Temporal and spatial variability of terrestrial diatoms at the catchment scale: controls on productivity and comparison with other soil algae

Jasper Foets [Corresp., 1, 2] , Carlos E Wetzel [1] , Adriaan J Teuling [2] , Laurent Pfister [1, 3]

[1] Environmental Research and Innovation Department, Luxembourg Institute of Science and Technology, Belvaux, Luxembourg

[2] Department of Environmental Sciences, Wageningen University and Research, Wageningen, Netherlands

[3] Faculty of Science, Technology and Medicine, University of Luxembourg, Belval, Luxembourg

Corresponding Author: Jasper Foets
Email address: jasper.foets@list.lu

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.

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4 Jasper Foets[1,2] Carlos E. Wetzel[1] Adriaan J. Teuling[2] Laurent Pfister[1,3]

5 [1] Environmental Research and Innovation Department, Luxembourg Institute of Science and

6 Technology, Belvaux, Luxembourg

7 [2] Department of Environmental Sciences, Wageningen University and Research, Wageningen,

8 Netherlands

9 [3] Faculty of Science, University of Luxembourg, Technology and Medicine, Belval,

10 Luxembourg

12 Corresponding Author:

13 Jasper Foets[1]

14 [1]Rue de Brill 41, Belvaux, L-4422, Luxembourg

15 Email address: jasper.foets@list.lu

Abstract

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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

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Introduction

Diatoms are microscopic, unicellular algae and form one of the most common and diverse algal

groups in both freshwaters and marine environments (Round, Crawford, Mann & 1990). They

are pigmented, photosynthetic and because of their high abundances, they play a large role in the

exchange of gasses between the atmosphere and biosphere. It has been estimated that they are

responsible for 20% of the total oxygen production on the planet (Scarsini et al., 2019). 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

39   (Smol & Stoermer, 2010). Generally, those environments are much harsher for diatoms than

40   aquatic habitats (Ress, 2012). Variables such as moisture and temperature can vary considerably

41   not only seasonally, but also over the course of a day or between two consecutive days. As a

42   consequence, diatoms are typically exposed to frequent and prolonged periods of desiccation.

43   Terrestrial diatom species have developed several features (both morphologically and

44   physiologically) to cope with the temperature variability and limited moisture conditions. For

45   instance, as the characteristic siliceous cell wall consists of many pores, they often decrease its

46   number or create structures to enclose them on the out- or inside to prevent water loss (Lowe et

47   al., 2007; Ress, 2012). This adaptability suggests that diatoms do not only survive on soils, but

48   that they eventually also could thrive and even be the dominant algal group in a terrestrial

49   environment.

50   As an integral component of the soil microbial community, diatoms play an important role in the

51   functioning of the soil ecosystem. By moving on or being attached to the soil surface, they

52   produce an extracellular matrix of mucopolysaccharides, which will bind soil particles and

53   eventually stabilize the soil (Booth, 1941; Paterson, 1986; Tolhurst, Gust & Paterson, 2002;

54   Jewson, Lowry & Bowen, 2006). This aggregation will subsequently reduce water loss by

55   evaporation, limit soil erosion and improve water infiltration, thereby providing a favorable

56   habitat for seed germination (Booth, 1941; Hoffmann, 1989). Moreover, they promote the release

57   of nutrients from insoluble compounds and the weathering of silicates by creating a slightly

58   acidic environment (Hoffmann, 1989; Wu et al., 2013). Schmidt, Dyckmans & Schrader (2016)

59   found that they even might be an important carbon input source for decomposing soil animals.

60   Their death and decay also provides organic matter, which other microorganisms and plants can

61   potentially utilize (Fritsch, 1907; Shubert & Starks, 1979; Starks, Shubert & Trainor, 1981). As

62   climate change is highly likely to increase the frequency and persistence of droughts in the near

63   future (Berg & Sheffield, 2018), there is growing concern about potential negative impacts that

64   this change could have on the viability and functions of terrestrial diatoms and subsequently also

65   on higher plants.

66   In temperate regions, the seasonal variability in meteorological (e.g. humidity), chemical (e.g.

67   nutrients) or biological conditions (e.g. vegetation height) is not causing any significant changes

68   in the composition of soil diatom communities according to Lund (1945) and Foets et al. (2020).

69   However, seasonal variations might prevail in the diatom biomass with possible maxima

70   observed in spring and autumn in response to changes in temperature and precipitation (Lund,

71   1945). A similar observation for soil algae was reported by Stokes (1940), who noticed sharp

72   increases in algal numbers after periods of rain and that the optimum moisture range for algal

73   growth lies between 40 and 60% of the moisture-holding capacity of the soil. This amount would

74   probably be ideal to produce a soil solution with enough available nutrients to support algal

75   growth (Pringsheim, 1950; Starks & Shubert, 1982). Furthermore, Davey (1991) and Grondin &

76   Johansen (1995) reported an increased algal biomass (in algal numbers and in chlorophyll a

77   concentration) after snow cover. Although Lund (1945) is the only study (of all aforementioned

78   studies) to date to have analyzed specifically the terrestrial diatom production, the temporal and

79   spatial scale of that study was relatively limited (i.e. one garden soil in optimal conditions).

80   There is a pressing need for a better understanding of the seasonal patterns in diatom production

81   across contrasted environmental settings. This stands as a prerequisite for their subsequent use in

82   future applications (e.g. as soil quality bio-indicator or as a hydrological tracer).

83   Algae and cyanobacteria use pigments and other compounds to regulate the spectral composition

84   and the intensity of incoming light (photon flux density). This allows them to maximize

85   photosynthetic efficiency whilst preventing photochemical degradation of cellular components

86   and indirect loss of function mediated by reactive oxygen species (Vincent, 2000). Two types of

87   pigments are involved in those processes. Chlorophylls, particularly a, b and c, are the central

88   components in light-harvesting, while carotenoids, including xanthophylls, are mainly engaged

89   in photoprotection (Demmig-Adams & Adams, 2000). In contrast to higher plants, which all

90   contain the same major carotenoids, algae have different characteristic carotenoids. So far,

91   around 1100 different natural carotenoids have been characterized and reported in the literature

92   (Fernandes et al., 2018). This diversity and specificity allows us to use certain carotenoids as so-

93   called biomarkers or chemotaxonomical markers to infer the production of different algal groups.

94   Furthermore, recent developments in High-Performance Liquid Chromatography (HPLC) allow

95   for rapid and accurate determinations of pigments and their derivatives in aquatic and sediment

96   samples. Pigment concentrations have now become a common tool in paleolimnological research

97   and water quality assessments (Verleyen et al., 2004; Reavie et al., 2017; Duong et al., 2019).

98   However, except for a few studies on soil primary production (Shubert & Starks, 1979; Starks &

99   Shubert, 1982; Davey, 1991; Davey & Rothery, 1992), no other attempts have been reported to

100   date to assess soil diatom production and its environmental controls.

