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Article

# Temporal Evolution of Diatoms in a Temporary Pond Situated in the Massif du Sancy Mountains (Massif Central, France) and Description of a New *Pinnularia* Species

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**Abstract:** A floristic survey was performed on a temporary pond, “Laqui du Seignavoux”, situated in the Mont-Dore massif of the French Massif Central, between 2017 and 2019. Except in 2017, each year, we performed four sampling campaigns from April/May to December. Water samples and diatoms were collected. The temporary pond evolved from a poorly mineralized ecosystem due to snow, inducing oligotrophic and oligosaprobic water, well oxygenated in spring, to a more mineralized, less oxygenated, eutrophic–hypereutrophic, and polysaprobic environment in summer. This change in abiotic conditions was found to be linked to the presence of cattle, beginning in May, that trample and excrete in the pond, leading to higher ammonium and nitrate concentrations. During this period, the dominant species were *Pinnularia* sp., *Nitzschia palea*, and *Nitzschia palea* var. *tenuirostris*. In spring and winter, different species of *Eunotia* dominated the community such as *Eunotia pseudogroenlandica*. Finally, light microscopy (LM) and scanning electron microscopy (SEM) observations on a new species of the genus *Pinnularia* from the temporary pond are presented and the ecological preferences are discussed.

**Keywords:** diatom communities; epipellic and epiphytic diatoms; mountain habitat; temporary pond; new species

## 1. Introduction

Lake and pond basins are formed by various glacial, tectonic, volcanic, and even episodic (e.g., occasional landslides) processes; however, specific information on the geomorphologic origins of small lake and pond basins in high-elevation regions is rather limited despite the fact that basin morphology can control the size of water bodies, their spatial distribution pattern, catchment area, etc. [1]. Although these waterbodies only occupy a small surface area in mountainous regions, forming a very important biodiversity component in the landscape, many aspects of which have been studied extensively in the past. One of the prime interests was to test the ecological impact of several physical and chemical characteristics of mountain (between 1500 and 3000 m) and lowland lakes on their

macroinvertebrate composition. Parameters such as surface area and water depth, elevation of the water body, water chemistry, catchment geology, and vegetation were amongst the most studied [2–8]. Other studies specifically concentrated on the geology and geomorphology of this particular ecosystem in high mountains [1], on the influence of seasonality and watershed on nutrients concentration [9], and on the relative contribution of scale-dependent catchment physical elements to ecosystem formation [10]. A few studies investigated the diatom diversity in pools in different mountains all around the world, such as in Japan and the Alpine, Pyrenean, Bulgarian, and Mediterranean massifs [11–17]. These studies focused on the environmental factors controlling benthic diatom diversity using taxonomic and ecological characterization. Some of the pools were characterized by an accumulation of decayed organic matter, forming layers of peat [12,15]. All studies underlined the potential contribution of these habitats to conservation of diatom biodiversity. Despite these published studies, little attention has been paid to macroinvertebrates [6,8] and diatoms [15,17] living in temporary ponds situated in mountains. Moreover, at present, no studies have been conducted for this type of pond in the French Massif Central.

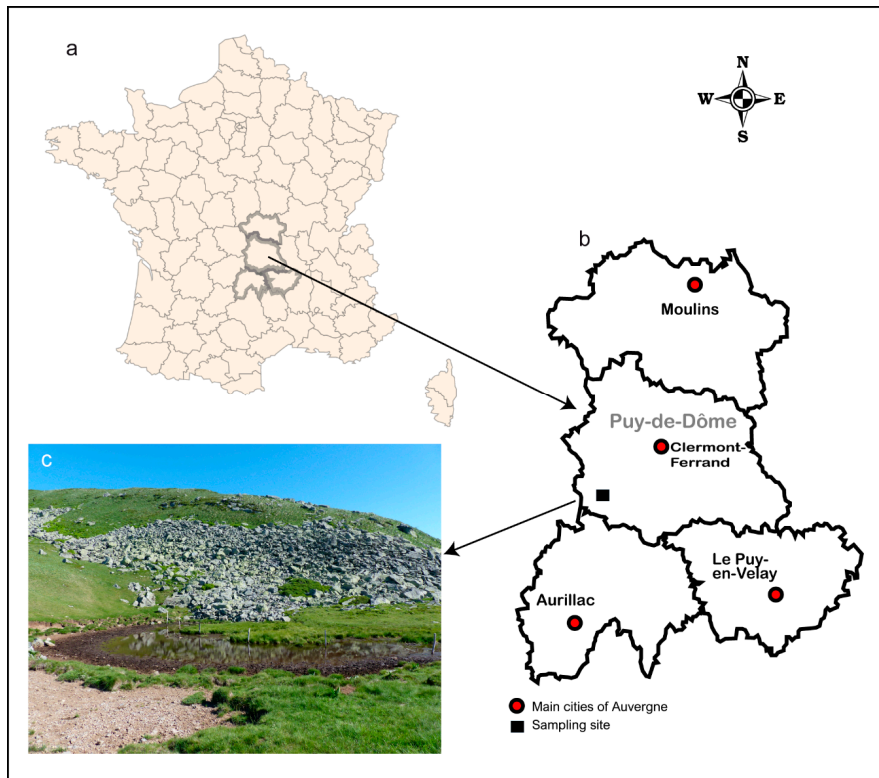
In the French Massif Central, two large stratovolcanoes are known: the Cantal and the Mont-Dore massifs. The youngest of these (Mont-Dore) covers an area of 500 km<sup>2</sup>, rests on the Variscan basement, and was active between 3.1 Ma and 200 ka [18]. Several temporary ponds are found in the Mont-Dore massif, one of them managed by a national natural reserve. The present study aims to estimate the current diatom diversity of this pond and to inform future management plans by documenting the consequences of anthropogenic pressure. Moreover, as few studies have been performed on temporary waterbodies, this study will also improve our knowledge on the algal composition and the diatom diversity in this type of habitats.

## 2. Materials and Methods

### 2.1. Study Site

The study area, “Laqui du Seignavoux”, is located in the Mont-Dore Massif (Figure 1), part of the French Massif Central, influenced by oceanic, continental, and Mediterranean climates. The area is known as the composite stratovolcano Mont-Dore, including two volcanic centers: Mont-Dore (*sensu stricto*) volcano, 2.5 to 1.6 m.y. old, in the northern part, and the more recent volcano Sancy, 0.95 to 0.25 m.y. old, in the southern part [19]. The latter is 1886 m high culminating at Puy de Sancy, the highest point of this massif. The crater of Puy de Sancy has been destroyed by erosion, lowering the volcano by 700 m. Now the volcano is composed of a system of ridges and plateaus separated by glaciated valleys, nowadays being filled with alluvium. Volcanic activities, glaciers, and erosion had created the actual relief of the massif.

On Mont-Dore Massif, the National Nature Reserve of Chastreix-Sancy was created in 2007 to protect its fragile environment. The temporary pond Laqui du Seignavoux is situated in this area, at an altitude of 1614 m a.s.l. (686973.747 E; 6490185.229 N in Lambert 93), and is continuously filled by precipitation and melting snow. The pond, with a surface area of around 500 m<sup>2</sup> and a depth of less than 1 m, is entirely snow-covered in winter (generally until April). From the beginning or middle of July (depending on the levels of precipitation), the pond dries out completely, only to be refilled in October with the return of rainy periods (Figure 2). From May until September, the land use in this part of the catchment is mainly dominated by grazing cattle that use this pond as a drinking place and as a result heavily trample half of its surface (Figure 2b).



**Figure 1.** Map of the studied site. (a,b) General location map of the study area where Laqui du Seignavoux is located (square); (c) photograph of the Laqui du Seignavoux in June 2017.



