



Assessing the ecological potential of reservoirs: a principal response curve (PRC) analysis approach

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Abstract Heavily modified water bodies (HMWB) have been seriously affected by human activities and natural processes promoting their imbalance, and impacting their functioning and biodiversity. This study explores a new approach of monitoring and assessing water quality in Mediterranean reservoirs using phytoplankton communities across a disturbance gradient, according to water framework directive. Phytoplankton and environmental data were sampled in 34 reservoirs over 8 years. Two types of reservoirs were analyzed: Type1 “run-of-river reservoirs” (located in the main rivers, with a low residence time); and Type2 “true reservoirs” (located in

tributaries, with high residence time). The transition from deeper and colder reservoirs (reference sites) to shallow and warmer (impaired sites) was clear in Type2, correlated to organic pollution and mineral gradients. Impaired sites from both types showed a higher richness of tolerant taxa. Principal response curve (PRC) provided a concise summary of phytoplankton temporal dynamics and assessed ecosystem health for Mediterranean HMWBs. PRC will provide a powerful tool for environmental quality assessment and be incorporated into monitoring and assessment programs. This approach can help policymakers to manage natural capital to achieve multiple objectives, mainly increasing ecosystem services, and improve

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readability and interpretation of spatial patterns in temporal changes.

Keywords Ecological potential · Heavily modified water bodies · Phytoplankton · PRC analysis · Reservoirs

Introduction

Ecosystems provide many benefits and services to human communities but are under severe pressure, reinforcing the need for monitoring (Gunkel et al., 2015). Therefore, there is a growing need for more effective integrated assessment and ecosystem-based management. Assessing the well-being of water resources and implementing mitigation measures has become a priority, reinforced by Water Framework Directive (WFD, Directive 2000/60/EC), which states the need for EU member states to maintain all water bodies free from pollution long after 2027. However, the progress of improving freshwater quality and reducing eutrophication is slow and remains behind targets. Indeed, a recent report (European Environment Agency, 2018) revealed that 60% of European water bodies fail to achieve good ecological and chemical status. Only by achieving its effective management, we will be able to address the new Green Deal EU strategy designed to combine existing policies (e.g., WFD, Nitrates Directive, and Biodiversity) with broader objectives related to climate change impacts, adaptation, and sustainable development goals in an integrated holistic framework (CNEGP, 2017; Bierozza et al., 2021). WFD is the first legislation establishing the innovative concept of the ecological status of surface waters based on Biological Quality Elements in addition to physical and chemical conditions (Boeuf & Fritsch, 2016). This is based on a reference system with the highest ecological status, including water bodies whose ecology, chemistry, hydrology, and morphology are in pristine conditions, with almost no impact by anthropogenic activities (reference approach). Setting a reference state is still a challenge for most water bodies. Heavily Modified Water Bodies (HMWB), such as reservoirs, must meet ‘good ecological potential’ (GEP) while maintaining their functions and ecosystem service delivery (i.e., hydroelectric powers, navigation, recreation, water storage/regulation, and flood

protection) (MEA, 2005). Similar to Good Ecological Status, this objective may differ slightly from the best possible condition, Maximum Ecological Potential (MEP). The MEP represents the maximum ecological quality that could be achieved for these systems once all mitigation measures that do not have significant adverse effects on its specified use or in the broader environment have been applied (mitigation measures approach) (GIG, 2007; EU, 2019). Therefore, to measure the distance to the target, the relative deviation from the biological reference condition needs to be considered, mainly in terms of altered species composition and abundance or biomass of key biota (Denys et al., 2014; Dehini & Gomes, 2022).

In aquatic ecosystems, phytoplankton communities are key elements that regulate biogeochemical cycles (Litchman et al., 2015; Padedda et al., 2017; Liu et al., 2021). This community is one of the most important primary producers (Domis et al., 2013) and a crucial biological element considered within the WFD. Because of their particular sensitivity to water quality and anthropogenic pressures, phytoplankton communities are broadly used as trophic and ecological indicators to assess water quality in HMWB (Fetahi, et al., 2014; Lyche Solheim et al., 2014; Zhang et al., 2020). Consumers like zooplankton, fish, and invertebrate communities, are not included in the quality classification of this water bodies typology, according to WFD (INAG, 2009; Almeida et al., 2020).

The implementation of the WFD has prompted the search for novel methodologies and biological indicators (Statzner et al., 2001; Simboura et al., 2005; Ekdahl et al., 2007) for the assessment of environmental quality because traditional approaches cannot entirely capture the natural variability of ecosystems. The development of indicator systems based on species-environment relationships has become a widely used approach for these tasks (Statzner et al., 2001; Dzioczek et al., 2006).

There is a growing need to describe the complexity of ecosystem dynamics, namely how communities change over space and time. However, despite the more holistic approach, many available tools provide ambiguous ordination diagrams, making unclear the detection of ecological impacts and the presentation of those impacts in an easily communicable way to policymakers, managers, and stakeholders who are usually not biologists (Pardal et al., 2004). The summary ordination plots and

diagrams from traditional ordination methods (e.g., redundancy analysis: RDA; principal component analysis: PCA; or multi-dimensional scaling: MDS) can remain uninterpretable to non-specialists and consequently are difficult to interpret (Warwick & Clarke, 1991). This is especially true when a time factor is present within the data because temporal trajectories are often non-linear in such plots. One approach to deal with time dependency's complexity and overcome such difficulties is applying a novel application of principal response curves (PRCs) (Van den Brink & Ter Braak, 1999).



PRC analysis was initially conceived for aquatic ecotoxicology, assessing the effect of toxicants on freshwater communities (Van den Brink & ter Braak, 1999; Brock et al., 2009), but potentially it has much broader applications (Pardal et al., 2004; Auber et al., 2017; Vendrig et al., 2017). It has been widely applied in aquatic and terrestrial ecology and ecotoxicology (e.g., Verdonschot et al., 2015; Sittenthaler et al., 2019), microbiology (e.g., Fuentes et al., 2014; Paliy & ShanKar, 2016) and soil science (e.g., Cardoso et al. 2008; Van Paassen et al., 2020). In these studies, the tested factor was the toxicant, and time was the dimension along which repeated treatments were performed, and patterns of change were identified (Auber et al., 2017). Therefore, PRC has advantages over traditional ordination techniques enabling a formal and robust statistical analysis of temporal (long-term) data series from spatial gradients, providing community-level insights into anthropogenic disturbances effects. Additionally, PRC provide a quantitative assessment of individual species' responses to stress agents. In this work, we selected reference sites (or less disturbed) as control. Other areas (treated or impacted areas) were compared to these reference sites, allowing changes in the environmental quality to be assessed over time. In general, Portuguese reservoirs have undergone significant eutrophication due to organic enrichment (Vasconcelos, 1991, 2001; Boavida & Gliwicz, 1996; INAG, 2006; Domingues & Galvão, 2007). This work assessed the phytoplankton composition across a disturbance gradient over 34 Portuguese hydropower reservoirs for eight years. We aimed to explore an efficient and user-friendly approach to monitor and assess water quality in two types of reservoirs using phytoplankton communities, a biological quality element used to classify the ecological potential (EP) according to the WFD.

