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Biodiversity and Litter Breakdown in a Karstic Freshwater System (Doberdò Lake, Northeast Italy) in Relation to Water Level Fluctuations and Environmental Features

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Abstract: The present study reports seasonal data about chemico-physical trends, macrobenthic invertebrates, and *Phragmites australis* decomposition rates within a karstic freshwater system in the western classic Karst. Results presented herein were compared with those obtained from the same system five years ago. Chemico-physical data showed higher temperatures and lower levels of pH and conductivity than in the past. The macrobenthic invertebrate community varied through the seasons and through the years, though they are dominated by few taxa, such as *Asellus* and *Gammarus*. These shredders were the most abundant functional feeding guild, playing a pivotal role in the decomposition of the vegetal organic matter. Decomposition rates k ranged between 0.009 and 0.016 days^{-1} and varied seasonally. k values were different from those previously reported for the investigated system. Differences are mainly due to changes in water supply, depending on decreasing rainfall regimes and hydrometric levels of the Soča River, which showed lower levels than in the previous study. Changes in water supply altered the fluctuating pulse that is typical of ephemeral karstic lakes, changing the system dynamics. Potential effects of climate change on local scale are also discussed.

Keywords: macrobenthic invertebrates; decomposition rates; karstic lake; leaf bag; environmental management

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1. Introduction

Karst is believed to account for about 21% of unknown global continental carbon sinks [1]. It consists of carbonate rocks, such as limestone and dolomite, interspersed with holes, caves, and channels that are often filled with water, which generally originates from precipitation that enters the soil and dissolves its CO_2 [2]. The absence of superficial waters is therefore contrasted by an impressive groundwater presence, and water and carbonate substrates interact through a series of complex structures and dynamic processes [3]. As waters move to and away from the surface, they can supply "disappearing" lakes and ponds [4], which are peculiar freshwater environments representing an interface between underground and surface waters [5,6]. Through a combination of intensity, timing, and extent of flood/drought events, water level variations affect many aspects of aquatic ecosystems and in particular ephemeral lakes, such as sedimentation and erosion of alluvial sediments, primary production, vegetation, mineralization, turnover rate, interaction among species, animal life cycles (i.e., spawning of fish and nesting of birds), and decomposition dynamics [7–10]. Water level fluctuations indirectly affect the organisms by changing the physical and chemical conditions [11] that control different levels of community structures [12,13]. These fluctuations favor the establishment of a variety of habitats that support different communities [14] and constitute a driving force and a limiting factor for ecosystem processes [8] by maintaining these environments in an early, relatively productive stage of development [15].

Among the communities hosted by these environments, macrobenthic invertebrates have a pivotal role. They cover all trophic roles of consumers [16,17] and constitute a trophic source for other invertebrates, fish, and birds [18]. Macrobenthic invertebrates are well-known bioindicators, often used when monitoring lotic and lentic systems [19–22], and are currently used in the context of ecological status assessment of surface waters in agreement with the European guidelines provided by the Water Framework Directive 2000/60/EC. However, less is known about communities of macrobenthic invertebrates in karstic ephemeral lakes. Smith et al. [5] studied the influence of habitat structure and flow permanence on invertebrate community within a karstic spring system; Tanaka et al. [6] analyzed macrobenthic invertebrate succession during leaf litter breakdown in perennial karstic rivers in western Brazil. However, regarding karstic ephemeral lakes, most of the studies are focused on vegetation [3,23], productivity [8,13], decomposition processes [10,24] and hydrology [25–27].

In aquatic systems, the vegetal organic matter breakdown is controlled mainly by intrinsic litter characteristics and environmental conditions: vegetal organic matter decomposition is affected by internal factors such as leaf characteristics [28,29] and by external factors such as water temperature and salinity [30–32], pH [33], nutrients [34–37], regional characteristics [31] such as climate [38], and solar radiation [39]. Microbial activity also contributes to litter decomposition, through bacterial [40,41] and fungal processes [42,43]. In general, regarding ephemeral freshwater systems, water temperature, high flood frequencies, and plant biomass with a high proportion of soluble substances promote fast decomposition processes, with high values of the decomposition rates [10]. Macrobenthic invertebrates also play a central role in litter breakdown, as they can accelerate the decomposition [44–49], adding their action to effects because of microbial activity and chemico-physical water parameters [49].

Regarding karstic systems, Bertoli et al. [24] reported that, in an ephemeral karstic lake, shredders and sources of water level fluctuations affect the *Phragmites australis* breakdown rate more than water temperature, which is one of the most important drivers in *P. australis* breakdown. This is due to the underground water supply, which impacts lake system processes and living communities, creating a buffer effect on the lake's water temperature [24]. Water level fluctuations represent a "pivotal disturbance factor", which is essential for the lake as it could also limit eutrophication [8,14]. However, climate change and human water use can cause changes in hydrological regimes, causing alterations in hydroperiod and seasonality: impacts can involve the shift in seasonal timing regarding the flood/drought cycle, changing the hydroperiod (duration of the wet period) [15,50–55]. Karstic environments are particularly sensitive to these effects and the loss of seasonality could lead to alteration of ecosystem services [15].

In this context, it was deemed of interest to investigate the macrobenthic invertebrate communities in a karstic lake (Doberdò Lake, Friuli Venezia Giulia, Northeast Italy) in relation to decomposition processes, variation of chemico-physical conditions of the water body, and changes in water supply. The main aims of the present study were to: (i) characterize seasonal trends of physico-chemical features and macrobenthic invertebrate assemblages in relation to the hydrological regime in a karstic ephemeral lake; (ii) analyze changes in seasonal variations of the organic matter breakdown rates using leaves of *Phragmites australis* and infer the effect of the environmental chemico-physical features and the macrobenthic invertebrate functional feeding guilds on the decomposition dynamics; and (iii) compare our results with previous investigations performed on the same study area, in relation to annual changes of the hydrological regime, after a 5-year period.

We expected to find trends affected by the origin of the water supply (groundwater from the Soča River and rainfall regime). In particular, we expected reduced differences among seasonal decomposition rates, which could be more affected by the origin of the water supply and by macrobenthic invertebrate functional feeding guilds than by chemico-physical parameters. In particular, we expected a low contribution from the temperature in the decomposition dynamics, as observed in the previous investigations [24]. These expectations are related to the underground origin of the Doberdò waters, which determines a buffer effect that inhibit the influence of the temperature on the decomposition process. On the other hand, we expected an effect from the variation of the lake hydrological level. Finally, we expected that macrobenthic invertebrate communities had a pivotal contribution in the decomposition of the vegetal organic matter and that this contribution could be higher than those of the chemico-physical features.