101   Previously, we have investigated the temporal and spatial variability in the composition of soil

102   diatom communities (Foets et al., 2020). There, we found that forests create a stable microhabitat

103   for diatoms, that the temporal variation of the communities is mainly controlled

104   by farming practices, and that they need one to two months to reestablish a new,

105   stable community after a significant change in the environment. Here, we leverage these findings

106   and investigate how diatom production changes over space and time in a mesoscale catchment

107   compared to green algae and cyanobacteria. The catchment scale was selected, since this is the

108   natural unit in which environmental variables such as radiation, temperature and soil moisture

109   show the largest variability. The aim of this study is mainly exploratory, but while analyzing the

110   data for Foets et al. (2020), we also developed two hypotheses. Our first hypothesis states that

111   soil diatom productivity (i.e. concentration of fucoxanthin) is controlled by meteorological

112   conditions. Our second hypothesis stipulates that the biomass changes depending on the type of

113   habitat, since communities in (anthropic) disturbed areas contain generally larger species (i.e.

114   higher pigment concentration) compared to undisturbed areas (Foets et al., 2020).

115   **Material and methods**

116   **Study area and weather conditions**

117   Our study site is located in the Attert River basin in Luxembourg, covering an area of

118   approximately 249 km² (Fig. 1). The western limit of the basin extends into Belgium

119   (49°46'13.0" N, 5°59'9.2" E). Additional information on the study area is given in Foets et al.

120   (2020).

121   We took monthly samples from December 2017 to November 2018. The summer season prior to

122   the start of our sampling campaign had been relatively wet (on average 88 mm/month; i.e. 32

123   mm/month more than in reference period 1981-2010), whereas the average monthly precipitation

124   during the sampling period was 56.8 ± 33.5 mm. The maximum monthly precipitation was

125   observed in December 2017 and January 2018 (111.9 and 123.5 mm, respectively). Monthly

126   precipitation reached a minimum in October 2018 (18 mm). July and August were the warmest

127   months, reaching mean monthly temperatures of 20.5 °C and 18.7 °C, respectively. Most

128   freezing days occurred in February and March 2018 (28 and 12, respectively) [Data obtained

129   from the 'Administration des Services Techniques de l'Agriculture' (ASTA)]. Overall, 2018 was

130 characterized by a cold winter and an exceptionally dry and warm summer period (Meteolux,

131 2019).

Meteorological data

133 Daily meteorological data for the entire study period was retrieved from two weather stations

134 (Useldange and Roodt) situated in the river basin. All data was taken from Useldange, except

135 daily precipitation. For that, we calculated Thiessen polygons to know which sites were closer to

136 which station. Besides, we derived air temperature for every site following the general equation:

137 -0.65°C x 100 m (Altitude). The station in Useldange is located at 280 m a.s.l. Additionally, 2°C

138 were deducted for the forested sites. Finally, the 7-day average prior to the sampling day was

139 taken for every meteorological variable.

Soil sampling and pigment analysis

141 In order to capture the intra-annual and spatial variability of the absolute algal abundances, we

142 took soil samples at the soil surface in 16 sites distributed across the Attert River basin (see Foets

143 et al., 2020). The sampling campaigns took place every three to five weeks for a period of 12

144 months (n = 192 samples). During each campaign, we made a description of the site in order to

145 keep track of any potential changes in the environment in between the sampling periods.

146 Characteristics such as type of land use, disturbances, height of the lower vegetation (we used

147 grass height as an indicator for mowing/grazing) were noted (see Table A1). In addition, we

148 measured soil moisture content and electrical conductivity (EC) 30 times, using a FieldScout

149 TDR 300 Soil Moisture Meter. Moreover, out of the four soil samples that we took per site, one

150 was to serve for pigment analysis and one was used for species community analysis (see Foets et

151 al., 2020).

152   We used metal cylinders (Ø: 5.6 cm, height: 4 cm) to collect small soil cores. Upon arrival in the

153   lab, all plant litter was carefully removed from the top soil layer to keep the sample undisturbed.

154   We then extracted algae by rinsing the superficial layer with Milli-Q water until a 50 mL falcon

155   tube was completely filled (see Barragán, Wetzel & Ector, 2018). The samples, consisting of soil

156   particles and water, were kept in the dark at 4°C until pigment extraction.

157   The following day, we centrifuged the samples for 10 minutes at 4000 rpm. We discarded the

158   supernatants and freeze-dried the residues for approximately 24 hours. Next, we extracted the

159   pigments two times with 5 mL methanol by shaking for 15 minutes at 25 Hz. with a mixer mill

160   (MM 400 Retsch). After centrifugation (4000 rpm., 10 min), organic phases were pooled and

161   extracts were concentrated by vacuum evaporation.

162   We separated and quantified the pigments by HPLC with Diode-Array Detection (HPLC-DAD,

163   1260 Infinity, Agilent). An Acquity UPLC HSS T3 column at 30°C was used (2.1x100 mm, 1.7

164   μm) with a mobile phase consisting of 50mM ammonium acetate solution,

165   acetonitrile/dichloromethane/methanol (75/10/15 v/v/v), and ethyl acetate. Pigments were eluted

166   at a flow rate of 300 μL min^{-1} with a ternary gradient. We identified the compounds of interest

167   by their retention time and compared the spectral data with certified standards provided by

168   Sigma-Aldrich and Carotenature. The detection limit for the standards was 30 ng per sample.

169   Quantification was done at 450, 464, 620 and 655 nm using external calibration. We expressed

170   chlorophyll and carotenoid concentrations in ng per sample (50 mL) and transformed them to μg

171   L^{-1} to make the concentrations comparable with other studies. The different pigments used in the

172   analysis, their affinity and interpretation are listed in Table 1.

173   **Physico-chemical analysis**

174   After the algal extraction, we removed the first cm of soil of each of the four samples. After

175   having dried the samples for one week at room temperature, we crushed the soils and sieved

176   them at 2 mm. We measured the pH according to the ISO 10390 (2005) standard. The

177   bioavailable part of nutrients (phosphorus (P), total nitrogen (TN), dissolved organic carbon

178   (DOC), silica (Si), magnesium (Mg), manganese (Mn), sodium (Na), potassium (K), iron (Fe)

179   and aluminum (Al)) were all extracted following the method described by Houba et al. (2000).

180   We added 150 mL of 0.01M $CaCl_2$ to 15 g of soil in a 250 mL glass bottle and shook this

181   mixture for two hours. Next, we centrifuged the samples for 15 minutes at 5000 rpm. The

182   supernatant was filtered through 0.7 µm glass microfiber filters and stored at 4°C prior to the

183   ICP-OES (5110 VDV radial, Agilent) analysis of P, Mg, Mn, K, Si and Na. DOC and TN

184   concentrations were determined directly after the extraction with a Torch Combustion TOC/TN

185   analyzer (Teledyne Tekmar). With a view to Fe and Al content analysis, we additionally filtered

186   10 ml of the supernatant with 0.45 µm syringe filters (Acrodisc®, Pall) and added 100 µL 1M

187   HCl before the ICP-MS (Thermo Elemental X7/Perkin-Elmer© DRC-e) analysis, but the free

188   metal ions concentrations were not estimated.

