The structuring role of macrophytes on aquatic communities : an international and multigroup approach

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Preface

This PhD thesis was conducted in the framework of the MadMacs project (Mass development of aquatic macrophytes; causes and consequences of macrophyte removal for ecosystem structure, function, and services) between November 2019 and October 2022 at the Université de Rennes 1 (France). The work was supervised by Professor Gabrielle Thiébaut, Dr. Christophe Piscart and Dr. Alexandrine Pannard. Chapter 1 and 3 was conducted in collaboration with researchers from the Norwegian Institute for Water Research (NIVA), the Norwegian University of Live Sciences (NMBU), the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), the Rhodes University in Grahamstown and the Federal University of Paraná. Fundings for the PhD were provided by the "Agence Nationale de la recherche" (ANR; N° ANR-18-IC4W-0004-06). This PhD thesis consists of three chapters, including two published articles (in Chapters 1 and 3), an submitted article (Chapter 2), and additional results (Chapters 1 and 3). The thesis is followed by a summary (résumé) in French.

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Introduction

1. General Introduction

Freshwater makes up only 0.01% of the world's water and covers less than 1% of the world's surface (Gleick, 1996). Despite such small distribution, freshwater systems are inhabited by a high diversity of organisms (Table 1). Balian et al. (2008) provided an overview of aquatic animals. A total of 125'530 animal species were found spread over all biogeographic regions. In other words, freshwater systems provide habitats to one out of ten known species worldwide (1'324'000 species; UNITED NATIONS ENVIRONMENT PROGRAMME, 2002). With 75'874 species worldwide, insects make up more than 60% of known aquatic species. For macrophytes, a total number of 2'614 species was recorded (Chambers et al., 2008).

Due to their small size, species identification and estimation of the global biodiversity are more complicated for phytoplankton. Although no global estimate for phytoplankton is available, the European freshwater information platform (www.freshwaterecology.info) has listed 2'514 species of phytoplankton alone for Europe.

Table 1: Summary of global aquatic biodiversity of macrophytes and animals. The table is based on data from Chambers et al. (2008) for macrophytes and Balian et al. 2008 for animals.

	Palearctic Region	Nearctic Region	Afrotropical Region	Neotropical Region	Oriental Region	Australasian region	Pacific and Oceanic Islands	Antarctic region	**World**
Macrophytes									
Pteridophyta	8	7	9	8	11	10	8	0	**171**
Spermatophyta	146	165	188	184	181	142	54	9	**2 443**
Total	**154**	**172**	**197**	**192**	**192**	**152**	**62**	**9**	**2 614**
Animal									
Annelids	870	350	186	338	242	210	10	10	**1 761**
Molluscs	1 848	936	483	759	756	557	171	0	**4 998**
Crustaceans	4 499	1 755	1 536	1 925	1’968	1 225	125	33	**11 990**
Arachnids	1 703	1 069	801	1 330	569	708	5	2	**6 149**
Collembolans	338	49	6	28	34	6	3	1	**414**
Insects	15 190	9 410	8 594	14 428	13 912	7 510	577	14	**75 874**
Vertebrates	2 193	1 831	3 995	6 041	3 674	694	8	1	**18 235**
Amphibia	160	203	828	1 698	1 062	301	0	0	**4 294**
Fish	1 844	1 411	2 938	4 035	2 345	261	0	0	**12 740**
Mammals	18	22	35	28	18	11	0	0	**124**
Birds	154	116	138	145	76	62	6	1	**567**
Other Vertebrates	35	101	91	163	191	70	2	0	**634**
Other	3 675	1 672	1 188	1 337	1 205	950	181	113	**6 109**
Total	**30 316**	**17 072**	**16 789**	**26 186**	**22 360**	**11 860**	**1 080**	**174**	**125 530**

Different organisms play different roles in aquatic systems and interact with each other (Dussart, 1966; Wetzel, 2011). As primary producers, phytoplankton and macrophytes form the base of all freshwater food webs. Via photosynthesis, they convert solar energy into chemical energy. This chemical energy is a food source for all other organisms unable to convert solar energy, the so-called heterotrophic organisms or consumers. Primary consumers like zooplankton, small macroinvertebrates, planktivorous fish and herbivorous fish depend directly on the primary producers as a food source. Secondary consumers like larger zooplankton species, predatory macroinvertebrates, and predatory fish consume primary consumers. They are then consumed by more giant predators, usually big predatory fish. Dead organic materials offer another food source. Decomposers such as detritivorous macroinvertebrates, zooplankton and fish break down organic matter into smaller pieces which can be further consumed by smaller decomposers such as bacteria and fungi, returning the energy and nutrients back to the food web. Besides these strict aquatic organisms, there is a variety of organisms linking terrestrial with aquatic systems, such as semiaquatic insects, birds or large mammals.

Nowadays, this biodiversity of freshwaters is threatened by global changes such as eutrophication, pollution, and invasive species. The 2022 Living Planet Index reported a decline of 83% in populations of freshwater species, a far more substantial decline than for terrestrial or marine species (WWF, 2022).

1.1. Macrophytes in aquatic systems

Chambers et al. (2008) define aquatic macrophytes as "aquatic photosynthetic organisms, large enough to see with the naked eye, that actively grow permanently or periodically submerged below, floating on, or growing up through the water surface". Aquatic macrophytes are represented in seven plant divisions: Cyanobacteria (blue-green algae), Chlorophyta (green algae), Rhodophyta

(red algae), Xanthophyta (yellow-green algae), Bryophyta (mosses and liverworts), Pteridophyta (ferns) and Spermatophyta (seed-bearing plants)". There are several classifications for macrophytes existing. In this thesis, I classified the macrophytes into two groups and four sub-groups based on the work of Arber (1920) and Sculthorpe (1967):

- Rooted macrophytes
    - Emergent macrophytes:

      Emergent plants pierce the surface. They, therefore, grow in and outside of the water.

Figure 1: Water-primrose as an example of an emergent macrophyte.

    - Floating-leaved macrophytes:

      Floating-leaved plants consist of floating leaves which are rooted in the sediment.

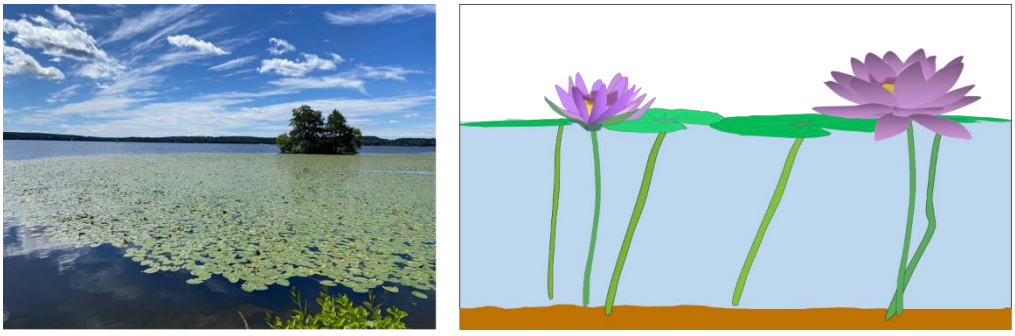

Figure 2: Water lily as an example of a floating-leaved macrophyte.

- Submerged macrophytes:

  Submerged macrophytes grow entirely underwater with roots attached to the sediment

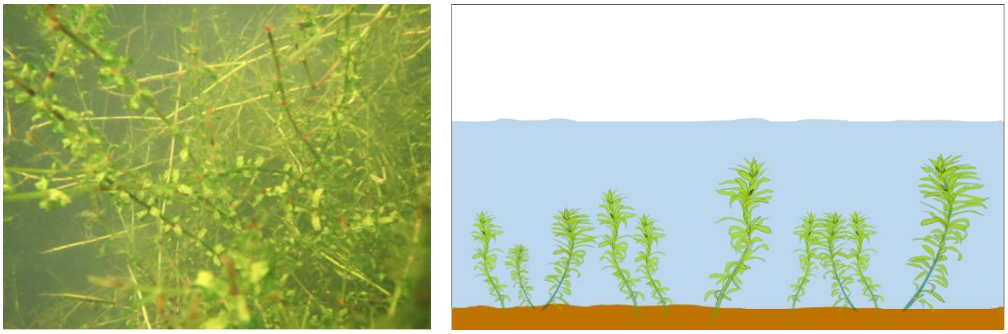

Figure 3: Western waterweed as an example of a submerged macrophyte.

- Free-floating macrophytes:

They are floating plants with their roots not attached to the sediment. In contrast to the other plant groups, free-floating macrophytes can move with water currents or wind.

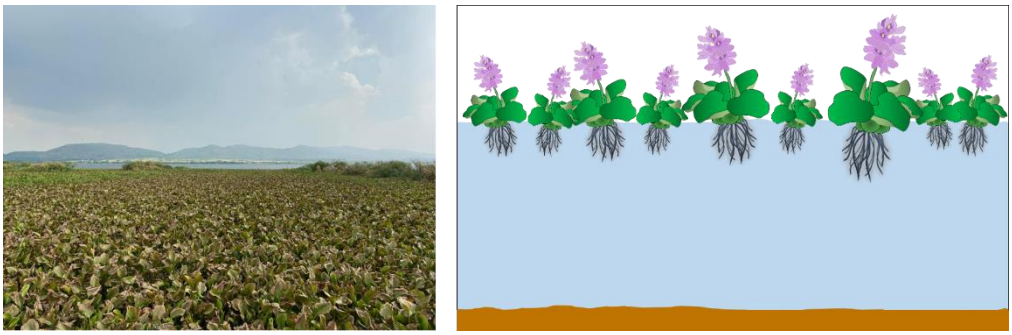

Figure 4: Water hyacinth as an example of a free-floating macrophyte.

1.2. The particular case of non-native species

The spread of invasive species was identified as one of the critical impacts on freshwater systems in global biodiversity scenarios for the year 2100 (Sala et al., 2000). The definition of invasive species is not always clear and can lead to confusion in terminology (Pyšek et al., 2004; Richardson et al., 2000). Pyšek et al. (2004) define invasive (plant) species as non-native species (i.e. species growing outside their native range) which grow outside of cultivation, are naturalized (i.e. they sustain stable populations), and have the potential to spread over a large area. Many non-native species fail to establish a stable population after introduction, and the timespan between the first introduction and the moment of rapid range expansion can be several years to decades (Hobbs & Humphries, 1995). However, every non-native species is a potential candidate to become an invasive species. Therefore, the early detection and management of non-native species facilitate the control of invasive species and reduce the costs of it (Hobbs & Humphries, 1995; Hussner et al., 2017).

In 2012, a total of 96 non-native macrophytes species from 30 different families were reported in Europe, 34 of which were present in France, the highest reported number together with Italy (Hussner, 2012). Currently, the European and Mediterranean Plant Protection Organization (EPPO; www.eppo.int) has listed ten macrophyte species as "pests recommended for regulation as quarantine pests". Six additional species are listed as "invasive alien species", and four are on the "observation list of invasive alien plants". The release and/or escape of imported alien macrophytes, used as ornamental plants in aquariums and ponds, into nature is considered the primary cause of the introduction of alien and invasive plants (Azan et al., 2015; Brunel, 2009).

Invasive macrophyte species originating from all continents except Antarctica can be found in Europe (Hussner, 2012). Invasive macrophyte species in Europe cover all biological types: submerged macrophytes (e.g. *Elodea nuttallii*), emergent macrophytes (e.g. *Ludwigia grandiflora*), floating-leaved macrophytes (e.g. *Hydrocotyle ranunculoides*) and free-floating macrophytes (e.g. *Pistia*

stratiotes). A successful invasion in a non-native environment requires the macrophyte species to go through both abiotic and biotic filters (Pulzatto et al., 2019). Abiotic conditions, such as nutrients, turbidity, conductivity or temperature, must be suitable for the plant to survive and reproduce (Pulzatto et al., 2019). Meanwhile, the potentially invasive species need to overcome the resistance from native species, parasitism and other biotic interactions (Elton, 1958; Levine et al., 2004; Petruzzella et al., 2018).

1.3. Role of macrophytes on the functioning of ecosystems

Macrophytes play a fundamental role in structuring aquatic ecosystems (Jeppesen et al., 1998). They have a substantial direct and indirect influence on aquatic systems' biotic and abiotic characteristics; they are often referred to as ecosystem engineers. Macrophytes offer habitat and increase structural complexity and diversity (Choi, Jeong, Kim, et al., 2014; Scheffer, 2004; Thomaz et al., 2008).

As primary producers, they form the base of aquatic food webs together with phytoplankton and macroalgae (Krause-Jensen & Sand-Jensen, 1998; Wetzel, 1992). Macrophytes are used as a place to live in, to seek refuge from predators, or as a food source used by a variety of organisms, including epiphytic algae (Cattaneo et al., 2008; Ferreiro et al., 2013; Kiss et al., 2003; Wijewardene et al., 2022), zooplankton (Choi, Jeong, Kim, et al., 2014; Sagrario et al., 2009), macroinvertebrates (Ferreiro et al., 2013; Smock & Stoneburner, 1980; Taniguchi et al., 2003), fishes (Petry et al., 2003; Vono & Barbosa, 2001) and waterbirds (Hansson et al., 2010; Klaassen & Nolet, 2007; van Altena et al., 2016). Macrophytes provide clean water by suppressing phytoplankton growth through high nutrient uptake, shading and the production of allelopathic chemicals (Hilt & Gross, 2008; Mulderij et al., 2007; Scheffer et al., 1993). As primary producers, macrophytes produce oxygen via photosynthesis leading to increased fluctuations (daily and seasonal) in dissolved oxygen levels (Caraco et al., 2006; Frodge et al., 1990). Furthermore, macrophytes play an essential role in the

nitrogen and carbon cycle (Carpenter & Lodge, 1986; Flanagan et al., 2006; Körner, 1999). In lotic systems, macrophytes decrease flow velocity (Naden et al., 2006; Sand-Jensen & Pedersen, 1999; Wharton et al., 2006) and thereby increase water levels (Green, 2005) and sedimentation rates (Schulz et al., 2003). Macrophytes support many ecosystem services provided by lakes and rivers, such as the provision of clean water, carbon sequestration or the provision of fertilizer or biomass fuel (Hilt et al., 2017; Janssen et al., 2021).

1.4. Biodiversity around macrophytes

Hilt et al. (2017; see also Figure 5) analyzed a total of 143 studies reporting comparisons of the biodiversity of different organism groups (waterfowl, fish, macrozoobenthos, zooplankton, macrophytes, phytoplankton or periphyton, and bacteria) in macrophyte and in phytoplankton dominated shallow lakes. Abundance and/or diversity were higher in most studies except for bacteria. However, for all organism groups, mixed results were found.

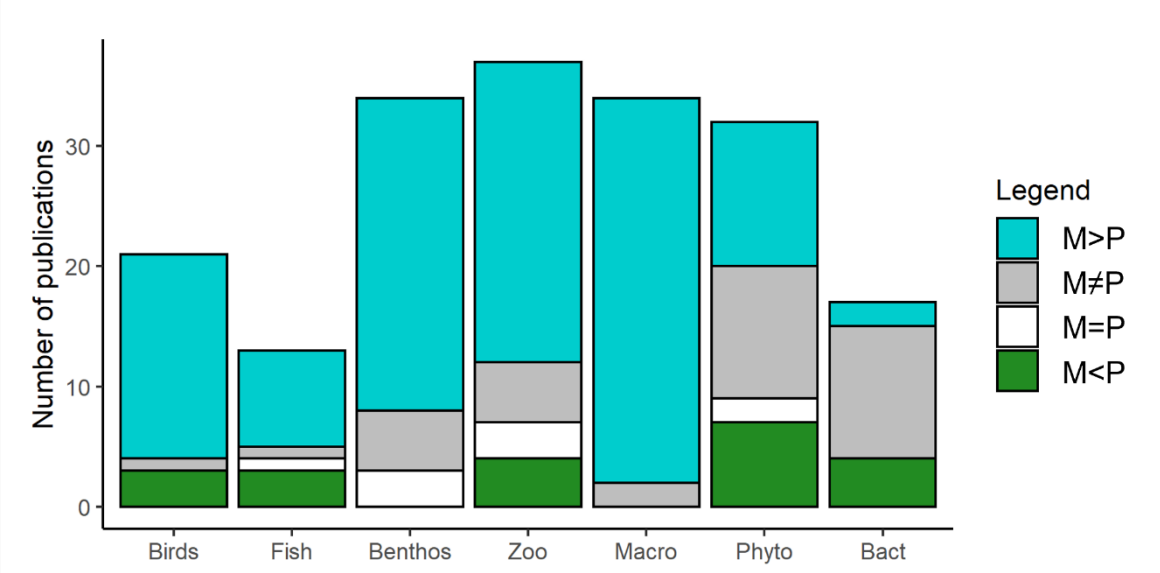

Figure 5: Number of studies reporting differences between macrophyte-dominated (M) and phytoplankton-dominated (P) lakes and ponds. M > P: macrophyte dominance provides higher biodiversity than phytoplankton dominance, M ≠ P: difference between macrophyte and phytoplankton dominance but no clear trend; M = P: no difference between macrophyte and phytoplankton dominance; M < P: macrophyte dominance provides lower biodiversity than phytoplankton dominance. (adapted from Hilt et al. 2017).

