Science of the Total Environment 969 (2025) 178983

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Environmental and overgrazing effects on diatom communities in high mountain mires

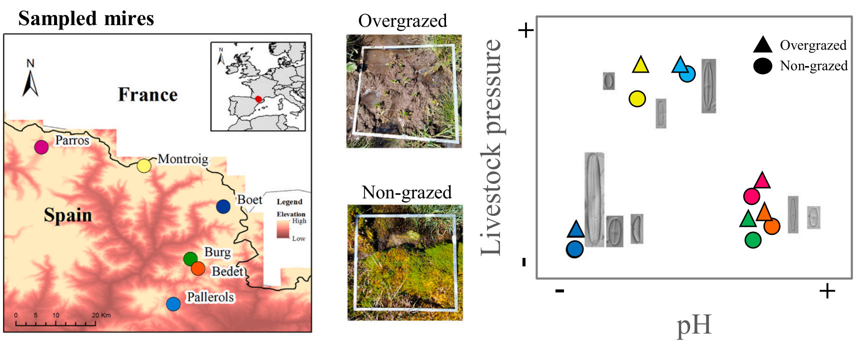
Fernanda Gonzalez-Saldias [a,b,*], Aaron Pérez-Haase [a,c], Eulàlia Pladevall-Izard [a,c], Joan Gomà [a,b]

[a] *Department of Evolutionary Biology, Ecology, and Environmental Sciences, University of Barcelona, Barcelona, Spain*
[b] *Freshwater Ecology, Hydrology, and Management laboratory (FEHM-lab), University of Barcelona, Barcelona, Catalonia, Spain*
[c] *Biodiversity Research Institute (IRBio), University of Barcelona, Barcelona, Spain*

HIGHLIGHTS

- The effects of livestock on diatom were studied in six mountain mires using fences.
- High mountain mires host a high diversity of diatoms.
- High livestock pressure leads to compositional changes in diatom communities.
- Diatoms showed resilience to livestock pressure but were influenced by pH.
- pH is the most important variable structuring these communities.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Fernando Pacheco

Keywords:
Bacillariophyceae
Anthropogenic pressure
Peatland
Pyrenees
pH

ABSTRACT

High mountain mires are affected by anthropogenic activities such as livestock, which have changed land use, fragmented habitats, and affected the structure and functioning of aquatic communities such as diatoms. Diatoms in mires exhibit high diversity, with a significant proportion of rare and threatened species, making them vulnerable to livestock pressure. Despite their common use as indicators of ecological status, little is known about how overgrazing and trampling affect diatom communities. To assess the impact of livestock pressure, we conducted experiments in six high-mountain mires in the Pyrenees, where livestock exclusion fences were installed. We identified a total of 370 species belonging to 60 genera, with a maximum diversity per site of 77 species. The variables most influencing diatom composition were pH, elevation, Bryophyta cover, water cover, livestock pressure, and mire area. Our results indicated an impact on diatom composition at the two locations with higher livestock pressure; however, we observed no effects on species richness or the Shannon index, suggesting that diatom communities are resistant to livestock pressure. The pH was the most important environmental variable for diatom community composition, demonstrating a positive relationship with diatom richness. Livestock pressure in high mountain mires causes habitat fragmentation and increased dry density of the soil, making diatom communities more vulnerable to environmental changes. However, these communities exhibit notable resistance to significant pressure, with pH emerging as the most critical factor influencing their structure. Our study showed that high livestock pressure affects diatom communities in mires. Considering that a

---

[*] Corresponding author at: Department of Evolutionary Biology, Ecology, and Environmental Sciences, University of Barcelona, Barcelona, Spain.
E-mail address: fernanda_gonzalezs@ub.edu (F. Gonzalez-Saldias).

https://doi.org/10.1016/j.scitotenv.2025.178983
Received 13 November 2024; Received in revised form 23 February 2025; Accepted 24 February 2025

high percentage of diatom species are red-listed as vulnerable or endangered, it is important to protect mires to avoid loss of diversity.

1. Introduction

Mires are permanently wet environments with slow decomposition of organic matter, low oxygen, low nutrient availability, and low temperature (Batzer et al., 2016; Rydin et al., 2013). In mountainous areas, mires often exhibit irregular shapes and rugged landscapes, forming small fragmented patches that resemble terrestrial islands (Ninot et al., 2017; Pérez-Haase et al., 2010). These environments feature variable water availability, pH (Poulícková et al., 2004), and vegetation coverage (Amigo et al., 2017; Ninot et al., 2017; Rydin et al., 2013). Due to these characteristics, mires provide diverse habitats and support a high level of biodiversity, including photosynthetic organisms (Batzer et al., 2016; Jiménez-Alfaro et al., 2012; Pérez-Haase and Ninot, 2017; Quiroga et al., 2022; Tsuraya et al., 2023).

Mires are sensitive habitats that are vulnerable to both natural and anthropogenic disturbances. Direct anthropogenic interventions, such as drainage for agricultural purposes, peat extraction, forestry activities, livestock grazing, and tourism, impact these ecosystems (Bergamini et al., 2009; Cambra, 2015; Joosten, 2003; Ramchunder et al., 2012). Additionally, grazing pressure, livestock trampling, and changes in land use can alter the pH, conductivity, and nutrient levels of mire vegetation (Cabezas et al., 2015; Wahren et al., 2001). These alterations affect the diversity and community structure of various organisms, including vascular plants and bryophytes (Boch et al., 2018; Grillo and Venora, 2011), macroinvertebrates (Herbst et al., 2012) and phytobenthic communities (Cid-Rodríguez et al., 2024; Niedermayr and Schagerl, 2010).

Diatoms, unicellular siliceous algae, are highly responsive to environmental changes and are commonly used as indicators for assessing water quality and ecological status (Cid-Rodríguez et al., 2024; Rimet, 2012; Smol and Stoermer, 2010). Their performance can be influenced by various environmental factors, such as light intensity, humidity, pH, electrical conductivity, dissolved oxygen levels, and nutrient concentrations (Delgado et al., 2012; Hargan et al., 2015; Soininen, 2007; Soininen and Teittinen, 2019). Among these factors, pH has emerged as one of the major drivers of the diatom community composition in mires (Cantonati et al., 2011; Poulíčková et al., 2013; Stanković et al., 2022).

Many mountainous regions support extensive summer cattle and horse grazing, often without herder supervision (Cid-Rodríguez et al., 2024; McDougall, 2007; Navarro et al., 2023). This unregulated grazing can severely impact mires, i.e., overgrazing by livestock reduces plant biomasses, and causes soil erosion, while trampling increases compaction (Grillo and Venora, 2011; Joosten et al., 2012). These disturbances can potentially harm microscopic communities, such as diatoms which are essential for ecosystem functioning. Diatoms in mires exhibit high species diversity, many of them rare and threatened (Cantonati and Lange-Bertalot, 2011; Mutinová et al., 2016). Consequently, their diversity may be negatively affected by livestock pressure.

There is an increasing interest in investigating the effect of changes in livestock regime on diatom communities in mires (Cid Rodriguez et al., 2022; Rossi et al., 2024), however, the primary drivers behind these changes remain unclear. We hypothesized that overgrazing and trampling livestock would decrease the diversity of diatom communities, due to habitat alterations. Additionally, we explored changes in physicochemical parameters affecting diatom community composition in six high mountain mires where fences excluded livestock.

