can be used as environmental markers in hydrological tracing, that more locations can be sampled, and that the number of stream sampling sites can be increased in the future. The work improved the use of terrestrial diatoms as hydrological tracers. Concerning future research, focus should be on investigating anthropic undisturbed habitats in unexamined or barely examined areas, exploring the combination of diatom research with research on suspended sediments, and introducing molecular techniques in terrestrial diatom research.

SAMENVATTING

In het afgelopen decennium zijn terrestrische diatomeeën, een groep van microscopisch kleine algen die op bodems voorkomen, bestudeerd voor het opsporen van waterbronnen en -routes over een breed scala van ruimtelijke schalen. Ondanks bemoedigende resultaten heeft het innovatieve tracerconcept zijn grenzen bereikt enerzijds door het gebrek aan ecologische kennis over terrestrische diatomeeën en anderzijds door beperkingen met betrekking tot de mogelijke staalnamelocaties. Om de toepasbaarheid van diatomeeën als hydrologische tracers te verbeteren, werd de temporele en ruimtelijke dynamiek van terrestrische diatomeeën onderzocht en werd een nieuwe, alternatieve bemonsteringsmethode getest om drift-diatomeeëngemeenschappen te bemonsteren.

Hoofdstuk 2 gaat verder in op het studiegebied en de gangbare staalname- en preparatiemethoden die worden gebruikt in de hoofdstukken 3 en 4. Het veldonderzoek werd uitgevoerd in het stroomgebied van de Attert (Luxemburg en België), waar elke vier weken bodemdiatomeeënmonsters werden verzameld op 16 locaties gedurende een periode van 14 maanden. Tegelijkertijd werden verschillende biologische, fysico-chemische en meteorologische variabelen gemeten, geanalyseerd of gehaald uit databases. In totaal resulteerde dit veldwerk in een dataset van gegevens over 224 bodemstalen.

In Hoofdstuk 3 werden de ruimtelijke en temporele variabiliteit van terrestrische diatomeeëngemeenschappen en hun gevoeligheid voor antropogene verstoringen bestudeerd. De resultaten geven aan dat bossen een stabiel microhabitat voor diatomeeën creëren en dat temporele variatie van de diatomeeëngemeenschappen voornamelijk wordt gecontroleerd door landbouwpraktijken in plaats van seizoensgebonden veranderingen in de omgevingsvariabelen. We vonden ook dat gemeenschappen één tot twee maanden nodig hadden om een nieuwe, stabiele gemeenschap te vormen na een aanzienlijke verandering in de omgeving. Bovendien werden indicatorsoorten toegewezen voor de verschillende verstoringsniveaus die tevens gebruikt kunnen worden voor het modelleren van de dynamiek van diatomeeëngemeenschappen.

In Hoofdstuk 4 werd de temporele en ruimtelijke dynamiek van diatomeeënabundanties op bodems bestudeerd en gerelateerd aan de abundanties van cyanobacteriën en groenalgen. De abundanties van de verschillende algengroepen werden afgeleid van pigmentanalyse die uitgevoerd werden met High-Performance Liquid Chromatography (HPLC). De resultaten toonden aan dat de productiviteit van diatomeeën voornamelijk wordt bepaalt door factoren die verband houden met de beschikbaarheid van bodemvocht, wat zich uit in seizoensvariaties in de abundanties. De concentratie van groenalgen bleef echter stabiel gedurende de hele

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onderzoeksperiode. Tevens bevatten antropisch verstoorde habitats in het algemeen minder levende diatomeeëncellen dan ongestoorde habitats en kunnen diatomeeën de dominante algengroep zijn in perioden met een hoge bodemvochtigheid. De resultaten laten dus zien dat diatomeeën een belangrijke rol kunnen spelen bij het functioneren van het bodemecosysteem en dat, zeker in het licht van klimaatveranderingen, meer aandacht zou moeten worden besteed aan deze algengroep.

De autecologie van diatomeeëntaxa die op bodems leven, werd onderzocht in Hoofdstuk 5. Daarin werd de data van verschillende, eerder uitgevoerde ecologische studies samengevoegd om robustere autecologische waarden te verkrijgen. Van de verschillende geanalyseerde omgevingsvariabelen konden enkel autecologische waarden vastgesteld worden voor pH en bodemvocht. Tevens verbeterden de resultaten van de statistische analyses aanzienlijk na het verwijderen van stalen die in antropisch verstoorde habitats waren verzameld, wat suggereert dat antropische verstoring de belangrijkste factor is die de aanwezigheid van diatomeeëntaxa bepaalt. Buiten het feit dat onze autecologische waarden overeenkomsten vertoonden met eerdere studies, wezen ze ook op een significante verbetering ten opzichte van de bestaande optimale waarden en toleranties voor pH en bodemvocht.