In this thesis, I focus on studying the effects of macrophytes on phytoplankton, zooplankton and macroinvertebrates (Figure 6). Out of these three groups, phytoplankton might be the most strongly impacted group by macrophytes. As macrophytes and phytoplankton are both primary producers, they compete for light and nutrients in the water column. A high density of phytoplankton represses submerged macrophytes and vice versa. Scheffer et al. (1993) described this relationship in a simple model based on three assumptions:

- An increase in nutrient level increases phytoplankton growth and therewith turbidity
- Macrophytes reduce turbidity by reducing turbulent energy and stabilizing sediments
- If the turbidity exceeds a critical threshold, macrophytes will disappear

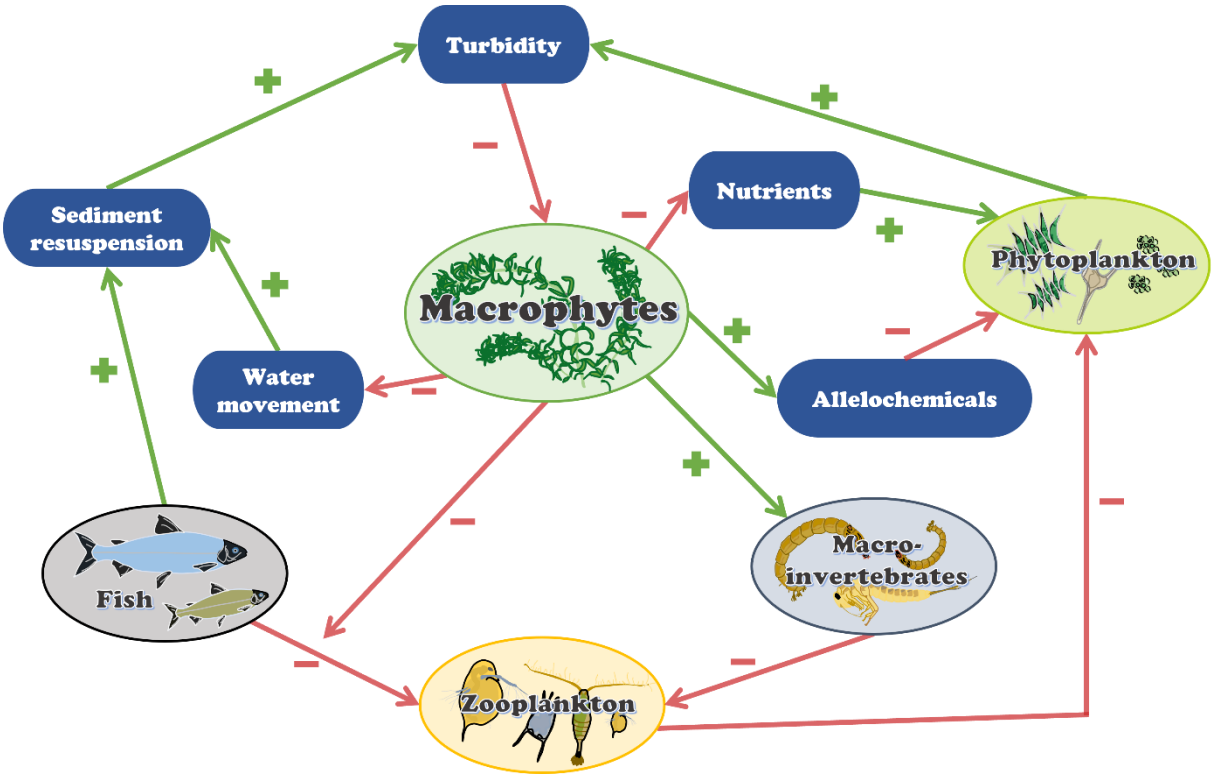

Figure 6: Schematic representation of biotic and abiotic interactions related to the presence of aquatic macrophytes and its effect on biodiversity (modified from Masclaux 2011).

This interaction leads to two different ecological states of a lake: macrophytes-dominated (clean-water state) or phytoplankton-dominated. Both states are stable, and a high impact (e.g., eutrophication) is needed to shift the entire system from one state to the other, but as soon as a threshold is reached, the state of the lake will change and become stable again. Since then, this model has been broadly tested and extended but still forms the foundation of macrophyte phytoplankton interaction (Scheffer & van Nes, 2007). Another negative effect of macrophytes on phytoplankton is the higher zooplankton abundance which is often correlated to macrophytes, which increases predation on phytoplankton (Timms & Moss, 1984; Van Donk & van de Bund, 2002). Further, the production of allelopathic chemicals in some plants can also limit phytoplankton growth (Hilt & Gross, 2008; Körner & Nicklisch, 2002; Švanys et al., 2014).

Compared to the negative effects on phytoplankton, macrophytes have more positive effects on zooplankton. For zooplankton, macrophytes have been shown to offer habitats and provide food, either direct or indirect, via biofilm (Thomaz & Cunha, 2010). Further, macrophytes offer a refuge from predators like fish or macroinvertebrates (Burks et al., 2002; Lauridsen & Lodge, 1996). In a process called diel horizontal migration (DHM), zooplankton migrates from the open-water zone towards the macrophytes during daylight to seek refuge and move away from macrophytes during the night to eat phytoplankton in the open water (Burks et al., 2002; Lauridsen & Lodge, 1996).

Macrophytes also influence macroinvertebrates in mainly positive ways. The provision of structural complexity and heterogeneity in the water column is a key determinant of macroinvertebrate richness (Scheffer, 2004; Thomaz et al., 2008). The creation of new niches for small macroinvertebrates (Labat et al., 2022; Taniguchi et al., 2003; Thomaz et al., 2008) as well as reduced predation pressure within macrophytes (Balci & Kennedy, 2003; Sato et al., 2014; Warfe & Barmuta, 2004) have been linked to increased abundance of macroinvertebrates among macrophytes. Macrophytes have been shown to be an essential food for macroinvertebrates. For herbivorous and omnivorous macroinvertebrates, they offer a direct food source (Cyr & Face, 1993; Wood et al., 2017), while grazers consume on the attached biofilm (Cyr & Face, 1993; Wolters

et al., 2019) and shredders and collectors consume plant litter (Casagranda et al., 2006; Saulino & Trivinho-Strixino, 2018). Anoxic conditions can occur under dense mats of macrophytes caused by increased dark respiration (Żbikowski et al., 2019) and reduced water mixing (Caraco & Cole, 2002; Vilas et al., 2017), causing problems for macroinvertebrates, especially benthic ones (Dodds & Whiles, 2019; Verberk & Bilton, 2013).

1.5. Mass development of macrophytes

Despite the many positive impacts of macrophytes on aquatic systems, macrophytes can also be problematic. Under favourable conditions, native and non-native species can proliferate and, within a short time, cover a large area with high density (Hussner et al., 2017; Verhofstad & Bakker, 2019). Mass development of macrophytes is usually dominated by one or two species and has been reported for different plant types (Hussner et al., 2017). Verhofstad et al. (2017) found that light availability and phosphorus availability in the sediment are the main drivers for mass development in shallow lakes in the Netherlands. Temperature changes, lack of disturbance, and biological competitors have also been named as possible reasons for mass development (Riis & Biggs, 2001). Mass developments of macrophytes are often considered a nuisance as they can cause many problems for humans. Dense mats of macrophytes can hinder commercial and recreational activities like fishing, navigation, swimming or other water sports (Dugdale et al., 2013; Güereña et al., 2015; Verhofstad & Bakker, 2019). Dense mats of macrophytes can lead to anoxic conditions in the water, harming other organisms (Janse & Van Puijenbroek, 1998; Verhofstad et al., 2017). The spread of one species of macrophytes can repress more diverse vegetation (Stiers et al., 2011). Reduced water flow due to plants can lead to an increase in water level leading to flooding risk for adjacent land (Dugdale et al., 2013; Riis & Biggs, 2001). Plant parts floating in the water can clog hydropower plants (Clayton & Champion, 2006; Dugdale et al., 2013). Mass developments are

expected to increase due to global change, and these problems will become more critical (Hussner et al., 2017; Thiemer et al., 2021).

1.6. Management of mass development of macrophytes

The problematic growth of macrophytes calls for the management of the plants. Management methods of macrophytes can be classified into three groups (see: Hussner et al., 2017 and references therein):

- Chemical Control:

Chemical control describes the application of herbicides on the macrophytes. Chemical control allows for large-scale treatments, is cost-effective and has a smaller environmental impact on the ecosystem when used correctly compared with other methods. Chemical control is more difficult to apply to submerged plants, and the use of chemicals is often strongly controlled by law (Hussner et al., 2017). The use of chemicals is not target-specific and can come with strong side effects for (similar) plants, but it also causes changes in water clarity and harms fish and macroinvertebrates (Hussner et al., 2017 and references therein). A sustainable use of herbicides would require broad testing on their environmental impacts before application.

- Biological Control:

Biological control describes the use of one/multiple organisms to manage macrophytes. For exotic plants, it is possible to import biocontrol agents, often insects, from their native range causing harm to the target species. Further, the import of pathogens or herbivores or the introduction of native herbivores might be an option. Biological control is inexpensive and can last, when successful, for decades. However, a species-specific control agent must be found to avoid an uncontrollable environmental impact of the imported

species, requiring extensive research and tests before a method is established (e.g. control of *Pontederia crassipes* by *Megamelus scutellaris* in South Africa (Coetzee et al., 2022)).

- Mechanical Removal:

Mechanical removal describes the removal of macrophytes by hand, mowing boats, or other harvesting machines. Mechanical removal is easy to control and has an immediate impact. Compared with the other methods, the plants are removed from the system with mechanical removal. Mechanical removal of macrophytes is cost-intensive, and success often stays for a short time, as plants are not removed entirely and regrow quickly.

In this thesis, I am interested in the mechanical removal of macrophytes as this method is used worldwide and can be applied in all systems and to all plant types. Various types of mechanical removal exist, and their usage depends on local conditions and the macrophyte species. Using an excavator, mowing boats or removing macrophytes by hand are three out of many methods (de Winton et al., 2013; Laranjeira & Nadais, 2008; Podraza et al., 2008). Compared to biological and chemical control, mechanical removal exports the macrophytes and nutrients out of the system (Hussner et al., 2017). However, with mechanical macrophyte removal, it is usually impossible to remove macrophytes entirely and often, plants remain in the sediment, or plant fragments end up in the water (Hussner et al., 2017; Thiemer et al., 2021). Therefore, macrophytes can regrow within weeks or years (Baattrup-Pedersen et al., 2018; Hussner et al., 2017; Pieterse & Murphy, 1990), and when plant fragments are spread in the water, this can even lead to an extension of the macrophyte mats or the invasion of new systems (Hussner et al., 2017). The low efficacy and the need to repeat the management lead to high costs (de Winton et al., 2013; Hussner et al., 2017). The management costs depend strongly on the system, the macrophyte species and the plant biomass (Hussner et al., 2017). In the USA, more than $100 million are spent yearly on macrophyte management. Alone the management of *Hydrilla verticillate* in Florida costs $14.5 million yearly (Pimentel et al., 2000). In

Europe, the management costs of a site with *Pontederia crassipes* are up to 4 million € per site (removal of 50 000 tons of plant material, EPPO, 2009) (removal of 50 000 tons of plant material, EPPO 2009). In the Netherlands, the management of *Cabomba caroliniana* costs 350 000 € for one site (Matthews et al., 2013).

Removal of macrophytes might not always be the best option, especially when removal only has a short-lasting success and plant regrow. However, the possible benefits of macrophytes are often overlooked and underestimated in decision-making for macrophyte management. A better understanding of the benefits and disbenefits of dense mats of macrophytes is needed to develop balanced and more sustainable management solutions in the future (Thiemer et al., 2021).

2. This thesis

2.1. Aim

Global biodiversity is declining rapidly, faster in freshwater systems than in terrestrial environments (WWF, 2022). Ongoing global environmental changes, rising temperatures, water pollution, habitat degradation, flow alterations, and the spread of NIS further threaten this valuable system (Dudgeon et al., 2006). To better protect aquatic biodiversity, it is vital to understand better their ecology and the interactions between different groups within the system. Based on this knowledge, more sustainable management strategies can be developed considering ecological, environmental, economic, and social perspectives. The spread of macrophytes and the mass development of dense mats of macrophytes is becoming a problem worldwide (Hussner et al., 2017; Verhofstad et al., 2017), and therefore the management of such macrophytes is essential.

Managing mass developments often come with high costs, especially in regions where chemical and biological removal can not be applied. Surprisingly, the consequences of this mechanical

removal are not well studied, and the possible benefits of the macrophytes providing different ecosystem services are overlooked by the public and in decision-making (Thiemer et al., 2021).

The overall aims of this thesis were to assess the role of macrophytes on the biodiversity of several taxonomic groups (i.e., phytoplankton, zooplankton, and macroinvertebrates) and the effect of the removal of dense mats of macrophytes on its surrounding biodiversity in a wide range of lakes and rivers in Europe and Africa.

2.2. Organization of the thesis

Chapter 1 addresses the impacts of dense mats of macrophytes on phytoplankton, zooplankton and macroinvertebrates. We expect to see positive effects of macrophytes on the abundance and diversity of macroinvertebrates and zooplankton. For phytoplankton, we expect adverse impacts. In addition, the sampling efficiency for macroinvertebrates within and outside of macrophyte stands was analyzed. We expect that the presence of macrophytes affects the sampling efficiency, and a higher number of samples in macrophytes is needed to reach comparable results.

Chapter 2 covers the role of invasive plants as a habitat and refuge. Based on a mesocosm study, we examine the role of invasive plants as a habitat for zooplankton and a refuge from fish predation. Using a semi-controlled mesocosm approach allows one to look specifically at the effect of different invasive macrophyte species with different growth forms without many other covariables, as in Chapter 1. We hypothesized that results are comparable with native macrophytes, for which it was shown that macrophyte stands play an essential role for zooplankton as a habitat and as a refuge from predation during daylight.

In **Chapter 3,** we analyze the effect of macrophyte removal on the surrounding biodiversity. We established a scoring method to analyze the short-term impact of macrophyte removal on surrounding biodiversity in a multi-national setup. Opposite to the hypothesis of Chapter 1, we expect a negative effect of plant removal on zooplankton and macroinvertebrate communities and a positive effect on phytoplankton. The findings of this short term are combined with results from a long-term comparison.

Chapter 1. Impact of macrophytes on biodiversity

1. Introduction

This chapter addresses the impacts of dense mats of macrophytes on phytoplankton, zooplankton and macroinvertebrate communities. The chapter is split into two main parts; a first part with a published article studying the effect of invasive macrophytes on macroinvertebrates and how they affect the sampling efficiency and a second part covering the same topic for phytoplankton and zooplankton.

2. Methods

2.1. Study Sites

This chapter includes work conducted at Lake Grand-Lieu (Lac de Grand-Lieu) in France, Lake Kemnade (Kemnader See) in Germany, Hartbeespoort Dam in South Africa and Lake Ebolowa in Cameroon (Figure 7). These sites were chosen to cover a diverse set of characteristics considering their geographical distribution and climate, the dominant macrophyte species (containing free-floating, emergent and submerged plants), different ecosystem types (lakes and reservoirs) and different trophic states. Local stakeholders performed macrophyte removal at each site.

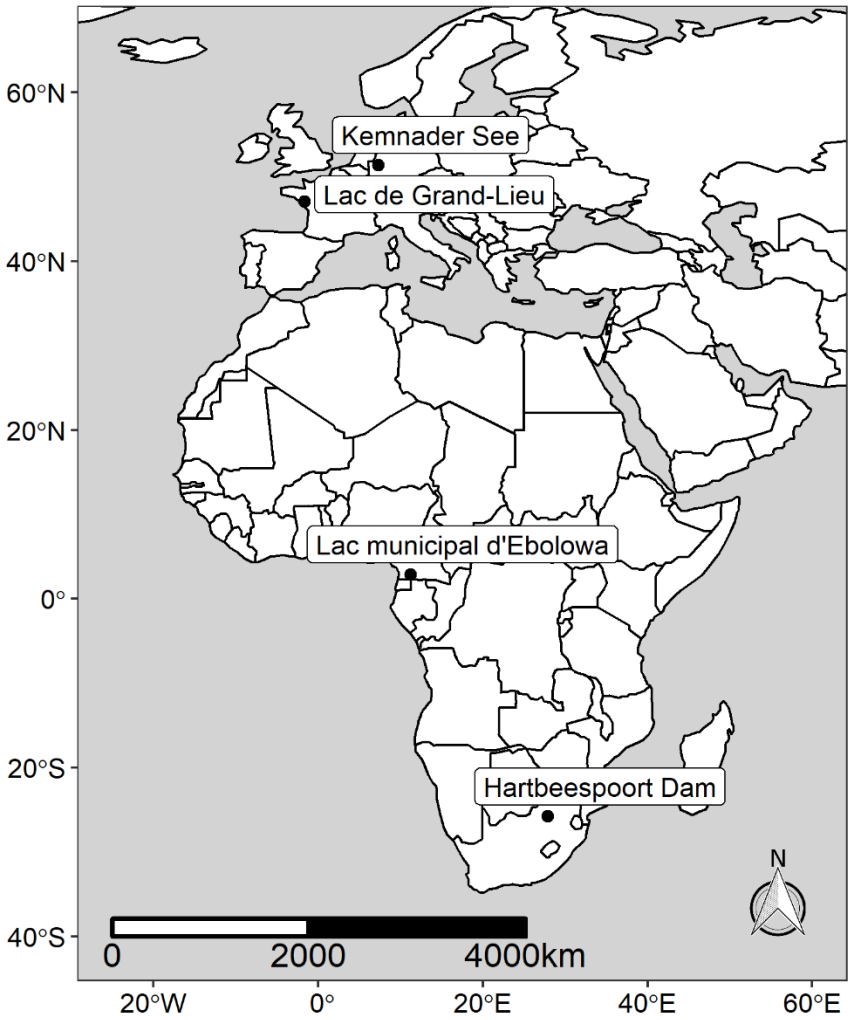

Figure 7: Map of the four study sites involved in Chapter 1.

2.1.1. Lake Grand-Lieu

Lake Grand-Lieu is a large, shallow lake in western France (Figure 8). When the water level reaches its highest in winter, it has a surface area of 62.92 km^2. It is a bird sanctuary and is considered a natural reserve of national importance because of its diverse flora and fauna. Since the 1990s, invasive plants *Ludwigia grandiflora* subsp. *hexapetala* (Hook. & Arn.) G.L.Nesom & Kartesz and *Ludwigia peploides* subsp. *montevidensis* (Spreng.) P.H.Raven have colonized the lake and the surrounding land (Fédération Départementale 44 Pêche, 2016). The two species are amphibious plants, growing as emergent plants in water and moist soil. Both species are native to South America (Thouvenot et al., 2013). They were introduced into France as ornamental plants in the 19th century and have been invading freshwater systems in large parts of the country ever since (Dutartre et al., 2007).

Figure 8: Pictures of the study site in Lake Grand-Lieu. The top row shows the impact site, on the left before removal and on the right after removal. The bottom row shows the control site on the left and impression during the removal on the right.

At Lake Grand-Lieu, a yearly removal campaign is organized by the "Syndicat de Bassin versant de Grand-Lieu" (http://www.sage-grandlieu.fr/), where plants are removed by hand or mechanically from the lake and its catchment. More than 1 200 tons of plant biomass has been removed since 2003 from the lake and the surrounding area, costing over 15 000€ yearly (Youenn, 2019, 2020). Managing these invasive species is costly and inefficient because regrowth regularly occurs, and macrophyte removal enhances the development of cyanobacteria in the lake, which has negative consequences for fishing and biodiversity.