2. Methodology

2.1. Field experiment

The study was conducted in six heavily pastured mires in the Central Pyrenees, where we installed livestock exclusion fences in 2018 (Fig. 1, Fig. S1). The mires exhibit diverse geological and environmental characteristics. All sites are currently subject to overgrazing and livestock presence, resulting in soil disturbance due to trampling. This area is characterized by presenting mostly bovine livestock pressure (>80 %), followed by equine and ovine (Pérez-Haase et al., 2019). These fences were deployed to exclude livestock grazing and trampling from a specific area in the mire to assess the effect of land use change on soil and communities. The area of the fences varies between mires (Table 1). We sampled these locations three years later in the summer of 2021 (Fig. 1).

2.2. Environmental variables and livestock intensity characterization

Environmental variables such as pH and conductivity were measured superficially using a portable pH meter (WTW ProfiLine pH 3310) and conductivity meter (WTW ProfiLine Cond 3310). Each mire comprised a non-grazed and an overgrazed area. Eight plots (50 × 50 cm, 0.25 m2) were established in each mire, four in non-grazed and four in overgrazed areas. Each plot was divided into 16 smaller squares of 12.5 × 12.5 cm. Five measurements of the environmental variables and calculated the average per plot. These measurements covered the four corners and one square in the center (see Fig. S1C). The average pH and conductivity values from each plot used were reported. We estimated the elevation and the area of each mire. We calculated the mire area by delineating each mire using infrared orthophotos from the Cartographic and Geological Institute of Catalonia with VISSIR 3.35 (http://srv.icgc.cat/vissir3/), accessed September 2023 (Table 1). Finally, the Growing Degree Day value (GDD) for each site was estimated using the Pyrenean Digital Climatic Atlas with a 30 m resolution (Batalla et al., 2018).

$$GDD = \sum\left(\left(\frac{(Tmax + Tmin)}{2}\right) - Tbase\right)*N$$

where $Tmax$ is the day's maximum temperature, $Tmin$ is the day's minimum temperature, $Tbase$ is equal to zero, and N is the number of days/month.

The vegetation in the study was typical of mountain fens, chiefly dominated by sedges (*Carex* spp.), on the herbaceous layer and peat mosses (*Sphagnum* spp.), or Bryophyta (*Campylium stellatum* (Hedw.) Lange & C.E.O.Jensen (1906), *Palustriella* spp., etc.) on the moss layer. We calculated a heterogeneity index to account for this environmental and vegetation variability. To estimate the heterogeneity, we used plots measuring 50 × 50 cm and defined five conditions based on the coverage percentage of five elements: water above the soil surface, muddy soil, *Sphagnum* mosses, Bryophyta, and sedges. We quantified the concentration of inorganic nitrogen (NH_4^+ and $NO_3^- + NO_2^-$) with FUTURA Automated Continuous Flow Analyzer in groundwater by measuring it in each mire, including both non-grazed ($n = 4$) and overgrazed areas ($n = 4$).

We quantified the livestock pressure intensity global in each mire by evaluating the trampling intensity, the herbivory intensity, and the number of livestock feces. We used 50 random plots (50 × 50 cm) outside the fence (overgrazed area) and calculated the average percentage of each category. The trampling intensity considered four levels: no trampled, compacted (soil trampled with vegetation cover), moderately trampled (deep hoof prints resulting in bare soil), and severely trampled (pitted soils with discontinuous vegetation cover). The degree of herbivory considered four levels: The first one without herbivory, the second one presented <10 % of the leaves consumed by livestock; the third one presents between 10 and 50 % of the vegetation consumed; and the fourth with >50 % of the vegetation consumed by the livestock. Finally, the four levels for the number of feces were: no feces (0), 1–2 feces, 3–5 feces, and >5 feces.

2.3. Diatom sampling, processing, and taxonomic identification

In each mire, we collected four samples from non-grazed and four from overgrazed areas to cover the heterogeneity of the diatom community (Fig. S1). In each collected sample, we used a plot (50 × 50 cm) to select five points (subsamples), ensuring comprehensive coverage of the potential diatom community's heterogeneity. We combined the subsamples in 50 mL flasks and preserved them in formalin (Fig. S1C). To characterize diatom community composition, we treated samples with 33 % w/v hydrogen peroxide heated to 100 °C for 24 h to remove the organic matter. We washed the suspension of clean frustule with distilled water and then mounted it on permanent microscope slides using Naphrax®. We identified and enumerated under a POLYVAR microscope with differential interference contrast, counting 500 valves per sample. The relative abundance of the diatom community was calculated for subsequence analyses. We followed the taxonomic identification of diatoms guidelines from monographs of Krammer, 2000a, 2000b; Lange-Bertalot, 2001, 2000; Lange-Bertalot et al., 2017, 2011.

2.4. Statistical analyses

Table 1

Descriptive information by each sampled site: geographical coordinates, elevation meters above the sea level (m.a.s.l.), and mire areas. The area of the fence represents the non-grazed area.

Mires	Coordinates		Elevation (m. a.s.l.)	Area (m^2)	Fence area (m^2)
	Latitude	Longitude			
Boet	42.618218	1.389382	1858	2928.69	883
Montroig	42.710535	1.209886	2010	2275.41	724
Pallerols	42.411081	1.237643	1669	6074.72	665
Bedet	42.478983	1.332213	1628	6113.28	758
Burg	42.500693	1.317491	2005	6471.51	842
Parros	42.752186	0.976974	1770	1524.56	520

First, we evaluated significant differences in the Richness and Shannon diversity index between non-grazed and overgrazed areas. We performed a one-way ANOVA when the assumptions of normality and homoscedasticity were fulfilled, otherwise, we conducted a Kruskal-Wallis test.

Second, we calculated the Bray-Curtis dissimilarity matrix using square root-transformed relative abundances. To evaluate the composition differences between non-grazed and overgrazed areas in each mire, we performed a PERMANOVA test. We then conducted Distance-based Redundancy Analyses (dbRDA) with a forward selection of variables on the diatom abundance dissimilarity matrix to elucidate the relationship between environmental variables and diatom community composition. To run this analysis, we included elevation, mire area, cover percentage of environmental heterogeneity, herbivory, trampling, and amount of feces as environmental variables. Subsequently, we took the selected variables to linear and polynomial regression analyses to assess the relationships between Richness, Shannon diversity, and the significant environmental variables identified in the dbRDA. The best model was selected according to the Akaike information criterion (AIC). To assess the effect of pH on diatom communities, a Mantel test was