2. Materials and Methods

2.1. Study Area

The present study was carried out at Doberdò Lake (Figure 1) (Municipality of Doberdò, Friuli Venezia Giulia, northeast Italy), located in the Regional Natural Reserve of Doberdò and Pietrarossa Lakes, in a Special Area of Conservation (SAC IT3340006) and in a Special Protection Area (SPA IT3341002). The lake is one of the most important examples of a karstic lake in Europe and cited as a site of international interest in the Friuli Venezia Giulia Region [3,54]. The lake covers the bottom of a base-level polje in a low elevation area characterized by the presence of many springs and several temporary lakes [54–57]. Water is supplied by springs in the western and northwestern portion of the Doberdò polje while true inlets and/or outlets are absent. Some estavelles alternatively discharge water as springs or shallow holes, while true shallow holes are found at the eastern section of the lake. Wide variations occur in the hydrological regime and in the extension of the wet surface area, which vary from 200 m^2 during dry periods to 400,000 m^2 during wet periods [23,25].

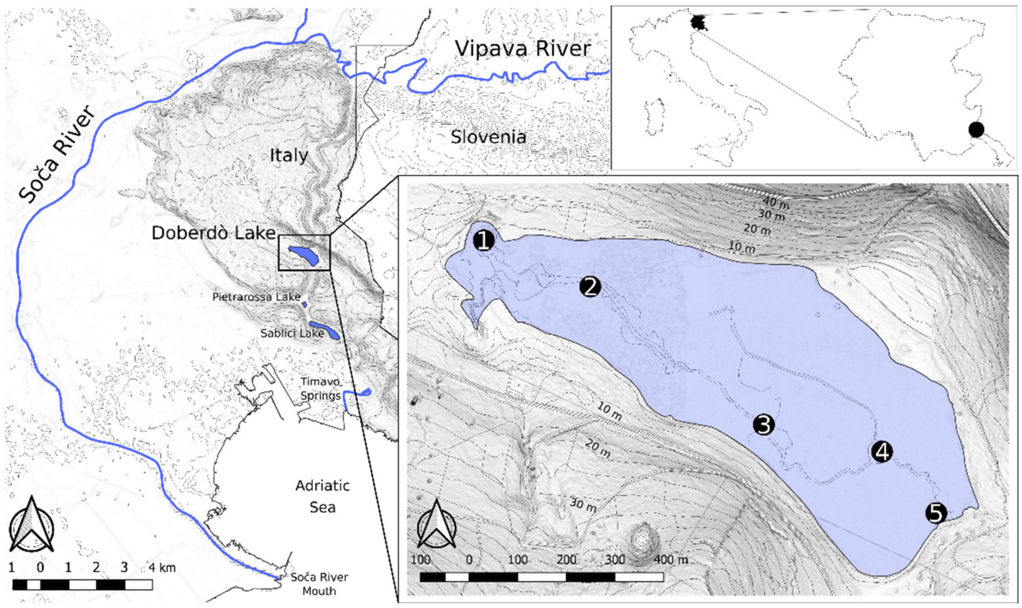

Figure 1. Study area and sampling sites at Doberdò Lake, Italy (source: Bertoli et al., 2020, modified) (Adapted with permission from Bertoli et al. [24]. 2020, Springer Nature Switzerland AG).

During the low-level phases, a small stream flows across the bottom of the polje from northwest to southeast, where shallow holes drain the basin. Water supply depends mainly on groundwater inflow from the Soča River and the Vipava Stream and on rainfall; despite proximity to the sea, tidal influence has never been documented [25]. Doctor [27] estimated that the Soča River supplies 75% of the flow to the smaller springs and water supply wells of the resurgence karstic zone and Calligaris et al. [57] described the Soča River as one of the main sources of supply for the Doberdò waters. Small variations in water level during the dry period are linked to hydrometric fluctuations of the Soča River caused by its regulation in Slovenia [25,26]. From the center to the border of the lake, vegetation is characterized by aquatic communities dominated by submerged and floating-leaved rooted plants (*Potamogeton* spp., *Myriophyllum* spp. and *Nuphar lutea*) and then by helophytic marsh vegetation dominated by tall sedges (*Carex elata*) and common reeds (*Phragmites australis*); behind these, a thin strip of mud soil along the lake shore hosts hygro-nitrophilous herbaceous communities. Toward the lake shores and landward, the zonation finally includes hygrophilous willow shrubs and woods (with *Salix cinerea*, *Salix alba*), and a meso-hygrophilous woodland with the field elm (*Ulmus minor*), black poplar (*Populus nigra*), and narrowed-leaved ash (*Fraxinus angustifolia* subsp. *oxycarpa*), with its mantle with *Ulmus minor* and *Paliurus spina-christi* [23]. The lake hosts a rich fish community, including *Scardinius hesperidicus* Bonaparte, 1845, *Squalius squalus* Bonaparte, 1837, *Leucos aula* Bonaparte, 1841, *Esox cisalpinus* Bianco & Delmastro, 2011, *Cobitis bilineata* Canestrini, 1865, *Padogobius bonelli* Bonaparte, 1846, and *Anguilla Anguilla* Linnaeus, 1758. Ichthyological aspects have a pivotal role for the geographical area including the Doberdò Lake, as in the region some species could overlap the extreme limits of their distribution areas [58].

Five sampling sites (Figure 1, Table 1) were selected considering depth, aquatic vegetation composition on the lakeshore and the bottom, flow characteristics, and water permanence. Same sites were previously reported by Bertoli et al. [24] in a former study about the Doberdò Lake. These sites have been chosen for the present work to check for significant changes in the lake after five years since the previous study.

Table 1. Coordinates and description of the sampling sites monitored at the Doberdò Lake during the present study.

Site	Latitude	Longitude
1	45°49′59.05″ N	13°33′15.05″ E
2	45°49′55.84″ N	13°33′27.69″ E
3	45°49′47.87″ N	13°33′40.79″ E
4	45°49′46.72″ N	13°33′52.40″ E
5	45°49′43.03″ N	13°33′57.92″ E

Site 1 was at the main spring, where the influence of groundwater and water depth are highest. Site 2 was a shallow water area along the course of the small central stream and site 3 was located within the stream near a small shallow hole. Site 4 was at the center of a large pool and site 5 was at the main shallow hole where floating hydrophytes were abundant. Geographic coordinates of each sampling site are reported in Table 1. The central portion of the lake is not subject to total drought even during low-level phases, even though some lake portions could be isolated from the main water body. Water level fluctuations were considered only in terms of water depth at the monitoring sites.

