Short-term effects of macrophyte removal on aquatic biodiversity in rivers and lakes

Benjamin Misteli [1]*, Alexandrine Pannard [1], Eirin Aasland [2], Sarah Faye Harpenslager [3], Samuel Motitsoe [4], Kirstine Thiemer [2,5], Stéphanie Llopis [1], Julie Coetzee [4], Sabine Hilt [3], Jan Köhler [3], Susanne C. Schneider [2,5], Christophe Piscart [1], Gabrielle Thiébaut [1]

[1] *Université de Rennes 1, UMR 6553 CNRS ECOBIO, 263 Avenue du Général, 35042 Rennes*

[2] *Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, 1430 Ås, Norway.*

[3] *Department of Community and Ecosystem Ecology, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 301, 12587 Berlin, Germany*

[4] *Rhodes University, Center for Biological Control, Department of Zoology and Entomology, P.O. Box 94, 6139 Grahamstown, South Africa*

[5] *Section for Nature-based Solutions and Aquatic Ecology, Norwegian Institute for Water Research, Økernveien 94, 0579 Oslo, Norway.*

Abstract

Mass development of macrophytes is an increasing problem in many aquatic systems worldwide. Dense mats of macrophytes can negatively affect activities like boating, fishing, or hydropower production and one of the management measures often applied is mechanical removal. In this study, we analyzed the effect of mechanical macrophyte removal on phytoplankton, zooplankton, and macroinvertebrate (pelagic and benthic samples) assemblages. Our study covered five sites in four countries in Europe and Africa with highly variable characteristics. In all sites, dense mats of different macrophyte species (*Juncus bulbosus* in a river in Norway; a mix of native macrophytes in a German river, *Elodea nuttallii* in a lake in Germany, *Ludwigia* spp. in a French lake and *Pontederia crassipes* in a South African lake) are problematic and mechanical removal was applied. In every country, we repeated the same BACI (Before-After-Control-Impact) design, including "before", "one week after", and "six weeks after" sampling in a control and an impact section. Repeating the same experimental design at all sites allowed us to disentangle common effects across all sites from site-specific effects. For each taxonomic group, we analyzed three structural and three functional parameters, which we combined in a scoring system. Overall, the removal of macrophytes negatively affected biodiversity, in particular, of zooplankton and macroinvertebrate assemblages. In contrast, plant removal had positive effects on the phytoplankton assemblages. Effects were more pronounced one week after removal than six weeks after. Consequently, we suggest a stronger consideration of the effect of plant removal on biodiversity to arrive at more sustainable management practices in the future.

Keywords: Aquatic plants, management, BACI, macroinvertebrates, zooplankton, phytoplankton

Highlights

• We studied the short-term impact of macrophyte removal on aquatic biodiversity.

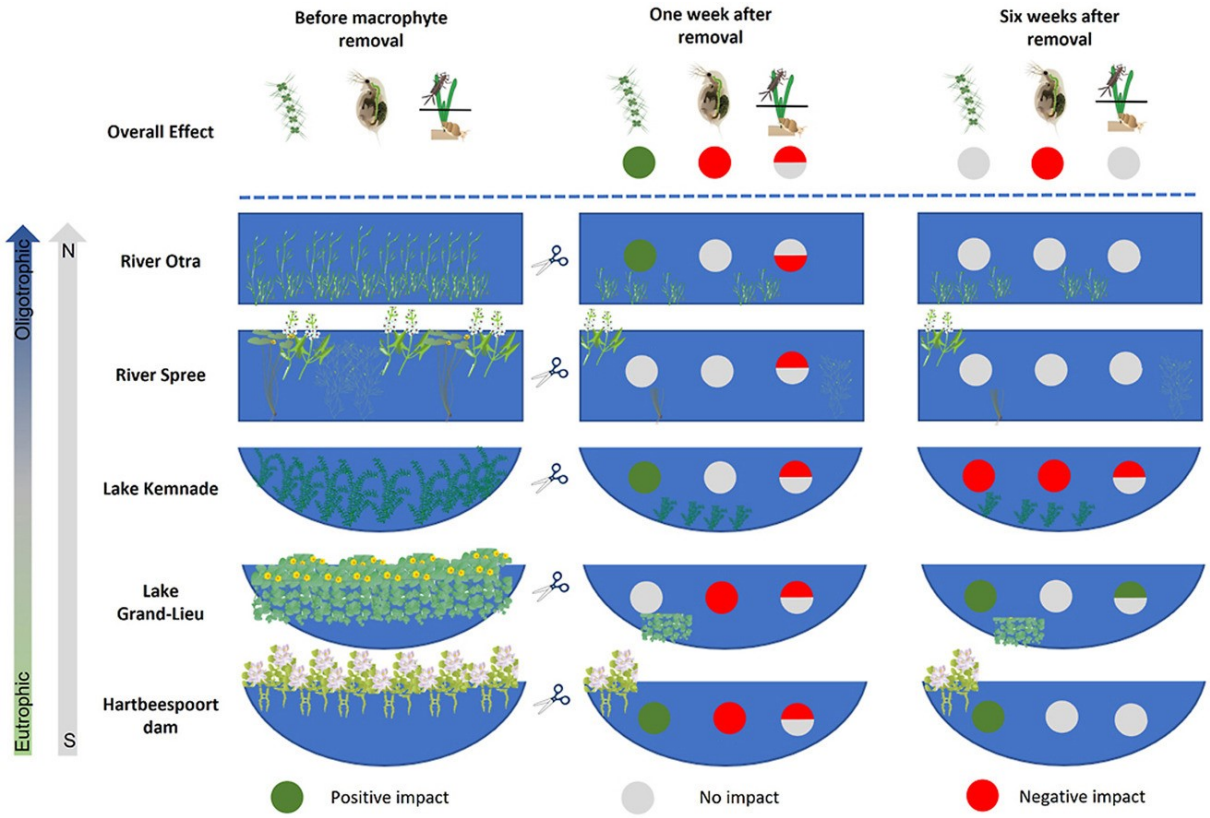
• Overall biodiversity was negatively impacted by plant removal.

• The removal negatively impacted zooplankton and macroinvertebrates.

• Positive effects were found for phytoplankton.

• The greatest impact was observed after one week, with resilience after six weeks.

Graphical Abstract



Introduction

Macrophytes play a crucial role in the functioning of aquatic and wetland systems and support a variety of ecosystem services (Hilt et al., 2017; Janssen et al., 2021). Under favorable environmental conditions (e.g., light, temperature, nutrients), exotic and native species can form dense stands within a short time (Hussner et al., 2017; Riis and Biggs, 2001) which can hinder commercial and leisure activities such as navigation, fishing, swimming and other water sports (Dugdale et al., 2013; Güereña et al., 2015; Verhofstad and Bakker, 2019). Furthermore, dense vegetation increases the risk of flood for adjacent land (Boerema et al., 2014), can clog hydropower stations (Dugdale et al., 2013), and represses a more diverse native vegetation (Stiers et al., 2011). Dense mats of floating plants create anoxic conditions (Janse and Van Puijenbroek, 1998). Mass development of macrophytes is thus often perceived as problematic, and managed through physical removal (Hussner et al., 2017; Thiemer et al., 2021), resulting in high financial costs for local authorities and taxpayers (de Winston et al., 2013). As mass developments are expected to increase in the future due to global change, their removal will become more important and balanced management strategies are needed (Hussner et al., 2017; Thiemer et al., 2021). However, studies on the effect of macrophyte removal on aquatic ecosystems are scarce including how this management strategy affects the diversity of phytoplankton, zooplankton and macroinvertebrates (Thiemer et al., 2021). Macrophyte removal could affect phytoplankton, zooplankton and macroinvertebrate assemblages, with consequences for ecosystem functioning. Macrophytes increase structural complexity and heterogeneity in the water column and offer habitats that would otherwise not be available (Thomaz et al., 2008). Dense macrophyte stands offer space, shelter and a source of food (directly or via epiphytic algae and bacteria) to macroinvertebrates (Ferreiro et al., 2014; Wolters et al., 2019), but they can also reduce the dissolved oxygen availability in the water, negatively affecting macroinvertebrates (Caraco et al., 2006; Stansbury et al., 2008). The effect of macrophytes on zooplankton depends on the interactions with other trophic groups. Studies have shown that zooplankton generally avoids macrophyte beds (Meerhoff et al., 2006), but in the presence of

predatory fish, zooplankton use macrophyte beds as a refuge to avoid predation during the day (Burks et al., 2002). Phytoplankton and macrophytes are in direct competition for nutrients and light (Scheffer et al., 1993; Xu et al., 2019). Therefore, the presence of macrophytes hinders the growth of phytoplankton (Scheffer et al., 1993; van Donk et al., 1993). Besides competition for nutrients, macrophytes have been shown to suppress phytoplankton even under nutrient saturation (Amorim and Moura, 2020; Vanderstukken et al., 2011). The production of allelochemicals by certain macrophyte species also has a strong impact on phytoplankton (Körner and Nicklisch, 2002; Švanys et al., 2014) especially cyanobacteria, making macrophytes a useful tool in cyanobacteria management (Bakker and Hilt, 2016; Wang et al., 2012).

Studies have shown reduced macroinvertebrate abundance after macrophyte removal in rivers (Grygoruk et al., 2015; Känel et al., 1998) and lakes (Habib and Yousuf, 2014; Miliša et al., 2006), while others demonstrated neutral (Buczyński et al., 2016; Ward-Campbell et al., 2017) or even positive (Bickel and Closs, 2009) effects of plant removal on macroinvertebrate abundance. Reduced taxonomic richness was found in a study covering a single river in Australia (Carey et al., 2018), while other studies in lakes and rivers did not find changes in richness (Bickel and Closs, 2009; Ward-Campbell et al., 2017). Shannon-diversity was shown to increase in a study in a river in the U.S. (Lusardi et al., 2018), while other studies in rivers did not detect a change in Shannon-diversity (Buczyński et al., 2016; Dabkowski et al., 2016). In lakes, several studies reported reduced Shannon-diversity (Habib and Yousuf, 2014; Miliša et al., 2006). Depending on the site and its characteristics, the effects are mixed, and it is difficult to detect a general response pattern for macroinvertebrates.