Our sampling was conducted in the northeastern part of the lake (47°08'02.1"N 1°40'28.0"W, Figure 9). In this part *Ludwigia* spp. cover a whole part of the lake and the surrounding terrestrial environment. In our study, we sampled two side arms of the lake.

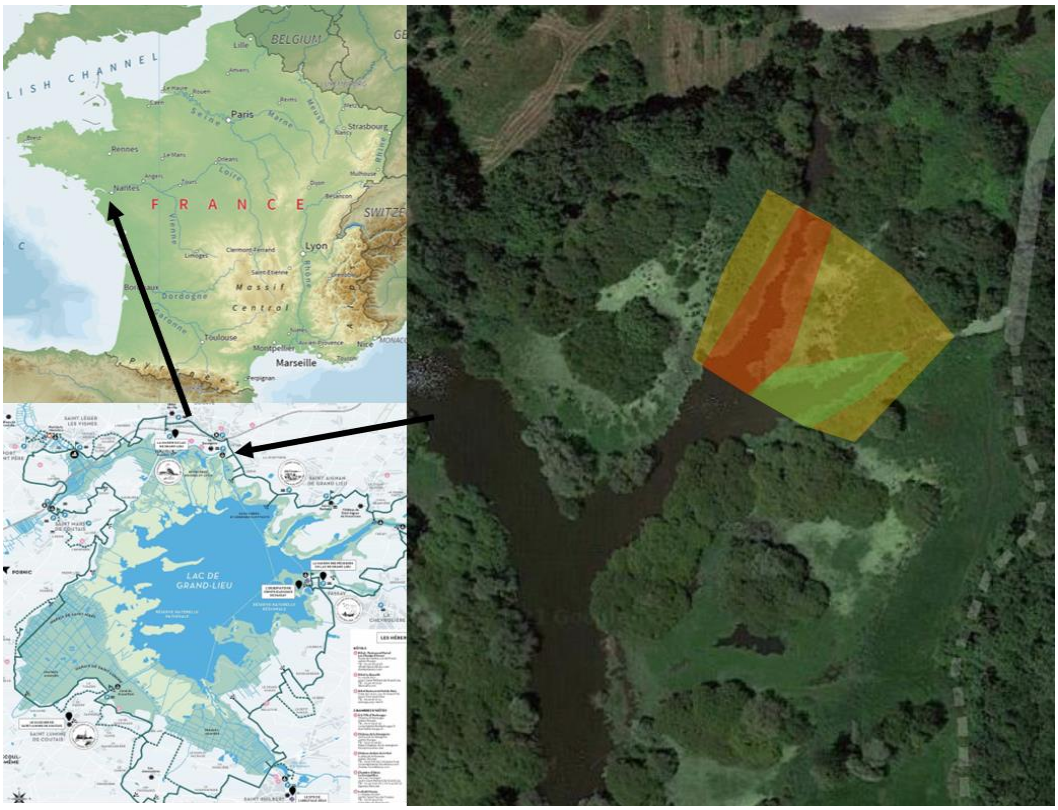

Figure 9: The location of our study site in the north of Lake Grand-Lieu, situated close to the city of Nantes in the northwest of France. The yellow shade indicates the lake areas invaded by Ludwigia spp. (aquatic as well as terrestrial) during the peak of the mass development, the aquatic form of Ludwigia spp. covered almost 80 ha, and the terrestrial form about 115 ha, of the entire lake and its surroundings. The red shade indicates our control site, and the green shade marks the impact site where plants were removed.

2.1.2. Lake Kemnade

Lake Kemnade is located in north-western Germany (51°24'57.6"N 7°15'36.0"E, Figure 10, Figure 11) and is the youngest in a series of reservoirs in the river Ruhr. The reservoir was created in 1979 and covered 125 ha with an average depth of 2.4 m. The lake is heavily used for recreation as it forms a natural island in a heavily populated area. Further, the lake is used for nutrient retention, water supply and hydropower production. Since 2000, the Ruhr reservoirs, including Lake Kemnade, have suffered from the mass development of *Elodea nuttallii (Planch.)* H St. John (Podraza et al., 2008).

Figure 10: Pictures of the study site: Top left: Study site above the water; Top right: Mowing boat on Lake Kemnade; Bottom left: typical day on the lake with sailing boat; Bottom right: Study site under the water

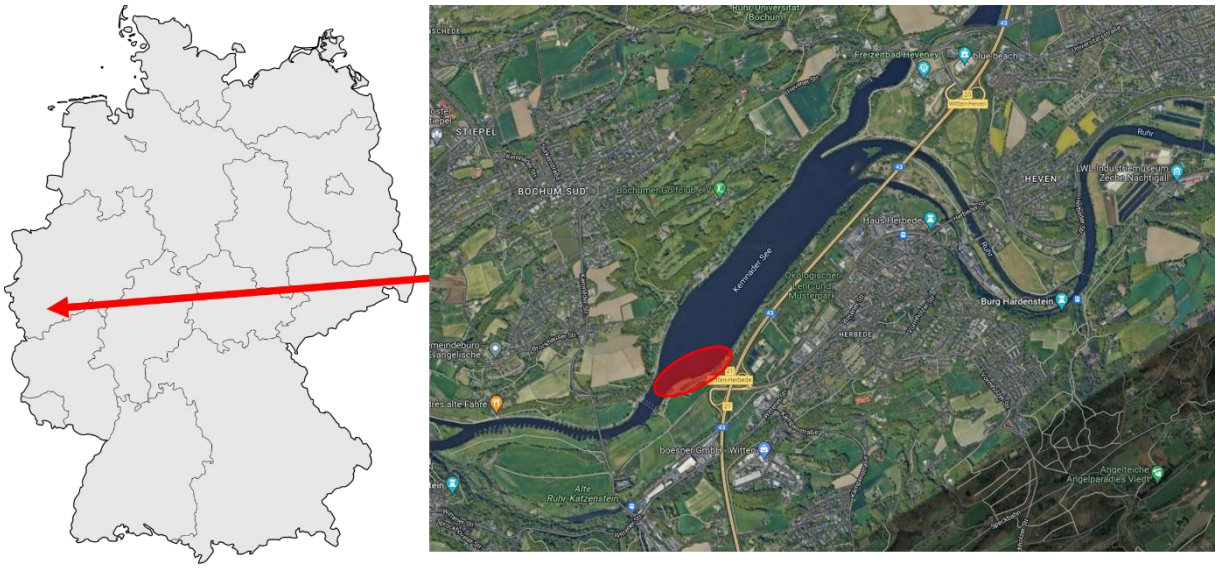

Figure 11: Map showing the location of Lake Kemnade within Germany (left) and the location of our study site (red oval) within the lake area.

E. nuttallii is a submerged macrophyte native to temperate North America and was brought to Europe as an ornamental plant and released by accident in the early 20th century (De Langhe & Delvosalle, 1967). Nowadays, it is widespread in many European countries and Asia, often forming dense monospecific mats (CABI, 2022). Mass development has become a problem for over 50 water sports clubs which depend on the lake for their activities. Further, loose plant parts are clogging the hydropower plants. The Ruhrverband, a non-profit-oriented water management company based on public law, is responsible for the management of the Ruhr and its reservoirs (www.ruhrverband.de/). To control the mass development, a mowing boat is in action every day between May and September (personal communication: Ruhrverband). The boat cuts the plants but does not entirely remove them, leading to fast regrowth as plants form side shoots (Podraza, 2017).

2.1.3. Hartbeespoort Dam

The Hartbeespoort Dam is a reservoir lake with a surface of 2000 ha, located 35km northwest of Johannesburg (Figure 12, Figure 13). The reservoir plays an essential role in the region for water supply for households, agriculture and mining and is a recreational hotspot. Nutrient enrichment through wastewater effluent, sewage spills and fertilizer runoff have led to severe eutrophication in the lake. Due to nutrient enrichment, water hyacinth and phytoplankton have started to proliferate (Harding, 2008).

Figure 12: Pictures of the study site: Top left: P. crassipes in our study site; Top right: aerial picture showing lake area covered by the P. crassipes; Bottom left: P. crassipes (brown color is a consequence of biological control applied to the lake); Bottom right: P. crassipes hindering boat and ship activities on the lake.

Pontederia crassipes (Mart.) Solms, also known as *Eichhornia crassipes* or water hyacinth, are free-floating macrophytes that originated in South America (EPPO, 2008). Because of their nice-looking flowers, they were introduced to South Africa as ornamental plants in the 1930s (EPPO, 2008). They became highly invasive and are now considered South Africa's most problematic macrophyte (Hill et al., 2020).

Plants covered up to 80% of the lake's surface in 1998 (Auchterlonie et al., 2021). Since the 1970s, multiple attempts (including chemical, biological and mechanical control) were made to control the water hyacinth without any (long-term) success (van Wyk & van Wilgen, 2002).

Currently, macrophyte growth is managed by a long-term biocontrol program led by the Centre for Biological Control from Rhodes University, our South African collaborator in the project (https://www.ru.ac.za/centreforbiologicalcontrol/).

Our sampling site was at Kurper Oord (Figure 13), which was invaded by water hyacinth in January 2020. Due to the location of the study site, the plants are not blown away by wind and form a permanent cover.

Figure 13: Map showing the location of Hartbeespoort Dam within South Africa (left) and the location of our study site (red oval) within the lake area.

2.1.4. Lake Ebolowa

Our second African lake is Lake Ebolowa (Lac municipal d'Ebolowa), located in the city of Ebolowa in Southern Cameroon (2°55'21.4"N 11°09'29.9"E, Figure 14). In 1962, the lake was built as a reservoir based on an extension of the riverbeds of the Mfoumou and Bengo'o river (Adjia et al., 2020). The lake has a maximal depth of 3m and covers a surface of 13.25ha with a volume of 980m^3. The lake was built to be a recreational hotspot in the city and for water sports and fishing. However, due to high nutrient inputs from urban wastewater in combination with high water temperature (mean 24.4°C), the lake has turned hypereutrophic (Adjia et al., 2020).

Consequently, poor water quality, cyanobacteria blooms and the spread of invasive macrophytes (between 2005 and 2012) became a problem, and the use of the lake was abandoned (Adjia et al., 2020).

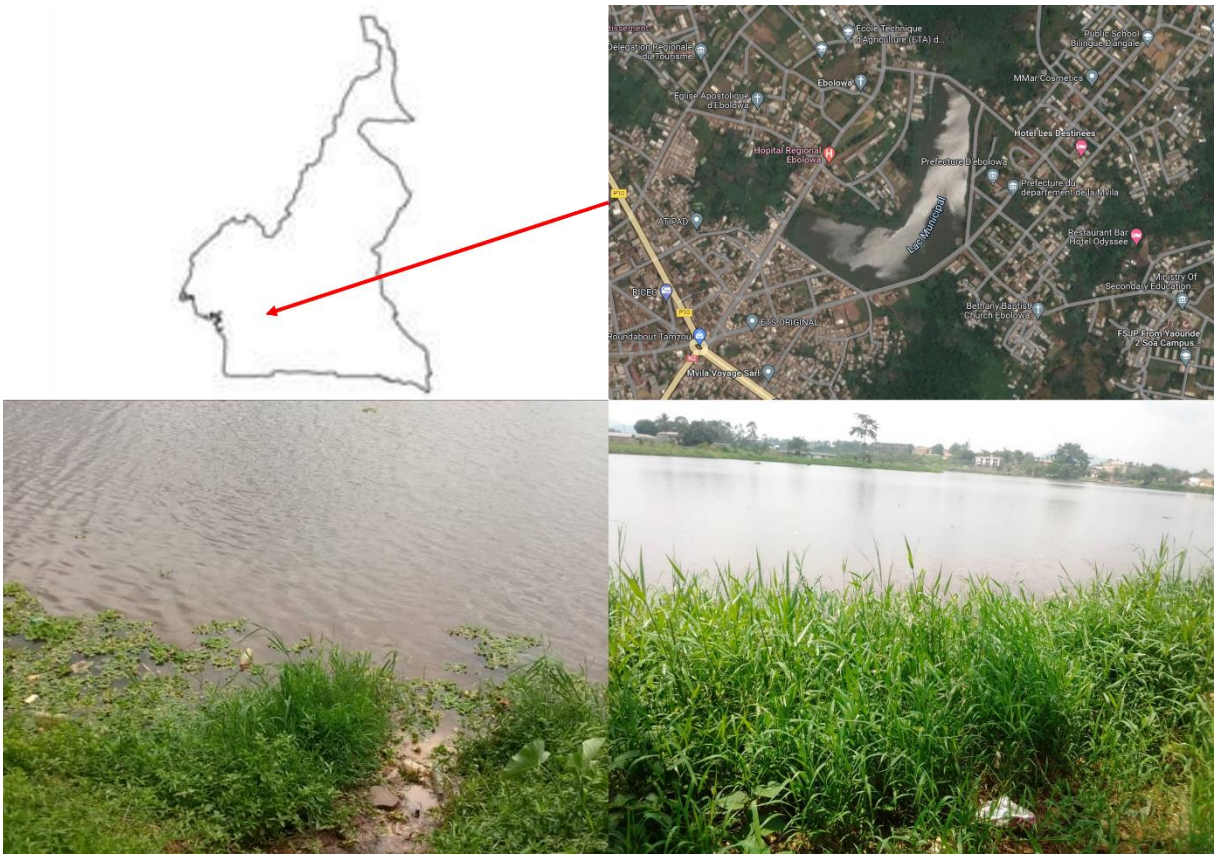

Figure 14: Map and pictures of the study site: Top row: Map showing the location of Lake Ebolowa within Cameroon and an aerial photo of the lake Bottom row: the two studied plant sections

Lake Ebolowa was the only lake in our study where macrophyte beds of two invasive species were analyzed: the emergent *Typha australis* Schumach. & Thonn. and the free-floating *Pistia stratiotes* L. (also known as water lettuce).

2.2. Biodiversity Sampling

For this chapter, we sampled two sections in every site. One covered by dense mats of macrophytes and another nearby without plants later called an open water section. Macroinvertebrates sampling consisted of two types: sediment samples and sweep samples. For macroinvertebrates, all sites were sampled. Phytoplankton and zooplankton were only sampled in Lake Grand-Lieu, Lake Kemnade and Hartbeespoort Dam. For all types of samples, five replicates were taken in all sites except Lake Ebolowa, where ten replicates were taken for every modality.

2.2.1. Phytoplankton

For phytoplankton, sub-surface water samples were taken. The sample volume (50 – 200ml) was adapted according to the phytoplankton density in each site, as determined before the sampling. All samples were fixed with acid Lugol and stored at 4°C. 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).

2.2.2. Zooplankton

For zooplankton, a known volume of water was filtered through a sieve with a 60 µm mesh. The filtered water volume depended on zooplankton density in each location and was adapted to ensure samples contained at least 400 individuals (Frontier, 1972). The zooplankton was then concentrated into 50 ml falcon tubes, fixed in 80% ethanol and stored at 4°C. After filtration, the zooplankton sample was narcotized with carbonated water and then conserved in 80% ethanol and stored at 4°C until identification. Zooplankton was subsampled for identification 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 identified until a total of at least 400 organisms were reached. If 400 organisms were not reached, the entire sample was identified.

2.2.3. Macroinvertebrates

Macroinvertebrates can live either in/on the sediment and/or on plants. Therefore, two different methods were applied to sample macroinvertebrates: the grab-sampling technique for sediment-dwelling macroinvertebrates and the sweep-sampling for communities living within the vegetation. Sweep-sampling was only performed in the macrophyte beds and not in the open water. Grab-samples were collected with an Ekman-Birge grab sampler. Samples were then washed through a 250 μm mesh sieve, and the remaining material was conserved in 96% ethanol. Sweep-samples were collected using a net with a 250 μm mesh size (400 μm for Lake Ebolowa). Sweep samples were collected using a hand net with a 250 μm mesh size and swept harshly through the plants in the case of submerged species or the roots for floating species for 30 seconds over $1m^2$. Samples were washed through a 250 μm mesh sieve and conserved in 96% ethanol. Macroinvertebrates were separated from sediment under a dissecting microscope and identified to the lowest taxonomic level possible. Identification was based on Tachet et al. (2000), Day and Steward (2001) and Durand and Lévêque (1980, 1981). For more detailed identification, we used additional references for Trichoptera (Waringer & Graf, 2011), Heteroptera (Savage, 1999), Odonata (Heidemann & Seidenbusch, 2002), Amphipoda (Piscart & Bollache, 2012) and Gastropoda (Glöer & Meier-Brook, 2003).

3. Article 1: How invasive macrophytes affect macroinvertebrate assemblages and sampling efficiency: results from a multinational survey

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Abstract

Macrophytes play an important role in the functioning and structuring of aquatic environments but rapid mass development of invasive macrophytes is causing global concerns. Macroinvertebrate richness and abundance are strongly influenced by macrophytes as macrophytes offer habitats and food resources, increase structural heterogeneity, and provide refuges. Meanwhile, the presence of macrophytes affects the efficiency of standard sampling methods for macroinvertebrates. These effects are not well studied but are leading to biased management decisions. To fill in this knowledge gap, we analysed macroinvertebrate communities from four lakes in four countries in Europe and Africa with mass development of invasive macrophytes. We compared macroinvertebrate communities in sediment samples from a plant-free part of the lake with those in sediment and sweep samples taken within macrophyte stands. We showed that taxa richness and density were higher in sediment samples beneath invasive macrophyte stands compared to plant-free habitats. Unique taxa were found in each sample type. Sampling efficiency of each sampling method varies greatly across lakes especially when replication is low. The taxonomic richness of macroinvertebrates within invasive macrophyte stands is often underestimated compared to open water sections with the same number of samples. To reach a high sampling coverage, a higher number of samples is necessary for sampling within invasive macrophytes. Our findings call for the development of a method that allows for comparable sampling within and outside of macrophyte stands. Such method will be the foundation for future research and management of aquatic systems.

Keywords: shallow lakes, aquatic plants, methods, habitat heterogeneity, sampling efficiency

Introduction

Macrophytes play a crucial role in the structuring and functioning of freshwater systems. In the last decades, growth and abundance of macrophytes have increased globally due to anthropogenic impacts like eutrophication (Verhofstad et al., 2017). The fact that invasive macrophytes can rapidly inhabit a complete aquatic system by forming dense mats is of great concern (Hussner et al., 2017; Strayer, 2010). The presence of dense macrophyte stands, often hinders or interferes with important socio-economic activities (e.g., boating, swimming, fishing, clogging of hydropower plants). Thus, in recent years, macrophytes have become an important target in water management and are often removed at high costs (Hussner et al., 2017; Thiemer et al., 2021).