In Hoofdstuk 6 werd het mogelijk gebruik van de Phillips sampler, een tijdgeïntegreerde massa-flux sampler, onderzocht om te zien of de sampler een representatief beeld kon geven van de diatomeeëngemeenschap van een hele stormloop. De representativiteit van de sampler werd getest door de samenstelling van de diatomeeëngemeenschappen te vergelijken met de gemeenschappen die door automatische samplers werden verzameld en dit voor drie neerslaggebeurtenissen. Ter controle werd ook nagekeken of de grootteverdeling van het gesuspendeerde sediment vergelijkbaar was tussen de twee methoden. De resultaten toonden aan dat de soortensamenstelling verschilde tussen de drie gebeurtenissen en dat voor de twee gebeurtenissen met het laagste debiet de Phillips sampler representatieve gemeenschappen bemonsterde. De gemeenschappen voor de derde gebeurtenis waren significant verschillend tussen de twee technieken, maar dit kon niet worden geverifieerd vanwege ruis in de sedimentgegevens die verzameld werden door de automatische samplers. Al met al blijkt de Phillips sampler een nuttige sampler te zijn om automatische samplers te vervangen en/of aan te vullen.

Het laatste hoofdstuk (7) vat de resultaten van de hoofdstukken 3 tot en met 6 samen, bespreekt deze en kijkt naar toekomstige onderzoekspistes die kunnen worden gevolgd. Over het algemeen laten onze resultaten toe om de het aantal staalnames te verminderen en de analyse van hydrologische tracerstudies die gebaseerd zijn op diatomeeën te versnellen. Ze

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geven ook aan dat er veel meer diatomeeën kunnen worden gebruikt als omgevingsindicatoren bij hydrologische tracering, dat er meer locaties kunnen worden bemonsterd en dat het aantal bemonsteringslocaties in de toekomst kan worden uitgebreid. In het algemeen verbeterde dit thesiswerk dus het gebruik van terrestrische diatomeeën als hydrologische tracers. Voor toekomstig onderzoek zou men moeten focussen op antropische onverstoorde habitats in niet-onderzochte of nauwelijks onderzochte gebieden, het combineren van diatomeeënonderzoek met onderzoek naar gesuspendeerde sedimenten, en het introduceren van moleculaire technieken in terrestrisch diatomeeënonderzoek.

ACKNOWLEDGEMENTS

Here, I would like to thank the people who made the completion of this work possible with their help and support.

First and foremost, I would like to thank my supervisors Carlos, Laurent and Ryan. Each of you contributed to the realisation of my PhD in different ways, all necessary for me to grow both professionally and personally. Moreover, you were all very reachable and on hand to help me when I needed it. This is not something obvious and I am really grateful for that. I am grateful to Carlos for his enthusiasm, energy, support and faith in me. Thank you for introducing me to the world of terrestrial diatoms and for being so involved during this research. I am grateful to Laurent for having given me the opportunity to be part of his DTU in hydrological sciences. Thank you for having supported and encouraged me during these four years .Thank you Ryan for guiding me smoothly through the PhD and for your very valuable advice during the writing process.

Thanks to the colleagues from the ERIN department for creating a great work environment, the support and fun moments in and out the office. Núria, Christophe, Jérôme and Jeff, thanks for the stimulating discussions and for the help in the lab- and fieldwork. Special thanks also to Luc for sharing numerous publications on diatoms, for the nice conversations and your support. Thanks Denis, Emmanuelle, Cédric and Johanna for the help in the lab. I am grateful to my office mates and to the other PhD's and Post-docs of the department, which made my stay in Luxembourg a pleasant one. Special thanks to Catherine for the many 'after works', nice discussions and help with writing the first paper, and Marta for being a guide during my first months in Luxembourg.

I thank all the people of the HWM group of Wageningen for feeling me very welcome every time I visited the group. It is a pity that I was not able to stay in Wageningen for the intended five months during my last year. I really would have enjoyed it. I would also like to thank the AEW group of Wageningen for allowing me to use the PhytoPAM. Specifically, I Thank Marlies and Frits, who both functioned as a contact point for all my related questions.

Thanks to my friends in Belgium for never forgetting to ask me how my work was going and for the nice and enjoyable moments together.

Heel veel dank gaat uit naar mijn familie en in het bijzonder mijn ouders, zus en bomma. Jullie hebben dan misschien niet zo veel van mijn onderzoek begrepen als ik zou willen, jullie bleven steeds geïnterresseerd en hebben me altijd blijven steunen. Ook kon ik altijd op jullie rekenen

Acknowledgements

wanneer ik het moeilijk had. Tenslotte, wil ik mijn vriendin Elien bedanken die mijn steun en toeverlaat was tijdens de laatste maanden van mijn doctoraat.

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<u>Foets, J.</u>, Wetzel, C.E., 2018. *Sellaphora harderi* (Hustedt) comb. nov. (Bacillariophyta, *Sellaphoraceae*), an overlooked terrestrial diatom. Notulae Algarum 1–5.

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- The influence of environmental factors on the structure and diversity of terrestrial diatom communities. 13th central European diatom meeting, 26-28 March 2019, Esch sur Alzette, Luxembourg

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Dr. ir. Peter Vermeulen

The presented research was carried out at the Luxembourg Institute of Science and Technology in association with the Hydrology and Quantitative Water Management Group of Wageningen University & Research.

This research is part of a Doctoral Education Unit in Hydrological Sciences and was financially supported by the Luxembourg National Research Fund (FNR) under grant agreement No. PRIDE15/10623093/HYDRO-CSI.

Financial support from Wageningen University for several PhD courses and for printing this thesis is gratefully acknowledged.

Cover design by Jasper Foets

Printed by Digiforce – ProefschiftMaken, Wageningen, NL