**Figure 2.** Field survey performed in April (a), July (b), November (c), and December (d) 2018.

## 2.2. Diatom Sampling and Physical and Chemical Analysis

The sampling site was georeferenced using a DGPS Geo7x (Trimble, Sunnyvale, CA, USA) in Lambert 93. Each month, several parameters were measured in situ: conductivity ( $\mu\text{S cm}^{-1}$ ), pH (pH unit), and water temperature ( $^{\circ}\text{C}$ ), using a multi-parameter WTW probe FC Multi 340i and dissolved oxygen (% and  $\text{mg L}^{-1}$ ) using a ProODO oxygen probe (Ysi, Yellow Springs, OH, USA). In addition, carbonate concentration ( $\text{HCO}_3^-$ ) was measured using a carbonate kit (Hach, Loveland, CO, USA). Additionally, during each campaign, a water sample was collected for further chemical analysis in the laboratory. After being filtered, the samples were analyzed using a high pressure ion chromatography method. For the cations analysis, a Thermo Scientific Dionex ICS1100 system was used, and for the anions, we used a Thermo Scientific Dionex Aquion system (Thermo Fisher Scientific, Courtaboeuf, France). The concentrations in different ions ( $\text{mg L}^{-1}$ ) were measured: lithium, sodium, ammonium, potassium, magnesium, calcium, fluoride, chloride, nitrite, nitrate, phosphate, sulphate, and carbonate.

An epipellic sample was taken in Laqui du Seignavoux on 19 June 2017. The sediments were recovered by scraping off the first millimeters of mud directly with the vial. In 2018 and 2019, four extra samples were taken: one in spring, one in summer (before drying up), and two in autumn (after the first precipitation event and after a first period of snow) (Table 1; Figure 2). During the surveys, diatoms were also recorded on macrophytes by washing them when they were present: in July and November 2018, and May and October 2019. In December 2018, it was impossible to sample diatoms due to the complete ice covering of the lake. In July 2019, following a drought, only a little water remained in the pond.

**Table 1.** Sampling dates, analyses done on the samples, and substrate collected for diatoms.

Sampling Dates	Diatom Sample	Water Sample	Substrate Collected
06/19/2017	Yes	Yes	Mud
04/21/2018	Yes	Yes	Mud
07/12/2018	Yes	Yes	Mud and macrophytes
11/08/2018	Yes	Yes	Mud and macrophytes
12/12/2018	No	Yes	Mud
05/16/2019	Yes	Yes	Mud and macrophytes
07/10/2019	Yes	Yes	Mud
10/07/2019	Yes	Yes	Mud and macrophytes
11/29/2019	Yes	Yes	Mud

## 2.3. Slide Preparation and Microscopy

Samples were prepared for light microscopy (LM) and scanning electron microscopy (SEM) observations following the method described in Prygiel and Coste [20] by cleaning a small sub-sample of epipellic and epiphytic raw material with hydrogen peroxide ( $\text{H}_2\text{O}_2$ , 35% *v/v*; Caldic France, Cournon d'Auvergne, France) and hydrochloric acid ( $\text{HCl}$  37% *v/v*; Fisher Scientific, Loughborough, England). The cleaned material was rinsed several times and the subsequently diluted with distilled water to avoid excessive concentrations of diatom valves on the slides. Finally, a drop of the diluted cleaned material was dried on coverslips and mounted in Naphrax. LM observations and morphometric measurements were performed using a DM2700M microscope (Leica, Wetzlar, Germany) with a 100x oil immersion objective using a differential interference contrast. Different taxonomical works were used [21–35]. The count data were converted into percentage relative abundance in respect to the total count ( $\approx 400$  valves) for each sample.

For SEM, subsamples were filtered with deionized water through a 3  $\mu\text{m}$  Isopore™ Membrane Filters polycarbonate (Merck Millipore Ltd., Tullagreen, Ireland), pieces of which were affixed to aluminum stubs and coated with platinum using a Modular High Vacuum Coating System BAL-TEC MED 020 (BAL-TEC AG, Balzers, Liechtenstein). An ultrahigh-resolution analytical field emission (FE)

scanning electron microscope, Hitachi SU-70 (Hitachi High-Technologies Corporation, Tokyo, Japan), operating at 5 kV and 10 mm working distance, was used for the analysis. SEM images were occasionally tilted up to an angle of 28°.

## 2.4. Data Analysis

### 2.4.1. Statistical Analyses on the Physical and Chemical Variables

Physical and chemical variables of the Laqui du Seignavoux were studied using principal component analyses (PCA). We did not retain the ions with a concentration below the detection limit—these being lithium and fluoride. Thus, the analysis was performed on dissolved oxygen (%), conductivity, and temperature. Pollutants were also determined such as  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , and other major ions, providing information on the geology of the area ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{HCO}_3^-$ ). Then, a focus was placed on the indicators of pollution— $\text{NH}_4^+$  and  $\text{NO}_3^-$ .

### 2.4.2. Diatoms Data Statistical Analyses

Taxa with a relative abundance higher than 1% in at least one sample were retained for further analysis in order to minimize the influence of rare taxa. Multivariate analysis was used to identify the evolution in the diatom community composition. First, detrended correspondence analysis (DCA) [36] was performed to determine the length of gradient of the first two axes, testing whether linear (<2 standard deviation (SD)) or unimodal (>2 SD) numerical techniques were appropriate [37]. For DCA, the following options were used: downweighting of rare species, rescaling of the axes, rescaling threshold of 1, number of segments 26, and number of runs 999. The preliminary DCA on all samples showed a gradient length that was 1.7 units of SD. Then, a PCA was subsequently performed to investigate species distribution [38].

Ecological traits were calculated according to Van Dam et al. [39] using OMNIDIA 6.0.8 software (Monbazillac, France) [40]. We considered trophic state and saprobity to assess the evolution of the community and the impact of the cattle. The analyses were performed using PC-ORD 7 software (Glenneden Beach, OR, USA) [41].

## 3. Results

### 3.1. Physical and Chemical Characteristics

Physical and chemical data obtained between 2017 and 2019 accounted for 76.4% of the cumulative percentage variance for PCA axes 1 and 2. The sample taken in July 2018, and also the one taken in July 2019 to a lesser extent, were situated in the right part of the first factorial plan. These samples were more mineralized with a high ammonium concentration. Then, a second PCA analysis was performed without the July 2018 sample, accounting for 68.9% of the cumulative percentage variance for PCA axes 1 and 2 (Figure 3). This analysis grouped, in the right part of the first factorial plan, the samples taken in April and December 2018 together with the samples taken in May, October, and November 2019. During these periods, the water was poorly mineralized and well-oxygenated. In the left part, all samples characterized as highly mineralized were positioned. In this group, June 2017 and July 2019 were apart due to high ammonium and carbonate concentrations and also high conductivity. At these periods, cattle were present at the pond. The sample taken in November 2018 was located in the upper and left part of the first factorial plan and was associated with a high nitrate concentration (Figure 3).

Conductivity and ammonium concentrations were very high in July 2018 ( $313 \mu\text{S cm}^{-1}$  and  $24 \text{ mg L}^{-1}$ , respectively) compared to June 2017 and July 2019 (Figure 4; Table 2) when the presence of cattle in and near the pond was observed. The nitrate concentration was the highest in November 2018 (reaching  $17 \text{ mg L}^{-1}$ ; Figure 4; Table 2).

Due to the presence of snow on the Laqui du Seignavoux at the end of the winter season (April 2018 and May 2019), the water contained only a few dissolved mineral elements. Oxygenation was high

(>65%), pH was slightly acidic, and the temperature was low (Table 2). Then, in summer, all values of the different ion concentrations and the water temperature increased, except for the nitrate concentration in July 2018. Finally, following several snowy episodes at the end of November, a return to the conditions observed in April and May, including a low water temperature, was noted (except in November 2018 when nitrate concentration was high).

