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Diversity, Composition and Environmental Relations of Periphytic Rotifer Assemblages in Lentic Freshwater Bodies (Flanders, Lower Belgium)

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Abstract: Periphytic rotifer assemblages from lentic habitats are understudied. To improve knowledge on the principal environmental determinants of their structure and composition, we examined summer periphyton from 184 freshwater bodies from a taxonomic and multi-trait-based perspective. Only the latter allowed consideration of all bdelloids. Alpha diversity decreased with electrolyte and aluminium concentration but increased with macrophyte richness, pointing at salinization, metal toxicity and loss of structural niche heterogeneity as potential threats for rotifer diversity. Replacement was the prominent component of beta diversity, with acidified sites showing the highest local contributions. Variation partitioning indicated that local conditions explained variation in species composition best, but general setting (soil type, land cover, connectivity) and spatial context were also not insignificant. Redundancy analysis related species composition more particularly to gradients of pH and trophic status, whereas the representation of functional groups was structured mainly by phytoplankton productivity. Mirroring shifts observed in the plankton, high phytoplankton productivity associated with larger size and more detritibacterivory. Dominance of collectors constrained variation in guild ratios, underlining the need for more refined functional approaches. To aid the use of periphytic rotifers in regional water quality assessment, we identified indicators and community thresholds for pH and trophic variables and determined optima and tolerances for individual taxa.

Keywords: meiofauna; macrophyte; trait; functional group; community-weighted mean; environmental threshold; indicator; eutrophication; salinisation; acidification

Citation: Denys, L.; De Smet, W.H. Diversity, Composition and Environmental Relations of Periphytic Rotifer Assemblages in Lentic Freshwater Bodies (Flanders, Lower Belgium). *Diversity* **2023**, *15*, 1214. https://doi.org/10.3390/d15121214

Academic Editors: Alan Christian, Evangelia Michaloudi, Sarma Nandini and S.S.S. Sarma

Received: 16 November 2023
Revised: 6 December 2023
Accepted: 10 December 2023
Published: 12 December 2023

1. Introduction

Rotifers are an important link in the functioning and food web of lentic freshwater bodies and wetlands, channelling energy and nutrients from detrital, heterotrophic and autotrophic food sources from the microbial scale to higher levels [1–4]. In the plankton, increased food availability in more nutrient-rich conditions affects rotifer abundance and size distribution as food concentration thresholds and reproductive potential increase with body size [5]. Augmenting concentrations of suspended detrital and bacterial matter with eutrophication induce replacement of a macrophagous feeding guild by a microphagous feeding guild, while concurrently, microphagous rotifers can profit from lessening of competitive exclusion and interference competition due to reduction of filter-feeding Cladocera in turbid water [6–11]. Furthermore, changing species interactions influence reliance on defensive morphologies or specialised avoidance techniques and affect life-history strategies [12–14]. Changed oxygen conditions [15] and the loss of shelter provided by submerged macrophytes [16] due to eutrophication also have their consequences. All together, these mechanisms result in notable differences between the species composition, assemblage structure and functional characteristics of planktonic rotifer communities of clear phytoplankton-poor freshwaters and turbid phytoplankton-dominated lakes and ponds.

Periphytic biofilms are an important and complex part of aquatic ecosystems [17,18] and their structure, composition, micro-environment and associated trophic relations are not independent of nutrient conditions [19–22]. Rotifers represent a major part of the non-algal periphyton biomass [23,24] and their diversity is generally higher in the littoral than in the plankton, especially when associated with firm substrates [23,25]. This is usually attributed to the nature of the periphytic microhabitat, e.g., allowing for adhesion and sessile growth, its more complex architecture and higher habitat heterogeneity, although a planktonic lifestyle also requires specific adaptations (e.g., [26–29]). Similar to planktonic rotifers, species living in the periphyton present morphological, dietary and behavioural adaptations, with feeding strategies that vary from indifferently collecting appropriately sized particles to selective active hunting. With a score of potential predators, including protozoa and metazoan invertebrates, as well as micropredatory fish and a variety of prey organisms, interspecific relations shaping periphytic rotifer communities are also likely to change within the biofilm according to ambient conditions. Yet, the assemblage composition of periphyton-inhabiting rotifers in surface waters remains underexplored and it is unclear whether changes in periphytic communities due to eutrophication mirror those occurring in the plankton, for instance in the prevalence of feeding guilds, or differ widely. Although some studies suggest that feeding guilds also reflect food availability in psammon communities [30,31], this is less straightforward for periphyton growths where predator–prey interactions may be alleviated by the relative shelter provided by plant architecture and microstructures. Additionally, food abundance and quality may be less constraining in more organically rich biofilms, allowing development of communities that are less dominance-oriented. Additionally, the pressure of macro-invertebrates grazing on periphyton may be less selective, although this also will be modulated by structural habitat complexity [32]. Furthermore, whilst physical-chemical analyses of the water column and trophic indicators such as algal pigments provide proximal insight into the ambient environment of zooplankton in lakes and ponds, this may not be the case for periphyton. For instance, Oh et al. [33] suggest that benthic rotifers, being independent of pelagic food sources, are not likely to be affected by water quality. Conversely, periphytic and planktonic assemblages mutually influence each other and, with free-living species easily becoming detached from their substrate and reproducing liberally in the water column and with many periphytic rotifers having a planktonic larval stage, at least partly shared environmental determinants may be expected.

Duggan [23] noted that, compared to the plankton, environmental factors shaping periphytic rotifer communities have drawn less attention and this has not changed very much in the last two decades. The focus of more comprehensive studies very much remained on (semi-)plankton (e.g., [34–36]) and periphyton-specific assessments were limited in extent. In spite of long-standing consideration in biological water quality assessment, particularly from plankton (e.g., [37–43]), the diversity and composition of periphytic rotifer assemblages in freshwaters along broader environmental gradients across wider regions still require further documentation, hampering their eventual application in water quality research. Notably, May and Wallace [44] could only report plankton studies in a recent review of long-term rotifer records in lentic systems. Apparent autecological characteristics, such as optima, depend on gradient lengths, as well as (bio)geographical characteristics, and are biased by region or selection of particular types of water bodies. For instance, distribution data from northern or high-altitude regions may not necessarily reflect the realised niche of taxa in the climatologically, geologically and generally more impacted conditions of the West European lowland. Broad-ranging regional analyses are therefore required to assess the potential for environmental inferences from periphytic rotifers.

Taxonomic difficulties and the cumbersome analysis of samples rich in algae and detrital matter are obvious reasons why there are few more extensive studies on periphytic rotifers. Moreover, the common use of net-haul or tube samples collecting a rather erratic mixture of planktonic and littoral communities (e.g., [41,45–48]), or collective treatment of different sample types [49–51], possibly confound habitat-specific relations, whilst biodi-

versity comparisons from water-column samples of waterbodies with strongly different proportions of periphytic and pelagic habitat [52] will invariably be biased. Additionally, many planktonic taxa also occur in the periphyton and the community structure of both habitats is reciprocally influenced [28,53], complicating the picture. Finally, it should be noted that smaller-scaled environmental patterns may well be underrated in studies based on incidence data [54,55] or much-simplified abundance estimates [50]. In particular for taxa with high taxonomic turnover during the season, consideration of (relative) abundance should be preferred [56,57]. Equally, functional relations are likely to become more evident when accounting for abundance [58–60].

In order to explore the principal environmental drivers of periphytic rotifer assemblage structure and composition, we analysed their diversity and species composition from a broad range of lentic water bodies across lower Belgium, a region with considerable natural geographic variation in freshwater conditions, as well as diverse land cover. We first focus on some general aspects of alpha and beta diversity, identifying their principal correlates in the study region, and assess the contribution of individual sites, including its replacement and abundance components, to total beta diversity. Then we examine the relative importance of local site characteristics, surrounding land cover, connectivity and spatial configuration of water bodies at the level of taxonomic species composition. Next, the relation of assemblage composition and multi-trait functional-group representation to environmental conditions is examined in more detail. Combination of a taxonomic with a trait-based approach may provide a more complete understanding of ecological relations and ecosystem status, but multiple traits have been rarely considered for littoral meiofauna from lentic waterbodies, especially rotifers, so far [61–63]. Hence, their complementarity is largely left unexplored. Finally, we consider the response of individual taxa and functional groups to gradients of relevant water quality variables, including proxies for trophic status and phytoplankton abundance, to identify potential indicator taxa and thresholds for major assemblage change.

2. Materials and Methods

2.1. Regional and Sample Site Characteristics

Biogeographical characteristics of lower Belgium, Flanders (51°00′ N 4°15′ E, 13.522 km^2, mainly below 200 m.a.s.l.; Figure 1), are summarised by Verbruggen et al. [64] and Franklin et al. [65]. Surface geology largely consists of Quaternary marine, fluvial and niveo-eolian deposits with well-developed clayey to sandy and, only locally, peaty topsoil. With an average yearly temperature of 9.8 °C and 780 mm.yr^{-1} of precipitation, the climate is mild and of a temperate maritime type (Cfb Köppen–Geiger type). The area is densely populated (current average ≈ 492 persons per km^2), and land use is generally intensive with c. 50% agricultural land and a third comprising built and infrastructural area. Consequently, anthropogenic pressures on surface waters (e.g., eutrophication, fish stocking, acidification, physical disturbance and alteration) are pervasive and often severe [66]. Standing waters are predominantly small [67], shallow and man-made with an agricultural, recreational or industrial origin; stratified water bodies mainly resulted from quarrying for sand, gravel and clay. Poorly buffered, more or less acid, naturally more nutrient-poor waters are largely restricted to the north-eastern part of Flanders, where leached cover sands occur, in the so-called Kempen region. With few exceptions, surface waters elsewhere are less dilute and usually also more nutrient-rich, with some brackish influences in the coastal polders and along the Dutch border to the west of the lower Scheldt River. Stony substrates are scarce or absent in the littoral areas of most lentic waters, but pond banks may be artificially reinforced for amenity reasons. Submerged or floating-leaved aquatic plants, submerged parts of helophytes and woody debris are the more usual natural substrates for periphytic communities, but occurrence of submerged macrophytes is frequently reduced due to turbidity or shading.

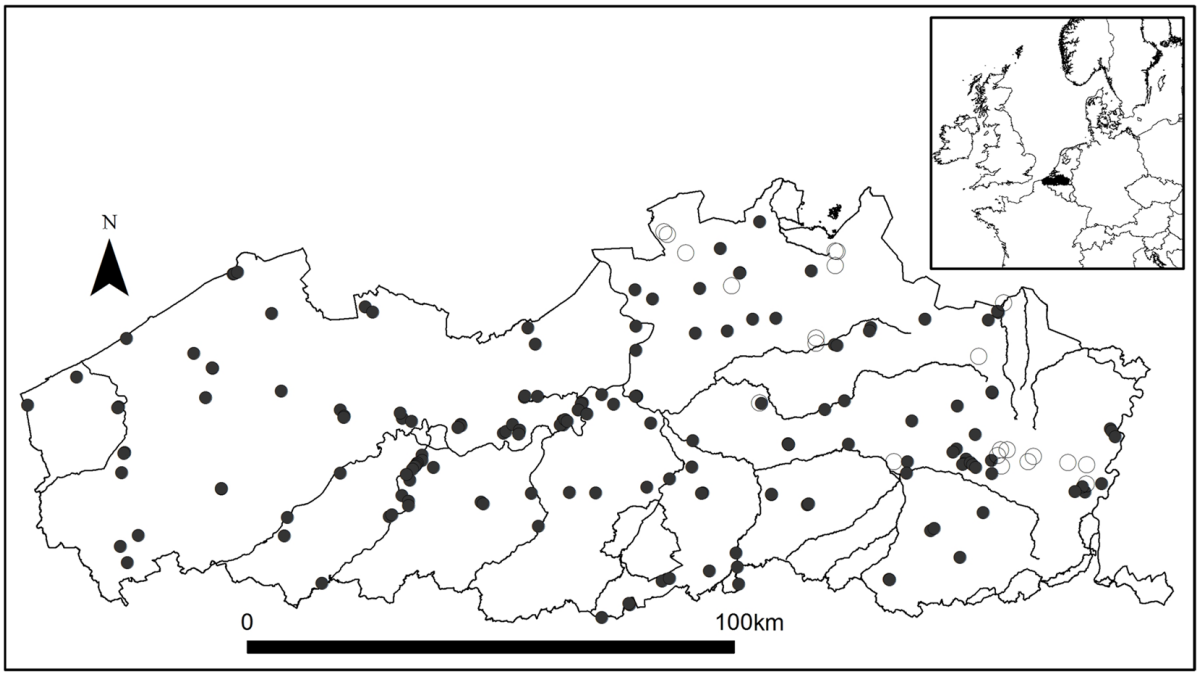

Figure 1. Study region with main rivers and distribution of sample sites (sites with median pH < 6.5 indicated by empty dots) and its location in Western Europe (insert).

Overall, 186 permanent water bodies throughout the region were sampled (Figure 1). These were selected to cover the range of freshwater conditions for as much as possible, as assessed previously from a comprehensive field survey [68]. Seemingly less-impaired sites were given special attention in site selection to improve the environmental scope as much as possible. Most sampled water bodies were shallow, small (max. 73 ha), ±alkaline and (hyper)eutrophic, but stratified pits and acid bog waters were also included (Table 1). Acid water bodies were restricted to the north-western part of the region. Surrounding land cover was highly varied, including mainly woodlands, agricultural land and semi-natural heaths. Emergent and submerged vegetation was often poorly developed. Summary statistics and linear correlations for all variables are provided in Tables S1 and S2.

Table 1. Summary characteristics of the sampled waterbodies. EC: electrical conductivity, TP: total phosphorus, TN: total nitrogen, chl *a*: chlorophyll *a*.

Variable	Unit	Minimum	Maximum	Median	Average	SD
surface	m^2	211	739,743	13,331	46,032	95,788
maximum depth	m	c. 0.5	c. 18	-	-	-
heath	%	0	90	0	4	15
deciduous	%	0	100	21	28	27
coniferous	%	0	94	0	5	15
poplar	%	0	94	0	13	21
field	%	0	55	0	8	14
pasture	%	0	97	12	20	24
emergent cover	%	0	100	1	10	21
submerged cover	%	0	100	1	21	35
pH	-	3.4	9.3	7.7	7.4	1.1
EC	$\mu S.cm^{-1}$	24	3520	460	520	393
TP	$mg.L^{-1}$	<0.07	2.89	0.13	0.29	0.45
TN	$mg.L^{-1}$	0.37	10.52	1.83	2.36	1.79
chl *a*	$\mu g.L^{-1}$	<1	310	21	42	56

2.2. Rotifer Sampling and Analysis

For rotifer analysis, a single sample was collected from May till June in either 1998 or 1999, consisting of a balanced mixture of the available permanently submerged plant substrates within a littoral stretch of c. 10 m. These included submerged and floating-leaved hydrophytes and helophytes (mostly commonly reed, *Phragmites australis* (Cav.) Trin ex Steud.). Where these were lacking, parts of riparian vegetation drooping into the water or fallen twigs were sampled; bryophytes were excluded from sampling. With a common natural substrate lacking, use of a composite sample moderated eventual substrate specificity. Samples were taken by cutting the plants with scissors at a depth of approximately 20–30 cm below the surface. Immediately after removing the cuttings from the water, the lower 5 to 7 cm was cut off and gathered in a polyethylene container. Immediately after collection, a little dilute formaldehyde was added for preservation.

All periphyton was removed by repeated rinsing with a strong jet of filtered water and rotifers were concentrated by sieving (40 µm mesh). Identification of monogononts was carried out mainly referring to Koste [69], Segers [70], Nogrady et al. [71], De Smet [72], De Smet and Pourriot [73] and Nogrady [74] by means of light microscopy (LM) and, if required, scanning electron microscopy of the trophi [75]. Identification of bdelloids followed Donner [76]. As many individuals as possible were counted, with a minimum of 151 and an average of 300 ± 53 (1 SD) animals per sample. The number of unidentifiable contracted bdelloids was tallied alongside as a separate OTU (on average these accounted for 650 ± 596 individuals per sample). If any additional taxa were encountered afterwards, these were added as 0.5 individuals to the counted total, or as unity for analyses requiring so. Two samples with an insufficient number of individuals were excluded from further analysis, leaving a total number of 184 samples and sites.

2.3. Water Chemistry

Water samples for laboratory analyses were taken from May until November in the same year as the rotifer samples and close to where these were collected, but not within dense vegetation. Samples were preferably from near the outflow, if present, or from a well-mixed site, as far from the bank as could be reached with waders or from a jetty. A horizontal 2 L Van Dorn sampler was filled at a depth of 0.5 m, or at half the water column where maximum depth was less than 1 m.

Samples were analysed in the laboratory no later than the next day. Major ions, inorganic nitrogen and phosphorus compounds, total phosphorus (TP) and Kjeldahl-nitrogen (KjN) were mostly analysed 3 times (±bimonthly). Chemical oxygen demand (potassium dichromate method; COD), COD after filtering over 0.45 µm (COD_f; a proxy for dissolved organic matter, DOM) and their difference, COD_p, accounting for particular organic matter (POM), biochemical oxygen demand (BOD), chlorophyll *a* (chl *a*), phaeopigments, potential gross oxygen production (pGOP; a proxy for phytoplankton productivity in the water column with ambient water quality), as well as absorbance at 254 nm (A254; aromatic organic compounds) and 440 nm (A440; humic substances, Gelbstoff, gilvin) were usually measured 4 to 5 times. Cations were analysed by ICP-AES (Thermo Jarrel Ash), anions and total phosphorus by segmented flow analysis (SAN^{plus}, Skalar, Breda, The Netherlands). pGOP and BOD were assessed by ex situ light/dark bottle incubation for 24 h at 20 °C and pigments via spectrophotometry according to Golterman et al. [77]. Total nitrogen (TN), organic nitrogen (TON) and total inorganic nitrogen (TIN) were calculated from components. Oxygen concentration and saturation, pH and specific conductivity (EC) were measured 5–6 times (±monthly) in the field using portable equipment (WTW Multiline P4 with CellOx 325, TetraCon 325 and SenTix 97/T, Xylem, Zaventem, Belgium). Temperature was measured as a control variable but not considered in analyses to avoid spurious results. Further details on the methodology and derived variables are given by Denys [78–80].

Because of the limited number of analyses, median concentrations with levels below analysis limits set at 50% of the measurement threshold were used as water-column variables; maxima were also considered for N and P compounds and chl *a*.

2.4. Other Variables

Maximum within-waterbody length and width, as well as shoreline length and surface area, were determined from air-survey orthophotos, and shoreline density (Dsl) was calculated as the ratio of the latter [81]. Maximum depth (4 classes: 0–1.5 m, 1.5–3 m, 3–6 m, >6 m) and bank slope (5 classes: <1/20, 1/20–≤1/5, 1.5–≤1/3, 1/3–≤1/1.5, >1/1.5) were noted as ordinal estimates in the field [78]. Major soil-texture (sand, loamy sand, loam, clay, peat) and land-cover types (grassland, field, fallow land, deciduous wood, poplar stand, coniferous wood, lentic water, lotic water, marsh, heath, coastal dune, built, infrastructure) were assessed by GIS as their proportion within a buffer of 50 m enclosing the water body from soil maps and the most recent 'biological valuation maps'—a detailed parcel-scale vegetation/land-cover map [82,83]—available at the time of sampling. Declerck et al. [84] concluded that land-use effects on smaller ponds in the region mainly operated at relatively small spatial scales, supporting the choice of a narrow buffer. The number of water bodies within 500 m (ponds) was determined from maps and orthophotos. Together with field observations, these sources also served to assess the number of in- and outflows (connections) for each site. Percentage of shoreline covered by woody vegetation (wooded shoreline) was estimated in the field, as were the percentage cover of submerged and emergent vegetation and the total number of macrophyte taxa (helophytes, floating and submerged macrophytes) within the water body.

Sites were further characterised by their latitude and longitude according to the Lambert projection (X and Y) and eigenvectors from principal coordinate analyses of Euclidian distances (dbMEMs) [85,86], which allows representation of non-linear spatial structures at different spatial scales by a set of orthogonal variables. dbMEMs were generated using dbmem in adespatial 0.3–16 [87] and their scale was determined as the range from semi-variograms fitted to a Gaussian or spherical model with the autofitVariogram function of automap 1.0–16 [88].

2.5. Trait Data, Functional Groups and Guild Ratio

To underpin functional groups, fifteen morphological, physiological and behavioural traits [89] were considered (Table 2). Grouping of traits within these categories was not always clear-cut. Habitat specificity may be considered an overarching trait [62], which we classified here as behavioural. Likewise, size is also a key trait relating to a range of organismal properties, including behavioural and physiological features. Although actually a life-history trait, obligate parthenogenesis, separating bdelloids from monogononts, was included as 'physiological' because it relates with rapid population response to environmental change and high colonisation potential; obviously, it could also be attributed to 'morphology' or 'behaviour'. Possession of toes was classified as behavioural, not morphological, because it links to temporary attachment capability and locomotion mode. Toe length, however, was considered its morphological counterpart. Note that for clarity we avoided to use semi-planktonic as a modality because it is not clearly defined and could apply to all non-sessile rotifers living on plant substrates. Literature data (i.e., the above-mentioned identification sources, [56,90–93]), internet databases [94] and personal observations were used to attribute trait modalities to taxa (Table S3). Parasites were classified as macrophagous, and unidentified bdelloids as periphytic, free living, solitary, collectors, microphagous, detritibacterivorous, ramate, obligate parthenogenetic and without defensive/protective structures, with size and toe features lacking. Modalities that were not mutually exclusive within a trait (life style, diet, food size) received equal weights when shared due to lack of quantitative data to underpin fuzziness. Note that microphagous (referring to food-item size) was not identical to collector (a mode of food acquisition), and the two were coded differently. Total length was $\log_{10}$ and toe length $\log_{10}(x + 1)$ transformed to reduce skewness.

Table 2. Traits with their different modalities, coding and trait-group attribution.

Trait	Coding	Modalities	Trait Group
organisation	fuzzy	colonial, solitary	morphological
food-particle size	fuzzy	microphagous (mostly <5 µm), macrophagous (mostly >5 µm)	morphological
trophi type	categorical	malleate, virgate, forcipate, cardate, incudate, malleoramate, uncinate, submalleate, malleovirgate, ramate	morphological
armoring	categorical	loricate, illoricate	morphological
spines	categorical	spined, unspined	morphological
mucus-secretion	categorical	with mucus, without mucus	morphological
tube formation	categorical	tube-dwelling, exposed	morphological
length	continuous	range midpoint	morphological
toe length	continuous	range midpoint	morphological
food acquisition	categorical	raptor, collector (single vs. multiple food items) [95,96]	behavioural
habitat (primary)	categorical	planktonic, periphytic	behavioural
substrate relation	categorical	adults sessile, adults free-living	behavioural
adhesion	categorical	toed, toeless	behavioural
diet	fuzzy	parasitic, predatory, cyanobacterivorous, algivorous, detritibacterivorous	physiological
obligate parthenogenetic	categorical	obligate parthenogenetic, heterogonic	physiological

Multivariate trait dissimilarity between assemblages was calculated as their Gower distance [97,98]. The contribution of individual traits was equalised by an iterative process accounting for fuzzy coding and the grouping of traits for trophi type and food acquisition [99] by means of the gawdis function in gawdis 0.1.3 [100] with 600 iterations. Due to redundancy with toe length, toe presence was not retained in the calculation of the distance matrix.

Functional groups (numbered FG1 to FGx) were identified by partitioning around medoids clustering (PAM) [101] of the taxa trait matrix using the Gower distance and optimising the number of groups with the silhouette method [102] as implemented with pam in cluster 2.1.2 [103]. Subsequently, the relative abundance of functional groups was calculated.

Community-weighted means (CWMs) were calculated for all traits and samples using untransformed counts, thus highlighting the functional importance of more dominant taxa [104]. Previously, the CWM approach was shown to be useful for analysing meiofaunal food-web relations in biofilms [105]. Two guild ratios (GRs) were determined: GRrc as the ratio of raptor and collector CWMs, i.e., rotifers preying on single particles vs. indifferently gathering multiple items [95,96], and GRmm as the ratio of macro- and microphagous CWMs; thus, GRs were positive numbers and did not account for body mass or size. With respect to Smith et al.'s GR [95], (raptor-microphagous)/total, the difference between GRrc, referring to ingestion mode ('how'), and GRmm, representing food-item size ('what'), is important to note. The terms of GRrc relate to trophi types, but those of GRmm require observations of behaviour or gut contents. Therefore, if Smith et al.'s GR [95] is inferred from the trophi it will be more comparable to GRrc.

2.6. Ordination and Variation Partitioning

For ordination and variation partitioning, all water-column variables, except pH and oxygen saturation, were $\log_{10}$-transformed to decrease outlier influence, adding a constant where necessary. Percentage variables (land cover, soil) were arcsine-square-transformed, shoreline length, surface area and Dsl $\log_{10}$-transformed, and the number of nearby water bodies (ponds) and hydrological connections were entered as their square root.

Variation partitioning by means of partial constrained redundancy analyses was applied to analyse the contribution of sets of variables acting at similar scales on species distribution [106,107]. Variation partitioning was based on Hellinger-transformed species data without unidentified bdelloids. Collinear environmental variables were ignored. After testing and removing the linear trend from the community spatial variables relation, a forward-selection procedure under a reduced model with 9999 Monte Carlo permutations was used for final variable selection [108]. Only variables with variance inflation factors <5 and a significant effect at $p < 0.05$ were considered but no multiple-testing correction was applied, maximising the variance explained by each variable group. Three variable groups were considered: local site variables, general setting features and spatial structure. Local variables included 24 remaining water-column variables, as well as the morphological and vegetation features of the water body itself, including the percentage of shoreline with woody vegetation. The setting included soil and vegetation/land-use variables for the peripheral 50 m buffer and both variables associated with connectivity. Spatial variables were X, Y and 45 dbMEMs. Adjusted R^2 values were permutation-based [109] (1000 permutations) and the resulting spatial component may be overoptimistic [110] with inflated type 1 error [111].

The relation of taxa identified to species level and of functional groups to environmental and sample characteristics was examined by redundancy analysis (RDA) with forward variable selection and 999 Monte Carlo permutations on Hellinger-transformed data. Partial RDA was used to assess the importance of individual variables retained with $p \leq 0.05$ after Holm adjustment. CWMs were related to the species–environment RDA as passive variables.

The forward.sel, varpart and rda functions from vegan 2.6–2 [112] were used for variation partitioning. Canoco 4.5 [113] and Canodraw 4.14 [114] were used for other ordinations and ordination plots, respectively.

2.7. Ordination and Variation Partitioning

Species pool, observed (S_obs), rarefied (S_rar) and estimated abundance-based Chao 1 species richness with bias-correction (S_chao1), Shannon entropy (H', base *e*), true Shannon diversity (exp(H'), H'_true) and Simpson diversity (D1 = 1-D) were derived from untransformed data, discounting unidentified bdelloids, with specpool, estimateR, rarefy and diversity in vegan 2.6–2 [112]. Sample completeness was calculated from S_chao1 and S_obs. The function specaccum (random addition, 100 permutations) from vegan yielded a species accumulation curve and the species pool was estimated with specpool (chao with small sample correction). Hill's N2, the effective number of occurrences, for taxa was determined from their percentage abundances with divparam from adiv 2.2 [115]. With the beta.div function in adespatial 0.3–16, the total variance of the assemblage dissimilarity matrix derived from $\log_{10}(x + 1)$ transformed count data, using the Ružička distance and square-rooted dissimilarities, was calculated as an estimate of beta diversity (BDtotal) [116,117]. The same function was used to obtain the relative contribution of individual samples, or local contribution, to beta diversity (LCBD), which represents a measure of assemblage uniqueness. Considering that rotifers will disperse easily within the study area and assuming that quantitative differences in dissimilarities would be less affected by spurious species observations, only abundances were considered for this analysis [57]. Samples with a more than average contribution to beta diversity were identified by a permutation test with 999 permutations at $p \leq 0.05$. BDtotal and LCBD values were calculated for monogononts and identified bdelloids only. Using beta.div.comp, also in adespatial, BDtotal was partitioned into a replacement (Repl) and a richness (RichDiff) or abundance difference component (AbDiff) according to Podani and Schmera [118] and Podani et al. [119] using the Jaccard distance for incidence and the Ružička distance on the $\log_{10}(x + 1)$ abundance data. Following Schmera et al. [120], this type of decomposition was preferred to the framework proposed by Baselga [121]. With LCBD.comp in the same package, LCBD values were further partitioned into their abundance (LCBDabun) and

replication difference (LCBDrepl) components, estimating the contribution of individual samples to spatial abundance and replacement gradients [57,122].

Using the frequency of taxa occurrences, Puchalski's originality index IFO = ($\Sigma_{i=1}{}^{s}$ $1/M_i$)/S_obs (where M_i is the number of samples with the taxon i; Puchalski cited in [123]) was calculated for each sample. IFO equals 1 if all taxa are unique to the sample.

2.8. Regression Analyses

Besides graphical and correlative data inspection, regression analysis was used to identify (subsets of) local and setting variables that statistically best explain the observed metric values. Dealing with overdispersion, we used negative binomial regression (NB2) with glm.nb in MASS 7.3–53 [124] to model species richness. D1, LCBD and proportions of functional groups, all ranging between zero and one, were analysed with beta regression [125] in betareg 3.1-4 [126] and the remaining metrics with generalised linear models (GLMs) in stats 4.2.1 [127]. Functional-group proportions were transformed according to Smithson and Verkuilen [128] to accommodate zeros. GLM and NB2 modelling was guided by Akaike's Information Criterium and/or deviance reduction using stepwise backward selection with stepAIC, dropterm and addterm from MASS. Likelihood ratio tests with lrtest from the lmtest 0.9–40 package [129] were used to compare models. Pseudo-R^2-values were calculated with PseudoR2 from DescTools 0.99.47 [130].