189   **Scanning electron microscope analysis**

190   We carried out a scanning electron analysis to get information regarding diatoms within the soil

191   matrix (e.g. their abundance, their way of living). Additional soil samples were taken in

192   November 2017 at an agricultural grassland site (site 9, see Foets et al. 2020). We carefully

193   placed a part of the soil samples on aluminum stubs and analyzed them with a Quanta 200 Field

194   Emission Gun Scanning Electron Microscope (FEG SEM) (Phillips-FEI). In addition, the

195   microscope was equipped with a Genesis XM 4i Energy Dispersive Spectrometer (EDS) system

196   for chemical analysis. For complementary EDS analysis, we used a large field detector, as our

197   examination was done in a low-vacuum environment (150 Pa).

198   **Statistical analyses**

199   Based on meteorological and discharge datasets, we calculated two soil moisture availability

200   proxies for the Attert River basin. First, we inferred daily water storage deficits (SD) from water

201   balance calculations as per Pfister et al. (2017). Second, we calculated daily differences between

202   precipitation input and potential evapotranspiration loss (PET). We used the FAO Penman-

203   Monteith equation for calculating PET (Allen et al., 1998). For these calculations, we assumed

204   the soil heat flux to be zero and the psychrometric constant equal to 0.065.

205   Correlations between continuous environmental variables and between pigment concentrations

206   were assessed using Spearman rank correlation. One of each correlated (i.e. $R^2 > 0.5$ or < -0.5)

207   environmental variable was retained for further discriminant analyses (see results section). Also,

208   possible spatial autocorrelation of the pigment measurements was assessed with a Mantel test.

209   For doing that, pigment data were transformed to a dissimilarity matrix (Bray–Curtis) and tested

210   if there was significant correlation (perm-999) with the geographical distance matrix. The latter

211   was obtained using the *rdist.earth* function from the R-package *fields* (Nychka et al., 2017).

212   Before carrying out the indirect and direct ordination analysis, pigment data was Hellinger-

213   transformed. Detrended correspondence analysis (DCA) on the pigment data revealed a gradient

214   length smaller than 2.0 S.D. (standard deviation) (Hill & Gauch, 1980). Therefore, we relied on a

215   redundancy analysis (RDA) as an exploration method.

216   We used the variation partitioning technique (Legendre, Borcard & Peres-Neto, 2005) to

217   quantify the relative contributions of three matrices of variables (soil chemistry, meteorology,

218   and site characteristics) to the explained variance and to test whether and to what extent their

219   contributions to the composition of the pigment concentrations could be separated. Testable

220   fractions were analyzed with RDA. For testing our first hypothesis, we carried out a multiple

221   regression analysis on the fucoxanthin data to identify the variables that are significantly related

222   to diatom abundances. A generalized mixed model (*lmer* function) was formed with 'site' as a

223   random variable to account for repeated measurements. Prior to model building, fucoxanthin

224   concentrations were log-transformed (x + 0.0001) to get normal distributed data and

225   environmental variables were rescaled. We did variable selection with backward elimination

226   using the *step* function from the *lmerTest* package (Kuznetsova, Brockhoff, Christensen, 2017).

227   Afterwards, the residuals of the final model were checked. For testing our second hypothesis, we

228   calculated diatom biovolume by multiplying the raw diatom community data given in Foets et al.

229   (2020) with the species size measurements given in Rimet & Bouchez (2012) and Omnidia

230   (version 6.0.8, 2018) (Lecointe, Coste & Prygiel, 1993). Next, we tested the variability of

231   biovolume and pigment concentrations over qualitative variables (e.g. month, soil and habitat

232   type) with analysis of variance (ANOVA), whilst accounting for repeated measurements, or the

233   non-parametric Kruskal-Wallis test. Homoscedasticity was checked with the Breusch-Pagan test.

234   Thereafter, we analyzed more specifically the significant results by relying on Tukey's range or

235   the pairwise Wilcoxon test, respectively. All aforementioned statistical analyses were performed

236   using the R statistical program (R v. 3.5.3.; http://www.r-project.org/) and additional functions

237   from the R-package *vegan* version 2.5-5 (Oksanen et al., 2019).

238   **Results**

239   **Soil Matrix**

240   Diatoms live at the top of the soil surface and with 15 cells per approximately 0.2 mm² (= 7500

241   cells cm⁻²) diatoms are relatively abundant even on a small area such as shown in Fig. 2B. The

242   occurring species are mainly *Nitzschia pusilla* (Kützing) Grunow emend. Lange-Bertalot,

243   *Sellaphora nana* (Hustedt) Lange-Bertalot, Cavacini, Tagliaventi & Alfinito and *Hantzschia*

244   *amphioxys* (Ehrenberg) Grunow. Bacilli-shaped bacteria are also present at the soil surface (Fig.

245   2C). However, because they are so small, it is impossible to see whether the bacteria are

246   cyanobacteria or not. Green algae are absent on the picture. This general observation is supported

247   by the average pigment concentrations measured on that particular site (site 9), which indicate

248   that diatoms on average (9.0 µg L^{-1}) are more abundant than green algae (2.6 µg L^{-1}) and

249   cyanobacteria (0.7 µg L^{-1}).

250   Besides the topography, the SEM picture also gives an idea of the chemical composition of the

251   top layer (Table 2). The contrast of the picture is based on the convention that "heavier"

252   elemental areas (= higher atomic number) are shown in light grey and low atomic number

253   elements in darker grey. Our subsequent analysis showed that the percentage of carbon and

254   oxygen in *H. amphioxys* is much higher than at the soil surface (difference of respectively ± 100

255   and 50%), while the silica concentration is approximately the same. Interestingly, the aluminum

256   concentration at the soil is high (11.0%) compared to the other elements and around a third of

257   that percentage is found in the diatom (3.4%). In addition to the composition mode, chemical

258   maps could be made displaying the chemical contrast in a colored pixel map (Fig. 2G - I). This

259   means that the higher the elemental concentration is, the higher the colored pixel density will be.

260   Since oxygen and silica had the highest concentrations, they gave the best pixel maps of all

261   present chemical elements. While the map of silica is only able to show us roughly the contours

262   of *H. amphioxys*, some differences in the external valve structure are visible on the oxygen map.

263   **Controlling environmental variables**

264   Chlorophyll a (cf. primary production (Table 1)) had the highest average concentration over the

265   sampling campaign ($12.7 \pm 13.5 \mu g$ L^{-1}), followed by lutein (cf. green algae) and fucoxanthin (cf.

266   diatoms), with concentrations of 5.7 ± 10.0 μg L^{-1} and 5.0 ± 8.2 μg L^{-1}, respectively. We

267   determined the lowest average concentrations in zeaxanthin (cf. cyanobacteria; 1.1 ± 1.5 μg L^{-1})

268   and diatoxanthin (0.6 ± 0.3 μg L^{-1}). However, the differences between the concentrations of

269   fucoxanthin and lutein were not significant when considering the whole dataset (Wilcoxon test, P

270   = 0.089), whereas the concentrations of zeaxanthin were significantly different for both

271   fucoxanthin and lutein (P < 0.001). This finding is confirmed when comparing the different

272   habitat types with each other (Fig. 3A). In addition, the figure shows that the type of habitat does

273   not have an influence on those three algal groups (Kruskal-Wallis, $df = 4$, P > 0.05). However,

274   diatom biovolume significantly differs between the different habitats with disturbed areas having

275   generally smaller communities than undisturbed habitats (ANOVA, $df = 4$, P < 0.001) (Fig. 3B).

