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The Effects of Cattle Grazing on the Composition of Diatom Assemblages in the Peatland Pools of the Southeastern Alps (Italy, Adamello-Brenta Nature Park)

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Abstract: Mires are significant C-sinks and important habitats for biodiversity conservation. Particularly in the Alpine peatlands, grazing and trampling cause mires to degrade, causing changes in plant communities that increase bulk density, peat erosion, and nutrient excesses. We took sixteen samples of epipelic and epiphytic diatoms from mire pools subjected to varying degrees of grazing in the Adamello-Brenta Nature Park (Trento, Italy). We identified >100 diatom species (ca. 70% included in Red List threat categories). We used nMDS to identify groups of diatom species. Their statistical significance was checked with an ANOSIM and, to identify the species that contributed most to the difference between the two groups, a BEST-SIMPER procedure was carried out. The multivariate analyses allowed us to identify significant differences among the most and least grazed sites, and to select the species that contributed most to this distinction. We confirmed that some species were more frequent and abundant in the most grazed sites and behaved as opportunists in the presence of a greater nutrient input, to the detriment of rare and sensitive species, which were more numerous and abundant in the less grazed sites. We provided useful information on grazing effects, underlining the importance of managing and protecting habitats of unique environmental value.

Keywords: diatoms; mire pools; peatlands; Red List; nutrients; grazing; trampling; sensitive species; opportunistic species; management

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1. Introduction

Mires are wetlands where layers of partially decomposed organic matter accumulate. In most wetland ecosystems, the rates of plant material production and decomposition are equal [1]. However, in peatlands, organic matter does not completely decompose due to the water saturation conditions, which create an anoxic environment where the decomposition process is slowed down [2]. Mires can only form under particular climatic conditions such as low temperatures, high humidity, and high precipitation [3]. Therefore, they are distributed in boreal and subarctic regions and in humid areas where the necessary conditions exist [4].

Despite covering only 3% of the Earth's surface, they represent one third of the global wetland areas [4] and provide important ecosystem services such as carbon storage, potable water supply, and opportunities for recreation [5]. Mires also host assemblages exclusive to these habitats, offering conservation value [6].

However, the peculiar peatland ecological conditions, such as the abundance of water, nutrient scarcity, and the close connection between *Sphagnum* mosses and the surface morphology of the ecosystem, make these habitats particularly vulnerable to changes in water quality and nutrient levels [7]. Moreover, peatlands are threatened by numerous factors, including climate change, air pollution, grazing, fires, artificial drainage, afforestation, and infrastructure development [8,9]. In Italy, peatlands are even more fragile due to their marginal distribution as compared to Europe [10]. Alpine mires, indeed, are glacial relicts [11] and cover areas which are as small as <0.1% of the entire nation [12].

Grazing is one of the main threats to the integrity of Alpine mires and to the biodiversity they possess [13]. There are numerous problems related to grazing: first, livestock trampling causes damage to the structure of the surface layers of peatlands and modifies the abundance and/or presence of plant species [10]. Furthermore, damage to vegetation and exposure of bare soil also cause peat erosion; in particular, poorly drained peatlands developed on flat or gently sloping terrain are the most vulnerable [14].

In addition to mechanical issues, there are also concerns related to nutrient enrichment. Indeed, peatlands are typically oligotrophic habitats and are extremely sensitive to nutrient inputs, especially ombrogenous ones [15].

Nitrogen inputs can lead to changes in vegetation composition [16] and can favor nitrophilic and fast-growing species at the expense of others [10]. Grazing can favor disturbance-tolerant species, such as tufted herbaceous plants and other grasses [4], and may lead to decreases in the presence of sensitive and rare species in bryophyte communities, which can be influenced by both nutrient inputs and trampling [13].

Recently, the effects of cattle grazing on mires were assessed using diatoms in peat cores, i.e., with a paleoecological approach [17].

Diatoms are unicellular algae of the Bacillariophyta division that colonize nearly all types of wet and aquatic habitats and their species-specific response to chemical and physical environmental changes make them especially useful in environmental monitoring [18], although their potential as bioindicators has not yet been fully exploited in peatlands [11].

Peatlands can host exceptionally rich diatom communities characterized by a high number of rare and threatened species [11]; this fact is likely still insufficiently documented [19]. Cantonati et al. [20] state that, according to the 2018 Red List [21], threatened taxa are concentrated in dystrophic and oligotrophic environments. Diatom diversity in European peatlands is very high, with approximately 403 taxa recorded, of which 14% belong to the genus *Pinnularia*, 10% to the genus *Eunotia*, and 10% to the genus *Navicula* [22]. In Italy, the presence of diatoms in peatlands has been poorly studied, except for in the Trentino-Alto Adige [23,24]. Only recently, Cantonati et al. [11] investigated diatom biodiversity in the Danta di Cadore mires, which constitute a Site of Community Importance of the European Union located in the southeastern Alps.

The present study aims to explore the suitability of mire pool diatoms as indicators of grazing-induced disturbance, with special attention paid to variations in the composition and structure of diatom communities in response to cattle grazing, and to the presence of threatened Red List species as an indicator of ecological integrity.

2. Materials and Methods

2.1. Study Area

This study was carried out in the Adamello-Brenta Nature Park (Southern Alps, Autonomous Province of Trento, Italy) in the summer of 2021. The Adamello-Brenta Nature Park protects an area of 620.5 km^2 and was recognized as "UNESCO Global Geopark" in 2015. It is divided by the Giudicarie Line, a major geologic fault zone in the Italian Alps, into two geologically and geomorphologically different groups: to the east of the line, the Brenta massif rises, made up of calcareous and dolomitic sedimentary rocks, while to the west there is the Adamello group, formed by intrusive igneous rocks, such as granite and tonalite. In the Adamello massif, the siliceous bedrock allows the persistence of surface flowing water and the presence of dystrophic habitats, such as high mountain lakes, streams, alpine peatlands, and springs [25].

Mires occupy approximately 177 ha of the park's surface and are mainly found in the upper Val Rendena [10]. The sampling sites for this study are located in the municipality of Pinzolo, found in the Adamello massif, and belong to the Special Area of Conservation "Torbiere Alta Val Rendena" (site identification code: IT3120167) (Figure 1).