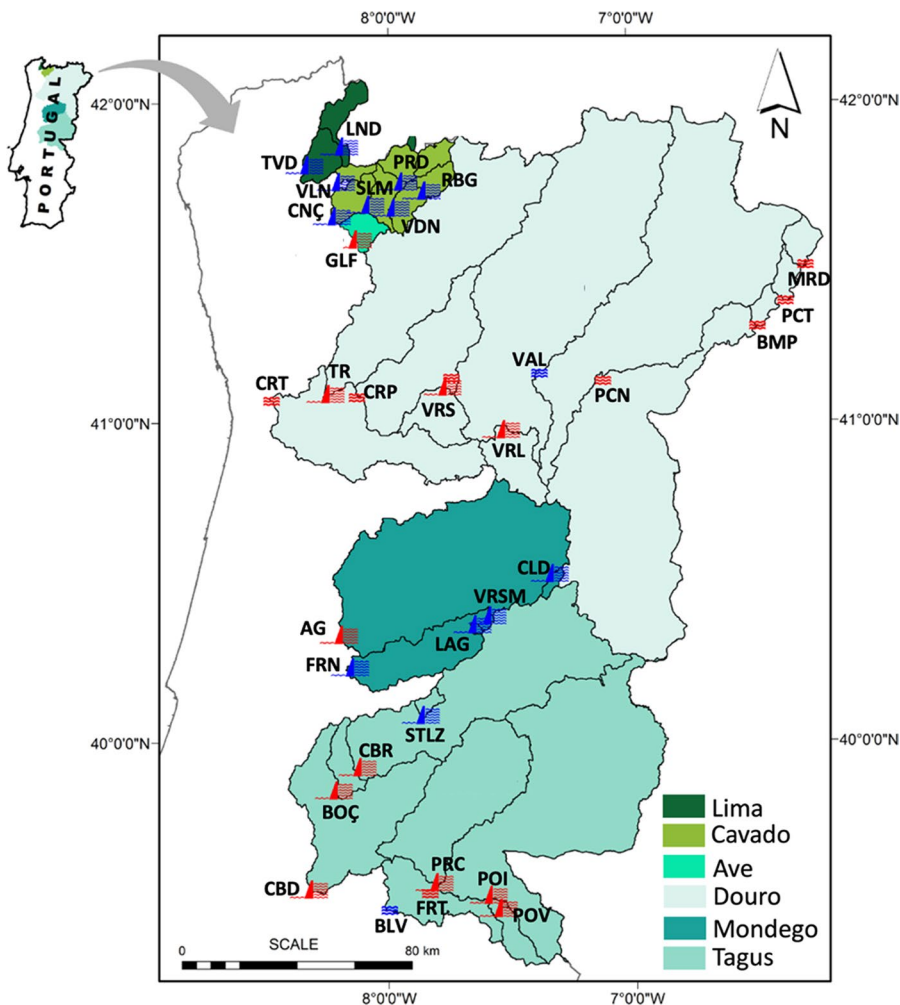
We hypothesized that differences in phytoplankton communities would be expected (1) in different types of reservoirs due to species spatial–temporal variations caused by the hydric resource use regime, (2) across the disturbance gradient in each typology: Type1 (run of river), lowland reservoirs located in the main rivers (Douro and Tagus), with a very low residence time; Type2 (true reservoirs), deeper high-altitude reservoirs, primarily located in tributaries, with a high residence time. It is expected that PRC analysis will provide a concise summary of phytoplankton communities' temporal dynamics and be a comprehensive tool to assess ecosystem health for Mediterranean HMWB.

Materials and methods

Study area

This study was carried out using data from 34 reservoirs in six catchments located in central and northern Portugal. The catchments were: Ave (1 reservoir), Cávado (6 reservoirs), Mondego (5 reservoirs), the Portuguese part of the international watersheds of Lima (2 reservoirs), Douro (11 reservoirs), and Tagus (9 reservoirs). The primary purpose of all these reservoirs is to provide hydroelectric power, although some secondary uses are also common, such as navigation, irrigation, water supply, and recreation. This extensive geographic area represents a wide range of physical and chemical characteristics, soil use, and anthropogenic pressure, including good and poor water quality conditions. Previous works have shown that phytoplankton community composition differed markedly between high altitude and lowland reservoirs (Cabecinha et al., 2009a). Based on the results of this work, we defined two types of reservoirs, comprising Type1 ($n=10$)—"Run-of-river" reservoirs located in the main rivers (Douro and Tagus), with a very short residence time (1–3 days); Type2 ($n=24$)—"True reservoirs" deeper high altitude reservoirs, located mainly in tributaries, with long residence time (65–300 days) (Fig. 1). "Run-of-river reservoirs" were generally situated at lower altitudes, had larger catchments, lower residence time, and were higher in water mineral content (hardness and conductivity) than higher altitude reservoirs (Tables 1, 2). In general, Type1 was more nutrient-rich, with

Fig. 1 Location of the 34 reservoirs studied and their distribution through six catchments: Ave, Cávado, Mondego, and the Portuguese part of the international watersheds of Lima, Douro, and the Tagus.  and  represent reservoirs of Type1 (Run-of-river reservoirs) and Type2 (True reservoirs); Red and Blue symbols represent impaired and reference sites on each type, respectively



watersheds dominated by industries and agriculture that occupied about 50% of the total area (> 15% of intensive agriculture). Type2 was characterized by watersheds with extensive natural areas (> 80%) and smaller agriculture areas (nearly 16%, but only 3% of intensive agriculture) (Cabecinha et al., 2009a, b, c, d).

Environmental parameters and chlorophyll a

The Laboratory of Environment and Applied Chemistry (LABELEC) conducted the sampling of environmental and biological parameters during the period from 1996 to 2004. The samples were taken four times per year, which corresponded to the seasons of spring (April/May), summer (July/August),

autumn (October/November), and winter (January/February). The sampling periodicity is indicated in Table 1. Not all reservoirs had the same sampling frequency; those with greater variability in physical, chemical and hydromorphological parameters were sampled more frequently (annually). The remaining reservoirs were sampled every 2 or 3 years. All samples were collected at 100 m from the reservoirs' crest at two different depths: (a) near the surface at approximately 0.5 m depth, and (b) near the bottom at 2 m above the bottom, (this depth was only considered for environmental parameters).

Turbidity, conductivity, water temperature, dissolved oxygen and pH were measured directly at the sampling sites using a YSI handheld multiparameter probe (Yellow Spring Instruments). The remaining

Table 1 Sampling periodicity and mean values of important physical and hydromorphological factors of the 34 reservoirs surveyed from 1996 to 2004