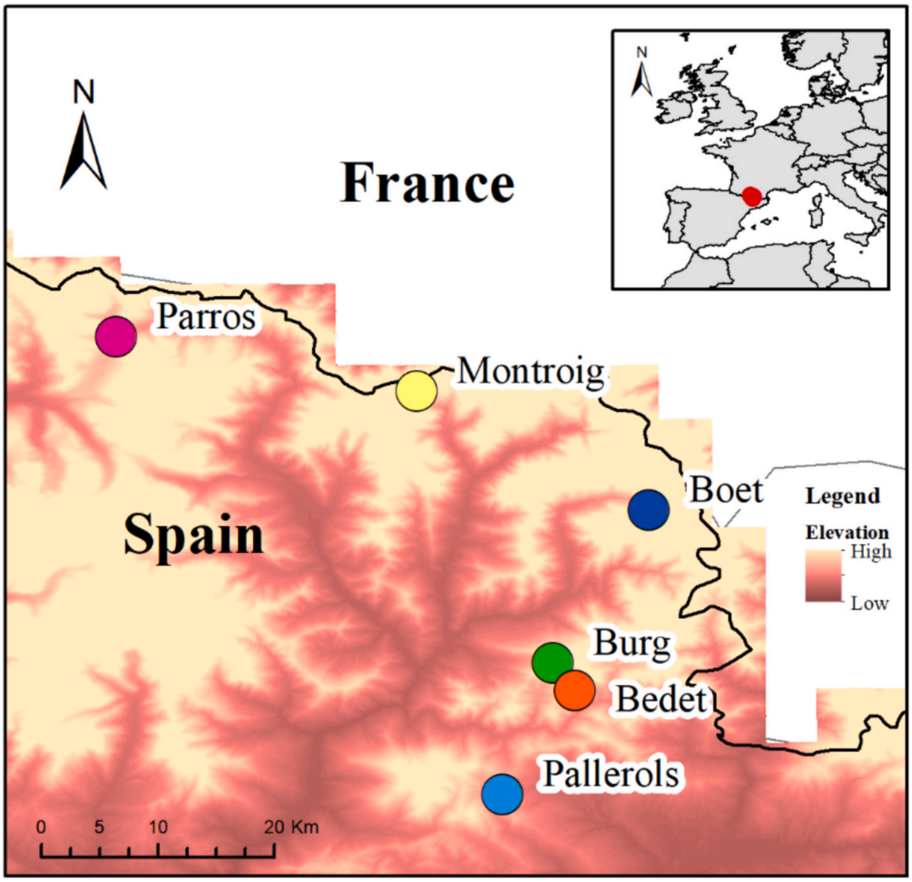


Fig. 1. Geographical location of the studied mires. Points correspond to the mires sampled in the Catalan Pyrenees in the summer of 2021.

performed between Bray-Curtis dissimilarity and pH.

All the statistical analyses were performed using the R program (v4.2.3) using the libraries vegan (Oksanen et al., 2022), dplyr (Wickham et al., 2023), and microbiome (Leo and Sudarshan, 2012).

3. Results

3.1. Environmental variability

The coverage of sedges, Bryophyta, muddy soil, *Sphagnum* mosses, and water varied among localities (Fig. S2, Fig. S3, Kruskal-Wallis, $p < 0.05$). Bedet exhibited the highest water coverage. Parros, Bedet, Pallerols, and Burg, showed a greater percentage of sedges. Boet had a higher *Sphagnum* cover and Montroig presented a higher percentage of Bryophyta and sedges. The only difference found between non-grazed and overgrazed areas was the mud cover percentage (Two-way ANOVA, $p = 0.005$, Table S2). For the other cover types, significant differences were found between mires and overgrazed areas except for the water cover percentage (Two-way ANOVA, $p < 0.05$ Table S2).

Studied mires displayed high variability in pH, ranging from 4.2 to 7.9 (Fig. 2A). Three mires were characterized as acidic: Boet, Montroig, and Pallerols (Fig. 2A), while Bedet, Burg, and Parros are calcareous mires. Specifically, Boet presented a significantly lower pH than all the other locations (ANOVA, $p < 0.001$). We also observed significant differences between Montroig and Pallerols compared to the other four mires (ANOVA, $p < 0.001$). Moreover, we found significant differences in pH comparing overgrazed from non-grazed areas in Boet and Pallerols (Kruskal-Wallis, $p < 0.001$), with lower pH on non-grazed areas, i.e. areas excluded from livestock pressure. We recorded the lowest conductivity in Pallerols (154.0 0 ± 63.77 μS/cm) and the highest in Boet (432.50 ± 345.11 μS/cm; Fig. 2B). We found significantly lower conductivity values in non-grazed than overgrazed areas (one-way ANOVA, $p < 0.001$) across most mires, except for Montroig and Burg (Fig. 2B). Additionally, ammonium concentrations varied several orders of magnitude between sampling sites. However, we detected no significant differences resulting from the exclusion treatment (Fig. 2C). Finally, we found groundwater nitrate and nitrite concentrations to be below the detection level (<0.378 mg/L), and therefore, we excluded these from further analysis.

Sampled data revealed a widespread impact of livestock pressure (Fig. 2D). We observed the highest percentages of severely trampled areas at the Montroig and Parros mires, while the Burg and Bedet exhibited the highest percentage of soil compaction and high herbivory pressure. Despite the evident effects of livestock farming, we found livestock feces were not abundant across the sites (Fig. 2D).

3.2. Diversity and composition of diatoms inside and outside the fence

We identified a total of 370 different species belonging to 60 genera across the studied sites (Table 2, Table S1). Only 17 species contributed >1 % of the total relative abundance (Fig. 5B), while 353 were <1 %, of which 70 species were extremely rare, being found in one plot. The most abundant species was *Achnanthidium minutissimum* (Kütz.) Czarnecki 1994 comprises 17.44 ± 15.48 % of the total individuals. This was followed by *Staurosira construens* var. *venter* (Ehrenb.) P.B. Hamilton 1992 (6.83 ± 11.76 %), *Eolimna minima* (Grunow) Lange-Bertalot 1998 (4.00 ± 5.26 %), and *Kobayasiella micropunctata* (H.Germain) Lange-Bertalot 1999 (2.87 ± 7.79 %, Table 2).

We observed great variability in local diversity within the diatom communities, with Richness and Shannon's diversity presenting similar trends (Fig. 3). Pallerols displayed the lowest and highest species richness per plot, ranging from 5 to 77 species (Fig. 3A). Boet had on average the lowest number of species richness per plot at 35.63 ± 5.76 species. However, we found no significant differences in species richness