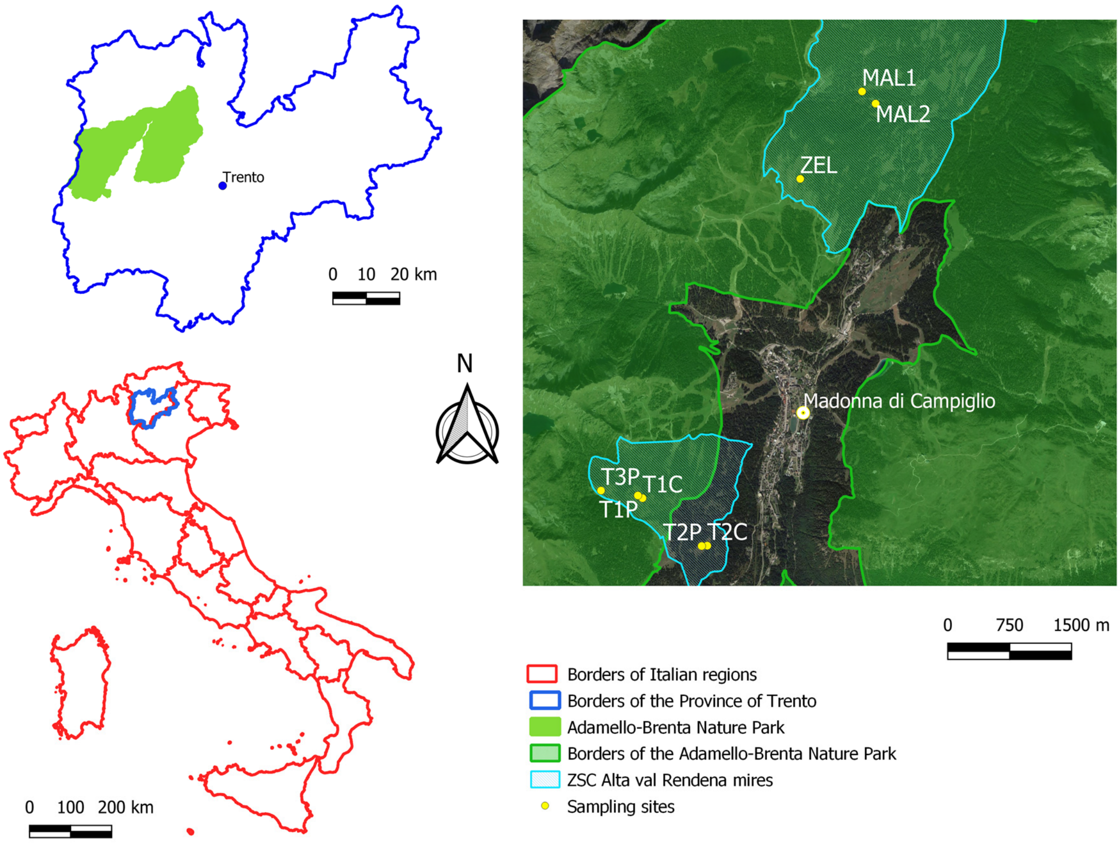

Figure 1. The location of the sampling sites as shown with QGIS v. 3.14.

The four mire pools studied, namely, Palù Marcia, Canton Ritorto, Zeledria, and Malghette, are subjected to different grazing intensities, which are estimated by counting the cow hoof prints within a circular area with a 5 m radius [13]. Zeledria (ZEL) is one of the most grazed sites: a few meters from the mires, there is an electrified fence for livestock, and the soil shows evident traces of very intense grazing. Canton Ritorto and Palù Marcia exhibit variable characteristics: three sites (T1P, T3P, T2P) are intensively grazed, and the other two (T1C, T2C) are considered to be poorly grazed. The level of disturbance is also low in Maghette (MAL1, MAL2), which is thanks to an electrified fence limiting livestock passage. Throughout the paper, highly grazed sites are indicated with 'HGSs', and barely grazed sites are indicated with 'BGSs'. The main features of the sampling sites are summarized in Table 1.

Table 1. Sampling site features.

Site Name	Site Code	Coordinates	Elevation m a.s.l.	Grazing Intensity	Hoof Prints	Sampling Date
Canton Ritorto	T1C	46°13′9.65″ N 10°48′13.10″ E	1777	low	12	29 July 2021
Canton Ritorto	T1P	46°13′10.73″ N 10°48′10.52″ E	1782	high	65	29 July 2021
Canton Ritorto	T3P	46°13′13.03″ N 10°47′49.73″ E	1800	high	37	29 July 2021
Malghette	MAL1	46°15′47.22″ N 10°50′22.53″ E	1718	low	11	26 August 2021
Malghette	MAL2	46°15′42.34″ N 10°50′30.02″ E	1711	low	0	26 August 2021

Table 1. *Cont.*

Site Name	Site Code	Coordinates	Elevation m a.s.l.	Grazing Intensity	Hoof Prints	Sampling Date
Palù Marcia	T2C	46°12′50.45″ N 10°48′49.11″ E	1689	low	19	29 July 2021
Palù Marcia	T2P	46°12′50.34″ N 10°48′45.93″ E	1694	high	87	29 July 2021
Zeledria	ZEL	46°15′13.52″ N 10°49′46.41″ E	1953	high	51	26 August 2021

2.2. Sample Collection, Laboratory Preparation and Taxonomic Identification

Samples of epipelic and epiphytic diatoms were collected from each mire pool. In total, 16 samples were collected (8 from surface sediments and 8 from bryophytes). For surface sediment samples, three squares of 36 cm^2 were sampled for each mire pool. Plastic squares with a central carved area of 3 × 3 cm were used to define the area to be sampled. The sediment was then sampled by drawing a plastic tube across the surface of the sediment, which we allowed to fill with a mixture of sediment and water. For the epiphytic diatom samples, submerged bryophytes were collected from each pool at three different points. Samples were fixed using formaldehyde to a final concentration of 4%.

In the laboratory, an aliquot of sediment or bryophytes was removed from each sample and then treated with hydrogen peroxide and hydrochloric acid. Cleaned valves were mounted in Naphrax® (Brunel Microscopes Ltd., Chippenham Wiltshire, UK) (synthetic resin dissolved in toluene with a refractive index of 1.74). Prepared materials, aliquots of the original samples, and slides were deposited in the diatom collection of the Museo delle Scienze of Trento (MUSE), Italy (sub-collection: cLIM008—mires, accession numbers: MUSE-LIM-DIAT 4090-4105). Permanent mounts were prepared for each sample, and a total of about 400 valves were counted at ×1000 magnification using a Zeiss Axioskop 2 microscope (Zeiss, Jena, Germany), considering the exact number of valves for each species and the number of fields of view (FOVs) observed. Relative and absolute abundances were calculated following the work of Cantonati et al. [26]. Micrographs of each species were taken with an Axiocam digital camera, and the taxonomic identification was performed based on the following references: [19,27–32]. Diatoms.org [33] and www.algaebase.org [34] were also consulted for species identification and nomenclature.

In addition to biological data, hydrochemical water samples were collected: we filled a 100 mL Kartell bottle for metal analysis and a 1L Kartell bottle for the study of the main nutrients and ions from each mire pool. The water samples were analyzed by the Environmental Protection Agency (APPA) of the Autonomous Province of Trento (PAT), Italy, following standard methods. Furthermore, electrical conductivity, pH, oxidation–reduction potential, temperature, dissolved oxygen, and turbidity were measured using a Hydrolab DS5 multiparameter probe (Table 2).

Table 2. Field parameters: temperature (T), pH, oxidation–reduction potential (Redox), dissolved oxygen (DO), and turbidity.