Apart from affecting important socio-economic activities, macrophytes also have a strong effect on the ecology of freshwater ecosystems, changing population dynamics of other aquatic organisms and their interactions, including macroinvertebrates (Burks et al., 2006). These effects on macroinvertebrates vary depending on the plant species, life form, structural complexity and density (Ferreiro et al., 2014; Taniguchi et al., 2003; Thomaz et al., 2008; Walker et al., 2013).

The most direct positive effect of macrophytes on macroinvertebrates is the provision of structural complexity and heterogeneity in aquatic systems (Scheffer, 2004; Thomaz et al., 2008). Complex structures have been shown to support higher invertebrate richness and abundance compared to less complex structures. This may be due to complex structures providing more microhabitats and creating new niches for small invertebrates (Labat et al., 2022; Morse et al., 1985; Taniguchi et al., 2003; Thomaz et al., 2008). Alternatively the higher abundance could be linked to reduced predation, as complex structures offer refugia for invertebrates (Balci and Kennedy, 2003; Dahl and Greenberg, 1997; Sato et al., 2014; Warfe and Barmuta, 2004).

Macrophytes are important primary producers and produce oxygen via photosynthesis. As a result, waterbodies with macrophytes often have increased fluctuations of dissolved oxygen (DO) concentrations (daily and seasonal) than open water sites (Caraco et al., 2006; Frodge et al., 1990), with periods of low DO availability at nights and during warm periods. Increased dark respiration

of macrophytes and of the attached biofilm (Żbikowski et al., 2019) as well as reduced oxygen exchange and transportation due to low mixing in macrophytes can lead to hypoxia (Caraco and Cole, 2002; Vilas et al., 2017). Many macroinvertebrate species depend directly on the availability of dissolved oxygen for their survival and low oxygen levels can critically change their diversity, community composition and biomass (Chapman et al., 2004; Dodds and Whiles, 2019; Verberk and Bilton, 2013).

Moreover, macrophytes act as food sources for herbivorous and omnivorous macroinvertebrates. Traditionally, herbivory on macrophytes has been considered insignificant (Lodge, 1991; Mann, 1988; Newman, 1991), but this assumption has been proven wrong. Different studies showed that herbivory on aquatic plants exceeds herbivory in terrestrial systems (Cyr and Face, 1993; Wood et al., 2017). Besides being a direct food source, macrophytes also provide habitats for epiphytic algae which are being grazed at high rates (Cyr and Face, 1993; Wolters et al., 2019). Typical herbivores that can be found living and grazing on macrophytes and epiphytic algae are larvae of aquatic insects such as Lepidoptera, Trichoptera, Diptera, Gastropoda, Oligochaeta and Crustacea (Cattaneo et al., 1998; Dehedin et al., 2013; Jacobsen and Sand-Jensen, 1992; Li et al., 2009).

Macrophytes can have both positive and negative effects on macroinvertebrates, affecting taxonomic richness and abundance compared to plant free sections. Such differences may not be well-captured by the standard sampling protocols. A great variety of methods are used to sample benthic macroinvertebrates in lentic systems, depending on the waterbody types. In wadable systems, a hand net can be used to sweep through the sediment (García-Criado and Trigal, 2005; Labat et al., 2022; Porst et al., 2016), while in deeper lakes, grab samples and core samples are the standard methods for sampling benthic macroinvertebrates (Blomqvist, 1991; Jónasson, 2004; Ntislidou et al., 2018; Sly, 1969). However, there is a lack of commonly accepted sampling methods and research on how to account for differences in the compositions between sections with and without plants. It can be expected that sediment samples alone are not enough to get a complete profile of macroinvertebrates in macrophyte beds, given that macrophytes offer an additional

habitat for macroinvertebrates. Therefore, complementary sampling within the plants should be performed (Indermuehle et al., 2010; Oertli et al., 2005). As macrophytes come in a wide variety of forms, a sweep net is commonly used for sampling within plants as it can be used within all plant types. Methodological studies on macroinvertebrate sampling mainly focus on comparing different sampling methods and their efficiency within macrophytes (Kornijów, 2014; Sychra and Adamek, 2010). Understanding the differences on macroinvertebrates sampling efficiency between macrophyte beds and plant-free sections are essential, especially when macroinvertebrate communities from these two sections are compared. However, research on this topic is scarce.

In this study we compare freshwater macroinvertebrate communities within dense stands of invasive macrophyte to those in plant-free sections in the same lake. Study lakes were situated along a latitudinal gradient, covering Germany, France, Cameroon and South Africa. We also compared sampling efficiency of sediment samples collected in plant-free sections, sediment samples within macrophyte stands and sweep samples collected in macrophytes. This methodological assessment provides essential information for future macroinvertebrate studies in systems with macrophytes. We hypothesize that: (i) the density and taxonomic richness of macroinvertebrates within invasive macrophyte stands are higher than those in open water sections; and therefore (ii) a higher number of samples is needed in stands of invasive macrophytes compared to plant-free sections to reach the same sample coverage of the macroinvertebrate community. Moreover, (iii) these two abovementioned effects are expected to be smaller in sections with free-floating plants as they offer less complex underwater structures compared to non-floating plants.

Materials and Methods

Study Lakes

In this study, we sampled five invasive macrophyte species in four lakes in Europe and Africa (see Figure 1), all of which differed in their chemical and physical parameters (Table 1). The first lake is the Hartbeespoort Dam in South Africa, invaded by the free-floating macrophyte *Pontederia crassipes* (Mart.), Solms (formerly known as *Eichhornia crassipes*). Since the 1960's the macrophyte infested the lake and covered up to 70% of the full lake. With a mix of mechanical, chemical and biological control measures since the 1990's the cover was reduced to 5% in the summer 2020 (Coetzee et al., 2021). In our second lake in Africa, the Lac municipal d'Ebolowa in Cameroon, we sampled beds of two different macrophytes. First the helophyte Typha australis Schumach. & Thonn. and second the free-floating *Pistia stratiotes* L. High nutrient input into the lake is leading to an eutrophication followed by a mass development of macrophytes and sedimentation a typical problem in other lakes in the region (Madjiki et al., 2013). In Europe we sampled Lac de Grand-Lieu in France invaded by the amphibious *Ludwigia grandiflora* subsp. hexapetala (Hook. & Arn.) G.L.Nesom & Kartesz and *Ludwigia peploides* subsp. montevidensis (Spreng.) P.H.Raven. Lac de Gran-Lieu is an important nature reserve and Ludwigia spp. became invasive in the 1970's, threatening the native biodiversity and hindering human activity (Youenn, 2020). The second lake in Europe is the Kemnader See in Germany invaded by submersed *Elodea nuttallii* (Planch.) H. St John. Kemnader See is an important recreational area in a densely populated area frequently used for all kinds of water activities. Since 2001 *E. nuttallii* grows in most parts of the lake hindering activities on the lake (Podraza et al., 2008). Our lakes cover a latitudinal gradient from 51°N to 25°S covering tropical (Lac municipal d'Ebolowa), subtropical (Hartbeespoort Dam) and temperate (both European lakes) climates. The lake surfaces range from 13 (Lac municipal d'Ebolowa) to 3700 ha (Lac de Grand-Lieu).

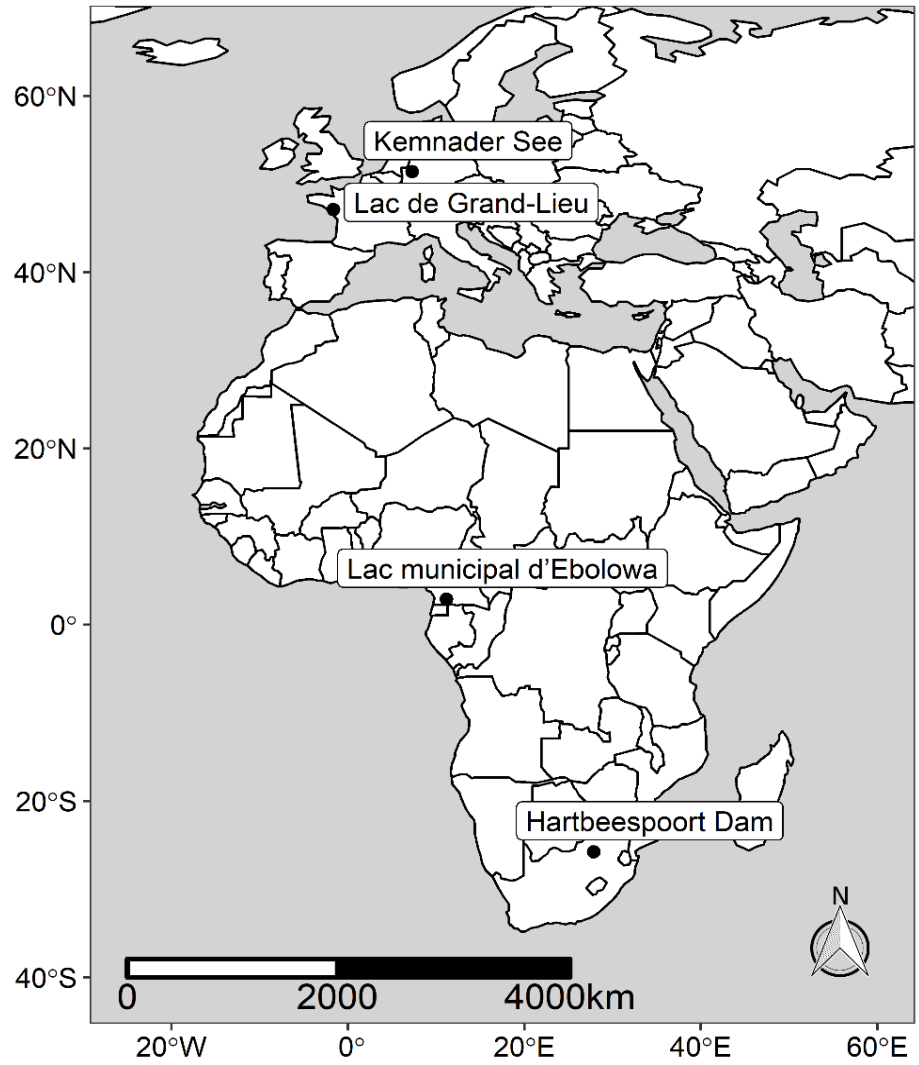

Figure 1: Location of the four lakes: Kemnader See in Germany, Lac de Grand-Lieu in France, Lac municipal d'Ebolowa in Cameroon and Hartbeespoort Dam in South Africa.

Macroinvertebrate Sampling and Processing

In each lake, two similar sections were selected for sampling: one with a dense macrophyte cover and one open water section without macrophytes. Replicates were distributed within the sections. Sediment samples were collected in both sections and additional sweep net samples were taken from vegetated sections to collect macroinvertebrates living within the macrophyte stands. The sample collection and processing varied slightly between lakes:

Table 1. Overview of study lakes characteristics

Lake	Country	Latitude Longitude	Altitude (m MSL.)	Description	Lake Surface (ha)	Depth* (m)	Trophic state	Macrophyte species	Life Form	Fish	Sampling date
Lac municipal d'Ebolowa	Cameroon	2° 55.3349' N 11° 9.4658' E	579	Artificial lake in the city	13	1.5	Hypereutrophic	*Typha australis* / *Pistia stratiotes*	helophyte/ free-floating	present	Mar-21
Hartbeespoort Dam	South Africa	25° 44.9572' S 27° 49.9966' E	1168	Reservoir lake	2060	4	Hypereutrophic	*Pontederia crassipes*	free-floating	present	Jan-20
Lac de Grand-Lieu	France	47° 8.0356' N 1° 40.4613' W	2	Natural shallow lake; Nature Reserve	3700	0.5	Eutrophic	*Ludwigia grandiflora* / *Ludwigia peploides*	amphibious	present	Jun-20
Kemnader See	Germany	51° 25.0188' N 7° 15.6079' E	71	Reservoir lake	1250	2,5	Eutrophic	*Elodea nuttallii*	submersed	present	Jul-20

*measured at the location of the sampling

- Lac de Grand-Lieu, Hartbeespoort Dam and Kemnader See:

  Sediment samples were taken using an Ekman grab sampler sampling an area of 15cm by 15cm. Macroinvertebrates living within macrophyte stands were sampled using a sweep net with 250 μm mesh size. The net was swept harshly through the plants for a duration of 30 seconds and over an area of 1 m². At each lake, we took five sediment samples from the open water section, and ten sediment samples plus ten sweep samples within the macrophytes.

- Lac municipal d'Ebolowa:

  The same method was used as described above, apart from using a 400 μm mesh sized sweep net (n=10). Also, this lake contained two different macrophyte types (T. australis and P. stratiotes), which were sampled separately and named as Ebolowa-Typha and Ebolowa-Pistia hereafter. For both vegetated sections the same open water section is used for comparisons. At both plant-free and the two vegetated sections, ten sediment samples were collected.

All samples were washed using a sieve with the same mesh size as the net used at that lake and were subsequently preserved in 96% ethanol. At the laboratory, macroinvertebrates were handpicked under a dissecting microscope. Macroinvertebrates were counted and identified to the lowest possible taxonomic level according to our expertise. Identification was based on Tachet et al. (2000), Day et al. (2001) and Durand and Lévêque (1981, 1980). For more detailed identification, we used additional references for Trichoptera (Waringer and Graf, 2011), Heteroptera (Savage, 1999), Odonata (Heidemann and Seidenbusch, 2002), Amphipods (Piscart and Bollache, 2012) and Gastropods (Glöer and Meier-Brook, 2003). Because sampled surface areas slightly differ between methods, all invertebrate densities were expressed as individuals per m², for better comparison.

Statistical Analysis

All statistical analysis was performed in R version 3.6.3 (R Core Team, 2021). Comparisons of density (log+1 transformed) and taxonomic richness between different sample types were made based on Kruskal-Wallis Rank Sum Test (Kassambara, 2020a) and pairwise Wilcox tests from the stats package (R Core Team, 2021). Non parametric tests were chosen due to non-normal distribution of some parameters and *p*-values were adjusted with the Holm-Bonferroni method for multiple testing (Holm, 1979). Macroinvertebrate communities were compared among lakes, macrophyte life forms (free-floating/non-floating) and sample types with permutational multivariate ANOVA (PERMANOVA) using adonis2 function from the vegan package (Oksanen et al., 2019). This analysis was based on a Bray-Curtis dissimilarity matrix (Bray and Curtis, 1957). Venn diagrams, created with ggvenn (Yan, 2021), were used to show overlaps and differences in the macroinvertebrate community by sample type. Because different sampling methods were used, we estimated the total taxonomic richness and computed rarefaction curves, including extrapolations, to compare sampling coverage of the different sample types based on Hill numbers (order q=0) (Chao et al., 2014) using the iNEXT package (Hsieh et al., 2020). These diversity estimators allow for an unbiased comparison of effective number of taxa (Chao et al., 2014). Plots were generated using ggplot2 (Wickham, 2016) and ggpubr for boxplots (Kassambara, 2020b).

Results

Comparison of taxonomic richness and density among sample types

Taxonomic richness differs among the three sample types (sediment samples in open water, sediment samples in macrophytes and sweep samples in macrophytes) in all lakes except Kemnader See based on the Kruskal-Wallis test results (Figure 2; Table S1). In all samples, but samples from Hartbeespoort Dam, we found significant differences between all sampling type combinations according to the pairwise Wilcox test results (Figure 2). In Hartbeespoort Dam,

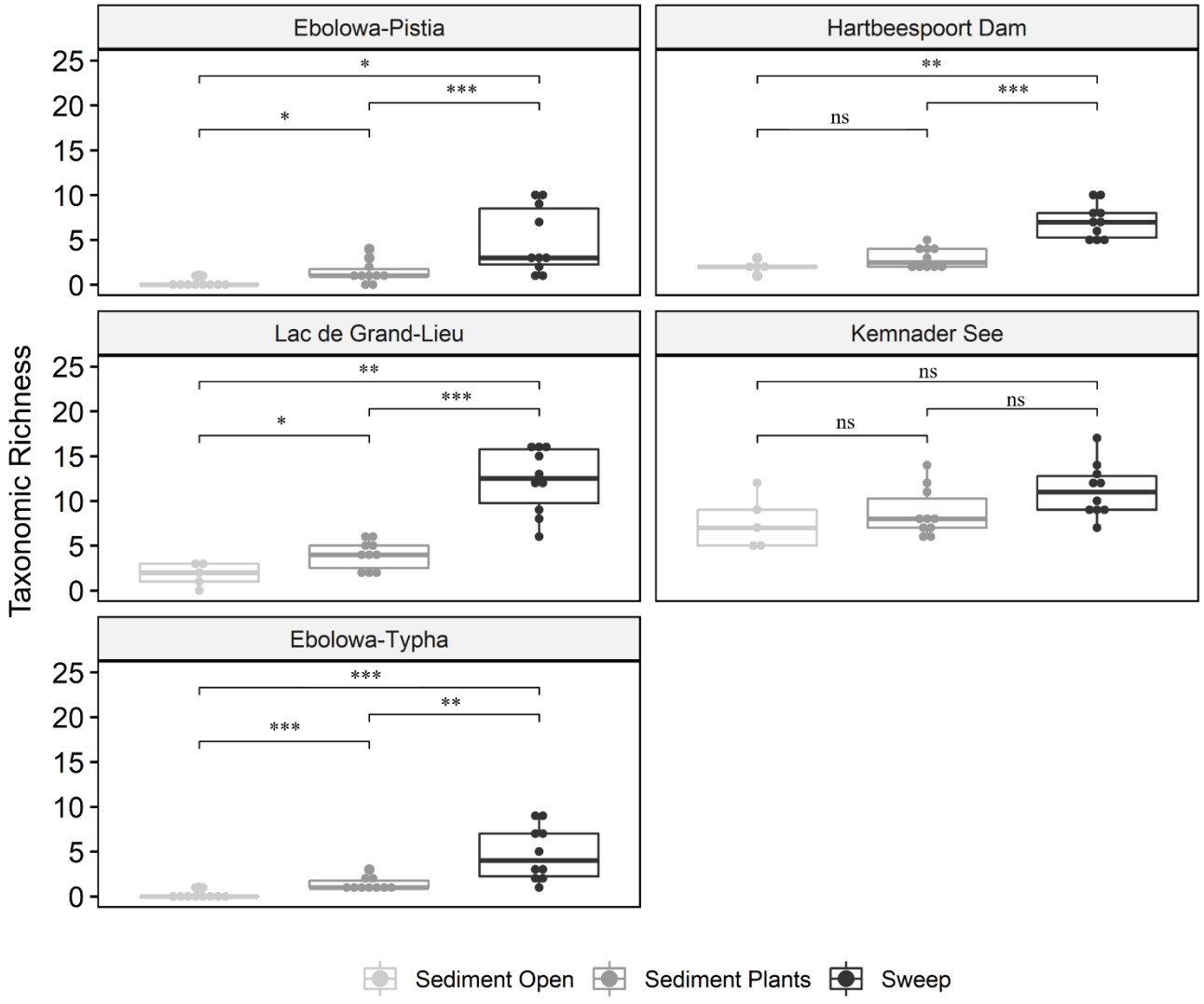

*Figure 215: Taxonomic richness of macroinvertebrates recovered from different sampling methods in each macrophyte. Results from pairwise Wilcox tests are shown for all sample type pairs. Significance level is indicated as: ns: p-value > 0.05, *: <0.05, **: <0.01, ***: < 0.001*

there is significantly higher taxonomic richness in sweep samples, but no differences between the two sediment sample types (Figure 2). For all macrophytes the highest estimated taxonomic richness was found in the sweep samples, followed by sediment samples in macrophytes and lowest in sediment samples in the open water (Table S2). Samples from free-floating plants (Ebolowa-Pistia, Hartbeespoort Dam) and non-floating plants (Lac de Grand-Lieu, Kemnader See, Ebolowa-Typha) showed similar patterns.