Only few studies are available on the effect of macrophyte removal on zooplankton and phytoplankton. Choi et al. (2014) showed an increase in abundance, richness and diversity of zooplankton after removing free-floating macrophytes in a lake in South Korea. Opposite results for abundance were found in other studies in a Mexican lake (Mangas-Ramírez and Elías-Gutiérrez, 2004) and a river in the U.K. (Garner et al., 1996). After plant removal, several studies showed a

clear increase in phytoplankton cell density (Wojciechowski et al., 2018) or in Chl-a concentration (Bicudo et al., 2007; James et al., 2002; Kuiper et al., 2017). However, other studies showed a short-term reduction in Chl-a concentration after plant removal (Alam et al., 1996; Morris et al., 2006). Increased turbidity due to the removal likely causes this short-term decrease (Thiemer et al., 2021). Furthermore, the removal of macrophytes increased the abundance of cyanobacteria in tropical lakes (Mangas-Ramírez and Elías-Gutiérrez, 2004; Wojciechowski et al., 2018), while Morris et al. (2006) did not find an effect on cyanobacteria in a shallow lake in Australia.

Existing studies on the effect of macrophyte removal on biodiversity often have a narrow scope (e.g. focusing on a single plant species, system or organism group) or a restricted sampling design (e.g. lacking a before-after comparison or a control site). Holistic studies considering multiple groups, species and systems are lacking, but are needed to disentangle general patterns from local differences (Thiemer et al., 2021). Additionally, trait-based analyses, such as functional evenness, functional richness or functional divergence (Villéger et al., 2008), can help to better predict ecological dynamics and increase comparability among different systems (Kremer et al., 2017).

In this study, we analyzed the effect of macrophyte removal in three lakes and two rivers with different trophic states (from oligotrophic to hypereutrophic) located in different climate zones (from temperate to tropical climate) along a latitudinal gradient from North Europe to South Africa. At each site, different macrophyte species (native or invasive) are considered problematic, and mechanical removal is part of the current management practice. We applied a BACI design (before-after-control-impact) and sampled macroinvertebrates, zooplankton, and phytoplankton before, and one week and six weeks after plant removal at control and impact sections. Using the same method and the same timespan between macrophyte removal and sampling enables us to compare results among sites. The high variability of our study systems covering different plant types, plant species, trophic levels, system types and climate zones allows us to disentangle general patterns from site-specific effects. We expected to find (i) negative effects of macrophyte removal on macroinvertebrate and zooplankton but positive effects on phytoplankton abundance and

diversity; and (ii) strongest impacts on all three groups one week after the removal and a partial recovery six weeks after removal. We applied a comprehensive approach considering six biodiversity and functional indices throughout, using a standardized scoring method.

Material and Methods

Study Locations

We sampled five aquatic systems in four countries in Africa and Europe (Figure 1). The studied systems differed in their physical features, the dominant vegetation and trophic status (Table 1). In all sites, dense mats of macrophytes are perceived as problematic and are removed mechanically as part of their management strategy.

The northernmost site was the oligotrophic river Otra in Norway. Our study was conducted in a dammed, slow-flowing part of the river, dominated by the native submerged macrophyte *Juncus bulbosus* L. causing problems for recreational use and hydropower production. Plant stands are usually mowed once every 3 years in early summer. The river Spree (Germany) is characterized by mass development of the native *Sagittaria sagittifolia* L., *Stuckenia pectinata* (L.) Börner and *Nuphar lutea* (L.) Sm. Dense macrophyte stands cause a water level increase of 20-50 cm in summer and increase the risk of flooding adjacent farmland during heavy rainfall events. Mechanical removal is therefore applied once per summer. Lake Kemnade (Kemnader See; Germany) is an important recreational area in a densely populated area. Dense stands of the non-native submerged species *Elodea nuttallii* (Planch.) H.St-John interfere with several recreational activities, and a mowing boat is active daily between May and September. Non-native, amphibious *Ludwigia grandiflora* subsp. *hexapetala* (Hook. & Arn.) G.L.Nesom & Kartesz and *Ludwigia peploides* subsp. *montevidensis* (Spreng.) P.H. Raven dominate in Lake Grand-Lieu (Lac de Grand-Lieu; France), an important nature reserve. The plants have negative effects on native vegetation and human activities such as professional fishing and boating. For 20 years, plants have been removed yearly, which is costly and ineffective due to fast regrowth. In the reservoir Hartbeespoort Dam (South Africa), the

floating macrophyte *Pontederia crassipes* Mart. (formerly *Eichhornia crassipes*; Pontederiaceae) covers significant parts of the lake (up to 60%), thereby hindering recreational and commercial activities. In all lakes, we used typical methods used in macrophyte management. All those methods are efficient at removing most of the macrophytes from the treated areas, a small part of the macrophytes, however, will always be left.

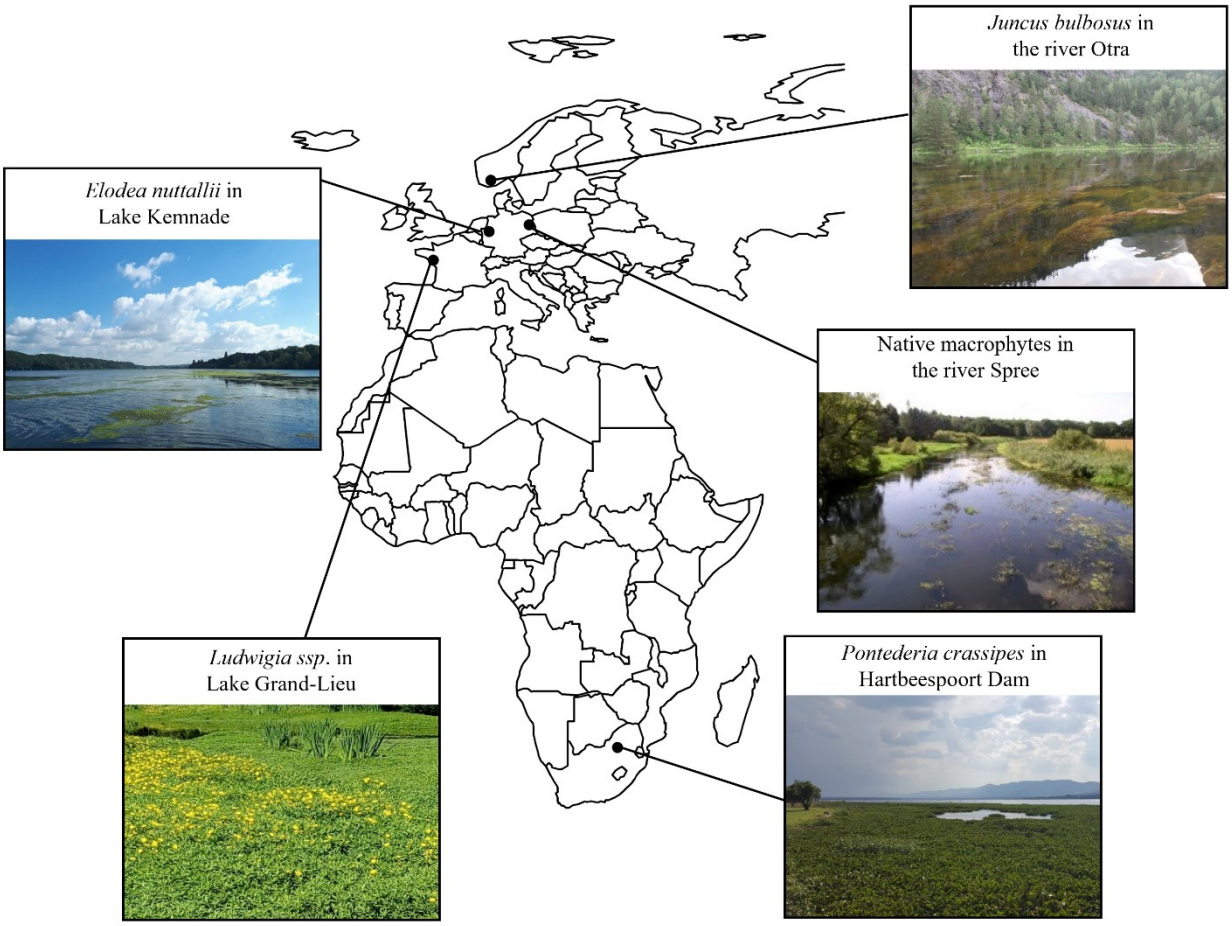


Figure 1: Location of the five study systems with mass macrophyte developments.

Table 1: Site characteristics. Depth and velocity are given for the sampling location. Climate zones according to the Köppen-Geiger classification (Kottek et al., 2006).

Site	Country	Latitude/ Longitude	Water body (size, depth, velocity)	Major problem of mass development	Trophic level	Climate zone	Dominant vegetation	Removal Method	Date of Removal	Size of Impact section
Otra	Norway	59.08864/ 7.550139	Regulated river (depth: 1.5 m, 0.1-0.5 m/s)	Plants hinder boating and fishing and clog the inlet of hydropower plants	Oligo-trophic	Subarctic climate (Dfc)	*Juncus bulbosus (native)*	Mechanical removal with a mowing boat	15.06.2020 - 22.06.2020	33000 m^2
Spree	Germany	52.43076/ 13.678259	River (part of river-lake system, depth: 1.25 m, 0.1 m/s)	Native macrophytes raise water levels and increase the risk of flooding adjacent agricultural land	Eutrophic	Warm-summer humid continental climate(Dfb)	*Sagittaria sagittifolia, Stuckenia pectinata* and *Nuphar lutea (all native)*	Mechanical removal with a mowing boat	10.-17.07.2019 27.–31.07.2020	60000 m^2
Lake Kemnade	Germany	51.41698/ -7.260132	Reservoir (125 ha, depth: 2 m)	Plants hindering sailing and shipping	Eutrophic	Temperate oceanic climate (Cfb)	*Elodea nuttallii (exotic)*	Mechanical removal with a mowing boat	28.-30.07.2020	5000 m^2
Lake Grand-Lieu	France	47.13393/ -1.674355	Shallow lake (64 km^2, depth 1 m)	Non-native macrophytes threatening biodiversity conservation	Hyper-eutrophic	Temperate oceanic climate (Cfb)	*Ludwigia peploides* and *L. grandiflora (both exotic)*	Removal by hand	06.-08.07.2020	550 m^2
Hartbeespoort Dam	South Africa	-25.74929/ 27.833276	Reservoir (2000 ha, depth: 4 m)	Plants causing problems for human lake uses	Hyper-eutrophic	Subtropical highland climate (Cwb)	*Pontederia crassipes* (exotic)	Removal by hand	20.-25.01.2020	625 m^2

Study Design

Our sampling was performed using a BACI Design (Before-After-Control-Impact, (Underwood, 1991)). We defined two sections of comparable size in every study site: one where macrophytes were removed (Impact section) and one where macrophytes were not removed (Control section). In lakes, the two sections were adjacent to each other. In the river Spree, the control section was upstream of the impact section, and in the river Otra, the two sections were located at the opposite shores. Both sections were sampled the week before plants were removed, and then one week and six weeks after plant removal. To reduce sampling bias, both sections were sampled on the same date and by the same people. In every sampling session in each section, five water samples were taken for phytoplankton, five water filtrations were performed for zooplankton, and five grab and sweep samples were taken for macroinvertebrates.