Site Code	Grazing Intensity	T (°C)	DO (mg/L)	DO (%)	Conductivity (µS/cm)	pH	Redox	Turbidity
MAL1	low	20.3	4.5	64	33	5.9	164	-
MAL2	low	20.5	6.2	88	19	6.1	171	1280
ZEL	high	24.8	7.8	124	7	5.5	242	11.8
T2C	low	21.7	7.2	100	18	4.5	264	16.4
T2P	high	24.3	4.1	61	21	5.0	248	37.0
T3P	high	28.2	7.5	115	12	5.1	267	1.9
T1C	low	10.2	4.3	48	35	5.7	109	3000
T1P	high	22.4	3.9	57	31	5.7	132	3000

2.3. Data Processing and Statistical Analysis

A threat status (used also as a measure of rarity) was assigned to all diatom species according to the current Red List data [21]. The work of Hofmann et al. [21] provided additional ecological attributes (aerial species) used in this study. The preferences of the individual taxa with respect to moisture were obtained from Van Dam et al. [35]. Furthermore, the list provided by Foets et al. [36] was used to identify "terrestrial" diatoms among the species we found.

The diatom relative abundance data obtained after the counts were arranged in a matrix. The species with zero relative abundance in all samples were discarded after a check of missing values. A log(x + 1) transformation was applied to homogenize the abundance values, and data quality was graphically tested using shade plots. A Bray–Curtis similarity matrix was generated after the inclusion of a dummy variable = 1 and used as an input for a nMDS (non-metric multi-dimensional scaling) biplot. The significance of the difference between groups identified via nMDS was tested through an analysis of similarities (ANOSIM, $\alpha = 0.05$, permutations = 999). The initial pool of species was reduced through a BEST analysis (Rho ≥ 0.9, $\alpha = 0.05$), followed by an analysis of similarity percentage (SIMPER, cut-off = 70%) to identify the species that contribute most to the difference between the two groups. The subset of species identified by the BEST analysis was assessed using a nMDS (after calculating the Bray–Curtis distances) and subsequently tested with an ANOSIM ($\alpha = 0.05$, permutations = 999). The results of the SIMPER analysis were plotted using bubble plots. All analyses were conducted using PRIMER software version 7 [37].

3. Results

3.1. Hydrochemistry

The field parameters measured during the sampling campaigns are available in Table 2.

A table of all hydrochemical values measured is available in Supplementary Materials Table S1.

Hydrochemical analyses produced results which are similar with regard to mires with high and low levels of grazing.

The pH ranges from slightly acidic to near neutral, with only one exception at the site T2C, which has the most acidic pH (pH = 4.7).

Conductivity and alkalinity values are low, indicating a limited ion content, which is consistent with the type of environment and the crystalline substratum on which the studied mires are located.

The concentrations of major cations and anions, such as Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, SO_4^{2-} and Cl^{-}, are within the expected range for ombrotrophic mires of Central and Northern Europe [38], with the exception of the potassium concentration at site MAL1 (3.4 mg L^{-1}). HCO_3^{-} values are closer to the concentrations found in ombrotrophic mires in Canada and the northern United States rather than in Europe [38].

Iron and aluminum contents are high; Vitt et al. [39] attribute these values to the chelation of metallic ions by dissolved organic matter (DOM).

Trace elements are typically present in inland waters at concentrations lower than 0.05 mgL^{-1} [40] and our results do not reveal any exception.

Regarding algal nutrients, silica values are consistently above the threshold limit for diatom growth (0.1 mg L^{-1}) [40].

Concentrations of nitrogen compounds (nitrates and ammonium ions) are low and comparable to those reported by Bourbonniere [38]. Elevated values of total nitrogen and total phosphorus are attributed to high concentrations of humic acids [11].

3.2. Assemblage Composition

We found 89 taxa, belonging to 31 genera, in the highly grazed sites (HGSs). The most represented genera were *Eunotia* (26); *Pinnularia* (16); *Encyonema* (5); *Nitzschia* (4); and *Gomphonema*, *Kobayasiella*, *Aulacoseira*, and *Chamaepinnularia* (3). *Frustulia crassinervia* is the most frequent species (with a relative abundance > 10%), and is present in 50% of the samples with a maximum relative abundance (MRA) of 27.9%. *Kobayasiella micropunctata* is the species with the highest MRA. *Aulacoseira alpigena* is present in 6 samples out of 8, with an MRA of 21.1%.

The most frequently occurring species, considering only presence/absence data, are (Figure S2) *Encyonema perpusillum*, *Nitzschia perminuta*, *Eunotia tenella*, and *Eunotia neocompacta* var. *vixcompacta*.

Among the species of the genus *Eunotia*, *E. neocompacta* var. *neocompacta*, *E. paludosa*, and *E. rhomboidea* are also frequent. *E. mucophila* is present in 5 out of 8 samples and reaches an MRA of 10.3%. The genus *Fragilaria* was sparsely found, although two species were observed and counted in the HGS, with low relative abundances. *Gomphonema* only has three species, among which *G. utae* and *G. coronatum* have never been counted and *G. hebridense* is present in only 2 samples, with an MRA of 4.2%. *Cymbopleura valaiseana* was found in only 3 samples with extremely low abundances (MRA = 0.5%). The only representative of the genus *Encyonopsis* is *E. falaisensis*.

Kobayasiella is widespread: *K. micropunctata* is the species with the highest maximum relative abundance among all the taxa counted in the 8 HGSs (MRA = 30.9%). *K. subtilissima* is present in 6 samples and reaches high abundances (MRA = 16.3%). The *Neidium ampliatum* species complex could be counted exclusively in the HGS, being present in 4 samples and with a relative abundance < 2%. Another species represented exclusively in the HGS is *Pinnularia microstauron*. The highest number of taxa in the HGS was found in the T1P sample of bryophytes (40), while the surface sediment sample from the T3P site had the lowest number of species (25) (Table S3).

A complete list of the taxa present in the HGS with additional information on abundance data is available in Supplementary Material Table S2.

In the barely grazed sites (BGSs), 101 taxa belonging to 28 genera were found. The most diversified in terms of species/variety representatives were *Eunotia* (26); *Pinnularia* (13); *Encyonema* (7); *Gomphonema* and *Nitzschia* (6); *Chamaepinnularia* (5); *Aulacoseira* (4); and *Kobayasiella*, *Encyonopsis*, and *Psammothidium* (3).

