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Macrobenthic Assemblages and the Influence of Microhabitat in a High-Mountain Lake (Northwest Italy)

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Abstract: High-mountain lakes are freshwater ecosystems situated above the tree line which are known for their remote locations and limited accessibility. These ecosystems host simplified biotic communities primarily concentrated in the littoral zone and dominated by benthic macroinvertebrates that serve as bioindicators of environmental pressures. A two-year monitoring investigation was performed in July 2022 and July 2023 at Nero Lake (Cesana Torinese, Northwest Italy). Five sites along the lakeshore were selected for sampling physicochemical water parameters and macrobenthos. All collected data were analysed to compare trends across years and within specific sites. The results revealed that Nero Lake exhibited consistent macrobenthic communities across the two years studied, but significant differences were observed in its microhabitats. This suggests that substrate type and physicochemical water parameters strongly influence community composition. Chironomidae larvae and Mollusca were the dominant species, showing distinct associations with different substrates and environmental factors from one year to another. These findings contribute to our understanding of the intricate relationships between benthic macroinvertebrates and their environments, highlighting the necessity of detailed, small-scale assessments to comprehend ecosystem dynamics and develop effective conservation strategies.

Keywords: alpine lakes; macroinvertebrates; microhabitat; substrate composition

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1. Introduction

Alpine lakes, commonly referred to as "high mountain lakes" [1], are freshwater ecosystems located above the tree line (e.g., at altitudes above 1500 m a.s.l.) which have peculiar characteristics due to their remoteness and limited accessibility. Indeed, these lakes share several characteristics, such as persistent low temperatures, low nutrient availability, oligotrophic conditions, and longs period of snow and ice cover. Despite these similarities, they can have different formation processes (e.g., the melting of glaciers, glacial erosion, etc.), different sizes and shapes, and different limnological characteristics [2].

These ecosystems have harsh environmental conditions that result in simple community structures with highly adapted species [3]. The biotic communities in these lakes are, therefore, rather simplified, with the majority concentrated in the littoral zone and consisting mainly of benthic macroinvertebrates (mainly Diptera Chironomidae and Oligochaetes) and amphibians, which account for most of the biodiversity [4]. These fragile biocenoses are highly susceptible to variations in physicochemical water parameters such as pH, nutrient concentrations, and dissolved oxygen [5–8].

These lentic systems are highly sensitive areas in which small changes reflect important effects on their biological and chemical processes, making them important "early warning systems" for global change [9]. Despite the almost complete absence of direct anthropic influence and their seclusion, alpine lakes are far from being considered pristine environments. Several threats, both old and emerging, cast a shadow over these fragile environments: (i) climate change, (ii) the retreat of glaciers, (iii) UV radiation, (iv) the long-range transport of contaminants and atmospheric deposition, (v) the impact of tourism, and (vi) the impact of alien species [2]. Indeed, alpine lakes are naturally fishless, although non-native fish species have been introduced for recreational purposes in recent decades [10], affecting lake communities [3]. These effects include reductions in or even the eradication of populations of other species, such as amphibians [11] and macroinvertebrates [12].

Benthic macroinvertebrates are a key element in alpine lake ecosystems [13] and represent a significant proportion of the total lake biomass [14]. They are considered excellent bioindicators [15,16] and can be useful for monitoring the immediate and long-term effects of anthropogenic activities on aquatic ecosystems [13,15,17]. They can be used to investigate the effects of climate change [9,18], for monitoring the accumulation of trace elements [19], and to assess the impact of introducing non-native fish [12]. A full understanding of the relationship between benthic communities and environmental parameters is essential for understanding which anthropogenic pressures benthic macroinvertebrates are most sensitive to [13].

In recent years, there has been increasing interest in the study of high-mountain lakes in terms of both their biota and their physicochemical characteristics [4,20–22]. In order to gain a deeper understanding of the specific processes occurring within these ecosystems, it is recommended that they be evaluated on a fine spatial scale [5,23]. This will facilitate a more comprehensive understanding of the ecosystems and the realization of future territorial policies. To date, no data have been published on the aquatic fauna of Nero Lake (Piedmont, Italy), with the sole exception of the catalogue of Trichoptera in the Western Alps by Cianficconi and Moretti [24]. In addition, Nero Lake has been subject to fish releases for fishing purposes in past years, and populations of brown trout, *Salmo trutta* (Linnaeus, 1758), and minnow, *Phoxinus lumaireul* (Schinz, 1840), can be found in its waters today [25].

In this context, it is of great interest to employ an approach that encompasses the entire lake as well as the various microhabitats within it. A two-year monitoring investigation of Nero Lake permitted the evaluation of three key areas: (i) the composition of its littoral benthic macroinvertebrate communities, (ii) possible differences in these communities' assemblages between two years, and (iii) site-specific factors influencing the littoral macroinvertebrate community. Global patterns define the main structure of the macrobenthic communities, while site-specific factors are responsible for shaping them [5].

Given the characteristics of Nero Lake and its location in the Western Alps, we hypothesise the following: (i) the macrobenthic community structure will show significant variation between the sampling sites, influenced by the specific environmental parameters of each site; (ii) the overall biodiversity and species richness will be comparable to other alpine lakes in the region; specifically, we expect to find a macrobenthic community primarily composed of insects from the order Diptera, particularly those belonging to the family Chironomidae, with smaller proportions of other organisms such as Mollusca, Crustacea, and Oligochaeta; and (iii) the physicochemical parameters of Nero Lake will be similar to those recorded in other alpine lakes with similar altitudes, substrates, depths, and dimensions.

In addition to addressing these hypotheses, the primary goal of this study is to establish a baseline understanding of the macrobenthic community and environmental parameters of Nero Lake. This baseline will serve as a point of reference for future long-term research, providing a scientific basis for monitoring ecological changes and assessing the impact of environmental stressors over time.

2. Materials and Methods

2.1. Study Area

Nero Lake is situated in the upper Susa Valley (Northwest Italy), beneath the municipality of Cesana Torinese, at an altitude of 2020 m a.s.l. The area is part of Natura 2000, a network of protected natural areas across the European Union that aims to conserve biodiversity and ensure the long-term survival of Europe's most valuable and threatened species and habitats. Nero Lake is designated a Site of Community Importance (SCI, IT1110058) [26] and a Special Protection Area (SPA) [27]. SCIs and SPAs are selected based on their ecological significance and contribute to the preservation of Europe's natural heritage. The only amphibian reported in the wetland area of Nero Lake is *Rana temporaria* (Linnaeus, 1758) [25]. The fish community consists of brown trout (*Salmo trutta*) and minnow (*Phoxinus lumaireul*), which were, respectively, introduced for recreational fishing and as forage fish for *S. trutta* [25]. Unfortunately, there are no data available indicating when the first fish were released into Nero Lake.

Nero Lake's morphology is the result of the erosive action of Quaternary Era glaciations and present-day hydrographic reticulation on a lithological substrate mainly consisting of easily erodible calcschists [28]. The main vegetation type is larch–cedar, but over half of the area is occupied by grasslands used for cattle grazing [29]. The lake has a surface area of approximately 1.65 hectares (0.02 km^2), a perimeter of 718 m, and a maximum depth of 6.13 m. On the southern side, there is a wetland area with floristic species of conservation interest, whereas on the northern side, extensive populations of *Potamogeton nodosus* (Poiret, 1816) and, secondarily, *Potamogeton alpinus* (Balbis, 1804), are present [30], as shown in Figure 1.