Sampling methods and processing

The same sampling method was used in each site and only slightly adjusted to the local conditions.

- Phytoplankton:

  For phytoplankton, sub-surface water samples were taken. According to the expected density of phytoplankton, the volume of sampled water ranged from 50 ml in hypereutrophic sites to 250 ml in oligotrophic sites. Samples were fixed with acidic Lugol and stored in a cold and dark place. All samples were sent to France for identification (Limnologie sarl, Rennes) and counted according to the NF EN 15204 French standard (AFNOR, 2006). Phytoplankton biomass was measured as Chl-a concentration after filtration on Whatman GF/F glass-fibre filters and extraction with dimethylformamide in a vibration shaker at 4 °C. Pigments were separated and quantified by HPLC (see Shatwell et al., 2012 for details).

- Zooplankton:

  With a 60 µm mesh, 20 to 80 l (depending on the characteristics of the system) of surface

water per sample were filtered. The sample volume required to collect enough individuals was pre-defined with a test sample, and the same sample size was used for the complete sampling. After filtration, the zooplankton sample was narcotized with carbonated water and then conserved in 80% ethanol and stored at 4° C before identification. Zooplankton was subsampled for identification and identified based on Bledzki & Rybak (2016). Subsamples of a known volume were randomly taken using a Hensen-Stempel pipette and placed in a Bogorov counting chamber. Subsamples were counted until a total of at least 400 organisms were reached. Finally, the abundance was calculated as individuals per liter. In the rivers Otra and Spree, the number of zooplankton collected was low, with many samples being completely empty (mean density of 0.285 individuals per liter in the Spree and 0.031 in the Otra). These sites were therefore excluded from further analysis, and the effect of removal was considered neutral.

- Macroinvertebrates:

  For macroinvertebrates, the sampling consisted of grab samples to collect macroinvertebrates associated with the sediment and sweep samples to collect macroinvertebrates associated with the macrophytes. Five grab samples were taken using an Ekman grab sampler, and samples were filtered using a sieve (250 μm mesh size). Five sweep samples were collected using a hand net with a 250 μm mesh size swept harshly through the plants in the case of submerged species, or through the roots for floating species for 30 seconds over 1 m^2. Both types of samples were stored in 80% ethanol. Macroinvertebrates were separated from sediment under a dissecting microscope and identified to the lowest taxonomic level possible.

Biological Indices

We used the number of individuals per sample (macroinvertebrates), individuals per liter (zooplankton), and Chl-a concentration (phytoplankton) to quantify abundance of

macroinvertebrates, zooplankton, and phytoplankton. Abundance values were log(N+1) transformed for analysis. If individuals were not all identified to the same level, only the lowest identification level was used to estimate taxonomic richness and Shannon-diversity to avoid overestimation. For example, when some individuals were identified to the species level, but others in the same genus could not be identified further, only the species level was included in the analysis. To assess the functional diversity, we used multidimensional functional diversity indices. A multi-dimensional space was created with every functional trait representing one dimension, and all taxa and their abundance were plotted in this space. Functional richness (the volume filled by the community of interest), functional evenness (the evenness of abundance distribution) and functional divergence (distribution of the abundance within the volume of the trait space) were used as indices to describe functional diversity (Villéger et al., 2008). Calculations were done with the mFD package in R (Magneville et al., 2022). Due to a lack of precision for available functional information, this analysis was performed at the genus level (or higher taxonomic level if not identified to genus). The following sources were used as a trait database for functional analysis: Tachet et al. (2000) for macroinvertebrates, Gavrilko et al. (2020) for zooplankton, and Padisák et al. (2009) plus Laplace-Tryture et al. (2021) for phytoplankton. Used traits are listed in Table S1. Calculating the functional parameters requires at least three taxa, so samples with a lower number of taxa were not analyzed. As the composition of the phytoplankton community plays a key role in management strategies, we did an additional analysis of the proportion of the cyanobacteria compared to the complete phytoplankton community based on cell count.

Statistical Analysis

All statistical analyses were performed in R version 4.1.2 (R Core Team, 2021). To test the overall effects of plant removal on each parameter across all five systems, linear mixed models (LMM) were performed with the function "lmer" from the package "lme4" (Bates et al., 2015). Statistical parameters for the linear mixed models are summarized in Table S2. "Before-After", "Control-

Impact", and their interaction were used as fixed factors, and site was included as a random factor (parameter ~ BA * CI + (1|Site)). In addition, each parameter was analyzed separately for each site with two-way ANOVAs using the "aov" function from the package "stats" (R Core Team, 2021). Test statistics can be found in Supplementary Information, Table S3.

Scoring

To summarize and compare the measured effects, we used a scoring system. Every parameter (abundance, taxa richness, Shannon-diversity, functional richness, functional evenness, and functional divergence) for every organism group (Zooplankton, Phytoplankton, Macroinvertebrates (Sweep and Grab Samples)) was scored with a value of -1, 0 or +1. If the model (linear mixed models for overall effects and ANOVA for effects by country) showed no significant difference ($p > 0.05$), the score was set to 0. Significant effects were scored with a -1 for a negative impact of macrophyte removal and +1 for positive impacts. The direction of impact was calculated based on the following formula:

$$Effect = (AFTERimpact - AFTERcontrol) - (BEFOREimpact - BEFOREcontrol)$$

Percentage differences were calculated based on the "Effect" value above compared to the value in the impact site before removal (BEFOREimpact). Therefore, these values can be lower than –100%. The scoring was done separately for one week and six weeks after sampling. An unweighted scoring together with the presentation of percentage differences were chosen, as the impact on the ecosystem between parameters is not comparable.

Results

Differences in aquatic biodiversity among sites

Differences in aquatic biodiversity were found between the five sites (Figure 2, Figure 3). For zooplankton, Lake Grand-Lieu showed the highest abundance with a mean of 4.022 (standard

deviation: 0.764) individuals per liter compared to 0.581 (0.133) in Hartbeespoort Dam and 0.178 (0.097) in Lake Kemnade. On the other hand, the zooplankton in Lake Grand-Lieu showed a lower functional richness than the other two sites (0.009 (0.016) compared to 0.175 (0.001) in Lake Kemnade and 0.127 (0.073) in Hartbeespoort Dam). Taxa richness, Shannon-diversity, functional evenness and functional divergence were more similar among sites.

For phytoplankton, variations in phytoplankton abundance among sites were high. The three lakes showed higher phytoplankton abundance compared to the rivers, with Hartbeespoort Dam (1330 (562) μg Chl*a*/l; only measured one week after the removal in control site) and Lake Grand-Lieu (166 (46) μg Chl*a* /l) having the highest estimates, followed by Lake Kemnade (29 (21) μg Chl*a* /l), which correlated with the declining order of their trophic status. Phytoplankton abundance was lower in the two rivers, with a higher value in the eutrophic river Spree (4.53 (1.56) μg Chl*a* /l) than the oligotrophic river Otra (1.12 (0.03) μg Chl*a* /l). As for zooplankton, taxa richness, Shannon-diversity, functional richness, functional evenness, and functional divergence did not follow clear trends, and variations were small.

Comparing grab and sweep samples of macroinvertebrates revealed differences in the macroinvertebrate distribution in the five sites. Hartbeespoort Dam (30 (14) individuals per sweep sample; 31 (22) individuals per grab sample) and Lake Grand-Lieu (166 (141); 66 (63)) consistently showed the lowest and second-lowest abundance in both sample types, respectively. For the remaining three sites, Lake Kemnade showed the highest abundance (3837 (1914); 897 (675)). The two rivers showed the second and third highest abundance for sweep samples, Otra (400 (452)) is ranked before Spree (237 (329)) and for the grab samples, Spree (229 (165)) followed by Otra (197 (151)). The taxa richness followed a different pattern than the abundance. For sweep samples, the highest taxa richness was found in Lake Grand-Lieu (12.1 (3.6) taxa found) and for grab samples in Otra (10.4 (2.9)). The lowest taxa richness in both sample types was found in Hartbeespoort Dam (6.8 (1.8) in sweep samples; 3.0 (1.2) in grab samples).