276   Results of the correlation analyses showed that the environmental descriptors, physico-chemical

277   and meteorological variables were not correlated between each other (R² not higher than 0.5 for

278   all the variables). DOC and TN (R² = 0.58) and EC and moisture (R² = 0.91) were correlated.

279   Furthermore, most of the meteorological variables were correlated with each other ($R^2 > 0.5$ or <

280   -0.5) and as a result, only radiation and SD of the meteorological variables were included in the

281   RDA analysis, while K and EC were removed. Concerning the different pigments, fucoxanthin

282   was correlated with diadinoxanthin (R² = 0.64), while lutein was positively correlated with

283   zeaxanthin (R² = 0.59) and with total chlorophyll a (R² = 0.58).

284   There was no indication of spatial autocorrelation in the pigment data according to the Mantel

285   tests (P > 0.05). The total variation explained by the RDA was 40%. The first two RDA axes

286   covered respectively 40 and 5% of the total explained variation. However, only the first axis

287 resulted as significant (P = 0.001 and 0.158 respectively) after permutation test. In the 2D

288 ordination, fucoxanthin and lutein are mainly related to the first axis, which is determined by

289 radiation, TN and SD, while the other carotenoids follow more the second axis, which is

290 correlated with soil moisture content (Fig. 4). Of those mentioned variables, most exhibit a

291 strong seasonal gradient (e.g. radiation, soil moisture and SD). The Venn diagram (Fig. 5) shows

292 that meteorological variables explain in total (including joint effects) 23.7% of the total variation

293 (F = 3.80, df = 7, P = 0.02), whereas the site characteristic variables and soil chemical factors

294 explain respectively 13.3 (F = 2.88, df = 5, P = 0.01) and 9.1% (F = 2.57, df = 11, P = 0.027).

295 However, only 37.7% of the variation could be explained. Furthermore, multiple regression

296 analysis revealed that diatom productivity (log-transformed) has a strong seasonal gradient with

297 radiation, SD, and vegetation height all significant (ANOVA, F > 8.73, P < 0.05), whereas pH is

298 an important factor (F = 31.49, P < 0.001) in creating a spatial gradient. Besides, diatom

299 productivity is positively correlated with pH and vegetation height, whereas a negative relation

300 with radiation and SD exist.

301 **Spatial and temporal variability pigment concentrations and diatom biovolume**

302 Diatom abundance shows a strong seasonal variability with a peak in February and March (Fig.

303 6B), which occurred after the two wettest months and coincides with the coldest period of the

304 sampling campaign. In the subsequent spring and summer period (April-September), abundances

305 are low with an average of 3.9 µg L^{-1} (± 3.8 µg L^{-1}) per month. A slight increase is again visible

306 in November. In contrast to the diatoms, green algae show less seasonality and their

307 concentrations remain relatively high throughout the whole campaign. Furthermore, chlorophyll

308 a reaches its highest concentrations in February and March following the pattern of fucoxanthin

309 (Fig. 6C). Also, a peak occurred in June reaching on average 24.0 µg L^{-1} while experiencing a

310   precipitation of 90.8 mm that month. The lowest average chlorophyll a concentrations were

311   measured at the beginning of the campaign in December and January (2.7 and 4.1 µg L^{-1}

312   respectively).

313   Since larger diatoms generally contain more pigments, the diatom biovolume was calculated to

314   check if the temporal variability of the fucoxanthin concentration was influenced by their cell

315   size. Opposed to the fucoxanthin concentration, the diatom biovolume did not change

316   significantly during the year (ANOVA, F = 2.29, df = 11, P >0.05) (Fig. 6C), meaning that the

317   temporal variability in the fucoxanthin concentration is not related to the absolute size of the

318   diatom community. Finally, in Fig. 6D the ratio between the photoprotective carotenoids in

319   diatoms (i.e. diadinoxanthin and diatoxanthin) and the light harvesting pigment fucoxanthin is

320   given. This ratio generally follows the pattern in radiation/temperature levels during the year. In

321   summer, diatoms sometimes had twice the amount of photoprotective pigments than fucoxanthin

322   during summer time, whereas in winter the average ratio was less than or equal to one in the first

323   three months of study campaign (Kruskal-Wallis, df = 11, P < 0.001).

324   **Discussion**

325   **Soil microbial community**

326   The SEM image of the soil surface showed a diverse microbial community. Both diatoms and

327   (cyano)bacteria were present. Surprisingly, green algae seemed absent. Generally, green algae

328   are an important part of the soil algal community (Starks, Shubert & Trainor, 1981; Rindi, 2011),

329   which our pigment data also confirms. However, for that agricultural grassland site our data

330   indicated that usually diatoms are more abundant than green algae during the period the sample

331   was taken (i.e. November). Besides, species of the algal classes Xanthophyta, Rodophyta and

332   Euglenophyta could also be present on soils. However, they generally occur in low abundances.

333   Therefore, we did not expect to see them on the picture. Despite that pigment concentrations

334   could be a good estimate for algal abundances, the amount of each pigment per algal cell is also

335   dependent on its size (i.e. larger cells have a higher amount of each pigment). Overall, terrestrial

336   diatoms and green algae have similar cell sizes (Rindi et al. 2011; Ress et al. 2012), whereas

337   cyanobacteria have on average smaller dimensions. Therefore, the pigment signal is lower for the

338   same number of living cells. This could explain why there are (cyano)bacteria present whilst

339   having a lower pigment concentration than green algae. Thus, for pigment analysis, size does

340   matter and this makes it difficult to compare pigment data with algal counts.

341   **Spatial and temporal variability in relation to environmental factors**

342   In this study, we analyzed 25 environmental variables and related them to the different

343   concentrations of fucoxanthin over the entire 12-month study period. Of those 25, we found that

344   pH, radiation, SD and height of the lower vegetation were significant in explaining 45% of the

345   variability in diatom productivity. The relation with pH was positive, suggesting that diatoms

346   prefer higher pH values. This is in agreement with an earlier work by Hoffmann (1989),

347   indicating that most diatoms reach their highest abundances on neutral to alkaline soils.

348   However, our maximum soil pH extended only to 8.11, meaning that we were unable to conclude

349   on an optimum occurring or not. Regarding radiation and SD, both variables can serve as a proxy

350   for soil moisture availability. The negative relation between those factors and fucoxanthin

351   concentration was therefore expected since water, which is often a limiting factor, is essential for

352   diatom survival and reproduction (Camburn, 1982; Van de Vijver, Ledeganck & Beyens, 2002).