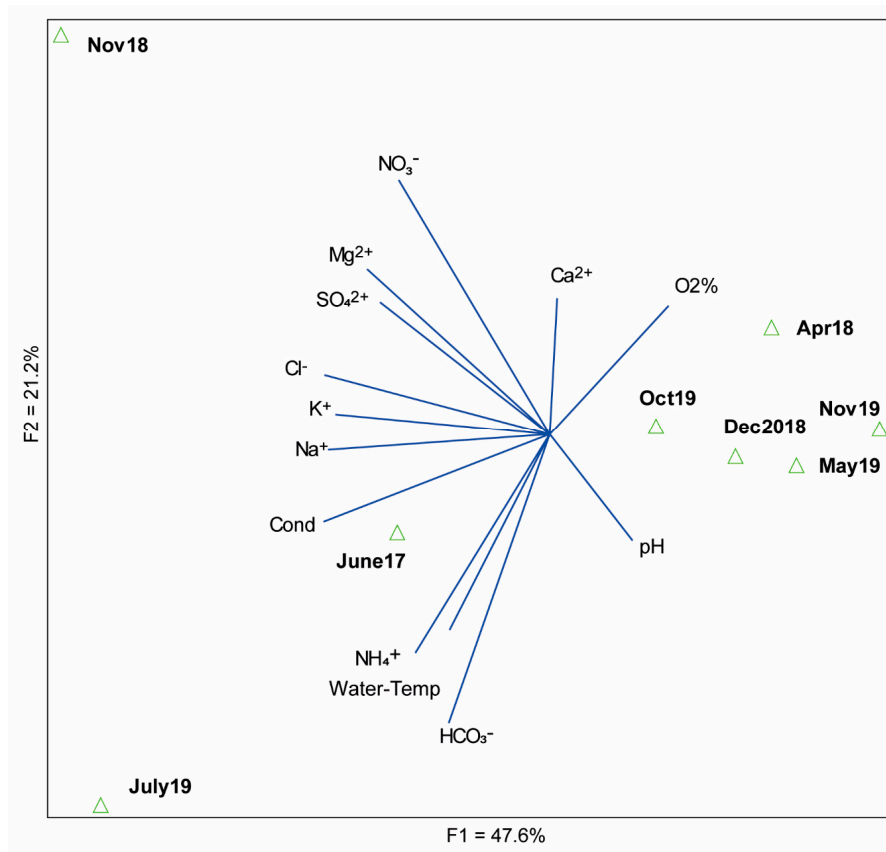


Figure 3. Principal component analysis performed on physical and chemical data.

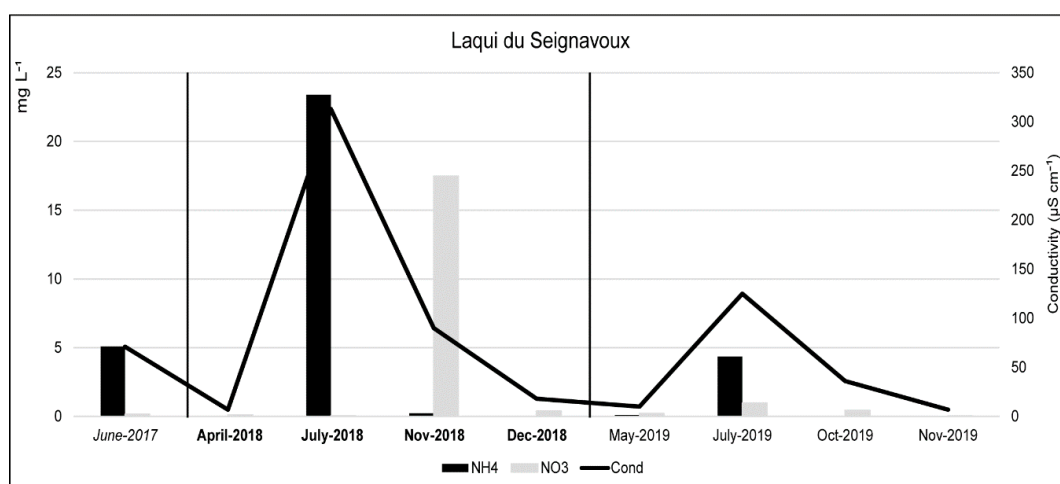


Figure 4. Concentrations of ammonium (mg L<sup>-1</sup>), nitrate (mg L<sup>-1</sup>), and conductivity (µS cm<sup>-1</sup>) measured in Laqui du Seignavoux for each campaign in 2017, 2018, and 2019.

**Table 2.** Concentrations (mg L<sup>-1</sup>) of the different ions measured for each sampling date (Water-T: water temperature in °C; Cond: conductivity in µS cm<sup>-1</sup>) (in bold: the highest concentrations and values).

Date	Na <sup>+</sup>	NH <sub>4</sub> <sup>+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	Cl <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	SO <sub>4</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>	Cond	pH	O <sub>2</sub> %	Water-T
06/19/2017	1.58	<b>5.09</b>	2.08	1.10	2.81	5.79	0.16	<0.04	3.93	20.0	<b>71</b>	6.25	<b>91</b>	<b>20.4</b>
04/21/2018	0.50	0.02	0.39	0.31	4.22	2.69	0.12	<0.04	0.92	8.3	7	6.03	65.4	1.4
07/12/2018	9.04	<b>23.40</b>	<b>57.41</b>	<b>1.72</b>	<b>9.03</b>	<b>50.88</b>	0.05	<0.04	<b>10.48</b>	<b>108.0</b>	<b>313</b>	7.04	3.8	<b>23.2</b>
11/08/2018	5.24	0.23	21.55	1.50	2.92	19.23	<b>17.47</b>	<0.04	5.16	3.2	<b>90</b>	6.34	69	4.8
12/12/2018	1.44	0.01	2.45	0.34	4.44	1.88	0.40	0.05	1.11	20.0	18	<b>7.7</b>	54.9	0.7
05/16/2019	0.68	0.11	1.62	0.24	1.01	0.92	0.22	<0.04	0.39	7.5	10	6.7	<b>93.1</b>	13.6
07/10/2019	5.69	<b>4.36</b>	20.63	0.50	1.00	<b>15.03</b>	<b>0.99</b>	<0.04	1.94	35.6	<b>125</b>	7.04	25.4	16.9
10/07/2019	1.48	0.01	5.03	0.44	2.32	2.51	0.43	0.06	3.66	13.2	36	7.57	<b>96</b>	12.1
11/29/2019	0.59	0.01	0.75	0.20	0.47	0.69	0.03	<0.04	0.21	3.6	7	<b>7.66</b>	88	1.3

### 3.2. Diatom Composition and Diversity

A total of 79 diatom species belonging to 28 genera was observed during all counts. The genera *Eunotia* (13% of all counted valves), *Gomphonema* (7%), and *Pinnularia* (63%) accounted for almost 83% of the entire community. On the basis of species richness, the genera *Eunotia* (12 taxa), *Gomphonema* (12 taxa), and *Pinnularia* (10 taxa) were the most diverse (Table 3).

**Table 3.** Dominant genera and the species associated and their percentage considering all counted valves (in bold: the highest percentages > 1%).

Genus	Taxa	% of All Counted Valves
EUNOTIA	<i>Eunotia bilunaris</i> (Ehrenberg) Mills	<b>3.10</b>
	<i>Eunotia bilunaris</i> (Ehrenberg) Mills abnormal form	0.04
	<i>Eunotia cisalpina</i> Lange-Bertalot & Cantonati in Cantonati & Lange-Bertalot	0.06
	<i>Eunotia curtgrundowii</i> Nörpel-Schempp & Lange-Bertalot	0.02
	<i>Eunotia exigua</i> (Brébisson ex Kützing) Rabenhorst	<b>1.77</b>
	<i>Eunotia groenlandica</i> (Grunow) Nörpel-Schempp & Lange-Bertalot	0.10
	<i>Eunotia incisa</i> W.Gregory	0.02
	<i>Eunotia islandica</i> Østrup	0.28
	<i>Eunotia minor</i> (Kützing) Grunow in Van Heurck	0.14
	<i>Eunotia palatina</i> Lange-Bertalot & W.Krüger in Werum & Lange-Bertalot	0.02
	<i>Eunotia pseudogroenlandica</i> Lange-Bertalot & Tagliaventi in Lange-Bertalot et al.	<b>7.24</b>
	<i>Eunotia subarcuatoides</i> Alles, Nörpel & Lange-Bertalot in Alles et al.	0.06
	<i>Eunotia tenella</i> (Grunow in Van Heurck) Hustedt in Schmidt et al.	0.24
	GOMPHONEMA	<i>Gomphonema angustatum</i> (Kützing) Rabenhorst
<i>Gomphonema</i> aff. <i>acidoclinatum</i>		0.10
<i>Gomphonema clavatum</i> Ehrenberg		0.02
<i>Gomphonema drutelingense</i> E.Reichardt		0.02
<i>Gomphonema hebridense</i> W.Gregory		0.06
<i>Gomphonema</i> aff. <i>innocens</i>		<b>6.51</b>
<i>Gomphonema</i> aff. <i>insigniforme</i>		0.02
<i>Gomphonema</i> aff. <i>micropus</i>		0.04
<i>Gomphonema lagenula</i> Kützing		0.06
<i>Gomphonema parvulum</i> Kützing		0.06
<i>Gomphonema sarcophagus</i> W.Gregory		0.02
<i>Gomphonema varioeduncum</i> Jüttner, Ector, E.Reichardt, Van de Vijver & E.J.Cox abnormal form		0.02
PINNULARIA	<i>Pinnularia</i> sp.	<b>62.26</b>
	<i>Pinnularia borealis</i> Ehrenberg	0.30
	<i>Pinnularia</i> aff. <i>carminata</i>	0.04
	<i>Pinnularia</i> cf. <i>kuetzingii</i>	0.04
	<i>Pinnularia microstauron</i> (Ehrenberg) Cleve	0.04
	<i>Pinnularia persudetica</i> Krammer	0.06
	<i>Pinnularia saprophila</i> Lange-Bertalot, H.Kobayasi & Krammer	0.02
	<i>Pinnularia</i> aff. <i>saprophila</i>	0.08
	<i>Pinnularia sinistra</i> Krammer	0.08
<i>Pinnularia viridiformis</i> Krammer	0.16	