2.9. Assemblage Composition along Selected Gradients

We used three approaches to document the distribution of taxa along principal water quality gradients suggested by variable selection for RDA. Firstly, Multi-level Pattern Analysis (MPA) [131], an extension of Indicator Species Analysis (Indval) [132], was used to identify taxa that preferentially occur in sample groups with, respectively, the 'lowest', 'low', 'high' and 'highest' values for a given variable, as delimited by its distribution quartiles (each comprising 46 observations). This analysis considers a species as a reliable indicator for a group or combination of groups based on its specificity, the product of the probability that a site belongs to this (combination of) group(s) if it occurs there, and its fidelity, the probability of finding the species at sites belonging to this (these) group(s). We used the multipatt function of indicspecies 1.7.11 [133] with 9999 permutations and Holm-adjusted p values ≤ 0.05 on $\log_{10}(x + 1)$-transformed numbers. Secondly, weighted-average optima and tolerances (standard deviations) according to Birks et al. [134] were calculated using percentage abundances for taxa with at least 3 occurrences with optimos.prime 0.1.2 [135]. Finally, Threshold Indicator Species Analysis (TITAN) [136] was used to identify taxa that contribute consistently to community change along selected environmental gradients by a decrease or increase in their abundance, as well as the areas of their major cumulative change. TITAN2 2.4.1 [137] was used with default settings but 500 permutations of the taxa data and 1000 resampling bootstraps. Reported change points are those based only on taxa filtered for purity and reliability of their response.

Differences in median values of functional-group representation between the quartile groups of samples were tested with the Kruskal–Wallis (K-W) test in stats 4.2.1 [124]. If $p \leq 0.05$ for the global test, between-group differences were explored further with the Conover–Iman all-pairs rank comparison test in PMCMRplus 1.9.6 [138].

3. Results

3.1. Species Composition

Overall, 217 taxa from 56 genera were identified at species level (Table S3): 214 monogononts and 3 bdelloids. The species accumulation curve remained unsaturated (Figure S1) and the Chao 1 estimator suggested that the species pool comprised at least 274 $\pm$ 21 taxa. *Cephalodella* (26 taxa) and *Lecane* (32 taxa) accounted for most species. Bdelloids were present in nearly every sample and often very numerous, but only three could be recognised at species level (*Dissotrocha aculeata*, *D. macrostyla* and *Rotaria neptunia*). There were 53 singletons (24.5%) and 24 species occurred only twice. Matrix fill of the taxa by site matrix

was low (8.6% without unidentified bdelloids, 9% if included). Three genera (*Cupelopagis*, *Hexarthra* and *Scaridium*) were limited to one site. Unidentified Bdelloidea were by far best represented, both by frequency and number (Table 3). *Lecane closterocerca* was next in line, being present in almost 84% of the samples and with the second highest average relative abundance (10%). *Lepadella patella*, *Colurella adriatica*, *Testudinella patina*, *Cephalodella gibba* and *C. auriculata* also occurred in more than half of the samples but their average percentage abundance remained below 5%. Three frequently occurring taxa (*Cephalodella* sp., *Collotheca* sp., *Ptygura* sp. 2) could not be identified reliably at species level.

Sixty-nine of the identified species were new to the Belgian fauna (Table S3).

Table 3. Taxa with a frequency of at least 10% (present in 19 samples) with their acronym used in figures, percentage frequency, total number of individuals counted, average relative abundance and its standard deviation.

Taxon	Acronym	Frequency (%)	Individuals	Average %	SD
Bdelloidea indeterminata	BDELinde	99.5	64,313	37.2	25.2
Brachionus quadridentatus	BRACQUAD	30.4	2553.5	3.0	11.3
Brachionus urceolaris	BRACURCE	13.0	357	0.5	4.1
Cephalodella auriculata	CEPHAURI	54.3	2070.5	2.4	8.7
Cephalodella forficula	CEPHFORF	16.3	189	0.2	0.9
Cephalodella gibba	CEPHGIBB	54.9	1214	0.9	2.1
Cephalodella gracilis	CEPHGRAC	23.4	389.5	0.4	1.5
Cephalodella hoodii	CEPHHOOD	27.7	245	0.2	0.8
Cephalodella intuta	CEPHINTU	21.7	298.5	0.3	1.2
Cephalodella megalocephala	CEPHMEGA	20.7	130	0.2	0.5
Cephalodella segersi	CEPHSEGE	32.6	1416.5	1.7	6.4
Cephalodella sp. 1	CEPHsp1	21.7	563.5	0.6	3.0
Cephalodella sterea	CEPHSTER	34.8	644.5	0.7	2.4
Collotheca sp.	COLLsp1	36.4	1132.5	1.4	5.2
Colurella adriatica	COLUADRI	52.7	1557.5	1.5	2.8
Colurella colurus	COLUCOLU	22.8	660.5	0.7	2.6
Colurella obtusa	COLUOBTU	26.6	128.5	0.1	0.4
Colurella uncinata	COLUUNCI	24.5	438	0.4	2.1
Euchlanis deflexa	EUCHDEFL	29.3	406	0.5	2.0
Euchlanis dilatata	EUCHDILA	32.1	1437.5	1.7	8.0
Keratella cochlearis	KERACOCH	23.4	193.5	0.2	0.6
Keratella quadrata	KERAQUAD	10.3	128	0.1	1.0
Lecane bulla	LECABULL	15.8	162.5	0.2	0.8
Lecane closterocerca	LECACLOS	83.7	8733	10.0	12.1
Lecane flexilis	LECAFLEX	34.8	634	0.9	4.1
Lecane hamata	LECAHAMA	25.0	402	0.5	1.8
Lecane luna	LECALUNA	15.2	432.5	0.5	2.3
Lecane lunaris	LECALUNR	45.1	2083.5	2.3	6.3
Lecane stichaea	LECASTIC	10.9	296	0.3	1.4
Lecane tenuiseta	LECATENU	20.7	342.5	0.4	1.5
Lepadella acuminata	LEPAACU	27.2	829.5	1.0	3.8
Lepadella ovalis	LEPAOVAL	35.3	1147.5	1.4	4.1
Lepadella patella	LEPAPATE	67.4	3059.5	3.8	8.0
Lepadella quadricarinata	LEPAQUAD	21.7	434.5	0.5	2.0
Lepadella triptera	LEPATRIP	12.0	120.5	0.2	0.7
Limnias ceratophylli	LIMNCERA	19.0	1388.5	1.7	7.8
Mytilina mucronata	MYTIMUCR	23.9	1071.5	1.2	3.8
Mytilina ventralis	MYTIVENT	17.4	350	0.4	2.1
Notommata cyrtopus	NOTOCYRT	10.3	76	0.1	0.3
Pleurotrocha petromyzon	PLEUPETR	14.1	306.5	0.3	1.4
Polyarthra dolichoptera	POLYDOLI	13.0	163	0.2	2.4
Proales fallaciosa	PROAFALL	26.1	243	0.2	0.9
Ptygura furcillata	PTYGFURC	24.5	175	0.2	0.6
Ptygura sp. 2	PTYGsp2	46.2	2313.5	2.1	6.3

Table 3. *Cont.*

Taxon	Acronym	Frequency (%)	Individuals	Average %	SD
Taphrocampa annulosa	TAPHANNU	12.0	256	0.2	1.1
Testudinella mucronata	TESTMUCR	29.3	763	0.7	2.6
Testudinella patina	TESTPATI	60.3	3726	4.5	11.7
Trichocerca brachyura	TRICBRAC	13.0	506	0.7	4.8
Trichocerca obtusidens	TRICRELI	31.0	1310.5	1.8	5.6
Trichocerca porcellus	TRICPORC	22.3	399.5	0.5	1.7
Trichocerca rattus	TRICRATT	34.2	1109.5	1.4	3.9
Trichocerca similis	TRICSIMI	10.9	198.5	0.3	3.0
Trichocerca weberi	TRICWEBE	12.5	136	0.2	1.1
Trichotria pocillum	TRIOPOCI	12.0	181.5	0.3	2.1

3.2. Alpha Diversity

The number of identified taxa per sample varied from 2 to 48, with an average of 18.5 ± 9 (Table 4). S_chao1 suggested the presence of at least 15.5 ± 42 extra taxa on average and high median sample completeness (94.9%), but some sample estimates seemed unrealistically high (up to 399 ± 138 taxa). For this reason, only S_obs and S_rar were considered for further analysis. Two samples had four singletons but 145 samples (78.8%) contained none, 30 had one and six had two. Species-rich assemblages also tended to have more unique species (R = 0.3, $p = 4 \times 10^{-5}$; Figure 2a). Shannon entropy and diversity were usually low (Table 4 and Figure S2). Average H'_true was 7.4 ± 4.3 taxa, varying from c. 1 to 26 taxa. Simpson diversity, however, was quite high for the majority of samples.

Table 4. Statistics for observed, rarefied and estimated number of taxa, sample completeness, Shannon entropy and diversity, Simpson diversity, LCBD, LCBD components (abundance) and IFO, all samples (monogononts and identified bdelloids only; CV: variation coefficient).

Metric	Average	SD	Minimum	P25th	P50th	P75th	Maximum	CV (%)
S_obs	18.6	9.0	2	12	19	24	48	48.6
S_rar	15.2	6.7	2	10	15.5	19	38	44.0
S_chao1	34.0	46.9	2	13	21	37.5	399	138.0
$S_{\_chao1}$-$S_{\_obs}$	15.5	41.9	0	0	1	15	351	271.2
sample completeness (%)	80.0	26.2	10.5	60.0	94.9	100	100	32.8
H'	1.84	0.60	0.15	1.55	1.89	2.25	3.28	32.3
H'_true	7.43	4.28	1.16	4.73	6.64	9.47	26.45	57.6
D1	0.72	0.17	0.04	0.65	0.77	0.84	0.96	24.0
LCBD	0.0054	0.0005	0.0046	0.0050	0.0055	0.0058	0.0063	8.5
LCBDrepl	0.0054	0.0015	1×10^{-5}	0.0049	0.0058	0.0063	0.0079	27.1
LCBDabun	0.0054	0.0046	0.0018	0.0025	0.0035	0.0062	0.0233	83.9
IFO	0.0586	0.0432	0.0112	0.0281	0.0449	0.0761	0.2576	73.7

In a NB2 regression model with local and setting variables, a combination of EC, Al and the number of macrophyte taxa provided the best estimate of observed species richness (Table 5). S_obs remained unaffected by EC up to c. 320 µS.cm^{-1}, decreasing steadily at higher values (Figure 3). Notably, the linear correlations of ortho-Pmax (R = −0.27, $p = 2 \times 10^{-4}$), TP and KjN (R = −0.25, $p = 6 \times 10^{-4}$) were slightly more negative than for EC (R = −0.23, p = 0.002; Table S4). The negative relation to aluminium appeared to be more gradual. Albeit also with considerable scatter, the number of taxa rose more or less linearly with the species richness of the vegetation (R = 0.37, $p = 2 \times 10^{-7}$). Furthermore, S_obs correlated negatively with individual major ions and most nitrogen compounds (Table S4). Rarefied species richness delivered very consistent results (Table 5).

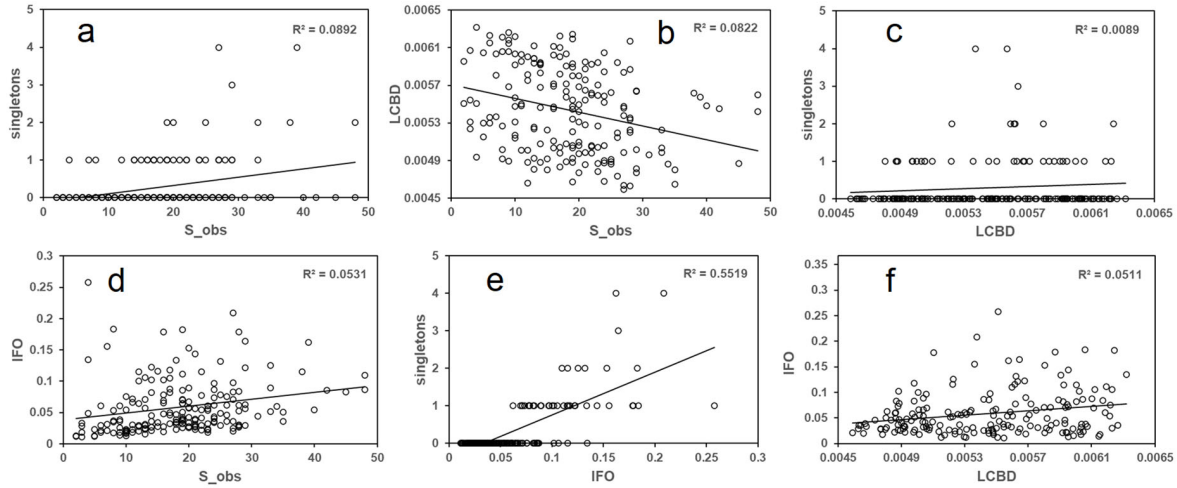

Figure 2. Scatter plots with linear trend of (**a**) observed number of taxa (S_obs) and singletons; (**b**) S_obs and LCBD; (**c**) LCBD and IFO; (**d**) S_obs and IFO; (**e**) IFO and singletons; (**f**) LCBD and IFO for monogononts and identified bdelloids. Plots for S_rar were very similar to those of S_obs due to almost perfect collinearity (R = 0.97).

Table 5. Regression models for observed and rarefied taxon richness, Shannon entropy, true Shannon diversity and Simpson diversity of monogononts and identified bdelloids with local and setting variables. Efron pseudo-R^2 followed by Veall–Zimmermann pseudo-R^2 for NB2 and GLM, pseudo-R^2 according to Zeileis et al. [126] for beta regression.

	S_obs			**S_rar**			**H′**			**H′_true**			**D1**		
model	NB2, log link			NB2, log link			GLM, gamma, identity link						beta, logit link, ML, Φ identity link		
D_0, *df*	243.5, 183			240.8, 183			26.72, 183			60.17, 183			-		
D, *df*	196.0, 180			196.6, 180			22.88, 179			48.89, 179					
pseudo-R^2	0.23, 0.21			0.21, 0.20			0.19, 0.20			0.21, 0,21			0.12		
log-L, *df*	−640.0, 5			−589.4, 5			−170.2, 6			−482.1, 6			94.2, 4		
	coeff. ± SE	*z*	*p*	coeff. ± SE	*z*	*p*	coeff. ± SE	*t*	*p*	coeff. ± SE	*t*	*p*	coeff. ± SE	*z*	*p*
φ	-	-	-	-	-	-	-	-	-	-	-	-	7.36 ± 0.73	10.01	$<2 \times 10^{-16}$
intercept	4.17 ± 0.38	11.00	$<2 \times 10^{-16}$	3.93 ± 0.34	11.56	$<2 \times 10^{-16}$	3.58 ± 0.52	6.89	9×10^{-11}	−0.02 ± 0.06	−0.34	0.73	3.66 ± 0.60	6.14	8×10^{-10}
EC	−0.35 ± 0.10	−3.45	6×10^{-4}	−0.33 ± 0.09	−3.61	3×10^{-4}	−0.48 ± 0.13	−3.79	2×10^{-4}	0.050 ± 0.012	3.88	1×10^{-4}	−0.62 ± 0.17	−3.70	2×10^{-4}
Al	−0.32± 0.10	−3.11	0.002	−0.29 ± 0.09	−3.19	0.001	−0.43 ± 0.13	−3.38	9×10^{-4}	0.039 ± 0.016	2.43	0.02	−0.57 ± 0.16	−3.50	5×10^{-4}
macrophytes	0.025 ± 0.006	4.18	3×10^{-5}	0.020 ± 0.005	3.68	2×10^{-4}	0.022 ± 0.008	2.77	0.006	0.023 ± 0.008	−2.77	0.006	-	-	-
wooded shore	-	-	-	-	-	-	0.17 ± 0.09	1.98	0.05	0.027 ± 0.010	−2.53	0.01	-		-

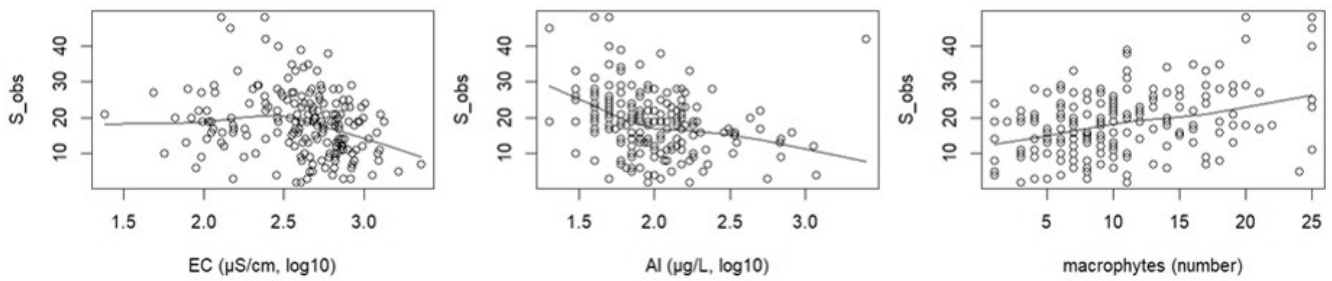

Figure 3. Scatter plots of environmental variables retained in the negative binomial model for observed species richness of monogononts and identified bdelloids with local and setting variables.

Four variables were retained in the GLMs for Shannon entropy and diversity. EC and aluminium obtained negative coefficients, whereas the number of macrophyte taxa and, marginally, the percentage of wooded shoreline, exerted a mildly positive influence (Table 5). The model for H′_true placed more emphasis on EC and wooded shoreline. Scatter plots indicated a stepped response of H′ to EC, similar to S_obs, and more linear patterns for the other covariates, which were somewhat neater for H′_true (Figure 4). Together with Na ($R = -0.24$, $p = 0.001$), EC, Al and the number of macrophytes presented the highest correlations with H′ (R = c. −0.25), but Cl, Mg, phosphorus compounds, NH_4 as well as TN and KjN, followed closely in this respect (Table S4). Correlations with TP, ortho-P and wooded shoreline increased slightly for true diversity.

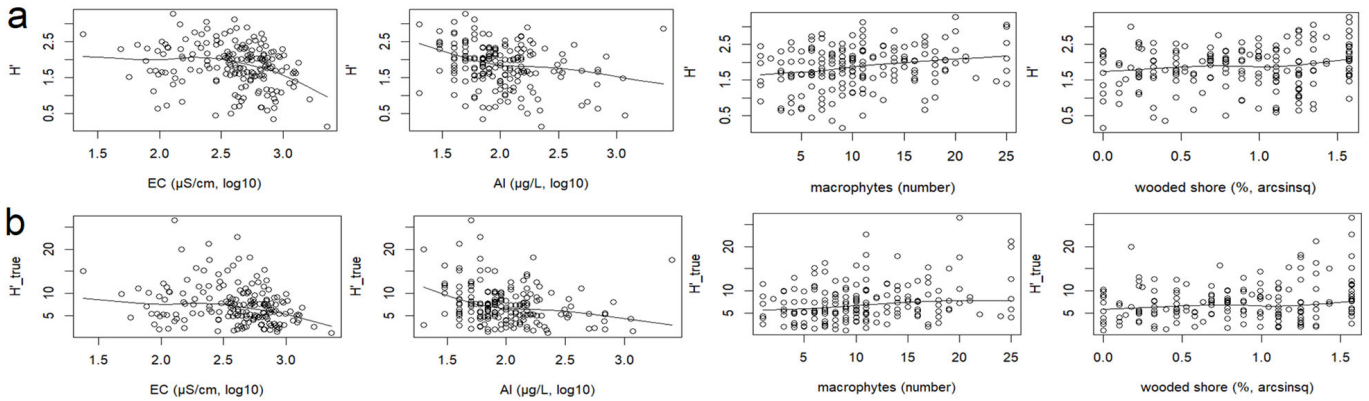

Figure 4. Scatter plots with loess smoother of environmental variables retained in the regression models for (**a**) Shannon entropy; (**b**) Shannon diversity of monogononts and identified bdelloids with local and setting variables.

EC and Al presented the highest correlations with D1 (R = 0.21, p = 0.003–0.005; Table S4) and only these variables were included in the regression model for Simpson diversity. This performed poorly, with higher values for EC tending to promote stronger dominance or, in the case of Al, mainly levelling-off its variation (Table 5 and Figure 5). Otherwise, correlations to variables were very similar to other alpha diversity metrics but coefficients were usually somewhat lower (Table S4).

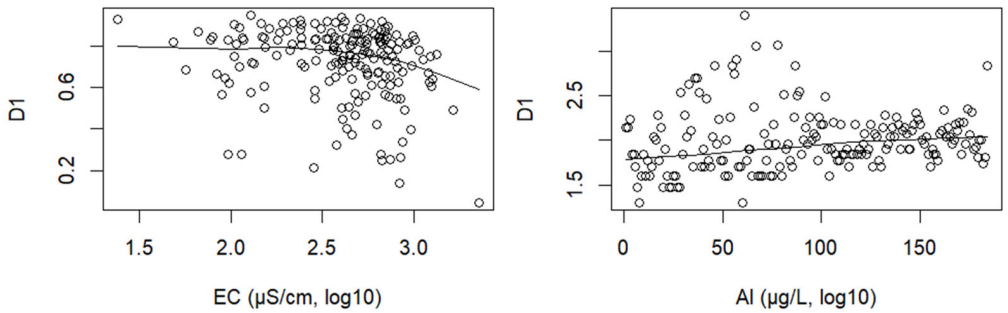

Figure 5. Scatter plots with loess smoother of environmental variables retained in the regression model for Simpson diversity of monogononts and identified bdelloids with local and setting variables.

3.3. Beta Diversity

Total beta diversity was only slightly higher for abundance than incidence data (Table 6). Replacement (species turnover) accounted for 61% of beta diversity with presence–absence data, increasing to three-quarters of the total with abundance, thus well exceeding differences attributable to loss or gain of species in both cases (Table 6).

Table 6. Decomposition of beta diversity (BDtotal) into replacement (Repl) and richness (RichDiff) or abundance difference (AbDiff) components with Jaccard (incidence) and Ružička (abundance) distance (monogononts and identified bdelloids, only).

	BDtotal	Repl	Repl/BDtotal	RichDiff	AbDiff	RichDiff/BDtotal
incidence	0.417	0.253	0.607	0.164	-	0.393
abundance	0.433	0.321	0.740	-	0.113	-

Abundance-based LCBD varied little between samples (Table 4). Although 25 samples (13.6%) contributed more to beta diversity than average at $p \leq 0.05$, none remained after Holm adjustment. With the exception of the loam belt along the southern border, higher values occurred scattered throughout the region (Figure S3a). LCBD values decreased slightly with the number of taxa ($R = -0.29$, $p = 8 \times 10^{-5}$; Figure 2b) but were unrelated to the number of singletons ($R = 0.09$, $p = 0.2$; Figure 2c).

From all local and setting variables, the combination of pH ($R = 0.30$, $p = 4 \times 10^{-5}$), Al ($R = 0.30$, $p = 2 \times 10^{-5}$) and the number of nearby ponds ($R = -0.19$, $p = 0.01$; Figure 2c) explained contributions to local beta diversity best with beta regression, albeit with restraint (Table 7). All sites with a pH below 4.9 had very high LCBD values (Figure 6a); LCBD varied much more strongly at pH $\geq$ 6. Whereas many circumneutral to alkaline sites presented a quite similar assemblage and contributed little to the regional variety, their LCBD could still attain the same level as in acid waters. Aluminium concentrations obtained a positive coefficient, although with considerable scatter throughout its range (Figure 6a). Judging from the negative coefficient for ponds, connectivity appeared to reduce LCBD slightly, but the pattern was not compelling and possibly only occurred at the highest pond densities (Figure 6a). Except for macrophyte number, shoreline slope and depth, all slightly negatively related to LCBD, most other variables showing some correlation to LCBD were not independent from the pH gradient (Tables S3 and S4). To reduce any pH-effects, LCBD was recalculated without the 22 samples with median pH < 6.5 (LCBD_non-acid). In this case, 20 samples (12.3%) contributed more than average to beta diversity without and zero with Holm correction. Higher values hardly occurred in the southern loam belt, as well as in the north-eastern cover-sand region (Figure S3b). Elimination of acid sites reduced the correlation with pH, Al, Ca, Na, K, Cl, morphological and land-cover variables, as well as ponds, and replaced the mildly negative correlation with COD_f by a positive one with Gelbstoff (Table S4). Aluminium retained a weakly positive correlation ($R = 0.20$, $p = 0.009$) and the sign switched for potassium from positive to negative ($R = -0.17$, $p = 0.04$). The number of macrophytes, potassium and Gelbstoff were retained as significant in the regression model, with only the latter showing a positive influence, but only at high concentrations (Table 7 and Figure 6).

Replacement contributed 3.85 times more to LCBD (all samples) than abundance differences and LCBDrepl was higher than LCBDabun for 67.6% of the sites. High LCBDrepl was mostly decisive for a more than average LCBD value (Figure S3c,d). LCBDabun varied much more than LCBDrepl and its maximum values were c. three times higher (Table 4). The distribution of LCBDrepl was heavily skewed to the right, but sites with high LCBDabun were less common (Supplementary Figure S2) with scattered occurrence throughout the region (Figure S3c). Both components correlated only marginally with LCBD (LCBDrepl $R = 0.15$, $p = 0.04$; LCBDabun $R = 0.19$, $p = 0.01$).

Table 7. Regression models for LCBD, LCBDrepl and IFO of monogononts and identified bdelloids with local and setting variables. Pseudo-R^2 according to Zeileis et al. [126].

	LCBD			**LCBD_Non-Acid**			**LCBDrepl**			**IFO**		
model	beta, logit link, ML, Φ identity link											
pseudo-R^2	0.16			0.14			0.09			0.30		
log-L, *df*	1170, 5			1007, 5			879.6, 5			397, 6		
	coeff. ± SE	*z*	*p*	coeff. ± SE	*z*	*p*	coeff. ± SE	*z*	*p*	coeff. ± SE	*z*	*p*
φ	30,617 ± 3194	9.59	$<2 \times 10^{-16}$	26,146 ± 2907	9.00	$<2 \times 10^{-16}$	1152.5 ± 121.9	9.46	$<2 \times 10^{-16}$	52.7 ± 5.6	9.38	$<2 \times 10^{-16}$
intercept	−5.15 ± 0.07	6.14	$<2 \times 10^{-16}$	−4.79 ± 0.08	−56.77	$<2 \times 10^{-16}$	−3.84 ± 0.35	−10.88	$<2 \times 10^{-16}$	0.77 ± 0.65	1.18	0.2
pH	−0.016 ± 0.006	−2.82	0.005	-	-	-	-	-	-	-	-	-
Al	0.048 ± 0.019	2.50	0.01	-	-	-	-	-	-	-	-	-
Ca	-	-	-	-	-	-	-	-	-	−0.32 ± 0.10	−3.17	0.002
K				−0.07 ± 0.02	−3.26	0.001						
Na	-	-	-	-	-	-	−0.225 ± 0.080	−2.80	0.005	-	-	-
TON	-	-	-	-	-	-	-	-	-	−0.66 ± 0.19	−3.55	4×10^{-4}
A440	-	-	-	$2 \times 10^{-4} \pm 7 \times 10^{-5}$	2.92	0.003	−0.0010 ± 0.0004	−2.87	0.004	-	-	-
oxygen saturation	-	-	-	-	-	-	−0.003 ± 0.001	−3.27	0.001	-	-	-
sand	-	-	-	-	-	-	-	-	-	0.14 ± 0.07	2.11	0.03
pasture	-	-	-	-	-	-	-	-	-	−0.33 ± 0.13	−2.58	0.01
ponds	−0.013 ± 0.004	−2.93	0.003	-	-	-	-	-	-	-	-	-
macrophytes	-	-	-	−0.004 ± 0.001	−3.48	5×10^{-4}	-	-	-	-	-	-

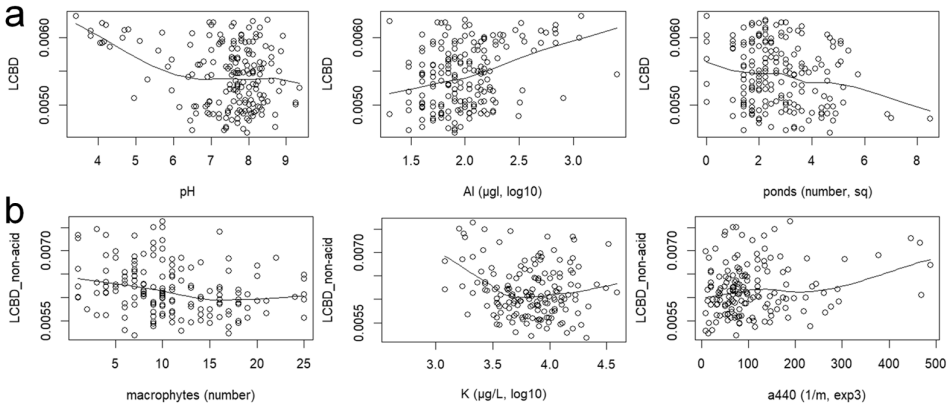

Figure 6. Scatter plots with loess smoother of environmental variables retained in the regression models for LCBD of monogononts and identified bdelloids with local and setting variables: (**a**) all samples; (**b**) sites with pH ≥ 6.5.

Overall, LCBDabun correlated negatively with S_obs ($R = -0.24$, $p = 9 \times 10^{-4}$), S_rar ($R = -0.28$, $p = 1 \times 10^{-4}$), H' ($R = -0.38$, $p = 1 \times 10^{-7}$) and D1 ($R = -0.44$, $p = 4 \times 10^{-10}$), and LCBDrepl positively with H' ($R = 0.21$, $p = 0.005$) and D1 ($R = 0.28$, $p = 2 \times 10^{-4}$). Scatter plots, however, revealed distinct curvilinear U- and inverted U-shaped patterns for LCBDabun and LCBDrepl, respectively, with species number and Shannon entropy (Figure 7a–d), and similar but more right-skewed and scattered distributions for Simpson diversity (Figure 7c,f). Thus, LCBDrepl tended to be highest and LCBDabun lowest at intermediate alpha diversity.