Brachysira brebissonii is the most frequent species (with relative abundance > 10%), being found in 5 out of 8 samples. In terms of presence/absence, the most frequent species is *Frustulia crassinervia*, present in 7 samples (Figure S1). Even *Eunotia boreoalpina*, *Encyonema neogracile*, *Eunotia neocompacta* var. *neocompacta*, *Gomphonema hebridense*, and *Kobayasiella micropunctata* are common. *Eunotia paludosa* shows the highest relative abundance among all species, dominating in the bryophyte sample T2C (MRA = 39.1%). The *Achnanthidium minutissimum* species complex and the genus *Caloneis*, represented by *C. tenuis* and *C. undulata*, were observed and counted exclusively in the BGS. *Aulacoseira alpigena* was present in 50% of the samples, with an MRA of 13.5%. *Chamaepinnularia* aff. *begeri* was observed and counted in 2 sites with an abundance higher than 6% and was completely absent in the HGS. *C. mediocris*, however, was only observed in 50% of the samples, while in the HGS it was one of the most frequent species (7 out of 8 samples). *C. schauppiana* was found exclusively in the BGS, except for a single observation at the T1P (grazed) site. *Eunotia* is a widely represented genus: *E. hexaglyphis*. *E. tetraodon*, *E. naegelii*, *E. juttnerae*, *E. triodon*, *E. praerupta*, and *E. nymmamaniana* were exclusively observed or were clearly more frequent in BGSs. *Gomphonema* has six species, of which *G. hebridense* is the most common, with an MRA higher than 10%.

The highest number of taxa among BGSs was found in the T1C site of surface sediment (49), and the lowest was found in the T2C sample of bryophytes (9) (Table S3).

A complete list of the taxa present in the BGS, with more information provided on abundance data, is available in the Supplementary Material Table S2.

3.3. Red List and Van Dam Index

Among the taxa found in the HGS, 96% are listed in the Red List [21]. Only 7 species are classified as D (data-deficient) and 22% of the taxa are considered "Not threatened".

The remaining taxa fall into the threatened categories (1, 2, 3, G, R, V): *Eunotia bactriana* and *Eunotia superpaludosa* are "threatened with extinction" (1). Additionally, 11 taxa belong to the category "severely endangered" (2) and 8 taxa to the category "endangered" (3). *Microfissurata paludosa* and *Chamaepinnularia schauppiana* are considered "extremely rare" (R) [11]. The results are similar for the BGS in terms of Red List information are available for 93% of the identified taxa. Overall, 21% of the species are classified as not threatened, there are not enough data (D) for 7%, and the remaining 65% fall within the 1, 2, 3, V, G, and R categories. Only one species is "threatened with extinction", i.e., *Eunotia hexaglyphis*. Additionally, 13 species are "severely endangered" and 12 are "threatened". *Staurosira spinarum* is considered "extremely rare" [11].

Light microscopy images of the rare and threatened species present or clearly frequent in the BGS are represented in Figure 2a–h, and the percentages of RL 2018 categories are represented in Figure S3.

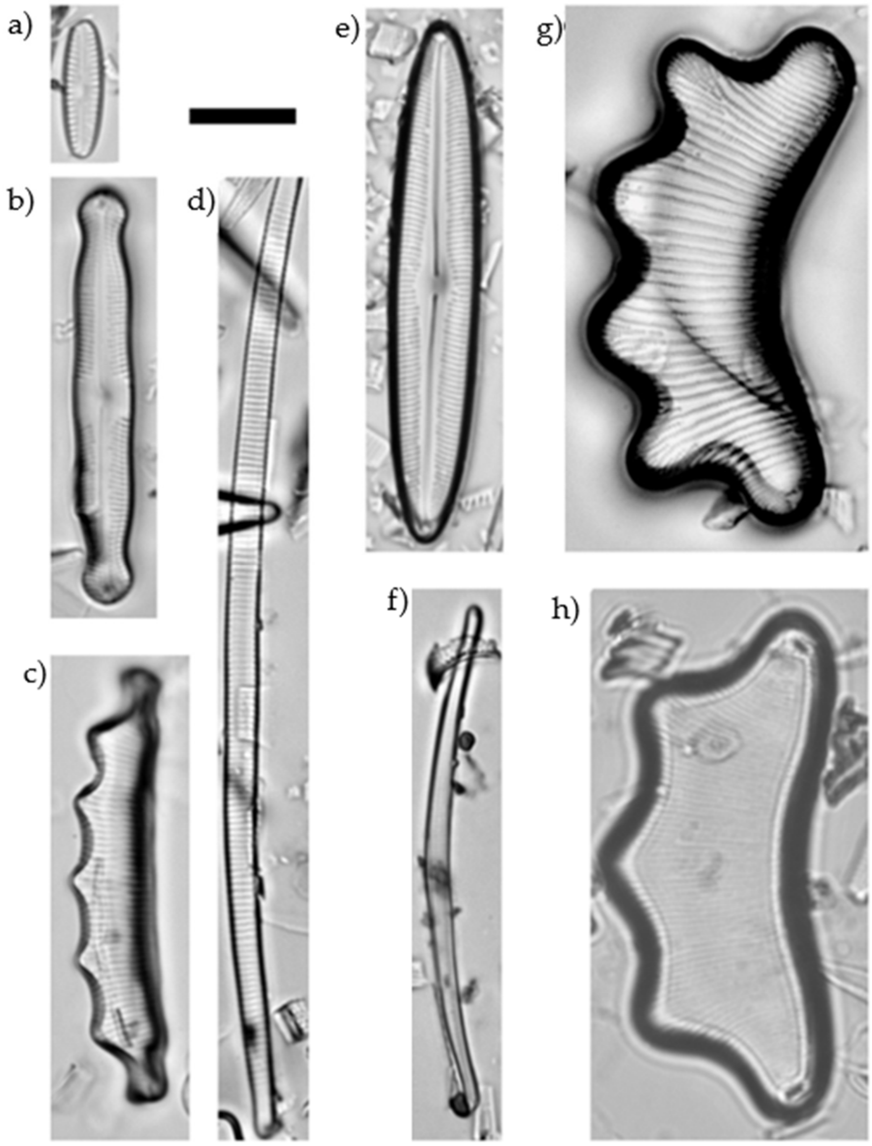

Figure 2. (**a**–**h**) Light microscopy images of the rare and threatened species present or clearly frequent in the BGS: (**a**) *Chamaepinnularia schauppiana*; (**b**) *Caloneis undulata*; (**c**) *Eunotia hexaglyphis*; (**d**) *Eunotia juttnerae*; (**e**) *Cymbopleura valaiseana*; (**f**) *Eunotia naegelii*; (**g**) *Eunotia tetraodon*; and (**h**) *Eunotia triodon*. Scale bar, 10 µm.

The index categories of Van Dam et al. [35] are available for 57% of the taxa found in the HGS: 26 species are acidobiontic or acidophilous; 29% of the species mainly occur in water bodies, and also rather regularly in wet and moist habitats (class 3, M index); and 40% are oligotraphentic or oligo-mesotraphentic. The results are similar for species in the BGS, with a prevalence of acidobiontic, acidophilous, oligotraphentic, and drought-resistant species. A complete list of the taxa, with more information on RL and other ecological indices, is available in the Supplementary Material Tables S4 and S5.

3.4. Analysis of Diatom Assemblage Composition

Some 27 species were removed after checking for missing values (Table S6).