**Table 4.** Distribution of the dominant species in 2018 and 2019 (relative abundance > 1%) (M = macrophytes) (in bold: the highest percentages observed each month).

Date	2018						2019				
	Apr	July	July-M	Nov	Nov-M	May	May-M	July	Oct	Oct-M	Nov
<i>Pinnularia</i> sp.	47.7	<b>79.4</b>	<b>72.3</b>	<b>69.8</b>	<b>75.2</b>	<b>81.8</b>	<b>56.2</b>	<b>58.7</b>	<b>73.2</b>	45.0	22.0
<i>Nitzschia palea</i> var. <i>tenuirostris</i>	3.0	<b>14.0</b>	<b>10.8</b>	9.1	2.1	4.7	1.4	5.6	3.8	3.2	2.1
<i>Gomphonema</i> aff. <i>innocens</i>	5.3	0.0	2.3	6.1	0.7	2.2	<b>22.7</b>	1.9	5.5	<b>14.4</b>	3.6
<i>Psammothidium helveticum</i>	<b>18.9</b>	3.3	6.3	6.6	1.6	3.2	1.9	12.9	8.1	3.2	2.9
<i>Eunotia pseudogroenlandica</i>	<b>10.1</b>	0.0	1.6	0.0	0.0	2.0	2.1	5.8	1.9	9.5	<b>53.9</b>
<i>Eunotia exigua</i>	<b>4.6</b>	0.0	0.2	0.0	0.0	1.2	<b>4.7</b>	0.7	0.5	<b>5.6</b>	2.1
<i>Eunotia bilunaris</i>	3.2	0.0	0.0	1.5	0.2	0.5	<b>8.4</b>	0.7	1.4	9.5	9.5
<i>Cocconeis euglypta</i>	0.0	0.0	0.0	0.0	<b>7.7</b>	0.0	0.0	0.0	0.0	0.0	0.0
<i>Neidium alpinum</i>	<b>1.8</b>	0.7	0.9	0.5	0.0	1.5	0.7	1.7	2.2	1.0	0.7

The results of the counts in 2018 and 2019 allowed a further analysis of the dominant taxa. *Pinnularia* sp. dominated almost all samples in both years with values often exceeding 60% of the total valve count. In April 2018, two other taxa played a subdominant role: *Eunotia pseudogroenlandica* and *Psammothidium helveticum*. Their abundance, however, decreased sharply in the other samples, being overtaken by *Nitzschia palea* var. *tenuirostris*. The macrophyte samples (marked with “M”) were slightly more diverse, with an increase of *Cocconeis euglypta* in November 2018. In 2019, the dominance of *Pinnularia* sp. was less explicit near the end of the year when the taxon was replaced by *Eunotia pseudogroenlandica*, reaching a relative abundance value of 54% whereas *Pinnularia* sp. represented only 22%. The macrophyte samples showed higher abundances of *Eunotia bilunaris* and were further characterized by a complete lack of *Cocconeis* taxa (contrary to 2018).

Despite its high abundance, it was impossible to identify the *Pinnularia* species on the basis of the currently available taxonomic literature. A detailed morphological analysis using both light and scanning electron microscopy observations showed that this unknown species should be described as a new species—*Pinnularia seignavouxensis* Beauger, Allain & Van de Vijver sp. nov.

***Pinnularia seignavouxensis* Beauger, Allain & Van de Vijver sp. nov. (Figure 5a–y)**

Description: Frustules in girdle view rectangular, occasionally two cells connected. Valves linear with almost straight, parallel margins and distinctly protracted, capitate to subcapitate, broadly rounded apices. Occasionally, valves with broadly rounded, rostrate apices observed. Valve dimensions ( $n = 40$ ): length 25–54  $\mu\text{m}$ , width 6–9  $\mu\text{m}$ . Axial area very narrow, linear, only very slightly widening towards the central area. Central area rather small, rounded, never forming a fascia due to series of shortened marginal striae. Valves with an asymmetrical central area, characterized on one side by more distantly spaced shortened striae present. Raphe filiform with straight raphe branches. Central raphe endings clearly unilaterally deflected, teardrop-shaped enlarged, with pores relatively close to each other. Terminal raphe fissures hardly discernible in LM; in SEM, hooked towards the opposite side than the central endings, continuing onto the valve mantle. Internally, central raphe endings obscured by silica flap, giving the impression of an internal continuous raphe. Terminal endings terminating onto small helictoglossae. Striae weakly radiate near the central area, gradually becoming distinctly convergent towards the apices, 14–16 in 10  $\mu\text{m}$ . Longitudinal lines absent.

Holotype: population deposited in Herbiers Universitaires de Clermont-Ferrand, France (CLF 103302).

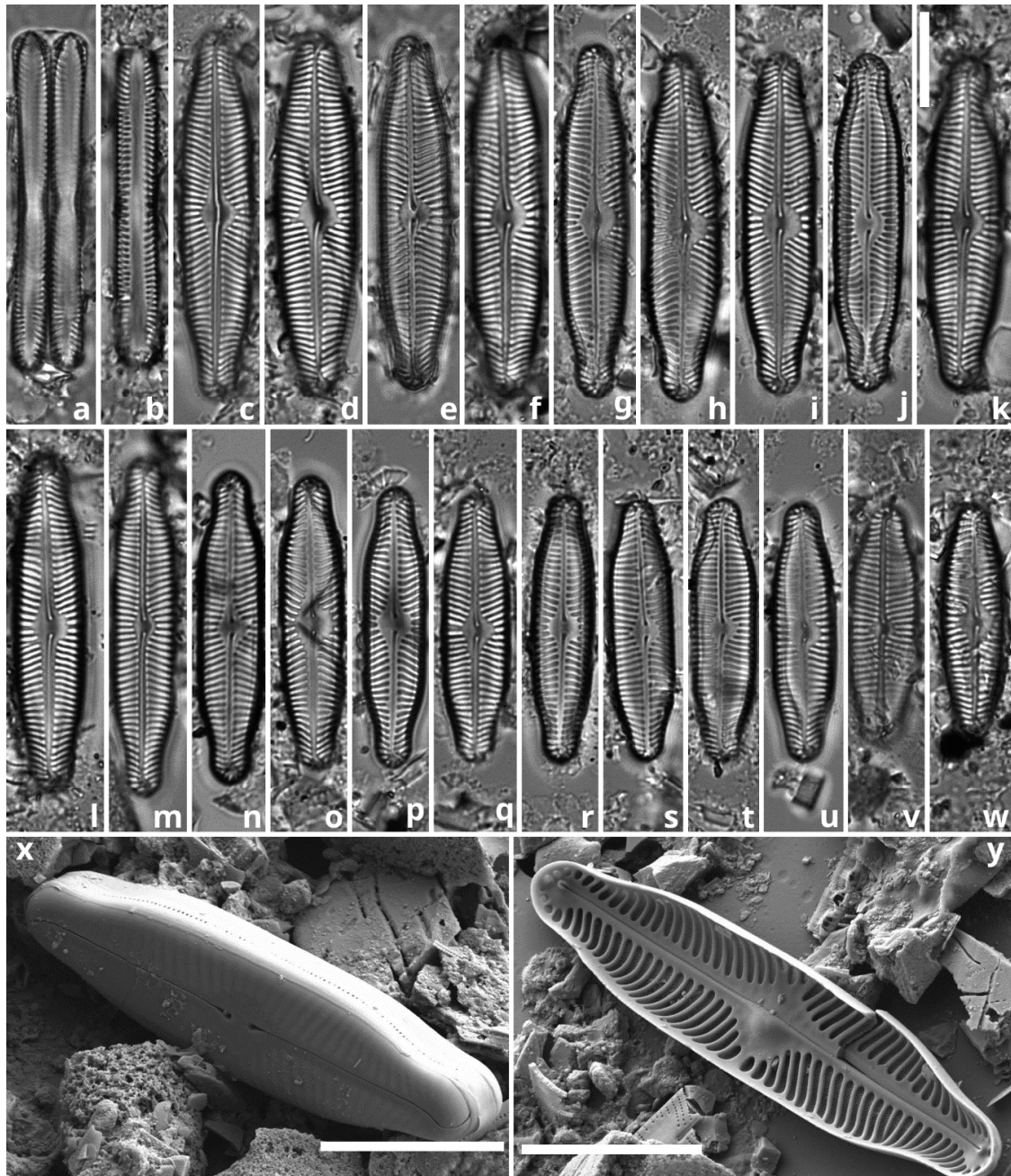
Isotype: populations deposited in Herbiers Universitaires de Clermont-Ferrand, France (CLF 103303) and Meise Botanic Garden, Belgium (BR 4600).