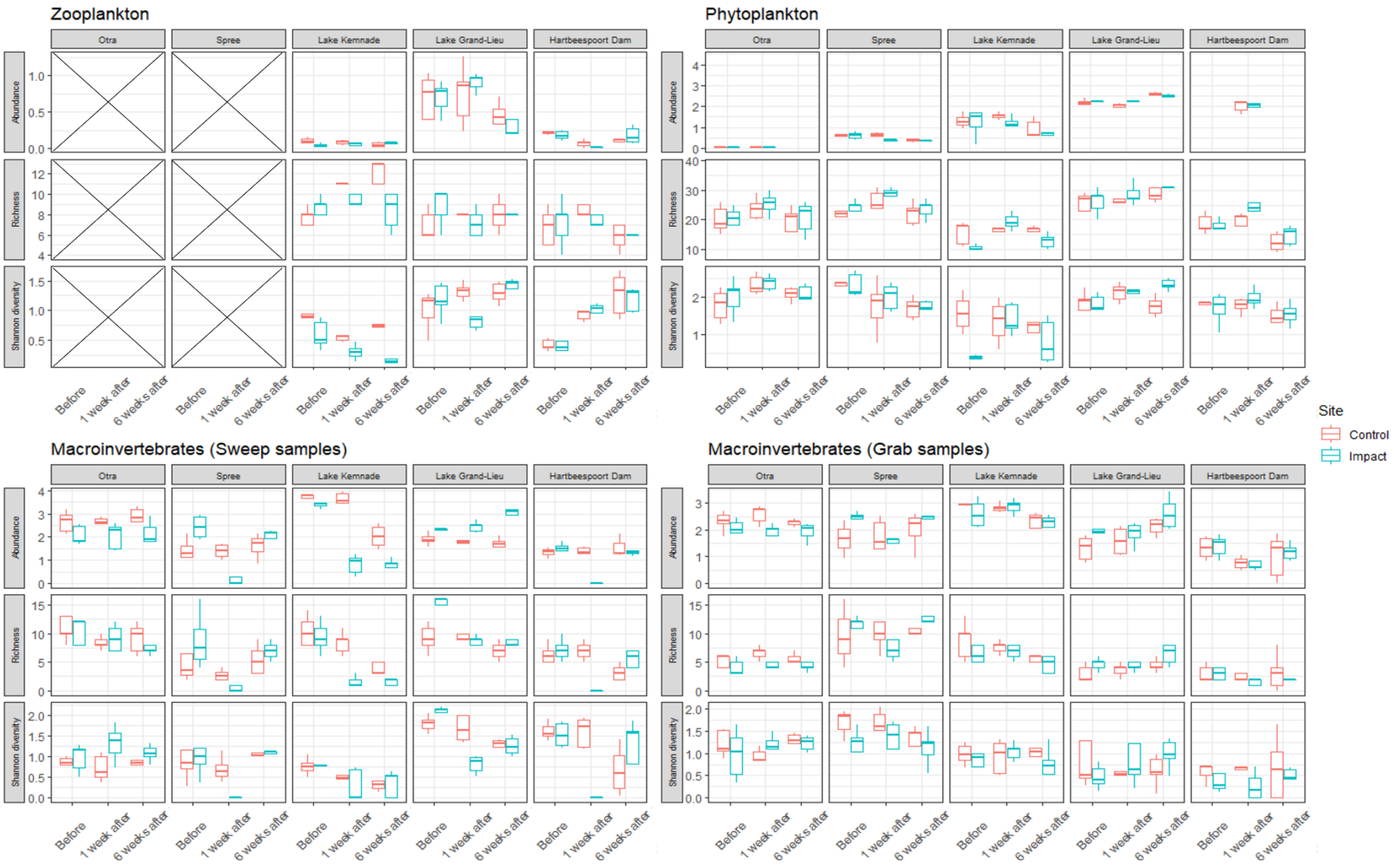


Figure 1: Abundance (log+1 transformed), species richness and Shannon diversity of zooplankton, phytoplankton, and macroinvertebrate assemblages from five sites before, one week after, and six weeks after macrophyte removal. Horizontal bold lines represent the median, boxes the 25% and 75% percentiles, and whiskers the minimum and maximum. n = 5 for each sampling time/session.

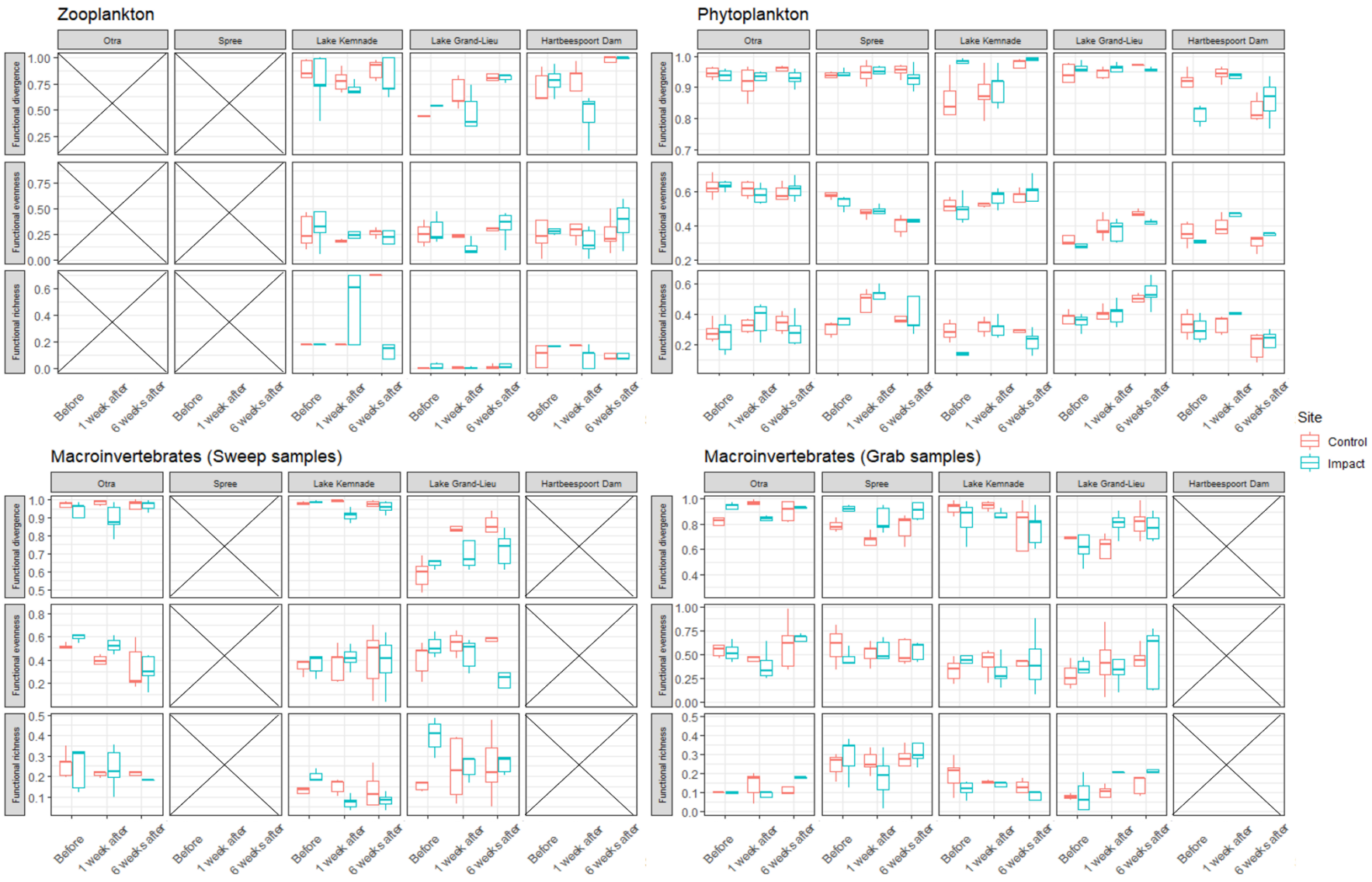


Figure 2: Functional divergence, functional evenness and functional of zooplankton, phytoplankton, and macroinvertebrate assemblages from five sites before, one week after, and six weeks after macrophyte removal. Horizontal bold lines represent the median, boxes the 25% and 75% percentiles, and whiskers the minimum and maximum. n = 5 for each sampling time/session.

Effects of macrophyte removal on aquatic biodiversity

We found adverse effects of removal on zooplankton assemblages after one week as well as six weeks (see Table 2, Table S2 and Table S3). In the overall model for one week after the removal, we found a negative impact on taxa richness (-25%; removal effect compared to before sampling in impacted site; see methods) and a negatively impacted functional divergence (-33%). After six weeks, only taxa richness (-25%) was affected in the overall model, while there was no longer an impact on functional divergence. Taking a closer look at each site after one week, we found negative effects only in Lake Grand-Lieu (taxa richness and Shannon-diversity), while in Lake Kemnade, we found negative (taxa richness) and positive (functional richness) effects. No effects on zooplankton were found in Hartbeespoort Dam.

Phytoplankton was the only group which was positively impacted by macrophyte removal. The overall model showed a positive effect on taxa richness (17%), Shannon-diversity (21%) and functional richness (24%) one week after the removal. After six weeks, the overall model showed no further negative effects. In Lake Kemnade, the river Otra and Hartbeespoort Dam, we found positive effects one week after the removal. After six weeks, only positive effects were found in Hartbeespoort Dam, while in Lake Kemnade, the impact became negative.

Macroinvertebrates associated with macrophytes (sweep samples) were most strongly affected by the removal. After one week, the overall model showed a decrease in abundance (-50%), taxa richness (-49%), Shannon-diversity (-48%), functional richness (-48%), and functional divergence (-38%). After six weeks, the overall model no longer showed an impact. The three lake sites were the most strongly affected. While we found some impacts in all three lakes one week after the removal, effects declined over time. We only found a negative impact on the abundance in Lake Kemnade, while we found a positively impacted abundance in Lake Grand-Lieu. The two river sites showed the least effect, and only the abundance was negatively impacted one week after removal in the river Spree, while no impact was found in the river Otra. Macroinvertebrates

associated with the sediment (grab samples) were not affected by plant removal in the overall model. Only in the river Otra did we find a negative impact on functional richness (-21%).

We combined all the above-mentioned results in our scoring (-1 for negative effects, +1 for positive effects). Both one-week-after and six-week-after sampling illustrated a negative overall impact of plant removal over all groups with a score of -3 (one-week-after) and -1 (six-week-after). However, no effect was consistent across all sites, and the impacts changed over time with site-specific differences. The strongest negative effect one week after the removal was found in Lake Grand-Lieu (-5) ahead of Hartbeespoort Dam (-2), river Spree (-1) and Lake Kemnade (-1). The river Otra was the only site with positive and negative effects equalizing each other (0). The scores changed strongly after six weeks. Lake Grand-Lieu showed no negative effects. In fact, this site had the highest positive impact, with a score of 2. Hartbeespoort Dam also had a positive score (1), while the two rivers, Otra and Spree, had no effect after six weeks. Lake Kemnade was the only site with a negative score after six weeks (-3).

Cyanobacteria

In addition to the scoring, we analyzed the proportion of cyanobacteria in the phytoplankton community to monitor cyanobacteria blooms after the removal of macrophytes (Table S4). We found a significant increase of the proportion of cyanobacteria only at Hartbeespoort Dam, with an increase of 45% after one week and 70% after six weeks. This increase in cyanobacteria was also visible during fieldwork as a green and foamy layer on the water. In Lake Kemnade, we found a 62% reduction in the proportion of cyanobacteria after one week and 44% after six weeks. There were no significant changes in the cyanobacteria proportion in any of the other sites.