2.2. Chemico-Physical Parameters

The main physical and chemical water parameters were monitored every two weeks at each sampling site from June 2021 to March 2022. Water conductivity (mS cm^{-1}), pH, temperature (°C), and dissolved oxygen (mg L^{-1}) were recorded using field meters (HI 9033 conductivity meter; HI 9125 pH/ORP meter; HI 9147 dissolved oxygen meter; Hanna Instruments Inc., Woonsocket, RI, USA). Parameters were measured at approximately mid-depth in the water column. Water depth was measured with a graduated rope. For each parameter, three values were recorded at each site, and mean values were calculated. To determine the influence of the underground water supply, values of the Soča River water level were retrieved. Levels about 72 h before monitoring chemico-physical features at the sampling sites were considered, in line with previous studies that reported a 72 h delay for the influence of Soča flood events at Doberdò Lake [25]. In addition, total rainfall data were considered. These data were referred to the Gradisca d'Isonzo locality, located about 10 km from the Doberdò Lake. Information regarding the Soča River water level and total rainfall were provided by the Regional Authority of the Friuli Venezia Giulia Region (Section of Water Resource Management). Finally, mean maximum air temperatures and total rainfall of the last three decades (1992–2022) were analyzed to check for long-term temporal patterns. Data were retrieved from the website of the Regional Environmental Agency of Friuli Venezia Giulia Region (https://www.clima.fvg.it/clima.php, accessed on 24 April 2022).

2.3. Macrobenthic Invertebrate Sampling through Leaf Bag Technique

The leaf bag technique [59] was used to study organic matter decomposition processes and for the macrobenthic invertebrate sampling operations. Leaf bags were prepared as described by Bertoli et al. [24] for the same sampling sites of the Doberdò Lake and in line with previous studies in other freshwater environments [60–62]. Leaves of *Phragmites australis* were chosen as it is one of the most abundant plant species in the Doberdò area and dominates the riparian vegetation. Senescent leaves were previously collected, removing them from the shoots at least 30 cm above the water surface, avoiding apical leaves and those that met water. Only intact leaves were taken. The leaves were air dried after collection and then stored in the dark in a dry laboratory room until use. Then leaves were cut into 10 cm long fragments (excluding basal and apical parts) and oven-dried to constant weight (60 °C for 72 h). Lots (3.0000 ± 0.0001 g dry weight) were put inside tubular cotton net bags of 5 mm mesh, which allows colonization of macrobenthic invertebrates and limits leaf material loss [60]. This mesh size does not allow us to estimate the role of the microbial decomposition separated from the macrobenthic invertebrates. However, we were interested in the whole effect, with a major focus on the macrobenthic invertebrates, using the same technique of the previous study [24].

The oven-dried leaves served to achieve initial conditions as uniform as possible [63], to obtain standardized samples, and to facilitate comparison. Sampling was performed during four seasons: late spring (late May–late June 2021), summer (July–August 2021), autumn (late October–early December 2021), and winter (February–early March 2022). Twelve leaf bags were submerged at each sampling site by placing them gently near the bottom and tethering them with strings to stones to prevent loss. The leaf bags were then collected after 15, 30, and 45 days of submersion. At each sampling time point, four leaf bags were retrieved from each sampling site, placed in separate glass boxes containing lake water, and rapidly brought to the laboratory, where the leaves were gently washed to remove sediments and macroinvertebrate colonizers. The invertebrates were stored in 70% ethanol solution until further analyses. The leaves from each bag were oven dried at 60 °C for 72 h and weighed ($x \pm 0.0001$ g dry weight). The remaining leaf mass at $t = 15$, 30, and 45 days of submersion is expressed in percentage, whereby the initial weight at $t = 0$ days (3.0000 ± 0.0001 g dry weight) is 100%. Macrobenthic invertebrate colonizers were identified at least to the genus level (if possible). Finally, each taxon was assigned to a functional feeding guild (FFG) according to Merritt and Cummins [64].

2.4. Statistical Analysis

Chemico-physical data collected during the present study were compared with those reported by Bertoli et al. [24] for the same sites in the 2016–2017 period. Two-way ANOVA and the honestly significant difference (HSD) Tukey post hoc test were used to check for temporal differences in physical and chemical parameters (factors "year" and "season"). Seasonal values of rainfall and hydrometric level of the Soča River observed during the periods 2016–2017 and 2021–2022 were also considered, as these factors heavily affect the Doberdò Lake dynamics. Data were checked for conformity to normality assumptions with a Shapiro–Wilks test and transformed where necessary ($\log(x + 1)$). Conformity to assumptions of variance homogeneity was checked using the Bartlett test. Principal coordinate analysis (PCA) was performed on a standardized data matrix to examine patterns of seasonal changes in physical and chemical parameters. The Pearson product-moment correlation coefficient (r) was used to test correlations between variables. For this analysis, data collected during the present study and those reported by Bertoli et al. [24] were considered.

Mean maximum air temperatures and total rainfall of the last three decades (1992–2022) were analyzed to check for long-term temporal patterns. For this purpose, a time series analysis was performed using the "hydroTSM" package for RStudio [65]. The augmented Dickey–Fuller test was used to check for the presence of time-dependent structures in the times series, considering a nonstationary condition as the null hypothesis.

Main community indices (number of observed taxa, Shannon–Wiener index, Evenness and Dominance) were calculated for the 2016–2017 and for the 2021–2022 macrobenthic invertebrate assemblages. Seasonal comparisons for the 2021–2022 dataset were performed using nonparametric Kruskal–Wallis test and the Conover–Iman test as a post hoc test. Values observed in the 2016–2017 seasons were compared using the Wilcoxon non-parametric test. The same test was used to compare values of the same season between the different years. Macrobenthic invertebrate investigations carried out by Bertoli et al. [24] for the 2016–2017 period regarded only spring and autumn, while the present study (2021–2022 period) describes the communities for all the four seasons. Therefore, all the comparisons between the 2021–2022 and 2016–2017 macrobenthic invertebrate datasets were performed only for spring and autumn. Considering the 2021–2022 dataset, one-way PERMANOVA [66,67] was performed to test significant seasonal differences in taxa composition between observed macrobenthic invertebrate communities (factor "season"). Comparisons with the 2016–2017 dataset were also performed, considering homologous seasons (factor "year"). Data were transformed ($\log(x + 1)$) prior to analysis to reduce the influence of very abundant taxa [68] and the multivariate homogeneity of group dispersions was checked using PERMDISP2 [69]. To minimize the influence of rare taxa, only organisms with relative abundances > 2% or present in at least two samples were included. The subsequent resemblance matrix was obtained using the Bray–Curtis measure. SIMPER analysis [70] was applied to identify the taxa that contributed most to the significant differences highlighted by PERMANOVA.

The percentage of the original mass remaining at the three sampling times (15, 30, and 45 days) was estimated and the decomposition rate was modeled as a negative exponential decay function, which is frequently used to describe decomposition [71,72]:

$$M_{\mathrm{t}} = M_0\, e^{-k\mathrm{t}}, \tag{1}$$

where M_{t} is the percent mass remaining at time t, M_0 is the initial percent mass, and k is the decomposition rate. Percentage values of the remaining mass were natural log transformed and ANCOVA was used to compare seasonal values of k as slopes of linear regression equations using time as a covariate [72,73]. Moreover, values of k measured during the present work were compared with those obtained in the period 2016–2017.