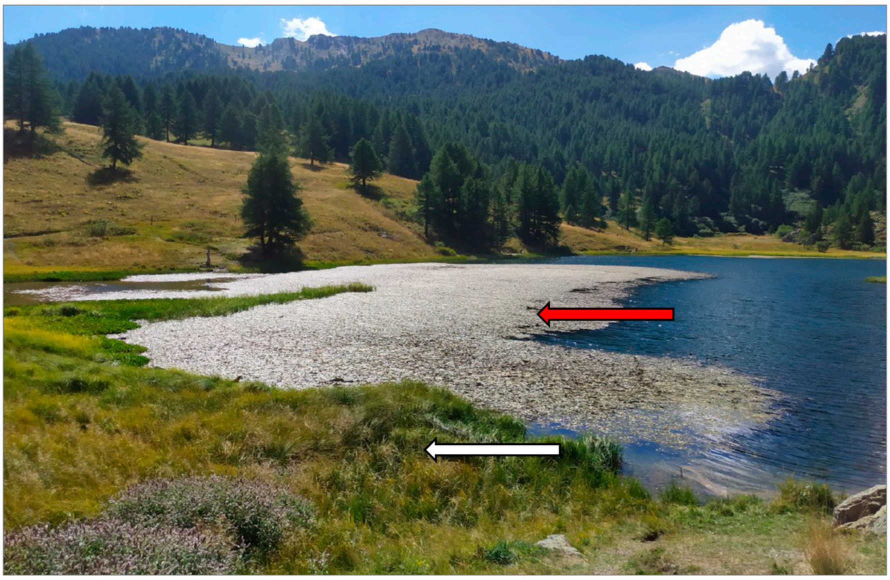

Figure 1. A view of the northern side of Nero Lake (Western Alps, Italy, 2020 m a.s.l.). A *Potamogeton* sp. population is indicated by a red arrow (from this perspective, *Potamogeton nodosus* and *Potamogeton alpinus* are indistinguishable). Marsh vegetation is indicated by a white arrow.

The presence of marsh vegetation is restricted to a narrow band of tall sedges, specifically *Carex vesicaria* (Linnaeus, 1753) and *Carex elata* (Allioni, 1785), which are often accompanied by *Menyanthes trifoliata* (Linnaeus, 1753) [31]. The scarcity of this vegetation is due to steep banks along the perimeter and excessive trampling by grazing and tourists [31]. Based on bathymetric surveys, five littoral sampling sites were identified for the analysis of physicochemical parameters and macrobenthic invertebrates, as shown in Table 1 and

Figure 2. Two sampling campaigns were conducted, the first in July 2022 and the second in July 2023.

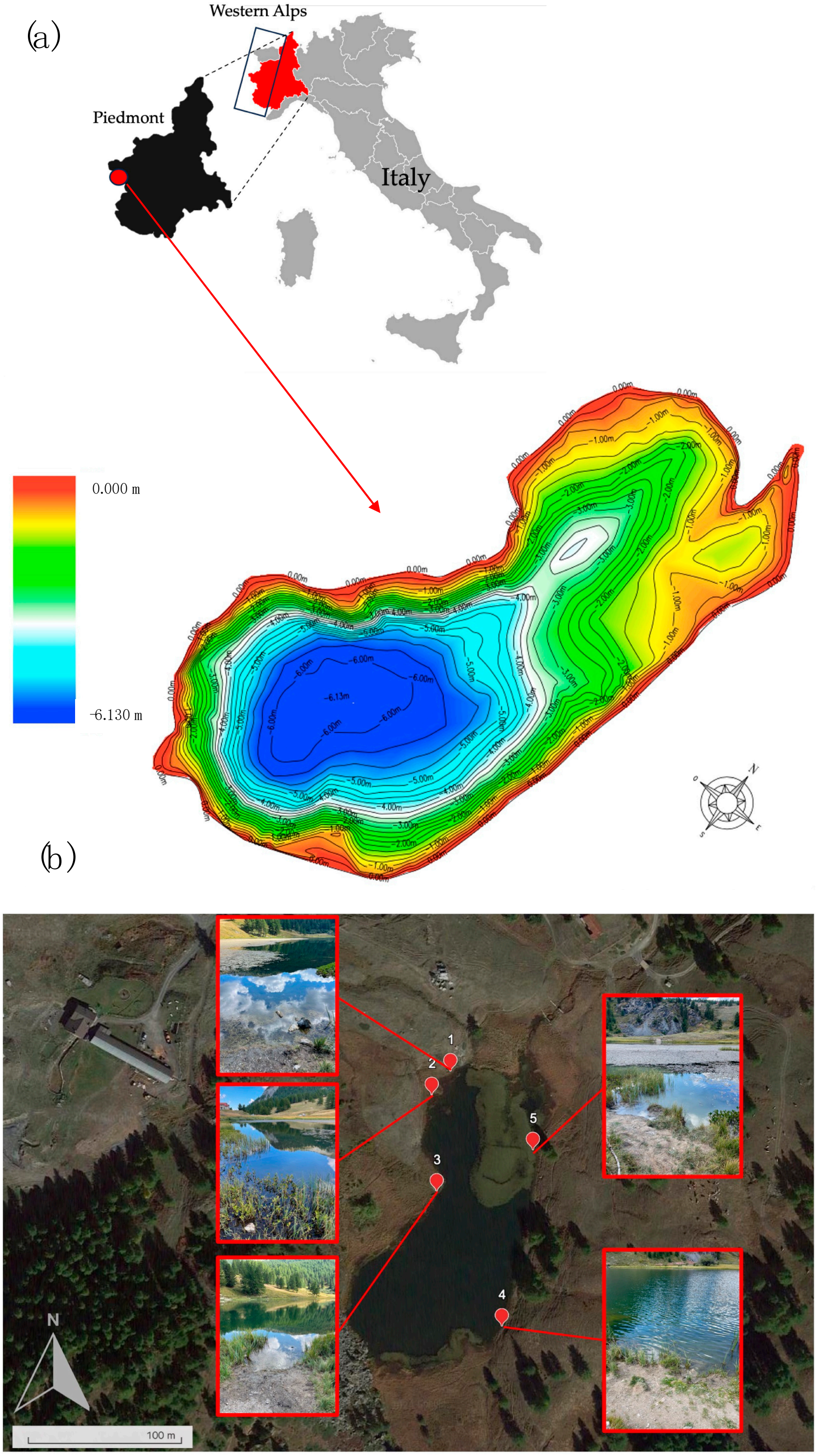

Figure 2. (**a**) Bathymetry of study area. Scale: 1:1000. (**b**) Aerial view of Nero Lake (Western Alps). Numbers reported on littoral areas represent five sampling sites.

Table 1. Geographical coordinates and descriptions of Nero Lake (Western Alps) sampling sites. See Figure 2 for sampling site numbers.

Site	Longitude	Latitude	Site Description
1	6°47′40″ E	44°54′06″ N	Northern side of lake. Bottom mainly composed of gravel; smaller presence of organic material compared to other sampling sites.
2	6°47′39″ E	44°54′06″ N	Northwestern side of lake. Sampling site located at a short distance from tributary coming from “Fattoria Lago Nero” and “Grange Servierettes” alpine pastures. Silt bottom with strong presence of *Menyanthes trifoliata*.
3	6°47′40″ E	44°54′04″ N	Western side of lake. Sampling site located on apex of debris cone. Bottom mainly comprises thick organic detritus and *Carex vesicaria*. Strong smell of hydrogen sulphide when handling substrate.
4	6°47′42″ E	44°54′01″ N	Southeastern side of lake, close to wetland area of southern shore. Silty bottom with strong presence of *Carex vesicaria*.
5	6°47′43″ E	44°54′05″ N	Northwestern side of lake. Silt bottom with submerged vegetation. Strong presence of *Carex vesicaria*, *Potamogeton* sp., and *Menyanthes trifoliata*.