Table 2: Scoring of the impact of macrophyte removal on biodiversity. -1: significant negative effect, 1: significant positive effect, 0: No (significant) effect, 0=values too low for analysis.*

		All Site		River Otra		River Spree		Lake Kemnade		Lake Grand-Lieu		Hartbeespoort Dam		Total (parameter)		Total (group)	
	Parameter	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**
Zooplankton	Abundance	0	0	0*	0*	0*	0*	0	1 (153%)	0	0	0	0	**0**	**1**	**-2**	**-1**
	Richness	-1 (-25%)	-1 (-25%)	0*	0*	0*	0*	-1 (-29%)	-1 (-54%)	-1 (-29%)	0	0	0	**-2**	**-1**		
	Shannon	0	0	0*	0*	0*	0*	0	0	-1 (-59%)	0	0	0	**-1**	**0**		
	F-richness	0	0	0*	0*	0*	0*	1 (169%)	-1 (-266%)	0	0	0	0	**1**	**-1**		
	F-evenness	0	0	0*	0*	0*	0*	0	0	0	0	0	0	**0**	**0**		
	F-divergence	-1 (-33%)	0	0*	0*	0*	0*	0	0	0	0	0	0	**0**	**0**		
Phytoplankton	Abundance	0	0	1 (59%)	NA	0	0	0	0	0	0	NA	NA	**1**	**0**	**5**	**1**
	Richness	1 (17%)	0	0	0	0	0	1 (70%)	0	0	0	0	0	**1**	**0**		
	Shannon	1 (21%)	0	0	0	0	0	1 (332%)	0	0	1 (43%)	0	0	**1**	**1**		
	F-richness	1 (24%)	0	0	0	0	0	1 (105%)	0	0	0	0	0	**1**	**0**		
	F-evenness	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0**		
	F-divergence	0	0	0	0	0	0	0	-1 (-12%)	0	0	1 (10%)	1 (15%)	**1**	**0**		
Macroinvertebrate	Abundance	-1 (-50%)	0	0	0	-1 (-86%)	0	-1 (-75%)	-1 (-28%)	0	1 (42%)	-1 (-100%)	0	**-3**	**0**	**-11**	**0**
	Richness	-1 (-49%)	0	0	0	0	0	-1 (-62%)	0	-1 (-47%)	0	-1 (-106%)	0	**-3**	**0**		
	Shannon	-1 (-48%)	0	0	0	0	0	0	0	-1 (-51%)	0	-1 (-99%)	0	**-2**	**0**		
	F-richness	-1 (-38%)	0	0	0	0*	0*	-1 (-65%)	0	-1 (-53%)	0	0*	0*	**-2**	**0**		
	F-evenness	0	0	0	0	0*	0*	0	0	0	0	0*	0*	**0**	**0**		
	F-divergence	0	0	0	0	0*	0*	-1 (-8%)	0	0	0	0*	0*	**-1**	**0**		
Macroinvertebrate	Abundance	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0**	**-1**	**0**
	Richness	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0**		
	Shannon	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0**		
	F-richness	0	0	0	0	0	0	0	0	0	0	0*	0*	**0**	**0**		
	F-evenness	0	0	0	0	0	0	0	0	0	0	0*	0*	**0**	**0**		
	F-divergence	0	0	-1 (-21%)	0	0	0	0	0	0	0	0*	0*	**-1**	**0**		
	Total (sitewise)	**-3**	**-1**	**0**	**0**	**-1**	**0**	**-1**	**-3**	**-5**	**2**	**-2**	**1**				

Discussion

Our results showed that removal of macrophytes affected the diversity of zooplankton, phytoplankton and macroinvertebrates in freshwater lakes and rivers. Although results differed among locations, we found common patterns. Overall, macrophyte removal had negative effects on the zooplankton and macroinvertebrate community and positive effects on the phytoplankton community. These findings are consistent with our first hypothesis. The effects were most pronounced one week after removal, with decreasing effects six weeks after the removal, confirming our second hypothesis.

Macrophyte removal had a stronger effect on macroinvertebrates living on or between plants than on those living in/on the sediment, as illustrated by different responses of the sweep-sampled and grab-sampled communities. This finding aligns with results reported by Känel et al. (1998), which show stronger effects of plant removal on species living directly on plants than species living in/on the sediment. While macroinvertebrates living within the plants (sweep samples) were negatively affected one week after macrophyte removal, in four out of five studied sites, negative effects only remained at Lake Kemnade six weeks after removal. The strong negative effects on macroinvertebrates associated with macrophytes immediately after removal could be explained by a high bycatch of macroinvertebrates together with the removed macrophytes (Dawson et al., 1991; Young et al., 2004). Lake Grand Lieu showed positive effects six weeks after the removal. The decrease in water level, as well as sediment disturbance after macrophyte removal might have increased the resuspension of sediment and small benthic invertebrates which we usually collect in grab samples but only in low density in sweep samples. The benthic macroinvertebrate community was only negatively affected in the river Otra. The removal practice in the Otra strongly affects the sediment, compared to the other sites where the removal has a smaller impact, which could explain that the Otra is the only site where we found negative effects on macroinvertebrates living in the sediment. The removal of floating plants was expected to have less impact on biodiversity as they only take up a small part of the water column. However, in Hartbeespoort Dam with floating *P.*

crassipes, comparable effects to the sites with other plant types were found. The removal of *P. crassipes* has been shown to strongly alter the water chemistry as it can reduce transparency and oxygen levels and increase nutrient availability drastically leading to lethal ammonia levels, all of which have negative effects on biodiversity (Mangas-Ramírez and Elías-Gutiérrez, 2004).

Removal of macrophytes had substantial effects on the zooplankton community. The taxa richness and functional divergence were negatively affected one week after plant removal, and taxa richness stayed reduced even after six weeks. This is in accordance with the higher diversity generally found in macrophyte beds compared to open water, associated with a higher habitat complexity (Choi et al., 2014; Kovalenko et al., 2012). In contrast to macroinvertebrates, no reduction in the abundance of zooplankton was found after macrophyte removal. Lake Kemnade even showed increased zooplankton abundance. The small zooplankton size compared to macroinvertebrates might help them avoid ending up as bycatch of macrophyte removal. Our results cannot confirm earlier studies (Garner et al., 1996; Mangas-Ramírez and Elías-Gutiérrez, 2004), which found a negative effect of macrophyte removal on zooplankton abundance. The increase in phytoplankton abundance might positively affect the zooplankton abundance due to higher availability of food.

Contrary to zooplankton and macroinvertebrates, we found positive effects on phytoplankton. The removal of macrophytes increased taxa richness, Shannon-diversity and functional richness one week after the plant removal. After six weeks, effects of plant removal on the measured diversity/indices were no longer found compared to the control sites. Other studies have found remarkable changes in Chl-a concentration shortly after plant removal, either positive (Bicudo et al., 2007; James et al., 2002) or negative (Alam et al., 1996; Morris et al., 2006). We could not confirm these results with our study. While short-term adverse effects can be explained by increased turbidity (lower light availability), positive effects can be explained by the decreased competition for light and nutrients. Short-term increase of phytoplankton richness and functional richness could be explained by an overlap of remaining plant-associated species and the newly established open water-associated species directly after the removal. Former studies showed

differences in the phytoplankton communities associated with macrophytes and open water sections (Gebrehiwot et al., 2017; Wojciechowski et al., 2018). In Hartbeespoort Dam, the only subtropical site in our study, we found a strong increase of cyanobacteria, aligning with studies with comparable results (Mangas-Ramírez and Elías-Gutiérrez, 2004; Wojciechowski et al., 2018). Allelopathic effects of *P. crassipes* could explain such increase (Liu et al., 2015). None of the other sites showed an increase in the cyanobacteria proportion, but in Lake Kemnade, the cyanobacteria proportion decreased. The reduction in cyanobacteria proportion in Lake Kemnade could be the consequence of the increased zooplankton abundance, which was shown earlier to have the potential to control cyanobacteria (Belfiore et al., 2021; Ger et al., 2014). In both Hartbeespoort Dam and Lake Kemnade, the effects were already evident after one week and remained until the sixth week.

While we found strong effects on assemblages one week after plant removal in the overall model, the effects did not persist six weeks later. Only zooplankton taxa richness remained reduced, while all other parameters that changed after the removal showed some resilience, and effects were not present after six weeks. Känel and Matthaei (1998) showed in a Swiss river that the effect of plant removal on macroinvertebrates fluctuated over time, and after 72 days, overall abundance was still affected. Furthermore, different taxa showed different response patterns. Our study did not last for 72 days, but already after six weeks, most effects had dissipated. We also found strong fluctuations in the values over time. Many existing studies only analyze one time point after the removal (e.g. Bickel and Closs, 2009: sampling after four months; Habib and Yousuf, 2014: sampling after 1-5 days), and such timing differences, to some extent, contribute to the different findings in these studies. In many cases, macrophytes regrow after removal (Bickel and Closs, 2009; Thiemer et al., 2021), which might aid recovery of communities. If frequent macrophyte removal is performed, this might hinder the development of a well-adapted community to both the macrophyte and clear water state.

Impacts of plant removal on the aquatic communities were system-specific, even though an overall

negative effect across systems was reported. The two least impacted sites were the two rivers. Fast recolonization via drift might help the communities to recover quickly (Baxter et al., 2017; Walks, 2007). The two river sites were the only sites where native vegetation grew in dense mats. Therefore, the effects of removal of native compared to exotic plants cannot be separated from the effects of system type. As different plant growth forms have different effects on other organisms (Walker et al., 2013), we could expect different effects of plant removal depending on the growth form and other plant characteristics (e.g., growth rate, dispersal ability, structural density). However, we could not identify such differences in our study. Another confounding factor in our study was the different ongoing macrophyte management strategies in our systems. All our sites were already managed prior to the experiment, and long-term effects of macrophyte removal in the past years could have affected our outcomes.

The monitoring of environmental impacts depends heavily on the choice of the proper experimental design. A simplified study design often results in an inaccurate estimate of the ecological response (Christie et al., 2019). The choice of a BACI (Before-After-Control-Impact) design turned out to be a good decision for our purpose. Values differed in the two sections already before the plants were removed, even if we chose two nearby sections as control and impact sections. Including a control site was important as we found high temporal variability in the control site without plant removal. Using only a CI (Control-Impact) or a BA (Before-After) design, as was often the case in former studies, might lead to wrong conclusions, and effects might be overlooked. While dense mats of macrophytes are often considered a nuisance due to their interference with human lake and river uses, their removal comes with adverse side effects for the ecosystem, including biodiversity. Biodiversity loss, especially in freshwater systems, is one of the biggest challenges of our time (Tickner et al., 2020) and saving biodiversity is part of the sustainable development goals defined by the United Nations (2015). A fact-based, unbiased understanding of macrophytes and their interaction with other organisms is key to developing management strategies to tackle this biodiversity loss. Future sustainable management strategies for mass develop of

macrophytes must consider not only the macrophytes as a problem but also other ecosystem services provided such as their role in promoting biodiversity.

Acknowledgements

We thank all members of the MadMacs project. An extra thank goes to Bertrand le Rouzic, Jean-Marc Paillisson, Guillaume Bouger, Augustin Soulard, Lena Schulz, Lisa Sanden, Constancia Levertz, and Nompumelelo Baso for all the help with the fieldwork in the different campaigns. Further we thank Limnologie SARL, Morgane Menvielle, Augustin Soulard and Lisa Sanden for their help with the labwork and Aijuan Liao for proofreading. This project would not have been possible without close collaboration with Jean-Marc Gillier and the local stakeholders National Nature Reserve of the Lake Grand-Lieu, Ruhrverband and the Department of Water and Sanitation South Africa. All data used for this manuscript will be made publicly available at the end of the project via the webpage of the Water JPI (http://www.waterjpi.eu/).