Reservoir	Code	Altitude (m)	Catchment area (km ²)	Dam area (km ²)	Mean Depth (m)	Residence time (days)	Ecological status class	Sampling periodicity
TYPE1								
Valeira	VAL	105.20	85,400	7.95	11.50	3.39	II	Triannual
Belver	BLV	46.15	62,802	2.86	5.61	–	II	Annual
Picote	PCT	480.00	63,750	2.44	26.92	3.27	III	Annual
Carrapatelo	CRP	71.89	92,050	9.52	16.72	5.76	IV	Triannual
Fratel	FRT	74.00	60,000	7.50	17.38	5.24	IV	Biannual
Pocinho	PCN	125.50	81,005	8.29	15.64	2.50	IV	Biannual
Régua	RG	73.50	90,800	8.50	12.06	2.10	IV	Triannual
Miranda	MRD	528.05	63,100	1.22	31.86	1.45	V	Biannual
Bemposta	BMP	402.00	63,850	4.05	30.83	9.52	V	Triannual
Crestuma-Lever	CRT	13.20	93,790	12.98	12.99	2.24	V	Annual
TYPE2								
Vilarinho das Furnas	VLN	569.50	77	3.46	34.52	202.99	I	Biannual
Caniçada	CNÇ	162.00	783	6.89	29.51	38.63	I	Annual
Lagoa Comprida	LAG	1600.00	6	15.71	18.16	–	I	Annual
Salamonde	SLM	280.00	642	2.42	31.14	21.75	I	Annual
St ^a Luzia	STLZ	655.60	50	2.46	24.34	–	I	Annual
Touvedo	TVD	50.00	1700	1.72	11.08	3.34	I	Annual
Paradela	PRDL	740.00	269	3.80	112.00	196.09	II	Biannual
Vale do Rossim	V.RSM	1436.46	5	0.37	27.00	–	II	Annual
Caldeirão	CLD	702.00	32	0.66	39.00	19.20	II	Annual
Fronhas	FRN	134.00	652	5.35	62.00	59.43	II	Biannual
Alto Lindoso	LND	338.00	1525	10.72	110.00	108.31	II	Annual
Alto Rabagão	RBG	880.00	101	22.10	94.00	594.12	II	Biannual
Venda Nova	VDN	700.00	356	4.00	97.00	63.32	II	Annual
Guilhofrei	GLF	335.63	122	16.30	49.00	–	III	Annual
Bouça	BOÇ	175.00	2525	1.85	65.00	7.62	IV	Biannual
Poio	POI	270.00	16	–	18.00	–	IV	Annual
Torrão	TR	65.00	3252	6.50	70.00	13.52	IV	Annual
Cabril	CBR	296.00	2340	20.23	136.00	138.93	IV	Triannual
Vilar	VLR	552.00	370	6.70	58.00	320.61	IV	Annual
Póvoa Meadas	POV	311.45	150	2.36	32.00	–	IV	Annual
Pracana	PRC	114.00	1410	5.50	60.00	105.43	IV	Biannual
Castelo de Bode	CBD	121.50	1340	32.91	115.00	191.10	V	Annual
Aguieira	AG	124.70	3100	20.00	89.00	50.59	V	Annual
Varosa	VRS	264.00	310	0.70	76.00	–	V	Annual

Ecological status varied from class I—High status to class V—Low status. Type1 (Run-of-river reservoirs) and Type2 (True reservoirs)

environmental variables were determined following the methodologies outlined by APHA (APHA, 1995).

A geographic information system database was created (ESRI, ArcGIS 9.0) with 12 spatial variables to determine the ecological status of each reservoir watershed. These variables were classified

Table 2 Mean values and standard deviation (SD) of important limnological properties of the five ecological status classes from the two types of Portuguese reservoirs surveyed from 1996 to 2004

Environmental variables	Response to perturbation	Type2																	
		Type1		Class II		Class III		Class IV		Class V									
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD								
Ephlimumion																			
Total Coliform (N/100 ml)	↑	989.2	437.7	506.9	448.2	1148.6	717.5	1156.8	722.9	513.4	1912.0	2536.4	10.782.8	1424.4	2715.5	3098.0	13.313.6	1313.5	4372.6
Faecal Coliform (N/100 ml)	↑	118.6	39.2	17.8	45.8	132.7	47.6	36.1	7.5	5.9	12.4	8.2	28.5	12.3	27.3	65.2	68.9	29.2	41.8
Chlorophyll a (mg/m ³)	↑	8.3	7.5	1.0	0.4	4.0	0.8	0.8	0.6	4.0	4.6	4.6	4.6	10.4	7.8	10.5	16.9	3.9	5.9
Surface water temperature (°C)	↑	15.8	99.7	16.3	5.3	16.2	5.6	15.8	78.6	15.4	4.8	16.3	5.3	17.2	5.8	18.0	5.3	18.4	5.2
Turbidity (NTU)	↑	3.4	102.2	4.1	6.5	3.3	3.9	4.6	80.5	1.3	3.7	1.2	1.5	1.6	1.0	2.9	2.7	1.6	2.4
pH	↓	7.8	104.6	8.1	0.5	7.9	0.6	7.9	82.4	6.8	0.5	7.1	0.6	6.8	0.3	7.8	1.1	7.7	1.1
Dissolved oxygen (mg/l)	↓	9.5	107.4	8.6	3.5	9.9	2.9	9.0	84.6	9.5	1.4	9.2	1.6	9.6	1.2	9.6	2.1	9.1	2.3
Conductivity (µS/cm)	↑	408.0	110.3	395.7	82.4	334.4	119.5	323.3	86.9	23.3	7.9	28.5	10.3	32.4	4.0	79.6	29.4	83.3	26.0
Hardness (mg CaCO ₃ /l)	↑	135.4	44.1	171.0	34.7	127.5	40.3	133.9	37.8	3.9	1.8	5.6	2.9	4.6	1.0	16.9	7.7	18.2	4.7
Ammonia-N (mg NH ₄ /l)	↑	0.2	30.0	0.1	0.1	0.2	0.1	0.2	17.9	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.2	0.3	1.0
Nitrate-N (mg NO ₃ /l)	↑	5.2	7.0	6.2	3.7	5.5	3.1	5.8	5.1	0.7	0.6	0.5	0.6	1.6	0.9	1.5	1.4	2.4	1.7
PO ₄ (mg/l)	↑	0.4	7.3	0.2	0.1	0.2	0.2	0.2	5.3	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.2
Total phosphorus (mg PO ₄ /l)	↑	0.6	7.5	0.3	0.1	0.4	0.2	0.3	5.4	1.2	4.2	0.0	0.0	0.0	0.0	0.2	0.2	0.2	0.2
Fe (µg/l)	↑	66.5	31.0	27.6	22.7	43.6	31.4	48.8	18.4	31.7	35.9	35.1	28.6	24.7	16.9	69.0	78.6	51.0	91.5
Mn (µg/l)	↑	21.0	12.1	8.9	8.3	14.5	12.4	14.3	7.4	12.0	22.0	12.8	13.4	8.0	5.1	19.0	15.4	13.6	13.2
Cl (mg/l)	↑	30.1	10.9	17.5	4.8	19.2	12.1	15.1	5.9	3.1	0.9	3.2	1.1	4.6	0.6	8.2	3.7	8.8	2.9
Chemical oxygen demand (mg O ₂ /l)	↑	10.0	7.6	2.2	1.8	11.5	4.8	8.6	5.5	4.7	2.9	5.0	2.8	4.8	2.0	12.4	9.0	7.5	5.6

Table 2 (continued)

Environmental variables	Response to perturbation		Type2																
	Type1		Class III		Class IV		Class V		Class I		Class II		Class III		Class IV		Class V		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
5-Day biochemical oxygen demand (mg O ₂ /l)	2.0	7.9	2.2	1.6	1.7	1.0	1.8	5.6	1.1	0.6	1.1	0.7	1.2	0.5	2.2	1.7	1.7	1.7	
Total silicon (mg SiO ₂ /l)	6.1	8.3	1.7	0.7	4.2	2.5	3.0	5.9	3.4	1.7	3.7	2.2	3.5	1.8	5.0	3.7	5.5	2.8	
Secchi disk depth (m)	2.0	9.1	10.0	4.0	2.1	1.2	1.7	6.4	4.2	1.8	4.0	1.9	2.8	1.2	2.1	1.2	3.3	1.8	
Hypolimnion																			
Chlorophyll a (mg/m ³)	6.9	9.7	0.7	0.4	1.0	1.3	0.6	6.8	1.5	1.6	2.0	2.8	3.9	5.0	4.6	9.9	0.9	1.6	
Water temperature (°C)	15.2	5.3	15.0	4.0	15.1	4.7	14.5	5.9	11.5	2.8	12.0	3.7	14.1	4.2	13.6	3.7	12.6	3.2	
Dissolved oxygen (mg O ₂ /l)	7.9	2.9	4.2	3.8	7.2	2.9	7.0	3.7	7.0	3.4	6.4	3.2	6.3	3.5	4.4	3.6	4.4	3.2	

Ecological status varied from class I—High status to class V—Low status. Response to perturbation: increase (↑), decrease (↓), and variable (†). Type1 (Run-of-river reservoirs) and Type2 (True reservoirs)

into four categories of anthropogenic stress measures that are prominent in the study area:

1. Land cover—6 land use/land cover variables using Corine Land Cover (CLC 1990, 2000—IGEOE, 2006). Road density (km/ha watershed) and urban areas, intensive and extensive agriculture, natural and semi-natural areas, and burned areas ratio;
2. Organic contamination load—Human population pressure (g BOD₅/hab.eq.day by ha watershed) and domestic animal pressure (g BOD₅/animal.eq.day by ha watershed) (INE, 2006);
3. Industrial contamination load- Point source pollution, including the number of quarries, mines, and transformation industries (number of point source/ha watershed) (INE, 2006);
4. Hydrometric variations—yearly water level changes were determined by the relative average and maximum theoretical water level differences.