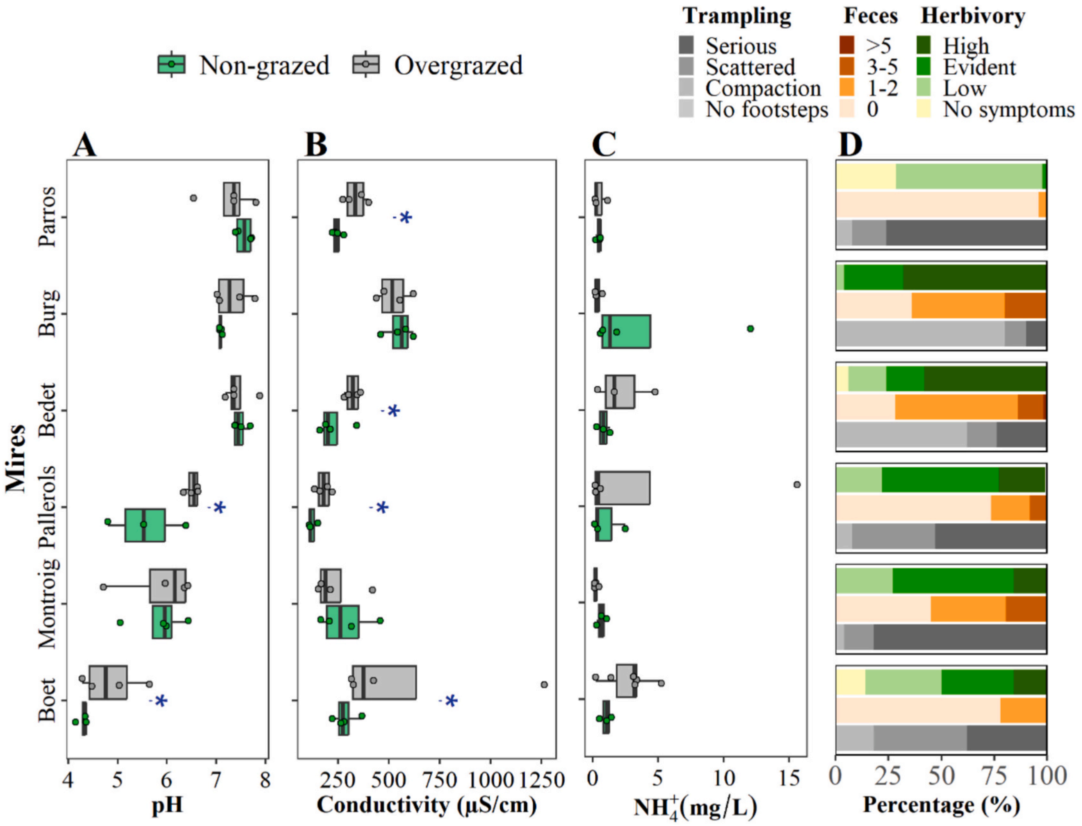


Fig. 2. Environmental characteristics of the study area: pH (A), conductivity (B), NH$_4^+$ concentration (C), and the percentage of livestock pressure (D) on sampled mires. * significant differences between treatments. *significant differences between treatments.

Table 2
The most abundant species of diatoms found in the sampled mires (only those with >1 % relative abundances) (mean ± standard deviation). Percentage of the total individuals.

Species	Relative abundance
Achnanthidium minutissimum (Kütz.) Czarnecki 1994	17.44 ± 15.48
Staurosira construens var. venter (Ehrenb.) P.B. Hamilton 1992	6.83 ± 11.76
Eolimna minima (Grunow) Lange-Bertalot 1998	4.00 ± 5.26
Kobayasiella micropunctata (H.Germain) Lange-Bertalot 1999	2.87 ± 7.79
Psammothidium subatomoides (Hustedt) Bukhtiyarova & Round 1996	2.83 ± 7.13
Pinnularia rupestris Hantzsch 1861	2.58 ± 8.46
Chamaepinnularia mediocris (Krasske) Lange-Bertalot 1996	2.57 ± 11.85
Eunotia faba (Ehrenberg) Grunow 1881	2.36 ± 6.25
Gomphonema parvulum (Kützing) Kützing 1849	2.24 ± 4.01
Caloneis tenuis (W.Gregory) Krammer 1985	2.23 ± 5.82
Navicula exilis Kützing 1844	2.20 ± 3.37
Gomphonema gracilis Ehrenberg 1838	1.65 ± 4.37
Odonthidium mesodon (Ehrenberg) Kützing 1849	1.62 ± 5.80
Staurosira confusa E.A.Morales 2005	1.61 ± 3.35
Aulacoseira tenella (Nygaard) Simonsen 1979	1.35 ± 4.16
Nitzschia acidoclinata Lange-Bertalot 1976	1.32 ± 1.87
Eunotia subarcuatoides Alles, Nörpel & Lange-Bertalot 1991	1.22 ± 3.21

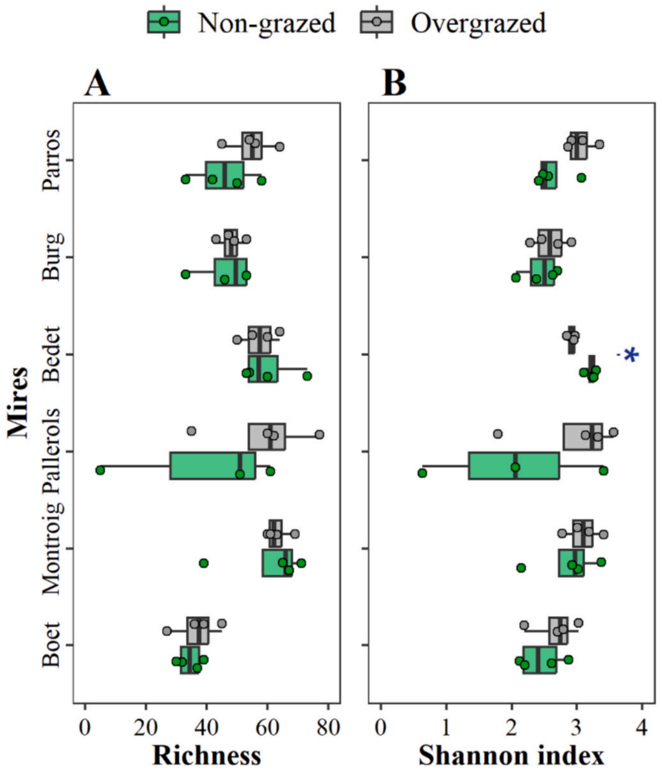


Fig. 3. Species richness (A) and Shannon index (B) of the sampled plots in non-grazed and overgrazed areas for each mire. * significant differences between treatments.

between non-grazed and overgrazed areas at any site (Fig. 3A). Similar to species richness, Palleros had the lowest Shannon index as well as the greatest variability (Fig. 3B). In contrast, Boet showed the lowest average Shannon index, but this did not significantly differ from the other mires. Noticeably, Bedet presented significant differences in the Shannon index between overgrazing and non-grazed areas, with higher values recorded in the non-grazed area (one-way ANOVA, $p < 0.01$).

According to the dbRDA analysis, we identified three distinct groups of diatom composition (Fig. 4). We identified significant differences between non-grazed and overgrazed areas in Montroig, along with a trend in Pallerols (PERMANOVA, $p = 0.030$ and $p = 0.050$ respectively). These two mires presented a greater dispersion in species composition.

3.3. *Effect of environmental variables on diatom communities*

The first two axes of the dbRDA explained 70 % of diatom compositional changes. The first axis (dbRDA1) was primarily influenced by pH, accounting for 53.8 % of the variation (Fig. 4). The second axis (dbRDA2) explained 16.2 % of the variability and aligned with variations in livestock pressure (herbivory), conductivity, water coverage, and Bryophyta coverage. The dbRDA analysis clustered the sites into three groups: the first group comprised only Boet plots and was associated with the lowest pH values. The second group included Montroig and Pallerols, which presented high herbivory levels, and a high percentage of Bryophyta coverage. The third group, consisting of Bedet, Burg, and Parros, correlated with feces abundance, larger mires, higher water coverage, and higher pH (Fig. 4).

We found that communities clustered into three groups based on different pH values. The first cluster, comprised of the Boet, was characterized by a higher relative abundance of *Psammothidium subatomoides* (Hustedt) Bukhtiyarova & Round 1996, *Pinnularia rupestris* Hantzsch 1861, and *Eunotia faba* (Ehrenberg) Grunow 1881. The second group, including Pallerols and Montroig, showed moderate differences in composition and was mainly represented by *S. construens* var. *venter, Chamaepinnularia mediocris* (Krasske) Lange-Bertalot 1996, and *K. micropunctata*. The last cluster was characterized by *E. minima* and *A. minutissimum* species. Likewise, the dominance pattern of diatom species changed with the pH (Fig. 5). The Mantel test showed that pH affects diatom community structure ($r = 0.73$, $p < 0.001$). Furthermore, diatom species richness has a significant and positive relationship with increasing pH ($R^2 = 0.238$, p-value $= 0.003$; Fig. 5C), whereas the Shannon diversity index did not show a relationship with pH.