Funding

This work is part of the MadMacs (Mass development of aquatic macrophytes – causes and consequences of macrophyte removal for ecosystem structure, function, and services) project in the framework of the collaborative international consortium of the 2017 call of the Water Challenges for a Changing World Joint Programme Initiative (Water JPI). The MadMacs project is supported by the French Agence National de Recherche (N° ANR-18-IC4W-0004-06), the Research Council of Norway (297202/E10), the German Federal Ministry of Education and Research (02WGR005), the South African Water Research Commission (K5/2951), and the Fundação Araucária in Brazil (N° 186/2019). Additional funding was provided by Krypsiv på Sørlandet, NIVA and NMBU.

References

AFNOR, 2006. BS EN 15204:2006: Qualité de l'eau Norme guide pour le dénombrement du phytoplancton par microscopie inversée (méthode Utermöhl) [Water quality Guide standard for phytoplankton enumeration by inverted microscopy (Utermöhl method)] (No. 2006- 09–29).

Alam, S.K., Ager, L.A., Rosegger, T.M., Lange, T.R., 1996. The Effects of Mechanical Harvesting of Floating Plant Tussock Communities on Water Quality in Lake Istokpoga, Florida. Lake Reserv. Manag. 12, 455–461. https://doi.org/10.1080/07438149609354285

Amorim, C.A., Moura, A.N., 2020. Effects of the manipulation of submerged macrophytes, large zooplankton, and nutrients on a cyanobacterial bloom: A mesocosm study in a tropical shallow reservoir. Environ. Pollut. 265, 114997. https://doi.org/10.1016/j.envpol.2020.114997

Bakker, E.S., Hilt, S., 2016. Impact of water-level fluctuations on cyanobacterial blooms: options for management. Aquat. Ecol. 50, 485–498. https://doi.org/10.1007/s10452-015-9556-x

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Baxter, C.V., Kennedy, T.A., Miller, S.W., Muehlbauer, J.D., Smock, L.A., 2017. Chapter 21 - Macroinvertebrate Drift, Adult Insect Emergence and Oviposition, in: Hauer, F.R., Lamberti, G.A. (Eds.), Methods in Stream Ecology, Volume 1 (Third Edition). Academic Press, Boston, pp. 435–456. https://doi.org/10.1016/B978-0-12-416558-8.00021-4

Belfiore, A.P., Buley, R.P., Fernandez-Figueroa, E.G., Gladfelter, M.F., Wilson, A.E., 2021. Zooplankton as an alternative method for controlling phytoplankton in catfish pond aquaculture. Aquac. Rep. 21, 100897. https://doi.org/10.1016/j.aqrep.2021.100897

Bickel, T.O., Closs, G.P., 2009. Impact of partial removal of the invasive macrophyte Lagarosiphon major (hydrocharitaceae) on invertebrates and fish. River Res. Appl. 25, 734–744. https://doi.org/10.1002/rra.1187

Bicudo, D.D.C., Fonseca, B.M., Bini, L.M., Crossetti, L.O., Bicudo, C.E.D.M., Araújo-Jesus, T., 2007. Undesirable side-effects of water hyacinth control in a shallow tropical reservoir. Freshw. Biol. 52, 1120–1133. https://doi.org/10.1111/j.1365-2427.2007.01738.x

Bledzki, L.A., Rybak, J.I., 2016. Freshwater Crustacean Zooplankton of Europe: Cladocera & Copepoda (Calanoida, Cyclopoida) Key to species identification, with notes on ecology, distribution, methods and introduction to data analysis. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-29871-9

Boerema, A., Schoelynck, J., Bal, K., Vrebos, D., Jacobs, S., Staes, J., Meire, P., 2014. Economic valuation of ecosystem services, a case study for aquatic vegetation removal in the Nete catchment (Belgium). Ecosyst. Serv. 7, 46–56. https://doi.org/10.1016/j.ecoser.2013.08.001

Buczyński, P., Zawal, A., Buczyńska, E., Stępień, E., Dąbkowski, P., Michoński, G., Szlauer-Łukaszewska, A., Pakulnicka, J., Stryjecki, R., Czachorowski, S., 2016. Early recolonization of a dredged lowland river by dragonflies (Insecta: Odonata). Knowl. Manag. Aquat. Ecosyst. 43. https://doi.org/10.1051/kmae/2016030

Burks, R.L., Lodge, D.M., Jeppesen, E., Lauridsen, T.L., 2002. Diel horizontal migration of zooplankton: costs and benefits of inhabiting the littoral. Freshw. Biol. 47, 343–365. https://doi.org/10.1046/j.1365-2427.2002.00824.x

Caraco, N., Cole, J., Findlay, S., Wigand, C., 2006. Vascular Plants as Engineers of Oxygen in Aquatic Systems. BioScience 56, 219–225. https://doi.org/10.1641/0006-3568(2006)056[0219:VPAEOO]2.0.CO;2

Carey, N., Strachan, S.R., Robson, B.J., 2018. Impacts of Indian waterfern (Ceratopteris thalictroides (L.) Brongn.) infestation and removal on macroinvertebrate biodiversity and conservation in spring-fed streams in the Australian arid zone. Aquat. Conserv. Mar. Freshw. Ecosyst. 28, 466–475. https://doi.org/10.1002/aqc.2828

Choi, J.-Y., Jeong, K.-S., La, G.-H., Joo, G.-J., 2014. Effect of removal of free-floating macrophytes

on zooplankton habitat in shallow wetland. Knowl. Manag. Aquat. Ecosyst. 414. https://doi.org/10.1051/kmae/2014023

Christie, A.P., Amano, T., Martin, P.A., Shackelford, G.E., Simmons, B.I., Sutherland, W.J., 2019. Simple study designs in ecology produce inaccurate estimates of biodiversity responses. J. Appl. Ecol. 56, 2742–2754. https://doi.org/10.1111/1365-2664.13499

Dabkowski, P., Buczynski, P., Zawal, A., Stepien, E., Buczynska, E., Stryjecki, R., Czachorowski, S., Smietana, P., Szenejko, M., 2016. The impact of dredging of a small lowland river on water beetle fauna (Coleoptera). J. Limnol. 75. https://doi.org/10.4081/jlimnol.2016.1270

Dawson, F., Clinton, E., Ladle, M., 1991. Invertebrates on cut weed removed during weed-cutting operations along an English river, the River Frome, Dorset. Aquac. Res. 22, 113–121. https://doi.org/10.1111/j.1365-2109.1991.tb00500.x

de Winston, M., Jones, M., Edwards, T., Özkundakci, D., Wells, W., McBrise, C., Rowe, D., Hamilton, D., Clayton, J., Champion, P., Hofstra, D., 2013. de Winton M, Jones H, Edwards T, Özkundakci D, Wells R, McBride C, Rowe D, Hamilton D, Clayton J, Champion P, Hofstra D (2013). Review of best management practices for aquatic vegetation control in stormwater ponds, wetlands, and lakes. (Auckland Council technical report No. TR2013/026).

Dugdale, T.M., Hunt, T.D., Clements, D., 2013. Aquatic weeds in Victoria: Where and why are they a problem, and how are they being controlled?

Ferreiro, N., Feijoó, C., Giorgi, A., Rosso, J., 2014. Macroinvertebrates select complex macrophytes independently of their body size and fish predation risk in a Pampean stream. Hydrobiologia 740, 191–205. https://doi.org/10.1007/s10750-014-1953-y

Garner, P., Bass, J. a. B., Collett, G.D., 1996. The effects of weed cutting upon the biota of a large regulated river. Aquat. Conserv. Mar. Freshw. Ecosyst. 6, 21–29. https://doi.org/10.1002/(SICI)1099-0755(199603)6:1<21::AID-AQC171>3.0.CO;2-Z

Gavrilko, D.E., Shurganova, G.V., Kudrin, I.A., Yakimov, B.N., 2020. Identification of Freshwater

Zooplankton Functional Groups Based on the Functional Traits of Species. (In Russ.). Povolzhskiy Journal of Ecology 0, 290–306. https://doi.org/10.35885/1684-7318-2020-3-290-306

Gebrehiwot, M., Kifle, D., Stiers, I., Triest, L., 2017. Phytoplankton functional dynamics in a shallow polymictic tropical lake: the influence of emergent macrophytes. Hydrobiologia 797, 69–86. https://doi.org/10.1007/s10750-017-3161-z

Ger, K.A., Hansson, L.-A., Lürling, M., 2014. Understanding cyanobacteria-zooplankton interactions in a more eutrophic world. Freshw. Biol. 59, 1783–1798. https://doi.org/10.1111/fwb.12393

Grygoruk, M., Frąk, M., Chmielewski, A., 2015. Agricultural Rivers at Risk: Dredging Results in a Loss of Macroinvertebrates. Preliminary Observations from the Narew Catchment, Poland. Water 7, 4511–4522. https://doi.org/10.3390/w7084511

Güereña, D., Neufeldt, H., Berazneva, J., Duby, S., 2015. Water hyacinth control in Lake Victoria: Transforming an ecological catastrophe into economic, social, and environmental benefits. Sustain. Prod. Consum. 3, 59–69. https://doi.org/10.1016/j.spc.2015.06.003

Habib, S., Yousuf, A.R., 2014. Impact of mechanical deweeding on the phytophilous macroinvertebrate community of an eutrophic lake. Environ. Sci. Pollut. Res. 21, 5653–5659. https://doi.org/10.1007/s11356-013-2470-7

Hilt, S., Brothers, S., Jeppesen, E., Veraart, A.J., Kosten, S., 2017. Translating Regime Shifts in Shallow Lakes into Changes in Ecosystem Functions and Services. BioScience 67, 928–936. https://doi.org/10.1093/biosci/bix106

Hussner, A., Stiers, I., Verhofstad, M.J.J.M., Bakker, E.S., Grutters, B.M.C., Haury, J., van Valkenburg, J.L.C.H., Brundu, G., Newman, J., Clayton, J.S., Anderson, L.W.J., Hofstra, D., 2017. Management and control methods of invasive alien freshwater aquatic plants: A review. Aquat. Bot. 136, 112–137. https://doi.org/10.1016/j.aquabot.2016.08.002

James, W.F., Barko, J.W., Eakin, H.L., 2002. Water Quality Impacts of Mechanical Shredding of

Aquatic Macrophytes. J Aquat Plant Manage 40, 7.