2.2. Physicochemical Water Parameters

At each site, primary physicochemical water parameters were measured using a multiparameter probe (Hanna Instruments®, Woonsocket, RI, USA, model HI98494—multiparameter pH/EC/OPDO with LDO optical sensor and Bluetooth) immersed at a depth of 0.2–0.3 m. The water temperature (°C), dissolved oxygen (mg L^{-1}), oxygen saturation (%), conductivity (µS cm^{-1}), and pH (unit) were recorded. Additionally, water samples (1 L) were collected in sterile bottles from each site for subsequent laboratory analyses. The containers were filled with water at a 0.2–0.3 m depth and placed inside a thermal container with ice to maintain a stable temperature during transport to the laboratory. The water samples were analysed in the laboratory for the main micronutrients using specific kits and a spectrophotometer (Hanna Instruments®, model: HI83300 Multiparameter Photometer) on the day of sampling. Ammonia/ammonium ion (NH_3/NH_4^+) concentrations (mg L^{-1}) were measured using an adaptation of the Nessler method (ASTM 2015), nitrate (NO_3^-) concentrations (mg L^{-1}) were obtained using an adaptation of the cadmium reduction method [32], and phosphate (PO_4^{3-}) concentrations (mg L^{-1}) were obtained using an adaptation of the ascorbic acid method [32]. Two technical replicates were performed for each parameter, and the mean value was reported.

2.3. Macrobenthic Invertebrate Sampling

To ensure thorough coverage of the lakeshore habitats, a detailed inspection of the entire perimeter of the lake was conducted by walking. During this process, various microhabitats, including a gravelly substrate, silt substrate, and submerged aquatic vegetation, were identified. It was found that the proportions of these microhabitats varied along the shoreline: approximately 20% of the shoreline consisted of gravelly substrate, while the remaining portion (80%) was composed of silty substrates and submerged vegetation. Based on these observations, five sampling sites in the littoral area of the lake were selected (see Figure 2 and Figure S1 in the Supplementary Materials for details). These sites had distinct features representing different combinations of submerged aquatic vegetation, substrate compositions, and shore characteristics such as the presence of affluents (see Table 1 for details). No sampling sites were selected on the south side of the lake due to its inaccessibility.

Macrobenthic invertebrates were sampled following the methodology used in previous works on alpine lakes [4,5,18,20]. Three replicates were collected for each sampling site. A Surber net (0.32 × 0.32 m, 250 μm mesh size; SCUBLA S.r.l., Remanzacco, Italy) was used to collect subsamples. The sampler was submerged in water to a depth of at least 0.3–0.4 m and the substrate was stirred by hand for a minimum of 2 min to transfer the organisms into the net. The samples were preserved in a 70% ethanol solution in plastic bottles and transported to the laboratory of the Regional Reference Centre for Aquatic Biodiversity (BioAqua; Avigliana, Turin, Italy). After sorting, the organisms were identified using a stereomicroscope (Stereomicroscope Binocular Zoom 0.8x-8x—LED illumination, Eurotek, Orma). Identification was conducted at the genus or family level using dichotomous keys by Campaioli et al. [33] and Tachet et al. [34]. Chironomidae larvae and Oligochaetes were identified using an optical microscope (Nikon Eclipse Ci, New York, NY, USA, model H550L, from ×4 to ×100 magnifications). The organism density per square meter (m^2) was calculated for each identified taxon at each sampling site.

2.4. Statistical Analysis

Due to the small sample size ($n \leq 20$), the Wilcoxon non-parametric test was used to determine significant differences between the two years' mean values of physicochemical water parameters at each sampling site. To describe the macrobenthic invertebrate assemblages, we calculated common community indices, such as the mean number of observed genera, the Shannon–Wiener index, and Evenness. We also compared annual density values observed for Nero Lake using the Wilcoxon non-parametric test.

Canonical Correspondence Analysis (CCA), a widely utilised technique in aquatic ecology, was used to examine the relationships between biological assemblages and their environments [35]. The significance of the overall CCA model was assessed using 999 permutations. The Kruskal–Wallis test was employed to explore differences in macroinvertebrate community densities between sites, and a post hoc test (a Conover–Iman test) was used to perform pairwise comparisons using the same rankings as those employed in the Kruskal–Wallis test [36–38].

A similarity matrix was created using the Bray–Curtis measure based on macrobenthic invertebrate littoral abundance data. We conducted a two-way PERMANOVA [39,40] to determine significant differences between the communities of the investigated sites (factor: "Sites"). Prior to the analysis, the data were transformed (log[x + 1]) to mitigate the influence of very abundant taxa [41]. All analyses were performed using 999 permutations.

A principal coordinate analysis (PCoA) was used to visualise the dissimilarities between sites [42]. All analyses were performed using RStudio version 2023.12.1 Build 402. Figures were generated using RStudio [43,44].

3. Results

3.1. Physicochemical Water Parameters

The mean values and standard deviations of the physicochemical water parameters at each sampling site are shown in Table 2. The parameter values did not differ significantly between the two sampling sessions except for oxygen saturation (p-value = 0.032), which was higher in July 2022, and conductivity (p-value = 0.008), which was higher in July 2023. The pH values were lower in the first year of sampling (p-value = 0.008). In terms of nutrient concentration, there were significant differences between ammonia and phosphate concentrations; the ammonia concentration was higher in July 2022 (p-value = 0.032), and the phosphate concentration was higher in 2023 (p-value = 0.008). Figure 3 shows the trends in the physicochemical parameters over the two years of sampling.