The nMDS results show a stress index of 0.1, indicating the good reliability of the analysis. Two main groups were identified: the group circled in red contains all the low-grazing sites, except for T2C. The other one, highlighted in blue, includes all the high-grazing sites (Figure 3a). The ANOSIM analysis confirmed the graphic differences detected in the plot (p-value = 0.031).

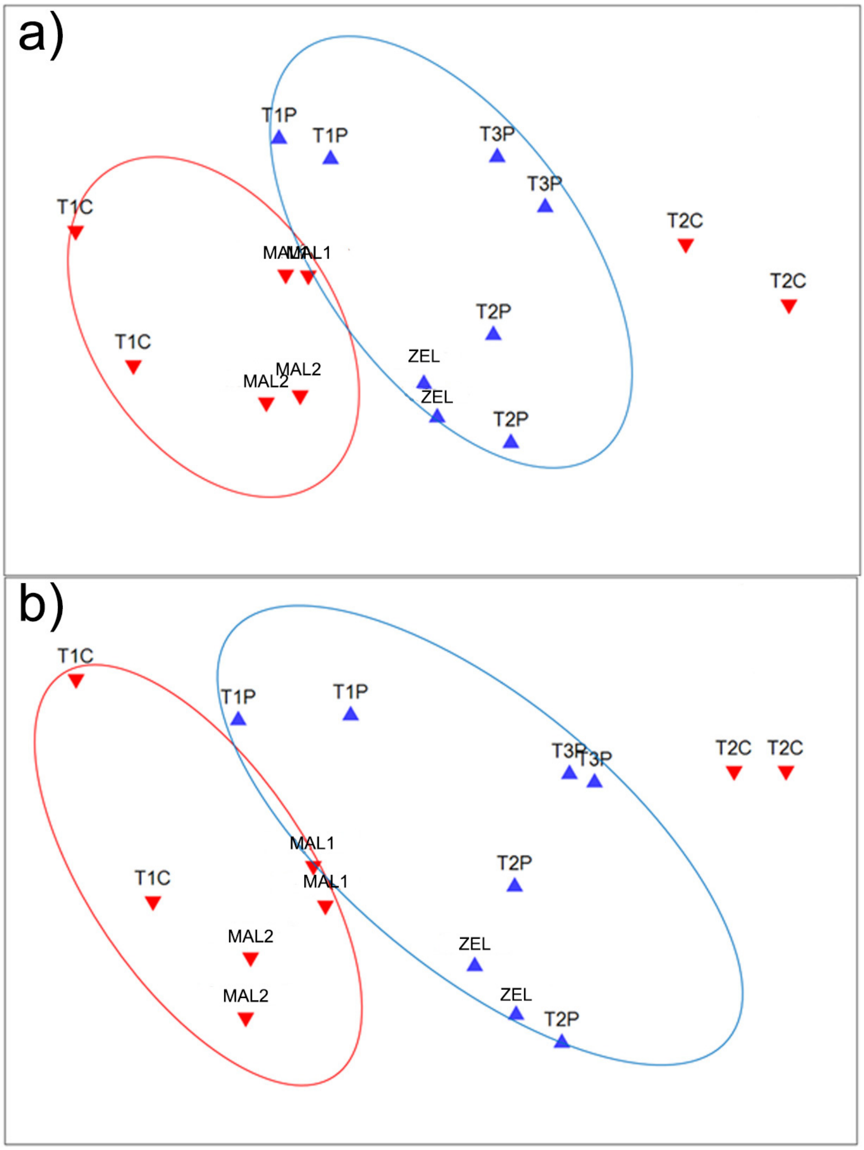

Figure 3. nMDS results: site groups identified before (**a**) and after the BEST analysis (**b**). The red triangles represent BGSs, and the blue ones are HGSs.

The BEST analysis divided a pool of 21 species (Rho = 0.926, *p*-value = 0.01) into subsets (Table S7).

The nMDS of the selected species has a slightly higher stress value (0.12). However, the pattern is entirely superimposable onto that represented in Figure 3a, indicating that the identified subset mirrors adequately the original matrix (Figure 3b).

The ANOSIM returned non-significant differences between groups (*p* value = 0.084).

The SIMPER analysis carried out on the 21 species reveals that 9 taxa contribute to 70% of the cumulative dissimilarity between the two groups previously identified. They can be arranged in terms of % of contribution as follows: *Eunotia tenella* (14.68%), *Encyonema neogracile* (11.46%), *Encyonema perpusillum* (9.27%), *Eunotia boreoalpina* (9.24%), *Nitzschia acidoclinata* (7.07%), *Eunotia minutula* (6.48%), *Pinnularia subcapitata* var. *subrostrata* (5.69%), *Brachysira confusa* (5.54%), and *Stenopterobia delicatissima* (4.72%). The bubble plots obtained for the nine taxa are shown in Figure 4a–i and the respective photos are shown in Figure 5a–i.