Type locality: Laqui de Seignavoux, (Puy-de-Dôme department, Auvergne-Rhône-Alpes region, France), 686973.747 E; 6490185.229N in Lambert 93, coll. date: 19 June 2017

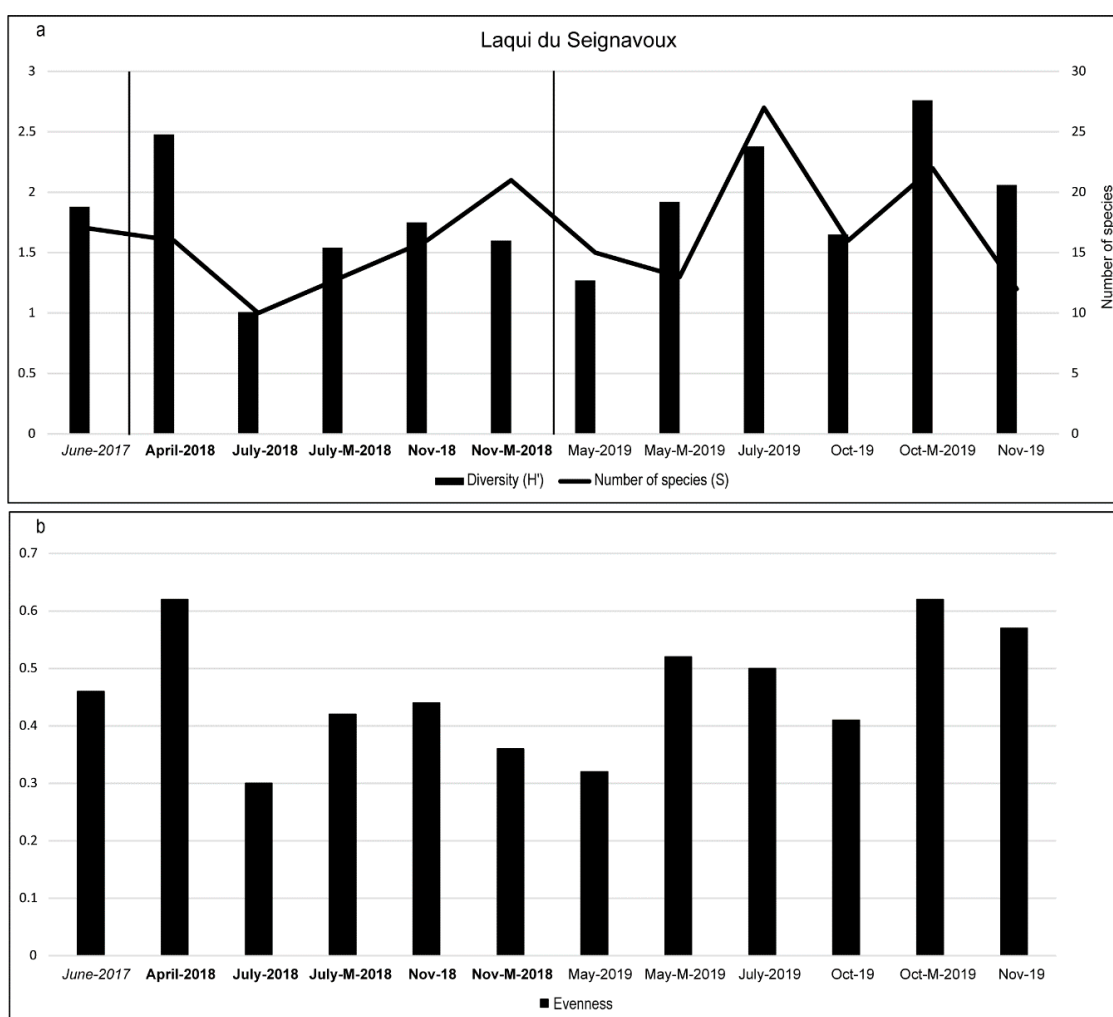
Etymology: the new species is named after the Laqui de Seignavoux pond where it was found.

An analysis of diatom diversity metrics was performed on the dataset (from 2017 to 2019), including the number of species (richness; S), Shannon–Weaver diversity ( $H'$ ), and species evenness ( $J'$ ) (Figure 6). S was the lowest in July 2018 and in May 2019 (on mud).  $H'$  was low, varying between 1.01 (July 2018) and 2.76 (October 2019), compared with S, which varied from 10 (July 2018) to

27 (July 2019) (Figure 6a). Moreover,  $J'$  ranged from 0.3 (in July 2018) to 0.62 (April 2018 and October 2019 on macrophytes), underlining that in July 2018, the community was dominated by one species (Figure 6b). The diatom communities on macrophytes appeared more diversified than the one observed on mud, regardless the sampling period, except in November 2018 (Figure 6a,b).