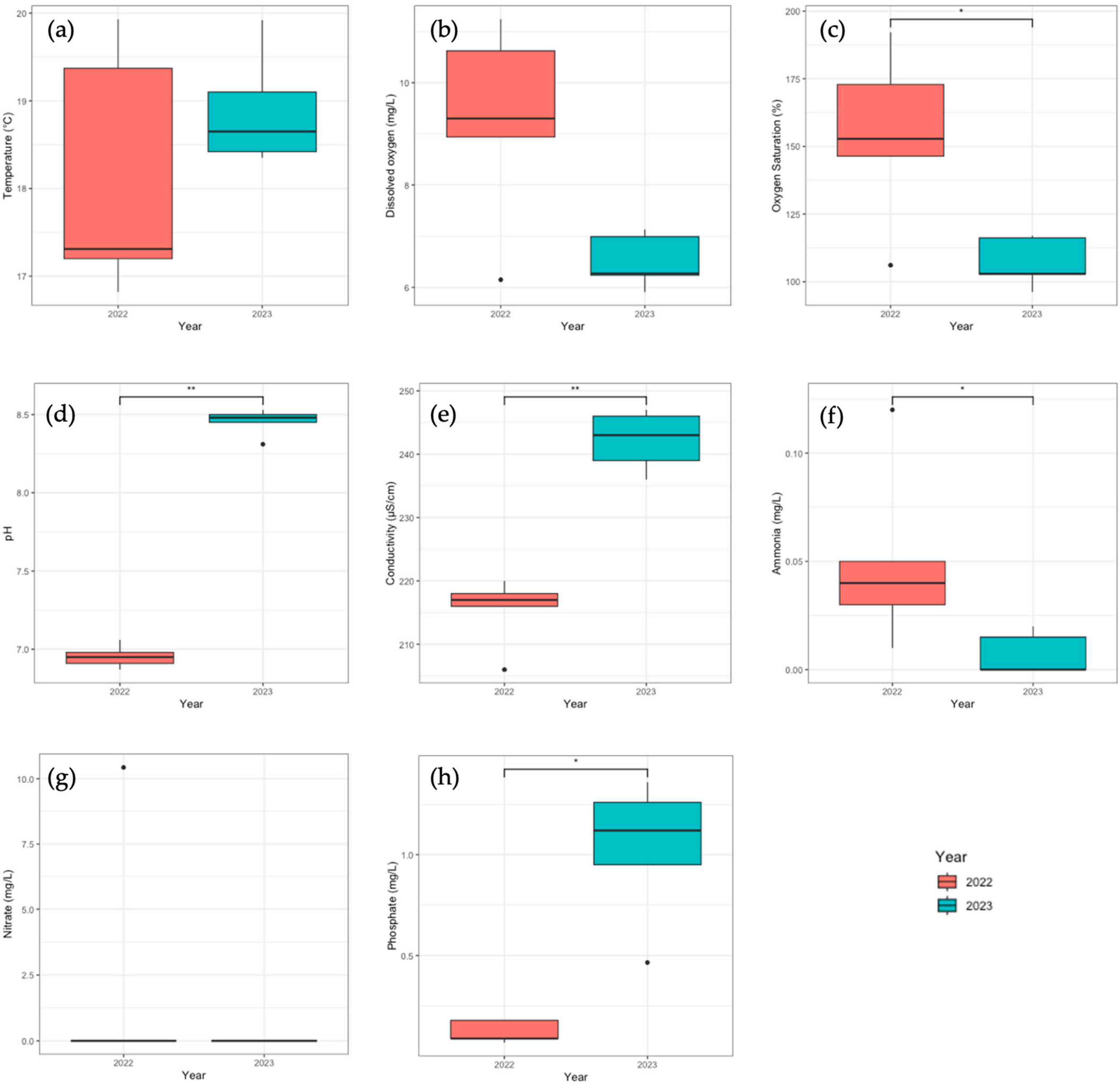

Figure 3. Box plots of (**a**) water temperature, (**b**) dissolved oxygen concentration, (**c**) oxygen saturation*, (**d**) pH*, (**e**) conductivity*, (**f**) ammonia concentration*, (**g**) nitrate concentration, and (**h**) phosphate concentration* in Nero Lake (Western Alps) in July 2022 and July 2023. Asterisks indicate significance (*p*-value < 0.05).

Table 2. Mean and standard deviation values of the physicochemical water parameters at each sampling site in July 2022 and July 2023 in Nero Lake (Western Alps).

Site	Year	Temperature (°C)	pH (Unit)	Dissolved Oxygen (mg L^{-1})	Oxygen Saturation (%)	Conductivity (µS cm^{-1})	Ammonia (mg L^{-1})	Nitrate (mg L^{-1})	Phosphate (mg L^{-1})
1	2022	17.2 ± 0.16	7.06 ± 0.02	8.94 ± 0.02	146.4 ± 0.16	217 ± 0.82	0.05 ± 0.03	10.43 ± 1.47	0.18 ± 0.03
	2023	19.92 ± 0.2	8.45 ± 0.02	6.99 ± 0.16	117 ± 1.63	243 ± 0.82	0.02 ± 0.02	0 ± 0	1.36 ± 0.65
2	2022	19.37 ± 0.16	6.95 ± 0.01	11.24 ± 0.02	192.2 ± 0.16	217 ± 0.82	0.03 ± 0.02	0 ± 0	0.09 ± 0.02
	2023	19.1 ± 0.2	8.5 ± 0.02	6.24 ± 0.16	102.9 ± 1.63	246 ± 0.82	0 ± 0	0 ± 0	1.26 ± 1.00

Table 2. *Cont.*

Site	Year	Temperature (°C)	pH (Unit)	Dissolved Oxygen (mg L^{-1})	Oxygen Saturation (%)	Conductivity (µS cm^{-1})	Ammonia (mg L^{-1})	Nitrate (mg L^{-1})	Phosphate (mg L^{-1})
3	2022	19.93 ± 0.02	6.87 ± 0.02	6.15 ± 0.01	106.1 ± 0.08	220 ± 0.82	0.11 ± 0	0 ± 0	0.07 ± 0.03
	2023	18.42 ± 0.2	8.31 ± 0.02	5.58 ± 0.34	96.2 ± 1.63	247 ± 0.82	0 ± 0	0 ± 0	0.95 ± 1.10
4	2022	16.82 ± 0.16	6.98 ± 0.02	10.62 ± 0.02	172.9 ± 0.08	216 ± 0.82	0.01 ± 0.01	0 ± 0	0.09 ± 0.02
	2023	18.65 ± 0.2	8.48 ± 0.02	6.27 ± 0.16	102.9 ± 1.63	236 ± 0.82	0.03 ± 0.02	0 ± 0	1.12 ± 0.61
5	2022	17.13 ± 0.08	6.91 ± 0.01	9.3 ± 0.08	152.8 ± 0.16	206 ± 0.82	0.03 ± 0.01	0 ± 0	0.18 ± 0.11
	2023	18.35 ± 0.2	8.53 ± 0.02	7.46 ± 0.34	116.2 ± 1.63	239 ± 0.82	0 ± 0	0 ± 0	0.47 ± 0.13

3.2. Macrobenthic Invertebrate Littoral Communities

The number of observed taxa, Shannon–Wiener Index, and Evenness did not differ significantly between the two sampling sessions (Wilcoxon non-parametric test, $p > 0.05$ for all comparisons), as shown in Figure 4. In 2022, 1225 macrobenthic invertebrates were collected and in 2023, 1890 macrobenthic invertebrates were collected.

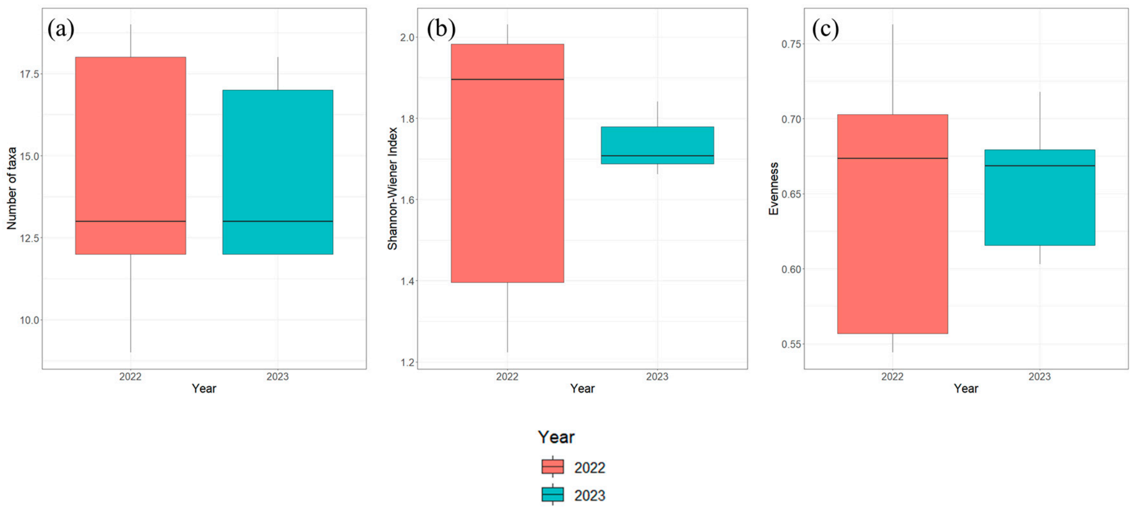

Figure 4. Box plots of (**a**) number of taxa identified, (**b**) Shannon–Wiener Index, and (**c**) Evenness for macrobenthic community in Nero Lake (Western Alps) in July 2022 and July 2023.