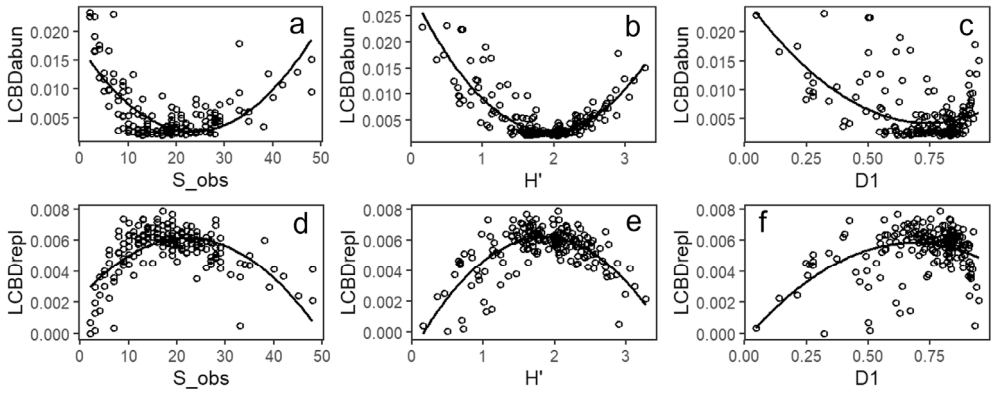

Figure 7. Scatter plots with loess smoother of (**a**–**c**) LCBDabun; (**d**–**f**) LCBDrepl against observed number of taxa (S_obs), Shannon entropy (H') and Simpson diversity (D1) of monogononts and identified bdelloids. See caption Figure 2 for S_rar.

LCBDabun correlated weakly positive with Gelbstoff (R = 0.18, p = 0.02), sodium (R = 0.17, p = 0.02) and coastal dune (R = 0.15, p = 0.05), but no satisfactory regression model could be fitted. Although LCBDrepl showed significant and occasionally somewhat stronger correlations with several ion concentrations, as well as morphometry and land-cover variables (Table S4), only Na ($R = -0.26$, $p = 4 \times 10^{-4}$), Gelbstoff ($R = -0.13$, p = 0.07) and oxygen saturation ($R = -0.12$, p = 0.11) were retained in a parsimonious model, the latter only as slightly modulating variables (Table 7 and Figure 8). Correlations with dimensions (e.g., surface $R = -0.18$, p = 0.02) and Dsl (R = 0.15, p = 0.04), nevertheless suggested somewhat lower values in large water bodies, whereas the relation with surrounding heathland was rather positive (R = 0.22, p = 0.003).

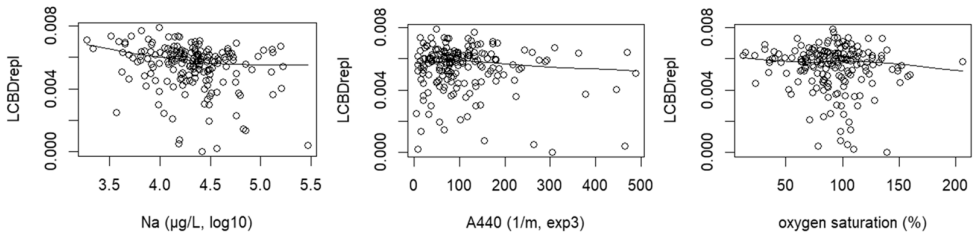

Figure 8. Scatter plots with loess smoother of environmental variables retained in the regression model for LCBDrepl of monogononts and identified bdelloids with local and setting variables.

3.4. Originality

IFO values ranged from 0.011 to 0.258 and were generally low (0.059 ± 0.043; Table 4). Although values generally increased somewhat with species richness (R = 0.23, p = 0.002), some species-poor to moderately rich assemblages (S_obs < 30) showed the highest values (Figure 2d). Originality was, of course, heavily influenced by the number of singletons (R = 0.74, $p < 2 \times 10^{-16}$; Figure 2e) and also correlated positively with LCBD (R = 0.23, p = 0.002; Figure 2f). The latter was likely linked to its negative correlation with major cations (e.g., Ca $R = -0.43$, $p = 2 \times 10^{-9}$; Mg $R = -0.37$, $p = 3 \times 10^{-7}$) and pH ($R = -0.4$, $p = 2 \times 10^{-8}$; Table S4), indicating the restricted occurrence of certain taxa in soft, more acid water.

The regression model from local and setting features retained TON ($R = -0.30$, $p = 4 \times 10^{-5}$) as the most influential variable, joined by Ca ($R = -0.43$, $p = 2 \times 10^{-9}$) and pasture ($R = -0.31$, $p = 2 \times 10^{-5}$) as negative covariates (Table 7 and Figure 9). Sand (R = 0.38, $p = 1 \times 10^{-7}$) was the only model variable with a significant positive coefficient. Notably, IFO correlated negatively with all variables related to phosphorus (e.g., TP $R = -0.29$, $p = 7 \times 10^{-5}$), phytoplankton (e.g., chl *a* $R = -0.16$, p = 0.03) and metabolism (e.g., BOD $R = -0.27$, $p = 2 \times 10^{-4}$), proxies for organic substances (e.g., COD $R = -0.22$,

$p = 0.003$), nitrite ($R = -0.24$, $p = 0.001$) and clay ($R = -0.24$, $p = 0.001$), but related positively with submerged cover ($R = 0.23$, $p = 0.002$), as well as heathland ($R = 0.33$, $p = 5 \times 10^{-6}$) and conifers ($R = 0.27$, $p = 2 \times 10^{-4}$).

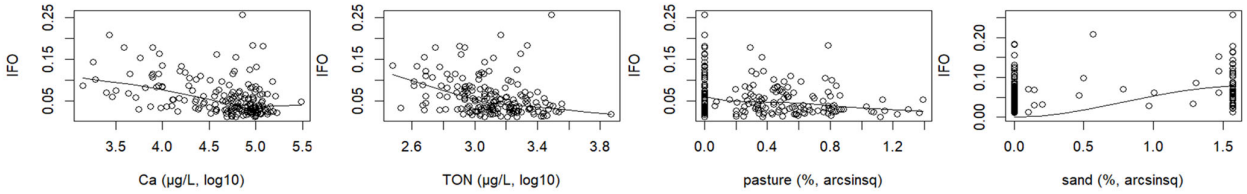

Figure 9. Scatter plots with loess smoother of environmental variables retained in the regression model for IFO of monogononts and identified bdelloids with local and setting variables.

3.5. General Species–Environment Relations

3.5.1. Variation Partitioning

Overall, the 28 selected variables explained 16% of the observed variation in species composition of the full dataset (Table 8 and Figure 10). The three main variable groups, as well as the physical site and water-column characteristics separately, all explained a significant part of the rotifer distributions. Local site variables accounted for a major part of the explained variation (12%), with their unique contribution amounting to 51% of the total. Setting and spatial structure each explained c. 5% with a similar but substantially lower unique proportion (c. 1.5%). Interactions due to covariance of the variable groups summed up to c. 5% of the variation, or almost 30% of the explained fraction. Interaction effects were of similar importance for local-setting and local-spatial scales, each accounting for c. 3% of species variation. More variation was shared by local features and spatial structure than by setting and spatial context. Interactions of local and setting variables with spatial coordinates represented more than twice as much of the total explained variation than the pure spatial component.

Table 8. Results of variation partitioning with the three sets of variables (monogononts and identified bdelloids, only).

Variation Component	Variable Set	*df*	R^2	$R^2_{adj.}$	*p*	Variables
total	local	13	0.19	0.12	0.001	pH, Al, Fe, TP, KjNmax, pGOP, CODf, CODp, A440, Dsl, emergent, submerged, wooded shore clay, loam, heath, coniferous, deciduous, ponds broad scale: MEM3, MEM4; intermediate scale: MEM6, MEM7, MEM14, MEM23; small scale: MEM39
	setting	6	0.08	0.05	0.001	
	spatial	9	0.10	0.05	0.001	
unique	local + setting	19	0.23	0.14	0.001	
	local + spatial	22	0.25	0.14	0.001	
	setting + spatial	15	0.15	0.08	0.001	
	all	28	0.29	0.16	0.001	
	local	13	-	0.08	0.001	
	setting	6	-	0.02	0.001	
	spatial	9	-	0.02	0.001	
	residuals	-	-	0.84	-	
interactions	local-spatial	13	-	0.09	0.001	
	local-setting	13	-	0.09	0.001	
	setting-spatial	6	-	0.03	0.001	
	setting-local	6	-	0.02	0.001	
	spatial-local	9	-	0.02	0.001	
	spatial-setting	9	-	0.03	0.001	

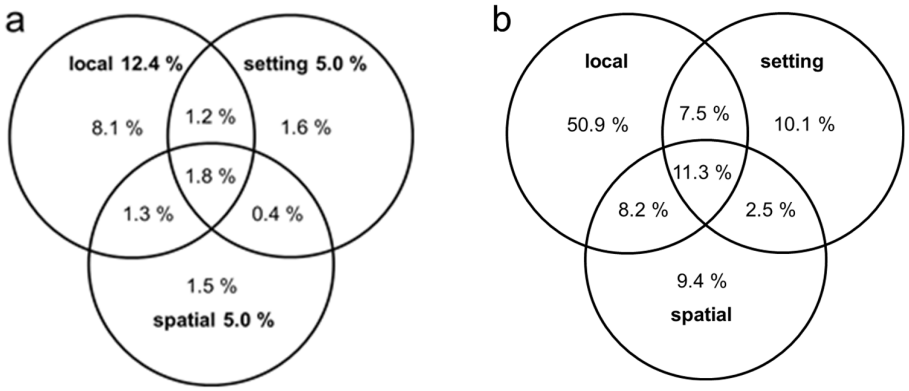

Figure 10. Visualisation of species variation partitioning of monogononts and identified bdelloids for the three sets of selected variables: (**a**) contribution to explained variation as percentage of total; (**b**) contribution as fractions of total explained variation.

pH, total phosphorus, potential oxygen production (pGOP), iron and both COD fractions emerged as the primary local variables (Table 8). Aluminium, A440, maximum Kjeldahl-nitrogen, Dsl and the percentage cover of emergent and submerged vegetation and wooded shoreline contributed marginally. In the group for setting, heathland, clay and the number of proximal ponds were most important, completed by coniferous and deciduous woodland and loam. The group of spatial variables included seven dbMEMs, X and Y. Two dbMEMs represented broad-scale structures (c. 33 km), four followed intermediately scaled patterns (9–18 km), and the smallest one operated at a scale of c. 1 km (Table S5). MEM3 represented an E–W oriented pattern, most strongly contrasting waterbodies along a central N–S axis with positive scores to those along the Middle Scheldt River towards the W and those towards the E with negative scores (Figure S4a). It correlated negatively with phosphorus compounds (TP $R = -0.26$, $p = 3 \times 10^{-4}$) and organic nitrogen (TON $R = -0.27$, $p = 2 \times 10^{-4}$), as well as with productivity-related variables and positively with larger dimensions (Table S5). This mainly results from the predominantly hypertrophic condition of ponds in the alluvium of the Schelde. The second broad-scale pattern (MEM4) was confined to the eastern part of Flanders, mainly setting apart some gravel pits along the Common Meuse River from smaller ponds distributed throughout this region (Figure S4b). Potassium ($R = -0.29$, $p = 5 \times 10^{-5}$) appeared to be its best environmental correlate, reflecting lower concentrations in the deeper pits connected to the Meuse groundwater system. Intermediately scaled patterns associated more with specific river catchments (MEM6, MEM7; Figure S4c,d), smaller clusters of water bodies (MEM14; Figure S4e), or differences between the coastal dunes and adjacent polders (MEM23; Figure S4f). MEM6 was more particularly related to iron ($R = 0.29$, $p = 5 \times 10^{-5}$), which associates with seepage influence, and MEM23 to dune ($R = 0.49$, $p = 2 \times 10^{-12}$) and sodium ($R = 0.28$, $p = 2 \times 10^{-4}$), reflecting a seaside position.

An RDA ordination to elucidate the pattern of identified taxa explicitly in relation to local and setting variables highlighted nine local variables of importance, all belonging to the local group and together explaining 16% of the species variation (Table 9). This set of variables differed slightly from the set retained in the general variation partitioning, principally because selection accounted for multiple testing and absence of spatial detrending (Table 10). Consequently, dissolved humic substances, maximum Kjeldahl-nitrogen and the percentage cover of emergent vegetation and wooded shoreline did not reappear as significant. Correspondingly, however, the constrained RDA identified pH as the principal structuring variable, followed by TP, aluminium and pGOP. All these variables retained a significant unique effect when covariates were partialled out, but individually accounted for only 1 to 2% of the species variation. Note that iron was the only variable that did not interact with any of the other selected variables, and that its unique effect exceeded that of aluminium and pGOP.

Table 9. General results of RDA on species composition (monogononts and identified bdelloids, only), with local and setting variables.

	Axis 1	Axis 2	Axis 3
eigenvalue (λ)	0.061	0.029	0.023
species–environment correlation	0.81	0.68	0.64
% variance species data	6.1	2.9	2.3
% variance species–environment	38.4	18.3	14.4
F-ratio		11.3	
sum λ, *F*-ratio, *p* all axes		0.159, 3.66, 0.001	

Table 10. Variables retained from the local and setting sets by forward selection and their contribution to explained variance of the species data by constrained and partial constrained RDA. Ordered by unique effect.

		Constrained—Marginal Effect				Partial Constrained—Unique Effect			
Variable	p_{Holm}	**% Data**	λ_1/λ_2	***F***	***p***	**% Data**	λ_1/λ_2	***F***	***p***
pH	0.014	4.7	0.49	9.0	0.001	2.0	0.20	3.5	0.001
TP	0.014	3.9	0.39	7.4	0.001	1.7	0.18	3.1	0.001
Fe	0.014	1.6	0.15	3.0	0.001	1.6	0.17	2.9	0.001
pGOP	0.014	2.4	0.23	4.4	0.001	1.3	0.13	2.4	0.001
Dsl	0.014	1.7	0.16	3.2	0.001	1.3	0.13	2.3	0.002
COD_p	0.014	1.8	0.17	3.4	0.001	1.2	0.12	2.0	0.004
COD_f	0.016	2.2	0.22	4.2	0.001	1.1	0.11	1.9	0.004
Al	0.049	2.7	0.29	5.1	0.001	1.0	0.11	1.8	0.007
submerged	0.049	1.8	0.17	3.3	0.001	1.0	0.10	1.7	0.012

Figure 11a shows the constellation of variables for the first two axes and the 42 best-fitting taxa. The first axis differentiated along a pH/TP gradient, with aluminium, as well as submerged cover, correlating negatively with this gradient. Moorland ponds subjected to anthropogenic acidification are affected by increased solubility of aluminium at very low pH. Likewise, submerged vegetation is lost in hypereutrophic conditions with high TP and pH. At the lower pH/TP end, *Lecane lunaris*, *L. stichaea*, *Euchlanis meneta* and *Bryceella stylata* attained the most positive scores (lowest pH), followed by a large group with e.g., many *Lecane* species (*L. flexilis*, *L. perpusilla*, *L. signifera*, *L. tryphema*, etc.), some *Cephalodella* (*C. auriculata*, *C. gracilis*, *C. inquilina*), *Aspelta circinator*, *Elosa worrallii*, *Keratella serrulata* and *Taphrocampa annulosa* grouping together at somewhat lower scores. Spurious occurrences can markedly influence species ordination scores; hence, the position of quite a few poorly represented taxa (*Aspelta circinator*: N2 = 3.6, *Bryceella styllata*: N2 = 2.1, *Elosa worrallii*: N2 = 1.2, *Keratella serrulata*: N2 = 1.6, *Lecane signifera*: N2 = 1.8, *L. tryphema*: N2 = 1.5, *Cephalodella inquilina*: N2 = 2.4) needs to be considered more prudently. *Lecane closterocerca*, *Cephalodella segersi*, *Mytilina mucronata* and *Testudinella patina* scored most negatively along axis 1, suggesting a close relation to the most alkaline conditions, which were also high in TP and non-particulate organic substances. To some extent, axis 1 indirectly reflected the raptor and collector CWMs, and hence also GRrc, and associated mouth-part types (Table S6), suggesting a stronger dependence on active foraging where pH and TP were low (Figure 11c).

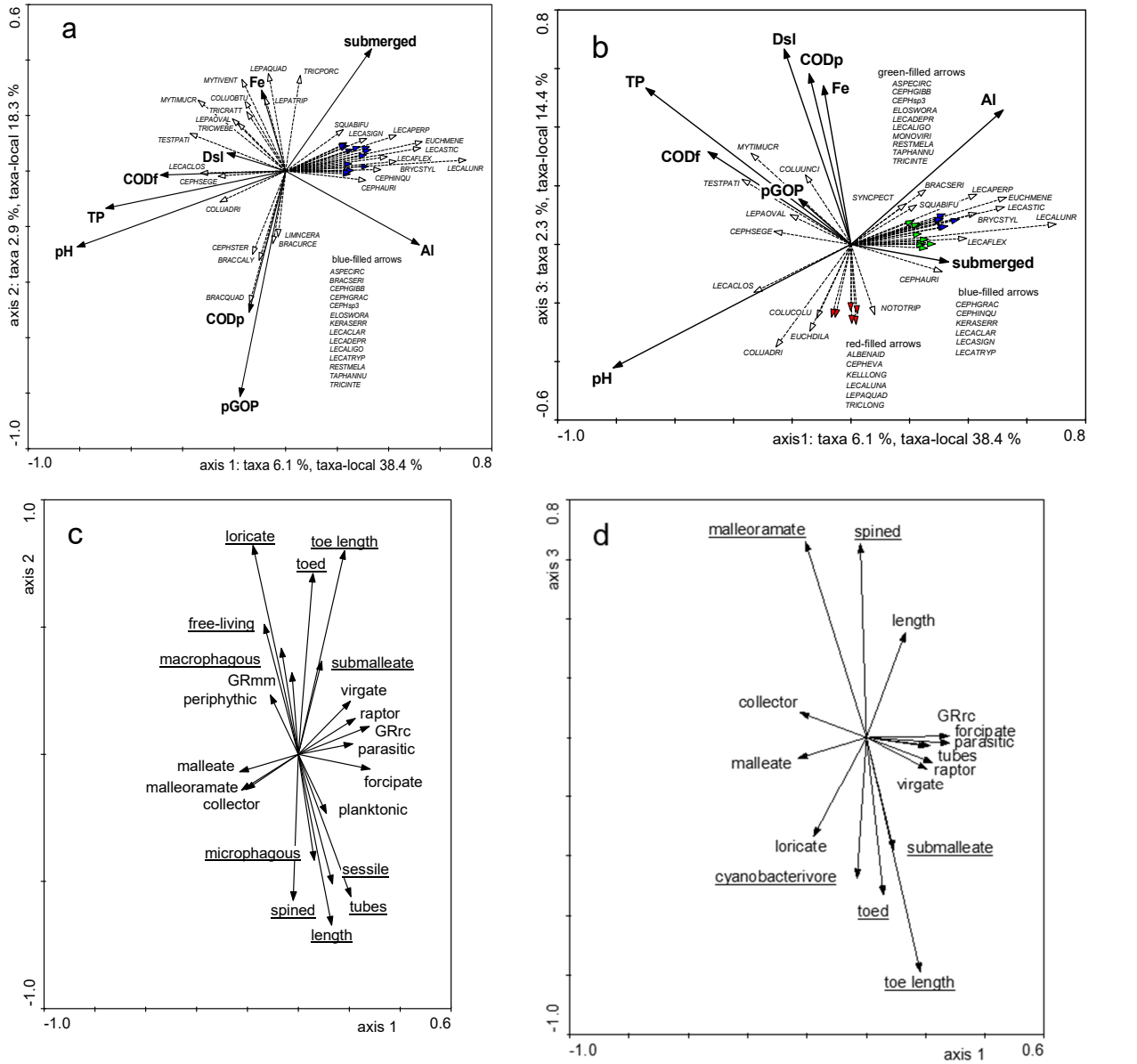

Figure 11. RDA ordination of taxa with selected local and setting variables. Taxa shown have a fit of at least 5%: (**a**) taxa and environmental variables, axes 1 and 2; (**b**) taxa and environmental variables, axes 1 and 3; (**c**) CWMs with Pearson R $p \leq 0.05$ for any of both axes as passive variables, axes 1 and 2; (**d**) CWMs and GRs with Pearson R $p \leq 0.05$ for any of both axes as passive variables, axes 1 and 3. Underlined variables $p_{Holm} \leq 0.05$.

The second axis was defined by high phytoplankton abundance, represented by potential oxygen production (pGOP) and particulate organic matter (COD_p), both leading to negative scores (Figure 11a). The CWMs for macro- and microphagous taxa and GRmm aligned rather well with this axis, which also differentiated for size, lorication and motility. The negative relation of submerged macrophyte cover to turbidity was also clearly reflected by a positive correlation with this axis. According to their ordination scores, *Brachionus quadridentatus*, *B. calyciflorus*, *B. urceolaris* (N2 = 3.1), *Cephalodella sterea*, *Limnias ceratophylli*, and, at higher pH, *Colurella adriatica*, were represented best at macrophyte-poor sites with abundant phytoplankton, whereas *Trichocerca porcellus*, *Lepadella triptera*, *L. quadricarinata* and *Squatinella bifurca* (N2 = 1.4) tended to occur more in macrophyte-rich, less turbid waterbodies. In the latter, the CWMs for microphagy, size, sessility, tube dwelling and spine formation tended to be lower. Noteworthy is the decoupling of TP from

the phytoplankton–macrophyte gradient in this ordination, suggesting that a considerable part of the phosphorus resided in 'non-autotrophic' organic matter. The third axis, which was still relatively strong compared to the second one, separated pH better from the other local variables (Figure 11b). Besides revealing more differentiated pH optima and particularly strong aluminium tolerance for *Brachionus sericus*, *Synchaeta pectinata* and *Squatinella bifurca*, it suggested that a distinct species group with *Albertia naidis* (N2 = 4.0), *Cephalodella eva*, *Kellicottia longispina* (N2 = 2.6), *Lecane luna*, *Lepadella quadricarinata*, *Notommata tripus* (N2 = 4.4) and *Trichocerca longiseta* (N2 = 2.6) to some extent characterised waterbodies with a less developed shoreline (lower Dsl) and lower concentrations of organic matter and iron. This likely reflected an inclination for these species to occur somewhat more in larger sand and gravel extraction pits, characterised by such conditions. At CWM level, the third axis mainly differentiated malleoramate and spined (positive) from toed and cyanobacterivore (negative) (Figure 11d).

PAM clustering suggested nine functional groups that closely followed generic alliance (Table 11). FG1 and FGs 4 to 9 were truly periphytic and, except for the large tube-dwellers of group 4, free-living. Groups 2 and 3 were partly or essentially planktonic, often lacking toes, thus moving about by swimming. Both groups were separated by their feeding mode (collector vs. raptor), diet and trophi. As expected, they were usually poorly represented, although dominance of FG2 was exceptionally noted. High relative abundance of this group usually involved *Brachionus quadridentatus*, the only species within this genus that we did not classify as planktonic, although it is not strictly periphytic. A planktonic habitat was more explicit for the members of FG3. Its highest percentage (34%) was entirely due to *Polyarthra dolichoptera*, a species with long serrated appendages. Groups 1, 5 and 9 were equipped with toes and, being raptors, often required larger food particles (FG1 and F9); only FG9 carried armour. These three groups showed moderate representation, with FG1 being least abundant. Smaller size characterised the loricate, microphagous toe-bearers of groups 6 and 8, which differed from each other only by malleoramate versus submalleate trophi. Both these groups were quite well represented. FG7, gathering all bdelloids and characterised by ramate trophi and obligate parthenogenesis, was the most abundant functional group.

Table 11. Abundance, composition and major characteristics of functional groups. Size values as average with SD for group members. * Bdelloids furthermore differ by absence of resting eggs and a longer life span.

Group	Trait Syndrome	Taxa
FG1 (32 taxa) 0.9 ± 2.1%, max. 19.4%	behaviour: periphytic, free-living, toes, size (306 ± 142 µm) morphology: mostly macrophagous, mostly virgate or forcipate, illoricate, spineless physiology: raptor, predatorial/algivorous, some parasitic, heterogonic	*Albertia*, *Asciaporrecta*, *Aspelta*, *Cephalodella parasitica*, *Cupelopagis*, *Dicranophorus*, *Encentrum*, *Eosphora*, *Erignatha*, *Itura*, *Lindia*, *Notommata* excl. FG5, *Pleurotrocha*, *Resticula*, *Scaridium*
FG2 (21 taxa) 4.6 ± 12.2%, max. 83.5%	behaviour: partly planktonic, free-living, mostly toeless, size (284 ± 148 µm) morphology: microphagous, mostly malleate, loricate physiology: collector, algivorous and detritibacterivorous, some cyanobacterivorous, heterogonic	*Anuraeopsis*, *Brachionus*, *Filinia*, *Kellicottia*, *Keratella*, *Notholca*, *Platyias*, *Trichotria*
FG3 (42 taxa) 0.4 ± 2.6%, max. 34.2%	behaviour: planktonic, free-living, usually toeless, size (336 ± 240 µm) morphology: macro- and microphagous, mostly virgate, mostly illoricate physiology: raptor, predatorial and algivorous, heterogonic	*Ascomorpha*, *Asplanchna*, *Conochilus*, *Harringia*, *Ploesoma hudsoni*, *Polyarthra*, *Synchaeta*, *Trichocerca capucina*, *T. pusilla*

Table 11. *Cont.*

Group	Trait Syndrome	Taxa
FG4 (15 taxa) 6.4 ± 13.0%, max. 91.9%	behaviour: periphytic, sessile, toeless, large (776 ± 549 µm) morphology: some colonial, microphagous, mostly malleoramate, tube-forming, large physiology: mostly collector, algivorous and detritibacterivorous, heterogonic	*Beauchampia*, *Cephalodella forficula*, *Collotheca*, *Floscularia*, *Limnias*, *Ptygura*, *Stephanoceros*
FG5 (42 taxa) 9.0 ± 12.6%, max. 80.9%	behaviour: periphytic, free-living, toes, rather small (207 ± 85 µm) morphology: microphagous, mostly virgate, illoricate, unspined physiology: mostly raptorial, detritibacterivorous, some algivorous, heterogonic	*Bryceella*, *Cephalodella* excl. FG4, *Hexarthra*, *Microcodon*, *Monommata*, *Notommata* cf. *cyrtopus*, *Notommata cyrtopus*, *Proales*, *Pseudencentrum*, *Taphrocampa*, *Wulfertia*
FG6 (30 taxa) 17.7 ± 17.9%, 98.1%	behaviour: periphytic, free-living, usually toes, small (138 ± 59 µm) morphology: microphagous, mostly malleate, loricate, unspined physiology: collector, detritibacterivorous, heterogonic	*Colurella*, *Elosa*, *Lepadella*, *Lophocharis*, *Mytilina*, *Squatinella*, *Testudinella*
FG7 (4 taxa, 1 OTU) 37.4 ± 25.4%, max. 95.4%	behaviour: periphytic, free-living, large? (664 ± 433 µm for identified taxa) morphology: microphagous, ramate, illoricate, unspined physiology: collector, detritibacterivorous, obligate parthenogenetic *	*Dissotrocha*, *Rotaria*, unidentified bdelloids
FG8 (40 taxa) 18.2 ± 17.0%, max. 99.8%	behaviour: periphytic, free-living, toes, small (160 ± 114 µm) morphology: microphagous, mostly submalleate, loricate, unspined physiology: collector, algivorous and detritibacterivorous, heterogonic	*Euchlanis*, *Lecane*
FG9 (19 taxa) 5.5 ± 11.0%, max. 60.8%	behaviour: free-living, mostly periphytic, loricate, many spined, toes, average size (243 ± 127 µm) morphology: macrophagous, virgate physiology: raptor, algivorous, heterogonic	*Ploesoma triacanthum*, *Trichocerca* excl. FG3

Generalisation to functional groups did not increase the variation in assemblage composition explained by local and setting variables in RDA (13.3%; Table 12) but allowed linking of unidentified bdelloids, now included for most traits, to the trait syndromes of the largely monogonont assemblage. Again, all selected variables belonged to the local group. pGOP, not pH, was now retained as the principal variable, both by marginal and unique contribution (Table 13), indicating precedence of nutritional resources over chemical environment in FG representation. Correlating negatively with both axes, high pGOP associated with stronger representation of the free-swimming FG2 and the bdelloid FG7, or lower relative abundance of FG8 and FG9 (Figure 12a). At the CWM level, increased phytoplankton productivity aligned with a more microphagous community (hence lower GRmm) of larger-bodied species, relying on parthenogenic reproduction, larger size and spine or tube formation rather than lorication or toe-supported adhesion and creeping (Figure 12b and Tables S7–S10). Toe length, correlating negatively with total length ($R = -0.54$, $p_{Holm} \leq 0.001$; Table S6) also correlated negatively. Calcium defined the second axis, replacing pH from the species ordination. Ca correlated strongly with pH ($R = 0.78$, $p \leq 0.001$) and even more so with Mg ($R = 0.87$, $p \leq 0.001$) and EC ($R = 0.92$, $p \leq 0.001$). Lower Ca concentrations led to higher axis 2 scores and associated particularly with FG8 and FG5, phytophagy and higher GRrc scores, whilst detritibacterivory was more commonplace at high calcium. Spinification rather aligned with high phytoplankton abundance and trophic status but not with low Ca. Only aluminium, with a vector opposite

to FG9 and FG6, and iron, for which the small detritibacterivores of FG6 showed stronger affinity, were variables of secondary importance. Iron aptly associates with organic colloids, which may represent an appropriate food source for the latter.

Table 12. General results of RDA on the representation of functional groups with selected local and setting variables.

	Axis 1	Axis 2	Axis 3
eigenvalue (λ)	0.079	0.038	0.013
species–environment correlation	0.58	0.47	0.34
% variance species data	7.9	3.8	1.3
% variance species–environment	59.2	28.6	10.1
F-ratio		15.3	
sum λ, *F*-ratio, *p* all axes		0.133, 6.9, 0.001	

Table 13. Variables retained by forward selection and their contribution to explained variance of functional-group representation by constrained and partial constrained RDA. Ordered by unique effect.