4. Discussion

We hypothesized that livestock activity would impact diatom communities due to strong environmental modifications such as soil compaction, vegetation changes, and increased nutrient levels from urine and feces. Surprisingly, our results suggest that high levels of livestock pressure affect the composition of diatom communities in mires, but do not significantly impact species richness. We also found that pH influences diatom species composition and richness, as demonstrated by the wide pH gradient present at the studied mires.

4.1. *Diatom of high mountain mires are resistant to livestock pressure*

Mires are described as geographically isolated (Ninot et al., 2017). This characterization suggests that these ecosystems may be sensitive to nutrient inputs from livestock feces and urine, as noted in another study (Cid-Rodríguez et al., 2024). However, our findings indicate that livestock did not significantly contribute to groundwater nitrogen levels in the studied Pyrenean mires. We found that the presence of livestock did not cause changes in pH at most locations; however, we observed an impact on the conductivity measurement (Fig. 2). These results suggest that, despite the widespread livestock pressure found, such as trampling, compaction, and herbivory, diatom communities were only perturbed in mires with the highest levels of livestock pressure.

Trampling has been found to significantly disturb the structure of the diatom community by altering the morphology of the mire, which promotes bare soil and increases soil drying, directly affecting them (Cid-Rodríguez et al., 2024; Hargan et al., 2015). Grazing also affects species composition, resulting in distinct community structures between sites with high livestock impact and those with lower impact (Cid-Rodríguez et al., 2024; Rossi et al., 2024). In our study, we observed significant differences in diatom community composition due to livestock in Montroig and Pallerols, both with a high livestock impact

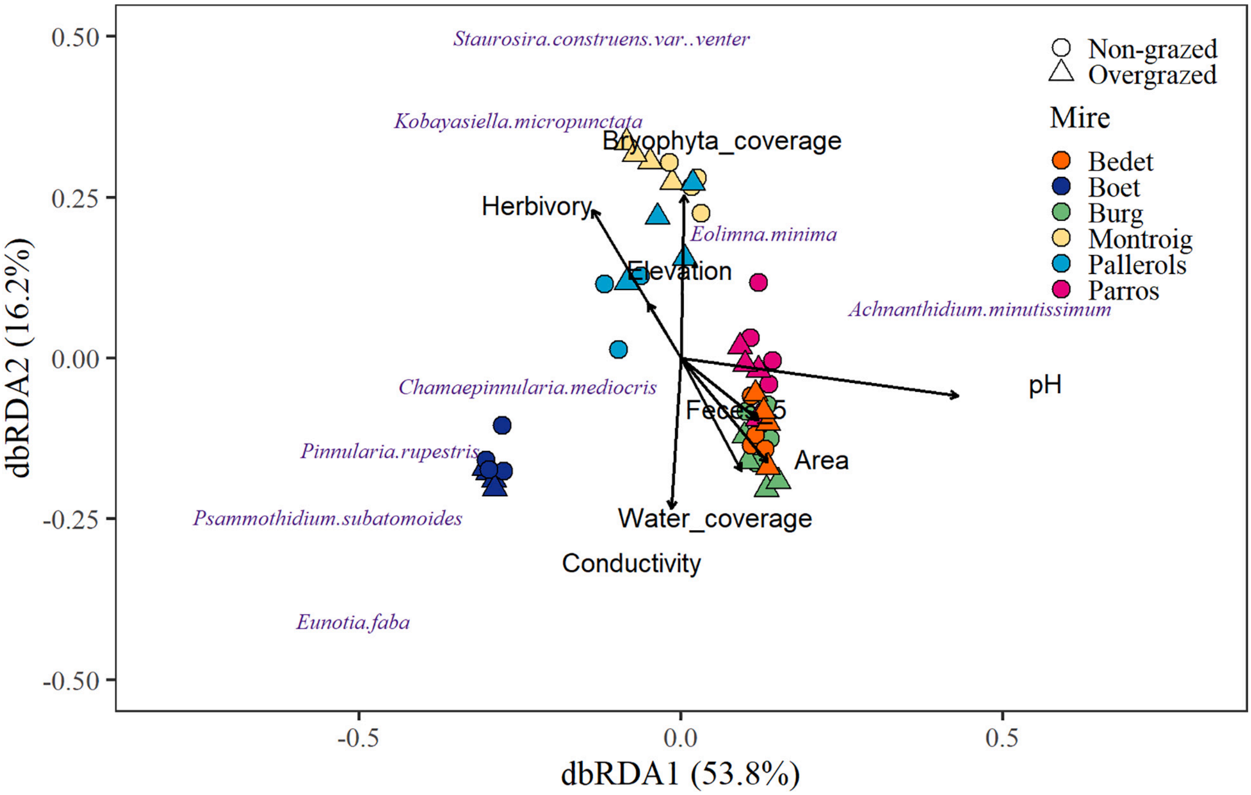


Fig. 4. Distance-based redundant analysis (dbRDA) of the diatom community with the environmental variables measured. The environmental variables selected through forward selection include the Bryophyta and water cover percentage, livestock pressure (measured by herbivory and feces greater than five), pH, conductivity, elevation and mire areas. Arrows represent environmental variables. In circle non-grazed and triangle overgrazed areas.

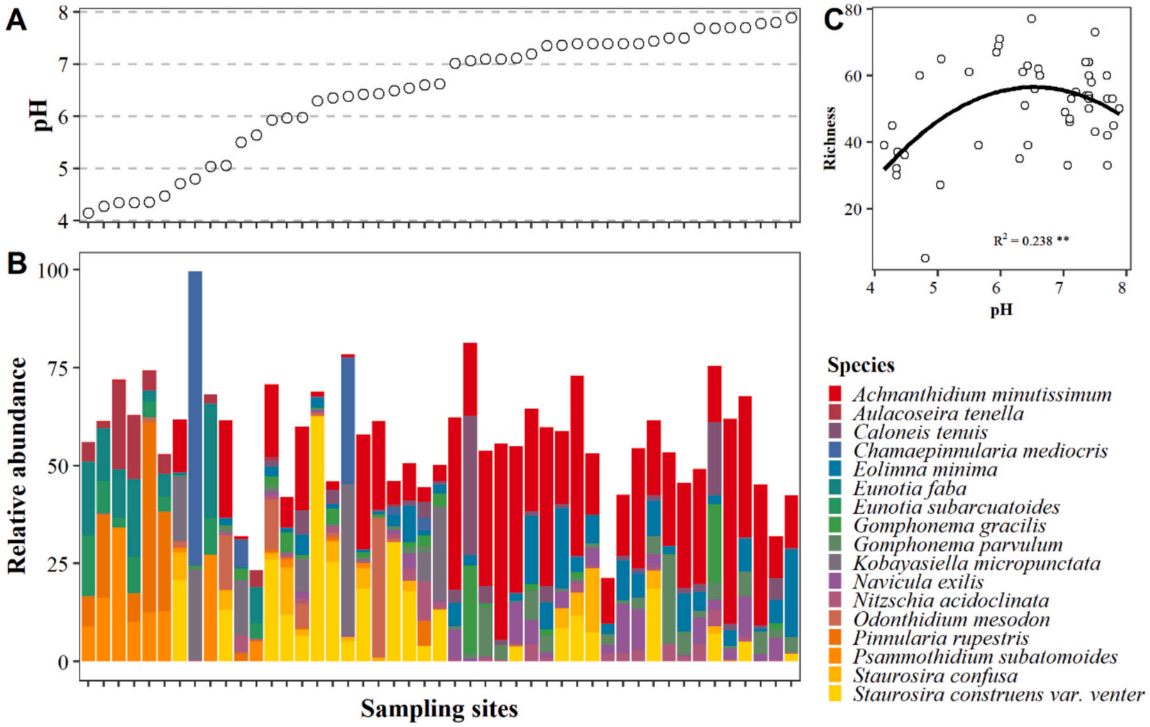


Fig. 5. A) pH gradient along the sampling plots, sorted from the lowest to highest pH. B) Representation of the relative contribution of the most abundant species (>1 % of the total relative abundances across sites) sorted from the lowest to highest pH. C) Polynomial regression of species richness with pH, $y = -33.125x^2 + 32.617x + 50.617$, $R^2 = 0.238$, p-value = 0.003.