The relevance of abiotic and biotic features as potential sources of variation in leaf decay rates was investigated using forward multiple stepwise regression analysis with the organic matter decomposition rate (k) as the dependent variable. To avoid multicollinearity, a nonredundant subset of variables was used as predictors from the initial list of parameters when evaluating the results of PCA and correlation analyses. If two or more variables were strongly correlated (threshold value $r > |0.4|$ and $p < 0.001$), they were excluded from the analyses, in agreement with previous investigations in Doberdò Lake [24] and with previous studies of leaf litter composition [49]. The number of shredders per leaf bag was defined as a biotic variable. Biotic and abiotic data were log transformed ($\log(x + 1)$) to fulfill assumptions of normality, which was checked with the same test as mentioned above. The relative importance of significant predictors to the variability explained by multiple stepwise regression was checked using the LMG method [74] and quantified as a percentage for significant regressors.

All analyses were performed using RStudio version 2021.9.0.351 [75,76], considering a p-level of 0.05 for significance. Figures were produced with RStudio and processed with Inkscape version 0.92 software.

3. Results

3.1. Physical and Chemical Parameters

Significant differences were detected by the two-way ANOVA for all considered chemico-physical features except for water depth, which did not show significant seasonal variations in both considered periods (Figure 2; Table S1). However, despite the ANOVA not allowing us to highlight significant variations, values observed in 2021–2022 were lower than those measured in 2016–2017 (Figure 2a). During each season, temperatures were significantly higher in 2021–2022 than in 2016–2017 except during autumn (Table 2, Figure 2b). Annual trends followed seasonality in both considered periods. However, in 2021–2022, wide variability was observed in spring, the values of which did not differ from those recorded in summer. Waters of the Doberdò Lake were generally well oxygenated at the monitored sites (Figure 2c), with similar levels observed in spring and summer for both study years. On the other hand, oxygen concentrations were higher in 2021–2022 than in 2016–2017 during autumn and in winter. Values of pH and conductivity (Figure 2d,e) were significantly higher during 2016–2017 than in 2021–2022. This trend was observed for each sampling season. The hydrometric levels of the Soča River and the rainfall regimes showed the same temporal trend, with levels significantly higher in 2016–2017 than those observed during the present work (Figure 2f,g).

Table 2. Results of PERMANOVA tests based on macrobenthic invertebrate assemblages observed in Doberdò Lake. Comparisons between communities observed in the present study and those observed in 2016–2017 are also reported. Significance is highlighted in bold.

Seasonal Comparison (Period 2021–2022)					
Source	**Sum of squares**	**d.f.**	**Mean squares**	***F***	***p***
Season	1.518	3	0.506	3.290	**0.001**
Residual	8.612	56	0.153		
Total	10.131	59			
Autumn 2016–2017 vs. Autumn 2021–2022					
Source	**Sum of squares**	**d.f.**	**Mean squares**	***F***	***p***
Season	0.745	1	0.745	4.915	**0.001**
Residual	4.245	28	0.151		
Total	4.990	29			
Spring 2016–2017 vs. Spring 2021–2022					
Source	**Sum of squares**	**d.f.**	**Mean squares**	***F***	***p***
Season	0.484	1	0.484	3.701	**0.001**
Residual	3.532	28	0.131		
Total	4.017	29			

PCA ordination highlighted that the first two axes explained 63.3% of observed variability (Figure 3) and the first three axes explained 76.3% (Table S2). Rainfall, hydrometric level of the Soča River, pH, and conductivity were strongly negatively related to the first axis PC1, which explained 41.6% of the observed variability. Data grouping associated to the 2016–2017 samplings were mainly located at the left side of the figure while grouping associated to the 2021–2022 sampling campaign were located on the right side of the plot.