		Constrained—Marginal Effect				Partial Constrained—Unique Effect			
Variable	p_{Holm}	**% Data**	λ_1/λ_2	***F***	***p***	**% Data**	λ_1/λ_2	***F***	***p***
pGOP	0.014	0.009	5.2	0.20	10.1	0.001	5.8	0.26	11.1
Ca	0.014	0.009	3.0	0.11	5.7	0.002	3.7	0.14	7.0
Fe	0.014	0.009	1.5	0.05	2.8	0.012	3.3	0.12	6.0
Al	0.014	0.009	2.8	0.11	5.2	0.001	2.4	0.09	4.5

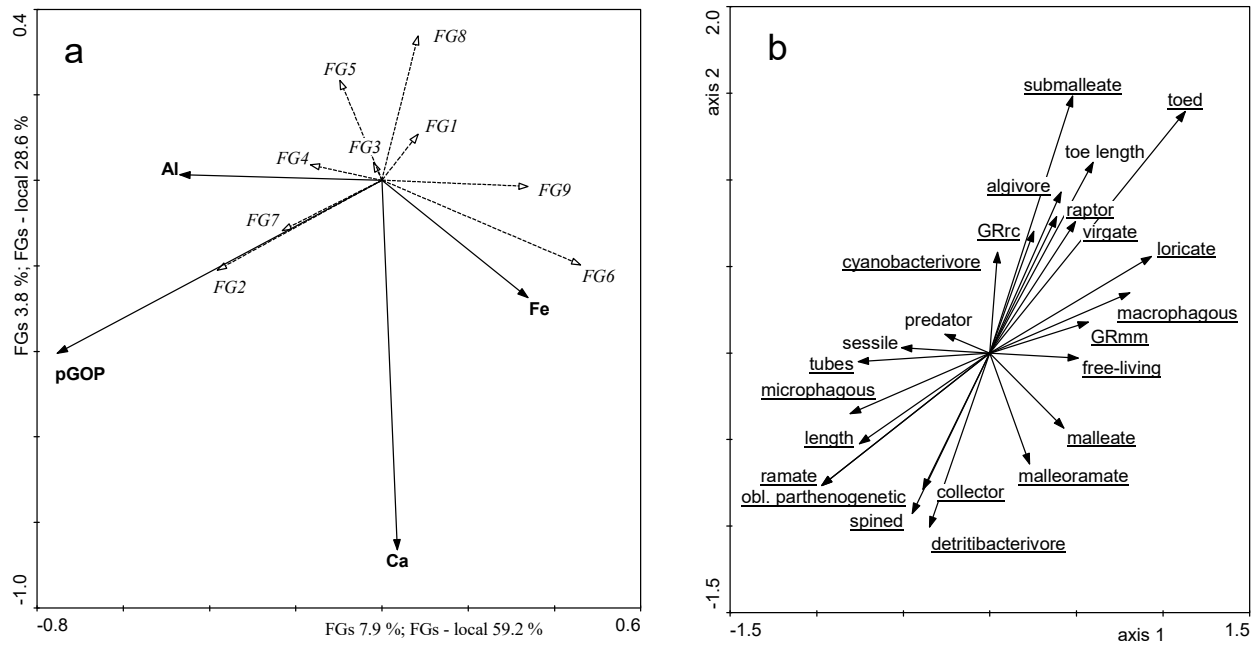

Figure 12. RDA ordination of functional groups with selected local and setting variables: (**a**) functional groups, axes 1 and 2; (**b**) CWMs and GRs with Pearson R $p \leq 0.05$ for any of both axes as passive variables, axes 1 and 2.

No appropriate beta regression models were obtained for FG1, FG3 and FG4. The first two FGs were very poorly represented, but even the proportion of the more abundant tube-forming FG4 hardly correlated with any of the measured site variables (Table S11). The models for FG5, FG8, FG9 and, particularly, FG7, with pGOP as the only variable, were

also very feeble and only FG2 and FG6 were captured reasonably well (Table 14). Several models included pGOP (positively FG2 and FG7, negatively FG6 and FG9), reflecting the RDA results, but less convincingly so for FG7 and FG9 (Figure 13). For the plankters of FG2, pGOP ($R = 0.33$, $p = 5 \times 10^{-6}$) and chl *a* ($R = 0.33$, $p = 1 \times 10^{-5}$) were even the most strongly associated variables. Although receiving the highest model coefficient, Ca showed only a weak slope for FG5 ($R = -0.24$, $p = 0.001$). The correlation with heathland ($R = 0.33$, $p = 5 \times 10^{-6}$) was actually stronger. The inclusion of wooded shoreline as relevant for FG5 was apparently due to a limiting rather than a linear relation. EC was retained for FG6 with a positive coefficient and as marginally negative for FG8. FG6 and FG9 tended to be low with higher Al and FG9 increased somewhat with Fe (Figure 13). The inclusion of EC in the model for FG6 concurred with positive correlations for a range of cations as well as chloride (Table S11), but in spite of a stronger association with aromatic organics (A254 $R = 0.37$, $p = 2 \times 10^{-7}$), the inverse relation with pGOP ($R = -0.26$, $p = 4 \times 10^{-4}$) appeared to be more consistent. Interestingly, this group of smaller-sized collectors responded differently to phosphorus compounds (e.g., ortho-P $R = 0.25$, $p = 5 \times 10^{-4}$), suggesting somewhat better representation in nutrient and organically rich yet less productive conditions. CODp retained the highest coefficient in the model for FG8, the *Lecane* group, but apparently controlling its proportion only at the highest concentrations. Negative correlations also occurred, however, with carbonates, phosphorus, silica, organic nitrogen and phytoplankton variables (Table S11), all hinting at less eutrophic conditions. Finally, next to pGOP, aluminium and iron played a contrasting role in the model for the algivores of FG9. Only the latter showed a significant linear correlation ($R = 0.26$, $p = 4 \times 10^{-4}$) and, together with a tendency for smaller dimensions and other correlating variables, pointed towards stronger development in seepage-fed alluvial ponds.

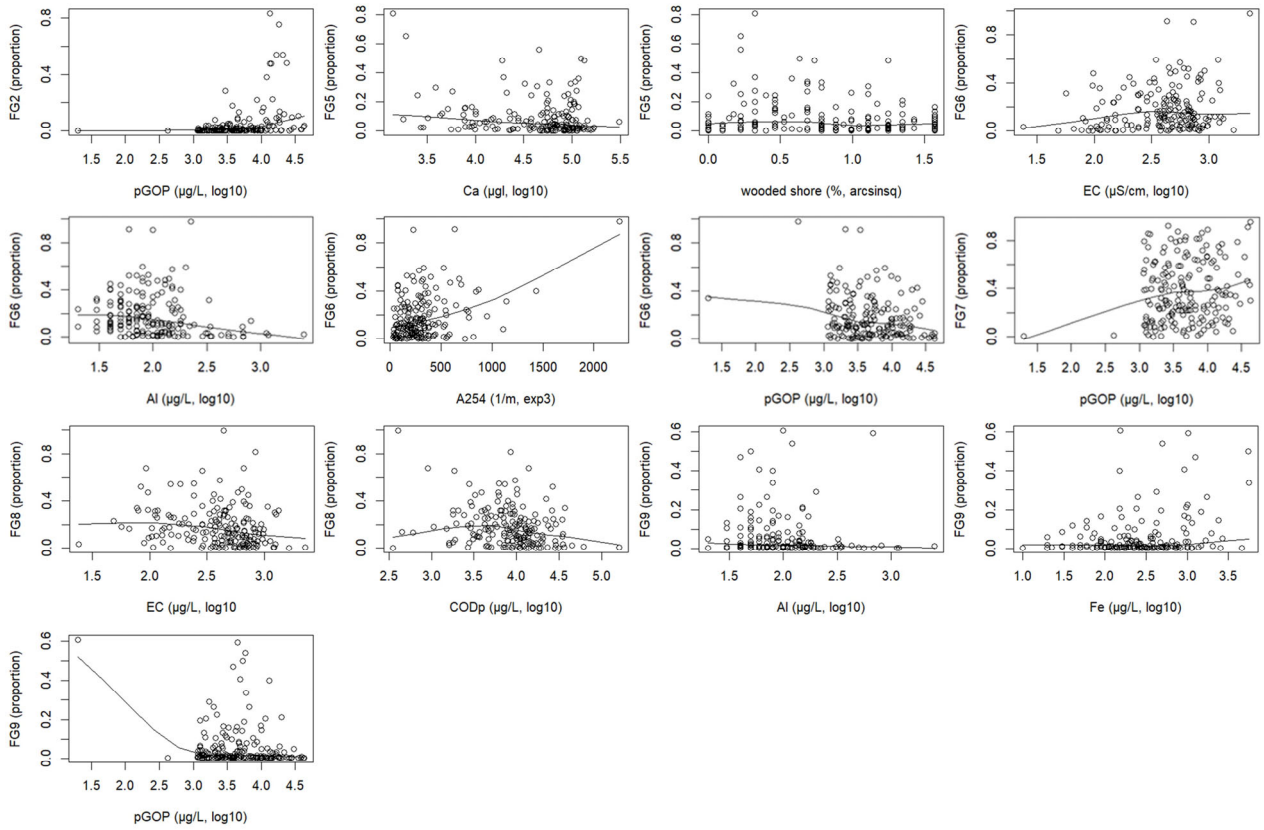

Figure 13. Scatter plots with loess smoother of environmental variables retained in the regression models for functional groups with local and setting variables.

Table 14. Regression models for GRrc, GRmm and functional-group proportions with local and setting variables. Efron pseudo-R^2 followed by Veall–Zimmermann pseudo-R^2 for GLM and pseudo-R^2 according to Zeileis et al. [126] for beta regressions.

	GRrc			**GRmm**			**FG2**			**FG5**			**FG6**			**FG7**			**FG8**			**FG9**		
model	glm, Gaussian, identity												beta regression, logit link, ml, φ identity link											
D_0, *df*	42.20, 183			10.35, 183																				
D, *df*	38.09, 181			9.02, 180																				
pseudo-R^2	0.10, 0.16			0.13, 0.12			0.20			0.09			0.29			0.05			0.12			0.12		
log-L, *df*	−116.18, 4			16.38, 5			424.7, 3			264.2, 4			158.7, 6			26.1, 3			132.9, 4			385.8, 5		
	coeff. ± SE	*t*	*p*	coeff. ± SE	*t*	*p*	coeff. ± SE	*z*	*p*	coeff. ± SE	*z*	*p*	coeff. ± SE	*z*	*p*	coeff. ± SE	*z*	*p*	coeff. ± SE	*z*	*p*	coeff. ± SE	*z*	*p*
φ							7.36 ± 0.96	7.65	2×10^{-14}	7.11 ± 0.82	8.68	$<2 \times 10^{-16}$	5.48 ± 0.58	9.46	$<2 \times 10^{-16}$	2.93 ± 0.27	10.77	$<2 \times 10^{-16}$	4.27 ± 0.44	9.61	$<2 \times 10^{-16}$	7.92 ± 1.00	7.90	3×10^{-15}
intercept	1.61 ± 0.34	4.67	6×10^{-6}	0.60 ± 0.19	3.14	0.002	−5.24 ± 0.61	−8.60	$<2 \times 10^{-16}$	0.46 ± 0.65	0.70	0.48	−0.15 ± 0.82	−0.19	0.85	−2.25 ± 0.61	−3.68	2×10^{-4}	2.16 ± 0.83	2.59	0.009	−1.26 ± 0.72	−1.74	0.08
EC	-	-	-	-	-	-	-	-	-	-	-	-	0.63 ± 0.20	3.07	0.002	-	-	-	−0.51 ± 0.21	−2.44	0.01	-	-	-
Al	-	-	-	-	-	-	-	-	-	-	-	-	−0.70 ± 0.21	−3.41	6×10^{-4}	-	-	-	-	-	-	−0.41 ± 0.21	−1.95	0.05
Fe	-	-	-	0.10 ± 0.04	2.94	0.004	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.37 ± 0.14	−2.60	0.009
Ca	−0.25 ± 0.07	−3.41	8×10^{-4}	-	-	-	-	-	-	−0.54 ± 0.14	−3.82	1×10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-
CODp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	−0.58 ± 0.17	−3.36	7×10^{-4}	-	-	-
A254	-	-	-	-	-	-	-	-	-	-	-	-	0.001 ± 0.206	−3.41	6×10^{-4}	-	-	-	-	-	-	-	-	-
pGOP	-	-	-	−0.13 ± 0.04	−3.50	6×10^{-4}	0.66 ± 0.16	4.13	4×10^{-5}	-	-	-	−0.55 ± 0.15	−3.72	2×10^{-4}	0.47 ± 0.17	2.87	0.004	-	-	-	−0.43 ± 0.16	−2.76	0.006
surface	-	-	-	−0.06 ± 0.03	−2.36	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
wooded shore	−0.19 ± 0.07	−2.71	0.007	-	-	-	-	-	-	−0.27 ± 0.014	−1.96	0.05	-	-	-	-	-	-	-	-	-	-	-	-

3.5.2. Guild Representation and Ratios

With only few exceptions, collectors were considerably more abundant than raptors in most samples, resulting in a median GRrc of only 0.12 and an average of c. 0.3 (Table 15). Raptors predominated by number in 10 samples, being more than twice as numerous in only two samples. Relative to macrophagy, microphagy, constituting more than 90% of the assemblage on average, was even more prevalent, and consequently GRmm varied less than GRrc. Both GRs were not interchangeable ($R = 0.45$, $p = 9 \times 10^{-11}$).

Table 15. General statistics for feeding-guild representation (%) and guild ratios. CV: variation coefficient.

CWM or GR	Average	SD	Minimum	P25th	P50th	P75th	Maximum	CV
raptor	17.0	16.7	0.0	4.5	11.0	25.9	81.5	101.8
collector	83.0	16.7	18.5	74.1	89.0	95.5	100.0	496.0
macrophagous	8.1	11.8	0.00	1.3	3.4	8.8	61.3	69.1
microphagous	91.8	11.8	0.39	91.0	96.6	98.7	100.0	780.4
GRrc	0.29	0.48	0.00	0.05	0.12	0.35	4.41	165.3
GRmm	0.12	0.24	0.00	0.01	0.04	0.10	1.59	49.5

The GLM model for GRrc, including only Ca ($R = 0.25$, $p = 7 \times 10^{-4}$) and wooded shoreline ($R = -0.20$, $p = 0.006$), remained quite weak (Table 14). As with the other variables correlating slightly negative at $p \leq 0.05$ with GRrc, heath ($R = 0.25$, $p = 7 \times 10^{-4}$) and pH excepted ($R = 0.23$, $p = 0.002$), few observations exerted strong leverage on the relation with wooded shoreline (Figure 14a and Table S4). GRmm also fitted poorly with pGOP ($R = -0.21$, $p = 0.004$) and Fe ($R = 0.21$, $p = 0.005$) as most important variables; surface area ($R = -0.20$, $p = 0.006$) and other size variables also correlated negatively (Figure 14b and Table S4), again with a limited number of sites determining relationships.

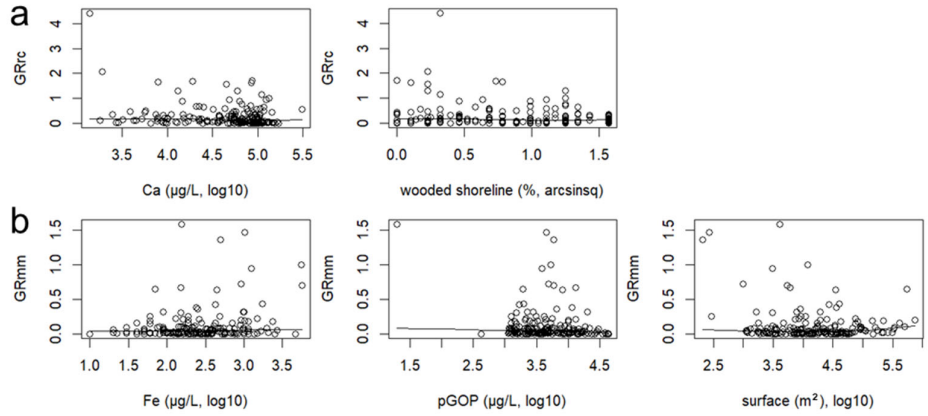

Figure 14. Scatter plots with loess smoother of environmental variables retained in the regression models for guild ratios with local and setting variables: (**a**) GRrc; (**b**) GRmm.

3.6. Assemblage Composition along Principal Environmental Gradients

RDA of the species composition and functional-group representation specified pH, TP and pGOP as the main local variables structuring the periphytic community. In the following paragraphs, the response of individual taxa (grouping all unidentified bdelloids as one OTU) to these gradients is examined in more detail, evaluating their indicator values for quartile ranges of these variables and abundance optima, as well as the areas of marked abundance change for responsive taxa. In addition, changes in the relative abundance of functional groups are explored. Although iron and aluminium were also selected as relevant variables, they remain beyond further consideration, as the former relates less to human impact and high aluminium concentrations were mainly limited to the most acid waters.

3.6.1. The pH Gradient

The four quartile intervals, i.e., pH < 7.1, 7.1–7.7, >7.7–8.1 and >8.1, discriminated primarily within the alkaline region. Indicator species analysis retained 48 taxa (22%) at $p \leq 0.05$ but only 16 at $p_{Holm} \leq 0.05$ (Table 16). The lowest quartile had nine reliable indicators, of which *Lecane stichaea*, *Taphrocampa annulosa*, *Dicranophorus luetkenii* and *Euchlanis meneta* presented the highest indicator values. Their WA optima ranged from pH 4.3 (*E. meneta*) to 6.5 (*D. luetkeni*) with tolerance ranges varying from 0.8 to 2 pH units (Table S12). *Lecane stichaea* (N2 = 7.1) and *Taphrocampa annulosa* (N2 = 8.2) were also rather well represented (Table S12), suggesting a more reliable estimation of their optima. The lowest optima within this interval (pH 4.1) were for the infrequently occurring *Bryceella stylata* and *Keratella serrulata*. *Lecane hamata* (N2 = 14.1) and *Lepadella acuminata* (N2 = 12.2) were well defined as circumneutral species, the latter however extending up to pH c. 8.0. In the more alkaline region, *Trichotria pocilum* (N2 = 3.4), *Colurella adriatica* (N2 = 38.9), *Euchlanis dilatata* (N2 = 9.1) and *C. colurus* (N2 = 11.4) were retained as reliable indicators with optima from pH 8.0 to 8.2. *Lecane flexilis* was rather common (N2 = 13.4) and might be bimodal in its distribution as it was not seen in at low pH. *Kellicottia longispina* was the only indicator attributed solely to the highest quartile and presented a rather narrow tolerance slightly above pH 8.

Table 16. Indicators for median pH quartiles with MPA indicator statistic and significance level and their WA optimum and tolerance range. $p_{Holm} \leq 0.05$ and tolerance $\leq$ 10% of gradient in bold.

pH Range	Lowest <7.1	Low 7.1–7.7	High >7.7–8.1	Highest >8.1	MPA Statistic	p	p_{Holm}	WA Optimum	WA Tolerance
Bryceella stylata	X	-	-	-	0.33	0.003	0.09	4.1	**3.9–4.2**
Keratella serrulata	X	-	-	-	0.29	0.01	0.21	4.1	**4.0–4.2**
Lecane perpusilla	X	-	-	-	0.35	0.002	0.07	4.3	3.8–4.9
Euchlanis meneta	X	-	-	-	0.45	<0.001	**<0.001**	4.3	3.9–4.7
Lecane clara	X	-	-	-	0.39	<0.001	**<0.001**	4.9	4.1–6.0
Lecane signifera	X	-	-	-	0.36	0.001	**0.04**	5.1	4.6–5.6
Lecane stichaea	X	-	-	-	0.57	<0.001	**<0.001**	5.2	4.3–6.3
Trichocerca bidens	X	-	-	-	0.43	<0.001	**<0.001**	5.4	4.5–6.4
Synchaeta pectinata	X	-	-	-	0.29	0.01	0.21	5.6	4.9–6.4
Aspelta circinator	X	-	-	-	0.36	0.002	0.07	5.9	5.2–6.8
Taphrocampa annulosa	X	-	-	-	0.49	<0.001	**<0.001**	6.3	5.6–7.0
Kellicottia bostoniensis	X	-	-	-	0.42	<0.001	**<0.001**	6.3	5.9–6.7
Lepadella triba	X	-	-	-	0.35	0.003	0.09	6.4	5.7–7.3
Conochilus hippocrepis	X	-	-	-	0.29	0.02	0.21	6.4	**6.3–6.5**
Microcodon clavus	X	-	-	-	0.30	0.007	0.15	6.5	5.7–7.5
Dicranophorus luetkeni	X	-	-	-	0.46	<0.001	**<0.001**	6.5	6.0–7.2
Trichotria tetractis	X	-	-	-	0.30	0.016	0.21	6.6	5.7–7.7
Ptygura sp. 1	X	-	-	-	0.38	0.004	0.10	6.7	6.1–7.4
Trichocerca intermedia	X	X	-	-	0.33	0.008	0.17	5.9	4.7–7.4
Lecane ludwigii	X	X	-	-	0.35	0.005	0.13	6.8	6.5–7.1
Lecane hamata	X	X	-	-	0.58	<0.001	**<0.001**	6.9	6.4–7.5
Testudinella incisa	X	X	-	-	0.32	0.05	0.23	7.1	6.7–7.6
Trichocerca porcellus	X	X	-	-	0.49	0.04	0.23	7.2	6.3–8.2
Lepadella acuminata	X	X	-	-	0.56	0.001	0.04	7.2	6.5–7.9
Beauchampia crucigera	-	X	-	-	0.27	0.03	0.23	7.3	6.9–7.7
Aspelta curvidactyla	-	X	-	-	0.31	0.006	0.14	7.3	6.9–7.8
Dicranophorus forcipatus	-	X	-	-	0.38	0.002	0.07	7.6	7.3–8.0
Euchlanis oropha	-	X	-	-	0.29	0.03	0.23	7.6	7.4–7.8
Platyias quadricornis	-	X	-	-	0.31	0.01	0.19	7.6	7.4–7.8
Cephalodella gracilis	X	X	X	-	0.51	0.01	0.21	5.5	4.3–7.2
Colurella uncinata	X	X	X	-	0.52	0.01	0.19	7.4	6.9–7.9
Lecane bulla	X	X	X	-	0.42	0.05	0.23	7.5	7.0–8.0
Mytilina mucronata	X	X	X	-	0.50	0.03	0.23	7.7	7.1–8.3
Rotaria neptunia	-	X	X	-	0.33	0.02	0.23	7.7	7.6–7.8

Table 16. *Cont.*

pH Range	Lowest <7.1	Low 7.1–7.7	High >7.7–8.1	Highest >8.1	MPA Statistic	*p*	p_{Holm}	WA Optimum	WA Tolerance
Trichotria pocillum	-	X	X	-	0.42	0.001	0.04	8.0	7.6–8.4
Brachionus urceolaris	-	X	X	X	0.39	0.04	0.23	7.6	7.4–7.9
Cephalodella hoodii	-	X	X	X	0.53	0.03	0.23	7.7	7.1–8.2
Cephalodella megalocephala	-	X	X	X	0.49	0.003	0.09	7.9	7.3–8.5
Euchlanis deflexa	-	X	X	X	0.56	0.01	0.19	7.9	7.4–8.4
Brachionus quadridentatus	-	X	X	X	0.57	0.006	0.14	7.9	7.5–8.4
Colurella adriatica	-	X	X	X	0.81	<0.001	<0.001	8.0	7.5–8.6
Ptygura furcillata	-	X	X	X	0.52	0.009	0.18	8.0	7.6–8.4
Cephalodella segersi	-	X	X	X	0.61	0.001	0.04	8.1	7.4–8.8
Euchlanis dilatata	-	X	X	X	0.62	<0.001	<0.001	8.1	7.6–8.5
Lecane luna	-	X	X	X	0.45	0.002	0.07	8.1	7.6–8.7
Lecane flexilis	X	-	X	X	0.59	0.02	0.23	6.3	4.8–8.3
Colurella colurus	-	-	X	X	0.58	<0.001	<0.001	8.2	7.8–8.6
Kellicottia longispina	-	-	-	X	0.29	0.02	0.23	8.2	8.1–8.4

TITAN identified 28 pure and reliable decreasers (13%) and 13 increasers (6%) along the pH gradient and suggested a gradual disappearance of taxa between pH 4 and 6.8, followed by a more pronounced area of decline up to c. pH 7.2, maximising at pH 6.9 (Figure 15 and Table 17). Note from the previous paragraph that this entire pH interval was represented by only one-quarter of the samples. The community change point for decreasers appeared to be rather well defined within a pH unit (pH 6.2–7.3). *Bryceella stylata* and *Lecane perpusilla* were the only indicators for change in very acid conditions, declining already below pH 5 in agreement with their WA optima near pH 4 and tolerance ranges. Interestingly, certain *Lecane* species showed very similar points of major change close to neutrality (*Lecane clara* and *L. lunaris* near pH 6.9, and *L. hamata*, *L. ludwigii* and *L. ungulata* near pH 7.4). Increasing taxa were fewer and mainly came to the foreground one by one between pH 7.3 and 8.0, maximising at pH 7.7 and with only very few taxa emerging in number only at higher pH (*Lecane luna*, *Cephalodella segersi*). Overall, the largest compositional changes were observed from slightly below neutrality up to pH 7.8.

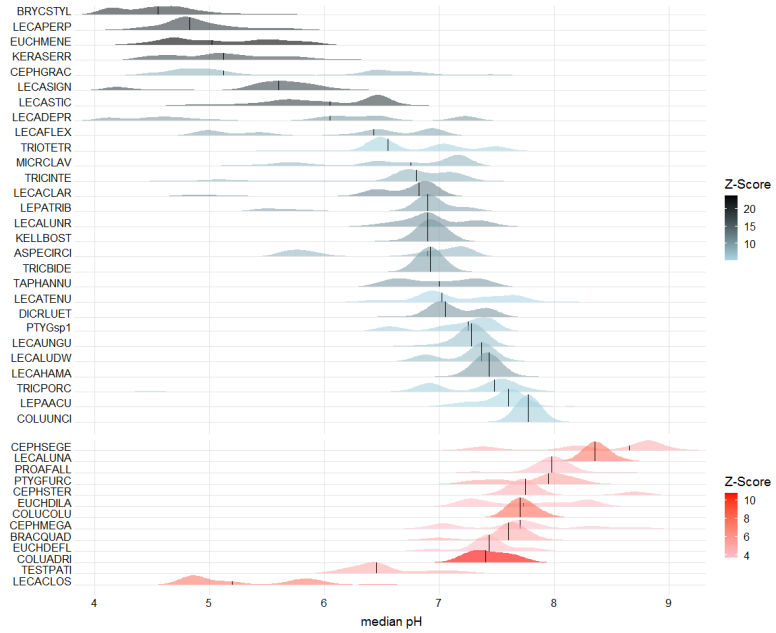

Figure 15. Probability density distribution of change points for pure and reliable decreasing (blue, negative *z* scores) and increasing taxa (red, positive *z* scores) along the pH gradient, arranged by median *z* score for 1000 bootstrap replicates (vertical lines). Acronyms as in Table 3.

Table 17. pH change points (cps) with confidence intervals for decreasing (fsumz−) and increasing (fsumz+) indicator taxa.

Median pH	cp	P5th	P10th	P50th	P90th	P95th
fsumz−	6.9	5.6	6.2	6.9	7.3	7.4
fsumz+	7.7	7.1	7.1	7.7	7.8	7.8

Only three functional groups showed differences in their relative abundance between the pH quartiles (Figure 16). The planktonic collectors of FG2 were marginally more abundant in the third quartile but hardly occurred at low as well as markedly alkaline pH, while FG8 and FG6, both representing smaller motile collectors, were somewhat better represented below pH 7 and between pH 7 and 7.8, respectively. Within-group variation was large and all distributions overlapped considerably.

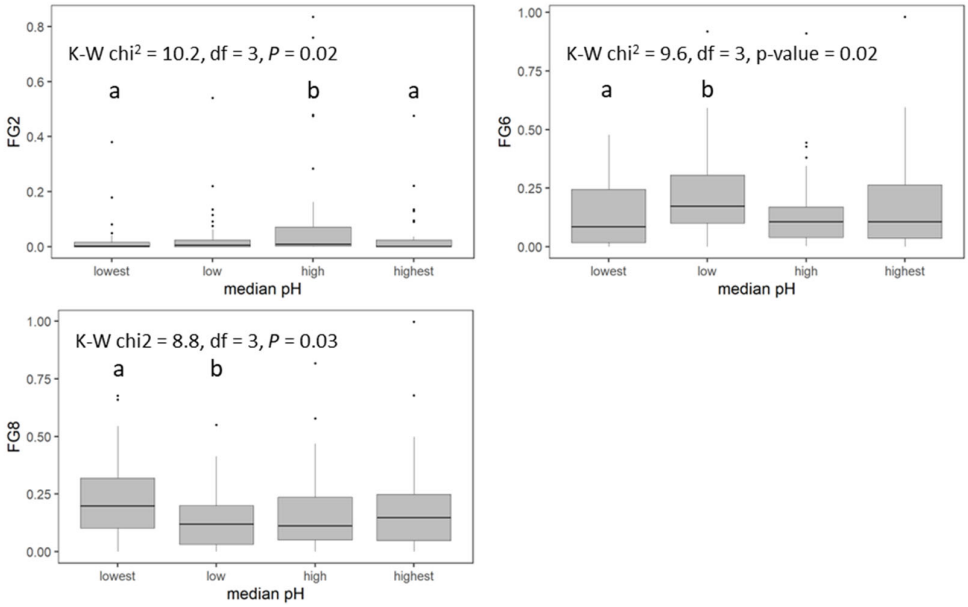

Figure 16. Tukey boxplots of the relative representation of functional groups by pH quartiles with significant differences (global Kruskal–Wallis test with lettering indicating differences at $p \leq 0.05$ for Conover–Iman all-pairs rank comparison test). pH groups: lowest <7.1, low 7.1–7.7, high >7.7–8.1, highest >8.1.