(Fig. 4). Strong selection for species was associated with habitat trophic state, with mires-specific differences (Fig. S4). Species in overgrazed areas have been associated with meso-eutraphentic environments, such as the *S. construens* var. *venter*. In the case of non-grazed areas oligotraphentic and mesotraphentic species were found (Cantonati et al., 2011; Van Dam et al., 1994), i.e., *C. mediocris* and *Odonthidium mesodon* (Ehrenberg) Kützing 1849 (Fig. S4). Diatom species that can live in disturbed mires will have adaptations that help them to survive desiccation periods promoted by the trampling effect, for example, mucilage production to maintain moisture, migration to moist refugia, or formation of resistant cells (McKew et al., 2011; Quevedo-Ortiz et al., 2024).

We encountered a high diversity of species in the mires, which exceeded that found in other freshwater systems, such as rivers and lakes, where communities are typically dominated by few species (Gomà et al., 2005; Wang et al., 2017). This richness can result from the pronounced environmental heterogeneity that promotes the formation of microhabitats, isolated mires, and dispersal limitation due to the species colonization capacity (Quevedo-Ortiz et al., 2024). The excess nutrients provided by livestock can dilute in the large water bodies of these environments, and vegetation can rapidly absorb them (Williams et al., 1999). Consequently, our study indicates that local environmental variables, rather than livestock farming, primarily influence the species composition of diatoms.

The high species richness of mires includes rare and endangered species. In our study, 45 % of the identified species were classified as vulnerable or endangered on the Red List (Cantonati et al., 2022) (Table S3). In line with this, other studies have reported that many species inhabiting mires are vulnerable or endangered on the Red List (Cantonati et al., 2022; Cid Rodriguez et al., 2022; Rossi et al., 2024). This highlights the need for a more accurate assessment of diatom endemism and vulnerable species, particularly in high-mountain mires, where these species are concentrated (Cantonati et al., 2022).

4.2. pH is the main driver of diatom compositional changes

In this study, we show that pH primarily affects the diatom community. Locations with low pH exhibit communities with low richness compared to others (Cambra, 2015; Poulícková et al., 2004). In contrast, communities with higher pH variation, ranging from acidic to neutral, display greater variability. Our data suggests that pH acts as an environmental filter. Localities with high environmental variability harbor more dissimilar communities, whereas those with lower pH values showed less dissimilarity (Fig. 4). These results highlight that pH influences both diatom species richness and composition in mires (Fig. 5C), with pH 5.5 identified as critical for diatoms (Fig. 5), leading to shifts in community structure (Hargan et al., 2015). A decrease in pH negatively impacts diatom richness, favoring acidophilic species (Cambra, 2015) or those resistant to pH variation (Cantonati and Lange-Bertalot, 2011). We observe the effect on diatom communities primarily when the pH range is wide (Poulícková et al., 2004) as habitats with narrower and more uniform ranges give rise to other environmental factors that may also be important for diatoms (Borics et al., 2003).

Diatom species composition and dominance changed along the pH gradient forming three distinct clusters (Fig. 4, Fig. 5). A group of acidobiontic and acidophilous species (pH 4.6 ± 0.5), a group of acidophilous and circumneutral characterized for inhabiting in a broader range of pH (6.0 ± 0.7), and an alkalibiontic cluster (7.4 ± 0.3). The first group represented members of the *Eunotia*, a genus described to thrive in acidic conditions (Battarbee et al., 2001; Cantonati et al., 2011; Ortiz-Lerín and Cambra, 2007; Poulícková et al., 2004), with *E. faba* and *E. subarcuatoides* Alles, Nörpel & Lange-Bertalot 1991 being the most abundant species in Boet mire (Fig. 5B). The second group was represented by *S. construens* var. *venter*, *C. mediocris*, and *K. micropunctata* (Fig. 4). For example, *S. construens var. venter* has been described to be pH tolerance, commonly found in stagnant waters (Hargan et al., 2015), although in this study its maximum abundance is around pH 6.5. Finally, the alkalibiontic group was mainly represented by *A. minutissimum* (Fig. 4), which was the most common and abundant species across a broad pH range with its maximum performance in neutral pHs (Fig. 5). This contrasts with other studies where *A. minutissimum* is considered to be indifferent to pH changes (Cantonati and Lange-Bertalot, 2011).

5. Conclusions

Overgrazing in high mountain mires generates compositional changes in diatom communities. This pressure leads to changes in vegetation cover and increased dry density, making diatom communities more susceptible to environmental changes. However, despite significant livestock pressure, the diatom communities have considerable resilience to intensive livestock activities, influenced by local variables such as pH. The pH is the most important variable that structures diatom communities.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.178983.

CRediT authorship contribution statement

Fernanda Gonzalez-Saldias: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Aaron Pérez-Haase:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Eulàlia Pladevall-Izard:** Writing – review & editing, Methodology. **Joan Gomà:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

FG-S was funded by the National Agency for Research and Development (ANID)/Scholarship Program/DOCTORADO BECAS CHILE/2020 - 72210270.

LIFE+ Resque Alpyr project (LIFE20 NAT/ES/000369), Interreg-POCTEFA Red-Bio (EFA088/01) and Bioocult project (OAPN 2413/2017).

We want to thank the staff of the Natural Park of Alt Pirineu and National Park of Aigüestortes i Estany de Sant Maurici for stimulating this research allocating funds and helping to set up the exclusion fences.

Data availability

I have shared the data as supplementary table S1

References

Amigo, J., Rodríguez-Guitián, M.A., Pradinho Honrado, J.J., Alves, P., 2017. The lowlands and midlands of northwestern Atlantic Iberia. In: Loidi, J. (Ed.), The Vegetation of the Iberian Peninsula, Plant and Vegetation, Volume 1. Springer International Publishing, Cham, pp. 191–250. https://doi.org/10.1007/978-3-319-54784-8_6.

Batalla, M., Ninyerola, M., Catalan, J., 2018. Pyrenean Digital Climate Atlas (PDCA). https://doi.org/10.5281/zenodo.1186639.

Battarbee, R.W., Jones, V.J., Flower, R.J., Cameron, N.G., Bennion, H., Carvalho, L., Juggins, S., 2001. Diatoms. In: Smol, J.P., Birks, H.J.B., Last, W.M., Bradley, R.S., Alverson, K. (Eds.), Tracking Environmental Change Using Lake Sediments: Terrestrial, Algal, and Siliceous Indicators, Developments in Paleoenvironmental Research. Springer Netherlands, Dordrecht, pp. 155–202. https://doi.org/10.1007/0-306-47668-1_8.