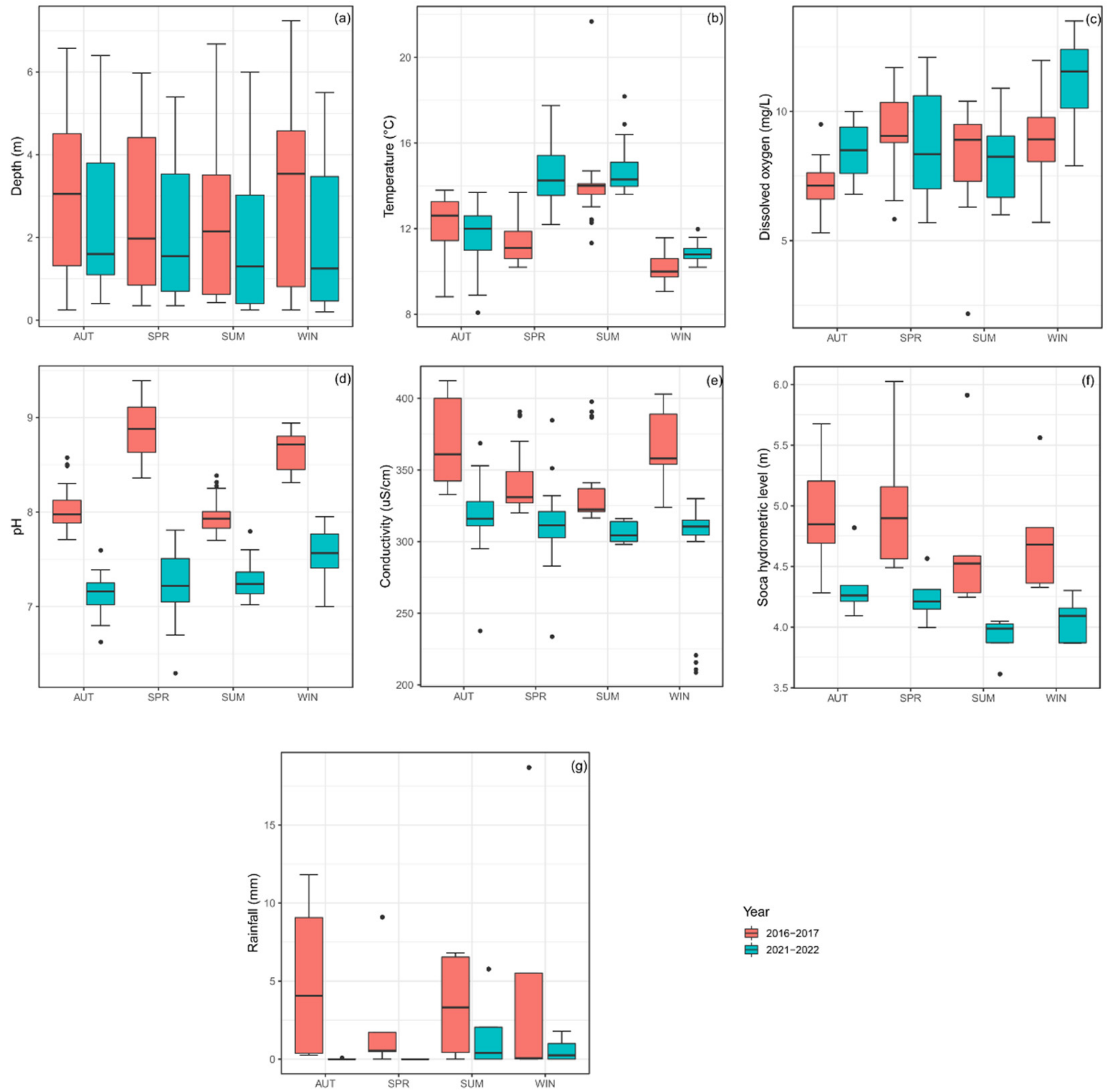

Figure 2. Seasonal trends of water depth (**a**), water temperature (**b**), concentration of dissolved oxygen (**c**), pH (**d**), conductivity (**e**), hydrometric level of the Soča River (**f**) and total rainfall (**g**) monitored during the study period in Doberdò Lake and comparison with data reported by Bertoli et al. [24] for the same sites in the period October 2016–September 2017 (n = 30 for each season). (data from 2016–2017 adapted with permission from Bertoli et al. [24]. 2020, Springer Nature Switzerland AG).

These findings suggest that the PC1 axis primarily identified a temporal gradient, depending on different rainfall regimes and hydrometric levels of the Soča River. Higher levels of pH and conductivity were also associated to these data groupings. The PC2 explained 21.6% of the observed variability. Water temperature was positively related to this axis, while dissolved oxygen concentration was negatively related (Table S2). Data groupings associated to winter samplings were mostly in the low section of the plot, as the most of spring samples, while autumn and summer data groupings took place in the central/upper section of the figure. However, most of the 2021–2022 spring samples were associated to high water temperatures and located in the upper section of the plot with summer data loads. These findings allow us to state that the second axis (PC2) represents a temperature gradient, mostly associated to seasonality. In addition, the right side of the plot is characterized by low rainfall and low levels of the Soča River, corresponding to reduced water supply for the lake. The water depth was negatively related to the PC3 (Figure 3; Table S2).

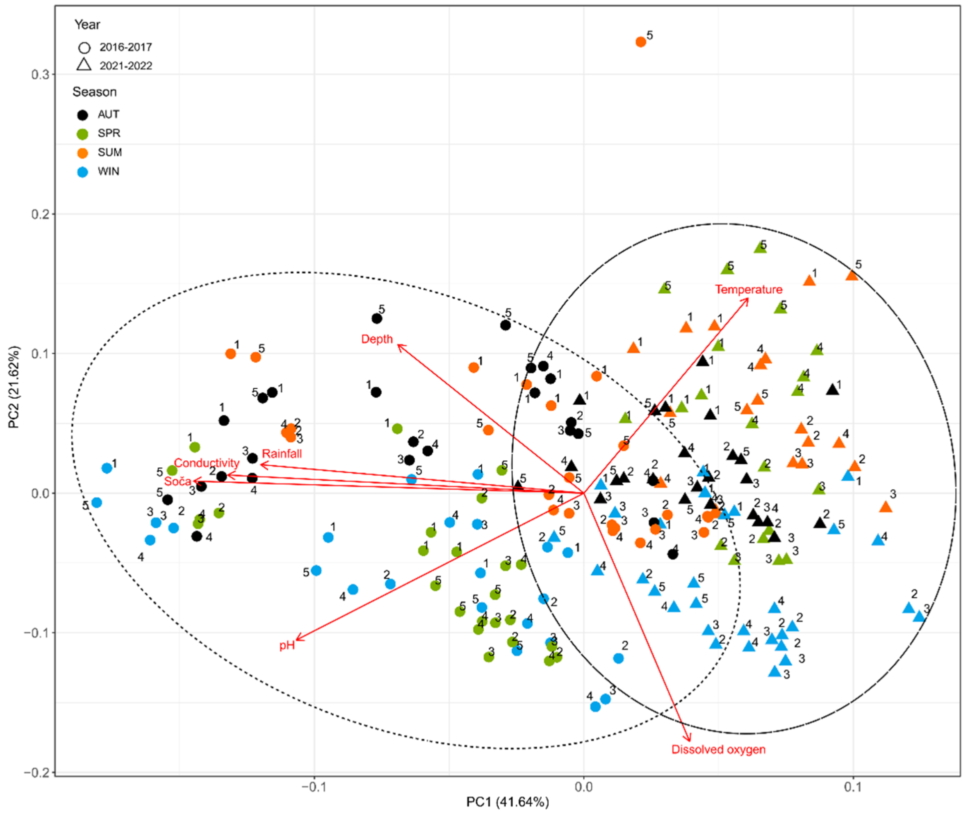

Figure 3. Principal component analysis (PCA) applied to physical and chemical parameters (Soča = Soča River hydrometric level; Rainfall = total rainfall) (n = 30 for each season). The ordination includes data collected during the present study and data reported by Bertoli et al. [24] (data from 2016–2017 adapted with permission from Bertoli et al. [24]. 2020, Springer Nature Switzerland AG).

The results of the time-series analyses are shown in Figure 4. Trends of total rainfall for the last three decades showed a slight but significant decreasing trend through the years (augmented Dickey–Fuller test = −3.479, p = 0.07) (Figure 4a). On the other hand, values of mean maximum air temperatures clearly increase during the considered time period (augmented Dickey–Fuller test = −5.423, p = 0.971) (Figure 4b).

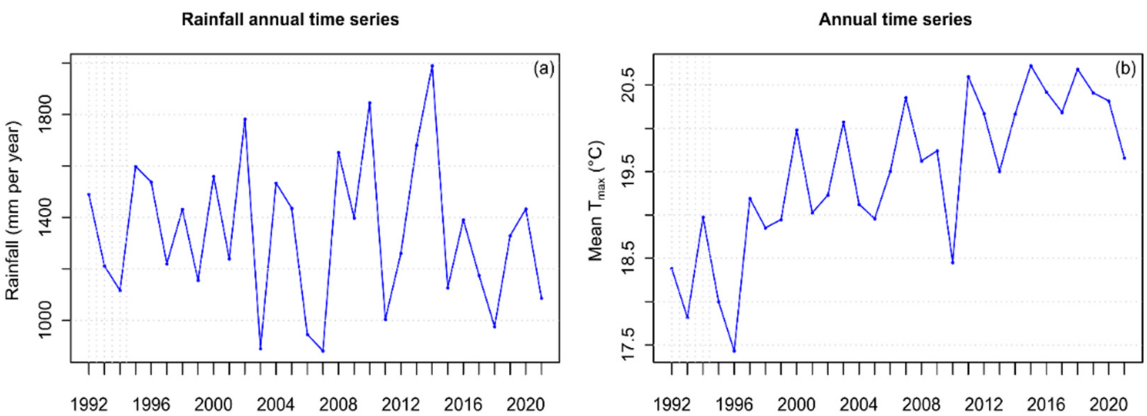

Figure 4. Trends of total rainfall (mm per year) (**a**) and mean maximum air temperatures (°C) (**b**) in the Gradisca d'Isonzo area. The site is located about 10 km from Doberdò Lake.