Most variables were represented on a per-unit-area basis (for more detailed information, see Cabecinha et al., 2009a,c). For each variable, a 5-point scale was developed (from 1—High status to 5—Low status). Thus, the aggregate of these 5-score scales represented the ultimate ecological quality of the reservoir's watershed and was categorised as follows: I—< 18; II—18 to 22; III—22 to 26; IV—26 to 30 and V—> 30 (see Table 2). Classes I and II represented reference reservoirs, and classes III, IV, and V represented impaired sites.

Phytoplankton analysis

The environmental parameters and phytoplankton samples were collected from 1996 to 2004 using a Van Dorn bottle. Phytoplankton community composition was analyzed using inverted microscopy, following Utermohl's method (Lund et al., 1958). To quantify and identify the phytoplankton, the samples were fixed in Lugol's solution (1% v/v) and, whenever possible, identified to the species level. The abundance of each taxon was estimated on a 5-score ordinal scale (0–20; 20–40; 40–60; 60–80; > 80%). A minimum of 50 random visual fields and at least 100 cells of the most common taxa were counted. Assuming that the cells were randomly distributed, the counting precision was $\pm 10\%$ (Venrick, 1978). EDP

Labellec provided all the data—Environment Laboratory is accredited according to the NP EN ISO/IEC 17025:2005 standard.

Statistical analysis

The spatial and temporal dynamics of phytoplankton communities along the perturbation gradient were analysed by the PRC ordination technique. PRC is based on RDA, the constrained form of Principal Component Analysis (Van den Brink & ter Braak, 1998, 1999; Cuppen et al., 2000; Van den Brink et al., 2000, 2003). The PRC method is a multivariate technique designed for data analysis from microcosm and mesocosm experiments. Due to its novelty, this method was mainly applied in aquatic ecotoxicology (Van den Brink & ter Braak, 1999; Van den Brink et al., 2003), with some incursions into ecology (Frampton et al., 2000, 2001). However, this approach has potential for a broader application in community ecology and the evaluation of ecosystem integrity.

The method analyzes differences in species composition between sites at each time point, similar to other ordination techniques. However, one advantage of this method is that any temporal changes in the 'control' (the reference sites in the present study) are constrained in the plot to a horizontal line. Thus PRC creates a graphical display with time (sampling dates) as a horizontal line and the basic response pattern (cdt) of each site (*d*) at each time (*t*) in relation to the control site on the vertical axis (by definition, the control site always has a cdt of zero for every time). When these coefficients are plotted for each time point, a principal response curve of the community is obtained for each site compared to the control site (Van den Brink & ter Braak, 1999). This allows an easily understandable representation of the temporal changes in the phytoplankton assemblages at each site with the reference control site. An additional advantage of the PRC technique is that it allows detecting effects at the species level. Derived species weight (bk) is the factor by which the basic response pattern is multiplied to attain the fitted response of species *k*. Species weights thus measure the affinity of a particular species to the community response pattern and can be used to estimate relative species abundance for each sampling date in each site compared to the control, using the expression $\exp(\text{bk} * \text{cdt})$. When the coefficients cdt are plotted against sampling date *t*,

the resulting PRC diagram displays a curve for each site that can be interpreted as the principal response curve of the community (Van den Brink & ter Braak, 1999).

In addition to providing a concise graphical summary of changes in community structure, PRC analysis allows an estimate of the variance in the data set explained per site. A PRC diagram aims to maximize the amount of variance due to sites; the higher the proportion of the variance displayed, the more closely will the fitted relative abundance of individual taxa inferred from the diagram matches the observed relative abundance. The null hypothesis assumes that the differences between impacted and reference sites are absent. To test these hypotheses Monte Carlo permutation tests were used (Van den Brink & ter Braak, 1999).

In the present study, the differences in phytoplankton community composition between the different ecological classes for the two Portuguese reservoir types were visualized by PRC (Principal Response Curves; Van den Brink and ter Braak (1998, 1999) using the CANOCO software package version 5.1 (ter Braak & Šmilauer, 2018). The analysis results in a diagram showing years on the x-axis and the first Principal Component of the differences in community structure between class I and the other classes on the y-axis. This yields a diagram showing the deviations in time between classes, with class I as reference (for Type2) and class II (for Type1). The species weights are shown in a separate diagram, indicating the species' affinity with this stated difference. The taxa with high positive weight are indicated to show a response similar to the deviations indicated by PRC, those with negative weight, one that is opposite to the response indicated by PRC. Taxa with a near-zero weight show a response very dissimilar to the response indicated by PRC or no response.

The data sets of the physical and chemical variables associated with the water column were also analysed using PRC. In these analyses, the variables were centered and standardized to account for differences in measurement scale (Kersting & Van den Brink, 1997). The variables related to chemical concentrations (TC_{olf}, FC_{olf}, NH₄⁺, Fe²⁺, Mn²⁺, Cl⁻, NO₃⁻, PO₄³⁻, TP, SiO₂) were Ln(ax + 1) transformed, a was determined following (Van den Brink et al., 2000) (see Table 3). This was done to down-weight high abundance values and approximate

normal distribution of the data (for rationale, see van den Brink et al., 1995). The scale used was adjusted to focus on inter-sample distances. Default values were chosen for all remaining options (ter Braak & Šmilauer, 2018).

This is one of the first examples in which monitoring data are analysed by PRC; how PRC can be used to analyse monitoring data is described more in detail in Van den Brink et al. (2008).

Results

From the 633 phytoplankton samples, a total of 250 taxa were identified. From these, 93 taxa occurred less than four times in each reservoir and were excluded from the dataset (see “Methods”). The 157 remaining taxa belonged to 6 divisions. The most important in terms of taxa number were Chlorophyta (41% of the taxa), Bacillariophyta (29%), and Cyanophyta (20%). There were 8 taxa of Crysophyta (5%) and 3 taxa of Pyrrophyta and Euglenophyta (representing each 2% of the total taxa).

The diversity of the phytoplankton communities in the two types of reservoirs is presented in Fig. 2a. These clearly show that the impaired sites of both types always had greater species richness than the less or non-impaired sites. Additionally, the diversity of the phytoplankton communities was determined to be associated only with well-known tolerant taxa, namely Chlorophyta, Cyanophyta, and mesoeutraphentic to hypereutraphentic diatoms (Fig. 2b, c). These figures clearly show that the greater species richness of impaired sites in both types of reservoirs was always associated with greater species richness of tolerant taxa. Over the past 8 years, there was a decline in biodiversity in 1998 and 1999, showing higher species richness (Fig. 2a).