3.6.2. The Total Phosphorus Gradient

The four median TP classes were lowest <70 µg.L^{-1}, low 71–110 µg.L^{-1}, high 111–300 µg.L^{-1} and highest >300 µg.L^{-1}. Some 22 taxa (10%) seemed reliable indicators for at least one of these classes, with again their number decreasing from the lowest to the highest quartile (Table 18). *Lecane stichaea*, *Euchlanis meneta* (N2 only 3.6) and *Taphrocampa annulosa* had the highest indicator scores for the lowest quartile. *Lecane lunaris* (N2 = 22.2) and *L. flexilis* (N2 = 13.4) extended slightly higher up the TP gradient, receiving high indicator statistics for concentrations below the observed median. Together with nine other species, their WA optimum lay below our analysis threshold (Table S12). *Trichocerca tenuior* was most indicative for the second quartile but its WA optimum was within the third quartile. The species was not uncommon (N2 = 8.7) and its tolerance range was quite wide. *Trichocerca porcellus*, *Lecane bulla* and *Keratella cochlearis* also occurred primarily in nutrient-rich conditions but avoided the highest interval and had rather modest optima, whilst *Brachionus quadridentatus*, *Cephalodella segersi* and *Mytilina mucronata* extended from eutrophic conditions up to the most hypertrophic waters. *Rotaria neptunia* was the only indicator limited to the highest quartile, with an extreme TP optimum of 1.1 mg.L^{-1}.

Table 18. Indicators for median TP quartiles with MPA indicator statistic and significance level and their WA optimum and tolerance range. $Pp_{Holm} \leq 0.05$ and tolerance $\leq 10\%$ of gradient in bold.

TP Range (µg.L^{-1})	Lowest <70	Low 71–110	High 111–300	Highest >300	Statistic	p	p_{Holm}	WA Optimum	WA Tolerance
Bryceella stylata	X	-	-	-	0.33	0.003	**0.02**	<0.07	**<0.07**
Lecane ungulata	X	-	-	-	0.36	0.001	**0.01**	<0.07	**<0.07**
Aspelta curvidactyla	X	-	-	-	0.35	0.002	**0.02**	<0.07	**<0.07**
Microcodon clavus	X	-	-	-	0.34	0.003	**0.02**	<0.07	**<0.07**
Notommata tripus	X	-	-	-	0.37	0.001	**0.01**	<0.07	**<0.07**
Euchlanis meneta	X	-	-	-	0.44	<0.001	**0.002**	<0.07	**<0.07**
Lecane stichaea	X	-	-	-	0.50	<0.001	**0.002**	<0.07	**<0.07**
Trichocerca bidens	X	-	-	-	0.35	0.004	**0.02**	<0.07	**<0.07–0.080**
Taphrocampa annulosa	X	-	-	-	0.49	<0.001	**0.002**	<0.07	**<0.07–0.085**
Lecane perpusilla	X	-	-	-	0.32	0.005	**0.02**	<0.07	**<0.07–0.101**
Cephalodella apocolea	X	X	-	-	0.39	0.001	**0.01**	<0.07	**<0.07**
Lecane lunaris	X	X	-	-	0.74	<0.001	**0.002**	<0.07	**<0.07–0.084**
Lecane flexilis	X	X	-	-	0.63	<0.001	**0.002**	<0.07	**<0.07–0.135**
Trichocerca tenuior	-	X	-	-	0.36	0.005	**0.02**	0.149	<0.07–0.402
Trichocerca porcellus	X	X	X	-	0.52	0.002	**0.02**	<0.07	**<0.07–0.155**
Lecane bulla	X	X	X	-	0.46	0.002	**0.02**	0.075	**<0.07–0.132**
Keratella cochlearis	X	X	X	-	0.52	0.002	**0.02**	0.094	<0.07–0.315
Testudinella mucronata	-	X	X	X	0.59	<0.001	**0.003**	0.126	**<0.07–0.252**
Brachionus quadridentatus	-	X	X	X	0.60	<0.001	**0.002**	0.263	0.106–0.656
Cephalodella segersi	-	X	X	X	0.59	0.002	**0.02**	0.478	0.155–1.476
Mytilina mucronata	-	-	X	X	0.56	<0.001	**0.002**	0.406	0.165–1.002
Rotaria neptunia	-	-	-	X	0.36	0.004	**0.02**	1.133	0.737–1.740

The TP gradient was not independent of pH/alkalinity in these data, and consequently many of the decreasers identified for pH were also among those showing a negative response to TP (Figure 17). Thirty taxa (14%) were considered pure and reliable decliners, versus only fourteen increasers. The narrow clustering of decreasers at the lower end of the gradient is somewhat deceptive, being due to the high analysis threshold for TP (70 µg.L^{-1}), which possibly obscured declines occurring at lower median concentrations. Hence, fsumz− was also situated in this range (Table 19). Increasers were again less in number and much more smeared out, some even appearing in number only at quite extreme TP concentrations (*Colurella uncinata*, *Rotaria neptunia*). Accounting for filtering criteria, their change point was situated at 110 µg.L^{-1}, though with a confidence interval skewed towards higher values.

Table 19. pH change points (cps) with confidence intervals for decreasing (fsumz−) and increasing (fsumz+) indicator taxa.

Median TP (µg.L^{-1})	cp	P5th	P10th	P50th	P90th	P95th
fsumz−	6.9	5.6	6.2	6.9	7.3	7.4
fsumz+	7.7	7.1	7.1	7.7	7.8	7.8

The most distinct, but still relatively minor, shifts in FG representation with increasing TP were a slight decline of the raptorial groups FG1 and FG5 from the first to the second quartile, and a somewhat more important drop of FG8, small loricate collectors, at extremely high concentrations (Figure 18). FG4 and, perhaps, FG9, reached their best representation in the second and third quartile, respectively. FG3 was too poorly represented to consider.

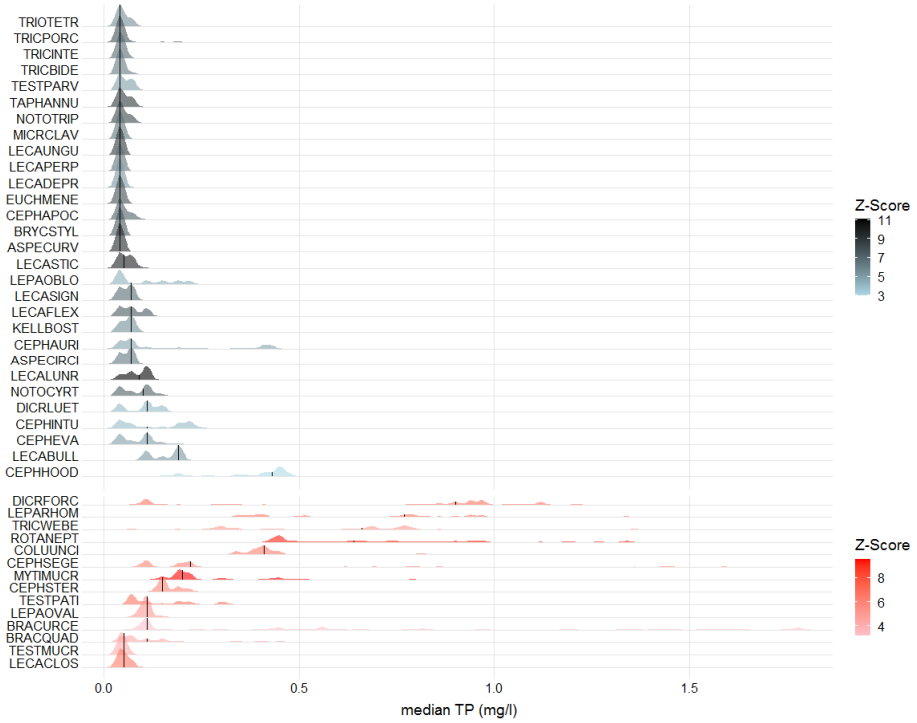

Figure 17. Probability density distribution of change points for pure and reliable decreasing (blue, negative *z* scores) and increasing taxa (red, positive *z* scores) along the TP gradient arranged by median *z* score for 1000 bootstrap replicates (vertical lines). Acronyms as in Table 3.

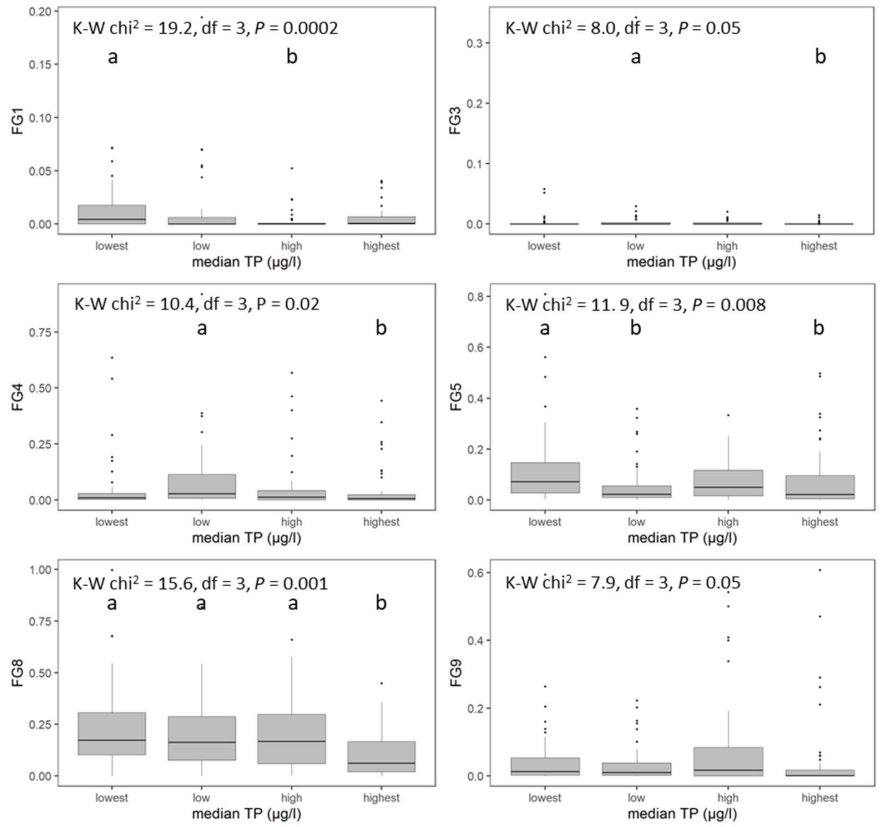

Figure 18. Tukey boxplots of the relative representation of functional groups by TP quartiles with significant differences (global Kruskal–Wallis test with lettering indicating differences at $p \leq 0.05$ for Conover–Iman all-pairs rank comparison test). TP groups: lowest < 70 µg.L^{-1}, low 71–110 µg.L^{-1}, high 111–300 µg.L^{-1}, highest >300 µg.L^{-1}.

3.6.3. The Phytoplankton Productivity Gradient

The number of potential indicator species (24; 12%) for the four intervals of potential oxygen production, resp. ≤1.23, >1.23–3.34, >3.34–8.95 and >8.95 mg.L^{-1}, was comparable to that for TP and their indicator value was somewhat lower on average (Table 20). After correction for sequential testing, *Lecane stichaea* remained the only robust indicator for the lower half of pGOP measurements. *Lepadella quadricarinata* (N2 = 12.7) and *Mytilina ventralis* (N2 = 10) were other taxa with tolerances limited to this region and a reasonable number of occurrences. *L. quadricarinata*, *Trichocerca porcellus* and *T. rattus* reliably characterised waterbodies up to the third quartile and only the latter extended into more elevated productivity levels. *Limnias ceratophylli*, *Brachionus quadridentatus* and *B. calyciflorus* identified conditions with the highest phytoplankton activity. Optima for the less abundantly occurring *Brachionus urceolaris* (N2 = 3.2) and *Pompholyx sulcata* (N2 = 4.2) were also among the highest (Table S12).

Table 20. Indicators for pGOP quartiles with MPA indicator statistic and significance level and their WA optimum and tolerance range. $p_{Holm} \leq 0.05$ and tolerance ≤ 10% of gradient in bold.

pGOP Range (mg.L^{-1})	Lowest ≤1.23	Low >1.23–3.34	High >3.34–8.95	Highest >8.95	Statistic	*p*	p_{Holm}	WA Optimum	WA Tolerance
Notommata tripus	X	-	-	-	0.31	0.02	0.19	0.8	0.2–2.7
Lecane stichaea	X	X	-	-	0.40	0.003	**0.05**	0.9	0.4–2.4
Aspelta circinator	X	X	-	-	0.31	0.02	0.21	1.5	0.8–2.8
Cephalodella apocolea	X	X	-	-	0.33	0.03	0.29	1.8	0.9–3.3
Taphrocampa annulosa	X	X	-	-	0.40	0.01	0.17	1.8	0.9–3.8
Lindia torulosa	-	X	-	-	0.24	0.05	0.29	1.1	0.6–2.1
Lepadella quadricarinata	X	X	X	-	0.53	<0.001	**0.005**	0.7	0.2–2.0
Mytilina ventralis	X	X	X	-	0.46	0.007	0.11	0.7	0.2–2.1
Trichocerca intermedia	X	-	X	-	0.31	0.03	0.29	1.1	0.3–3.8
Lepadella triptera	X	X	X	-	0.38	0.05	0.29	1.5	0.4–6.1
Trichotria pocillum	X	X	X	-	0.39	0.03	0.29	1.8	0.9–3.8
Trichocerca porcellus	X	X	X	-	0.51	0.002	**0.04**	1.6	0.7–3.8
Trichocerca rattus	X	X	X	-	0.60	0.002	**0.03**	2.2	0.8–6.1
Colurella obtusa	X	X	X	-	0.53	0.01	0.18	2.3	0.7–7.5
Trichocerca bidens	X	-	X	-	0.34	0.01	0.15	2.9	1.5–5.7
Euchlanis incisa	-	X	X	-	0.311	0.05	0.29	3.3	1.6–7.0
Lecane hamata	-	X	X	X	0.51	0.03	0.29	4.0	1.7–9.2
Cephalodella intuta	-	X	X	X	0.48	0.04	0.29	4.3	1.8–10.1
Stephanoceros fimbriatus	-	-	X	X	0.33	0.05	0.29	6.5	3.7–11.4
Limnias ceratophylli	-	-	X	X	0.49	0.001	**0.03**	9.5	4.0–23.0
Brachionus quadridentatus	-	-	X	X	0.69	<0.001	**0.002**	14.3	7.5–27.5
Brachionus urceolaris	-	-	X	X	0.41	0.01	0.15	15.2	8.3–27.6
Pompholyx sulcata	-	-	-	X	0.26	0.04	0.29	9.9	4.4–22.2
Brachionus calyciflorus	-	-	-	X	0.35	0.001	**0.02**	21.8	12.1–39.5

TITAN showed that the assemblage changed most abruptly at low pGOP values (Table 21). The community change point for decreasers, culminating at 2.6 mg.L^{-1}, was better constrained than the one for increasers, which appeared more gradually and already within the range where other taxa declined (Figure 19).

Table 21. pGOP change points (cps) with confidence intervals for decreasing (fsumz−) and increasing (fsumz+) indicator taxa.

Median pGOP	cp	P5th	P10th	P50th	P90th	P95th
fsumz−	2.6	1.3	1.3	2.6	4.9	5.6
fsumz+	3.9	3.0	3.2	4.2	9.0	9.3

Figure 19. Probability density distribution of change points for pure and reliable decreasing (blue, negative *z* scores) and increasing taxa (red, positive *z* scores) along the pGOP gradient arranged by median *z* score for 1000 bootstrap replicates (vertical lines). Acronyms as in Table 3.

Seventeen taxa (8%) showed a consistent decline along the gradient, with *Lepadella quadricarinata*, *Trichocerca porcellus*, *T. rattus*, *T. weberi*, *Lecane stichaea*, *Mytillina ventralis* and *Aspelta circinator* attaining the highest *z* scores. The first ones to diminish were *Mytilina mucronata* and *Trichocerca weberi*, shortly followed by a series of six taxa, from *Notommata cyrtopus* to *Lepadella quadricarinata* (Figure 19). Only eleven taxa (5%) were robust increasers: *Brachionus quadridentatus*, *B. calyciflorus*, *B. urceolaris*, *Keratella tecta*, *K. quadrata*, *Cephalodella sterea*, *C.* sp. 1, *Limnias ceratophylli* and *Lecane hamata*. Their emergence was smeared out along the gradient. With the exception of Bdelloidea indet., the first taxa showing a more concerted increase were *Limnias ceratophylli*, *Testudinella mucronata* and *Cephalodella* sp. 1, with *Keratella quadrata* and *Colurella adriatica* following thereafter. *Keratella tecta*, and even more so *Brachionus calyciflorus*, indicated the onset of the most productive conditions.

The proportion of five FGs differed among the pGOP quartiles (Figure 20). For three of them, FG1, FG4 and FG8, a post hoc test was unable to detect differences between individual groups and only a somewhat reduced abundance of FG1 and FG8 and increase in FG4 seemed to occur above the median. FG2 showed a more pronounced increase above 9 mg.L^{-1}, whereas FG6 decreased rather gradually from the lowest to the highest quartile. None of these changes was very substantial. The abundance of FG3 and FG7 did not differ between classes.

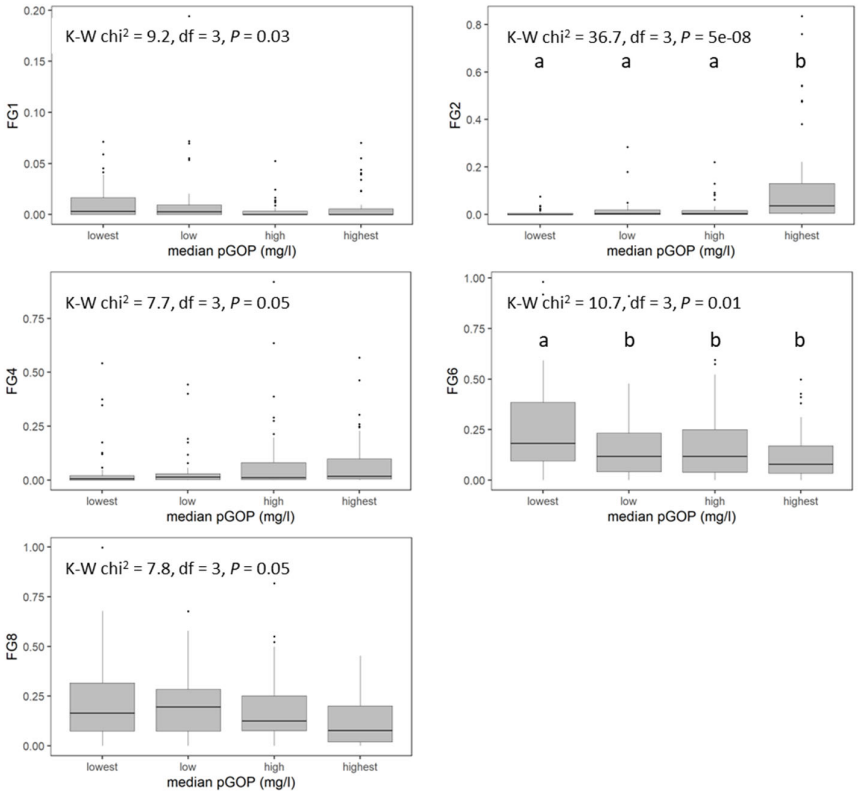

Figure 20. Tukey boxplots of the relative representation of functional groups by pGOP quartiles with significant differences (global Kruskal–Wallis test with lettering indicating differences at $p \leq 0.05$ for Conover–Iman all-pairs rank comparison test). pGOP groups: lowest $\leq$1.23 mg.L^{-1}, low >1.23–3.34 mg.L^{-1}, high >3.34–8.95 mg.L^{-1}, >8.95 mg.L^{-1}.

4. Discussion

With a total of 217 morphotaxa in 184 samples from a single habitat type (freshwater periphyton) and season (summer), the number of taxa compared quite favourably to comprehensive multi-seasonal inventories of even much larger biodiverse areas (e.g., [139]). Within a single sampling event, the number of taxa that could be tallied amounted to two-thirds of the current total number of rotifer species recorded in Belgium [140]. With many of the unobserved taxa occurring only in particular microhabitats or more saline conditions, this demonstrates the broad coverage of our survey. As our sampling did not account for any temporal differences, our results nevertheless remain indicative in this respect and more intensive sampling would undoubtedly have yielded an even more extensive species inventory [141,142], as also indicated by the species-accumulation curve. Nevertheless, higher taxonomic diversity is likely in less-impaired European regions. Ejsmont-Karabin and Karpowicz [29], for example, observed 148 taxa in the summer periphyton of 30 stratified Masurian lakes, sampled over the period 2009–2020, whereas the same number of (more varied) water bodies only accounted for 125.5 $\pm$ 8.8 taxa in Flanders. Overall, about a third of all individuals could not be identified to species level, also limiting the representativity of our diversity analyses, as well as rendering estimations of unobserved species richness less valid. Although terrestrial environments are considered as their principal habitat and diversity centre [143,144], bdelloids are commonly well represented in aquatic biofilms [24,145,146]. In our study, they numerically predominated on monogononts in 61% of the samples, leaving ample room for undetected diversity. Analyses of live samples complemented by genetic assays will be necessary to fill this particular knowledge gap. Our analyses also do not include the symbiont *Asciaporrecta difflugicola* De Smet, 2006, described from Alaska [147], where it was found living inside the test of *Difflugia labiosa* Wailes, 1919. Following its description, it was searched for in our most

Difflugia-rich samples and noted inhabiting the Difflugiidae *D. labiosa*, *D. urceolata* Carter, 1864 and *Netzelia tuberculata* (Wallich, 1864) in at least six of the examined water bodies. This shows that the occurrence of additional symbionts also cannot be fully excluded.

Species composition of monogononts agreed well with other periphyton studies, e.g., by the presence of numerous *Lecane* and *Trichocerca* species [23,29,148–150], although with 12% of all taxa, *Cephalodella* was particularly well represented. Most of the species are distributed world-wide [94,151,152]. One presumably non-native species was encountered: *Kellicottia bostoniensis*, a Nearctic-Neotropical species [151], occurred in relatively nutrient-poor, slightly acid conditions with high DOM. Almost one-third of the identified species were new records for Belgium, the majority commonly occurring throughout Europe [152].

Prevalence of singletons was within the typical range for temperate regions [153] and epiphyton (e.g., [29]). Average species richness and Shannon entropy were low compared to other periphyton surveys [29,146]. Four of the five most speciose samples (upper 0.25 percentile: >40 taxa) consisted entirely or predominantly of fine-leaved submerged plants (although one was from submerged twigs), suggesting that this type of vegetation, offering high structural complexity and potential for clogging with fine material, presented high niche availability. More complex artificial macrophytes support higher rotifer diversity [145] and more taxa occurred on emergent reeds, sedges and elodeids than on more simply structured nymphaeids in Polish riverine periphyton [146].

Aluminium and electrolyte concentration negatively affected observed species richness and other metrics of structural diversity. Both these variables operated in different parts of the regional water quality gradient, ranging from extremely acid and mineral-poor to alkaline and mineral-rich. In Flanders, atmospheric pollution typically resulted in high aluminium concentrations in acidification-sensitive soft-water ponds [154,155]. Reduced species richness of planktonic rotifers due to acidification is well documented from regions elsewhere [34,156,157] and toxicity tests revealed strong sensitivity of some species, possibly non-predatory ones especially, to aluminium [158]. Langley et al. [46] noted higher species richness in net-haul samples from urban ponds with increasing carbonate content and distance to roads—a possible source of, among others, metal pollution.

Species richness and Shannon diversity started to decline progressively at EC values of about 320 $\mu S.cm^{-1}$, which is lower than thresholds so far reported for (semi-)planktonic communities [159–161] and close to values considered in connection to extirpation of salt-sensitive macro-invertebrates (e.g., [162]). Consequently, a relatively minor increase in mineral loading could already lead to reduced rotifer diversity in waterbodies with lower ionic content. Notably, Onandia et al. [36] identified fertiliser-driven salinisation as an important process reducing alpha diversity of planktonic pond rotifers at genus level in Northeast Germany, but there are other common activities in the region besides fertilisation that can generate salt inputs (road salts, salt addition to fishing baits, etc.), whereas seawater intrusion occurs in coastal and estuarine areas.

Although pH presents a natural range in the region from c. 4.5 in dystrophic ponds to 8.5 where soils are richest in carbonates, and an even broader range was covered in this survey, pH was not of immediate importance for species richness. Mineral concentrations have increased in many waterbodies through intensive agricultural land use, increased leaching and pollution. Thus, the combined impacts of anthropogenic acidification and increased mineral loading may well have lowered diversity at both ends of the regional conductivity gradient, obscuring any effect of pH.

Species richness and diversity increased with the number of macrophyte taxa, a rough proxy for structural habitat diversity. Macrophyte species richness relates to general ecological status, being subject to, e.g., bank reinforcement, eutrophication and fish stocking. Substrate specificity and taxon-richness differences of rotifer assemblages in relation to macrophyte species identity within water bodies have been widely demonstrated [24,163–165]. Concurrent with the aforementioned observations of Lucena-Moya and Duggan [145] and with Viera et al. [166], who demonstrated higher rotifer richness on artificial leaves with veins than on those with a smooth surface, a positive relation between macrophyte

species richness and rotifer diversity was not unexpected. Declerck et al. [167] postulated that for many organism groups negative species-richness responses to lake productivity would be mediated by the turbidity-induced decline in submerged plant cover. As neither submerged plant cover, nor phytoplankton-related variables (pigments, pGOP, CODp, etc.) emerged as primary covariates of diversity measures in our study, and considering also that macrophyte species richness did not correlate with waterbody morphometry, we presume that structural complexity, or heterogeneity of habitable space [168,169], was a more important driver than merely plant abundance for rotifer richness. Together with similar observations for other biota (e.g., [170–173]) and functional aspects [174–176], this adds to the importance of vegetation characteristics such as species richness or complexity for conservation.

Although spanning a broad range of trophic conditions, alpha diversity related poorly with trophic status in our data. Other studies observed the lowest taxon richness for planktonic rotifers in the least productive conditions [41,177], suggesting limitation by low levels of food resources. Halabowski et al. [146] found that species richness also increased with nutrient concentrations for periphytic rotifers. Dodson et al. [178], however, observed a unimodal response of species richness to primary productivity in a plankton survey of 33 lakes, but no consistent relation in whole-lake enrichment experiments. They suggested food competition and adverse conditions as possible causes for a decrease in species number. Onandia et al. [36] and Ejsmont-Karabins et al. [179] also reported a decrease in plankton-species number at higher trophic levels, which Ejsmont-Karabins et al. [179] explained by a left-truncated humpback relation. We observed negative correlations for species richness and Shannon diversity with phosphorus compounds but lack analytical detail for TP to examine the relationship at lower concentrations, whereas a linear decline occurred with ortho-P. Other variables associated with productivity (chl *a*, pGOP, oxygen saturation) were, however, unrelated—unimodal nor linear—to diversity metrics, making control by food resources less likely. In addition to phosphorus, we noted negative correlations with reduced nitrogen levels (NH_4^+, KjN, TON, TN). Wen et al. [180] also observed lower values for species richness and other diversity metrics of planktonic rotifers at high ammonium concentrations, attributing this to ammonia toxicity, whereas Karpowicz et al. [181] found fewer species in anoxic than oxic water layers of Polish lakes. Overall, multiple interacting variables, in our case substrate heterogeneity, salinity, metal and ammonia toxicity, in particular, appear to influence species richness, resulting in a relationship that could easily be mistaken for a 'partial humpback' when attributed to a single covariate. In our correlational study, we are, however, unable to isolate the possible influence of several other mechanisms commonly associated with loss of macrophytes and anthropogenic disturbance, such as extreme abiotic conditions, contamination with micropollutants or competition related to food quality. Further research in more controlled and in natural, less impaired conditions is required to elucidate the actual relationship of periphytic rotifer diversity with productivity.

Supporting beta in addition to alpha diversity can strengthen strategies to improve overall biodiversity [182–185]. Accounting for abundance added little to total beta diversity, indicating that abundance of less frequently occurring taxa usually remained low. Predominance of the replacement (substitution) over the richness-difference component, and even more so when accounting for abundance, is the usual pattern [186], and suggested that communities were more strongly structured by environmental filtering and species interactions (historical events presumably being negligible at the scale of our study) [57,187] than by assembly processes influencing the number of taxa (colonisation, extinction, introduction, number of niches). This again reflected the extensive gradients covered in this study as well as the high dispersal capacity of rotifers [188]. High assemblage turnover also implies that regional biodiversity of this group will mainly depend on a large variety of water bodies supporting different communities and less on exceptionally species-rich sites [121,189].

The contribution of individual sites to total beta diversity (LCBD) showed limited variation. Elimination of taxa sensitive to anthropogenic pressures as well as unlimited

dispersal and mass effects will foster homogeneity. Since 55% of the ponds were directly connected to another water body and flooding events may result in additional connections, the latter cannot be dismissed (e.g., [190]). As commonly observed for other groups with many common species relative to rare ones [185,191,192], the LCBD of periphytic rotifers correlated negatively with their species richness. Consequently, focusing entirely on high species richness would also imply a risk of neglecting sites with unique conditions. The negative relation was sustained as species number increased, with the most speciose assemblages being least unique. The most species-rich assemblages are more likely to share more taxa [193]. Castro et al. [194] noted that near-pristine conditions may yield a positive relation, warranting further examination of this relation in less impaired regions. Various processes influence the nature of this relation, however, and there is much variation among biota [195], as illustrated, e.g., by a negative correlation for pelagic and littoral plankton rotifers in floodplain lakes of the Brazilian Pantanal [196] and a positive one for spiders along an Indian river [197]. The higher uniqueness of species-poor assemblages coincided with low pH, high aluminium and low pond density, evidencing the distinct community composition of low pH sites. The influence of individual variables on LCBD changed along the pH gradient, however. When only sites with circumneutral and alkaline pH were considered, a high number of macrophytes became a homogenising factor. This agrees well with the notion of a large fraction of shared taxa in the most species-rich assemblages and a plenitude of occupied niches in water bodies where diverse vegetation is present. The apparent effects of potassium and Gelbstoff on LCBD in non-acid conditions are less evidently explained but seem to coincide with generally higher concentrations of potassium (>c. 3 $mg.L^{-1}$) and lower colour at pH > 7.5. Potassium correlates with many of the measured variables and is a common fertiliser component. The negative relation of LCBD to pond density suggests that distance-dependent processes, i.e., dispersal limitation, are not entirely lacking. It was determined mainly by sites with either a very low (0–1 within 500 m) or a very high ($\geq$16 within 500 m) number of nearby ponds, hardly sloping in between. Ponds within large pond clusters were limited to the non-acid pond set (max. 72 ponds within 500 m versus 23 for pH < 6.5), whereas those with a pH < 6.5, being situated mainly in sandy infiltration areas, were generally somewhat more isolated, both by density (average 6.5 $\pm$ 5.9 versus 9.7 $\pm$ 9.9 ponds within 500 m) and number of hydrological connections (1.4 $\pm$ 2.1 versus 0.9 $\pm$ 1.0 in- and outflows). This explains why the number of nearby ponds was a significant variable for LCBD with the complete dataset but not for non-acid water bodies, illustrating the context dependency of connectivity-related signals.