3.2. Macrobenthic Invertebrates

An amount of 52,408 macrobenthic invertebrate specimens was collected during the study period: 29,401 during spring, 8994 during summer, 6801 during autumn, and 7212 during winter. Main community indices are reported in Figure 5. In 2021–2022, significant seasonal differences were observed for all indices (Kruskal–Wallis test, $H > 9.45$, d.f. = 3, $p < 0.01$ for al comparisons). Spring values for the number of observed taxa, Shannon–Wiener index, and evenness were significantly lower than those observed in other seasons (Conover–Iman test, $p < 0.05$ for all comparisons) (Table S3). Values of the Shannon–Wiener index were also significantly different between summer and winter and between autumn and winter (Conover–Iman test, $p < 0.05$ for all comparisons), highlighting an increasing trend (Figure 5b). Dominance showed an opposite trend, significantly decreasing from spring to summer and from summer to autumn. Values of all indices differed between different years for both considered seasons, autumn and spring (Wilcoxon test: $p < 0.05$ for all comparisons), except for evenness in autumn and for the number of taxa in spring. In the 2016–2017 period, indices did not differ seasonally (Table S4).

Regarding the community composition during the present study, Malacostraca were the most abundant organisms in spring (93.2%) and summer (54.2%) (Table S5). In these seasons, *Asellus* showed the highest percentages (78.2% and 52%, respectively, in spring and summer) while values for *Gammarus* were generally lower (14.6 and 2.3%). Crustaceans decreased in number during autumn (30.2%) and winter (29.2%) but remained among the most abundant organisms in the leaf bags, and *Asellus* was always the most observed genus (22.5–23.7%). Due to the groundwater origin of the water supply of Doberdò Lake, *Niphargus* was collected, with frequencies lower than the other crustaceans (0.3–3% during the whole study period). Hexapoda was the second group in terms of frequencies and showed a higher number of taxa than the other invertebrate groups. During spring, Hexapoda percentages were lower than the other seasons (3.2%) and began to increase from summer (20.6%), through autumn (42%) to winter (45.7%). *Palpomyia* (order Diptera, family Ceratopogonidae) and the Chironomidae subfamilies (Tanypodinae, Chironominae, and Orthocladiinae) were the most abundant insects observed throughout the study period (Table S5).

Other taxa showed frequencies generally lower than 1%, except for the trichopteran *Hydroptila* in winter (4.8%). Oligochaeta were generally less frequent in the leaf bags (0.3–3.8%). Hirudinea and Tricladida were always present within the samples, showing variations in frequency ranges during the study period (2.1–17.8% for leeches and 0.6–9.9% for flatworms).

For the 2021–2022 dataset, PERMANOVA highlighted a significant effect for the factor "season" (Table 2).

The SIMPER test showed that the percentage contribution to the dissimilarity for the season factor was higher for *Asellus*, *Palpomyia*, and *Gammarus* than the other taxa. Hydracarina, Chironominae, *Limnodrilus*, and *Erpobdella*, and the Tricladida taxa also showed significant contributions (Table 3). Comparisons of datasets collected in different years also highlighted significant differences (Table 2), which were again mainly related to the *Asellus*, *Gammarus*, *Palpomyia*, and Chironomidae subfamilies (Table 3).

3.3. Decomposition Rates

Decomposition of *Phragmites australis* organic matter in Doberdò Lake fit with negative exponential models for each monitored season (Figure 6; Table S6). Significant seasonal differences were detected for decomposition rates k (days^{-1}), except between spring and summer. Comparisons with k values reported by Bertoli et al. [24] for the same area differed significantly both for spring (ANCOVA: $F_{(1,158)} = 13.620$; $p < 0.001$) and autumn (ANCOVA: $F_{(1,158)} = 8.907$; $p < 0.01$). Stepwise multiple regression allowed us to highlight that water temperature showed the highest relative importance in decomposition rates, followed by shredders, Soča River levels, and dissolved oxygen concentrations, while the rainfall was at least significant and showed the lowest relative importance (Table 4).