For both reservoir types, the PRC analysis shows a clear spatial gradient related to eutrophication (Figs. 3, 4). In our analysis, for Type1 reservoirs, in respect of the differences in phytoplankton communities between the different ecological classes, sampling date accounted for 29% of the total variation in species composition could be attributed to differences between sampling dates, 12% exclusively to the differences between years, 4% exclusively to differences between season, while the interaction between season and year explained the remaining

Table 3 Environmental variables, respective codes, and transformations are used in the principal response curve (PRC) analysis

Environmental variables	Code	Transformation
Epilimnion		
Total Coliform (<i>N</i> /100 ml)	TotColf	Ln($2x + 1$)
Faecal Coliform (<i>N</i> /100 ml)	FecColf	Ln($2x + 1$)
Chlorophyll a (mg/m^3)	Cpl_a	None
Surface water temperature ($^{\circ}\text{C}$)	Temp	None
Turbidity (NTU)	Turb	None
pH	pH	None
Dissolved Oxygen (mg/l)	DO	None
Conductivity ($\mu\text{S}/\text{cm}$)	Cond	None
Hardness ($\text{mg CaCO}_3/\text{l}$)	Hard	None
Ammonia-N ($\text{mg NH}_4/\text{l}$)	NH ₄	Ln($44.44x + 1$)
Nitrate-N ($\text{mg NO}_3/\text{l}$)	NO ₃	Ln($200x + 1$)
PO ₄ (mg/l)	PO ₄	Ln($333.33x + 1$)
Total Phosphorus ($\text{mg PO}_4/\text{l}$)	TotP	Ln($333.33x + 1$)
Fe ($\mu\text{g}/\text{l}$)	Fe	Ln($0.7x + 1$)
Mn ($\mu\text{g}/\text{l}$)	Mn	Ln($x + 1$)
Cl (mg/l)	Cl	Ln($1.67x + 1$)
Chemical Oxygen Demand ($\text{mg O}_2/\text{l}$)	COD	None
5-Day biochemical oxygen demand ($\text{mg O}_2/\text{l}$)	BOD ₅	None
Total silicon ($\text{mg SiO}_2/\text{L}$)	SiO ₂	Ln($20x + 1$)
Secchi disk depth (m)	SD	None
Hypolimnion		
Chlorophyll a (mg/m^3)	Cpl_a-Hp	None
Water temperature ($^{\circ}\text{C}$)	Temp-Hp	None
Dissolved oxygen ($\text{mg O}_2/\text{l}$)	DO-Hp	None

13%. Differences in species composition between the reservoirs with different ecological status explained 46% ($P \leq 0.001$) of the variation in species composition; 20% ($P \leq 0.001$) of the latter is displayed in the diagram (Fig. 3a). The remaining 25% of the total variance is related to differences between reservoirs with the same ecological status.

The most affected taxa were the diatoms *Melosira distans* (Ehrenberg) Kützing and *Fragilaria capucina* Desmazières, both with negative weights, indicating a reduced abundance compared to that in the reference site (Fig. 3a). In contrast, the taxa with the highest positive weight (i.e., which increased in abundance) were the diatoms *Cyclotella meneghiniana* Kützing and *Melosira ambigua* (Grunow) O.Müller (Fig. 3a). However, other taxa are also shown to have increased in time (e.g., *Diatoma vulgare* Bory, *Nitzschia* sp., *Navicula cryptocephala* Kützing, and *Ulnaria ulna* (Nitzsch) Compère. Thus, the sign of the species scores indicates the direction of the changes in

abundance, while the magnitude of the score reflects the size of the changes.

Also, for Type I reservoirs, the differences between reference and impaired sampling sites measured by physical and chemical parameters are shown in Fig. 3b. 39% of the total variation in environmental variables could be attributed to differences between sampling dates, 17% exclusively to the differences between years, 12% exclusively to differences between seasons, while the interaction between season and year explains the remaining 10%. Differences in environmental variables between the reservoirs with different ecological status explained 41% ($P > 0.05$) of the variation in species composition; 25% ($P > 0.05$) of the latter is displayed in the diagram (Fig. 3b). The remaining 20% of the total variance is due to differences between reservoirs with the same ecological status. The Monte Carlo permutation test indicated no significant differences between reservoirs of the different classes. The major differences

Fig. 2 Comparison of species richness from reference and impaired sites of both reservoir types (a). **b** and **c** For reference and impaired sites, the comparison between the diversity of the phytoplankton communities associated only with well-known tolerant taxa, namely Chlorophyta, Cyanophyta, and mesoeutraphentic to hypereutraphentic diatoms, of Type1 (Run-of-river reservoirs) and Type2 (True reservoirs), respectively