353   We also noticed that SD was a better factor for explaining the temporal and spatial variability in

354   the concentration of fucoxanthin than precipitation and soil moisture content. This is probably

355   because it takes both variables into account. In this case, soil moisture was a point measurement

356   and does not give any information of the antecedent conditions, while precipitation (average of

357   seven days prior to sampling day) does not inform on how much water is still prevailing in the

358   upper soil layers. Finally, we found a positive relation between vegetation height and diatom

359   abundances. This could indicate that the capacity of a higher vegetation to better keep moisture

360   and protect against UV-radiation is important for diatom growth – particularly in warm and dry

361   conditions (Ress, 2012; Zhang, Lv & Pan, 2013). Despite having analyzed many different

362   environmental factors, we were still not able to explain a large part of the variation.

363   We observed maxima in the chlorophyll a concentration in winter and in June, following

364   month(s) of high precipitation. Stokes (1940) reported the same observation and considered the

365   antecedent moisture conditions as responsible for the high abundances in summer. Regarding the

366   peak during winter, he considered snow cover as being important for algal growth and

367   conjectured that a blanket of snow keeps the soil moist and warmer than the surrounding

368   atmosphere – creating favorable conditions for algal development. However, the results of Davey

369   (1991) and Grondin & Johansen (1995) refute the latter, since the disappearance of snow led

370   instead to a steep increase in the algal concentration. Although we encountered snowfall during

371   February and March, the snow layer did not prevail for long. Thus, our winter peak could be

372   rather the result of changes between the presence and absence of snow cover, so that soil algae at

373   one point in time are protected against harsh winter conditions and at another point, they are able

374   to maintain photosynthesis. An additional reason for the winter peak could be that under those

375   low light conditions soil algae maximizes their chlorophyll a content to optimize their

376   photosynthetic activity and decrease their chlorophyll content under high-irradiance conditions in

377   summer (Neidhardt et al., 1998; Quesada & Vincent, 1993; Bohne & Linden, 2002). Indeed,

378   according to Kuczynska, Jemiola-Rzeminska & Strzalka (2015) those changes are usually fast

379   since they are engaged in basic processes (e.g. photosynthesis and photoprotection), which are

380   essential for cell life. Thus, seasonality in chlorophyll a concentration is probably the result of

381   the algal growth in combination with their physiological state. In addition, chlorophyll a

382   comprises different algal groups which could respond in a different way to varying

383   environments.

384   Indeed, diatoms and green algae reacted differently to seasonal conditions. While diatoms

385   exhibited very high abundances in winter and lower abundances during summer, the abundances

386   of green algae remained high throughout the sampling period. This indicates that green algae are

387   more tolerant to dry and warm environmental conditions than diatoms. The reasons for this could

388   lie in the mucilage production and the cell aggregation ability (e.g. forming filaments) of soil

389   green algae. It is known that for green algae the extracellular matrix is well resistant against

390   periods of desiccation and could contain a considerable amount of moisture (up to 97% of the

391   total weight) (Boney, 1980; Shephard, 1987; Rindi, 2011). Furthermore, by forming filaments,

392   they support a high self-protection against water loss (Karsten & Holzinger, 2014). Although

393   many soil diatom species have developed adaptations to fluctuating moisture availabilities in

394   terrestrial habitats (including the production of a mucopolysacharide matrix), the adaptations

395   made by terrestrial green algae seem to be more efficient (Shephard, 1987; Ress, 2012).

396   Despite the fact that diatom abundances are strongly related to moisture availability, the latter

397   obviously does not affect the diversity and composition of their assemblages. Johansen (1993)

398   observed high diatom diversity in arid environments, whereas, in a related study by Foets et al.

399   (2020), diatom composition and diversity did not change significantly during the year. In

400   addition, the latter also showed that diatom communities have a strong spatial component and are

401 controlled by the amount of anthropic disturbance with larger species often being more tolerant

402 (cf. *H. amphioxys*, *Pinnularia borealis* Ehrenberg). Such observations were also visible in the

403 results of our biovolume calculations. However, there were no spatial differences in fucoxanthin

404 concentrations, meaning that overall less living diatom cells are present in disturbed compared to

405 undisturbed areas. On the contrary, the temporal variation in the measured fucoxanthin

406 concentration is not influenced by the diatom biovolume, since the results of the biovolume did

407 not show any temporal variation. Additionally, like chlorophyll, fucoxanthin has an important

408 function in light-harvesting and its concentration is therefore affected in the same way as

409 chlorophyll by different irradiance levels (Xia et al., 2013; Kuczynska, Jemiola-Rzeminska &

410 Strzalka, 2015). Because of that, we would expect the differences in the number of living diatom

411 cells between the summer and winter period to be smaller than what we would expect from the

412 results derived from the pigment analysis. Besides the fact that the size and physiological state of

413 diatoms are important for pigment concentration, we could conclude that the distribution of

414 diatom communities is mainly controlled by spatial factors related to the amount of disturbance

415 and that diatom abundances are generally linked to temporal factors related to (soil) moisture

416 availability.

417 **Recommendations and perspectives**

418 Pigment analyses proved to be a useful tool in identifying microbial communities and deriving

419 their abundances (Leavitt & Hodgson, 2001). Here, we applied the same algal extraction method

420 as described in Barragán, Wetzel & Ector (2018) for diatom community analysis. However, it is

421 recommended by the authors that only samples of bare soil or with only low presence of grass or

422 other vegetation are taken. This is because dense vegetation does not allow a proper rinsing of

423 the soil substrate and subsequently hinders the detachment of algal communities. As we followed

424   this recommendation, we believe that we equally extracted the different algal groups.

425   Furthermore, we used average diatom biovolume measurements taken from aquatic specimens

426   (including terrestrial species occurring in those habitats). However, previous studies of Van de

427   Vijver & Beyens (1997), Ress (2012) and Stanek-Tarkowska et al. (2013) all reported that cell

428   sizes could be significantly different (both larger and smaller) between terrestrial and aquatic

429   habitats. We did not incorporate this, but as those changes are not unidirectional, we believe that

430   there would only be a minimal effect on some of the results. Besides, we were not able to

431   compare our pigment concentrations with other studies, since many different preparation,

432   extraction and analytical methods are used and subsequently pigment concentrations are

433   expressed in different units (e.g. $\mu g\ L^{-1}$, $mg\ cm^{-2}$, $mg\ g^{-1}$ soil) (Leavitt & Hodgson, 2001;

434   Cartaxana & Brotas, 2003; Schagerl & Künzl, 2007; Kuczynska, Jemiola-Rzeminska & Strzalka,

435   2015).

436   Moreover, only few studies exist on the ecology of terrestrial diatoms compared to aquatic

437   diatoms. This is rather surprising, since terrestrial diatoms and other algae are arguably affected

438   more directly than aquatic algae by climatic changes and can be expected to respond in an

439   immediate way (Rindi, 2011; Ress, 2012; Souffreau et al., 2013). Therefore, future research

440   should give more attention to terrestrial microbial communities, certainly in the light of climate

441   change.