Batzer, D., Wu, H., Wheeler, T., Eggert, S., 2016. Peatland invertebrates. In: Batzer, D., Boix, D. (Eds.), Invertebrates in Freshwater Wetlands: An International Perspective on Their Ecology. Springer International Publishing, Cham, pp. 219–250. https://doi.org/10.1007/978-3-319-24978-0_7.

Bergamini, A., Peintinger, M., Fakheran, S., Moradi, H., Schmid, B., Joshi, J., 2009. Loss of habitat specialists despite conservation management in fen remnants 1995–2006. Perspect. Plant Ecol. Evol. Syst. 11, 65–79. https://doi.org/10.1016/j.ppees.2008.10.001.

Boch, S., Allan, E., Humbert, J.-Y., Kurtogullari, Y., Lessard-Therrien, M., Müller, J., Prati, D., Rieder, N.S., Arlettaz, R., Fischer, M., 2018. Direct and indirect effects of land use on bryophytes in grasslands. Sci. Total Environ. 644, 60–67. https://doi.org/10.1016/j.scitotenv.2018.06.323.

Borics, G., Tóthmérész, B., Grigorszky, I., Padisák, J., Várbíró, G., Szabó, S., 2003. Algal assemblage types of bog-lakes in Hungary and their relation to water chemistry, hydrological conditions and habitat diversity. In: Naselli-Flores, L., Padisák, J., Dokulil, M.T. (Eds.), Phytoplankton and Equilibrium Concept: The Ecology of Steady-state Assemblages, Developments in Hydrobiology. Springer, Netherlands, Dordrecht, pp. 145–155. https://doi.org/10.1007/978-94-017-2666-5_13.

Cabezas, J., Galleguillos, M., Valdés, A., Fuentes, J.P., Pérez, C., Perez-Quezada, J.F., 2015. Evaluation of impacts of management in an anthropogenic peatland using field and remote sensing data. Ecosphere 6, 1–24. https://doi.org/10.1890/ES15-00232.1.

Cambra, J., 2015. Micro-scale distribution of algae in a Pyrenean peat-bog, Spain. Hidrobiológica 25, 213–222.

Cantonati, M., Lange-Bertalot, H., 2011. Diatom monitors of close-to-pristine, very-low alkalinity habitats: three new Eunotia species from springs in Nature Parks of the south-eastern Alps. J. Limnol. 70. https://doi.org/10.4081/jlimnol.2011.209.

Cantonati, M., Lange-Bertalot, H., Decet, F., Gabrieli, J., 2011. Diatoms in very-shallow pools of the site of community importance Danta di Cadore Mires (south-eastern Alps), and the potential contribution of these habitats to diatom biodiversity conservation. Nova Hedwigia 93, 475–507. https://doi.org/10.1127/0029-5035/2011/0093-0475.

Cantonati, M., Hofmann, G., Spitale, D., Werum, M., Lange-Bertalot, H., 2022. Diatom Red Lists: important tools to assess and preserve biodiversity and habitats in the face of direct impacts and environmental change. Biodivers. Conserv. 31, 453–477. https://doi.org/10.1007/s10531-021-02339-9.

Cid Rodriguez, M., Cantonati, M., Spitale, D., Zaccone, C., 2022. Impact of Cattle Grazing on Peat Properties and Diatoms. https://doi.org/10.5194/egusphere-egu22-9910.

Cid-Rodríguez, M., Cantonati, M., Spitale, D., Galluzzi, G., Zaccone, C., 2024. Using diatoms and physical and chemical parameters to monitor cow-pasture impact in peat cores from mountain mires. Sci. Total Environ. 926, 171779. https://doi.org/10.1016/j.scitotenv.2024.171779.

Delgado, C., Pardo, I., García, L., 2012. Diatom communities as indicators of ecological status in Mediterranean temporary streams (Balearic Islands, Spain). Ecol. Indic. 15, 131–139. https://doi.org/10.1016/j.ecolind.2011.09.037.

Gomà, J., Rimet, F., Cambra, J., Hoffmann, L., Ector, L., 2005. Diatom communities and water quality assessment in mountain rivers of the upper segre basin (La Cerdanya, Oriental Pyrenees). Hydrobiologia 551, 209–225. https://doi.org/10.1007/s10750-005-4462-1.

Grillo, O., Venora, G., 2011. Ecosystems Biodiversity. BoD – Books on Demand.

Hargan, K.E., Rühland, K.M., Paterson, A.M., Finkelstein, S.A., Holmquist, J.R., MacDonald, G.M., Keller, W., Smol, J.P., 2015. The influence of water-table depth and pH on the spatial distribution of diatom species in peatlands of the Boreal Shield and Hudson Plains, Canada. Botany 93, 57–74. https://doi.org/10.1139/cjb-2014-0138.

Herbst, D.B., Bogan, M.T., Roll, S.K., Safford, H.D., 2012. Effects of livestock exclusion on in-stream habitat and benthic invertebrate assemblages in montane streams. Freshw. Biol. 57, 204–217. https://doi.org/10.1111/j.1365-2427.2011.02706.x.

Jiménez-Alfaro, B., Fernández-Pascual, E., Díaz González, T.E., Pérez-Haase, A., Ninot, J.M., 2012. Diversity of rich fen vegetation and related plant specialists in mountain refugia of the Iberian Peninsula. Folia Geobot. 47, 403–419. https://doi.org/10.1007/s12224-012-9125-1.

Joosten, H., 2003. Wise Use of Mires: Background and Principles 239–250.

Joosten, H., Tapio-Bistrom, M.L., Tol, S., 2012. Peatlands – guidance for climate changes mitigation through conservation, rehabilitation and sustainable use. Mitig. Clim. Agric. Ser. 5.

Krammer, K., 2000a. Diatoms of Europe: Diatoms of the European Inland Waters and Comparable Habitats. Gantner.

Krammer, K., 2000b. Diatoms of Europe: Cymbopleura, Delicata, Navicymbula, Gomphocymbellopsis, Afrocymbella. A.R.G. Gantner Verlag K.G.

Lange-Bertalot, H., 2000. Diatoms of Europe: The Genus Pinnularia. A.R.G. Gantner Verlag K.G.

Lange-Bertalot, H., 2001. Diatoms of Europe: Navicula Sensu Stricto, 10 Genera Separated From Navicula Sensu Lato, Frustulia. A.R.G. Gantner Verlag K.G.

Lange-Bertalot, H., Bąk, M., Andrzej, W., Tagliaventi, N., 2011. Eunotia and Some Related Genera.

Lange-Bertalot, H., Hofmann, G., Werum, M., Cantonati, M., 2017. Freshwater Benthic Diatoms of Central Europe. Over 800 Common Species Used in Ecological Assessment (English edition with updated taxonomy and added species.).

Leo, L., Sudarshan, S., 2012. Microbiome R Package.

McDougall, K.L., 2007. Grazing and fire in two subalpine peatlands. Aust. J. Bot. 55, 42–47. https://doi.org/10.1071/BT06096.

McKew, B.A., Taylor, J.D., McGenity, T.J., Underwood, G.J.C., 2011. Resistance and resilience of benthic biofilm communities from a temperate saltmarsh to desiccation and rewetting. ISME J. 5, 30–41. https://doi.org/10.1038/ismej.2010.91.