Species replacement was the main component in the individual contribution of sites to regional beta diversity, suggesting emphasis on multiple sites for metacommunity conservation [122,198]. Both components showed opposed unimodal trends with increasing alpha diversity and, as expected [184,199], the abundance difference component of LCBD was highest at low as well as high species richness and Shannon entropy, whereas the substitution part was highest at intermediate alpha diversity. Hence a trade-off existed between alpha diversity and the functionally predominant part of beta diversity. Contrary to the incidence-based LCBD components of diatoms, zooplankton and macroinvertebrates in a Hungarian cluster of similar ponds [200], there were many ponds with high LCBDrepl (right-skewed) and few with higher LCBDabun (left-skewed), the latter originating from species thinning, especially at lower pH. Although relationships were weak, replacement-induced LCBD decreased with higher sodium, chloride and potassium content and tended to be higher within heathland, whereas LCBDabun related positively to sodium and humic substances. Inverse relations of both components to environmental correlates were noted previously, e.g., for diatoms [198]. In our case, results suggested that, although particular attention to non-acidified humic ponds may be warranted to support high-abundance populations of certain specialists, retaining low concentrations of monovalent ions overall might be more rewarding to ensure maximal assemblage turnover at regional scale. Da Silva Brito et al. [196] tentatively attributed less variation (as total LCBD) in rotifer plankton at higher conductivities to selection and dominance of a limited number of opportunistic

taxa. The decrease in alpha diversity and heterogeneity due to replacement differences at higher mineral concentrations observed here indicates a similar, albeit perhaps less intense, process in the periphyton. In any case, the result emphasises that the potential impact of salinisation on rotifer diversity is not limited to species erosion but may also include homogenisation.

LCBD, as well as its richness and replacement components, were poorly explained by environmental conditions in this study, indicating a strong influence of stochasticity or unaccounted processes and presumably also reflecting the broader environmental tolerances of many taxa. Consequently, possibilities for steering beta diversity effectively by environmental management and planning may be limited. Considering that LCBD tended to decrease with pond density, it does not seem that regional rotifer beta diversity will benefit much from pond restoration through digging or hydrological measures where ponds have disappeared, unless in the unlikely event that this would result in additional between- and within-waterbody vegetation heterogeneity. Obviously, this may be different at smaller spatial scales and other aspects of biodiversity, as well as functions and services provided by lentic waterbodies, will usually benefit also from such actions [201], so it should nevertheless be considered a no-regret measure where appropriate. Although environmental correlates suggested that stronger human impact reduced heterogeneity in non-acid conditions, the lack of a relation between LCBD or its components and any of the proxies for primary productivity indicated that management measures affecting vegetation composition more immediately than eutrophication control might be more effective to improve regional heterogeneity within this group. Yet, the contrary may be true regarding the incidence of infrequently occurring taxa, as highlighted by high values of the originality index for dilute, unproductive water bodies with little organic matter, situated on sand in a non-agricultural setting.

Analysis of species number, Shannon diversity, LCBD and IFO indicated that rather few of the sampled sites presented particularly species-rich or compositionally exceptional assemblages or stood out by their representation of rare taxa. All these criteria may be used, preferably in combination, to pinpoint sites of higher conservation value, although little concordance is expected in view of their limited correlation. Moreover, caution is required because degradation and disturbance easily lead to ecological uniqueness relative to less impaired conditions [36,57,202], as also clearly shown by the present study. Rotifer assemblages from the most acid water bodies, particularly those with high aluminium concentrations, were all quite unique, but in the absence of pristine conditions, the acidification footprint should be borne in mind and restoration, rather than conservation, may be more in order here.

In spite of the range of measured variables, by far the largest proportion of the variation in the species distribution of periphytic rotifers remained unaccounted for. This is a common feature in variation partitioning of species assemblages and may be attributed to a variety of statistical and biological reasons, such as stochastic and temporal variation, sparse data matrices, more complex responses than accounted for by RDA, inadequate estimation of variables, unaccounted for environmental gradients or species interactions [203,204]. Focusing on the explained variation [205], community composition and structure would be more strongly influenced by local conditions and water quality in particular, than by site setting or spatial factors at regional scale, thus identifying environmental filtering as the predominant process shaping assemblage composition. This is in line with results for planktonic rotifers [36,206]. Setting and spatial variables explained similar fractions, but their unique contribution was quite limited, albeit significant. In a survey across heterogenous landscapes and conditions, a large fraction of shared variation among differently scaled influences comes as no surprise. Interactions of the setting and spatial variable sets were focused on local conditions, reflecting stronger links between the complex of pH/alkalinity, trophic conditions, organic matter content and macrophyte vegetation on the one hand, and soil/land cover or geographic context on the other, than between the setting features and spatial context of water bodies. Although the results of variation partitioning with

MEMs depend on the sampling pattern [85,207], the significance of spatial structures and their scale nevertheless indicated the presence of biogeographic patterns within the region. Several of the selected site- and setting-specific variables, including pH, soil type and vegetation, also relate to broad-scale landscape gradients, particularly differences between the coastal and alluvial polders in the west, the southern loam belt and the eastern cover-sand areas. The unique spatial signal, which may concur, e.g., with climatological, historical or migration characteristics, was however quite weak ($\leq$1.5%), in accordance with fairly unrestricted dispersal at the scale of the study region. Nevertheless, some structure was present at 1–10 km scales, in line with the findings of Barta et al. [208] regarding the relevance of pond clusters for rotifer metacommunity structure. Acknowledging the shortcomings of the partitioning technique in accurately estimating the contribution of environmental and spatial variables to assemblage variation [110,209], the analysis suggested that primary concern for biodiversity management of this group would primarily lie with local environmental conditions, albeit that these will inevitably depend on surroundings and geography. Results concerning the local contribution to beta diversity and originality of assemblages are also in line with predominance of environmental filtering, partly modulated by or originating from human impact, in determining assemblage composition.

Considering local and setting variables only, the unique effects of non-chemical features were outweighed by water-column variables at the species level. This agrees with general expectations where between-waterbody variation is stronger in water chemistry than in physical conditions (e.g., [210]). pH and TP explained most of the variation in community turnover. The importance of the pH/alkalinity gradient was widely documented in studies of various habitats [25,49,211,212], although more often as being only of secondary importance in a multivariate context (Table S13). Its precedence in this study was likely due to the broad pH range in our dataset. The relevance of trophic variables for assemblage composition, here TP as well as pGOP, confirms that the long-recognised relation of rotifers to trophic status [8,38,50,213] extends to the epiphytic compartment. Species-specific responses to aluminium were already considered in relation to alpha diversity, but a few studies also demonstrated iron toxicity experimentally at concentrations within the range encountered in our survey [157,214,215]. Several physiological mechanisms may be involved [216], particularly at low oxygen/high Fe_2^+ concentrations, but effects at assemblage level do not appear to have been reported so far. Organic matter variables also associated with taxonomic shifts, contributing independently to the explained variation. The response to organic particulates closely followed that to pGOP, whereas it aligned more with TP and pH for dissolved substances. Oxygen production strongly depends on plankton and detritus content. Variables often mentioned in the context of organic pollution, such as BOD, oxygen saturation and nitrogen compounds ([38], Table S13), were not found to be of importance for assemblage turnover. Organic substances in the investigated water bodies mainly originate from breakdown of biomass that was produced in situ or within the vicinity, not from sewage, and most taxa associated with more nutrient-rich conditions have a wide tolerance for oxygen [181,217]. Furthermore, submerged cover and shoreline density also exerted weak effects. Contrary to their importance for rotifer (semi-)plankton, the role of submerged macrophyte abundance and morphometry for periphyton is likely more indirect.

Our combination of morphological, behavioural and physiological traits yielded nine functional groups, of which seven may be considered autochthonous for the periphyton and two as more or less planktonic. In comparison, Obertegger and Wallace [62] clustered all 138 rotifer genera into ten groups using a somewhat different trait selection (e.g., including corona type but not diet) and non-fuzzy coding. For the 54 genera in common, only one of their clusters matched entirely with one of our functional groups (cluster 1 and FG7, bdelloids). Three of their clusters referred to a single FG (nr 2 Collothecidae—FG4, nr 4 *Albertia*—FG1 and nr 8 *Asplanchna* and *Polyarthra*—FG3) and all others included members of up to four of our groups. Except for FG7, all functional groups were spread over several clusters. A species-specific classification, as suggested by Obertegger and Wallace [62] and

applied here, led to differentiation within *Cephalodella*, *Encentrum* (incl. *Pseudencentrum*), *Notommata*, *Ploesoma* and *Trichocerca*. Although planktonic rotifers may have been passively trapped in the periphyton layer, cf. *Polyarthra*, it cannot be excluded that active swimmers were also capable to sustain themselves within the voids of less dense periphyton canopies, or even commuted between periphyton and open water [23]. Notably, Spojlar et al. [24] recorded the highest numbers of the planktonic *Keratella cochlearis* on *Mentha aquatica* with the lowest periphyton density. By number, and presumably biomass, motile, microphagous species were the most important functional group, reflecting the abundance of small organic particles as the major food resource and the importance of rotifers in upcycling bacterial production and detrital matter in the periphytic food web, as well as the need for an active feeding strategy to search and maintain themselves within the most favourable 'feeding grounds' of the layered periphyton growth.

Even though it allowed accounting for all bdelloids, simplification of assemblage structure to functional groups slightly increased residual variation in redundancy analysis with local and setting variables, indicating that some information was lost. Additionally, the number of significant variables was reduced from nine to four. The variables pertaining to functional-group representation were a subset of those selected on a taxonomic basis. Although both levels of organisation showed considerable overlap in environmental determinants, the 'trophic response', represented by pGOP, became prominent and associated more directly to phytoplankton abundance when considering functional groups. Calcium, replacing pH, was the next most important structuring variable, followed by iron and, finally aluminium. Although we acknowledge that difficulties in trait assignment [62], lack of relevant traits (e.g., reproductive traits) or crudeness of certain classifications may have weakened underlying relations to some extent. This, together with only limited shifts in the relative abundance of individual functional groups along pH- and productivity-related gradients, suggests greater importance of the micro-environment and/or species interactions within the periphyton layer in the relative prevalence of successful trait syndromes. Our summer sampling favoured more mature biofilms where such processes would probably have gained in importance relative to water-column quality compared to an early development stage. Functional-group representation primarily related to differentiation by feeding mode, suggesting that food abundance and assortment were, to a certain extent, important. Availability of food items within the periphyton layer depends on its structure and developmental stage [3,218,219], which in turn relates to substrate age. Rotifer abundance increases with macrophyte age [163,164], supporting the idea that the functional importance of rotifers also increases as biofilms develop. On the other hand, Onandia et al. [36] observed that environmental conditions, in their case EC, internal nutrient recycling and primary productivity, explained much less variation in the rotifer plankton assemblage of temporary hypertrophic kettle-holes in autumn than in spring, and point to increased importance of biotic factors (competition, predation) during the season as a likely explanation. A comparison of periphyton assemblages between seasons in permanent water bodies with specific attention to biofilm characteristics and grazing would be relevant to further examine the relative importance of food versus species interactions in structuring assemblage composition.

Body length, detritibacterivory and (predator-inducible) spinification increased with phytoplankton productivity (cf. FG2 particularly *Brachionus* and FG7), whereas in less productive conditions, lorication, smaller size, macrophagy and raptorial features (trophi types, toes) were more commonplace (FG6, FG8, FG9). This parallels the larger body size and higher percentage of bacterivores with increasing food availability for planktonic rotifers [5,180,220,221]. Living at the interface, periphytic rotifers are well placed to respond to nutritional changes within the biofilm and profit from higher concentrations of suspended bacteria and detrital matter in more eutrophic conditions [222–225]. However, although longer toes might improve the capability to feed on planktonic algae and bacteria [47], toe length decreased as pGOP increased, again agreeing rather with more proximate food particles. It is not clear whether spine formation and lorication play a similar defensive

role in periphyton as in the plankton, where mesozooplankton exerts the main predation pressure [226,227]. So far, evidence for size-selective predation on periphytic rotifers is scarce, pointing rather to size and mobility as influencing vulnerability [228]. Possession of spines is also not independent from body size [229]. Small spines were proposed to facilitate burrowing into sediments [230] and this may also be an asset in thicker, more clogged periphyton canopies. Where food availability is less, smaller loricated species may rely more on inducible 'hidden' defences involving carapace strengthening [13], facing fitness costs only when deployment is required.

Although the relative abundance of the microphagous swimmers of FG2 increased slightly, higher productivity translated rather poorly into guild ratios or functional-group representation. GRrc remained insensitive and even GRmm responded rather erratically. Biomass-based GRs [9,96] might have led to a stronger response, but so far there appears to be little justification for the additional effort required to obtain the necessary data [11]. Guild ratios are probably too-coarse metrics for periphyton, which is usually collector-dominated and overwhelmed by microphagous rotifers, restraining their variation: GRrc was >1 for only 5.4% and GRmm for only 2.1% of all samples (not unimportantly with bdelloids contributing much more than in plankton). Less arbitrary fuzzy coding of micro- versus macrophagy could offer further improvement here. Possibly, more refined food-niche classifications, such as suggested for planktonic taxa [231], might also have improved sensitivity of functional groups, but these already encompassed the essentials. The response to productivity was also not consistent with the micro-/macrophagous split for all functional groups, and the fraction of small but microphagous taxa (FG6, and possibly FG8) even declined with pGOP. In a tropical eutrophic lake, Jiménez-Santos et al. [225] observed year-round dominance of raptorial over microphagous sessile rotifers by the number of individuals. Sessile taxa made up more than 50% of the assemblage in only four of our samples. The proportion of tube-dwelling taxa, particularly *Limnias ceratophylli*, and the CWM of tube formation, were slightly stimulated by increased phytoplankton abundance. In contrast, an experimental study with *L. ceratophylli* and *L. melicerta* suggested that sessile species are susceptible to higher densities of unicellular algae that may hamper their filter feeding [232]. The different outcome is possibly explained by differing and more variable food conditions in the wild.

There have been few attempts to estimate apparent autecological characteristics for rotifers (e.g., Duggan et al. [40] for the New Zealand Trophic Lake Index) and, consequently, differences in environmental optima between regions have not received much attention. Bērziņš and Pejler [49] graphically presented pH distributions for a large number of taxa with more than 50 observations in south and central Sweden. Compared to the pH at maximum abundance in Sweden, the median pH at maximum relative abundance in lower Belgium was at least 0.4 pH units higher for 68% of the species in common, whereas this was the case for 43% of the taxa when compared to the weighted-average optimum (Figure S5), with larger differences for, e.g., *Lindia torulosa* (+2.1 pH units for both comparisons), *Cephalodella intuta* (+2.6 and +1.6 pH units, respectively), *Kellicottia longispina* (+1.6 pH units) and *Trichocerca longiseta* (+2.3 and +2.0 pH units, respectively). On the other hand, species such as *Cephalodella gibba* (−3.6 and −1.3 pH units, respectively) and *Euchlanis meneta* (−2.0 and −1.8 pH units, respectively), occurred in markedly more acid conditions. It is not likely that the apparent shift to mostly higher pH optima from Sweden to Belgium suggested by our comparison merely results from methodological differences, such as in abundance estimation, or the length of the regional pH gradients, the latter being similar. Considerable intraspecific regional differences in environmental optima of biota are not uncommon (e.g., [233–236]) and it appears that rotifers also require an appropriate reference frame.

Since the classic work of Bērziņš and Pejler [49], many rotifers tend to be considered rather as pH generalists. Accounting for relative abundances, we nevertheless identified 22% of the observed taxa as useful pH indicators in our study area, most of them in non-alkaline waters, with more marked assemblage change occurring from circumneutrality to

c. pH 7.8. Only about half as many species showed some potential for indicating trophic status, either from the perspective of primary productivity (pGOP) or nutrients (TP). *Aspelta circinator*, *Cephalodella apocolea*, *Euchlanis meneta*, *Lecane lunaris*, *L. stichaea*, *Notommata cyrtopus*, *Taphrocampa annulosa* and *Trichocerca porcellus* declined with increasing pGOP as well as TP, but only *Brachionus urceolaris*, *B. quadridentatus* and *Testudinella mucronata* matched with a positive response. Major assemblage change points were situated at relatively low trophic status. With regard to TP, the decline of sensitive taxa at (median) TP concentrations below or near 70 $\mu g.L^{-1}$ suggests a sensitivity to nutrient enrichment in the same range as various other eutrophication indicators, e.g., chrysophyte biovolume, macrophyte coverage, cyanobacterial biovolume, macrophyte maximum colonisation depth and zooplankton biomass [237,238]. There appear to be no comparable studies relating to potential gross oxygen production, but the changepoint of 2.6 $mg.L^{-1}$ O_2 for decreasing taxa corresponds roughly to a median chl *a* concentration of c. 16 $\mu g.L^{-1}$ in our data. This is close to the chl *a* thresholds for macrophyte dominance, maximum depth of submerged macrophytes, cyanobacteria dominance and a good–moderate status boundary of 21–23 $\mu g.L^{-1}$ in shallow Central European lakes [239], as well as to thresholds for major changes in macrophyte coverage (18.5 $\mu g.L^{-1}$) and species richness (21.1 $\mu g.L^{-1}$) in a set of shallow lakes from Belgium and The Netherlands [240]. Hence, it seems that major changes in periphytic rotifer assemblages may concur with a more general regime shift.

As the use of the number of representatives from *Trichocerca* or *Lecane* has been suggested to assess trophic status from plankton, e.g., with the quotients #*Brachionus*/#*Trichocerca* [38] or #*Brachionus*/#*Lecane* [241], it is noteworthy that some of these taxa (cf. *Trichocerca bidens*, *T. intermedia*, *T. porcellus*, *T. rattus*, *T. tenuior*, *T. weberi*, *Lecane bulla*, *L. hamata*) were judged 'indicative' for very broad TP or pGOP intervals, or even presented quite elevated optima for these variables. Although informative about the relative position of taxa along gradients in our dataset, we nevertheless caution about relying on the apparent optima and tolerances reported here for quantitative inferences without additional corroboration. A physiological mechanism linking rotifers directly to these variables is lacking and interdependencies of nutrient and phytoplankton variables with vegetation characteristics, other interactions and indirect relations or unaccounted influences can lead to marked variation in species–environment relationships, especially for proxies of trophic status [242,243]. Furthermore, depending entirely on morphotaxa, it remains unknown to what extent cryptic diversity, which is common among rotifers (e.g., [244–246]), influences assumptions based on autecological profiles (see [247] for a current status report). Inferential precision will suffer if autecological differentiation is missed and taxon-based analyses, such as TITAN, may overlook potential indicators and lead to less clearly defined change points. Unfortunately, reliable exploration of more complex species response curves (as suggested, for instance, by the multimodal density profile of *Brachionus quadridentatus* for pGOP, Figure 18) requires more and more evenly distributed data than are available from this study [248,249].

5. Conclusions

The sensitivity of periphytic (monogonont) rotifer diversity to increased ionic concentrations makes their assemblages in water bodies with low mineral content vulnerable to even mild salinisation. The apparent influence of certain metals requires special attention and warrants further examination. Qualitative vegetation characteristics influenced alpha and beta diversity, presumably by controlling niche availability and heterogeneity, whereas submerged cover exerted only a limited effect on assemblage composition. Although special attention was given to include sites that appeared to be less impaired, few presented exceptional assemblages. Moreover, sites contributing more than average to beta diversity were often those that supported a distinct, yet species-poor, fauna as a result of anthropogenic acidification.

Environmental filtering, in particular water chemistry, explained most of the variation in assemblage composition. Although less important, morpho-structural features of

water bodies, their general setting and spatial context do not appear to be insignificant. Compositional changes depended most strongly on gradients in pH/alkalinity and trophic conditions.

Even though allowing a more complete appreciation of the assemblage, i.e., inclusion of all bdelloids, functional grouping did not support the same information as a species-based classification chiefly restricted to monogononts. Similar to planktonic assemblages, compositional shifts occurred along the productivity gradient at functional level, e.g., in size distribution and food acquisition, but these remained less pronounced. This was presumably due to the less specialised dietary requirements of abundantly occurring taxa, affluence of small food particles promoting dominance of detritibacterivores and more constant availability of different food types in periphyton growths, reducing limitation by food resources.

Consideration of traits allows more explicit consideration of functional structure, roles and processes (e.g., [250]) and enables comparisons from biogeographical realms down to specific ecosystems, independent of species identity. To date, in rotifer ecology this was mainly concerned with zooplankton feeding, but further elaboration of trait databases spanning a larger variety of traits as well as non-planktonic taxa offers opportunities to study a much broader range of aspects. We explored some of them using multiple traits, community-weighted means of their modalities as well as functional groups but were limited to a single community snapshot. Studies on the seasonal dynamics of trait representation in periphyton, as well as on their year-to-year variation, are needed to assess the potential of such approaches better, e.g., with respect to ongoing environmental change.

Autecological environmental attributes of rotifers also require more attention, as well as regionalisation. Although our study focused mainly on smaller-sized water bodies and the regional species pool of periphytic rotifers included only a limited number of potentially useful environmental indicators, significant assemblage shifts appeared to align with environmental criteria suggested to underpin acceptable ecological status for shallow freshwater in the Atlantic part of Europe. This encourages further investigation of the potential use of periphytic assemblages in ecological water quality assessment, notwithstanding the laboriousness of their analysis.

Overall, the variables considered in this study explained only a limited amount of the observed variation in diversity and assemblage composition. In addition to stochasticity and seasonal variation as possible causes, functional responses indirectly highlighted that the structure of the periphyton layer and its stage of development also need to be considered. Hence, micro-environmental conditions and species interactions within periphyton growths, as well as the effects of grazing and physical disturbance, remain important caveats in periphytic rotifer ecology that need to be addressed in future studies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15121214/s1, Figure S1: Species accumulation curve with 2 SD confidence band; Figure S2: Frequency distribution of observed species richness (a), Shannon diversity (b), Simpson diversity (c), LCBD (d), LCBDrepl (e), LCBDabun (f) and IFO (g) for all samples; Figure S3: Geographic distribution of LCBD (a), LCBD_non-acid (b), LCBDabun (c) and LCBDrepl (d). Higher than average ($p \leq 0.05$, unadjusted) values for LCBD (a,c,d) or LCBD_non-acid (b) in red; Figure S4: Geographical pattern of selected MEM scores in variation partitioning of species composition; Figure S5: Differences between the median pH at maximum percentage in lower Belgium and the pH at maximum abundance in Sweden (a) and between the weighted-average pH optimum in lower Belgium and the pH at maximum abundance in Sweden (b) for 81 species in common. Swedish data graphically reported by Bērziņš and Pejler [49]; Table S1: General statistics for major site variables (SD standard deviation, CV variation coefficient); Table S2: Linear correlations between measured variables with $p \leq 0.05$ (unadjusted); Table S3: Observed taxa with acronym and trait modalities. Nomenclature according to [94]; Table S4: Linear correlations ($p \leq 0.05$, unadjusted) between diversity metrics, IFO, GRrc, GRmm and local environmental variables, setting variables and MEMs. * $p_{Holm} \leq 0.05$–0.01, ** $p_{Holm} \leq 0.01$–0.001, *** $p_{Holm} \leq 0.001$; adjusted by group. Note that MEMs for all and non-acid sites differ; Table S5: Range of selected dbMEMs and their Pearson correlations

to site and setting variables. p 0.05–>0.001 plain text, p 0.001–>0.0001 underlined, $p \leq 0.0001$ bold (unadjusted); Table S6: Pearson correlations among CWMs and GRs. For traits with two mutually exclusive commodities, only one is shown. p_{Holm} 0.05–>0.001 plain text, 0.001–>0.0001 underlined, ≤0.0001 bold; Table S7: Pearson correlation of morphological CWMs (excl. food size and trophy type) with site variables. For traits with two mutually exclusive commodities (organisation, loricate, spined, mucus, tubes, toed) only one is shown. p 0.05–>0.001 plain text, p 0.001–>0.0001 underlined, $p \leq 0.0001$ bold (unadjusted); Table S8: Pearson correlations of morphological CWMs (food size and trophy type) with site variables. p 0.05–<0.001 plain text, p 0.001–<0.0001 underlined, $p \leq 0.0001$ bold (unadjusted); Table S9: Pearson correlation of physiological CWMs with site variables. For traits with two mutually exclusive commodities (reproduction), only one is included. p 0.05–>0.001 plain text, p 0.001–>0.0001 underlined, $p \leq 0.0001$ bold (unadjusted); Table S10: Pearson correlation of behavioural CWMs with site variables. For traits with two mutually exclusive commodities (habitat, motility, feeding), only one is included. p 0.05–>0.001 plain text, p 0.001–>0.0001 underlined (unadjusted); Table S11: Linear correlations of local and setting variables to relative abundance of functional groups. p 0.05–>0.001 plain text, p 0.001–>0.0001 underlined, $p \leq 0.0001$ bold (unadjusted); Table S12: Number of observations (N), Hill's N2, estimated optima and tolerance limits for median pH, TP and pGOP. Bold if confidence interval ≤10% of entire gradient; Table S13: Physical-chemical, morphological and vegetation variables influencing species composition of freshwater rotifer assemblages in multivariate multi-site studies from different parts of the world; variables ranked by relative importance (if several within same group, most important one only). [1] includes Ca, Mg, hardness; [2] includes Al, Fe; [3] includes COD, DOC; [4] includes pGOP, pigments; [5] includes Secchi depth; [6] wavelength not reported; [7] includes Dsl, depth, area; phosphorus and nitrogen stand for all their forms. Blue: (semi-)plankton or mixed origin; green: epiphyton; red: mosses. * Separate analyses for substrate and water chemistry. ** Analysis at family level. References [251–255] are cited in the supplementary materials.

Author Contributions: Conceptualisation and methodology, L.D.; data curation and formal analysis, L.D. and W.H.D.S.; rotifer identification and counting, W.H.D.S.; sample collection, L.D.; writing, original draft preparation, L.D. and W.H.D.S.; visualisation, L.D.; review and editing, L.D. and W.H.D.S. All authors have read and agreed to the published version of the manuscript.

Funding: Data collection was funded by the Flemish Government project VLINA C97/02 at the Biology Department of the University of Antwerpen.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Apart from privacy restrictions, the datasets generated during the current study are available from the corresponding authors on reasonable request.