The density of the taxa that compose the littoral macrobenthic community, illustrated in Figure 5, did not show any significant differences between the two years (Wilcoxon non-parametric test, $p > 0.05$ for all comparisons). The densities of each taxon are reported in detail in Tables S1 and S2 (Supplementary Materials).

Hexapoda and Mollusca were the most abundant taxa in Nero Lake (with 180–1730 ind./m^2 and 3–1430 ind./m^2, respectively), followed by Oligochaeta (0–550 ind./m^2), Crustacea (0–493 ind./m^2), and Hirudinea (0–55 ind./m^2). The Crustaceans and Molluscs showed low variability in both years and were almost entirely (100% and 99%, respectively) represented by the amphipod *Gammarus* sp. (Fabricius, 1775) and by a snail belonging to the Valvatidae family, *Valvata piscinalis* (Müller, 1774). In both years, the most representative (77%) family of Hexapoda was Chironomidae (Diptera order), which was divided into the subfamilies Chironominae (Chironomini and Tanytarsini), Orthocladiinae, Tanypodinae, Diamesinae, and Coryoneurinae. In both years, the Chironomidae community was dominated by the tribe Chironomini (41%) and by the subfamily Tanypodinae (44%). The other subfamilies and tribes were present in much smaller proportions.

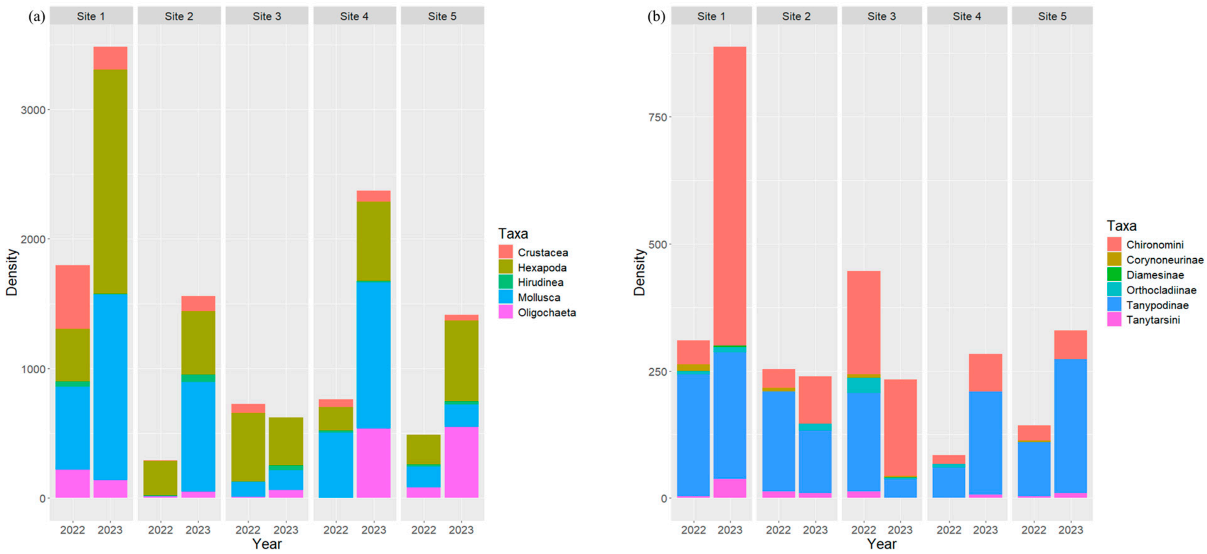

Figure 5. Density of (**a**) main identified taxa of macrobenthic invertebrates and density of (**b**) main identified taxa of Diptera Chironomidae in Nero Lake (Western Alps) in July 2022 and July 2023.

As there were no statistically significant differences in the composition and density of the macroinvertebrate community between the two sampling years, any differences between sampling sites were examined separately for each year using the Kruskal–Wallis test. In July 2022, only the density of Hirudinea showed significant differences between sampling sites (p-value = 0.039). In July 2023, Crustacea, Mollusca, and Chironomini were significantly different between sites (p-value = 0.018, p-value = 0.031, and p-value = 0.040, respectively). For each of these taxa, a post hoc Conover–Iman test was performed. In 2022, Hirudinea were abundant at sites 1, 4, and 5, while they had significantly lower densities at sites 2 and 3. In 2023, Mollusca were abundant at sites 1, 2, and 4 and scarcely present at sites 3 and 5. In the same year, Crustaceans exhibited high densities at site 1 and low densities at sites 2, 4, and 5 and were absent at site 3. Chironomini showed high densities at sites 1 and 3 and lower densities at the other sites. The differences between sites are shown in detail in the Supplementary Materials in Tables S3–S6.

3.3. Relationship between Macrobenthic Invertebrates and Environment

In the Canonical Correspondence Analysis (CCA), the overall model was found to be statistically significant in both 2022 (F = 3.170; p = 0.01) and 2023 (F = 2.807; p = 0.023). The model explained a significant proportion of the variation in species composition based on environmental variables; the first two axes collectively explain 74.63% of the variance in 2022 and 88.8% of the variance in 2023, as shown in Table 3.

Table 3. The eigenvalues and proportion of variance explained by each axis in the CCA analysis in July 2022 and in July 2023 (Nero Lake, Western Alps).

Axis	Year	Eigenvalue	Proportion of Variance Explained
1	2022	0.288	46.81
	2023	0.161	66.28
2	2022	0.171	27.82
	2023	0.055	22.52
3	2022	0.152	24.66
	2023	0.022	9.23
4	2022	0.004	0.72
	2023	0.005	1.98

The CCA revealed some groupings of macroinvertebrate taxa according to environmental variables, as shown in Figure 6. In 2022, Mollusca and Hirudinea exhibited an association with dissolved oxygen and silt. In contrast, Crustacea were associated with gravelly substrates and the presence of nutrients (nitrates and phosphates). Finally, Hexapoda and Oligochaeta were associated with the presence of dissolved ammonia, higher temperatures, and conductivity. In 2023, Mollusca and Crustacea were influenced by the amount of dissolved nutrients in the water (ammonia and phosphates) and were associated with silt and higher values of pH. Oligochaeta were apparently associated with silt and higher pH values. Hexapoda and Hirudinea seemed to be associated with higher dissolved oxygen concentrations and gravelly substrates.