442   **Conclusions**

443   In this study, we investigated the temporal and spatial variability of soil diatom abundances and

444   compared them with the abundances of green algae and cyanobacteria. The abundances were

445   derived from pigment analysis using HPLC. Our results supported our first hypothesis that

446   diatom abundances show seasonal succession and that their abundances are mainly controlled by

447 factors influencing the (soil) moisture availability (e.g. radiation and storage deficit). However,

448 our second hypothesis was rejected, since the effect of habitat type, which played a key role in

449 shaping the diatom communities (Foets et al., 2020) and subsequently their biovolume, was not

450 seen in the pigment concentrations. This led to the conclusion that overall less diatom cells are

451 present in disturbed than in undisturbed habitats. Contrary to diatoms, green algal productivity

452 remained stable over the course of the study period. Also, we observed that diatoms could have

453 higher abundances than green algae and cyanobacteria at periods of the year with high soil

454 moisture. Concerning future studies, more focus should be on the ecology of terrestrial diatoms

455 and other algae, since they are affected more directly than aquatic algae by climatological

456 conditions (Rindi, 2011; Hinder et al., 2012).

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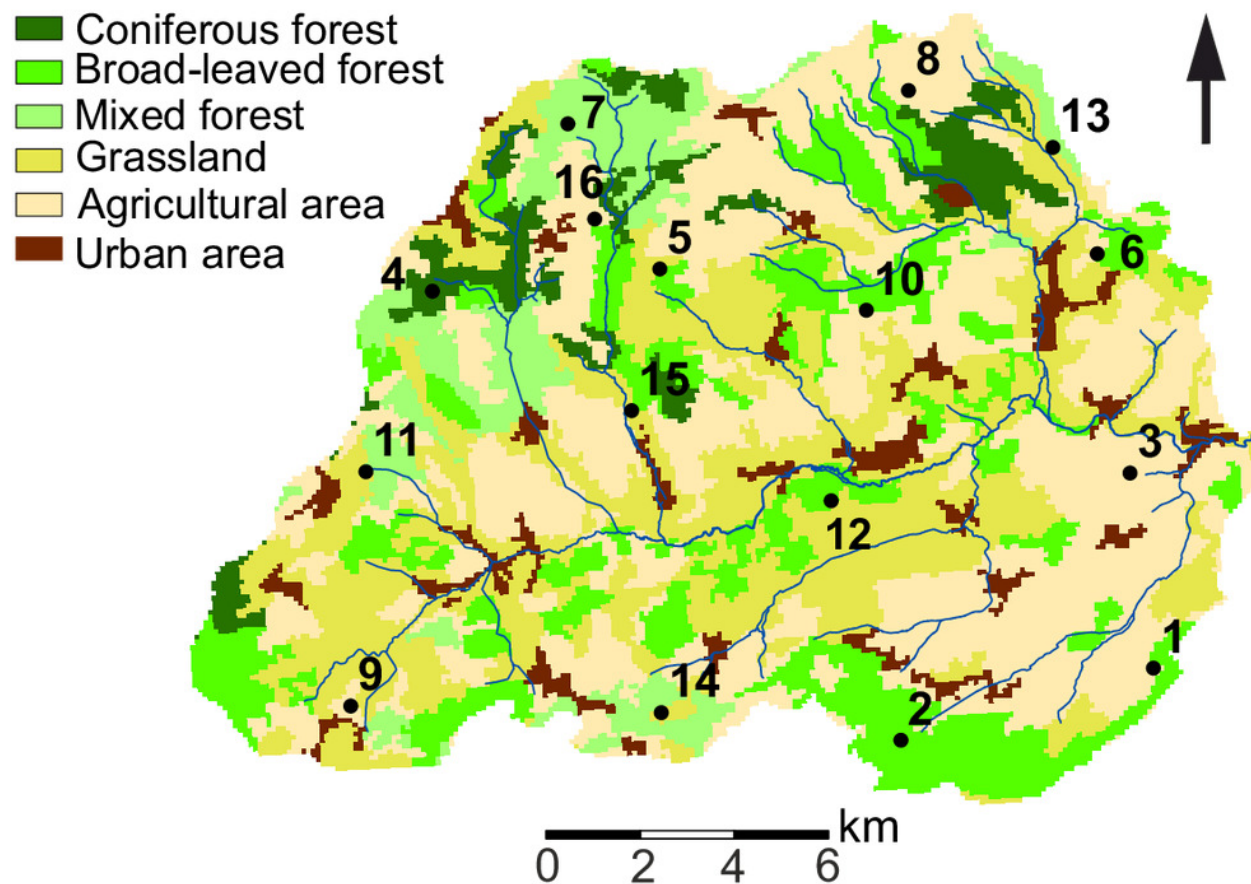
Figure 1

Land use characteristics of the Attert River Basin in Luxembourg and Belgium.

The map shows the 16 sampling locations. Source: modified from Corine Land Cover (2018).

Summary of proxies employed in this study.

Cy, Cyanophyta (cyanobacteria); E, Euglenophyta; CL, Chlorophyta (~ green algae); B, Bacillariophyceae (~ diatoms); R, Rhodophyta; X, Xanthophyceae. *, major carotenoid in most species of the class. Table derived from Verleyen et al. (2004) and Takaichi (2011).

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	Ze	Cy*, R*, B, E, Cl	Cyanobacteria production
Lutein	Lu	Cl*	Green algal production
(Dd +Da) Fx^{-1}			Mean irradiance per diatom

Element distribution for two example points.

Weight percentages are derived from the scanning electron analysis from two points indicated in Fig. 2F. 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.

Element	EDS 1 (%)	EDS 2 (%)
C	22.7	12.5
N	3.0	
O	36.2	24.5
Mg	0.4	0.8
Al	3.4	11.0
Si	24.9	26.3
P	0.4	0.3
S	0.4	0.3
K	3.2	6.1
Ca	1.4	2.9
Fe	4.0	5.5
Ti		0.8

Figure 2

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 Fig. 4 in Foets et al., 2020) sampled in November 2017. (A) Soil sample on aluminum stub. (B) SEM image. (C) Close-up of the dashed rectangle showing bacilli-shaped bacteria. (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 analyzed 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* (Hustedt) Lange-Bertalot, Cavacini, Tagliaventi & Alfinito; 2, *Nitzschia pusilla* (Kützing) Grunow emend. Lange-Bertalot; 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, E), Jean-Luc Biagi (B, F-I).