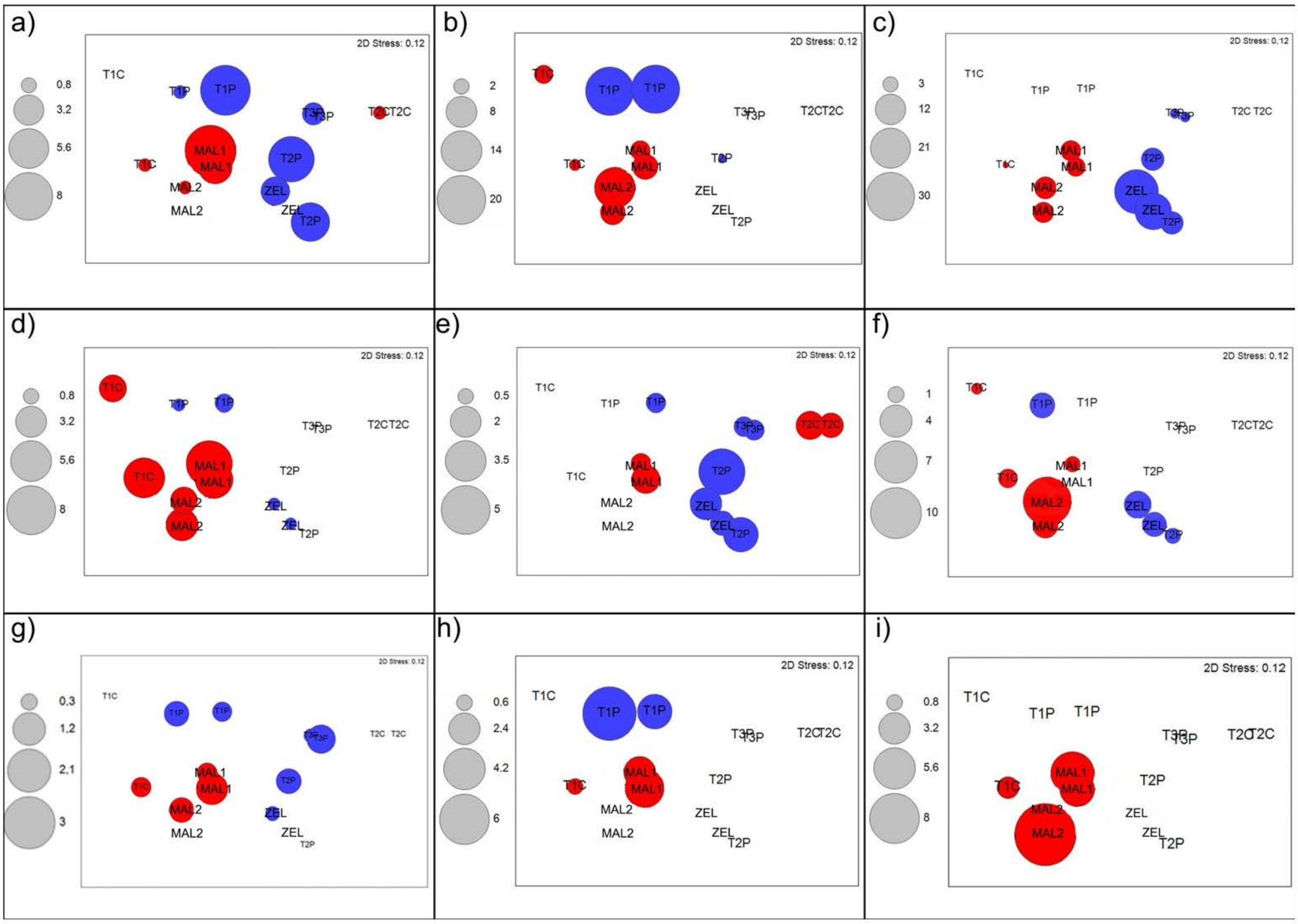

Figure 4. (**a**–**i**). Bubble plots of the species that contribute to 70% of the cumulative dissimilarity between the two groups: (**a**) *Encyonema perpusillum*; (**b**) *Encyonema neogracile*; (**c**) *Eunotia tenella*; (**d**) *Eunotia boreoalpina*; (**e**) *Eunotia minutula*; (**f**) *Nitzschia acidoclinata*; (**g**) *Pinnularia subcapitata* var. *subrostrata*; (**h**) *Stenopterobia delicatissima;* (**i**) *Brachysira confusa.* The red circles represent the abundances of the species in the BGS, and the blue ones represent the abundances of the species in the HGS. For each bubble plot, the reference values of the abundances are represented on the left.

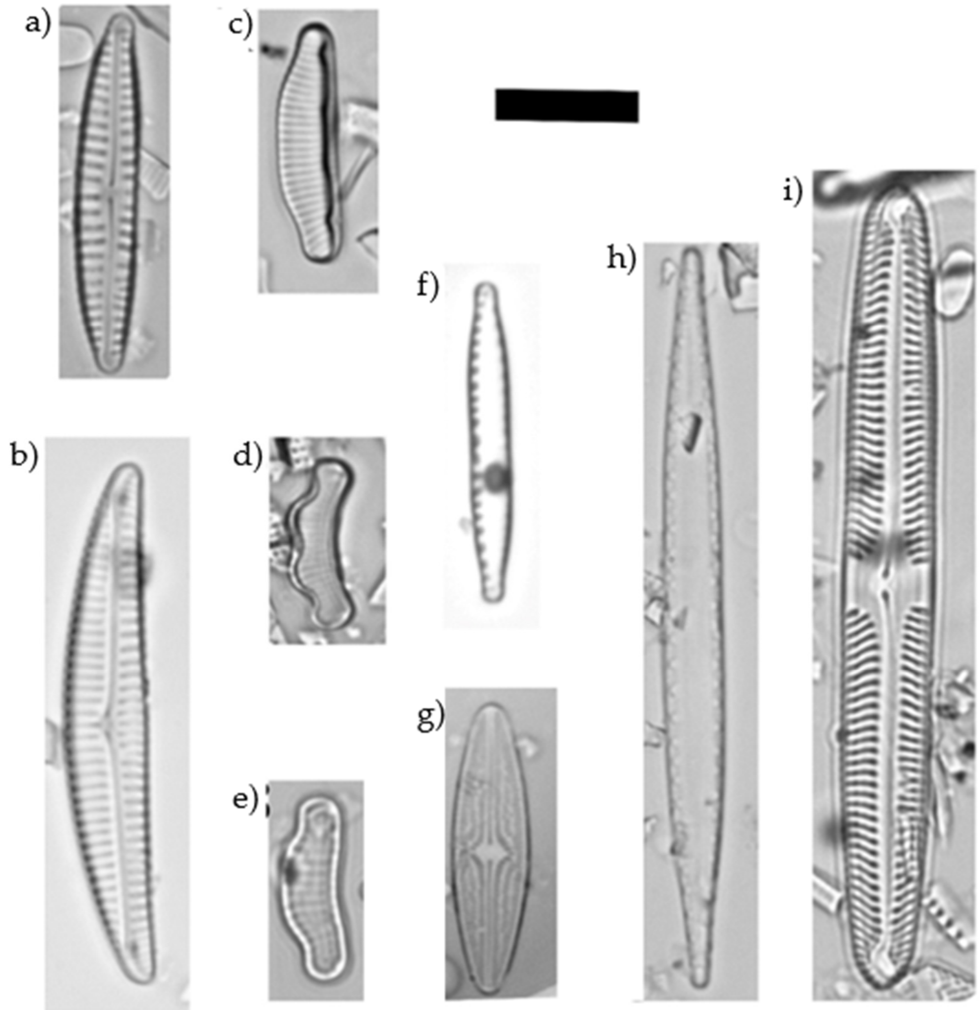

Figure 5. (**a**–**i**). Light microscopy images of the species that contribute to 70% of the cumulative dissimilarity between the two groups: (**a**) *Encyonema perpusillum*; (**b**) *Encyonema neogracile*; (**c**) *Eunotia boreoalpina*; (**d**) *Eunotia minutula*; (**e**) *Eunotia tenella*; (**f**) *Nitzschia acidoclinata*; (**g**) *Brachysira confusa*; (**h**) *Stenopterobia delicatissim*; (**i**) *Pinnularia subcapitata* var. *subrostrata a*. Scale bar, 10 µm.

4. Discussion

This study proved that different grazing intensities cause changes in mire pool diatom assemblages. Multivariate analyses found a significant difference between high- and low-grazing sites and the species that most contribute to this distinction.

The only exception to the expected pattern is site T2C (Figure 2a,b). However, this pool presented the most acidic conditions (pH = 4.7). pH is one of the main factors that influence diatom assemblage composition in freshwater ecosystems and in mires [41–43]. As pH increases, the species richness of communities tends to increase [43,44]. The most acidic peatlands often have lower species richness, and this is confirmed also by our study: the T2C pool displayed the lowest species numbers in both the bryophyte and surface sediment samples. Moreover, in the epibryon, the dominant species is *Eunotia paludosa*, which notoriously prefers *Sphagnum* moss and acidic conditions [11,45].