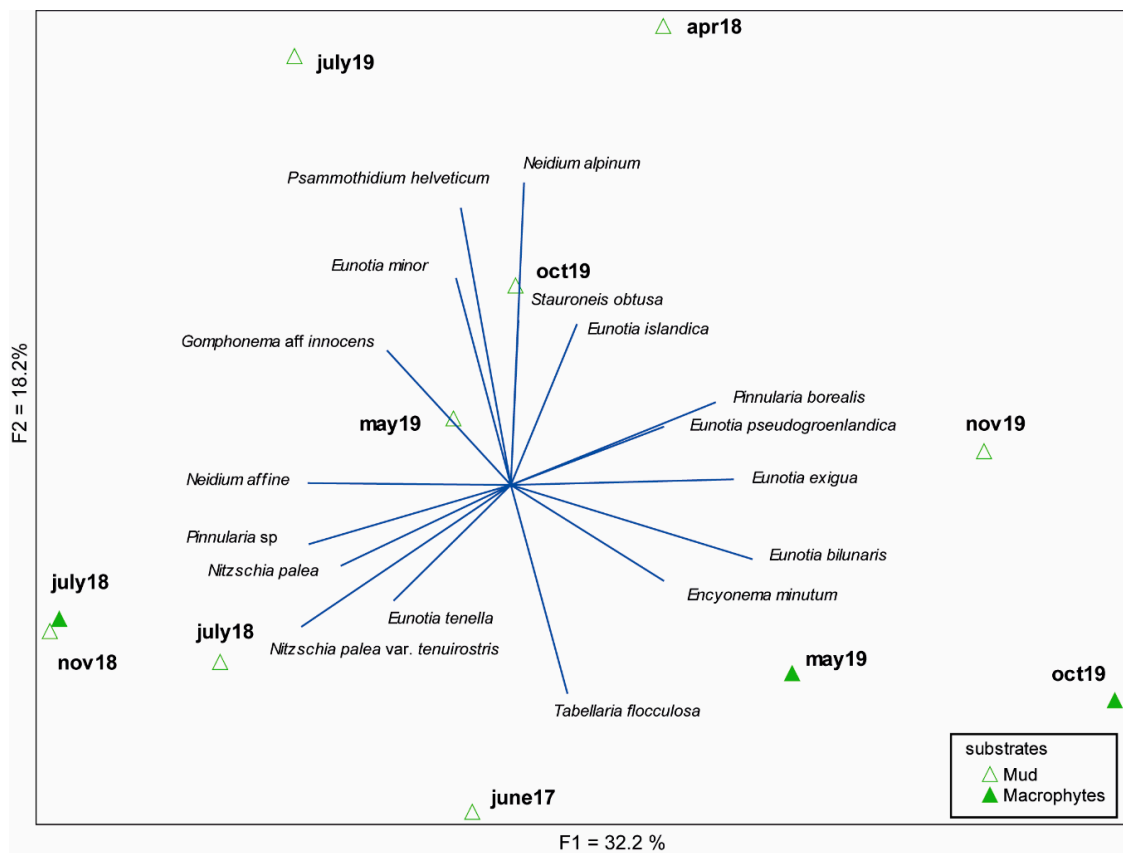
**Figure 5.** *Pinnularia seignavouxensis*, type population from Laqui de Seignavoux pond. (a–w): Light microscopy (LM) observations. (x,y): SEM illustrations of the individuals. Scale bar (white line) = 10  $\mu\text{m}$ .



**Figure 6.** (a) Shannon–Weaver diversity and number of species (richness) for each sampling period (M = macrophytes); (b) species evenness for each sampling period (M = macrophytes).

An overall PCA (Figure 7) performed using the dominant diatom species in the survey (relative abundance > 1% in at least one sample) accounted for 48.4% of the cumulative percentage variance for PCA axes 1 and 2 (eigenvalues: 5.87 and 4.78, respectively). The analysis showed that the sample collected in November 2018 from macrophytes can be considered as an outlier. The sample was dominated by several species such as *Cocconeis euglypta*, *Lemnicola hungarica*, *Sellaphora atomoides* C.E.Wetzel & Van de Vijver, and *Staurosira venter* (Ehrenberg) Cleve & J.D.Möller, which are almost absent in the other samples. Therefore, a second PCA was performed without the sample “November 2018 from macrophytes”. It accounted for 50.4% of the cumulative percentage variance for PCA axes 1 and 2 (eigenvalues: 5.79 and 3.29, respectively). The left lower part of the first factorial plan grouped the samples collected in June 2017, July 2018 (both mud and macrophytes), and November 2018 (plus May 2019). These samples were associated with *Eunotia tenella* (Grunow) Hustedt, *Neidium affine* (Ehrenberg) Pfitzer, *Nitzschia palea* (Kützing) W.Smith, *N. palea* var. *tenuirostris*, and *Pinnularia* sp. (i.e., *P. seignavouxensis*). The sample of July 2019, situated in the left upper part, was dominated by *Gomphonema* aff. *innocens*, *Psammothidium helveticum*, and *Eunotia minor* (Kützing) Grunow in Van Heurck. On the right lower part, the samples of May and October 2019, taken on macrophytes, were grouped and associated with *Encyonema minutum* (Hilse) D.G.Mann, *Eunotia bilunaris*, *E. exigua*, and *Tabellaria flocculosa* (Roth) Kützing. Finally, the samples of April 2018, October 2019, and November 2019 were associated with *Eunotia islandica* Østrup, *E. pseudogroenlandica*,

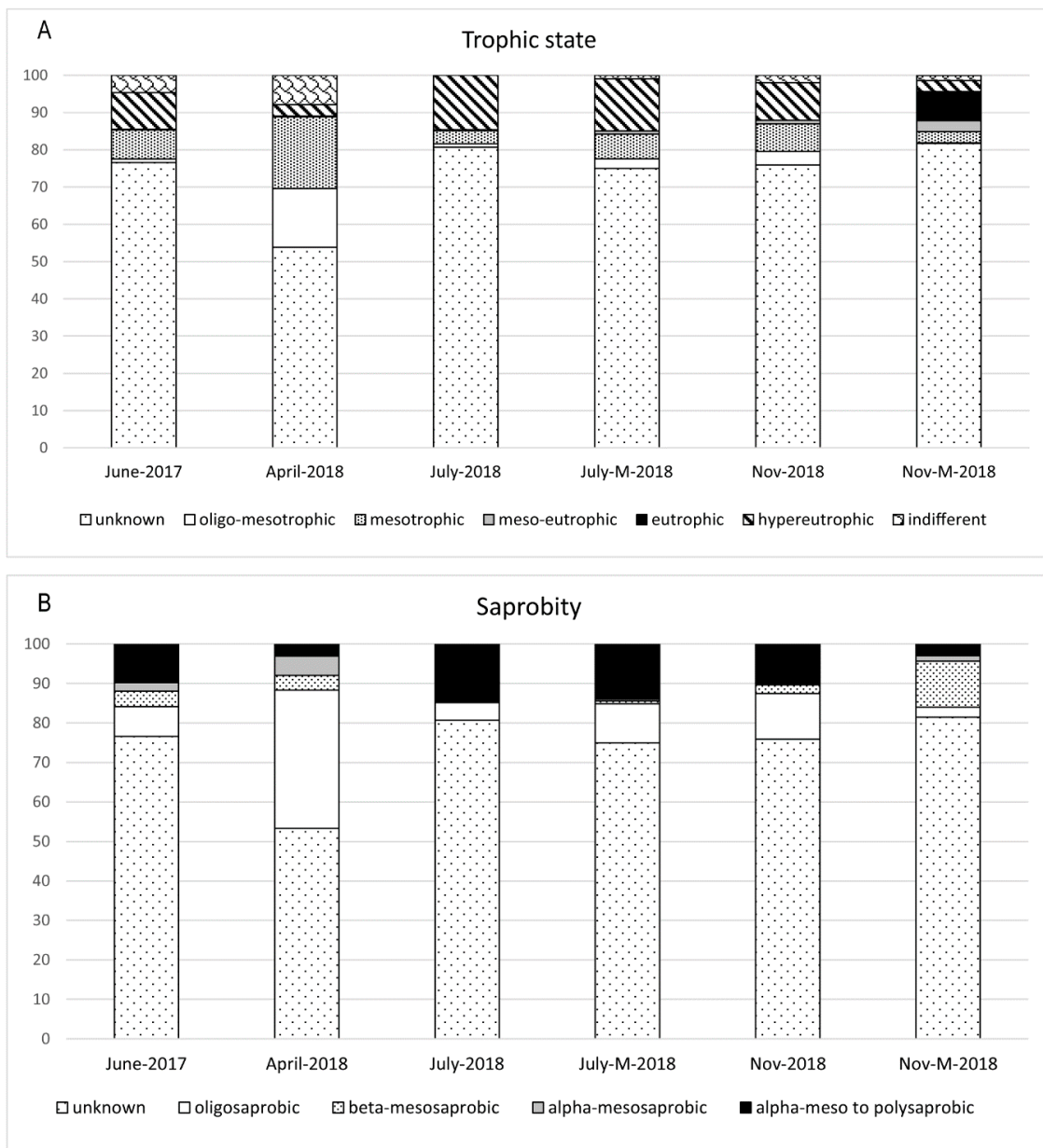
*Neidium alpinum*, *Pinnularia borealis* Ehrenberg, and *Stauroneis obtusa* Lagerstedt on the right upper part of the first factorial plan.