Mutinová, P.T., Neustupa, J., Bevilacqua, S., Terlizzi, A., 2016. Host specificity of epiphytic diatom (Bacillariophyceae) and desmid (Desmidiales) communities. Aquat. Ecol. 50, 697–709. https://doi.org/10.1007/s10452-016-9587-y.

Navarro, C.J., Carilla, J., Acosta, O.O., Nieto, C., Ovejero, R., Grau, H.R., 2023. Herbivore rewilding does not promote biodiversity in Argentine Andean peatlands. Anthropocene 42, 100382. https://doi.org/10.1016/j.ancene.2023.100382.

Niedermayr, R., Schagerl, M., 2010. Structuring factors of the phytobenthos community along a mountain headwater (Kalkalpen National Park, Austria). Fundam. Appl. Limnol. Arch. Hydrobiol. 177, 93–104. https://doi.org/10.1127/1863-9135/2010/0177-0093.

Ninot, J.M., Carrillo, E., Ferré, A., 2017. The Pyrenees. In: Loidi, J. (Ed.), The Vegetation of the Iberian Peninsula, Plant and Vegetation, Volume 1. Springer International Publishing, Cham, pp. 323–366. https://doi.org/10.1007/978-3-319-54784-8_8.

Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M.D., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M.-H., Cunha, E.R., Smith, T., Stier, A., Braak, C.J.F.T., Weedon, J., 2022. vegan: Community Ecology Package.

Ortiz-Lerín, R., Cambra, J., 2007. Distribution and taxonomic notes of Eunotia Ehrenberg 1837 (Bacillariophyceae) in rivers and streams of Northern Spain. Limnética 415–434.

Pérez-Haase, A., Ninot, J.M., 2017. Hydrological heterogeneity rather than water chemistry explains the high plant diversity and uniqueness of a Pyrenean mixed mire. Folia Geobot. 52, 143–160. https://doi.org/10.1007/s12224-017-9291-2.

Pérez-Haase, A., Ortuño, E.C. i, Sugrañes, J.M.N.i, 2010. Diversitat de comunitats vegetals a les molleres de la Vall d`Aran (Pirineus centrals). Acta Bot. Barc. 61–112.

Pérez-Haase, A., Ninot, J.M., Batriu, E., Pladevall-Izard, E., Espuny, J., Anadon-Rosell, A., Mercadé, A., Ferré, A., Carrillo, E., 2019. Habitats of community interest in the mires of Aigüestortes and Alt Pallars: distribution, impacts and threats. In: Carrillo, E., Ninot, J.M., Buchaca, T., Ventura, M. (Eds.), LIFE+ LimnoPirineus: Conservation of Aquatic Habitats and Species in High Mountains of the Pyrenees [WWW Document]. URL. http://www.lifelimnopirineus.eu/en/project/publications. (Accessed 5 August 2024).

Poulícková, A., Hájková, P., Krenková, P., Hájek, M., 2004. Distribution of diatoms and bryophytes on linear transects through spring fens. Nova Hedwigia 411–424. https://doi.org/10.1127/0029-5035/2004/0078-0411.

Poulíčková, A., Bergová, K., Hnilica, R., Neustupa, J., 2013. Epibryic diatoms from ombrotrophic mires: diversity, gradients and indicating options. Nova Hedwigia 351–365. https://doi.org/10.1127/0029-5035/2013/0091.

Quevedo-Ortiz, G., Fernández-Calero, J.M., Cañedo-Argüelles, M., von Schiller, D., Fortuño, P., Bonada, N., Gomà, J., 2024. An experimental study to assess resistance and resilience strategies of freshwater diatoms to cope with drying in Mediterranean temporary rivers. Hydrobiologia. https://doi.org/10.1007/s10750-024-05585-4.

Quiroga, M.V., Casa, V., García, P.E., Küppers, G.C., Mataloni, G., 2022. Diversity patterns across aquatic communities from peat bogs in changing environmental scenarios. In: Mataloni, G., Quintana, R.D. (Eds.), Freshwaters and Wetlands of Patagonia: Ecosystems and Socioecological Aspects. Springer International Publishing, Cham, pp. 117–135. https://doi.org/10.1007/978-3-031-10027-7_6.

Ramchunder, S.J., Brown, L.E., Holden, J., 2012. Catchment-scale peatland restoration benefits stream ecosystem biodiversity. J. Appl. Ecol. 49, 182–191. https://doi.org/10.1111/j.1365-2664.2011.02075.x.

Rimet, F., 2012. Recent views on river pollution and diatoms. Hydrobiologia 683, 1–24. https://doi.org/10.1007/s10750-011-0949-0.

Rossi, F., Galassi, D.M.P., Angeli, N., Cantonati, M., Di Cicco, M., 2024. The effects of cattle grazing on the composition of diatom assemblages in the peatland pools of the Southeastern Alps (Italy, Adamello-Brenta Nature Park). Land 13, 1780. https://doi.org/10.3390/land13111780.

Rydin, H., Jeglum, J.K., Bennett, K.D., 2013. The Biology of Peatlands, 2e. OUP Oxford.

Smol, J.P., Stoermer, E.F., 2010. The Diatoms: Applications for the Environmental and Earth Sciences. Cambridge University Press.

Soininen, J., 2007. Environmental and spatial control of freshwater diatoms—a review. Diatom Res. 22, 473–490. https://doi.org/10.1080/0269249X.2007.9705724.

Soininen, J., Teittinen, A., 2019. Fifteen important questions in the spatial ecology of diatoms. Freshw. Biol. 64, 2071–2083. https://doi.org/10.1111/fwb.13384.

Stanković, I., Udovič, M.G., Šušnjara, M., Alegro, A., Levkov, Z., 2022. Diversity and ecology of benthic diatoms in a mire with narrow pH gradient. Fottea 22, 39–55. https://doi.org/10.5507/fot.2021.015.

Tsuraya, F., Rahmansyah, M.S., Fikri, F., Hasanah, R., 2023. Identification of microalgae in peat swamp waters. Biol. Environ. Pollut. 3, 43–53. https://doi.org/10.31763/bioenvipo.v3i2.654.

Van Dam, H., Mertens, A., Sinkeldam, J., 1994. A coded checklist and ecological indicator values of freshwater diatoms from The Netherlands. Neth. J. Aquat. Ecol. 28, 117–133. https://doi.org/10.1007/BF02334251.

Wahren, C.H.A., Williams, R.J., Papst, W.A., 2001. Vegetation change and ecological processes in alpine and subalpine *Sphagnum* bogs of the Bogong High Plains, Victoria, Australia. Arct. Antarct. Alp. Res. 33 (3), 357–368. https://doi.org/10.1080/15230430.2001.12003440.

Wang, J., Meier, S., Soininen, J., Casamayor, E.O., Pan, F., Tang, X., Yang, X., Zhang, Y., Wu, Q., Zhou, J., Shen, J., 2017. Regional and global elevational patterns of microbial species richness and evenness. Ecography 40, 393–402. https://doi.org/10.1111/ecog.02216.

Wickham, H., François, R., Henry, L., Müller, K., Vaughan, D., 2023. dplyr: A Grammar of Data Manipulation. R Package Version 1.1.4.

Williams, B., Silcock, D., Young, M., 1999. Seasonal dynamics of N in two Sphagnum moss species and the underlying peat treated with 15NH415NO3. Biogeochemistry 45, 285–302. https://doi.org/10.1023/A:1006134515648.