Acknowledgments: Lieve Clement and Alfons Das performed laboratory chemical analyses. Marleen Coenen, Veerle Moons, Jo Packet, Dimitri Van Pelt, Bianca Veraart and Lucy Weiss contributed to data collection and sampling. Jo Packet prepared the maps for Figure 1. Hendrik Segers provided the current total of observed taxa in Belgium. We also thank the referees for their constructive comments and the publishers for the APC waiver.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gilbert, J.J.; Bogdan, K.G. Rotifer grazing: In situ studies on selectivity and rates. In *Trophic Interactions within Aquatic Ecosystems*; Meyers, D.G., Strickler, J.R., Eds.; Westview Press Inc.: Boulder, CO, USA, 1984; pp. 97–133.
2. Arndt, H. Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates)—A review. *Hydrobiologia* **1993**, *255*, 231–246. [CrossRef]
3. Weitere, M.; Erken, M.; Majdi, N.; Arndt, H.; Norf, H.; Reinshagen, M.; Traunspurger, W.; Walterscheid, A.; Wey, J.K. The food web perspective on aquatic biofilms. *Ecol. Monogr.* **2018**, *88*, 543–559. [CrossRef]
4. Liu, Y.; Tackx, M.; Dauta, A.; Julien, F.; Buffan-Dubau, E. Rotifers stimulate the specific N–NO_3 uptake rate in lotic phototrophic biofilms. *Freshw. Biol.* **2021**, *66*, 1245–1256. [CrossRef]
5. Stemberger, R.S.; Gilbert, J.J. Body size, food concentration, and population growth in planktonic rotifers. *Ecology* **1985**, *66*, 1151–1159. [CrossRef]
6. Pejler, B. Zooplanktic indicators of trophy and their food. *Hydrobiologia* **1983**, *101*, 111–114. [CrossRef]
7. Gilbert, J.J. Suppression of rotifer populations by *Daphnia*: A review of the evidence, the mechanisms, and the effects on zooplankton community structure. *Limnol. Oceanogr.* **1988**, *33*, 1286–1303. [CrossRef]

8. Karabin, A. Pelagic zooplankton (Rotatoria + Crustacea) variation in the process of lake eutrophication II. Modifying effect of biotic agents. *Ekol. Pol.* **1985**, *33*, 617–644.
9. Obertegger, U.; Manca, M. Response of rotifer functional groups to changing trophic state and crustacean community. *J. Limnol.* **2011**, *70*, 231–238. [CrossRef]
10. Goździejewska, A.M.; Kruk, M. Zooplankton network conditioned by turbidity gradient in small anthropogenic reservoirs. *Sci. Rep.* **2022**, *12*, 3938. [CrossRef]
11. Arcifa, M.S.; Barretto de Souza, B.; Simões de Morais-Junior, C.; Goulart Corrêa Bruno, C. Functional groups of rotifers and an exotic species in a tropical shallow lake. *Sci. Rep.* **2020**, *10*, 14698. [CrossRef]
12. Snell, T.W. Blue-green algae and selection in rotifer populations. *Oecologia* **1980**, *46*, 343–346. [CrossRef]
13. Yin, X.; Jin, W.; Zhou, Y.; Wang, P.; Zhao, W. Hidden defensive morphology in rotifers: Benefits, costs, and fitness consequences. *Sci. Rep.* **2017**, *7*, 4488. [CrossRef]
14. Zhang, H.; Hollander, J.; Hansson, L.-A. Bi-directional plasticity: Rotifer prey adjust spine length to different predator regimes. *Sci. Rep.* **2017**, *7*, 10254. [CrossRef] [PubMed]
15. Mikschi, E. Rotifer distribution in relation to temperature and oxygen content. *Hydrobiologia* **1989**, *186*, 209–214. [CrossRef]
16. Basiǹska, A.; Kuczýnska-Kippen, N.; Świdnicki, K. The body size distribution of *Filinia longiseta* (Ehrenberg) in different types of small water bodies in the Wielkoposka region. *Limnetica* **2010**, *29*, 171–182. [CrossRef]
17. Vadeboncoeur, Y.; Steinman, A.D. Periphyton function in lake ecosystems. *Sci. World* **2002**, *2*, 1449–1468. [CrossRef]
18. Gubelit, Y.I.; Grossart, H.-P. New methods, new concepts: What can be applied to freshwater periphyton? *Front. Microbiol.* **2020**, *11*, 1275. [CrossRef] [PubMed]
19. Hillebrand, H.; Kahlert, M. Effect of grazing and nutrient supply on periphyton biomass and nutrient stoichiometry in habitats of different productivity. *Limnol. Oceanogr.* **2001**, *46*, 1881–1898. [CrossRef]
20. Van der Grinten, E. Dynamic Species Interactions in Phototrophic Biofilms. Ph.D. Thesis, University of Amsterdam, Amsterdam, The Netherlands, 2004.
21. Blanco, S.; Romo, S.; Fernández-Aláez, M.; Bécares, E. Response of epiphytic algae to nutrient loading and fish density in a shallow lake: A mesocosm experiment. *Hydrobiologia* **2008**, *600*, 65–76. [CrossRef]
22. Traunspurger, W.; Wilden, B.; Majdi, N. An overview of meiofaunal and nematode distribution patterns in lake ecosystems differing in their trophic state. *Hydrobiologia* **2022**, *847*, 2665–2679. [CrossRef]
23. Duggan, I.C. The ecology of periphytic rotifers. *Hydrobiologia* **2001**, *446*, 139–148. [CrossRef]
24. Špoljar, M.; Fressl, J.; Dražina, T.; Meseljević, M.; Grčič, Z. Epiphytic metazoans on emergent macrophytes in oxbow lakes of the Krapina River, Croatia: Differences related to plant species and limnological conditions. *Acta Bot. Croat.* **2012**, *71*, 125–138. [CrossRef]
25. Wallace, R.L.; Snell, T.W.; Ricci, C.; Nogrady, T. Rotifera: Volume 1. Biology, ecology and systematics. In *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World*; No. 23; Segers, H., Ed.; Backhuys Publishers: Leiden, The Netherlands, 2006.
26. Irvine, K.; Balls, H.; Moss, B. The entomostracan and rotifer communities associated with submerged plants in the Norfolk Broadland—Effects of plant biomass and species composition. *Int. Rev. Ges. Hydrobio.* **1990**, *75*, 121–141. [CrossRef]
27. Jersabek, C.D. Distribution and ecology of rotifer communities in high-altitude alpine sites—A multivariate approach. *Hydrobiologia* **1995**, *313*, 75–89. [CrossRef]
28. Duggan, I.C.; Green, J.D.; Thompson, K.; Shiel, R.J. Rotifers in relation to littoral ecotone structure in Lake Rotomanuka, North Island, New Zealand. *Hydrobiologia* **1998**, *387*, 179–197. [CrossRef]
29. Ejsmont-Karabin, J.; Karpowicz, M. Rotifera in lake subhabitats. *Aquat. Ecol.* **2021**, *55*, 1285–1296. [CrossRef]
30. Lokko, K.; Virro, T. The structure of psammic rotifer communities in two boreal lakes with different trophic conditions: Lake Võrtsjärv and Lake Saadjärv (Estonia). *Oceanol. Hydrobiol. St.* **2014**, *43*, 49–55. [CrossRef]
31. Lokko, K.; Virro, T.; Kotta, J. Seasonal variability in the structure and functional diversity of psammic rotifer communities: Role of environmental parameters. *Hydrobiologia* **2017**, *96*, 287–307. [CrossRef]
32. Walsh, E.J. Habitat specific predation susceptibilities of a littoral rotifer to two invertebrate predators. *Hydrobiologia* **1995**, *313*, 205–211. [CrossRef]
33. Oh, H.-J.; Jeong, H.-G.; Nam, G.-S.; Oda, Y.; Dai, W.; Lee, E.-H.; Kong, D.; Hwang, S.-J.; Chang, K.-H. Comparison of taxon-based and trophi-based response patterns of rotifer community to water quality: Applicability of the rotifer functional group as an indicator of water quality. *Anim. Cells Syst.* **2017**, *21*, 133–140. [CrossRef]
34. Siegfried, C.A.; Blomfield, J.A.; Sutherland, J.W. Planktonic rotifer community structure in Adirondack, New York, USA lakes in relation to acidity, trophic status and related water quality characteristics. *Hydrobiologia* **1989**, *175*, 33–48. [CrossRef]
35. Bielańska-Grajner, I.; Gładysz, A. Planktonic rotifers in mining lakes in the Silesian Upland: Relationship to environmental parameters. *Limnologica* **2010**, *40*, 67–72. [CrossRef]
36. Onandia, G.; Maassen, S.; Musseau, C.L.; Berger, S.A.; Olmo, C.; Jeschke, J.M.; Lischeid, G. Key drivers structuring rotifer communities in ponds: Insights into an agricultural landscape. *J. Plankton Res.* **2021**, *43*, 396–412. [CrossRef] [PubMed]
37. Kolkwitz, R.; Marsson, M. Ökologie der tierischen Saprobien. Beiträge zur Lehre von der biologischen Gewässerbeurteilung. *Int. Rev. Ges. Hydrobio. Hydrogr.* **1909**, *2*, 126–152. [CrossRef]
38. Sládeček, V. Rotifers as indicators of water quality. *Hydrobiologia* **1983**, *100*, 169–201. [CrossRef]

39. Mäemets, A. Rotifers as indicators of lake types in Estonia. *Hydrobiologia* **1983**, *104*, 357–361. [CrossRef]
40. Duggan, I.C.; Green, J.D.; Shiel, R.J. Distribution of rotifers in North Island; New Zealand; and their potential use as bioindicators of lake trophic state. *Hydrobiologia* **2001**, *446*, 155–164. [CrossRef]
41. Duggan, I.C.; Green, J.D.; Shiel, R.J. Distribution of rotifer assemblages in North Island, New Zealand, lakes: Relationships to environmental and historical factors. *Freshwater Biol.* **2002**, *47*, 195–206. [CrossRef]
42. Čeirāns, A. Zooplankton indicators of trophy in Latvian lakes. *Acta U. Latv.* **2007**, *723*, 61–69.
43. Ejsmont-Karabin, J. The usefulness of zooplankton as lake ecosystem indicators: Rotifer trophic state index. *Pol. J. Ecol.* **2012**, *60*, 339–350.
44. May, L.; Wallace, R.L. An examination of long-term ecological studies of rotifers: Comparability of methods and results; insights into drivers of change and future research challenges. *Hydrobiologia* **2019**, *844*, 129–147. [CrossRef]
45. Pontin, R.M.; Langley, J.M. The use of rotifer communities to provide a preliminary national classification of small water bodies in England. *Hydrobiologia* **1993**, *255*, 411–419. [CrossRef]
46. Langley, J.M.; Kett, S.; Al-Khalili, R.S.; Humphrey, C.J. The conservation value of English urban ponds in terms of their rotifer assessment. *Hydrobiologia* **1995**, *313*, 259–266. [CrossRef]
47. Green, J. Associations of planktonic and periphytic rotifers in a tropical swamp, the Okavango Delta, Southern Africa. *Hydrobiologia* **2003**, *490*, 197–209. [CrossRef]
48. Martins, B.A.; Coelho, P.N.; Nogueira, M.G.; Perbiche-Neves, G. Composition and richness of monogonont rotifers from La Plata River Basin, South America. *Biota Neotrop.* **2020**, *20*, e20201001. [CrossRef]
49. Bērziņš, B.; Pejler, B. Rotifer occurrence in relation to pH. *Hydrobiologia* **1987**, *147*, 107–116. [CrossRef]
50. Bērziņš, B.; Pejler, B. Rotifer occurrence and trophic degree. *Hydrobiologia* **1989**, *182*, 171–180. [CrossRef]
51. Malekzadeh Viayeh, R.; Špoljar, M. Structure of rotifer assemblages in shallow waterbodies of semi-arid northwest Iran differing in salinity and vegetation cover. *Hydrobiologia* **2012**, *686*, 73–89. [CrossRef]
52. Pociecha, A.; Wilk–Woźniak, E.; Mróz, W.; Bielańska–Grajner, I.; Gadzinowska, J.; Walusiak, E. Biodiversity of rotifers in urban water reservoirs of Southern Poland. *Oceanol. Hydrobiol. St.* **2015**, *44*, 335–342. [CrossRef]
53. Kuczyńska-Kippen, N. Zooplankton structure in architecturally differentiated macrophyte habitats of shallow lakes in the Wielkopolska Region; Poland. *Oceanol. Hydrobiol. St.* **2006**, *35*, 179–191.
54. Edmondson, W.T. Ecological studies of sessile Rotatoria: Part I. Factors affecting distribution. *Ecol. Monogr.* **1944**, *14*, 31–66. [CrossRef]
55. Van Duinen, G.A.; Zhuge, Y.; Verberk, W.C.E.P.; Brock, A.M.T.; van Kleef, H.H.; Leuven, R.S.E.W.; van der Velde, G.; Esselink, H. Effects of rewetting measures in Dutch raised bog remnants on assemblages of aquatic Rotifera and microcrustaceans. *Hydrobiologia* **2006**, *565*, 187–200. [CrossRef]
56. Pourriot, R. Food and feeding habits of Rotifera. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* **1977**, *8*, 243–260.
57. Legendre, P. Interpreting the replacement and richness difference components of beta diversity. *Global Ecol. Biogeogr.* **2014**, *23*, 1324–1334. [CrossRef]
58. Grime, J.P. Benefits of plant diversity to ecosystems: Immediate, filter and founder effects. *J. Ecol.* **1998**, *86*, 902–910. [CrossRef]
59. Klimešová, J.; Janeček, Š.; Horník, J.; Doležal, J. Effect of the method of assessing and weighting abundance on the interpretation of the relationship between plant clonal traits and meadow management. *Preslia* **2011**, *83*, 437–453.
60. Mouillot, D.; Graham, N.A.J.; Villéger, S.; Mason, N.W.H.; Bellwood, D.R. A functional approach reveals community responses to disturbances. *Trends Ecol. Evol.* **2013**, *28*, 167–177. [CrossRef]
61. Cifoni, M.; Boggero, A.; Galassi, D.M.P.; Di Lorenzo, T. An overview of studies on meiofaunal traits of the littoral zone of lakes. *Water* **2021**, *13*, 473. [CrossRef]
62. Obertegger, U.; Wallace, R.L. Trait-based research on Rotifera: The holy grail or just messy? *Water* **2023**, *15*, 1459. [CrossRef]
63. Liu, P.; Wang, T.; Li, H.; Zhang, X.; Wang, L.; Jeppesen, E.; Han, B.-P. Functional diversity and redundancy of rotifer communities affected synergistically by top-down and bottom-up effects in tropical urban reservoirs. *Ecol. Indic.* **2023**, *155*, 111061. [CrossRef]
64. Verbruggen, C.; Denys, L.; Kiden, P. Belgium. In *Palaeoecological Events during the Last 15000 Years. Regional Syntheses of Palaeoecological Studies of Lakes and Mires in Europe*; Berglund, B.E., Birks, H.J.B., Ralska-Jasiewiczowa, M., Wright, H.E., Eds.; J. Wiley & Sons Ltd.: Chichester, UK, 1996; pp. 553–574.
65. Franklin, A.; Peeters, M.; Leentjes, V. A country profile. In *Biodiversity in Belgium*; Peeters, M., Franklin, A., Van Goethem, J.L., Eds.; Royal Belgian Institute of Natural Sciences: Brussel, Belgium, 2003; pp. 21–48.
66. Denys, L. Stilstaande zoete wateren. In *Natuurrapport 2001. Toestand van de Natuur in Vlaanderen: Cijfers Voor Het Beleid*; Kuijken, E., Boeye, D., De Bruyn, L., De Roo, K., Dumortier, M., Peymen, J., Schneiders, A., Van Straaten, D., Weyembergh, G., Eds.; Mededelingen van het Instituut voor Natuurbehoud 18; Instituut voor Natuurbehoud: Brussel, Belgium, 2001; pp. 79–87.
67. Denys, L.; Packet, J.; Scheers, K.; Smeekens, V.; Wils, C.; De Knijf, G.; Leyssen, A. Profielschets van stilstaande wateren in Vlaanderen. Een nieuw digitaal bestand voor het natuur- en biodiversiteitsonderzoek. *Natuur. Focus* **2019**, *18*, 128–135.
68. Denys, L.; Moons, V.; Veraart, B. (Eds.) *Ecologische Typologie en Onderzoek Naar een Geïntegreerde Evaluatiemethode voor Stilstaande Wateren op Regionale Schaal: Hoekstenen Voor Ontwikkeling, Herstel en Opvolging van Natuurwaarden*; Final report VLINA 97/02; University of Antwerp: Antwerpen, Belgium, 2000.
69. Koste, W. (Ed.) *Rotatoria. Die Rädertiere Mitteleuropas. Überordnung Monogononta*; Gebrüder Borntraeger: Berlin, Germany, 1978.

70. Segers, H. Rotifera 2. The Lecanidae (Monogononta). In *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World 6*; Dumont, H.J., Nogrady, T., Eds.; SPB Academic Publishing: Amsterdam, The Netherlands, 1995.
71. Nogrady, T.; Pourriot, R.; Segers, H. Rotifera 3: The Notommatidae and the Scaridiidae. In *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World 8*; Dumont, H.J., Ed.; SPB Academic Publishing BV: Amsterdam, The Netherlands, 1995.
72. De Smet, W.H. Rotifera 4: The Proalidae (Monogononta). In *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World 9*; Dumont, H.J., Ed.; SPB Academic Publishing: The Hague, The Netherlands, 1996.
73. De Smet, W.H.; Pourriot, R. Rotifera 5: The Dicranophoridae (Monogononta) and the Ituridae (Monogononta). In *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World 12*; Nogrady, T., Ed.; SPB Academic Publishing: The Hague, The Netherlands, 1997.
74. Nogrady, T. Rotifera 6: Asplanchnidae, Gastropodidae, Lindiidae, Microcodidae, Synchaetidae, Trochosphaeridae and *Filinia*. In *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World 18*; Nogrady, T., Segers, H., Eds.; SPB Academic Publishing: Amsterdam, The Netherlands, 2002.
75. De Smet, W.H. Preparation of rotifer trophi for light and scanning electron microscopy. *Hydrobiologia* **1998**, *387*, 117–121. [CrossRef]
76. Donner, J. *Ordnung Bdelloidea (Rotifera, Rädertiere)*; Bestimmungsbücher zur Bodenfauna Europas 6; Akademie Verlag: Berlin, Deutschland, 1965.
77. Golterman, H.L.; Clymo, R.S.; Ohnstad, M.A.M. *Methods for Physical and Chemical Analysis of Fresh Waters*; Blackwell Scientific Publications: Oxford, UK, 1978.
78. Denys, L. Relation of abundance–weighted averages of diatom indicator values to measured environmental conditions in standing freshwaters. *Ecol. Indic.* **2004**, *4*, 255–275. [CrossRef]
79. Denys, L. Calibration of littoral diatoms to water-chemistry variables in standing freshwaters of lower Belgium (Flanders): Inference models for sediment assemblages from historical samples. *J. Paleolimnol.* **2006**, *35*, 763–787. [CrossRef]
80. Denys, L. Water-chemistry transfer functions for epiphytic diatoms in standing freshwaters and a comparison with models based on littoral sediment assemblages (Flanders, Belgium). *J. Paleolimnol.* **2007**, *38*, 97–116. [CrossRef]
81. Osgood, R.A. Shoreline density. *Lake Reserv. Manage.* **2005**, *21*, 125–126. [CrossRef]
82. De Blust, G.; Paelinckx, D.; Kuijken, E. Up-to-date information on nature quality for environmental management in Flanders. In *Ecosystem Classification for Environmental Management*; Klijn, F., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1994; pp. 223–249.
83. Vriens, L.; Bosch, H.; De Knijf, G.; De Saeger, S.; Guelinckx, R.; Oosterlynck, P.; Van Hove, M.; Paelinckx, D. *De Biologische Waarderingskaart. Biotopen en hun Verspreiding in Vlaanderen en het Brussels Hoofdstedelijk Gewest*; Mededelingen van het Instituut voor Natuur–en Bosonderzoek. INBO.M.2011.1; Instituut voor Natuur–en Bosonderzoek: Brussel, Belgium, 2011.
84. Declerck, S.; De Bie, T.; Ercken, D.; Hampel, H.; Schrijvers, S.; Van Wichelen, J.; Gillard, V.; Mandiki, R.; Losson, B.; Bauwens, D.; et al. Ecological characteristics of small farmland ponds: Associations with land use practices at multiple spatial scales. *Biol. Conserv.* **2006**, *131*, 523–532. [CrossRef]
85. Borcard, D.; Legendre, P. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecol. Model.* **2002**, *153*, 51–68. [CrossRef]
86. Dray, S.; Legendre, P.; Peres-Neto, P.R. Spatial modelling: A comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecol. Model.* **2006**, *196*, 483–493. [CrossRef]
87. Dray, S.; Bauman, D.; Blanchet, G.; Borcard, D.; Clappe, S.; Guenard, G.; Jombart, T.; Larocque, G.; Legendre, P.; Madi, N.; et al. Package Adespatial. Multivariate Multiscale Spatial Analysis. 2022. Available online: https://github.com/sdray/adespatial (accessed on 1 April 2022).
88. Hiemstra, P. Package 'Automap'. Automatic Interpolation Package. 2022. Available online: https://cran.r-project.org/web/packages/automap (accessed on 5 May 2022).
89. Martini, S.; Larras, F.; Boyé, A.; Faure, E.; Aberle, N.; Archambault, P.; Bacouillard, L.; Beisner, B.E.; Bittner, L.; Castella, E.; et al. Functional trait-based approaches as a common framework for aquatic ecologists. *Limnol. Oceanogr.* **2020**, *66*, 954–964. [CrossRef]
90. Pourriot, R. Recherches sur l'écologie des Rotifères. *Vie Milieu (Suppl.)* **1965**, *21*, 1–224.
91. Stemberger, R.S. *A Guide to the Rotifers of the Laurentian Great Lakes. Report EPA–600/4–79–021*; U.S. Environmental Protection Agency: Cincinnati, OH, USA, 1979.
92. Pejler, B.; Bērziņš, B. On the ecology of Dicranophoridae. *Hydrobiologia* **1993**, *259*, 129–131. [CrossRef]
93. Fontaneto, D.; De Smet, W. Rotifera. In *Handbook of Zoology: Gastrotricha, Cycloneuralia and Gnathifera 3*; Schmidt-Rhaesa, A., Ed.; W. de Gruyter GmbH: Berlin, Germany, 2015; pp. 217–300.
94. Jersabek, C.D.; Leitner, M.F. The Rotifer World Catalog. 2013. Available online: http://www.rotifera.hausdernatur.at/ (accessed on 11 September 2019).
95. Smith, H.A.; Ejsmont-Karabin, J.; Hess, T.M.; Wallace, R.L. Paradox of planktonic rotifers: Similar structure but unique trajectories in communities of the Great Masurian Lakes (Poland). *Verh. Int. Ver. Limnol.* **2009**, *30*, 951–956. [CrossRef]
96. Obertegger, U.; Smith, H.A.; Flaim, G.; Wallace, R.L. Using the guild ratio to characterize pelagic rotifer communities. *Hydrobiologia* **2011**, *662*, 157–162. [CrossRef]
97. Gower, J.C. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* **1966**, *53*, 325–338. [CrossRef]

98. Pavoine, S.; Vallet, J.; Dufour, A.-B.; Gachet, S.; Daniel, H. On the challenge of treating various types of variables: Application for improving the measurement of functional diversity. *Oikos* **2009**, *118*, 391–402. [CrossRef]
99. De Bello, F.; Botta-Dukát, Z.; Lepš, J.; Fibich, P. Towards a more balanced combination of multiple traits when computing functional differences between species. *Methods Ecol. Evol.* **2021**, *12*, 443–448. [CrossRef]
100. De Bello, F.; Botta-Dukát, Z.; Lepš, J.; Fibich, P. Package 'Gawdis'. Multi-Trait Dissimilarity with More Uniform Contributions. 2021. Available online: https://github.com/pavel-fibich/gawdis (accessed on 12 May 2021).
101. Kaufman, L.; Rousseeuw, P.J. *Finding Groups in Data: An Introduction to Cluster Analysis*; Wiley–Interscience: Hoboken, NJ, USA, 1990.
102. Rousseeuw, P.J. Silhouettes: A graphical aid to the interpretation and validation of cluster analysis. *Comput. Appl. Math.* **1987**, *20*, 53–65. [CrossRef]
103. Maechler, M.; Rousseeuw, P.; Struyf, A.; Hubert, M.; Hornik, K. cluster: Cluster Analysis Basics and Extensions, r Package Version 2.1.2. 2021. Available online: https://CRAN.R-project.org/package=cluster (accessed on 17 April 2021).
104. Májeková, M.; Paal, T.; Plowman, N.S.; Bryndová, M.; Kasari, L.; Norberg, A.; Weiss, M.; Bishop, T.R.; Luke, S.H.; Sam, K.; et al. Evaluating functional diversity: Missing trait data and the importance of species abundance structure and data transformation. *PLoS ONE* **2016**, *11*, e0149270. [CrossRef]
105. Neury-Ormanni, J.; Vedrenne, J.; Wagner, M.; Jan, G.; Morin, S. Micro-meiofauna morphofunctional traits linked to trophic activity. *Hydrobiologia* **2020**, *847*, 2725–2736. [CrossRef]
106. Borcard, D.; Legendre, P.; Drapeau, P. Partialling out the spatial component of ecological variation. *Ecology* **1992**, *73*, 1045–1055. [CrossRef]
107. Borcard, D.; Legendre, P.; Avois-Jacquet, C.; Tuomisto, H. Dissecting the spatial structure of ecological data at multiple scales. *Ecology* **2004**, *85*, 1826–1832. [CrossRef]
108. Blanchet, F.G.; Legendre, P.; Borcard, D. Forward selection of explanatory variables. *Ecology* **2008**, *89*, 2623–2632. [CrossRef] [PubMed]
109. Peres-Neto, P.; Legendre, P.; Dray, S.; Borcard, D. Variation partitioning of species data matrices: Estimation and comparison of fractions. *Ecology* **2006**, *87*, 2614–2625. [CrossRef] [PubMed]
110. Gilbert, B.; Bennett, J.R. Partitioning variation in ecological communities: Do the numbers add up? *J. Appl. Ecol.* **2010**, *47*, 1071–1082. [CrossRef]
111. Bauman, D.; Vleminckx, J.; Hardy, O.J.; Drouet, T. Testing and interpreting the shared space-environment fraction in variation partitioning analyses of ecological data. *Oikos* **2019**, *128*, 274–285. [CrossRef]
112. Oksanen, J.; Simpson, G.; Blanchet, F.; Kindt, R.; Legendre, P.; Minchin, P.; O'Hara, R.; Solymos, P.; Stevens, M.; Soecs, E.; et al. Vegan: Community Ecology Package. R Package Version 2.6-2. 2022. Available online: https://CRAN.R-project.org/package=vegan (accessed on 17 April 2022).
113. Ter Braak, C.J.F.; Šmilauer, P. *Canoco for Windows 4.56*; Plant Research International: Wageningen, The Netherlands, 2009.
114. Ter Braak, C.J.F.; Šmilauer, P. *Canoco Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination*; Microcomputer Power: Ithaca, NY, USA, 2002.
115. Pavoine, S. Package Adiv. Analysis of Diversity. *Version: 2.2.* 2022. Available online: https://cran.r-project.org/web/packages/adiv/ (accessed on 6 October 2022).
116. Legendre, P.; Borcard, D.; Peres-Neto, P.R. Analyzing beta diversity: Partitioning the spatial variation of community composition data. *Ecol. Monogr.* **2005**, *75*, 435–450. [CrossRef]
117. Legendre, P.; De Cáceres, M. Beta diversity as the variance of community data: Dissimilarity coefficients and partitioning. *Ecol. Lett.* **2013**, *16*, 951–963. [CrossRef]
118. Podani, J.; Schmera, D. A new conceptual and methodological framework for exploring and explaining pattern in presence–absence data. *Oikos* **2011**, *120*, 1625–1638. [CrossRef]
119. Podani, J.; Ricotta, C.; Schmera, D. A general framework for analyzing beta diversity, nestedness and related community-level phenomena based on abundance data. *Ecol. Complex.* **2013**, *15*, 52–61. [CrossRef]
120. Schmera, D.; Podani, J.; Legendre, P. What do beta diversity components reveal from presence-absence community data? Let us connect every indicator to an indicandum! *Ecol. Indic.* **2020**, *117*, 106540. [CrossRef]
121. Baselga, A. Partitioning the turnover and nestedness components of beta diversity. *Global Ecol. Biogeogr.* **2010**, *19*, 134–143. [CrossRef]
122. Ruhí, A.; Datry, T.; Sabo, J.L. Interpreting beta-diversity components over time to conserve metacommunities in highly dynamic ecosystems. *Conserv. Biol.* **2017**, *31*, 1459–1468. [CrossRef]
123. Ejsmont-Karabin, J. Rotifer occurrence in relation to age, depth and trophic state of quarry lakes. *Hydrobiologia* **1995**, *313*, 21–28. [CrossRef]
124. Ripley, B.; Venables, B.; Bates, D.M.; Hornik, K.; Gebhardt, A.; Firth, D. Support Functions and Datasets for Venables and Ripley's MASS. Version 7.3-53. 2020. Available online: https://www.rdocumentation.org/packages/MASS/versions/7.3-58.3 (accessed on 7 March 2023).
125. Ferrari, S.; Cribari–Neto, F. Beta regression for modelling rates and proportions. *J. Appl. Stat.* **2004**, *31*, 799–815. [CrossRef]
126. Zeileis, A.; Cribari-Neto, F.; Gruen, B.; Kosmidis, I.; Simas, A.B.; Rocha, A.V. Package 'betareg'. Version 3.1–4. 2022. Available online: https://cran.r-project.org/web/packages/betareg/index.html (accessed on 9 February 2021).