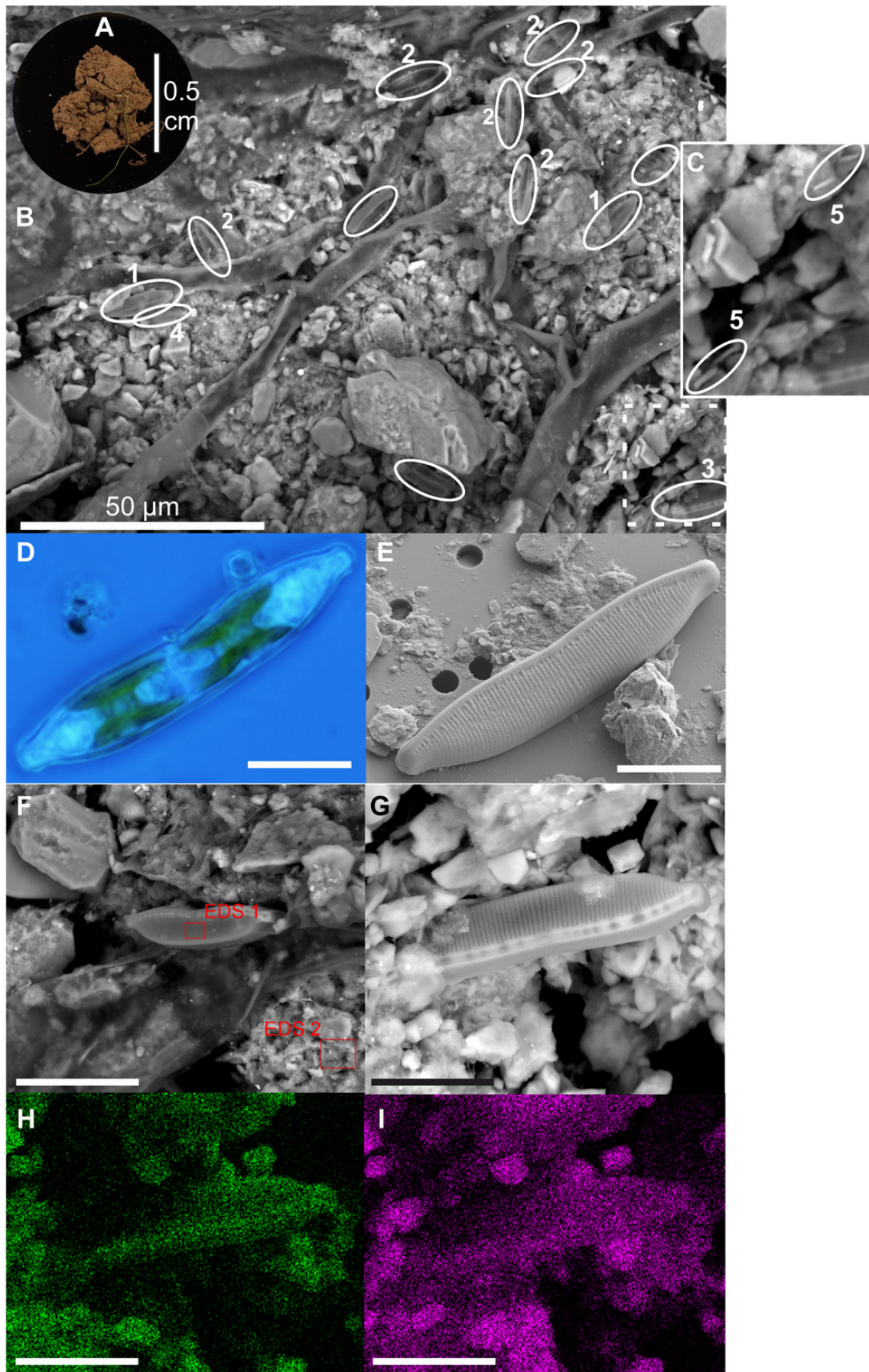



Figure 3

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 & 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).

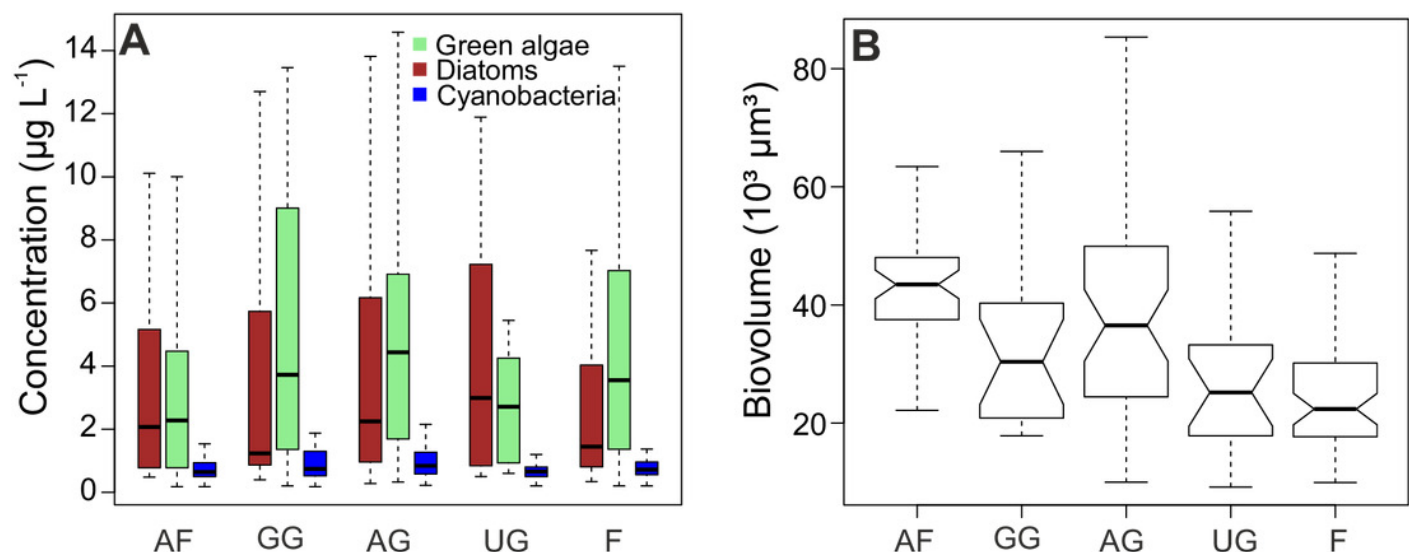



Figure 4

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; Fe, iron; TN, total nitrogen; SD, storage deficit; P, phosphorus; Al, Aluminum; radiation, global radiation; moisture, soil moisture content.