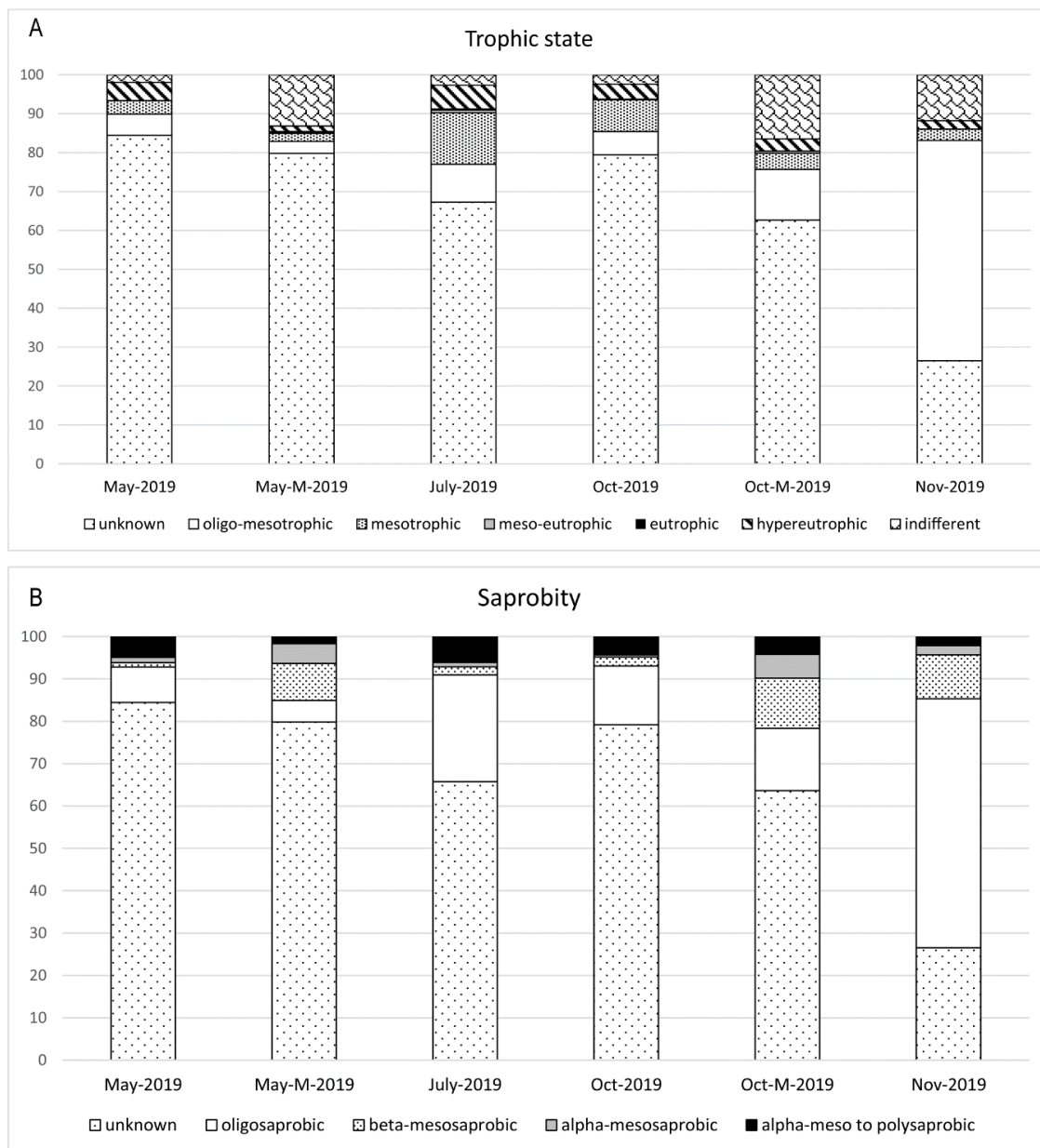
**Figure 7.** Principal component analysis performed on diatoms observed at each sampling period (relative abundance > 1% in at least one sample).

The ecological traits trophic state and saprobity underlined differences between the seasons. However, in the Laqui du Seignavoux, as the dominant species *Pinnularia seignavouxensis* is a newly described taxon with at present no determined ecology, the highest percentage was associated with “unknown” ecology.

In 2017, more than 10% of the counted taxa belonged to hypereutrophic and alpha-mesosaprobic to polysaprobic species (Figure 8A). In 2018, most oligotrophic species present in April decreased and were replaced by eutrophic and hypereutrophic species in July and November (>10%) (Figure 8A). During these months, an increase of alpha-mesosaprobic to polysaprobic species (>10%) (Figure 8B) was also observed. In 2019, eutrophic and hypereutrophic species increased in July (6.1%), whereas they represented only 4.68% and 1.41% in May, respectively, considering respectively mud and macrophytes) (Figure 9A). These percentages decreased in October (3.8 and 3.2% respectively) then 2.2% in November. For saprobity (Figure 9B), the same pattern was observed as for the trophic state.



**Figure 8.** Ecological traits retained: trophic state (A) and saprobity (B) obtained in 2017 and 2018 (M = macrophytes).



**Figure 9.** Ecological traits retained: trophic state (A) and saprobity (B) obtained in 2019 (M = macrophytes).

#### 4. Discussion

The “Massif du Sancy” and more precisely the National Natural Reserve of Chastreix-Sancy is a protected area with rare and endangered diatom species [42]. In this reserve, the temporary pond Laqui du Seignavoux forms a particular habitat in the nature reserve where cattle are present in spring and summer when it is often dry. The pond, therefore, forms an extreme habitat for diatoms as they have to support various important stress factors such as freezing during winter months and desiccation combined with high temperatures in summer. A study on the survival capacities of 34 terrestrial and aquatic diatom species to freezing, heating, and desiccation underlined that vegetative cells are very sensitive to desiccation and freezing, with habitat-dependent tolerance to temperature extremes [43].

The surveys performed between 2017 and 2019 allowed not only the assessment of the diatom community at different seasons but also documented the impact of human activities in June 2017, July 2018, and July 2019. The entire study period was dominated by a new *Pinnularia* species, *P. seignavouxensis* Beauger, Allain & Van de Vijver. On the basis of a comparison analysis, the new

species can be separated from similar *Pinnularia* species. *Pinnularia seignavouxensis* shows a clear resemblance to *Pinnularia microstauron* var. *nonfasciata* [26], mainly due to its similar central area, but can be distinguished by its distinct lower valve width (6–9  $\mu\text{m}$  in *P. seignavouxensis* compared to 8–11  $\mu\text{m}$  in *P. microstauron* var. *nonfasciata*), a considerably higher stria density: 14–16 in 10  $\mu\text{m}$  for the new species whereas only 11–13 striae in 10  $\mu\text{m}$  were observed in *P. microstauron* var. *nonfasciata*. Another similar taxon is *Pinnularia angliciformis* Van de Vijver & Beyens, described from the sub-Antarctic region [44]. Differences, however, include a more irregular central area forming often a fascia in *P. angliciformis* and a lower stria density (10–12 in 10  $\mu\text{m}$ ) in *P. angliciformis*. There are also ecological differences between *P. seignavouxensis* and several related taxa. *Pinnularia seignavouxensis* shows a broad tolerance to nutrients, as large populations of this species were present under eutrophic to hypertrophic conditions with an ammonium concentration exceeding 25  $\text{mg L}^{-1}$ , whereas *P. microstauron* var. *nonfasciata* is typical for oligotrophic, oligosaprobic waters with a low electrolyte content. In December 2019, the number of individuals of *Pinnularia seignavouxensis* decreased and was replaced by *Eunotia pseudogroenlandica*, underlining the fact that the unknown species preferred meso- to hypereutrophic water. *Pinnularia angliciformis* could be present sometimes in enriched pools with low to moderate conductivity at Crozet Archipelago [44] as the new species. These differences justify the separation of *P. seignavouxensis* as a new species. A more careful morphological and ecological documentation of this new *Pinnularia* species is very important to facilitate the detection of further changes in diatom composition following external environmental influences such as the decrease (or increase) of pressure from cattle.