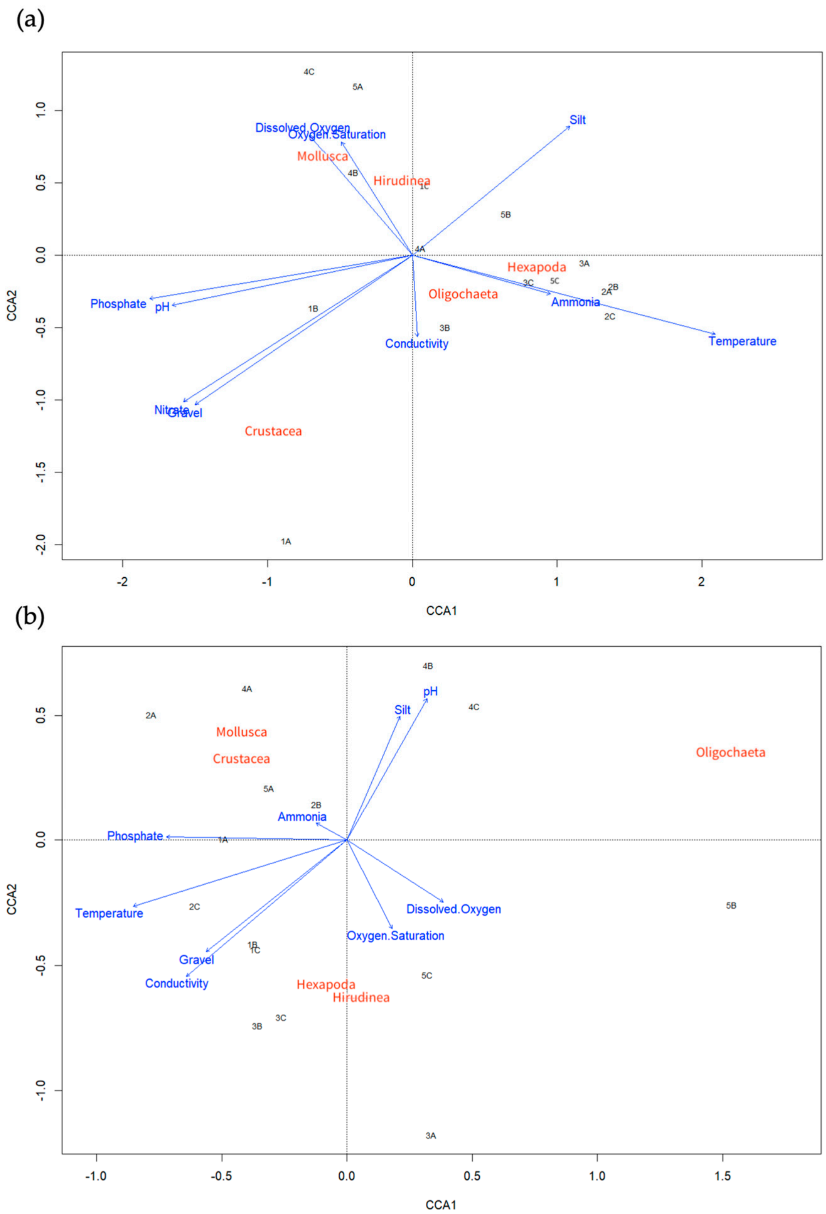

Figure 6. A Canonical Correspondence Analysis (CCA) plot showing the correspondence (~influence) of main environmental factors (blue arrows) with the macrobenthic invertebrate composition (in red) of the sampling sites in July 2022 (**a**) and July 2023 (**b**) in Nero Lake (Western Alps). The analysis was based on the fifteen replicates, three for each sampling site (numbers in black from 1 to 5 indicate the sampling site, and letters A to C indicate the replicate for each site).

The results of the two-way PERMANOVA indicate that there are statistically significant differences between the invertebrate communities at the different sampling sites in the two years (Table 4).

A principal coordinate analysis (PCoA) was employed to illustrate the dissimilarities in terms of species composition and abundance between sites. The resulting plots (Figure 7) demonstrate that the biological characteristics of the sites exhibited varying levels of overlap between the two sampling years. In 2022, the intra-site variability in the microbenthic community was lower, accompanied by a lower level of overlap. Conversely, in 2023, the sites exhibited greater variability and a higher level of overlap between them.

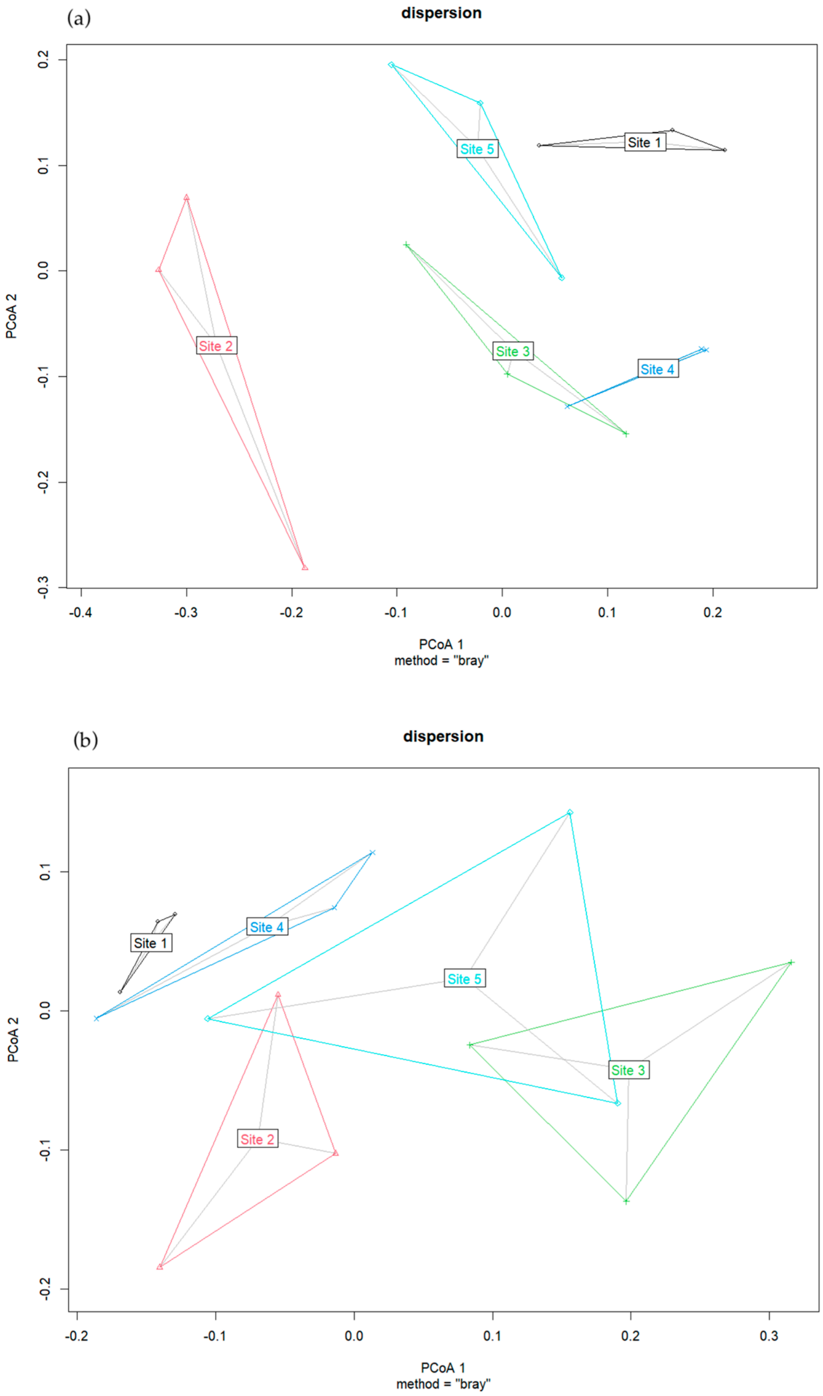

Figure 7. Principal coordinate analysis (PCoA) of species data in Nero Lake (Western Alps) using Bray–Curtis distance. (**a**) July 2022; (**b**) July 2023. Objects are sites, and distances quantify similarity in terms of species composition and abundance.