Macroinvertebrate communities found in each type of samples differed not only in taxonomic richness but also in their density. Figure 3 (and Table S3) shows the density of macroinvertebrates found in different sample types in each macrophyte. Significant differences of macroinvertebrate density among different sample types were found in all lakes (Table S4). The two types of sediment samples were different in Ebolowa-Pistia, Kemnader See and Ebolowa-Typha. Within plants sediment samples were different from sweep samples in all macrophytes except Ebolowa-Pistia. Open-water sediment samples differed from sweep samples only in Ebolowa-Typha. In all lakes the highest density was found in sediment samples taken within macrophyte stands, except in Hartbeespoort Dam where mean density was highest in sediment samples from the open water area. The lowest density was found either in sediment samples from the open water (Ebolowa-Pistia, Kemnader See, Ebolowa-Typha) or in sweep samples (Hartbeespoort Dam, Lac de Grand-Lieu). There were no differences in density between samples collected from within stands of free-floating and non-floating macrophytes.

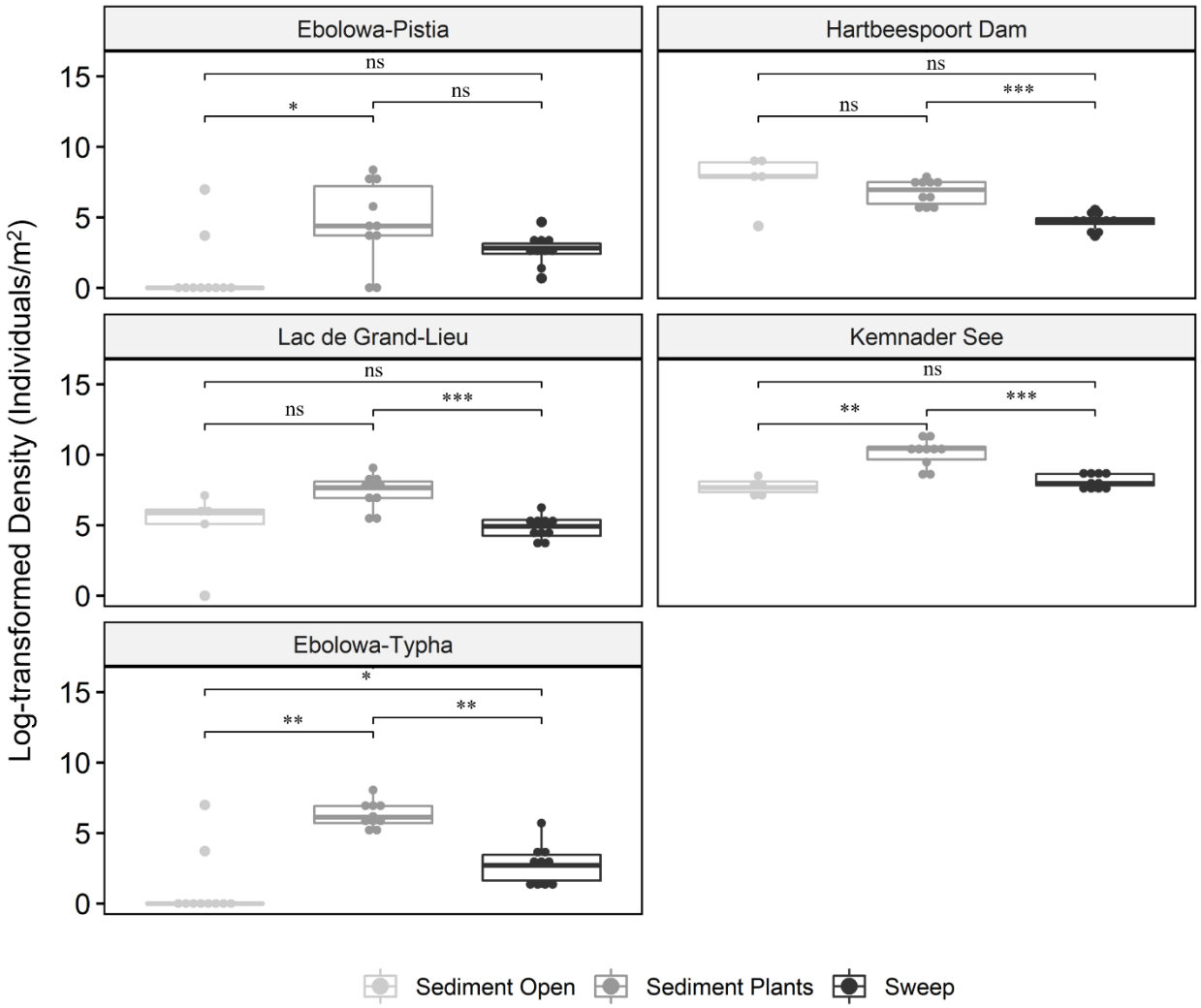

*Figure 3: Log-transformed density of macroinvertebrates recovered from different sampling methods in each sampling site. Results from pairwise Wilcox tests are shown for all sample type pairs. Significance level is indicated as: ns: p-value > 0.05, *: <0.05, **: <0.01, ***: < 0.001*

Comparisons of species assemblages

Based on the permutational multivariate ANOVA (PERMANOVA) analysis, 33.3% ($F = 35.8$, $p < 0.001$) of the dissimilarities among macroinvertebrate communities was explained by the lake (see Table S5). The interaction between lake and sample type accounted for 11.8% ($F = 6.3$, $p < 0.001$) of the dissimilarities. Further, the sample type accounted for 9.0% ($F = 14.6$, $p < 0.001$) of the dissimilarities, plant life form for 5.4% ($F = 17.5$, $p < 0.001$) and their interaction for another 3.1% ($F = 5.0$, $p < 0.001$).

Presence and absence of taxa per sample type

The distribution of macroinvertebrate taxa in the three sample types varies among lakes (Figure 4, Table S6). In general, around 90% of the diversity in all lakes was covered solely by sweep samples (exclusive and shared taxa with other sample types), except in Kemnader See where the sweep samples cover 76% of all taxa. The distribution of species in Kemnader See samples was more even than any other site. In all lakes, we found species exclusive to the sweep samples. In Lac de Grand-Lieu and Kemnader See, some taxa were only present in the sediment samples in open water. Taxa exclusively present in sediment samples within plants were reported in Ebolowa-Pistia, Hartbeespoort Dam, Kemnader See and Ebolowa-Typha.

Common taxa found in all sample types included Oligochaeta (Ebolowa-Pistia, Hartbeespoort Dam, Kemnader See, Lac de Grand-Lieu), amphipods and isopods (Kemnader See), Hydracarina (Acari; Kemnader See), Sphaeriidae (Bivalvia; Lac de Grand-Lieu), Chironomidae (Diptera; Lac de Grand-Lieu, Kemnader See), *Caenis* sp. (Ephemeroptera; Kemnader See), Nemathelminthes (Lac de Grand-Lieu) and the gastropods *Stagnicola* sp. (Ebolowa-Pistia, Ebolowa-Typha), *Physella acuta* (Kemnader See), *Gyraulus* sp. (Kemnader See) and *Potamopyrgus antipodarum* (Kemnader See). Several unique species were found within the open water samples: *Corbicula fluminea* (Bivalvia), *Chelicorophium sowinskyi* (Amphipoda), *Limnius* sp. (Coleoptera) and *Acroloxus lacustris* (Gastropoda) in Kemnader See and Ceratopogonidae (Diptera) in Lac de Grand-Lieu. Most taxa found in sediment samples were also found in the sweep samples. The five taxa restricted to sediment samples within plants were the two Diptera genus *Chironomus* sp. (Ebolowa-Pistia) and *Chaoborus* sp. (Hartbeespoort Dam), the two Gastropoda *Potadoma* sp. (Ebolowa-Pistia, Ebolowa-Typha) and *Melania tuberculate* (Ebolowa-Pistia, Ebolowa-Typha) as well as Nemathelminthes (Lac de Grand-Lieu). In sweep samples, we found a long list of exclusive taxa from many different groups (Table S6).

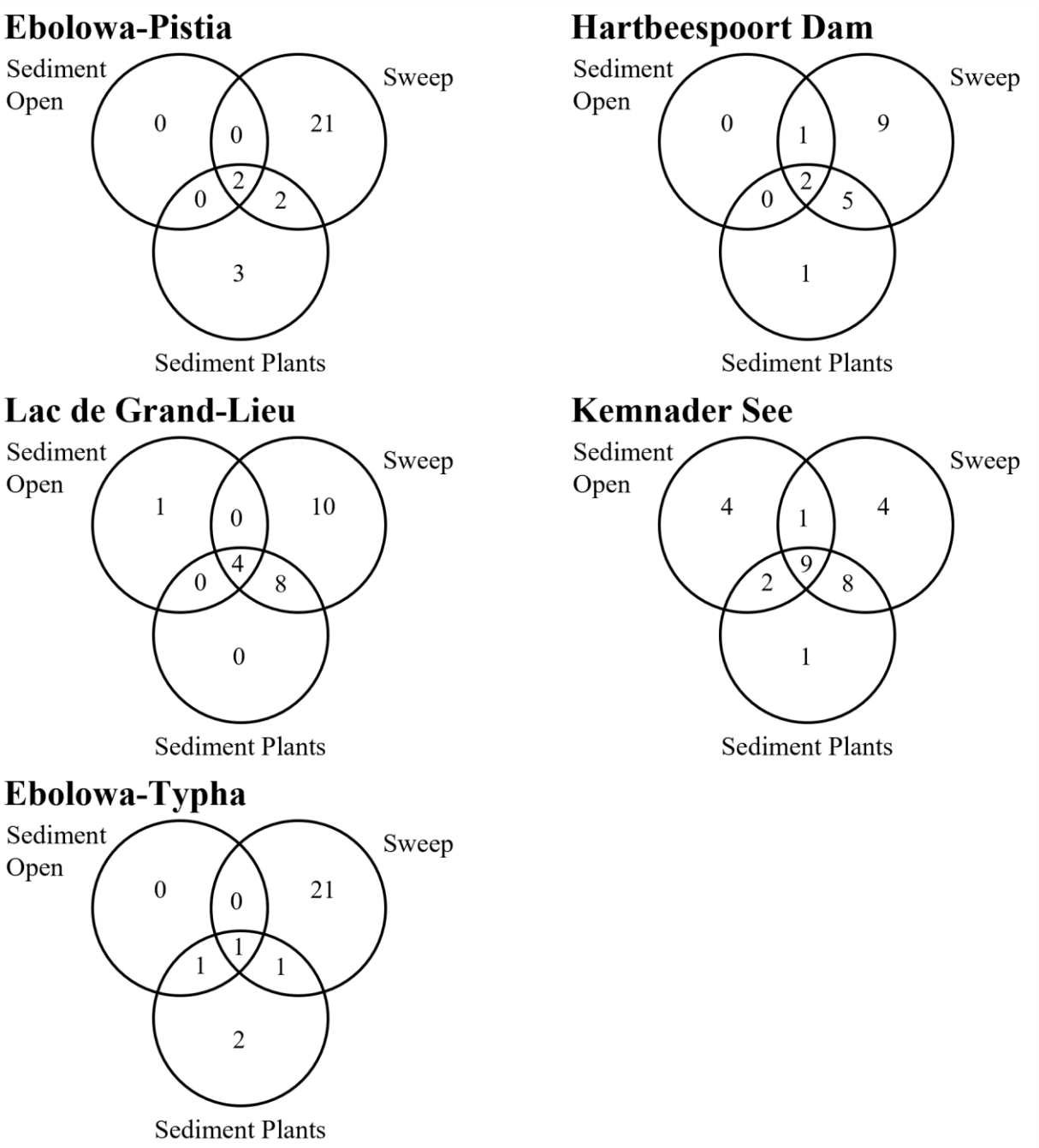

Figure 4: Venn diagrams showing taxa distributions in the three sample types.

Macroinvertebrates diversity found in floating plants and in submersed plants differed, although both sampled macrophytes were located in Lac municipal d'Ebolowa and the total number of taxa was close (Figure 5). Ten taxa (Coleoptera: *Curculionidae*, *Cybister lateralimarginalis, Dryops* sp., *Hydrophilus* sp.; Gastropoda: *Galba truncatula*, *Gyraulus* sp., *Physa* sp.; Heteroptera: *Ranatra linearis*, *Notonecta* sp.; Arthropoda: *Chaoborus* sp.) only occurred in the sweep samples from the free-floating plants (Ebolowa-Pistia), and nine taxa (Diptera: *Simulini* sp.; Gastropoda: *Planorbis* sp.; Coleoptera: *Dytiscus* sp., *Chaetarthria* sp., Scirtidae; Heteroptera: *Belostoma* sp.; Odonata: *Coenagrion speciosa*, *Platycnemis* sp.; Ephemeroptera: *Heptagenia* sp.) were only found in the sweep samples of the non-floating plant (Ebolowa-Typha).

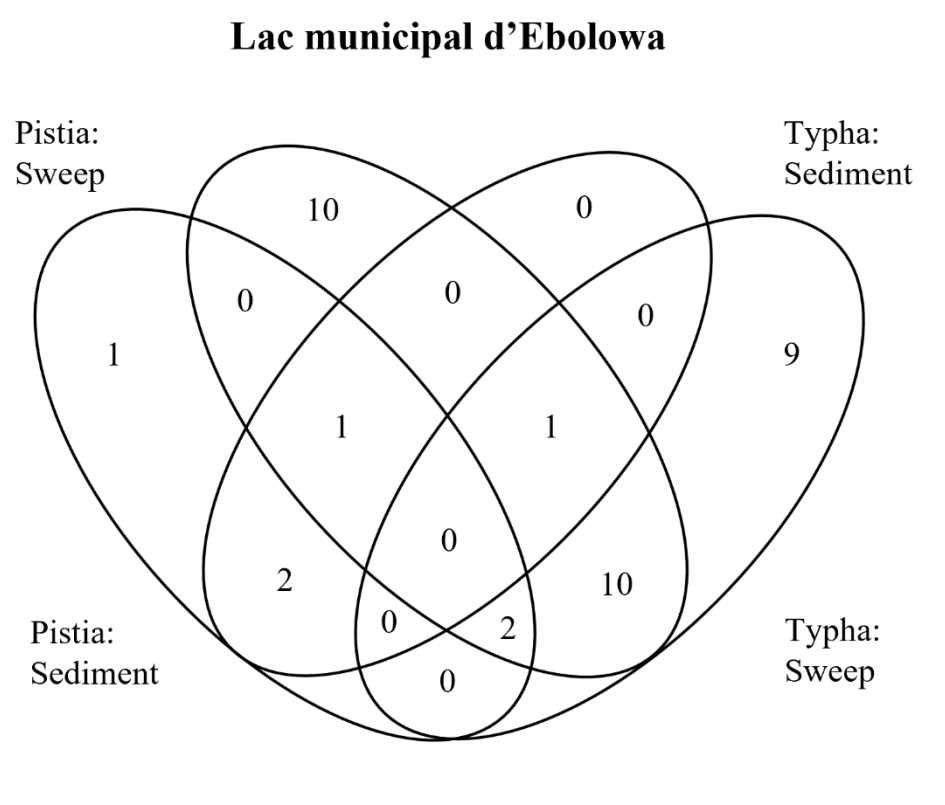

Figure 5: Venn diagram showing the taxa number in samples from within floating plants (Ebolowa-Pistia) and within submersed plants (Ebolowa-Typha) in Cameroon

Differences in sampling coverage by sampling type

Rarefaction curves (Figures 6) showed that reaching a high sample coverage (% of taxa present in the habitat found) requires a high number of samples. For a low number of samples, the curves show considerable differences in the sampling coverage of each sample type. For instance, with a total of five samples, we reach a coverage gap between the lowest and highest sampling coverage by sample type of 5% in Kemnader See, 15% in Hartbeespoort Dam, 20% in Lac de Grand-Lieu, 22% in Ebolowa-Typha and 23% in Ebolowa-Pistia. To reach a sample coverage of 95%, based on the extrapolation curves, for open water sediment (Sediment Open) between 5 (Hartbeespoort Dam) and 15 samples (Ebolowa-Pistia), for sediment of the plant zone (Sediment Plants) between 10 (Hartbeespoort Dam, Kemnader See) and 26 (Ebolowa-Typha) samples and for within macrophytes (Sweep) between 11 (Lac de Grand-Lieu) and 28 (Ebolowa-Typha) samples are needed. Within lakes, the number of samples which are needed to reach 95% coverage varies by sample type. The differences between number of samples needed per sampling type to reach 95% sample coverage are quite big. The gap between the first sample type to reach 95% coverage to the last one reaches from 11 samples (Ebolowa-Pistia, Kemnader See, Lac de Grand-Lieu) up to 18 samples (Hartbeespoort Dam). Considering the mean number (averaged over all sites) of samples necessary to reach a 95% coverage of the community we need 9.8 sediment sediment samples in open water sections, 17.2 sediment samples in macrophytes and 18.4 sweep samples.