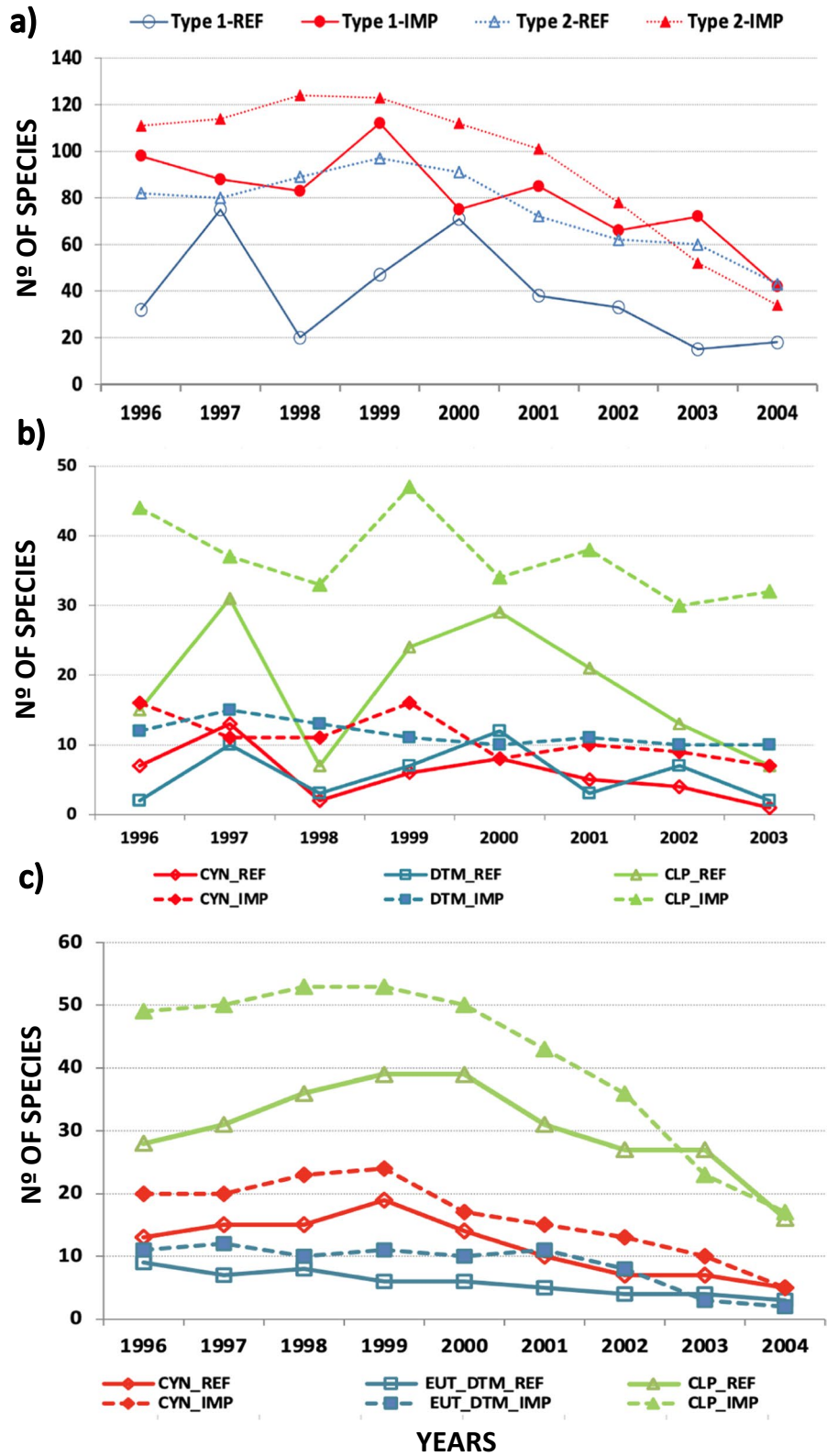


Fig. 3 Diagram showing the first component of the PRC in taxa composition of the phytoplankton (a) and measured physical and chemical parameters (b) between the Type1 (Run-of-river reservoirs) having different ecological status (II through V). The taxa and parameter weights shown in the right part of the diagram represent the affinity of each taxon and parameter, respectively, with the response shown in the diagram. For clarity, only species with a weight larger than 1 or smaller than -1 and parameters with a weight larger than 0.25 or smaller than -0.25 are shown. In sampling date, 1, 2, 3, and 4 represent each year's spring, summer, autumn, and winter

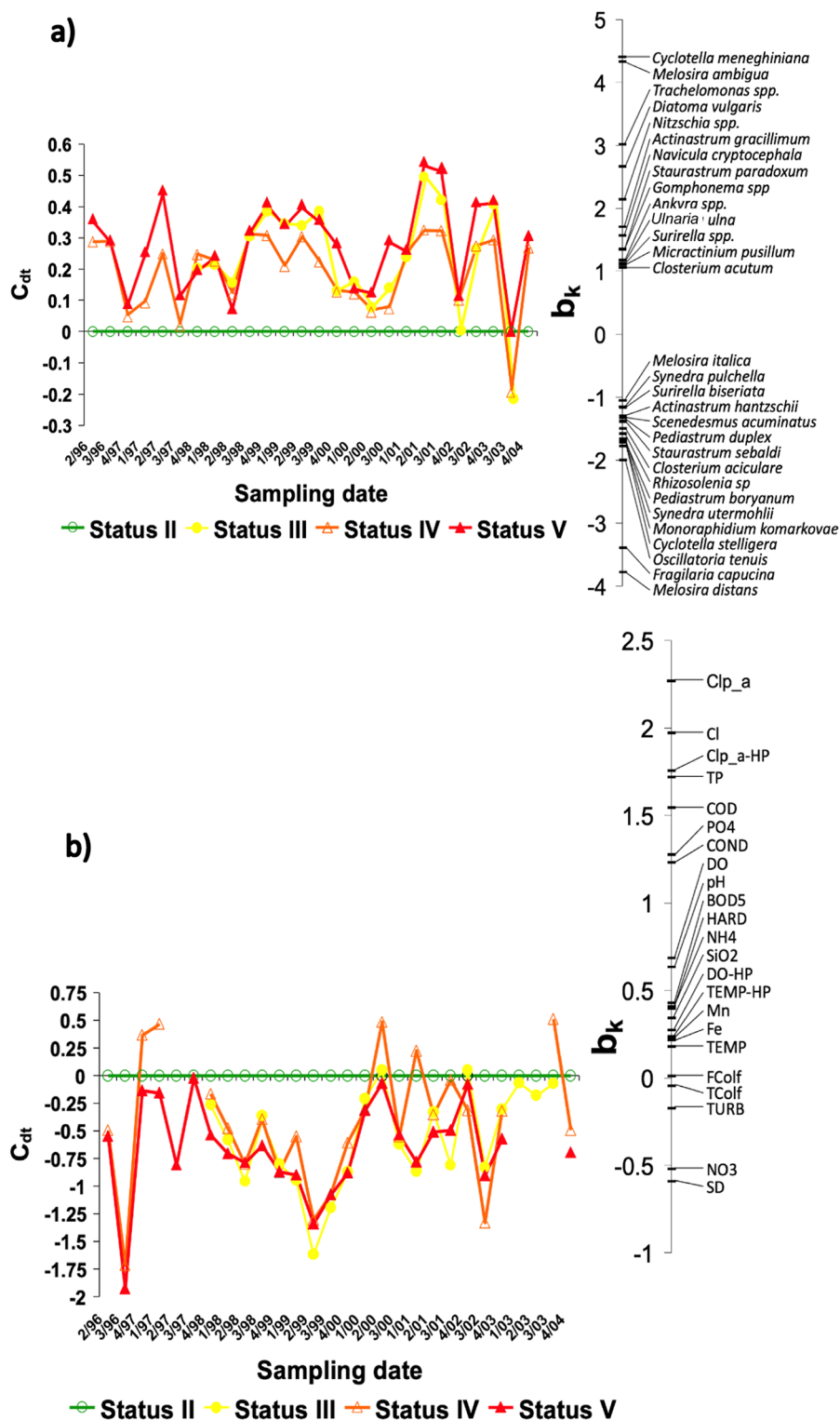
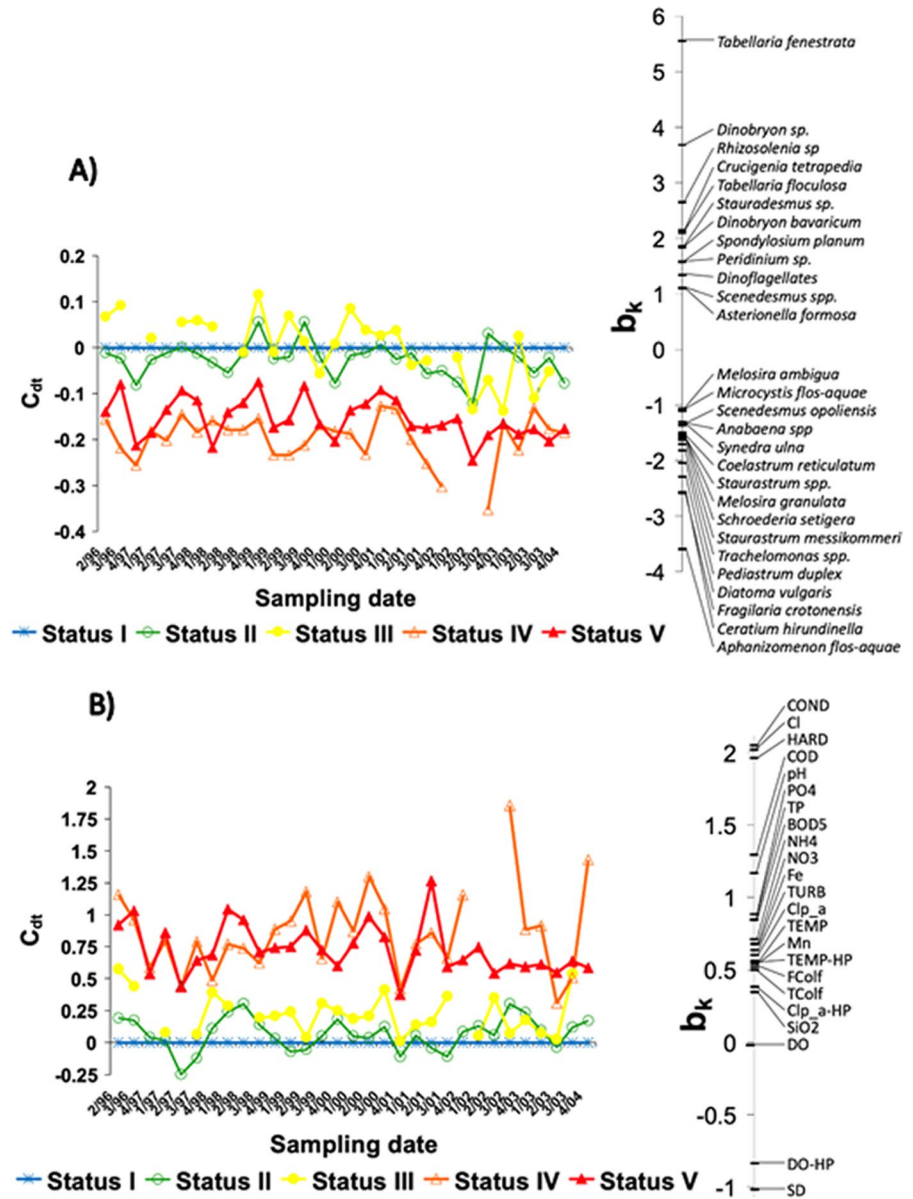