In spring (April 2018 and May 2019), the amount of open water was influenced by snowmelt and rain. The presence of snow, particularly in April, created conditions with low conductivity and high oxygenation, as is particular to this period of the year. This resulted in a higher presence of several oligotrophic to mesotrophic species such as *Eunotia exigua*, *E. pseudogroenlandica*, *Neidium alpinum*, and *Psammothidium helveticum* [32,33,39]. Moreover, *P. helveticum* is typical for slightly acidic rivers and ponds with low electrolyte content [33].

In summer (June 2017, July 2018, and 2019), the presence of cattle, who trample and excrete in the water, increased the eutrophication of the pond. The conductivity was high, particularly in 2018, combined with a low oxygenation, which was partly also influenced by a steep rise in water temperature. The pH became slightly alkaline. Nováková [45] highlights pH as the most determining environmental variable for the algal assemblages, underpinning in particular that the number of species increased with an increasing pH. This hypothesis did not match with the 2018 results in the present study since the highest number of taxa and the highest value of the Shannon diversity were found in April 2018. Only in 2019 did the increase in diversity correspond to the conclusion in Nováková [45].

Moreover, particularly in the summer of 2018 (and in extent also in the summers of 2017 and 2019), the influence of cattle led to an increase in ammonium from  $<1 \text{ mg L}^{-1}$  to 23.4  $\text{mg L}^{-1}$ , together with an increase in other ions related to the influence of animals. This increase of ammonium could have two reasons: (1) the direct impact of the cattle with their urine and excrements as they defecate occasionally directly into the pond or in its immediate surroundings (Figure 2b), or (2) the result of a process of incomplete degradation of organic matter [46]. The sediment depth in the pond (reaching 40 cm in the deepest part), the reduced water volume, and the lack of water input contribute to the bacteriological decomposition of soluble organic substances, increasing in this way the ammonium nitrogen concentration only in this shallow pond compared to another pond [47]. Associated with this high ammonium concentration, different mesotrophic to hypereutrophic species were present: *Neidium affine*, *Nitzschia palea*, *N. palea* var. *tenuirostris*, and *Pinnularia seignavouxensis* [33,39]. *Nitzschia palea* var. *tenuirostris* and *N. palea* are considered polluo-tolerant species [33,39].

Linked to the nitrogen cycle, it is well known that ammonium is transformed into nitrites and nitrates by oxidation, an oxygen-consuming process [46]. The presence of both ammonium and nitrates was observed in July 2019 when only very limited water was present and also when livestock could not use the pond. During this period, the nitrogen cycle was complete. At this period, nitrites and nitrates

never reached the same concentrations as in 2018. It could be due to consumption by plants and/or due to the shorter duration of the cattle trampling that did not lead to the same concentration as in 2018.

In November 2018, after a dry period (no water in the pond), the nitrate concentration was high. This high nitrate presence could be linked to the oxidation of ammonium following the re-impoundment linked to the nitrogen cycle. The ammonium and also organic matter could have been trapped in the sediments and the re-impoundment could have resuspended the particle in the water, leading to an increase in the nitrate concentration from  $<1 \text{ mg L}^{-1}$  in July to  $17.5 \text{ mg L}^{-1}$  in November. The conductivity of the water was still high ( $90 \text{ } \mu\text{S cm}^{-1}$ ) and the water was well oxygenated.

In winter, the situation was different with relation to the physico-chemistry. Between October and November, the process of denitrification was ongoing until nitrates were depleted [48], as observed since the end of November. Indeed, on 12 December 2018 and 29 November 2019, the concentrations returned to the initial state of April. The nitrates could have been consumed by the last aquatic plants in combination with a dilution effect due to a higher precipitation, which resulted in a return of the oligotrophic conditions. Unfortunately, only in November 2019 was a sample taken, showing low conductivity ( $7 \text{ } \mu\text{S cm}^{-1}$ ), low water temperature ( $1.7 \text{ } ^\circ\text{C}$ ), and high oxygenation (88%). On the basis of the dominance of *Eunotia pseudogroenlandica* (53%), the water was characterized as oligotrophic as this, usually rare, species is known to be oligotrophic and oligosaprobic, living in poor electrolyte aquatic environments [31,33,39]. Cantonati et al. [15] observed the species in very shallow pools of the southeastern Alps, and Pavlov and Levkov [49] found it in a small temporary lake in Macedonia at an altitude higher than 2000 m a.s.l., where it was observed in high abundance as an epiphyte on moss-vegetation. However, in the Laqui du Seignavoux, *E. pseudogroenlandica* was observed on both substrates: macrophytes and mud. *Eunotia bilunaris* was also present as this species predominantly occurs in oligo- to dystrophic waters with low electric conductance and low pH [32].

Among the necessary nutrients that could be used by diatoms, nitrogen and phosphorus are two parameters that are mostly required for the development of diatoms in aquatic ecosystems. The importance of nutrients in structuring benthic diatom communities was demonstrated in different studies [50–52]. During the survey from June 2017 to December 2019, the phosphate concentration never exceeded the detection limit. Only in December 2018 and October 2019 did it exceed the limit ( $0.05$  and  $0.06 \text{ mg L}^{-1}$ , respectively). However, these concentrations are still below the threshold level of  $0.5 \text{ mg L}^{-1}$ , a value that constitutes a pollution indicator [46]. However, it is well known that there is a relationship between the N/P ratio and the abundance of diatoms, as well as the fact that the ratio value affects the algal composition [53,54]. In December 2018, the ratio value was 8, but no diatom sampling was performed as it was also the onset of the ice cover phase. In October 2019, the ratio value was also low (7.1), followed by an increase of the relative abundance of *Pinnularia seignavouxensis*, although no major changes in the diatom composition could be observed. As the pond is situated in an area where there is only little human impact, nitrates are the only nutrient source for diatoms and their presence is mainly linked to the frequency of cattle. This could explain the increase in species richness and Shannon diversity in summer and autumn. It will be interesting in the future to use artificial substrate to manage the evolution of the diatom abundance and to evaluate its relationship with the N/P ratio in this temporary pond.

The samples taken on macrophytes contributed to completing the diatom biodiversity observed in the pond. In July and November 2018, *Cocconeis euglypta* and *Gomphonema aff. innocens* were observed as being associated with other taxa such as *Lemnicola hungarica* (Grunow) Round & Basson (relative abundance  $< 1\%$ ). *Cocconeis euglypta* and *Lemnicola hungarica* are considered epiphytic species [55], and in our study, they were only observed on plants. In November, with the high nitrate concentration, different meso-eutrophic and beta-mesosaprobic species were present, such as *Sellaphora atomoides* and *Staurosira venter* [33,39,56], but were only observed on macrophytes. In May and October 2019, *Cocconeis* was absent and apparently replaced by *Eunotia bilunaris* on the macrophytes, the latter being a well-known epiphyte [32]. *Tabellaria flocculosa* was also observed and seemed to be associated with *Eunotia exigua* in October. Both species were observed together in shallow and moderately acidified



mountain lakes in Bulgaria [14]. However, at this period, the pH was alkaline contrary to June 2017, when these species were also associated.

## 5. Conclusions

The temporary pond Laqui du Seignavoux evolved from a poorly mineralized, well-oxygenated ecosystem in spring with “pristine conditions” due to snowmelt, inducing oligotrophic and oligosaprobic water, to a more mineralized, less oxygenated, eutrophic–hypertrophic, and polysaprobic environment in summer. This change in abiotic conditions is most likely linked to the presence of cattle in and around the pond in summer. With ammonium concentrations that could be higher than 20 mg L<sup>-1</sup>, the pond presented conditions that could be considered as “toxic”. Among the dominant species, we observed a decrease in oligotrophic taxa present in winter and spring, in favor of others more resistant to these extreme conditions such as *Nitzschia palea* or *N. palea* var. *tenuirostris*. As this study was performed to help in the management of the pond by the national nature reserve of Chastreix-Sancy, the main conclusion was to support the removal of the cattle from the site. In the future, with the climatic change and the management of the reserve, if cattle will not trample and excrete in the pond, it will be interesting to verify the evolution of the diatom communities and biodiversity, with a particular attention to *Pinnularia seignavouxensis*, in an environment where only wild animals will be present.

**Author Contributions:** A.B. conceived the research; A.B., E.A., and O.V. performed fieldwork; A.B., E.A., C.E.W., L.E., and B.V.d.V. analyzed the data, and wrote and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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