In the other sites, species richness is comparable to that found in other studies [11,42], with high values of T1P and T1C. Moreover, studying karst pools affected by an increased concentration of ammonium due to grazing, Novak and Zelnik [46] found a negative correlation between this disturbance and species richness.

4.1. Observation on the Species Selected Through the SIMPER Analysis

The species selected through the SIMPER analysis are all indicators of good habitat quality. This result is not surprising considering that the examined mires are not excessively degraded and still support a certain degree of integrity [17].

This study aims to highlight that intense grazing, even if it does not completely degrade the habitat, favors some species rather than others and has negative effects on assemblage composition.

Eunotia tenella is a species typical of springs and habitats that are not necessarily dystrophic. It is frequently found in Central Europe [32]. Kokfelt et al. [47] hypothesized that *E. tenella* is favored by low pH values, partially aerated conditions, and greater availability of light and nutrients, conditions which occur when the surface vegetation of the mire dies and begins to decompose. *E. tenella* can also be abundant in disturbed environments, such as waterways polluted by acid and material mine waste [48,49]. Our analysis confirms that the species tolerates disturbed environments and takes advantage of nutrient increases. In terms of the HGS, the bubble plot highlights its almost ubiquitous presence and high abundances, especially in the Zeledria samples, which are from a site where grazing is particularly intense.

Encyonema neogracile is a species typical of oligotrophic and electrolyte-poor habitats and is present in acidic or circumneutral waters. It is an indicator of excellent ecological quality [32] and, in this study, the species is found to prefer undisturbed habitats and is widespread in BGSs, except for at the T2C site, where the low pH probably does not favor the species, which is more abundant in slightly acidic and circumneutral mires with higher pH values [42].

Encyonema perpusillum is common in mires [50,51]. It is typical of oligotrophic environments and acidic freshwater habitats, being found on siliceous substrate and characterized by a low electrolyte content [32]. *Encyonema perpusillum* is more abundant and frequent in the HGS, suggesting that the species becomes opportunistic when a nutrient increase occurs.

Although *Eunotia boreoalpina* is not necessarily exclusive to dystrophic habitats, it is common in undisturbed sites. The species is locally abundant in central European mountains, on siliceous substrates, and in aquatic environments that are moderately acidic and poor in electrolytes, such as springs, lakes, streams, and peatlands [32]. The results show that it suffers from intense grazing.

Nitzschia acidoclinata is related to oligo-dystrophic environments and shows a trend of preferring minimally impacted sites, even if it is also present in more disturbed ones. The species is classified as mesotraphentic [35]; therefore, it may live in waters with higher nutrient contents. Lange-Bertalot et al. [32] define it as an oligosaprobous species that colonizes freshwaters poor in electrolytes, although it has also been found in more alkaline environments and the authors hypothesize that the ecological range of the species may be wider than assumed. An MRA of 2.8 is found in the HGS; in the BGS, it measures at 9. In the HGS, indeed, *N. perminuta* is widespread and reaches an MRA value greater than 15%. This species is quite common in mires, and is not considered threatened [21]. An experimental study, moreover, showed that *N. perminuta* dominates communities when a certain amount of phosphate is added [52].

Eunotia minutula is a rare species in Europe and lives in oligotrophic and dystrophic habitats [30]. Its ecology is still poorly known [53] and our study shows that the species can take advantage of nutrient increases.

Pinnularia subcapitata var. *subrostrata* is poorly studied. Hofmann et al. [21] classify it as a species typical of oligotrophic waters, being moderately acidic and rich in humic acids; the distribution and conservation data of the species are considered insufficient, and the species is classified as "D" in the 2018 Red List. Krammer [28] defines it as a Nordic–Alpine species that is typical of oligotrophic and water that is not very mineralized, particularly from Lapland. In our study, it is also ubiquitous and widespread in HGSs, suggesting that it can tolerate disturbance.

Brachysira confusa has been recently described [54] within the framework of the revision of the *B. brebissonii–intermedia* complex; it includes morphotypes with rounded apices, and was typically identified as *B. brebissonii* in the past. This paper can contribute to the development of a more in-depth understanding of its ecology, linking this species to undisturbed habitats: it was found exclusively in BGSs.

Stenopterobia delicatissima is quite rare and is considered severely endangered [21]. It is an element typical of mires, preferring oligotrophic and habitats rich in humic acids. It is an indicator of excellent ecological quality [32]. The species prefers undisturbed sites, although it is also present in the T1P grazed site with a relative abundance greater than 5%. The large cell size could favor this species in the presence of a nutrient excess, in line with the hypotheses that larger species become more abundant when there is greater nutrient availability [55] and that cell size varies along a trophic gradient [56].

4.2. Observations on the Diatom Assemblages

The generalist species that are clearly more frequent in the HGS are *Encyonema perpusillum*, *Nitzschia perminuta*, and *Eunotia neocompacta* var. *vixcompacta*.

In the BGS, specialist and sensitive species are dominant, and no species is present in all eight samples. *Frustulia crassinervia* is the most widespread species: it prefers low pH, conductivity, and nutrient values [32]. Other studies confirm that the species is an indicator of good water quality [57]. Other common species include *Eunotia boreoalpina*, *Encyonema neogracile*, *Eunotia neocompacta* var. *neocompacta*, *Gomphonema hebridense*, and *Kobayasiella micropunctata*. The abundance of *Eunotia neocompacta* var. *neocompacta* in the HGSs is particularly low; on the contrary, *Eunotia neocompacta* var. *vixcompacta* is more widespread in this group. Of the two varieties, the nominal one is rare and is considered severely endangered [21,53].

The threatened categories are present with higher percentages in the BGS due to the disturbance that causes the disappearance of the most sensitive and endangered species. In general, the percentage of threatened species is particularly high, which is in line with the fragility of the oligotrophic and dystrophic habitats of which the most characteristic species are typical [20]. Earlier studies obtained similar results, highlighting the high percentages of rare and threatened species according to the Central European Red List [11].

Chamaepinnularia mediocris, for example, is a widely distributed species in mires [11,58,59]. In HGSs, it is one of the most frequent species in terms of presence/absence. *Chamaepinnularia schauppiana*, conversely, is a rare [11,60] indicator of oligotrophy [27], and its total absence in the HGS is significant, except for one observation in the T1P site, where the species however was never counted. On the contrary, its presence is more widespread in BGSs. *Achnanthidium minutissimum* is a species complex that is particularly discussed in the literature and which is still undergoing taxonomic definition [61]. The species is often related to conditions of oligotrophy and is considered an indicator of nutrient-poor waters [62]. The species is dominant in pools that are not subjected to grazing [46], suggesting that the disturbance creates problems for species with a low growth form profile, sensu Passy [63], due to the increase in nutrients to which they are not adapted. Additionally, in our study, the *Achnanthidium minutissimum* species complex is linked to undisturbed habitats and is exclusively present in BGSs.