Fig. 4 Diagram showing the first component of the PRC of the differences in taxa composition of the phytoplankton (a) and measured physical and chemical parameters (b) between the Type2 (True reservoirs) having different ecological status (I through V). The taxa and parameter weights shown in the right part of the diagram represent the affinity of each species and parameter, respectively, with the response shown in the diagram. For clarity, only taxa with a weight larger than 1 or smaller than -1 and parameters with a weight larger than 0.25 or smaller than -0.25 are shown. In sampling date, 1, 2, 3, and 4 represent each year's spring, summer, autumn, and winter



between reference and impaired sites were observed in 1998 and 1999. For these impaired sites, compared to the less disturbed ones, lower levels of chlorophyll a, Cl, and phosphorus (TP and PO_4^{3-}) are indicated together with higher levels of NO_3^- and Secchi disk depth. Larger or smaller differences between class I and other ecological classes seem to be associated with higher or lower abundances of diatoms *Melosira ambigua* and *Cyclotella meneghiniana* (Fig. 3a), probably related to higher or lower levels of nutrients, namely NO_3^- .

The PRC diagram showing the differences in taxa composition between the Type2 reservoirs having different ecological status is shown in Fig. 4a. 9% of the total variation in species composition could be attributed to differences between sampling dates, 4% exclusively to the differences between years, 2% exclusively to differences between season, while the interaction between season and year explains the remaining 3%. Differences in species composition between the reservoirs with different ecological status explained

27% ($P \leq 0.001$) of the variation in species composition; 21% ($P \leq 0.001$) of the latter is displayed in the diagram (Fig. 4a). The remaining 64% of the total variance is due to differences between reservoirs with the same ecological status.

The PRC diagram shows fewer differences between phytoplankton communities of reservoirs belonging to status II and III than the reference sites. Contrarily, for reservoirs of status IV and V, more impaired, these differences were more significant. The most affected taxa were *Tabellaria fenestrata* (Lyngbye) Kützing (diatom) and the *Dinobryon* sp. (Crysoophyta), both with positive weights, indicating a reduced abundance compared to that in the reference site (Fig. 4a). In contrast, the taxon with the highest negative weight (i.e., increased in abundance) was the Cyanophyta *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault (Fig. 4a). Other species' abundance seems to have also increased in time (e.g., *Microcystis flos-aquae* (Wittrock) Kirchner and *Anabaena* spp. (Fig. 4a).

The differences between reservoirs with different ecological status and reference sampling sites measured by physico-chemical parameters are shown in Fig. 4b. 18% of the total variation in environmental variables could be attributed to differences between sampling dates, 7% exclusively to the differences between years, 8% exclusively to differences between season, while the interaction between season and year explains the remaining 3%. Differences in environmental variables between the reservoirs with different ecological status explained 31% ($P \leq 0.001$) of the variation in species composition; 52% ($P \leq 0.001$) of the latter is displayed in the diagram (Fig. 4b). The remaining 51% of the total variance is due to differences between reservoirs with the same ecological status. As in PRC based in phytoplankton communities, the PRC based on physical and chemical parameters shows fewer differences between reservoirs of status II and III when compared to the reference sites and more significant differences for reservoirs of status IV and V. For these impaired sites, compared to the reference, lower levels of dissolved oxygen in the hypolimnion and Secchi disk depth are indicated together with higher conductivity, hardness and levels of Cl, nutrients (NO_3^- , TP and PO_4^{3-}) (Fig. 4b).

Discussion

The 34 studied reservoirs were identified and delimited into two types of dammed water bodies, characterized by different hydromorphological features, water chemistry characteristics, and a specific species composition (Cabecinha et al., 2009a). Community structure changes with pollution or stress. In the WFD, high ecological status through biological parameters is defined as a slight or minor deviation from the reference community. A PRC methodology was used to assess the importance of this deviation and measure the degradation of ecological status in the two types of reservoirs over time. The PRC analyses showed significant differences between the reference and impaired sites of each dam Type, reflecting the levels of disturbance over the study period.

In explaining the variance in species composition and environmental variables, time, namely differences in sampling dates, assumed higher importance in Type1 than in Type2 reservoirs. This was expected since Type1 is “riverine reservoirs” that resemble a river more than a lake, with short hydraulic retention times (Anderson et al., 2015; Munasinghe et al., 2021), good mixing, and relatively higher water velocities. Type2 are “artificial lake reservoirs” where water storage and release cycles are long and operate on at least seasonal cycles, but generally on multi-year cycles (Klaver et al., 2007; Hsu et al., 2015).

Most disturbed reservoirs of Type1 had larger watersheds belonging to international basins like Douro and Tagus, dominated by agriculture and having significant urban areas. These reservoirs were positively associated with an anthropogenic pressure gradient (Fig. 3b) and associated with mesoeutrophic to hypereutrophic taxa (Van Dam et al., 1994; Tavassi et al., 2004), namely *Melosira ambigua*, *Cyclotella meneghiniana*, *Diatoma vulgare*, *Navicula cryptocephala* and *Ulnaria ulna* (Fig. 3a). These species are known to be tolerant or need periodic high concentrations of N, contrarily to *Melosira distans* (Van dam et al., 1994; Barinova & Chekryzheva, 2014).

Most impacted reservoirs of Type2 lay in densely populated, industrialized, or agricultural areas, receiving high organic matter inputs and industrial discharge. These findings clearly show that human activity significantly affects the trophic status of aquatic ecosystems. Therefore, these sites were positively correlated with water mineral content

(Cl, hardness, and conductivity), nutrients (P and N), and organic pollution gradients (Fig. 4b). These disturbed sites were dominated by tolerant taxa, namely blue-green algae such as *Aphanizomenon flos-aquae* and *Anabaena* spp. These blue-green algae belonged to a genus whose ability to produce toxins that can affect various organisms, including humans, is known to increase in eutrophic conditions (Vasconcelos, 2001; Visser et al., 2016; Lürling et al., 2018). These taxa appear associated with meso- to hypereutrophic taxa like *Fragilaria crotonensis* Kitton, *Diatoma vulgare*, *Pediastrum duplex* Meyen, *Melosira ambigua*, and *Aulacoseira granulata* (Ehrenberg) Simonsen (Fig. 4a) (Van Dam et al., 1994; Tavassi et al., 2004). Reference sites of Type2 presented in general large forested areas and were mainly dominated by intolerant taxa, *Tabellaria fenestrata*, *Tabellaria flocculosa* (Roth) Kützing, and Crysophytes like *Dinobryon* sp. and *Dinobryon bavaricum* Imhof (Fig. 4a).