Table 4. Results of PERMANOVA based on macrobenthic invertebrates sampled in Nero Lake (Western Alps) in July 2022 and July 2023. Asterisks indicate significance (p-value < 0.05).

Source	Year	d.f.	Sum of Squares	R^2	F	p
Site	2022	4	0.647	0.659	4.836	0.001 ***
	2023	4	0.331	0.601	3.761	0.003 ***
Residual	2022	10	0.335	0.341		
	2023	10	0.220	0.399		
Total	2022	14	0.982	1.000		
	2023	14	0.552	1.000		

4. Discussion

4.1. Physicochemical Water Parameters

In high-altitude alpine lakes, the water temperature usually does not exceed 12 °C but can reach 15 °C in exceptional cases [4,45,46]. During the two sampling periods at Nero Lake, water temperatures of 19 °C were reached, which is higher than the data reported in the literature for this type of environment. Climate change is more pronounced in alpine environments; in the last 30 years, temperatures in alpine environments have increased by an average of 1.5–2.0 °C in comparison to 0.5 °C at the global scale [47,48]. It is therefore likely that the temperature of the water in Nero Lake has increased in recent years. The lake may also be more vulnerable to high summer temperatures in the area due to its small size and shallow depth. Unfortunately, long time series of data are not available for this lake, so it is not possible to determine with certainty the extent of this increase.

The dissolved oxygen concentration and oxygen saturation were high in both years, in line with values recorded in other alpine lakes [10,46,49,50]. The highest values were recorded in 2022, coinciding with a lower mean water temperature; indeed, the dissolved oxygen concentration is inversely proportional to water temperature. Conductivity was significantly higher in 2023, as expected, since conductivity is dependent on temperature [51].

The average pH value was comparable to those reported in the literature for alpine lakes with substrates similar to that of Nero Lake [46,52]. The lowest value recorded was 6.87 at sampling Site 3, which is still higher than typical values for lakes under acidified conditions [53,54].

The concentration of ammonia (NH_4^+) was significantly higher in 2022, and the highest values recorded were at Site 1, Site 2, and Site 3. This difference could be due to the presence of organic pollution, particularly from grazing cattle [55]. During the first year of sampling, several cows grazed in the area, and their faeces were observed on the banks of the lake, especially at the first three sampling sites. In 2023, the cows were still present in the area, but an electrified fence (placed all around the lake about 10 metres from the water) prevented them from approaching the banks. Fencing is an effective method of preventing livestock from approaching a lake's banks and reduces the amount of faeces deposited directly into the water [55]. This could explain the significant reduction in the amount of ammonia in water in 2023.

Nitrate (NO_3^-) was only detected in 2022 at Site 1, where the average concentration was 10.43 mg L^{-1}, whereas it was not detected in 2023. This result could be due to increased organic pollution in 2022 at Site 1, which was among the sites most affected by the presence of cows. Phosphate concentrations differed between the two years, with significantly higher values observed in 2023. The 2022 values are in line with those recorded in other remote alpine lakes [5,56,57]. The significantly greater presence of phosphates in 2023 could be related to the greater coverage of aquatic vegetation and plants on the lakeshore, leading to a higher amount of dissolved nutrients [57]. High levels of phosphorus, as well as nitrate, can cause algal blooms [58] and alter the balance of trophic networks in alpine lakes [59]. Further research is needed to understand whether this increase in nutrients in the waters of Nero Lake is part of a trend or an isolated event.

This analysis of physicochemical water parameters provided valuable insights into the environmental characteristics of Nero Lake. The results of this study indicate that, as anticipated, Nero Lake shares similarities with other alpine lakes in the Western Alps region at similar altitudes and with comparable geological characteristics [4,5,46,52]. The findings from the two-year monitoring period reveal only minor differences in physicochemical parameters, which is consistent with expectations for the relatively brief duration of the study period. The physicochemical parameters, such as temperature and pH, exhibited uniformity across the sampling sites, consistent with expectations for a small lake like this. However, variability was observed in parameters such as the concentration of dissolved nutrients, suggesting localised influences from the surrounding environment. Runoff from adjacent land areas could introduce excess nutrients into the lake, leading to spatial heterogeneity in nutrient concentrations. Additionally, the shallow nature of the lake may exacerbate nutrient retention, further contributing to localised nutrient enrichment (the maximum depth is 6.13 m and is reached in only a small portion of the lake, see Figure 2). These findings underscore the importance of considering the influence of landscape characteristics on water quality dynamics in Nero Lake.

Nero Lake is not immune to human impacts. Its shallow nature exacerbates its vulnerability as it reduces the volume of water available for dilution and could amplify the effects of nutrient inputs. While our findings do not indicate that Nero Lake is at immediate risk of acidification in comparison with other alpine lakes [53], it is essential to emphasise its potential future vulnerability to this threat. The dimensions of the basin, coupled with other anthropogenic pressures, underscore the need for vigilance to prevent the onset of acidification in the future.

Overall, Nero Lake exhibits characteristics typical of small and shallow alpine lakes, but the presence of anthropogenic pressures highlights the need for proactive management strategies to mitigate potential environmental threats and preserve its biodiversity.

4.2. Macrobenthic Invertebrate Littoral Communities and Their Relationship to the Environment

The number of taxa discovered along the shores of Nero Lake, ranging from 9 to 19 per sampling site, is consistent with values found in other high-altitude lakes [5,9,13,52]. The Evenness and Shannon–Wiener index values are also consistent with those found in other studies of small Alpine lakes [4,5,9].

The macrobenthic community of Nero Lake did not change significantly over the two years of sampling, maintaining a certain stability in both composition and abundance, although significant variations were recorded in some of the physicochemical parameters of the water. It is unlikely that these variations had a significant impact on the overall abundance and composition of macroinvertebrates. It would be interesting to follow the evolution of the lake over time in order to detect any long-term changes in its biotic community.

In contrast, at the microhabitat level, macrobenthic communities showed statistically significant differences between sampling sites in the same year. This could indicate that slight differences in substrate and physical–chemical parameters between sites may influence the presence and abundance of macroinvertebrates, even in such a small lake and with such short distances between sampling sites. Moreover, a Canonical Correspondence Analysis (CCA) showed that different groups of invertebrates appear to be associated with different environmental features at the microhabitat level.

Alpine lakes are inhabited mainly by stenothermic macrobenthic fauna which are resistant to harsh environmental and climatic conditions [60]. The literature reports that the most common macroinvertebrates found in these lakes are the larvae of Diptera Chironomidae and Oligochaetes [4,9,20,61]. As predicted, Chironomidae were the most prevalent among Hexapoda at every sampling site in both years. Odonata such as *Enallagma* sp., typical of alpine wetlands, aquatic Coleoptera (e.g., Dytiscidae, Elmidae), Baetidae (e.g., *Baetis* sp.), and Megaloptera (e.g., *Sialis* sp.) were observed in smaller proportions at every sampling site, in accordance with the observations of other authors [4,5,9,62]. Among Chironomidae, the Chironomini tribe and Tanypodinae subfamily dominated the community at every sampling site in both years; both groups are typical of Alpine lakes at relatively low altitudes [61]. Based on the distribution of Chironomidae, alpine lakes can be divided into groups with similar characteristics [62]. In the case of Nero Lake, the predominance of Chironomini means that it falls into the category of relatively warm lakes which are rich in dissolved phosphate and other micronutrients and have an alkaline or at least slightly acidic pH. The less-represented groups, such as Orthocladiinae, Diamesinae, and Tanytarsinae, prefer colder and deeper lakes or those located at higher altitudes [62].