Janse, J.H., Van Puijenbroek, P.J.T.M., 1998. Effects of eutrophication in drainage ditches. Environ. Pollut., Nitrogen, the Confer-N-s First International Nitrogen Conference 1998 102, 547–552. https://doi.org/10.1016/S0269-7491(98)80082-1

Janssen, A.B.G., Hilt, S., Kosten, S., de Klein, J.J.M., Paerl, H.W., Van de Waal, D.B., 2021. Shifting states, shifting services: Linking regime shifts to changes in ecosystem services of shallow lakes. Freshw. Biol. 66, 1–12. https://doi.org/10.1111/fwb.13582

Känel, B., Matthaei, C., Uehlinger, U., 1998. Disturbance by aquatic plant management in streams: Effects on benthic invertebrates. Regul. Rivers-Res. Manag. - REGUL RIVER 14, 341–356. https://doi.org/10.1002/(SICI)1099-1646(199807/08)14:43.0.CO;2-2

Körner, S., Nicklisch, A., 2002. Allelopathic Growth Inhibition of Selected Phytoplankton Species by Submerged Macrophytes1. J. Phycol. 38, 862–871. https://doi.org/10.1046/j.1529-8817.2002.t01-1-02001.x

Kottek, M., Grieser, J., Beck, C., Rudolf, B., Rubel, F., 2006. World Map of the Köppen-Geiger climate classification updated. Meteorol. Z. 259–263. https://doi.org/10.1127/0941-2948/2006/0130

Kovalenko, K.E., Thomaz, S.M., Warfe, D.M., 2012. Habitat complexity: approaches and future directions. Hydrobiologia 685, 1–17. https://doi.org/10.1007/s10750-011-0974-z

Kremer, C.T., Williams, A.K., Finiguerra, M., Fong, A.A., Kellerman, A., Paver, S.F., Tolar, B.B., Toscano, B.J., 2017. Realizing the potential of trait-based aquatic ecology: New tools and collaborative approaches. Limnol. Oceanogr. 62, 253–271. https://doi.org/10.1002/lno.10392

Kuiper, J.J., Verhofstad, M.J.J.M., Louwers, E.L.M., Bakker, E.S., Brederveld, R.J., van Gerven, L.P.A., Janssen, A.B.G., de Klein, J.J.M., Mooij, W.M., 2017. Mowing Submerged Macrophytes in Shallow Lakes with Alternative Stable States: Battling the Good Guys? Environ. Manage. 59, 619–634. https://doi.org/10.1007/s00267-016-0811-2

Laplace-Treyture, C., Derot, J., Prévost, E., Le Mat, A., Jamoneau, A., 2021. Phytoplankton morpho-functional trait dataset from French water-bodies. Sci. Data 8, 40. https://doi.org/10.1038/s41597-021-00814-0

Liu, R., Ran, X., Bai, F., Xu, J., Yang, S., Shi, J., Wu, Z., 2015. Use of chlorophyll a fluorescence to elucidate the toxicity target of N-phenyl-2-naphthylamine on photosynthetic system of Cylindrospermopsis raciborskii (Cyanobacteria). Phycologia 54, 12–19. https://doi.org/10.2216/14-050.1

Lusardi, R.A., Jeffres, C.A., Moyle, P.B., 2018. Stream macrophytes increase invertebrate production and fish habitat utilization in a California stream. River Res. Appl. 34, 1003–1012. https://doi.org/10.1002/rra.3331

Magneville, C., Loiseau, N., Albouy, C., Casajus, N., Claverie, T., Escalas, A., Leprieur, F., Maire, E., Mouillot, D., Villéger, S., 2022. mFD: an R package to compute and illustrate the multiple facets of functional diversity. Ecography 2022. https://doi.org/10.1111/ecog.05904

Mangas-Ramírez, E., Elías-Gutiérrez, M., 2004. Effect of mechanical removal of water hyacinth (Eichhornia crassipes) on the water quality and biological communities in a Mexican reservoir. Aquat. Ecosyst. Health Manag. 7, 161–168. https://doi.org/10.1080/14634980490281597

Meerhoff, M., Fosalba, C., Bruzzone, C., Mazzeo, N., Noordoven, W., Jeppesen, E., 2006. An experimental study of habitat choice by Daphnia: plants signal danger more than refuge in subtropical lakes. Freshw. Biol. 51, 1320–1330. https://doi.org/10.1111/j.1365-2427.2006.01574.x

Miliša, M., Kepčija, R.M., Radanović, I., Ostojić, A., Habdija, I., 2006. The impact of aquatic macrophyte (Salix sp. and Cladium mariscus (L.) Pohl.) removal on habitat conditions and macroinvertebrates of tufa barriers (Plitvice Lakes, Croatia). Hydrobiologia 573, 183–197. https://doi.org/10.1007/s10750-006-0271-4

Morris, K., Bailey, P.C.E., Boon, P.I., Hughes, L., 2006. Effects of plant harvesting and nutrient enrichment on phytoplankton community structure in a shallow urban lake. Hydrobiologia 571, 77–91. https://doi.org/10.1007/s10750-006-0230-0

Padisák, J., Crossetti, L.O., Naselli-Flores, L., 2009. Use and misuse in the application of the phytoplankton functional classification: a critical review with updates. Hydrobiologia 621, 1–19. https://doi.org/10.1007/s10750-008-9645-0

R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Riis, T., Biggs, B.J.F., 2001. Distribution of macrophytes in New Zealand streams and lakes in relation to disturbance frequency and resource supply—a synthesis and conceptual model. N. Z. J. Mar. Freshw. Res. 35, 255–267. https://doi.org/10.1080/00288330.2001.9516996

Scheffer, M., Hosper, S.H., Meijer, M.-L., Moss, B., Jeppesen, E., 1993. Alternative equilibria in shallow lakes. Trends Ecol. Evol. 8, 275–279. https://doi.org/10.1016/0169-5347(93)90254-M

Shatwell, T., Nicklisch, A., Köhler, J., 2012. Temperature and photoperiod effects on phytoplankton growing under simulated mixed layer light fluctuations. Limnol. Oceanogr. 57, 541–553. https://doi.org/10.4319/lo.2012.57.2.0541

Stansbury, J., Kozimor, L., Admiraal, D., Dove, E., 2008. Water quality modeling of the effects of macrophytes on dissolved oxygen in a shallow tailwater reservoir. Lake Reserv. Manag. 24, 339–348. https://doi.org/10.1080/07438140809354844

Stiers, I., Crohain, N., Josens, G., Triest, L., 2011. Impact of three aquatic invasive species on native plants and macroinvertebrates in temperate ponds. Biol. Invasions 13, 2715–2726. https://doi.org/10.1007/s10530-011-9942-9

Švanys, A., Paškauskas, R., Hilt, S., 2014. Effects of the allelopathically active macrophyte Myriophyllum spicatum on a natural phytoplankton community: a mesocosm study. Hydrobiologia 737, 57–66. https://doi.org/10.1007/s10750-013-1782-4

Tachet, H., Richoux, P., Bournaud, M., Usseglio-Polatera, P., 2000. Invertébrés d'eau douce: Systématique, biologie, écologie [Freshwater invertebrates: systematics, biology and ecology]. CNRS ÉDITIONS, Paris.

Thiemer, K., Schneider, S., Demars, B., 2021. Mechanical removal of macrophytes in freshwater ecosystems: Implications for ecosystem structure and function. Sci. Total Environ. 782, 146671. https://doi.org/10.1016/j.scitotenv.2021.146671

Thomaz, S.M., Dibble, E.D., Evangelista, L.R., Higuti, J., Bini, L.M., 2008. Influence of aquatic macrophyte habitat complexity on invertebrate abundance and richness in tropical lagoons. Freshw. Biol. 53, 358–367. https://doi.org/10.1111/j.1365-2427.2007.01898.x

Tickner, D., Opperman, J.J., Abell, R., Acreman, M., Arthington, A.H., Bunn, S.E., Cooke, S.J., Dalton, J., Darwall, W., Edwards, G., Harrison, I., Hughes, K., Jones, T., Leclère, D., Lynch, A.J., Leonard, P., McClain, M.E., Muruven, D., Olden, J.D., Ormerod, S.J., Robinson, J., Tharme, R.E., Thieme, M., Tockner, K., Wright, M., Young, L., 2020. Bending the Curve of Global Freshwater Biodiversity Loss: An Emergency Recovery Plan. BioScience 70, 330–342. https://doi.org/10.1093/biosci/biaa002

Underwood, A.J., 1991. Beyond BACI: Experimental designs for detecting human environmental impacts on temporal variations in natural populations. Mar. Freshw. Res. 42, 569–587. https://doi.org/10.1071/mf9910569

United Nations, 2015. Resolution adopted by the General Assembly on 25 September 2015: 70/1. Transforming our world: the 2030 Agenda for Sustainable Development (No. A/RES/70/1).

van Donk, E., Gulati, R.D., Iedema, A., Meulemans, J.T., 1993. Macrophyte-related shifts in the nitrogen and phosphorus contents of the different trophic levels in a biomanipulated shallow lake. Hydrobiologia 251, 19–26. https://doi.org/10.1007/BF00007160

Vanderstukken, M., Mazzeo, N., Van Colen, W., Declerck, S. a. J., Muylaert, K., 2011. Biological control of phytoplankton by the subtropical submerged macrophytes Egeria densa and

Potamogeton illinoensis: a mesocosm study. Freshw. Biol. 56, 1837–1849. https://doi.org/10.1111/j.1365-2427.2011.02624.x

Verhofstad, M.J.J.M., Bakker, E.S., 2019. Classifying nuisance submerged vegetation depending on ecosystem services. Limnology 20, 55–68. https://doi.org/10.1007/s10201-017-0525-z

Villéger, S., Mason, N.W.H., Mouillot, D., 2008. New Multidimensional Functional Diversity Indices for a Multifaceted Framework in Functional Ecology. Ecology 89, 2290–2301. https://doi.org/10.1890/07-1206.1

Walker, P.D., Wijnhoven, S., van der Velde, G., 2013. Macrophyte presence and growth form influence macroinvertebrate community structure. Aquat. Bot. 104, 80–87. https://doi.org/10.1016/j.aquabot.2012.09.003

Walks, D.J., 2007. Persistence of plankton in flowing water. Can. J. Fish. Aquat. Sci. 64, 1693–1702. https://doi.org/10.1139/f07-131

Wang, H., Zhong, G., Yan, H., Liu, H., Wang, Y., Zhang, C., 2012. Growth Control of Cyanobacteria by Three Submerged Macrophytes. Environ. Eng. Sci. 29, 420–425. https://doi.org/10.1089/ees.2010.0286

Ward-Campbell, B., Cottenie, K., Mandrak, N., McLaughlin, R., 2017. Maintenance of agricultural drains alters physical habitat, but not macroinvertebrate assemblages exploited by fishes. J. Environ. Manage. 203, 29–39. https://doi.org/10.1016/j.jenvman.2017.07.032

Wojciechowski, J., Burda, T.M., Scheer, M.B., Costa, E.A.D. da, Fernandes, L.F., 2018. Potential effects of mechanically removing macrophytes on the phytoplankton community of a subtropical reservoir. Acta Bot. Bras. 32, 588–594.