Aulacoseira alpigena is a cosmopolitan species that prefers oligotrophic waters characterized by low conductivity [64]. It is one of the few centric species found in mires and, although it is often considered planktonic, it displays benthic behavior in this specific habitat [57]. Among the species of the genus *Aulacoseira*, the less common *A. nivalis* and *A. nivaloides* appear almost exclusively in the BGS, while *A. alpigena* is widespread among the HGS and also reaches conspicuous relative abundances.

The genus *Caloneis* was found exclusively in BGSs: *C. undulata* is a "severely endangered" species [21] commonly found in mires with few individuals [53]. The genus *Eunotia* has some species that are present only in undisturbed sites, such as *Eunotia hexagliphys*, which is considered at risk of extinction [21], or *E. triodon*, *E. tetraodon*, *E. praerupta*, and *E. nymmaniana*, which are classified as "severely endangered".

Therefore, for BGSs, it is possible to conclude that there are many rare and sensitive species that are clearly found more frequently than in HGSs.

For the HGS, there is a prevalence of common and generalist species. A particularly widespread species, for example, is *Eunotia mucophila*, which reaches a relative abundance greater than 10%. This species was described from the mucilage of red algae (*Batrachospermum* [30]); therefore, it lives in a microhabitat that is quite rich in nutrients [30]. Even Van Dam et al. [35] classified it as an oligomesotraphentic species. The three taxa exclusive to the HGS are *Pinnularia microstauron* and the two varieties of this species. The species' nominal variety has a trophic index equal to 7 [35], according to which the species is widespread in oligotrophic to hypertrophic environments. Furthermore, *P. microstauron* has good motility, which can favor it in the presence of moderate nutrient enrichment. This is consistent with Passy's [63] hypothesis, according to which mobile species can select the most suitable microhabitat and exploit its resources. *Neidium ampliatum* was also exclusively found in the HGS. This is a group that is still taxonomically debated, and the species is classified as oligomesotraphentic [35]. Both *P. microstauron* and *N. ampliatum* are species with large biovolumes that can be favored by increases in nutrients. The situation is similar for the genus *Kobayasiella*, which is clearly more frequent and abundant in the HGS. *Kobayasiella subtilissima*, the largest of the three species found, reaches high abundances in the HGS, suggesting that the species tolerates disturbance and can take advantage of nutrient enrichment. The species, although considered severely endangered [21], has already been found often and with high abundances in other studies. Additionally, it does not seem to be very rare in mires [42,58].

4.3. Bioindication Value of Diatoms in Mires: Environmental Monitoring and Paleoecological Research

Recently, the effects of cattle grazing on mires were assessed using a paleoecological approach [17], proving that grazing had a significant impact on the diatom species composition present in the top 15 cm of the peat cores. Also, in the paleolimnological study, the assemblages of the control sites were different from those of the grazed sites. Cid-Rodríguez et al. [17] hypothesized that a high grazing intensity causes an increase in common and generalist species and that this trend can be also observed in our study on present-day mire pool diatom assemblages. The paleoecological approach shows some common results with our study: *Encyonema perpusillum* and *Aulacoseira alpigena* are considered indicators of highly grazed sites, strengthening the hypothesis that the species become opportunistic when a nutrient increase occurs. On the other hand, both approaches recognized rare species like *Brachysira confusa*, *Chamaepinnularia schauppiana*, and *E. nymmaniana* as being typical of BGSs.

Both studies demonstrate that diatom communities effectively respond to grazing disturbance and highlight that there is an impact related to grazing, although it is not a devastating disturbance, confirming the effectiveness of these algae as useful environmental indicators. In general, apart from the variation caused by grazing, diatom assemblages identify the sites analyzed as oligotrophic, acidic, or weakly acidic and subject to desiccation. These are characterized by highly specialized, often rare diatom species [11].

In the hydrochemical analysis, no differences appeared between the groups of high- and low-grazing sites. An increase in the concentration of nutrients, such as nitrates and phosphates, was hypothesized [13], but the concentration of nutrients in mires varies strongly because of complex interactions between microbial activities, removal by plants, and spatial variability [65]. Therefore, the absence of correlation between the intensity of grazing and the concentration of nutrients is a result that can be expected [13]. Also, Cremonese et al. [66], when monitoring the hydrochemical characteristics of the Mont Avic Nature Park mires and evaluating the impact of grazing, found comparable results. In relation to nitrogen compounds, the authors did not observe any trends that were correlated with the presence/absence of grazing manure. The result is similar for the evaluation of the other chemical and physical aspects considered and the cited authors believe that the intrinsic variability of most peat water quality descriptors constitutes the main limit to the identification of any impact on water chemistry [66].

Given the inadequacy of hydrochemical analysis in showing the possible presence of grazing or the intensity of disturbance, diatoms can be a good proxy, exploiting the ability of biological data to integrate the variability of chemistry over time into models. Lavoie et al. [67] suggest that the period of the integration of chemical data into assemblages varies according to the trophic state and the nutrient concentration of the streams. They also suggest that diatom communities from oligotrophic streams are more sensitive to and directly affected by nutrient increases. The mires analyzed in this study, which are oligotrophic environments, mirror the same features of oligotrophic streams, and the composition of diatoms assemblages can be a valid method of evaluating the quality and integrity of these habitats, although their potential as bioindicators has not yet been fully exploited in mires [11].

5. Conclusions

Although there is an urgent need to examine the state of the conservation of peatlands in high mountain grazing areas, the effects of this disturbance have been poorly investigated [13]. Grazing favors the most common and/or opportunistic species, such as *Encyonema perpusillum*, *Eunotia tenella*, *Eunotia neocompacta* var. *vixcompacta*, and *Kobayasiella subtilissima*, which benefit from the increase in nutrients to the detriment of rarer and more sensitive species (e.g., *Brachysira confusa*, *Chamaepinnularia schauppiana*, *Encyonema neogracile*, *Eunotia hexagliphys*, *Stenopterobia delicatissima*). Diatoms are a promising proxy for identifying a disturbance and monitoring its effects [15,57]. Assessing the integrity of high mountain peatlands is essential due to their fragility in Italy [7]. Habitat 7110*, to which two of the four mires studied belong, is of community interest and is particularly at risk. Its distribution is mainly linked to the Alps and the habitat is threatened by numerous factors, such as climate changes, variations in the overall hydrological system (water capture, variation in the water table, drainage, and reclamation), flooding (to obtain lakes that can be used in firefighting or for artificial snow, etc.), the impact of road salt (near roads and inhabited centers), deforestation, peat extraction, the grazing or transit of livestock and game, and, locally, trampling along paths open to the public [68].

Conserving these habitats is fundamental to protecting their rich biodiversity and to ensuring the integrity and functioning of mires as main carbon-sink habitats. Given the rich diversity of the diatoms hosted by mires pools [11], with percentages of rare or threatened species greater than 60%, and considering the known fragility of peatlands [7], greater protection of these crucial habitats is highly recommended.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/land13111780/s1.

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