In 2022, Hexapoda (and thus Chironomidae) in Nero Lake showed a preference for warmer microhabitats, with a predominance of silty substrates and the presence of dissolved ammonia. The following year, they showed a completely different tendency at every sampling site, being associated with gravelly substrates and well-oxygenated waters. Chironomidae are abundant in very different microhabitats, ranging from soft and fine sediments and plant detritus layers to gravelly substrates [63], with different taxa preferring different substrates. During the sampling process in Nero Lake, different proportions of species may have been caught, which could explain the different associations with the microhabitats we found. Similarly, species with different requirements for dissolved oxygen levels [64] may have been caught in the two years, with the more demanding species being more prevalent in 2023.

Surprisingly, the second most abundant taxon in Nero Lake comprised Molluscs rather than Oligochaetes. Molluscs were almost entirely represented by *Valvata piscinalis* (Valvatidae). This gastropod inhabits a wide range of aquatic environments from rivers [65] to alpine lakes [66]. It feeds on organic detritus and is often found in both soft substrates and more gravelly areas [66]. The presence of dissolved organic matter in the water, even in large quantities, does not disturb it [67]. This would explain the association found in Nero Lake between this mollusc and the presence of dissolved ammonia and phosphates. These nutrients were found to be higher in Sites 1, 2, and 4, where the highest densities of molluscs were detected. Nero Lake is relatively rich in nutrients and organic matter due to the large amount of aquatic vegetation covering its shores and submerged littoral zone and the proximity of grazing livestock. These conditions create a suitable environment for the presence of large quantities of *V. piscinalis*, which can reach high densities of over 600 individuals per square metre in eutrophic lakes [68]. In Nero Lake, these densities exceeded 1000 individuals per square metre.

The Oligochaeta community was predominantly composed of Lumbriculidae, which prefer soft substrates [69,70]. This would explain the association found between silty substrates and the abundance of Oligochaetes in 2023. Steingruber et al. [71] reported that as pH increases, the number of Oligochaeta taxa and their abundance also increase, as observed in Nero Lake in July 2023. The association between Oligochaeta and high ammonia values that we identified in 2022 contrasts with that described by Bartels et al. [61], who reported a marked preference for phosphate-rich environments. Further investigations will be required to find any possible association concerning both water parameters and Oligochaeta distribution.

The genus *Gammarus*, representing almost the entirety of Crustacea in Nero Lake, is commonly found in small alpine lakes, and some species are adapted to survive near-zero temperatures and long periods of ice cover on the lake surface [72]. Commonly, the Gammaridae inhabiting alpine lakes are *Gammarus lacustris* (Sars, 1863). However, in 2017, Alther et al., [72] described a new species endemic to the Alps, *Gammarus alpinus* (Alther, Fiser & Altermatt, 2016), based on morphological and genetic characteristics. It would be interesting to conduct genetic analyses on samples from Nero Lake to determine which of the two species they belong to (or if both are present), especially since *G. alpinus* is influenced by climate change, alien species (i.e., fish introduced for fishing purposes), and eutrophication, all of which are stress factors present in Nero Lake. Crustaceans were associated with opposite substrate characteristics in the two years under consideration: in 2022, they were mainly associated with gravelly substrates, while in 2023, they were mainly associated with soft, silty substrates. If different species of *Gammarus* coexist in Nero Lake, spatial separation at the microhabitat level could occur due to differences in feeding behaviours and refuge preferences [73]. This could explain the opposite associations found in the two years. To test this hypothesis, it would be necessary to determine whether one or more species of *Gammarus* are present. Gammaridae are members of the functional feeding groups (FFGs) [74] of detritivores and shredders [75]. This may explain their association with areas with higher dissolved nutrients during both years.

The Hirudinea observed in Nero Lake belonged to the family Glossiphonidae (e.g., *Glossiphonia*, *Helobdella*), which is typical of both lentic and lotic habitats [34] and is also present in alpine lakes [76]. As with crustaceans, leeches were observed to be associated with different substrates in 2022 (silt) and 2023 (gravel). This difference could be attributed to the varying availability of prey in the different microhabitats of Nero Lake from one year to the next, or to shifts in diet. In fact, Glossiphonidae are generalist predators that can prey on Oligochaeta, Gasteropoda, Amphipoda, and Diptera Chironomidae, depending on availability and period [77]. The association between leeches and dissolved oxygen concentration in both years is worthy of further investigation. It may be linked to the fact that Glossiphonidae do not possess either haemoglobin or accessory respiratory organs [78]. This may explain their preference for more oxygenated water in Nero Lake. This result is in line with that reported by César et al. [79], who found that some species of the genus *Helobdella* prefer high dissolved oxygen concentrations.

Nero Lake exemplifies the general characteristics of alpine lakes. However, there are some peculiarities that need further investigation. A particularly noteworthy aspect of this study is the examination of the lake through a small-scale analysis, for identifying potential interconnections between biotic and abiotic communities. It would be of interest to apply this approach not only to the macrobenthic community, but also to other assemblages such as diatoms.

5. Conclusions

This study provided an insight into the composition of the benthic macroinvertebrate community in Nero Lake, Cottian Alps, in relation to environmental parameters. Although significant variations in some of these parameters were recorded between the two sampling years, these variations did not cause significant changes in the overall macrobenthic community. It was interesting to note that despite the short distances between sites, there were significant differences in community composition on a small scale. This highlights the importance of microhabitats and physicochemical parameters in modelling communities. Small-scale analyses are essential to providing a more complete picture of wildlife populations and any changes that may be occurring over time, whether due to natural causes or as a direct result of human impact. This is particularly important in a lake which, as in the studied case, is of a small size, is threatened by eutrophication, and is subject to anthropogenic impacts (e.g., the presence of livestock, the introduction of non-native fish species). As this is a Site of Community Interest, it is particularly important to investigate the impact of non-native species on biodiversity. To achieve this, it would be necessary to investigate the *Salmo trutta* population, particularly their stomach contents, to understand how much they affect the macrobenthic community via predation.

It should be acknowledged that this work provides only a partial picture of the complexity of a high-altitude lake ecosystem. Samples taken during the summer show the dominant species during that limited season but may not reflect the complexity of the lake. Therefore, long-term monitoring will be needed to provide a broader view and deeper knowledge so that any signs of alterations can be identified and prompt action taken.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d16060329/s1, Figure S1: Photos of the five sampling sites selected on Nero Lake (Western Alps) in July 2022 and July 2023; Table S1: Densities (ind./m^2) of identified taxa in July 2022 (Nero Lake, Western Alps); Table S2: Densities (ind./m^2) of identified taxa in July 2023 (Nero Lake, Western Alps); Table S3: Results of the post hoc Conover-Iman test performed on Hirudinea data (July 2022, Nero Lake, Western Alps); Table S4: Results of the post hoc Conover-Iman test performed on Crustacea data (July 2023, Nero Lake, Western Alps); Table S5: Results of the post hoc Conover-Iman test performed on Mollusca data (July 2023, Nero Lake, Western Alps); Table S6: Results of the post hoc Conover-Iman test performed on Chironomini data (July 2023, Nero Lake, Western Alps).

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