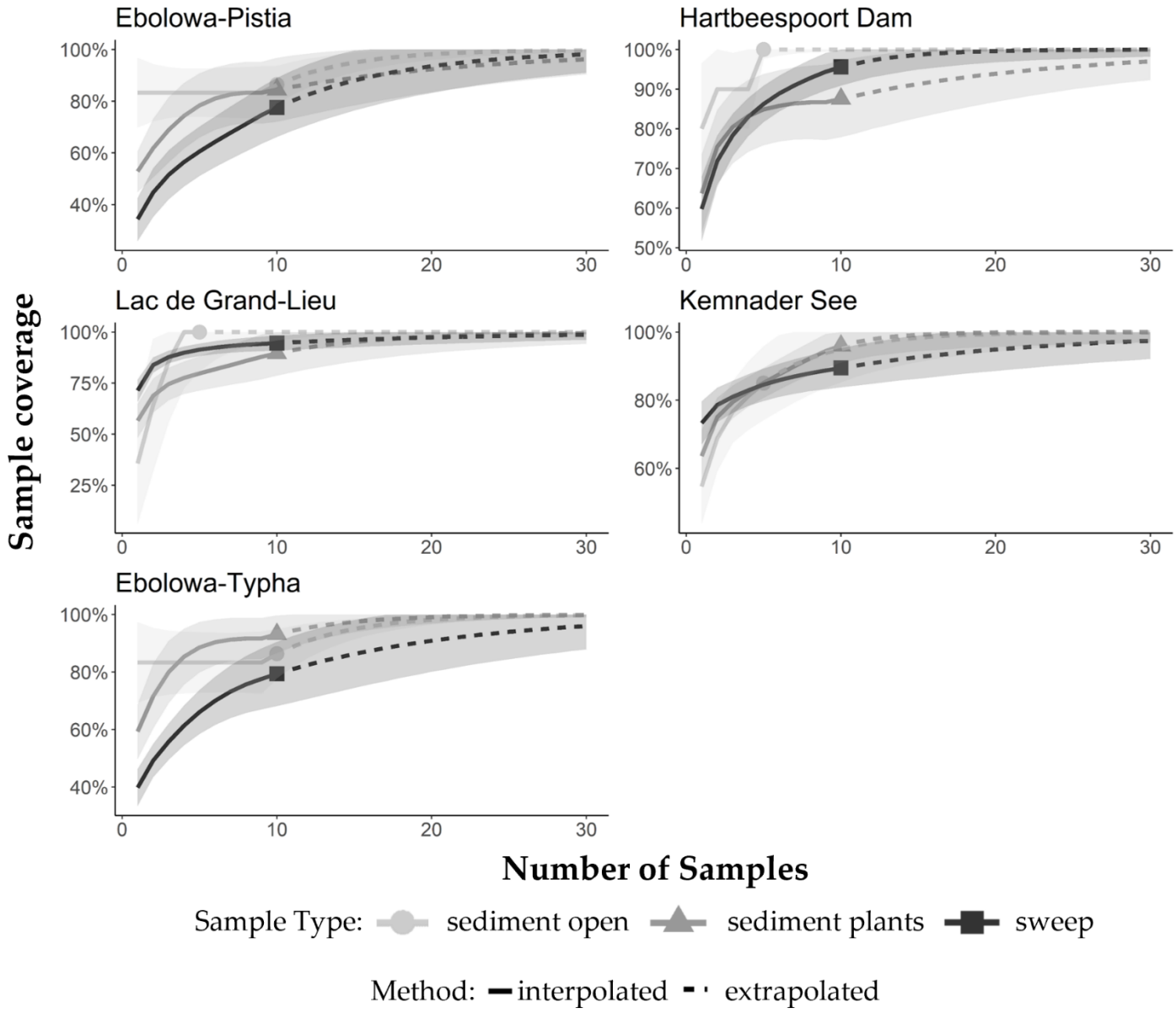

Figure 6: Rarefaction curves showing the sampling coverage depending on the number of samples. Solid line indicates interpolated data and dashed lines extrapolated data. Colour and symbol represent different sample types.

Discussion

This study aims to understand the role of dense mats of macrophytes as a habitat for macroinvertebrates and to provide the first methodical analysis of sampling efficiency of different sampling methods inside and outside of macrophyte stands. The mix of different plant species in four lakes with different characteristics allows us to disentangle global from local effects.

The effect of invasive macrophytes on macroinvertebrate assemblages

Differences in density between sediment samples and sweep samples are biased by the method used, but the density in the two sediment sample types is well comparable. We found a significant increase in macroinvertebrate density in Kemnader See, Ebolowa-Pistia and Ebolowa-Typha. Lac de Grand-Lieu showed a trend to a higher density, whereas in Hartbeespoort Dam, the density tends to be lower within the macrophytes. The increase in macroinvertebrate density in the sediment under macrophyte beds can not only be explained by increased habitat heterogeneity by the presence of macrophytes but also by the availability of food sources. Macrophytes offer a direct food source as plant litter accumulates under macrophyte beds. In addition, macrophytes can increase the growth of periphyton on the sediment, and plant litter offers an extra surface where periphyton can grow (Smock and Stoneburner, 1980; Vermaire et al., 2013; Wolters et al., 2019). Macrophytes, therefore, not only offer a food source for Shredders but also for Gatherers and Scrapers on the sediment (Hill, 1985). Sediment samples from Hartbeespoort Dam were the only ones showing a slightly lower macroinvertebrate density in macrophyte mats. This pattern could be the result of low DO levels within macrophyte stands. Former studies have shown that reduced DO levels under floating beds of *P. crassipes* can lead to decreased macroinvertebrate density (Casco et al., 2014; Coetzee et al., 2014). The fact that mainly Oligochaeta and Hirudinea were found in the area also supports the hypothesis as these two taxa are known for their tolerance for low oxygen levels (Glasby et al., 2021). Taxonomic richness in the sediment samples was also higher

between the plants compared to the plant-free section. Increased macroinvertebrate density combined with the above-described increase in habitat and food resources can be expected to cause this increase in richness. In Hartbeespoort Dam and Kemnader See, we did not find a significant increase in taxonomic richness. Low DO levels under the macrophytes and the lower density we found in Hartbeespoort Dam could explain the small effect of macrophyte presence on taxonomic richness. In Kemnader See, allelopathic chemicals by *Elodea nuttallii* (Erhard and Gross, 2006) or a higher sediment heterogeneity outside the macrophytes, which we observed during the sampling, could explain that taxonomic richness is not increased in macrophytes. In sweep samples we found a higher taxonomic richness (measured as well as estimated richness) and different taxa than in sediment samples. Among the many contrasting characteristics of open water and macrophyte stands, the most fundamental one is structural heterogeneity. The presence of macrophytes offers a complex structure in the water column, providing more diverse habitats and therefore leading to increased macroinvertebrate diversity. This result aligns with findings from earlier studies (Ferreiro et al., 2014; Labat et al., 2022; Scheffer, 2004; Thomaz et al., 2008; Walker et al., 2013). As for the taxa richness in the sediment, we did not find an increased richness in Kemnader See, which might be caused by the same reasons explained above. Both higher taxonomic richness and density within macrophytes confirm our first hypothesis.

The impact of different plant types

No clear difference was found comparing samples from floating and non-floating plants regarding taxa richness. Non-floating macrophytes generate more complex underwater structures than free-floating plants, which grow on the water surface and build roots which don't reach down to the sediment (Meerhoff et al., 2003; Thomaz et al., 2008). Hypothesis (iii) can therefore not be confirmed. Even if they don't grow down to the sediment, plant litter accumulates under the plant cover. This plant litter seems to be enough to have comparable effects to the presence of rooted macrophytes. The effects of plant types in our study may be masked by other factors like climate

and/or eutrophic levels as they have more profound effects on the aquatic ecosystem across a larger geographic scale (Kosten et al., 2009; Pan et al., 2015). The two lakes with floating plants (Lac municipal d'Ebolowa, Hartbeespoort Dam) are hypereutrophic and situated in a subtropical or tropical climate. In contrast, the lakes with non-floating plants share no common feature. To fully understand the impacts of plant types on macroinvertebrate communities at a larger scale, including more lakes and different macrophytes species of different growth forms, should be included for further investigations. Nonetheless, the comparisons between samples from within floating and submersed plants in our lake in Cameroon provide insights into the effects of plant types on the macroinvertebrate communities at a local scale. Only 43% of the taxa were found in both plants, while the rest remained exclusive to only one of the two plants. This finding is in line with research from Walker et al. (2013), which showed that different macrophyte species accommodate different macroinvertebrate communities and taxa. Structures provided by macrophytes differ strongly depending on plant life type and species (Thomaz et al., 2008). Aquatic systems with a diverse macrophyte community could be expected to support higher macroinvertebrate diversity than systems dominated by monospecific stands, as they provide even higher levels of structural heterogeneity (Yofukuji et al., 2021).

Presence and absence of macroinvertebrates in different sample types

Most of the macroinvertebrate taxa found in plants were only found in sweep samples, and only a few taxa were unique to sediment samples in our study. The presence of macrophytes offers new and different habitats for macroinvertebrates. Increased habitat heterogeneity from macrophytes offers a niche for a broad variety of invertebrate taxa (Morse et al., 1985; Thomaz et al., 2008). Macroinvertebrate communities were mostly dominated by one or two taxa, although sweep samples also contained several other taxa with intermediate abundance. In most lakes, the same abundant taxa were found in all sample types. However, in samples around macrophytes, especially sweep samples, we found many additional taxa with high abundance known to be herbivorous

or/and detritivorous. This includes Gastropoda, Lepidoptera, Ephemeroptera or Isopoda, which feed on plant tissue or the algal biofilm growing on it (Tachet et al., 2000). In addition, a higher density of Coleoptera, Odonata and Hydracarina associated with plants was also detected. All of these taxa are known to be predators to which the increased prey density (macroinvertebrates as well as zooplankton) in macrophytes offers a rich hunting ground (Sagrario et al., 2009). The presence of macrophytes therefore increases the complexity of the macroinvertebrate communities based on the fact that many of those taxa were found in the sweep samples only. Yet, the presence of unique taxa to only one of the three sampling types is reported for all three types of habitats. This finding has important implications for the proper sampling strategy for waterbodies with macrophytes. Sweep samples need to be added to the classical sediment sampling when macrophytes are present as they contain a high diversity exclusive to this sampling type. However, as both sediment samples in open water sections and macrophytes can contain exclusive taxa, sediment samples should be taken in both open water and under macrophyte stands.

The effect of invasive macrophytes on the sampling efficiency

Sampling efficiency differs between sample types. Such gaps are biggest when collecting only a low number of samples (usually less than 5) which is standard for many research projects and biomonitoring programs. The taxa richness within macrophytes tends to be underestimated compared to open water sections if only a low number of samples are taken. In current macroinvertebrate sampling protocols, such differences in sampling efficiency are overlooked (for example in Oertli et al., 2005; Indermuehle et al., 2010). A project aiming to achieve a complete profile of the macroinvertebrates living in a certain waterbody with macrophytes, requires combined usage of different sampling methods, and the number of samples needs to be high enough to avoid differences in sampling coverage. However, in practice, taking a higher number of samples is difficult to achieve as it usually increases expenses and workload. For research and biomonitoring, the reduction of labor and costs is crucial. However, from a scientific point of

view, a higher sample number leads to better comparable results. Finding the right balance between high precision and low labor and costs is a difficult task. Our study shows differences in sampling efficiency with low number of samples. If high numbers of samples are not possible, a more comprehensive methodical research to develop more comparable sampling strategies is needed to provide a reliable and strong knowledge foundation for making management decision. These findings could be used as part of the guideline for using a commonly accepted method when sampling aquatic systems with macrophytes. Factors like trophic state, climate, system size, plant type and plant species, leading to different patterns among lakes, need to be studied further. Similar findings can also be applied to sampling in rivers inhabited by macrophytes, but such notions remain to be validated by further studies covering more diverse freshwater bodies.

Conclusion

Under increasing anthropogenic pressure, climate change and the increasing spread of invasive species, aquatic systems are under tremendous pressure and aquatic biodiversity is declining. To reduce the decline of aquatic diversity, good management is the key. All lakes in our study are colonized by an invasive species. Before the invasion of plants, only small parts of the lakes were colonized by macrophytes, and the invasion with macrophytes created new habitats for macroinvertebrates and other organism groups. Even if invasive macrophytes are often considered as problematic and are removed (Hussner et al., 2017; Thiemer et al., 2021), we found positive effects for macroinvertebrates. As long as invasive plants don't repress a more diverse native vegetation, an invasion could be seen as beneficial from a macroinvertebrate perspective. Our findings call for a more flexible management plan for waterbodies with invasive macrophytes, taking these positive effects of invasive macrophytes into account. Moreover, a good management strategy needs to be based on robust scientific data. With this study, we have shown that the presence of macrophytes affects the sampling efficiency of the standard sampling methods, which makes it challenging to reach comparable results of macroinvertebrate sampling. Therefore, further research is needed to tackle this bias.

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Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: B.M., G.T., A.P. and C.P.; methodology: B.M., G.T., A.P. and C.P; validation: L.K.F.; formal analysis: B.M., F.L. and A.P. investigation: B.M., N.C.B., L.K.F. S.F.H. S.N.M. resources: C.P.; data curation: B.M., L.K.F., F.L. and S.F.H.; writing—original draft preparation: B.M.; writing—review and editing: B.M., N.C.B., L.K.F., S.F.H., F.L., S.N.M., G.T., A.P. and C.P.; visualization: B.M.; supervision: G.T., A.P., and C.P.; project administration: G.T.; funding acquisition: G.T. All authors have read and approved the published version of the manuscript.

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Supporting Information

Table S1: Summary of Kruskal-Wallis rank sum test comparing taxonomic richness among different sampling types by lake. If the test was significant (p-value < 0.05), pairwise Wilcoxon rank sum tests (p-values, adjusted with Holm-Bonferroni method) were performed. p-values < 0.05 are highlighted in bold.

Lake	Kruskal-Wallis	Sediment Open / Sediment Plants	Sediment Plants / Sweep	Sediment Open / Sweep
Ebolowa-Pistia	**0.0001**	**0.0143**	**0.0006**	**0.0148**
Hartbeespoort Dam	**0.0001**	0.1246	**0.0007**	**0.0049**
Lac de Grand-Lieu	**0.0001**	**0.0345**	**0.0007**	**0.0051**
Kemnader See	0.053	-	-	-
Ebolowa-Typha	**<0.0001**	**0.0010**	**0.0028**	**0.0003**

Table S2: Estimated taxonomic richness based on Hill numbers (order q=0).

Lake	Sediment open	Sediment plants	Sweep
Ebolowa-Pistia	3.9	13.4	39.25
Hartbeespoort Dam	4.0	14.4	20.4
Lac de Grand-Lieu	3.0	12.4	28.6
Kemnader See	18.8	19.0	32.2
Ebolowa-Typha	3.9	6.9	40.2

Table S3. Mean density in individuals/m2 (± SE) by site and sampling type

Lake	Sediment Open	Sediment Plants	Sweep Plants
Ebolowa-Pistia	112 ± 108	936 ± 470	24 ± 10
Hartbeespoort Dam	4368 ± 1668	1184 ± 276	118 ± 18
Lac de Grand-Lieu	432 ± 207	2616 ± 797	165 ± 44
Kemnader See	2576 ± 705	35836 ± 8537	3836 ± 605
Ebolowa-Typha	112 ± 108	816 ± 280	43 ± 28

Table S4: Summary of Kruskal-Wallis rank sum test comparing density (log+1 tranformed) among different sampling types by lake. If the test was significant (p-value < 0.05), pairwise Wilcoxon rank sum tests (p-values, adjusted with Holm-Bonferroni method) were performed. p-values < 0.05 are highlighted in ***bold****.*

Lake	Kruskal-Wallis	Sediment Open / Sediment Plants	Sediment Plants / Sweep	Sediment Open / Sweep
Ebolowa-Pistia	**0.0072**	**0.032**	0.054	0.34
Hartbeespoort Dam	**0.0011**	0.0858	**0.0010**	0.0799
Lac de Grand-Lieu	**0.0019**	0.0799	**0.0006**	0.3710
Kemnader See	**0.0003**	**0.0013**	**0.0006**	0.2064
Ebolowa-Typha	**0.0001**	**0.0024**	**0.0013**	**0.0138**

Table S5: Results of Permutational multivariate analysis of variance (adonis2 function, vegan package, 999 permutations)

	Df	SumOfSqs	R2	F	Pr(>F)
Plant life form	1	2.159	0.05427	17.4866	0.001
Sample type	2	3.594	0.09036	14.5565	0.001
Lake	3	13.263	0.33342	35.8096	0.001
Plant life form : Sample type	2	1.249	0.03140	5.0591	0.001
Sample type : Lake	6	4.698	0.11812	6.3429	0.001
Residual	120	14.815	0.37243		
Total	134	39.778	1.00000		

Table S6: Presence and absence of taxa in each sample type within each macrophyte. The corresponding order of sample type within each macrophyte is grab open/ grab plants/ sweep samples In each cell, "-" indicates that the taxon is absent in the sample and "0" indicates that it is present.