When reservoirs become more eutrophic, the diversity of phytoplankton assemblage decreases, ultimately leading to the dominance of Cyanobacteria (see Fig. 4a). The PRC diagram allowed analysing the changes in the community structure over time. In general, the most significant deviations from impaired sites to reference reservoirs of Type2 are associated with seasonality (namely in summer periods) and bloom formations. Bloom formation (as observed in 1997, 2002, and 2003—see Fig. 4a) could result in surface scums, producing unpleasant taste and odour, becoming an unsatisfactory food source for many organisms in the food web, and production of toxins (Reynolds & Petersen, 2000; Lürling et al., 2018).

Rarely will a single factor be responsible for the mass appearance of Cyanobacteria, but a combination of several of them, including hydrodynamic effects, oxygen depletion in the water column, anoxic conditions at the sediment–water interface, elevated temperatures, and low TN/TP-ratios (Dokulil & Teubner, 2000; Vasconcelos, 2001; Islam et al., 2012). This corroborates the results obtained in this study (see Fig. 4). Besides nutrients, the morphology of reservoirs is of decisive importance for cyanobacterial development. The dominance of colony-forming species such as *Microcystis* and *Aphanizomenon* is more commonly in deeper reservoirs (Dokulil & Teubner, 2000; Xiao et al., 2018; Christensen et al., 2019), as Type2 reservoirs that belong to the IV ecological status class.

For HMWBs, the reference conditions on which status classification is based are within the range of “Maximum Ecological Potential,” representing the maximum ecological quality that could be achieved for these systems (Lyche Solheim, 2005; GIG, 2007). Therefore, only sites showing nearly undisturbed physical and chemical, hydromorphological, and biological conditions were chosen as reference sites, as explained in the “Materials and methods” section (see “Environmental parameters and chlorophyll a”).

It was challenging to find many reference sites for Type1 reservoirs, with only 10 sampling sites. Only 2 sites were selected as reference sites. Most “run-of-river” reservoirs in Portugal lie in densely populated regions, representing rather impacted sites. Additionally, all these reservoirs belong to international river watershed, subject to significant anthropogenic pressures due to upstream intensive agriculture practiced in Spain, reflected by the mesoeutrophic taxa that characterized these sites. This might indicate that the Type1 reservoirs investigated here as “best available” do not represent good reference sites. The PRC method could be helpful in this “scenario”, hence enables researchers to contrast the time series of impacted sites or treated sites with the time series of reference sites, but also has the advantage that an external particular starting position can be introduced as a reference, namely less impacted reservoirs or flushed lakes in other European countries.

However, according to Auber et al. (2017), when community/ecosystem dynamics have a gradual change, the PRC is less adapted since the community reference has less sharp differences, although contiguous, community structures that will difficult to determine a baseline and generally the discretization of time.

Many multivariate methods have been used to analyse biological time series of communities, with the ordination method PCA being the most frequently used. (e.g. Li & Kafatos, 2000; Cabecinha et al., 2018). Other methods, like non-metric multidimensional scaling and clustering, have also been proposed but have some disadvantages compared to ordination, as discussed by (Van den Brink & ter Braak, 1998; Van den Brink et al., 2008; Pardal et al., 2004). The PRC analysis results in a diagram where the time vector runs straight from left to right and differences are displayed on the y-axis. This presentation mode is very powerful, especially for non-experts, since this is

the same type of display we would use to disseminate univariate information (Van den Brink et al., 2008; Auber et al., 2017).

Biomonitoring data sets often comprise biological and environmental data, like physical and chemical data, land-use data, etc. Trends and relationships between the biological and environmental data set can be displayed in a triplot, showing samples, species, and environmental variables. When interested in relationships between biology and environmental variables and their changes in time, one can imagine that these triplots could get very complex and only show a part of the variation (Van den Brink et al., 2008). A possibility to obtain a clearer overview is to perform separate PRC analyses on the biological and environmental data sets, as shown in Figs. 3 and 4. In this way, the dynamics of the differences between the sites for biological communities and environmental factors are displayed in separate diagrams. Their relationships over time can be easily inferred.

This ordination technique can summarize complex responses because it is not restricted to a single dimension [as (dis)similarity analysis]. When combined with Monte Carlo permutation testing is a graphical summary of the structure present in the obtained data set and the statistical significance of hypothesized differences (Van den Brink et al., 2003; ter Braak & Šmilauer, 2018).

Pardal et al. (2004) summarize how PRC analysis can be applied to several common environmental scenarios, independently of the number of analysed sites, namely in a standard disturbance gradient to a recovery scenario of the environmental quality after management or mitigation measures that might lead to a better environmental quality or even the establishment of threshold values/levels necessary for qualitative evaluation of ecosystem health. Therefore, PRC analysis could be a powerful tool to reach and implement WFD objectives since it allows to know in time the ecological status of a site and compare the deviations with the reference. Consequently, to assess “Maximum Ecological Potential” for all reservoirs until 2015, as a requisite of WFD, several mitigation measures could be implemented, and the monitoring results easily analysed, interpreted, and compared by PRC. These mitigation measures could reduce the nutrient load from the catchment to the reservoir, altering the hydrodynamic conditions (e.g., artificial

mixing or intermittent turbulence of the water column), or even apply in reservoir eco-technologies.

The PRC diagrams also provide meaningful information on the species contributing to these trends. Combining this with knowledge about the ecological requirements of these species will provide decision-makers with a diagnostic tool for the ecological functioning of their water systems, e.g., as required by the EU Water Framework Directive.

Conclusions

In this study, PRC analysis was used effectively to explore a suitable way of monitoring and assessing the water quality of two types of Mediterranean reservoirs using phytoplankton communities. This method was used to analyse changes in species composition and environmental variables between sites with different ecological status over time, allowing to estimate the degree of impairment at a particular site by contrasting it with a reference site, as proposed by WFD.

For both reservoirs’ types, the PRC analysis showed significant differences between the reference and impaired sites, reflecting the levels of disturbance that they experienced over the study period, mostly associated with a clear spatial gradient related to eutrophication.

This methodology proved to be capable of concisely summarizing the complex data set of the phytoplankton community while permitting information to be displayed with visual clarity and easy to interpret by non-experts, namely decision-makers, politicians, and the general public. This PRC application clearly provides a highly accurate synthesis of community-level and environmental parameters dynamics at numerous sampling sites to easily characterize and quantify the spatio-temporal dynamics of ecosystems.

Moreover, PRC gives higher readability and interpretation of spatial patterns in temporal changes useful for spatial management decisions, reinforcing the relevance of this application for the ecosystem approach.

In conclusion, we believe that PRC will provide a powerful tool for environmental quality assessment in the future and should be incorporated into monitoring and assessment programs along with the existing range of univariate and multivariate tools presently

used. Also, this approach can help policymakers to manage the natural capital to achieve multiple objectives, namely the ecosystem services provided by nature that can contribute to SDG targets.

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Author contributions In the overall context of this paper, the contributions are as follows: E.C. and P.V.B. conceived, designed, and drafted the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability The data that support the findings of this study are available from [third party name] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [third party name].

Declarations

Conflict of Interest The authors have no conflicts of interest to declare. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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