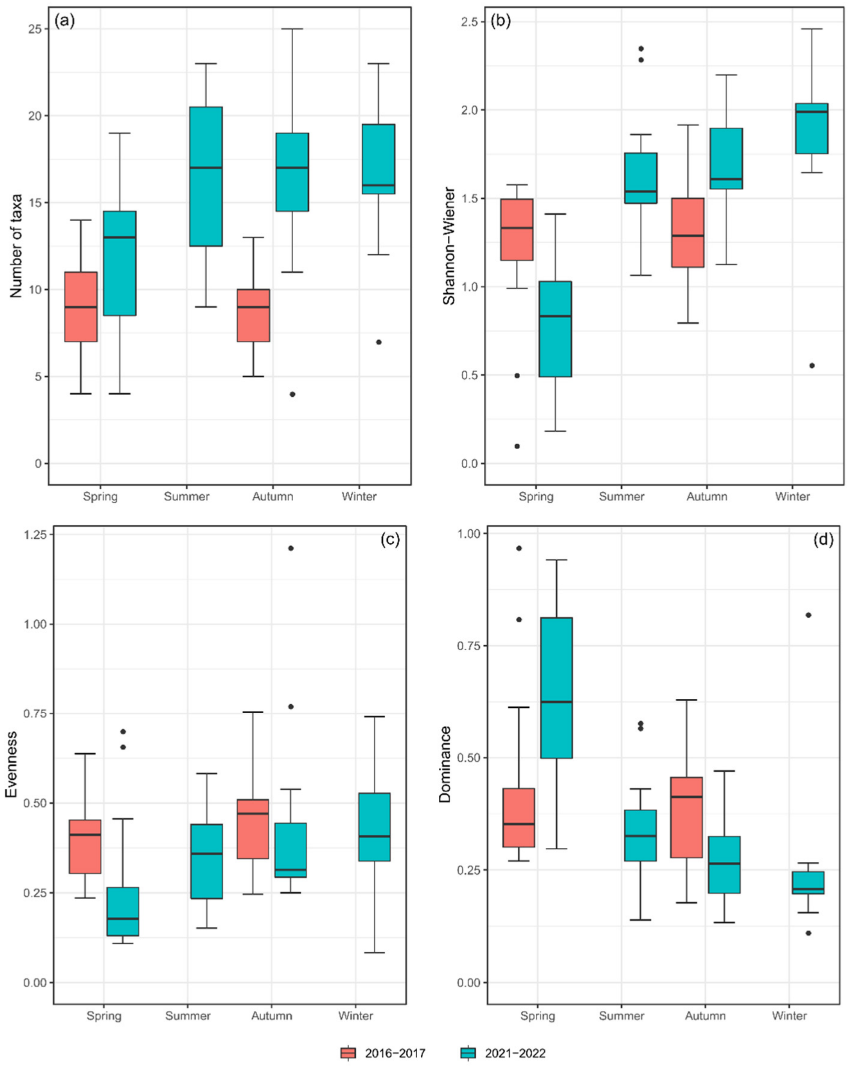

Figure 5. Main community indices ((**a**): Number of taxa; (**b**): Shannon Wiener index; (**c**): Evenness; (**d**): Dominance) measured for the macrobenthic invertebrate assemblages observed in Doberdò Lake via leaf bag technique and comparisons with data collected by Bertoli et al. [24] in same sampling sites in spring and autumn (2016–2017 period) (data from 2016–2017 adapted with permission from Bertoli et al. [24]. 2020, Springer Nature Switzerland AG).

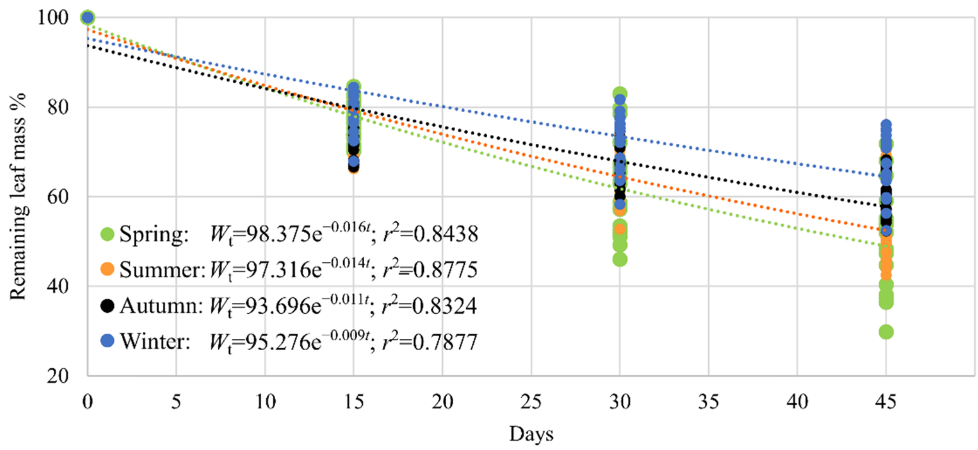

Figure 6. Comparison of *Phragmites australis* leaf mass decay calculated for Doberdò Lake between seasons in the present study. The initial weight at t = 0 days (3.0000 ± 0.0001 g dry weight) was set at 100%.

Table 3. Main taxa contributing to observed dissimilarities highlighted by the SIMPER test. Only taxa showing a contribution higher than 3% are shown.

Year 2021–2022, Seasonal Comparisons				2016–2017 vs. 2021–2022—Autumn				2016–2017 vs. 2021–2022—Spring			
Taxon	**Av. Dissim**	**Contrib. %**	**Cumul. %**	**Taxon**	**Av. Dissim**	**Contrib. %**	**Cumul. %**	**Taxon**	**Av. Dissim**	**Contrib. %**	**Cumul. %**
Asellus	4.79	8.38	8.38	*Asellus*	5.475	9.108	9.108	*Asellus*	6.197	11.390	11.390
Gammarus	4.60	8.04	16.41	*Gammarus*	4.835	8.043	17.150	*Gammarus*	5.625	10.340	21.720
Palpomyia	4.12	7.19	23.61	*Palpomyia*	4.601	7.653	24.800	Chironominae	5.225	9.601	31.330
Hydracarina	3.41	5.96	29.56	*Erpobdella*	4.226	7.030	31.830	*Palpomyia*	3.852	7.078	38.400
Chironominae	3.17	5.53	35.10	Tanypodinae	3.109	5.173	37.010	*Tubificinae*	3.703	6.804	45.210
Limnodrilus	2.91	5.08	40.18	Orthocladiinae	2.773	4.613	41.620	*Erpobdella*	3.497	6.425	51.630
Erpobdella	2.50	4.36	44.54	Tubificinae	2.711	4.509	46.130	Leptoceridae	2.600	4.777	56.410
Polycelis	2.32	4.05	48.59	*Glossiphonia*	2.554	4.249	50.380	*Sphaerium*	2.271	4.174	60.580
Dugesia	2.06	3.60	52.19	*Dugesia*	2.417	4.022	54.400	*Glossiphonia*	2.180	4.005	64.590
Dendrocoelum	1.92	3.36	55.55	*Dendrocoelum*	2.239	3.725	58.130	Orthocladiinae	1.914	3.516	68.100
Leptoceridae	1.86	3.24	58.80	*Haliplus*	2.225	3.701	61.830	*Bithynia*	1.785	3.280	71.380
Orthocladiinae	1.84	3.21	62.00	*Polycelis*	1.816	3.022	64.850	*Polycelis*	1.729	3.177	74.560
Tubifex	1.78	3.10	65.11					*Dugesia*	1.683	3.093	77.650
Bithynia	1.77	3.09	68.20								

Table 4. Stepwise multiple regression analysis between organic matter decomposition rates (*k*) and biotic/abiotic features. Only significant regressors are reported (rainfall = total rainfall; Soča = Soča River hydrometric level; DO = dissolved oxygen concentration).

	β	St. Error β	Estimate	St. Error	t Value	p-Level	Relative Importance (%)
Intercept	0.000	0.013	−0.087	0.013	−6.683	0.000	
Temperature	0.492	0.002	0.015	0.002	7.539	0.000	47.56
Shredders	0.320	0.000	0.001	0.000	5.846	0.000	22.56
Soča	−0.348	0.005	0.007	0.005	6.097	0.000	22.42
DO	0.185	0.001	0.005	0.001	3.024	0.003	5.79
Rainfall	−0.080	0.001	0.002	0.001	1.527	0.047	1.67

4. Discussion

4.1. Chemico-Physical Parameters

Despite the statistical analyses not allowing us to highlight significant differences in water depth between the 2021–2022 and 2016–2017 values, water levels observed in the present study were slightly lower than those observed five years ago, indicating a reduced water supply. In fact, rainfall, and Soča River hydrometric levels, which represent the main water sources for Doberdò Lake, were significantly lower during 2021–2022 than in 2016–2017 (Figure 2e,g), leading to changes in the hydrological regime of the karstic lake, with consequences to the ecosystem dynamics. This is also highlighted by the PCA, which showed a clear pattern between the 2016–2017 and 2021–2022 study periods. PCA loads associated with the 2016–2017 samplings showed a clearer seasonality than those associated with the 2021–2022 sampling campaigns. Moreover, as in the old samples the effect of great flood events was easily detectable (left side of the graph), the influence of flooding was absent for the 2021–2022 loads. Chemico-physical trends observed during 2021–2022 in Doberdò Lake were comparable with those previously reported by other authors [24,57]. However, significant differences with trends disclosed in other studies highlight the importance of the water supply sources affected by human actions and climatic changes.