Wolters, J.-W., Reitsema, R.E., Verdonschot, R.C.M., Schoelynck, J., Verdonschot, P.F.M., Meire, P., 2019. Macrophyte-specific effects on epiphyton quality and quantity and resulting effects on grazing macroinvertebrates. Freshw. Biol. 64, 1131–1142. https://doi.org/10.1111/fwb.13290

Xu, X., Zhou, Y., Han, R., Song, K., Zhou, X., Wang, G., Wang, Q., 2019. Eutrophication triggers

the shift of nutrient absorption pathway of submerged macrophytes: Implications for the phytoremediation of eutrophic waters. J. Environ. Manage. 239, 376–384. https://doi.org/10.1016/j.jenvman.2019.03.069

Young, R.G., Keeley, N.B., Shearer, K.A., Crowe, A.L.M., 2004. Impacts of diquat herbicide and mechanical excavation on spring-fed drains in Marlborough, New Zealand, SCIENCE FOR CONSERVATION. Department of Conservation, Wellington.

Supplementary Information:

Table S1: List of traits included in the functional analysis for the three organism groups. Letter in brackets describes the format of each trait: (Q) quantitative traits , (N) nominal traits , (F) fuzzy traits

Zooplankton	Phytoplankton	Macroinvertebrates
Body length (Q)	Life form (N)	Maximal potential size (F)
Trophic group (N)	Mean width (Q)	Life cycle duration (F)
Feeding type: active prey capture (N)	Mean length (Q)	Potential number of cycles per year (F)
Feeding type: Primary filtration (N)	Biovolume (Q)	Aquatic stages (F)
Feeding type: Secondary filtration (N)	Size class based on the individual length (O)	Reproduction (F)
Feeding type: Gathering (N)	Reynolds Functional Groups (N)	Dispersal (F)
Locomotion type: Swimming (N)	Main nutrition mode (N)	Resistance forms (F)
Locomotion type: Crawling (N)	Motility (N)	Respiration (F)
Locomotion type: Attachment (N)	Flagellum (N)	Locomotion and substrate relation (F)
	Aerotope (N)	Food (F)
	Contractile vacuole (N)	Feeding habits (F)
	Mucilage (N)	Transversal distribution (F)
	Akinete (N)	Longitudinal distribution (F)
	Heterocyte (N)	Altitude (F)
	Plast (N)	Substrate (F)
	Siliceous skeleton (N)	Current velocity (F)
	Ornamentation (N)	Trophic status (F)
	Chlorophyll-b (N)	Salinity (F)
	Chlorophyll-c (N)	Temperature (F)
	Xanthophyll (N)	Saprobity (F)
	Phycobilin (N)	
	Toxin (N)	

Table S2: Detailed statistical results for linear mixed effect models.

		1 week				6 weeks			
	Parameter	**Df (numerator)**	**Df (Denominator)**	**F-statistic**	**p-value**	**Df (numerator)**	**Df (Denominator)**	**F-statistic**	**p-value**
Zooplankton	Abundance	1	54	0.024	0.878	1	54	1.773	0.189
	Richness	1	54	8.068	**0.006**	1	54	5.303	**0.025**
	Shannon	1	54	2.932	0.095	1	54	0.825	0.368
	F-richness	1	53.006	0.585	0.448	1	53.007	3.895	0.054
	F-evenness	1	55	1.110	0.297	1	55	0.273	0.603
	F-divergence	1	53.022	5.323	**0.025**	1	53.015	0.401	0.529
Phytoplankton	Abundance	1	72.002	0.111	0.740	1	53.002	0.910	0.345
	Richness	1	95.991	5.717	**0.019**	1	95.999	0.648	0.423
	Shannon	1	95.996	4.466	**0.037**	1	96.003	2.405	0.124
	F-richness	1	95.984	4.614	**0.034**	1	95.999	0.933	0.336
	F-evenness	1	96.004	1.085	0.300	1	96.009	1.229	0.270
	F-divergence	1	95.937	0.012	0.915	1	95.998	0.278	0.599
Macroinvertebrate	Abundance	1	89.006	20.324	**<0.001**	1	90.016	0.334	0.565
	Richness	1	88.996	19.503	**<0.001**	1	90.014	0.791	0.376
	Shannon	1	89.011	10.671	**0.002**	1	90.01	0.603	0.440
	F-richness	1	64.632	5.725	**0.020**	1	66.562	3.005	0.087
	F-evenness	1	65.406	0.042	0.840	1	66.977	0.546	0.463
	F-divergence	1	64.136	1.482	0.228	1	66.084	0.004	0.952
Macroinvertebrate	Abundance	1	90.016	1.686	0.197	1	90.029	0.023	0.881
	Richness	1	90.029	0.023	0.881	1	89.999	0.168	0.683
	Shannon	1	90.005	1.687	0.198	1	90.023	0.900	0.345
	F-richness	1	66.02	0.002	0.964	1	68.034	2.540	0.115
	F-evenness	1	66.081	0.397	0.532	1	68.138	0.423	0.518
	F-divergence	1	66.014	0.252	0.617	1	68.136	0.183	0.671

Table S3: P-values corresponding to Table 2. F-richness/-evenness/-divergence stands for Functional richness/evenness/divergence

		All Site		River Otra		River Spree		Lake Kemnade		Lake Grand-Lieu		Hartbeespoort Dam		Total (parameter)		Total(group)	
	Parameter	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**
Zooplankton	Abundance	0.88	0.19	NA	NA	NA	NA	0.16	0.01	0.47	0.48	0.17	0.21	**0**	**1**	**-2**	**-1**
	Richness	<0.01	0.03	NA	NA	NA	NA	<0.01	<0.001	0.05	0.21	0.55	0.80	**-2**	**-1**		
	Shannon	0.09	0.37	NA	NA	NA	NA	0.83	0.21	<0.01	0.65	0.77	0.54	**-1**	**0**		
	F-richness	0.45	0.05	NA	NA	NA	NA	0.03	<0.01	0.12	0.43	0.01	0.08	**1**	**-1**		
	F-evenness	0.30	0.60	NA	NA	NA	NA	0.85	0.76	0.05	0.94	0.68	0.40	**0**	**0**		
	F-divergence	0.02	0.53	NA	NA	NA	NA	0.69	0.80	0.13	0.92	0.08	0.39	**0**	**0**		
Phytoplankton	Abundance	0.74	0.34	<0.01	NA	0.19	0.95	0.59	0.81	0.10	0.21	NA	NA	**1**	**0**	**5**	**1**
	Richness	0.02	0.42	0.53	0.79	0.64	0.63	<0.01	0.36	0.50	0.87	0.23	0.20	**1**	**0**		
	Shannon	0.04	0.12	0.54	0.31	0.47	0.88	<0.01	0.11	0.74	<0.01	0.15	0.45	**1**	**1**		
	F-richness	0.03	0.34	0.39	0.56	0.83	0.56	0.01	0.09	0.52	0.15	0.27	0.29	**1**	**0**		
	F-evenness	0.30	0.27	0.23	0.91	0.64	0.10	0.44	0.41	0.59	0.83	0.05	0.44	**0**	**0**		
	F-divergence	0.91	0.60	0.25	0.79	0.83	0.24	0.07	0.02	0.75	0.23	0.02	<0.01	**1**	**0**		
Macroinvertebrate	Abundance	<0.001	0.57	0.98	0.63	<0.001	0.22	<0.001	<0.01	0.29	<0.01	<0.001	0.31	**-3**	**0**	**-11**	**0**
	Richness	<0.001	0.38	0.51	0.71	0.16	0.71	<0.01	0.51	<0.01	0.11	<0.001	0.29	**-3**	**0**		
	Shannon	<0.01	0.44	0.17	0.89	0.15	0.97	0.32	0.61	<0.01	0.79	<0.001	0.06	**-2**	**0**		
	F-richness	0.02	0.09	0.63	0.82	NA	NA	<0.01	0.10	0.03	0.10	NA	NA	**-2**	**0**		
	F-evenness	0.84	0.46	0.40	0.25	NA	NA	0.93	0.81	0.06	0.10	NA	NA	**0**	**0**		
	F-divergence	0.23	0.95	0.18	0.23	NA	NA	<0.001	0.09	0.05	0.15	NA	NA	**-1**	**0**		
Macroinvertebrate	Abundance	0.20	0.88	0.28	0.98	0.07	0.47	0.13	0.23	0.40	0.76	0.85	0.83	**0**	**0**	**-1**	**0**
	Richness	0.34	0.68	0.44	0.86	0.44	0.71	0.38	0.68	0.35	0.75	0.36	0.47	**0**	**0**		
	Shannon	0.20	0.35	0.18	0.63	0.55	0.34	0.63	0.27	0.18	0.06	0.37	0.91	**0**	**0**		
	F-richness	0.96	0.12	0.23	0.39	0.19	0.70	0.14	0.37	0.17	0.26	NA	NA	**0**	**0**		
	F-evenness	0.53	0.52	0.69	0.52	0.48	0.26	0.11	0.69	0.75	0.81	NA	NA	**0**	**0**		
	F-divergence	0.62	0.67	<0.01	0.37	0.35	0.63	0.72	0.56	0.13	0.92	NA	NA	**-1**	**0**		

Total (sitewise)	**-3**	**-1**	**0**	**0**	**-1**	**0**	**-1**	**-3**	**-5**	**2**	**-2**	**1**	

Table S4: P-values for analysis of the Cyanobacteria proportion.

		All Site		**River Otra**		**River Spree**		**Lake Kemnade**		**Lake Grand-Lieu**		**Hartbeespoort Dam**	
Parameter		**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**	**1 week**	**6 weeks**
Cyanobacteria proportion	p-Value	0.980	0.864	0.077	0.369	0.955	0.693	0.005	0.035	0.913	0.091	0.037	0.007
	% Change	0	0	0	0	0	0	-62	-44	0	0	45	70