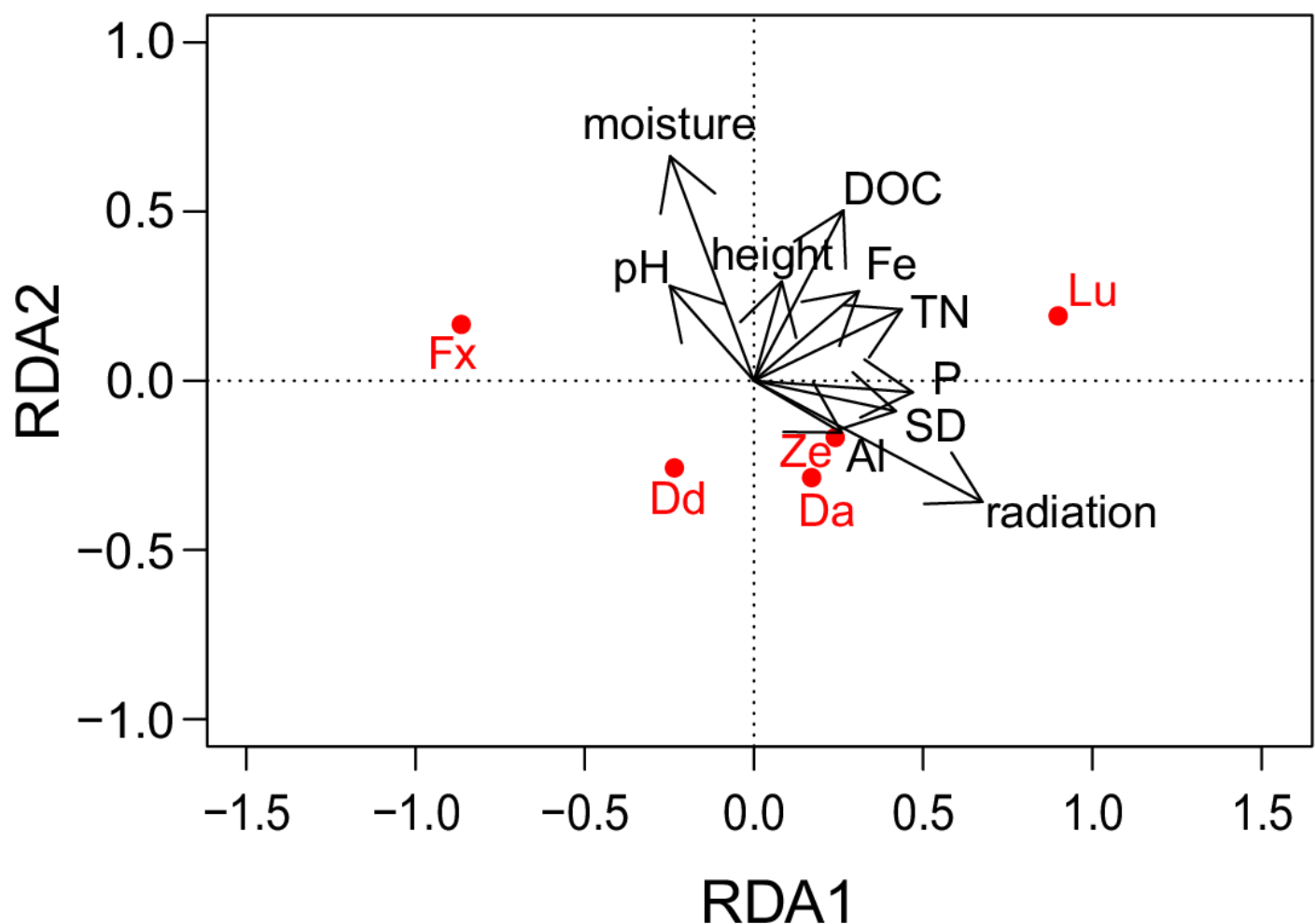



Figure 5

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 frozen 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.

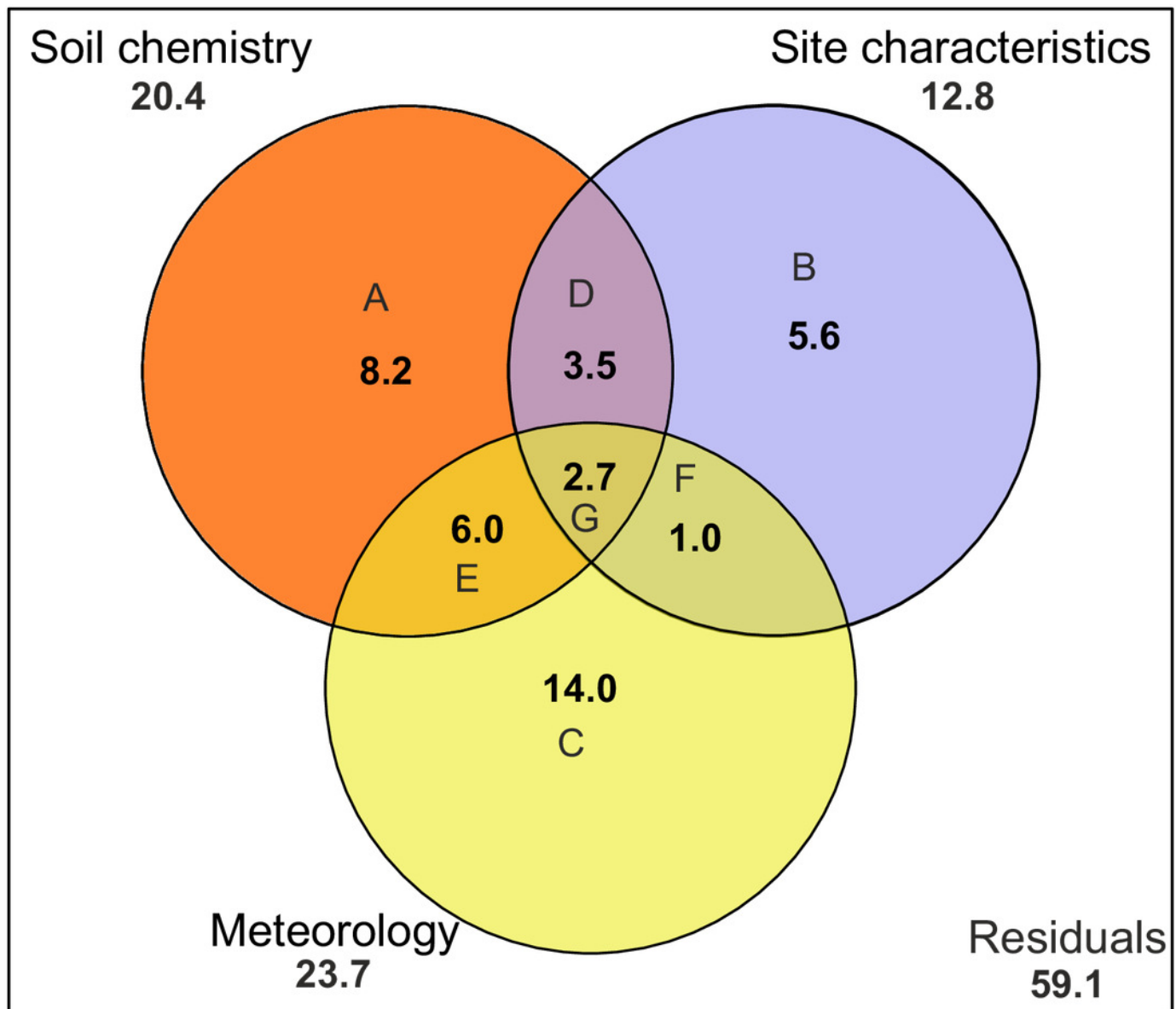



Figure 6

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 algal and diatom abundances. (C) Total algal production (TChla) and diatom biovolume. Diatom biovolume has been calculated according to Rimet & Bouchez (2012). (D) Temporal variability of the ratio between the photoprotective (Da+Dd) and light-harvesting (Fx) pigments of diatoms.