127. R Core Team and Contributors Worldwide The R Stats Package. Version 4.2.1. 2022. Available online: https://stat.ethz.ch/R-manual/R-devel/library/stats/ (accessed on 23 June 2023).
128. Smithson, M.; Verkuilen, J. A better lemon squeezer? Maximum-likelihood regression with beta-distributed dependent variables. *Psychol. Methods* **2006**, *11*, 54–71. [CrossRef]
129. Hothorn, T.; Zeileis, A.; Farebrother, R.W.; Cummins, C.; Millo, G.; Mitchell, D. lmtest Version 0.9–40. Testing Linear Regression Models. 2022. Available online: https://cran.r-project.org/package=lmtest (accessed on 21 March 2021).
130. Signorell, A.; Alfons, A.; Anderegg, N. 112 Others DescTools. Tools for Descriptive Statistics. Version: 0.99.47. 2022. Available online: https://cran.r-project.org/web/packages/DescTools/index.html (accessed on 23 October 2022).
131. De Cáceres, M.; Legendre, P.; Moretti, M. Improving indicator species analysis by combining groups of sites. *Oikos* **2010**, *119*, 1674–1684. [CrossRef]
132. Dufrêne, P.; Legendre, P. Species assemblages and indicator species: The need for a flexible asymmetrical approach. *Ecol. Monogr.* **1997**, *67*, 345–366. [CrossRef]
133. De Cáceres, M.; Jansen, F.; Dell, N. Package Indicspecies. Relationship between Species and Groups of Sites. 2022. Available online: https://emf-creaf.github.io/indicspecies/ (accessed on 4 March 2022).
134. Birks, H.J.B.; Line, J.M.; Juggins, S.; Stevenson, A.C.; ter Braak, C.J.F. Diatoms and pH reconstruction. *Phil. T. R. Soc. B.* **1990**, *327*, 263–278. [CrossRef]
135. Sathicq, M.B.; Nicolosi Gelis, M.M.; Cochero, J. Optimos Prime Helps Calculate Autoecological Data for Biological Species. Version: 0.1.2. 2020. Available online: https://cran.r-project.org/web/packages/optimos.prime (accessed on 14 October 2022).
136. Baker, M.E.; King, R.S. A new method for detecting and interpreting biodiversity and ecological community thresholds. *Methods Ecol. Evol.* **2010**, *1*, 25–43. [CrossRef]
137. Baker, M.E.; King, R.S.; Kahle, D. Package Threshold Indicator Taxa Analysis. Version: 2.4.1. 2020. Available online: https://CRAN.R-project.org/package=TITAN2 (accessed on 7 December 2020).
138. Pohlert, T. PMCMRplus package. Calculate Pairwise Multiple Comparisons of Mean Rank Sums. Extended. 2022. Available online: https://cran.r-project.org/web/packages/PMCMRplus/ (accessed on 17 August 2022).
139. Brown, P.D.; Schröder, T.; Ríos-Arana, J.V.; Rico-Martinez, R.; Silva–Briano, M.; Wallace, R.L.; Walsh, E.J. Patterns of rotifer diversity in the Chihuahuan Desert. *Diversity* **2022**, *12*, 393. [CrossRef]
140. Segers, H. (Royal Belgian Institute of Natural Sciences, Brussels, Belgium). Personal communication.
141. Dumont, H.J.; Segers, H. Estimating lacustrine zooplankton species richness and complementarity. *Hydrobiologia* **1996**, *341*, 125–132. [CrossRef]
142. Muirhead, J.R.; Ejsmont-Karabin, J.; MacIsaac, H.J. Quantifying rotifer species richness in temperate lakes. *Freshwater Biol.* **2006**, *51*, 1696–1709. [CrossRef]
143. Ricci, C. Ecology of bdelloids: How to be successful. *Hydrobiologia* **1987**, *147*, 117–127. [CrossRef]
144. Fontaneto, D.; Ficetola, G.F.; Ambrosini, R.; Ricci, C. Patterns of diversity in microscopic animals: Are they comparable to those in protists or in larger animals? *Global Ecol. Biogeogr.* **2006**, *15*, 153–162. [CrossRef]
145. Lucena-Moya, P.; Duggan, I.C. Macrophyte architecture affects the abundance and diversity of littoral microfauna. *Aquat. Ecol.* **2011**, *45*, 279–287. [CrossRef]
146. Halabowski, D.; Bielánska-Grajner, I.; Lewin, I.; Sowa, A. Diversity of rotifers in small rivers affected by human activity. *Diversity* **2022**, *14*, 127. [CrossRef]
147. De Smet, W.H. Asciaporrectidae, a new family of Rotifera (Monogononta: Ploima) with description of *Asciaporrecta arcellicola* gen. et sp. nov. and *A. difflugicola* gen. et sp. nov. inhabiting shells of testate amoebae (Protozoa). *Zootaxa* **2006**, *1339*, 31–49. [CrossRef]
148. Pejler, B. On the taxonomy and ecology of benthic and periphytic Rotatoria. Investigations in northern Swedish Lapland. *Zool. Bidrag. Uppsala* **1967**, *33*, 327–422.
149. Arora, J.; Mehra, N.K. Species diversity of planktonic and epiphytic rotifers in the backwaters of the Delhi segment of the Yamuna River; with remarks on new records from India. *Zool. Stud.* **2003**, *42*, 239–247.
150. John, B.; Kumar, R.S. Periphytic rotifers: A comprehensive study on species composition, substrate specificity and habitat preference down a tropical riverine system. *Maced. J. Ecol. Environ.* **2021**, *23*, 5–16. [CrossRef]
151. Segers, H. Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution. *Zootaxa* **2007**, *1564*, 1–104. [CrossRef]
152. De Ridder, M.; Segers, H. Monogonont Rotifera recorded in the world literature (except Africa) from 1960 to 1992. *Studiedoc. KBIN* **1997**, *88*, 1–481.
153. Walsh, E.J.; Arroyo, M.L.; Schröder, T.; Wallace, R.L. Species richness and species turnover (complementarity) of Rotifera in selected aquatic systems of Big Bend National Park; Texas. In *Proceedings of the Sixth Symposium on the Natural Resources of the Chihuahuan Desert, Region*; Hoyt, C.A., Karges, J., Eds.; Chihuahuan Desert Research Institute: Fort Davis, TX, USA, 2014; pp. 185–204.
154. Vangenechten, J.H.D.; Van Puymbroeck, S.; Vanderborght, O.L.J. Physico-chemistry of surface waters in the Campine region of Belgium, with special reference to acid moorland pools. *Arch. Hydrobiol.* **1981**, *90*, 369–396.
155. Denys, L.; van Straaten, D. A survey of the acid water diatom assemblages of two heathland relics in the Belgian northern Campine (Groot and Klein Schietveld, Brasschaat) with an assessment of their conservational value. *Diatom Res.* **1992**, *7*, 1–13. [CrossRef]

156. MacIsaac, H.J.; Hutchinson, T.C.; Kellar, W. Analysis of planktonic rotifer assemblages from Sudbury; Ontario; area lakes of varying chemical composition. *Can. J. Fish. Aquat. Sci.* **1987**, *44*, 1692–1701. [CrossRef]
157. Brett, M.T. The rotifer communities of acid-stressed lakes of Maine. *Hydrobiologia* **1989**, *186*, 181–189. [CrossRef]
158. Santos-Medrano, G.E.; Rico-Martínez, R. Lethal effects of five metals on the freshwater rotifers *Asplanchna brigthwellii* and *Brachionus calyciflorus*. *Hidrobiológica* **2013**, *23*, 82–86.
159. Kling, G.W.; O'Brien, W.J.; Miller, M.C.; Hershey, A.E. The biogeochemistry and zoogeography of lakes and rivers in arctic Alaska. *Hydrobiologia* **1992**, *240*, 1–14. [CrossRef]
160. Kaya, M.; Fontaneto, D.; Segers, H.; Altindağ, A. Temperature and salinity as interacting drivers of species richness of planktonic rotifers in Turkish continental waters. *J. Limnol.* **2010**, *69*, 297–304. [CrossRef]
161. Bielańska-Grajner, I.; Cudak, A. Effects of salinity on species diversity of rotifers in anthropogenic water bodies. *Pol. J. Environ. Stud.* **2014**, *23*, 27–34.
162. Cormier, S.M.; Zheng, L.; Flaherty, C.M. A field-based model of the relationship between extirpation of salt-intolerant benthic invertebrates and background conductivity. *Sci. Total Environ.* **2018**, *633*, 1629–1636. [CrossRef]
163. Pontin, R.M.; Shiel, R.J. Periphytic rotifer communities of an Australian seasonal floodplain pool. *Hydrobiologia* **1995**, *313*, 63–67. [CrossRef]
164. Duggan, I.C.; Green, J.D.; Thompson, K.; Shiel, R.J. The influence of macrophytes on the spatial distribution of littoral rotifers. *Freshwater Biol.* **2001**, *46*, 777–786. [CrossRef]
165. Kuzyńska-Kippen, N. Habitat choice in rotifera communities of three shallow lakes: Impact of macrophyte substratum and season. *Hydrobiologia* **2007**, *593*, 27–37. [CrossRef]
166. Vieira, L.C.G.; Bini, L.M.; Velho, L.F.M.; Mazão, G.R. Influence of spatial complexity on the density and diversity of periphytic rotifers; microcrustaceans and testate amoebae. *Fund. Appl. Limnol.* **2007**, *170*, 77–85. [CrossRef]
167. Declerck, S.; Vandekerkhove, J.; Johansson, L.; Muylaert, K.; Conde-Porcuna, J.M.; Van der Gucht, K.; Pérez-Martínez, C.; Lauridsen, T.; Schwenk, K.; Zwart, G.; et al. Multi-group biodiversity in shallow lakes along gradients of phosphorus and water plant cover. *Ecology* **2005**, *86*, 1905–1915. [CrossRef]
168. Taniguchi, H.; Nakano, S.; Tokeshi, M. Influences of habitat complexity on the diversity and abundance of epiphytic invertebrates on plants. *Freshwater Biol.* **2003**, *48*, 718–728. [CrossRef]
169. Pierre, J.I.S.; Kovalenko, K.E. Effect of habitat complexity attributes on species richness. *Ecosphere* **2014**, *5*, 1–10. [CrossRef]
170. Thomaz, S.M.; da Cunha, E.R. The role of macrophytes in habitat structuring in aquatic ecosystems: Methods of measurement; causes and consequences on animal assemblages' composition and biodiversity. *Acta Limnol. Brasil.* **2010**, *22*, 218–236. [CrossRef]
171. Alfonso, G.; Beccarisi, L.; Pieri, V.; Frassanito, A.; Belmonte, G. Using crustaceans to identify different pond types. A case study from the Alta Murgia National Park, Apulia (South–eastern Italy). *Hydrobiologia* **2016**, *782*, 53–69. [CrossRef]
172. Zelnik, I.; Gregorič, N.; Tratnik, A. Diversity of macroinvertebrates positively correlates with diversity of macrophytes in karst ponds. *Ecol. Eng.* **2018**, *117*, 96–103. [CrossRef]
173. Yofukuji, K.Y.; Cardozo, A.L.P.; Quirino, B.A.; Aleixo, M.H.F.; Fugi, R. Macrophyte diversity alters invertebrate community and fish diet. *Hydrobiologia* **2021**, *848*, 913–927. [CrossRef]
174. Engelhardt, K.A.; Ritchie, M.E. Effects of macrophyte species richness on wetland ecosystem functioning and services. *Nature* **2001**, *411*, 687–689. [CrossRef]
175. Fleming, J.P.; Wersal, R.M.; Madsen, J.D.; Dibble, E.D. Weak non-linear influences of biotic and abiotic factors on invasive macrophyte occurrence. *Aquat. Invasions* **2021**, *16*, 349–364. [CrossRef]
176. Wang, H.; Zhang, X.; Shan, H.; Lv, C.; Ren, W.; Wen, Z.; Tian, Y.; Weigel, B.; Ni, L.; Cao, T. Biodiversity buffers the impact of eutrophication on ecosystem functioning of submerged macrophytes on the Yunnan-Guizhou Plateau; Southwest China. *Environ. Pollut.* **2022**, *314*, 120210. [CrossRef] [PubMed]
177. Fernández, R.; Alcocer, J.; Oseguera, L. A Regional pelagic rotifer biodiversity in a tropical karst lake district. *Diversity* **2020**, *12*, 454. [CrossRef]
178. Dodson, S.I.; Arnott, S.E.; Cottingham, K.L. The relationship in lake communities between primary productivity and species richness. *Ecology* **2000**, *81*, 2662–2679. [CrossRef]
179. Ejsmont-Karabin, J.; Górniak, A.; Karpowicz, M. Diversity of rotifer communities in lakes of the Suwalki Landscape Park. *Limnol. Rev.* **2016**, *16*, 207–211. [CrossRef]
180. Wen, X.; Zhai, P.; Feng, R.; Yang, R.; Xi, Y. Comparative analysis of the spatiotemporal dynamics of rotifer community structure based on taxonomic indices and functional groups in two subtropical lakes. *Sci. Rep.* **2017**, *7*, 578. [CrossRef]
181. Karpowicz, M.; Ejsmont-Karabin, J.; Kozłowska, J.; Feniova, I.; Dzialowski, A.R. Zooplankton community responses to oxygen stress. *Water* **2020**, *12*, 706. [CrossRef]
182. Wiersma, Y.E.; Urban, D.L. Beta diversity and nature reserve system design in the Yukon, Canada. *Conserv. Biol.* **2005**, *19*, 1262–1272. [CrossRef]
183. Socolar, J.; Gilroy, J.; Kunin, W.E.; Edwards, D.P. How should beta-diversity inform biodiversity conservation? *Trends Ecol. Evol.* **2016**, *31*, 67–80. [CrossRef] [PubMed]
184. Hill, M.J.; White, J.C.; Biggs, J.; Briers, R.A.; Gledhill, D.; Ledger, M.E.; Thornhill, I.; Wood, P.J.; Hassall, C. Local contributions to beta diversity in urban pond networks: Implications for biodiversity conservation and management. *Divers. Distrib.* **2021**, *27*, 887–900. [CrossRef]

185. Xia, Z.; Heino, J.; Yu, F.; He, Y.; Liu, F.; Wang, J. Spatial patterns of site and species contributions to β diversity in riverine fish assemblages. *Ecol. Indic.* **2022**, *145*, 109728. [CrossRef]
186. Soininen, J.; Heino, J.; Wang, J. A meta–analysis of nestedness and turnover components of beta diversity across organisms and ecosystems. *Global Ecol. Biogeogr.* **2018**, *27*, 96–109. [CrossRef]
187. Leprieur, F.; Tadesco, P.A.; Hugueny, B.; Beauchard, O.; Dürr, H.H.; Brosse, S.; Oberdorff, T. Partitioning global patterns of freshwater fish beta diversity reveals contrasting signatures of past climate change. *Ecol. Lett.* **2011**, *14*, 325–334. [CrossRef]
188. Li, F.; Tonkin, J.D.; Haase, P. Local contribution to beta diversity is negatively linked with community wide dispersal capacity in stream invertebrate communities. *Ecol. Indic.* **2020**, *108*, 105715. [CrossRef]
189. Hill, M.J.; Heino, J.; Thornhill, I.; Ryves, D.B.; Wood, P.J. Effects of dispersal mode on the environmental and spatial correlates of nestedness and species turnover in pond communities. *Oikos* **2017**, *126*, 1575–1585. [CrossRef]
190. Strecker, A.L.; Brittain, J.T. Increased habitat connectivity homogenizes freshwater communities: Historical and landscape perspectives. *J. Appl. Ecol.* **2017**, *54*, 1343–1352. [CrossRef]
191. Vilmi, A.; Karjalainen, S.M.; Heino, J. Ecological uniqueness of stream and lake diatom communities shows different macroecological patterns. *Divers. Distrib.* **2017**, *23*, 1042–1053. [CrossRef]
192. Da Silva, P.G.; Hernández, M.I.M.; Heino, J. Disentangling the correlates of species and site contributions to beta diversity in dung beetle assemblages. *Divers. Distrib.* **2018**, *24*, 1674–1686. [CrossRef]
193. Maloufi, S.; Catherine, A.; Mouillot, D.; Louvard, C.; Couté, A.; Bernard, C.; Troussellier, M. Environmental heterogeneity among lakes promotes hyper β–diversity across phytoplankton communities. *Freshwater Biol.* **2016**, *61*, 633–645. [CrossRef]
194. Castro, E.; Siqueira, T.; Melo, A.S.; Bini, L.M.; Landeiro, V.L.; Schneck, F. Compositional uniqueness of diatoms and insects in subtropical streams is weakly correlated with riffle position and environmental uniqueness. *Hydrobiologia* **2019**, *842*, 219–232. [CrossRef]
195. Szabó, B.; Lengyel, E.; Padisák, J.; Stenger-Kovács, C. Benthic diatom metacommunity across small freshwater lakes: Driving mechanisms; β-diversity and ecological uniqueness. *Hydrobiologia* **2019**, *828*, 183–198. [CrossRef]
196. Da Silva Brito, M.T.; Heino, J.; Pozzobom, U.M.; Landeiro, V.L. Ecological uniqueness and species richness of zooplankton in subtropical floodplain lakes. *Aquat. Sci.* **2020**, *82*, 43. [CrossRef]
197. De, K.; Singh, A.P.; Sarkar, A.; Singh, K.; Siliwal, M.; Uniyal, V.P.; Hussain, S.A. Relationship between species richness; taxonomic distinctness; functional diversity; and local contribution to β diversity and effects of habitat disturbance in the riparian spider community of the Ganga River; India. *Ecol. Process.* **2023**, *12*, 13. [CrossRef]
198. Benito, X.; Vilmi, A.; Luethje, M.; Carrevedo, M.L.; Lindholm, M.; Fritz, S.C. Spatial and temporal ecological uniqueness of Andean diatom communities are correlated with climate; geodiversity and long–term limnological change. *Front. Ecol. Evol.* **2020**, *8*, 260. [CrossRef]
199. García-Navas, V.; Martínez-Núñez, C.; Tarifa, R.; Molina-Pardo, J.L.; Valera, F.; Salido, T.; Camacho, F.M.; Rey, P.J. Partitioning beta diversity to untangle mechanisms underlying the assembly of bird communities in Mediterranean olive groves. *Divers. Distrib.* **2021**, *28*, 112–127. [CrossRef]
200. Vad, C.F.; Péntek, A.L.; Cozma, N.J.; Földi, A.; Tóth, A.; Tóth, B.; Böde, N.A.; Móra, A.; Ptacnik, R.; Ács, É.; et al. Wartime scars or reservoirs of biodiversity? The value of bomb crater ponds in aquatic conservation. *Biol. Conserv.* **2017**, *209*, 253–262. [CrossRef]
201. Cuenca-Cambronero, M.; Blicharska, M.; Perrin, J.-A.; Davidson, T.A.; Oertli, B.; Lago, M.; Beklioglu, M.; Meerhoff, M.; Arim, M.; Teixeira, J.; et al. Challenges and opportunities in the use of ponds and pondscapes as nature-based solutions. *Hydrobiologia* **2023**, *850*, 3257–3271. [CrossRef]
202. Ossyssek, S.; Hofmann, A.M.; Geist, J.; Raeder, U. Diatom Red List species reveal high conservation value and vulnerability of mountain lakes. *Diversity* **2022**, *14*, 389. [CrossRef]
203. Økland, R.H. On the variation explained by ordination and constrained ordination axes. *J. Veg. Sci.* **1999**, *10*, 131–136. [CrossRef]
204. García-Girón, J.; Heino, J.; García-Criado, F.; Fernández-Aláez, C.; Alahuhta, J. Biotic interactions hold the key to understanding metacommunity organisation. *Ecography* **2020**, *43*, 1–11. [CrossRef]
205. Vandvik, V.; Birks, H.J.B. Partitioning floristic variation in Norwegian upland grasslands into within-site and between-site components: Are the patterns determined by environment or by land-use? *Plant Ecol.* **2001**, *162*, 233–245. [CrossRef]
206. De Bie, T.; De Meester, L.; Brendonck, L.; Martens, K.; Goddeeris, B.; Ercken, D.; Hampel, H.; Denys, L.; Vanhecke, L.; Van der Gucht, K.; et al. Body size and dispersal mode as key traits determining metacommunity structure of aquatic pond organisms. *Ecol. Lett.* **2012**, *15*, 740–747. [CrossRef] [PubMed]
207. Brind'amour, A.; Mahévas, S.; Legendre, P.; Bellanger, L. Application of Moran Eigenvector Maps (MEM) to irregular sampling designs. *Spat. Stat.-Neth.* **2018**, *26*, 56–68. [CrossRef]
208. Barta, B.; Szabó, A.; Szabó, B.; Ptacnik, R.; Vad, C.F.; Horváth, Z. How pondscapes function: Connectivity matters for biodiversity even across small spatial scales in aquatic metacommunities. *Ecography* **2023**, e06960. [CrossRef]
209. Viana Duarte, S.; Keil, P.; Jeliazkov, A. Disentangling spatial and environmental effects: Flexible methods for community ecology and macroecology. *Ecosphere* **2022**, *13*, e4028. [CrossRef]
210. Tolonen, K.T.; Karjalainen, J.; Hämäläinen, H.; Nyholm, K.; Rahkola-Sorsa, M.; Cai, Y.; Heino, J. Do the ecological drivers of lake littoral communities match and lead to congruence between organism groups? *Aquat. Ecol.* **2020**, *54*, 839–854. [CrossRef]
211. Myers, F.J. The distribution of rotifera on Mount Desert Island. *Am. Mus. Nov.* **1931**, *494*, 1–12.

212. Hájková, P.; Bojková, J.; Fránková, M.; Opravilová, V.; Hájek, M.; Kintrová, K.; Horsák, M. Disentangling the effects of water chemistry and substratum structure on moss-dwelling unicellular and multicellular micro-organisms in spring-fens. *J. Limnol.* **2011**, *70* (Suppl. S1), 54–64. [CrossRef]
213. Saksena, D.N. Rotifers as indicators of water quality. *Acta Hydrochim. Hydrobiol.* **1987**, *15*, 481–485. [CrossRef]
214. Couillard, Y.; Ross, P.; Pinel-Alloul, B. Acute toxicity of six metals to the rotifer *Brachionus calyciflorus*, with comparisons to other freshwater organisms. *Environ. Toxicol.* **1989**, *546*, 451–462. [CrossRef]
215. Hernández-Flores, S.; Santos-Medrano, G.E.; Rubio-Franchini, I.; Rico-Martínez, R. Evaluation of bioconcentration and toxicity of five metals in the freshwater rotifer *Euchlanis dilatata* Ehrenberg, 1832. *Environ Sci. Pollut. Res. Int.* **2020**, *27*, 14058–14069. [CrossRef] [PubMed]
216. Han, C.; Kim, H.-J.; Lee, J.-S.; Sakakura, Y.; Hagiwara, A. Species-specific effects of iron on temperate and tropical marine rotifers in reproduction, lipid, and ROS metabolisms. *Chemosphere* **2021**, *277*, 130317. [CrossRef] [PubMed]
217. Bērziņš, B.; Pejler, B. Rotifer occurrence in relation to oxygen content. *Hydrobiologia* **1989**, *182*, 171–180. [CrossRef]
218. Saikia, S.K.; Nandi, S.; Majumder, A. Review on the role of nutrients in development and organization of periphyton. *J. R. B.* **2013**, *3*, 780–788.
219. Casartelli, M.R.; Ferragut, C. The effects of habitat complexity on periphyton biomass accumulation and taxonomic structure during colonization. *Hydrobiologia* **2018**, *807*, 233–246. [CrossRef]
220. Ejsmont-Karabin, J.; Feniova, I.; Kostrzewska-Szlakowska, I.; Rzepecki, M.; Petrosyan, V.G.; Dzialowski, A.R. Use of rotifer trophic state indices to show the effect of hydrobionts and nutrients on water trophic status in mesocosms. *Oceanol. Hydrobiol. St.* **2020**, *49*, 123–131. [CrossRef]
221. Liang, D.; Wang, Q.; Wei, N.; Tang, C.; Sun, X.; Yang, Y. Biological indicators of ecological quality in typical urban river-lake ecosystems: The planktonic rotifer community and its response to environmental factors. *Ecol. Indic.* **2020**, *112*, 106127. [CrossRef]
222. Bird, D.F.; Kalff, J. Empirical relationships between bacterial abundance and chlorophyll concentration in fresh and marine waters. *Can. J. Fish. Aquat. Sci.* **1984**, *41*, 1015–1023. [CrossRef]
223. Chrzanowski, T.H.; Sterner, R.W.; Elser, J.J. Nutrient enrichment and nutrient regeneration stimulate bacterioplankton growth. *Microb. Ecol.* **1995**, *29*, 221–230. [CrossRef] [PubMed]
224. Muylaert, K.; Declerck, S.; Geenens, V.; Van Wichelen, J.; Degans, H.; Vandekerkhove, J.; Van der Gucht, K.; Vloemans, N.; Rommens, W.; Rejas, D.; et al. Zooplankton, phytoplankton and the microbial food web in two turbid and two clearwater shallow lakes in Belgium. *Aquat. Ecol.* **2003**, *37*, 137–150. [CrossRef]
225. Jiménez-Santos, M.A.; Sarma, S.S.S.; Nandini, S.; Wallace, R.L. Sessile rotifers (Rotifera) exhibit strong seasonality in a shallow, eutrophic Ramsar site in Mexico. *Invertebr. Biol.* **2019**, *138*, e12270. [CrossRef]
226. Williamson, C.E. Predator-prey interactions between omnivorous diaptomid copepods and rotifers: The role of prey morphology and behavior. *Limnol. Oceanogr.* **1987**, *32*, 167–177. [CrossRef]
227. Yoshida, T.; Urabe, J.; Elser, J.J. Assessment of 'top-down' and 'bottom-up' forces as determinants of rotifer distribution among lakes in Ontario, Canada. *Ecol. Res.* **2003**, *18*, 639–650. [CrossRef]
228. Sanchez-Avila, A.S.; Wallace, R.L.; Walsh, E.J. Motility and size of rotifers as risk factors for being consumed by the passive protistan predator *Actinosphaerium* sp. *Hydrobiologia* **2023**. [CrossRef]
229. Gilbert, J.J. The cost of predator-induced morphological defense in rotifers: Experimental studies and synthesis. *J. Plankton Res.* **2013**, *35*, 461–472. [CrossRef]
230. Ricci, C.; Balsamo, M. The biology and ecology of lotic rotifers and gastrotrichs. *Freshwater Biol.* **2001**, *44*, 15–28. [CrossRef]
231. Gilbert, J.J. Food niches of planktonic rotifers: Diversification and implications. *Limnol. Oceanogr.* **2022**, *67*, 2218–2251. [CrossRef]
232. Sarma, S.S.S.; Jiménez–Santos, M.A.; Nandini, S.; Wallace, R.L. Demography of the sessile rotifers, *Limnias ceratophylli* and *Limnias melicerta* (Rotifera: Gnesiotrocha), in relation to food (*Chlorella vulgaris* Beijerinck, 1890) density. *Hydrobiologia* **2017**, *796*, 181–189. [CrossRef]
233. Odland, A.; Birks, H.J.B.; Line, J.M. Ecological optima and tolerances of *Thelypteris limbosperma*, *Athyrium distentifolium*, and *Matteuccia struthiopteris* along environmental gradients in Western Norway. *Vegetatio* **1995**, *120*, 115–129. [CrossRef]
234. Bennett, J.R.; Cumming, B.F.; Ginn, B.K.; Smol, J.P. Broad-scale environmental response and niche conservatism in lacustrine diatom communities. *Global Ecol. Biogeogr.* **2010**, *19*, 724–732. [CrossRef]
235. Wagner, V.; Chytrý, M.; Zelený, D.; von Wehrden, H.; Brinkert, A.; Danihelka, J.; Hölzel, N.; Jansen, F.; Kamp, J.; Lustyk, P.; et al. Regional differences in soil pH niche among dry grassland plants in Eurasia. *Oikos* **2017**, *126*, 660–670. [CrossRef]
236. Soininen, J.; Jamoneau, A.; Rosebery, J.; Leboucher, T.; Wang, J.; Kokociński, M.; Passy, S.I. Stream diatoms exhibit weak niche conservation along global environmental and climatic gradients. *Ecography* **2018**, *41*, 346–353. [CrossRef]
237. Søndergaard, M.; Jeppesen, E.; Jensen, J.P.; Amsinck, S.L. Water Framework Directive: Ecological classification of Danish lakes. *J. Appl. Ecol.* **2005**, *42*, 616–629. [CrossRef]
238. Poikane, S.; Phillips, G.; Birk, S.; Free, G.; Kelly, M.G.; Willby, N.J. Deriving nutrient criteria to support 'good' ecological status in European lakes: An empirically based approach to linking ecology and management. *Sci. Total Environ.* **2019**, *650*, 2074–2084. [CrossRef]
239. Poikane, S.; Portielje, R.; van den Berg, M.; Phillips, G.; Brucet, S.; Carvalho, L.; Mischke, U.; Ott, I. Defining ecologically relevant water quality targets for lakes in Europe. *J. Appl. Ecol.* **2014**, *51*, 592–602. [CrossRef]

240. Lauridsen, T.L.; Jeppesen, E.; Declerck, S.A.J.; De Meester, L.; Conde-Porcuna, J.M.; Rommens, W.; Brucet, S. The importance of environmental variables for submerged macrophyte community assemblage and coverage in shallow lakes: Differences between northern and southern Europe. *Hydrobiologia* **2015**, *744*, 49–61. [CrossRef]
241. Smolak, R.; Walsh, E.J. Rotifer species richness in Kenyan waterbodies: Contributions of environmental characteristics. *Diversity* **2022**, *14*, 583. [CrossRef]
242. Sayer, C.D.; Davidson, T.A.; Jones, J.I.; Langdon, P.G. Combining contemporary ecology and palaeolimnology to understand shallow lake ecosystem change. *Freshwater Biol.* **2010**, *55*, 487–499. [CrossRef]
243. Juggins, S.; Anderson, N.J.; Ramstack Hobbs, J.M.; Heathcote, A.J. Reconstructing epilimnetic total phosphorus using diatoms: Statistical and ecological constraints. *J. Paleolimnol.* **2013**, *49*, 373–390. [CrossRef]
244. Mills, S.; Alcántara-Rodríguez, J.A.; Ciros-Pérez, J.; Gómez, A.; Hagiwara, A.; Hinson Galando, K.; Jersabek, C.D.; Malekzadeh-Viayeh, R.; Leasi, F.; Lee, J.-S.; et al. Fifteen species in one: Deciphering the *Brachionus plicatilis* species complex (Rotifera; Monogononta) through DNA taxonomy. *Hydrobiologia* **2017**, *796*, 39–58. [CrossRef]
245. García-Morales, A.E.; Domínguez-Domínguez, O. Cryptic molecular diversity in the morphologically variable rotiferan *Brachionus quadridentatus* (Rotifera: Monogononta). *Rev. Biol. Trop.* **2019**, *67*, 1114–1130. [CrossRef]
246. García-Morales, A.E.; Domínguez-Domínguez, O. Cryptic species within the rotifer *Lecane bulla* (Rotifera: Monogononta: Lecanidae) from North America based on molecular species delimitation. *Rev. Mex. Biodivers.* **2020**, *91*, e913116. [CrossRef]
247. Walczyńska, A.; Fontaneto, D.; Kordbacheh, A.; Hamil, S.; Jimenez-Santos, M.A.; Paraskevopoulou, S.; Pociecha, A.; Zhang, W. Niche differentiation in rotifer cryptic species complexes: A review of environmental effects. *Hydrobiologia* **2023**. [CrossRef]
248. Coudun, C.; Gégout, J.-C. The derivation of species response curves with Gaussian logistic regression is sensitive to sampling intensity and curve characteristics. *Ecol. Model.* **2006**, *199*, 164–175. [CrossRef]
249. Michaelis, J.; Diekmann, M.R. Biased niches—Species response curves and niche attributes from Huisman–Olff–Fresco models change with differing species prevalence and frequency. *PLoS ONE* **2017**, *12*, e0183152. [CrossRef]
250. Obertegger, U.; Flaim, G. Taxonomic and functional diversity of rotifers, what do they tell us about community assembly? *Hydrobiologia* **2018**, *823*, 79–91. [CrossRef]
251. Wallace, R.L.; Walsh, E.J.; Arroyo, M.L.; Starkweather, P.L. Life on the edge: Rotifers from springs and ephemeral waters in the Chihuahuan Desert, Big Bend National Park (Texas, USA). *Hydrobiologia* **2005**, *546*, 147–157. [CrossRef]
252. Joniak, T.; Kuczyńska-Kippen, N.; Gąbka, M. Effect of agricultural landscape characteristics on the hydrobiota structure in small water bodies. *Hydrobiologia* **2017**, *793*, 121–133. [CrossRef]
253. Moreno-Gutiérrez, R.M.; Sarma, S.S.S.; Sobrino-Figueroa, A.S.; Nandini, S. Population growth potential of rotifers from a high altitude eutrophic waterbody, Madín reservoir (State of Mexico; Mexico): The importance of seasonal sampling. *J. Limnol.* **2018**, *77*, 441–451. [CrossRef]
254. Yin, L.; Ji, Y.; Zhang, Y.; Chong, L.; Chen, L. Rotifer community structure and its response to environmental factors in the Backshore Wetland of Expo Garden, Shanghai. *Aquacult. Fish.* **2018**, *3*, 90–97. [CrossRef]
255. Jekatierynczuk-Rudczyk, E.; Ejsmont-Karabin, J. Rotifers of inter-forest springs. *Diversity* **2023**, *15*, 153. [CrossRef]