Group	Taxa	Ebolowa-Pistia	Hartbeespoort Dam	Lac de Grand-Lieu	Kemnader See	Ebolowa-Typha
Ephemeroptera	*Baetis* sp.	-/-/-	-/-/-	-/0/0	-/-/-	-/-/-
	Cloeon sp.	-/-/-	-/-/-	-/-/-	-/0/0	-/-/-
	Caenis sp.	-/-/-	-/-/-	-/-/-	0/0/0	-/-/-
	Heptagenia sp.	-/-/-	-/-/-	-/-/-	-/-/-	-/-/0
Lepidoptera	Pyralidae	-/-/-	-/0/0	-/-/-	-/0/0	-/-/-
Odonata	*Libellula* sp.	-/-/0	-/-/-	-/-/0	-/-/-	-/-/0
	Orthetrum sp.	-/-/0	-/-/-	-/-/-	-/-/-	-/0/0
	Coeanagion speciosa	-/-/0	-/-/-	-/-/-	-/-/-	-/-/0
	Coenagrionidae	-/-/-	-/-/0	-/-/-	-/-/-	-/-/-
	Ischnura elegans	-/-/-	-/-/-	-/0/0	-/-/-	-/-/-
	Platycnemis pennipes	-/-/-	-/-/-	-/-/-	-/0/0	-/-/-
	Platycnemis sp.	-/-/-	-/-/-	-/-/-	-/-/-	-/-/0
Trichoptera	*Stactobia moselyi*	-/-/-	-/-/-	-/-/-	-/0/0	-/-/-
	Ecnomus sp.	-/-/-	-/-/-	-/-/-	0/-/0	-/-/-
Heteroptera	*Notonecta glauca*	-/-/0	-/-/0	-/0/0	-/-/-	-/-/-
	Hydrocyrius sp.	-/-/0	-/-/-	-/-/-	-/-/-	-/-/0
	Ranatra linearis	-/-/0	-/-/-	-/-/-	-/-/-	-/-/-
	Plea pulla	-/-/-	-/-/0	-/-/-	-/-/-	-/-/-
	Appasus capensis	-/-/-	-/-/0	-/-/-	-/-/-	-/-/-
	Corixinae	-/-/-	-/-/-	-/-/0	-/0/0	-/-/-
	Belostoma sp.	-/-/-	-/-/-	-/-/-	-/-/-	-/-/0
Coleoptera	*Amphiops* sp.	-/-/0	-/-/-	-/-/-	-/-/-	-/-/0
	Noterus sp.	-/-/0	-/-/-	-/-/-	-/-/-	-/-/0
	Macroplea sp.	-/-/0	-/-/-	-/-/-	-/-/-	-/-/0
	Curculionidae	-/-/0	-/-/-	-/-/-	-/-/-	-/-/-
	Laccophilus sp.	-/-/0	-/-/-	-/-/-	-/-/-	-/-/0
	Cybister lateralimarginalis	-/-/0	-/-/-	-/-/-	-/-/-	-/-/-
	Dryops sp.	-/-/0	-/-/-	-/-/-	-/-/-	-/-/-
	Hydrophilus	-/-/0	-/-/-	-/-/-	-/-/-	-/-/-
	Scirtidae	-/-/-	-/0/0	-/-/-	-/-/-	-/-/0
	Helodidae	-/-/-	-/-/0	-/-/-	-/-/-	-/-/-
	Hydrophilidae	-/-/-	-/-/-	-/-/0	-/-/-	-/-/-
	Limnius sp.	-/-/-	-/-/-	-/-/-	0/-/-	-/-/-
	Dytiscus sp.	-/-/-	-/-/-	-/-/-	-/-/-	-/-/0
	Chaetarthria sp.	-/-/-	-/-/-	-/-/-	-/-/-	-/-/0
Megaloptera	*Sigara* sp.	-/-/-	-/-/-	-/0/0	-/-/-	-/-/-
Diptera	*Chaoborus*	-/-/0	-/0/-	-/-/-	-/-/-	-/-/-
	Chironomini	-/0/0	-/-/-	-/-/-	-/-/-	-/-/0
	Syrphidae	-/-/0	-/-/-	-/-/-	-/-/-	-/-/0
	Chironomus	-/0/-	-/-/-	-/-/-	-/-/-	-/-/-
	Chironomidae	-/-/-	-/0/0	0/0/0	0/0/0	-/-/-
	Syrphidae	-/-/-	-/0/0	-/-/-	-/-/-	-/-/-

Group	Taxa	**Ebolowa-Pistia**	**Hartbeespoort Dam**	**Lac de Grand-Lieu**	**Kemnader See**	**Ebolowa-Typha**
Diptera (Continued)	Rhagionidae	-/-/-	-/-/0	-/-/-	-/-/-	-/-/-
	Ephydridae	-/-/-	0/-/0	-/-/-	-/-/-	-/-/-
	Psychodidae	-/-/-	-/-/0	-/-/-	-/-/-	-/-/-
	Ceratopogonidae	-/-/-	-/-/-	0/-/-	0/0/-	-/-/-
	Sciomyzidae	-/-/-	-/-/-	-/-/0	-/-/-	-/-/-
	Dolichopodidae	-/-/-	-/-/-	-/0/0	-/-/-	-/-/-
	Stratiomyidae	-/-/-	-/-/-	-/-/-	0/0/-	-/-/-
	Simulini	-/-/-	-/-/-	-/-/-	-/-/-	-/-/0
Decapoda	*Procambarus clarkii*	-/-/-	-/-/-	-/0/0	-/-/0	-/-/-
Amphipoda	*Crangonyx pseudogracilis*	-/-/-	-/-/-	-/-/0	-/-/-	-/-/-
	Dikerogammarus villosus	-/-/-	-/-/-	-/-/-	0/0/0	-/-/-
	Chelicorophium sowinskyi	-/-/-	-/-/-	-/-/-	0/-/-	-/-/-
	Gammarus roeselii	-/-/-	-/-/-	-/-/-	-/0/0	-/-/-
Isopoda	*Asellus aquaticus*	-/-/-	-/-/-	-/-/0	0/0/0	-/-/-
	Proasellus meridionalis	-/-/-	-/-/-	-/-/0	-/-/-	-/-/-
Acari	Hydracarina	-/-/-	-/0/0	-/0/0	0/0/0	-/-/-
Gastropoda	*Gyraulus* sp.	-/-/0	-/-/-	-/-/-	0/0/0	-/-/-
	Physa sp.	-/-/0	-/-/-	-/-/-	-/-/-	-/-/-
	Stagnicola sp.	0/0/0	-/-/-	-/-/-	-/-/-	0/0/0
	Aplexa hypnorum	-/-/0	-/-/-	-/-/-	-/-/-	-/-/0
	Radix sp.	-/0/0	-/-/-	-/-/-	-/-/-	-/-/0
	Potadoma sp.	-/0/-	-/-/-	-/-/-	-/-/-	-/0/-
	Melania tuberculata	-/0/-	-/-/-	-/-/-	-/-/-	-/0/-
	Galba truncatula	-/-/0	-/-/-	-/-/-	-/-/-	-/-/-
	Oxyloma patentissima	-/-/-	-/-/0	-/-/-	-/-/-	-/-/-
	Physela acuta	-/-/-	-/-/-	-/-/0	0/0/0	-/-/-
	Acroloxus lacustris	-/-/-	-/-/-	-/-/0	0/-/-	-/-/-
	Ferrissia californica	-/-/-	-/-/-	-/0/0	-/-/-	-/-/-
	Menetus dilatus	-/-/-	-/-/-	-/-/0	-/-/-	-/-/-
	Potamopyrgus antipodarum	-/-/-	-/-/-	-/-/-	0/0/0	-/-/-
	Valvata piscinalis	-/-/-	-/-/-	-/-/-	-/0/0	-/-/-
	Radix auricularia	-/-/-	-/-/-	-/-/-	-/-/0	-/-/-
	Planorbis sp.	-/-/-	-/-/-	-/-/-	-/-/-	-/-/0
Bivalvia	Sphaeriidae	-/-/-	-/-/-	0/0/0	-/0/0	-/-/-
	Corbicula fluminea	-/-/-	-/-/-	-/-/-	0/-/-	-/-/-
Oligochaeta	Oligochaeta	0/0/0	0/0/0	0/0/0	0/0/0	0/0/-
Hirudinea	*Haementeria costata*	-/-/0	-/-/-	-/-/-	-/-/-	-/-/0
	Hirudinea	-/-/-	0/0/0	-/-/-	-/-/-	-/-/-
	Glassiphoniidae	-/-/-	-/-/-	-/-/-	-/-/0	-/-/-
Platyhelminthes	Platyhelminthes	-/-/-	-/-/0	-/-/-	-/-/0	-/-/-
Nemathelminthes	Nemathelminthes	-/-/-	-/-/-	0/0/0	-/0/-	-/-/-

4. How invasive macrophytes affect zooplankton and phytoplankton assemblages and their sampling efficiency

4.1. Introduction

The presence of dense mats of macrophytes also affects zooplankton and phytoplankton. Increased habitat heterogeneity (Thomaz & Cunha, 2010) and the provision of food mainly via the epiphytic biofilm (Thomaz & Cunha, 2010), are beneficial for a diverse zooplankton community and, therefore, lead to increased zooplankton richness in macrophyte beds (Declerck et al., 2005). In addition, macrophytes provide refuges for zooplankton from predation by fish and macroinvertebrates (Burks et al., 2002; Lauridsen & Lodge, 1996). Many zooplankton taxa use macrophyte beds during day times as a refuge and move to the open water at night, leading to a high density of zooplankton in macrophytes during the day (Burks et al., 2002; Declerck et al., 2005; Lauridsen & Lodge, 1996).

Conversely, phytoplankton is negatively affected by the presence of macrophytes. Phytoplankton biomass has been shown to be negatively correlated with macrophyte biomass (Muylaert et al., 2010). Macrophytes are in direct concurrence for nutrients and light with phytoplankton. If dense mats of macrophytes are present, reduced nutrients and less light are available for the growth of phytoplankton (Scheffer et al., 1993). In addition, increased zooplankton abundance in macrophyte-dominated lakes increases the grazing on phytoplankton (Schriver et al., 1995). Macrophytes change the aquatic environment, leading to a shift in phytoplankton communities and a reduction in phytoplankton richness (Muylaert et al., 2010; Takamura et al., 2003).

We have shown that additional macroinvertebrate samples are needed to reach the same sampling coverage in macrophyte beds compared to open water sites (Misteli et al., 2022). As the

zooplankton community also profits from increased habitat heterogeneity provided by macrophytes, similar results can be expected. On the contrary, as the phytoplankton community around macrophytes may harbor a lower richness and does not benefit from increased habitat heterogeneity, no differences in sampling coverage and even less sampling effort can be expected for phytoplankton sampling within macrophyte beds (Muylaert et al., 2010; Takamura et al., 2003). We compare the zooplankton and phytoplankton communities within dense mats of invasive macrophytes to those in open-water sections in the same lake. We also compare the sampling efficiency of samples collected in plant-free sections with samples collected within macrophytes stands. This methodological assessment provides essential information for future analysis of zooplankton and phytoplankton communities in systems with macrophytes. For zooplankton, we hypothesize that: (i) the density and taxonomic richness of zooplankton within macrophyte stands are higher than those in open water areas; (ii) to reach the same sample coverage of the zooplankton community as in plant-free areas, a higher number of samples is needed in stands of invasive macrophytes, which provide a more heterogeneous habitat in comparison with plant-free areas. For phytoplankton, we expect to find (iii) low or no impact of macrophytes on taxonomic richness in macrophyte beds compared to open water sites as phytoplankton usually does not show changes on a small scale; and (vi), therefore, no difference in sampling efficiency should be found.

4.2. Methods

For this study, we analyzed phytoplankton and zooplankton samples sampled in Hartbeespoort Dam (South Africa), Lake Grand-Lieu (France), and Lake Kemnade (Germany). At all sites, we took five zooplankton and phytoplankton samples within macrophytes and five in a nearby open water part according to the protocol described above (2.2. Biodiversity Sampling).

All statistical analyses for this section were performed in R version 3.6.3 (R Core Team, 2021). Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity was used to compare the community composition. NMDSs were performed with the metaMDS function combined with permutational multivariate ANOVA (PERMANOVA) adonis2 function, both from the *vegan* package (Oksanen et al., 2019). Pairwise t-tests were performed to compare richness, density (log (x+1) transformed), and Shannon-diversity in and outside macrophyte beds. The function t.test provided by the package *stats* was used for t-tests (R Core Team, 2021). Rarefaction curves, including extrapolations, created with the *iNEXT* package (Hsieh et al., 2020), were used to compare sampling coverage in macrophyte beds and open water. Plots were generated using *ggplot2* (Wickham, 2016) and ggpubr for boxplots (Kassambara, 2020).

4.3. Results

4.3.1. Zooplankton

Zooplankton communities are different within macrophyte stands and in open water in all three sites (Figure 15). Zooplankton density is significantly higher (pairwise t-test: p-value=0.008) in the macrophytes in Hartbeespoort Dam (Table 2). A similar trend is seen in the two other sites, but the difference is not significant (Lake Grand-Lieu: 0.051, Lake Kemnade: 0.313). Zooplankton richness is higher in macrophyte beds in Lake Grand-Lieu (p-value=0.006), while the other two sites do not show significant differences between the macrophyte beds and the open water.

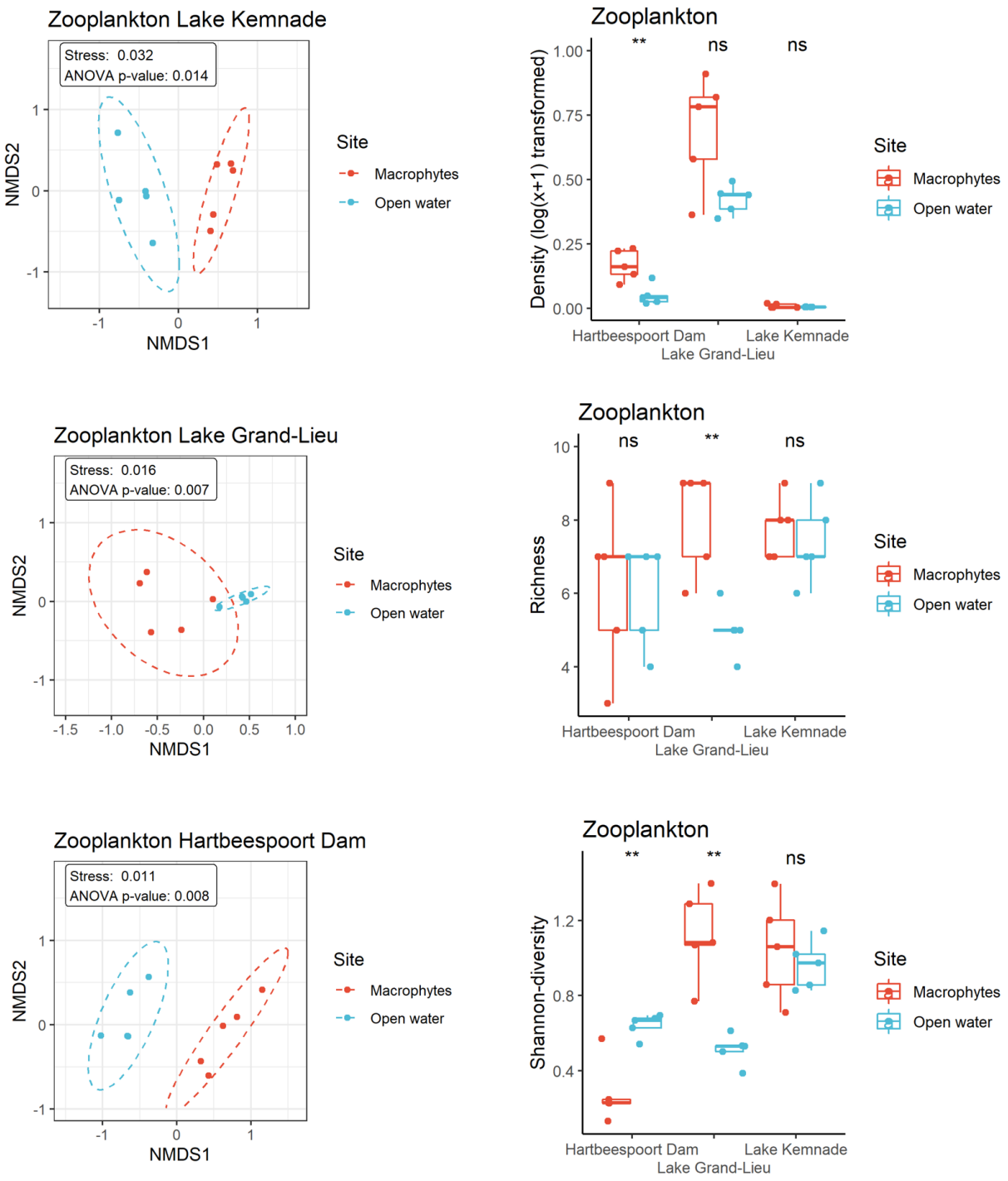

*Figure 15: Differences in zooplankton communities in macrophytes and open water. Left: NMDS showing the community compositions in macrophytes (red) and open water (blue) in all three study sites. Right: Differences in log (x+1)-transformed density, richness and Shannon-diversity of zooplankton in macrophytes (red) and open water (blue). Asterisk indicate significance level from pairwise t-tests: * = p<0.05, ** = p<0.01, ns = p>0.05, all statistics are summarized in Table 2*

Table 2 Statistical summary of t-tests accompanying Figure 15.

Study Site	Density			Richness			Shannon-diversity		
	t-score	Df	p-value	t-score	Df	p-value	t-score	Df	p-value
Hartbeespoort Dam	3.67	6.937	**0.008**	0.167	6.68	0.873	-4.502	5.047	**0.006**
Lake Grand-Lieu	2.647	4.527	0.051	4.243	5.882	**0.006**	5.359	4.916	**0.003**
Lake Kemnade	1.151	4.07	0.313	0.632	7.339	0.54	0.604	5.704	0.569

Shannon-diversity is increased in macrophyte beds in Lake Gran-Lieu (p-value=0.003), while Hartbeespoort Dam shows a lower Shannon-diversity in macrophyte beds (p-value=0.006). Lake Kemnade does not show significant differences between the two sections regarding Shannon-diversity.