Rainfall regimes are fluctuating across the decades in the Region Friuli Venezia Giulia and in the study area, with drought years alternating with wet periods. However, a slightly decreasing trend could be observed (Figure 4a). Similar trends were observed in other karstic zones, such as central Slovenia, for the Cerknica Lake area, where the precipitation rate showed a slight decrease and a variation in temporal distribution and short-term rates were observed. Moreover, air temperatures markedly increased across the last 150 years in the same geographic zone [15]. Increasing trends for air temperatures could also be observed for the study area considered herein (Figure 4b). Since 1961, mean air temperatures for the Friuli Venezia Giulia plain showed an increasing trend equal to 0.3 °C per decade, but during the last decades the pattern accelerated [77]. Even a small change in a relevant factor can impact ecosystem complexity in the long term [15,78] and meteorological changes add their effect to those that are due to human activity (i.e., water diversion), negatively affecting the amount of water available for the water supply. This could lead to the loss of seasonality, which affects plant life cycles and their production that is related to the water level. These effects have been observed in Cerknica Lake [15], another well-known example of an ephemeral karstic lake located in Slovenia. Our findings seem to be in line with those considerations, as highlighted by the PCA results. We conclude that reduced rainfall and low Soča River levels affected the chemico-physical condition of Doberdò Lake, which showed a reduced seasonality in the drought/flood cycle.

4.2. Macrobenthic Invertebrates

Composition of macrobenthic invertebrate communities in Doberdò Lake varied through the seasons and through the years, as highlighted by the PERMANOVA. In a natural condition, variations should be related to the ephemeral nature of the studied environment and to the alternance between wet and drought phases. In fact, the level fluctuations represent a pulse disturbance that interfere with the succession of the ecosystem and support its long-term stability in a stage between youth and maturity [8]. This could explain the lower diversity observed in the 2016–2017 period than in 2021–2022 (Figure 5). Macroinvertebrate community abundance and diversity is usually higher in perennial habitats, although in some intermittent ones abundances could be high because of the ability to rapidly colonize water bodies following the resumption of flow [5]. The trends observed for the community indices highlighted that, in both the considered periods, the system showed a certain degree of stability, except for in the spring of 2021, when dominance was very high. This fact could likely be related again to the reduced water supply observed in the recent period, which interrupts the buffer effect related to water flow from underground. This buffer effect smooths temperature fluctuations and seasonal extremes, as observed in Cerknica Lake [8,15]. High temperatures observed in the spring 2021 period correspond to a reduced rainfall (Figure 2b,e). However, temperatures were significantly higher than in 2016–2017 during the whole year. High dominance values found in the spring season are related to the high presence of crustaceans, which also represent the main shredder taxa in the lake (*Asellus*, *Gammarus*), and that could be related to the great amount of algae observed during this season in association with lower oxygenation levels. The crustacean contribution to variability was always higher than that of the other taxa, as highlighted by the SIMPER test. Crustaceans, Diptera, Tricladida, and Hirudinea are the most abundant invertebrate groups in Doberdò Lake. These organisms are strictly related to the presence of a rich aquatic vegetation, which provides food especially for shredders and scrapers [78–81] and refuges for all the taxa [80,82,83]. Vegetation could also favor a large presence of predators [81,83,84], which in Doberdò Lake are mainly represented by Hirudinea, *Sialis*, *Palpomyia*, Tricladida, and Odonata. Shredders are the main FFG in spring and summer, while other FFG (especially predators) become more frequent in the samples during other seasons (Table S5). Results of the SIMPER test seem to support these conclusions.

Changes in hydrological regime affect also macrobenthic invertebrates that are involved in the decomposition of the organic matter.

4.3. Decomposition Rates

In the 2016–2017 period, *P. australis* breakdown rates were mainly positively affected by shredders, and negatively by fluctuations of the Soča River and rainfall. The buffer effect that is due to water fluctuations in the karstic system reduced the importance of water temperature in the decomposition process [24]. Interestingly, during the present study, this pattern changed with water temperature that showed a high relative importance in the decomposition of *P. australis* leaves, as expected. In fact, temperature is recognized as the main factor leading the decomposition process [29,85]. We suppose that changes in the hydrological regime of Doberdò Lake, with reduced water supply, changed the decomposition dynamics, increasing the influence of water temperature as the “buffer effect” of groundwater was reduced. Values observed for decomposition rate k during the period 2021–2022 showed a clear seasonal trend and were significantly higher than values measured before. These values are also higher than those reported by Dolinar et al. [10] for Cerknica Lake, confirming that changes in temperature regimes and rainfall heavily affect the system dynamics of delicate environments such as ephemeral karstic lakes. In this context, the water demand that is due to human activities increase the observed effects, as the Soča River is known to be impacted by hydropeaking and water diversion [86]. These impacts are also known to affect the hydrological regime of Doberdò Lake.

5. Conclusions

The present study reports seasonal composition of macrobenthic invertebrate communities within a karstic lake in the western portion of the classic Karst (northeastern Italy).

Seasonal trends of physico-chemical features and *P. australis* decomposition rates in relation to changes in water supply caused by seasonal and multi-annual temporal variations were also analyzed. Finally, data presented herein were compared with those obtained for the same sites five years ago: the observed differences were mainly due to changes in water supply, depending on decreasing rainfall regimes and decreasing hydrometric levels of the Soča River. Our results warn about potential impacts for freshwater ephemeral environments located in karstic landscapes. These environments could be considered as biodiversity reservoirs, but they are threatened by climate changes and human water use. Alterations in the peculiar hydrological regimes of these unique environments could cause the loss of seasonality, affecting plant life cycles and production dynamics, related to water level fluctuations.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d14060460/s1: Table S1: Two-way ANOVA of chemical and physical parameters by year and season.; Table S2: Correlations between parameters and PCA axes (first three axes are shown); Table S3: Results of the Kruskal Wallis nonparametric test and of the Conover-Iman test for the seasonal comparisons between the values of the community indices calculated from the 2021–2022 dataset; Table S4: Results of the Wilcoxon nonparametric test for the comparisons of the community indices values between autumn 2016–2017 and 2021–2022, between spring 2016–2017 and 2021–2022 and between values seasonally observed (spring and autumn) in the 2016–2017-period; Table S5: Seasonal percentage frequencies of macrobenthic invertebrate taxa collected via leaf bags technique in Doberdò Lake; and Table S6: Results of the ANCOVA application to compare organic matter decomposition rates between the sampling seasons in the Doberdò Lake.

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