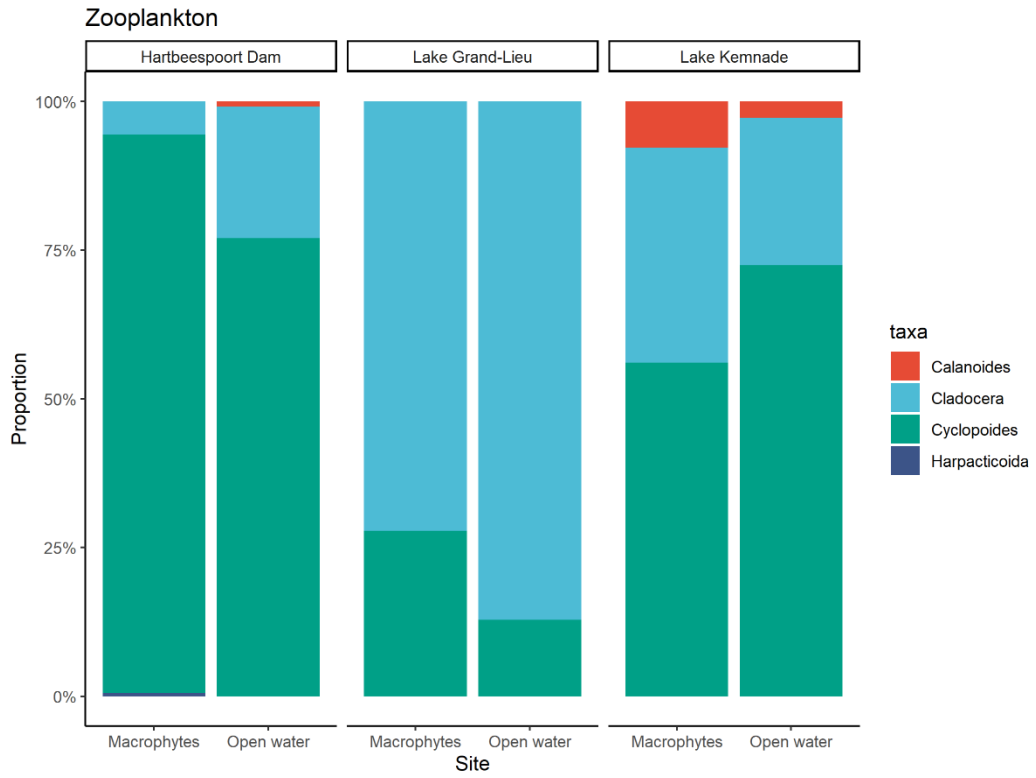

Figure 16: Barplot showing the zooplankton community composition in macrophytes and open water for the three study sites.

Table 3: Table showing the community composition (Mean (in percentage) ± SD) of zooplankton in macrophytes and open water in the three study sites.

Country	Hartbeespoort Dam		Lake Grand-Lieu		Lake Kemnade	
Section	Macrophytes	Open water	Macrophytes	Open water	Macrophytes	Open water
Calanoides	0.05 ± 0.11	0.93 ± 0.60	0 ± 0	0 ± 0	7.80 ± 3.83	2.72 ± 1.83
Cyclopoides	93.91 ± 5.05	77.02 ± 2.80	27.77 ± 14.49	12.82 ± 3.11	56.05 ± 28.48	72.45 ± 5.06
Harpacticoida	0.479 ± 0.43	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Cladocera	5.56 ± 5.25	22.06 ± 2.91	72.23 ± 14.49	87.18 ± 3.11	36.16 ± 32.14	24.84 ± 3.28

In Hartbeespoort Dam, Cyclopoides dominate both zooplankton communities in macrophytes and open water (Figure 16, Table 3). The macrophyte community consists of fewer Cyclopoides compared to the open-water one, and the open-water community has more Cladocera and Calanoides. In addition to Calanoides, Cyclopoides, and Cladocera, Harpacticoides are also found within the macrophytes. In Lake Grand-Lieu, Cladocera also have a higher proportion in open water than macrophytes, but Cladocera are more dominant than Cyclopoides in both sections. In Lake Kemnade, Cyclopoides dominate macrophytes and open water but have a higher proportion in open water, and Calanoides and Cladocera are more abundant in the macrophytes.

Figure 17 shows the sampling coverage reached depending on the number of samples for the three sites. Considering the aim to reach a 95% sample coverage, only two samples are needed in the open water of Lake Grand-Lieu and Hartbeespoort Dam, while in macrophytes, three and nine samples are needed, respectively. However, in Lake Kemnade, only two samples are needed within macrophytes and eleven samples in the open water. Assuming a number of five samples taken in each site, we will reach a sampling coverage of 100% in the open water in Lake Grand-Lieu and 96.4% within the macrophytes. In Lake Kemnade, we will reach 91.5% in the open water and 100% in the macrophytes, and in Hartbeespoort Dam, 100% in the open water and 91.1% within the macrophytes.

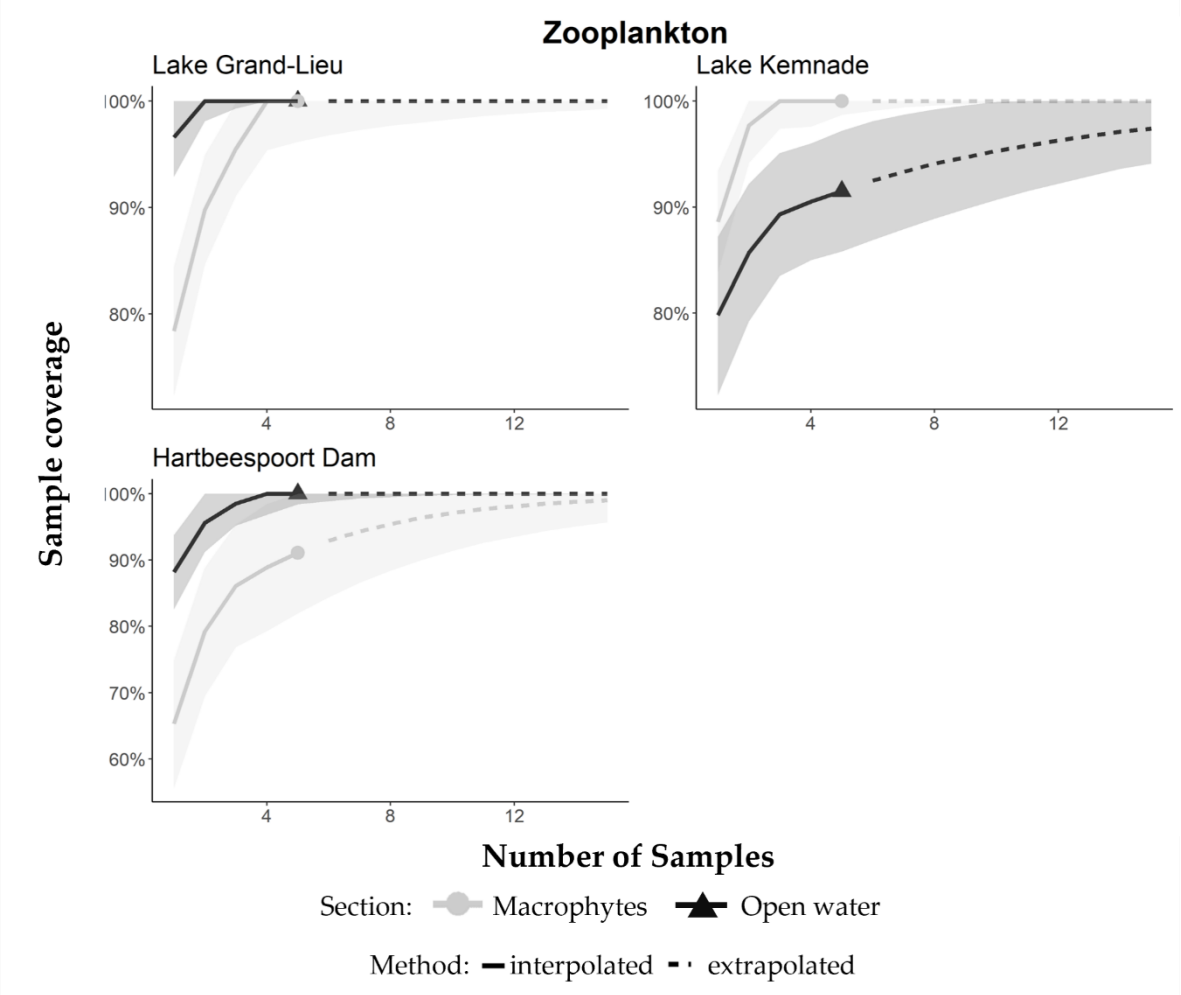

Figure 17:Rarefaction curves showing the sampling coverage as the number of samples increases. Solid lines demonstrate interpolated data and dashed lines extrapolated data. Color and symbol represent the different sections: light gray/round: Macrophytes; black/triangles: Open water

4.3.2. Phytoplankton

For phytoplankton, we did not find a difference between the macrophytes and open-water communities in Lake Grand-Lieu (Figure 18). Only in Lake Kemnade (ANOVA: p-value=0.012) and Hartbeespoort Dam (p-value=0.012) did we find a different community in macrophytes and open water (Figure 18). Considering the species richness and Shannon-diversity, we do not find any difference in open-water sections compared to macrophytes (Figure 18, Table 4).

The communities in all lakes are dominated by Chlorophytes and Cyanobacteria (Figure 19, Table

5). Chlorophytes in Lake Kemnade and Hartbeespoort Dam are more abundant in the open water, while Cyanobacteria are more abundant within macrophytes. In Lake Grand-Lieu, the two sections show a similar proportion of Chlorophytes and Cyanobacteria. Besides the dominant species, Diatoms, Charophytes, Chrysophytes, Cryptophytes, Dinophytes, and Euglenophytes are also present with a low percentage. They can be found in both open water and macrophytes (if present) except Dinophytes (only present within macrophytes in Lake Kemnade and Hartbeespoort Dam). To reach a sampling coverage of 95%, a total of six (Hartbeespoort Dam) or seven (Lake Grand-Lieu, Lake Kemnade) samples are needed in the open water (Figure 20). In macrophytes, the variability is higher. While Lake Kemnade five samples are sufficient to recover 95% of the community within macrophytes, eight and twelve are needed for Hartbeespoort Dam and Lake Grand-Lieu, respectively. With five samples, we will reach between 90.8% (Lake Grand-Lieu), and 93.8% (Hartbeespoort Dam) sampling coverage in open water and between 85% (Lake Grand-Lieu) and 96.3% (Lake Kemnade) are reached in macrophyte beds.

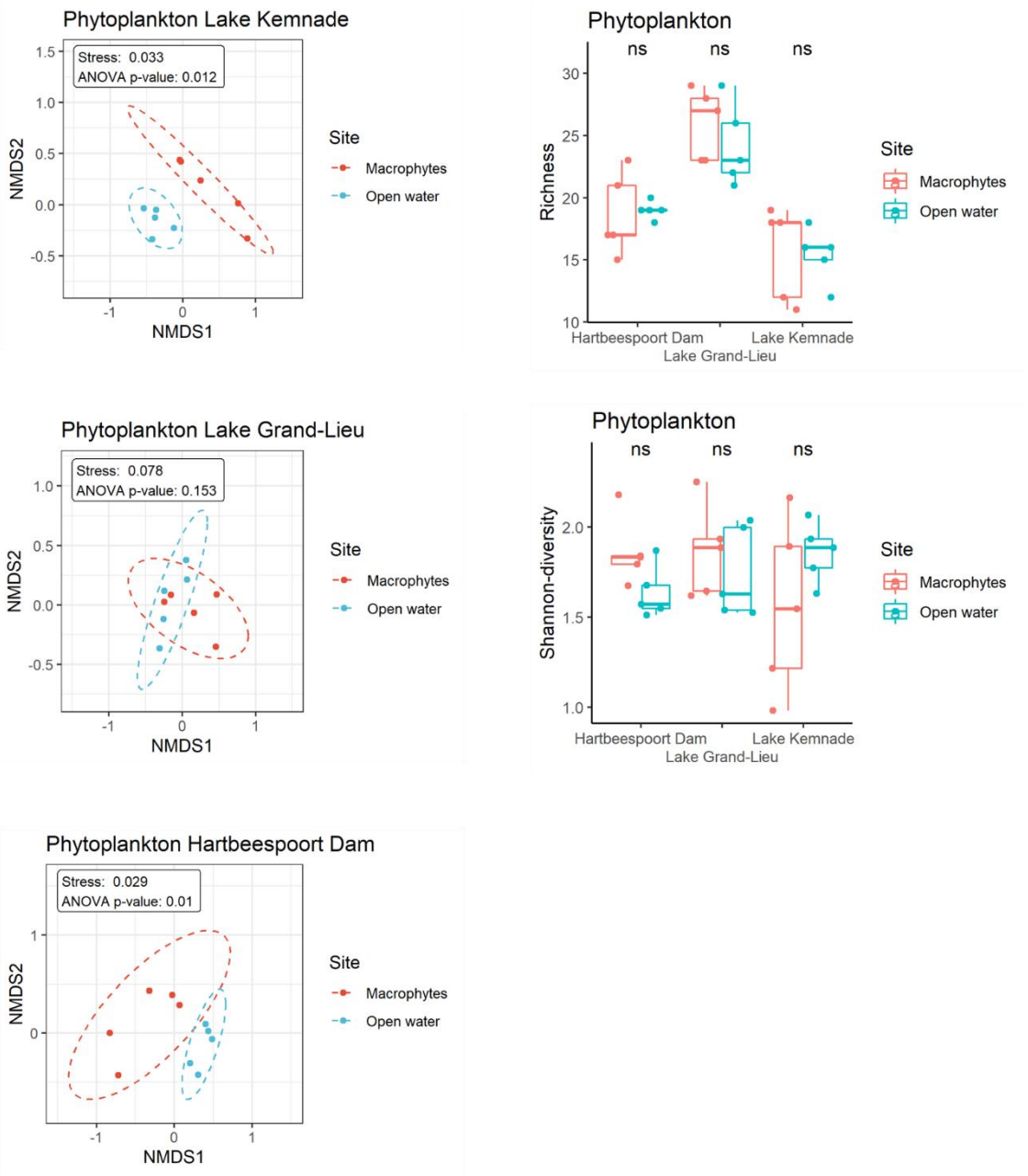

Figure 18: Differences in phytoplankton communities in macrophytes and open water. Left: NMDS showing the community compositions in macrophytes (red) and open water (blue) in all three study sites. Right: Differences in richness and Shannon-diversity of phytoplankton in macrophytes (red) and open water (blue). Asterisk indicate p-values from t-tests: ns = p>0.05, all values summarized in Table 4.

Table 4: Statistical summary of t-tests accompanying Figure 18.

Study Site	Richness			Shannon-diversity		
	t-score	Df	p-value	t	Df	p-value
Lake Kemnade	0.102	6.413	0.922	-1.309	4.919	0.248
Lake Grand-Lieu	0.931	7.837	0.38	0.751	7.997	0.474
Hartbeespoort Dam	-0.266	4.37	0.802	2.156	7.509	0.065

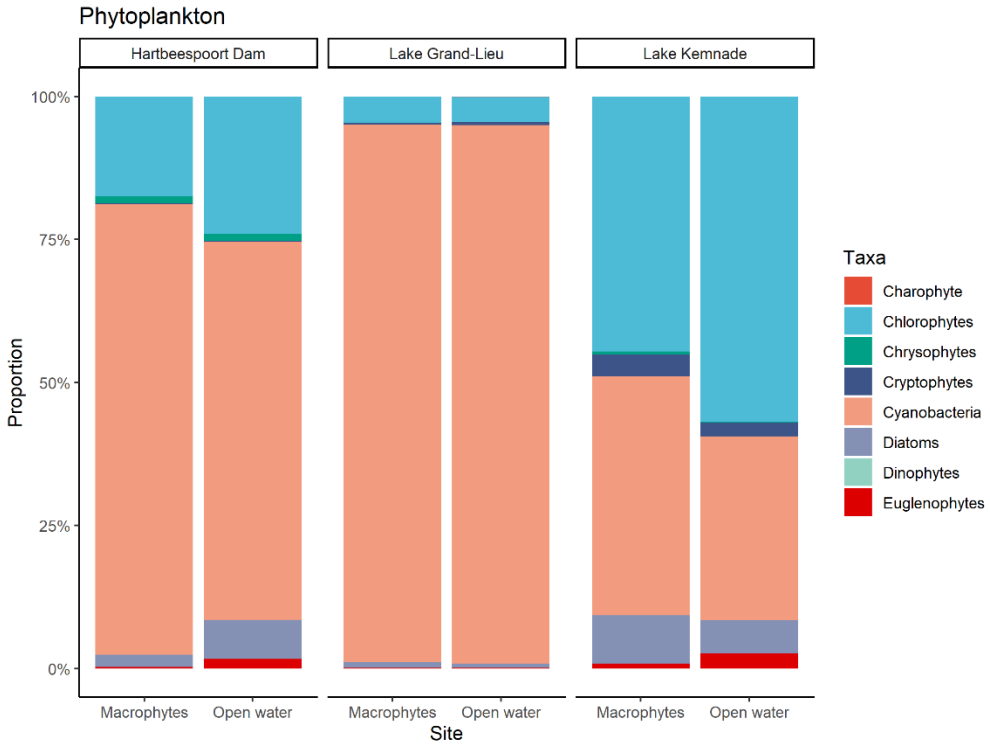

Figure 19: Barplot showing the phytoplankton community composition in macrophytes and open water for the three study sites.

Table 5: Table showing the community composition of zooplankton (Mean (in percentage) ± SD) in macrophytes and open water in the three study sites.

Country	Hartbeespoort Dam		Lake Grand-Lieu		Lake Kemnade	
Site	Macrophytes	Open water	Macrophytes	Open water	Macrophytes	Open water
Cyanobacteria	78.79 ± 7.38	66.14 ± 8.05	94.00 ± 2.03	94.18 ± 0.74	41.79 ± 33.50	32.13 ± 18.13
Chlorophytes	17.45 ± 8.98	24.00 ± 9.08	4.54 ± 1.76	4.33 ± 0.82	44.61 ± 30.63	56.87 ± 16.07
Diatoms	2.08 ± 1.261	6.84 ± 2.70	0.96 ± 0.59	0.70 ± 0.25	8.45 ± 4.50	5.79 ± 2.20
Charophytes	0 ± 0	0 ± 0	0.02 ± 0.03	0.10 ± 0.07	0 ± 0	0 ± 0
Chrysophytes	1.25 ± 1.40	1.27 ± 0.75	0.08 ± 0.11	0.06 ± 0.04	0.52 ± 0.27	0.16 ± 0.15
Cryptophytes	0.14 ± 0.19	0.12 ± 0.05	0.28 ± 0.14	0.49 ± 0.14	3.76 ± 2.88	2.42 ± 0.15
Dinophytes	0.03 ± 0.03	0 ± 0	0.01 ± 0.02	0.01 ± 0.02	0.05 ± 0.11	0 ± 0
Euglenophytes	0.27 ± 0.25	1.63 ± 1.57	0.12 ± 0.11	0.14 ± 0.07	0.83 ± 0.86	2.63 ± 1.21

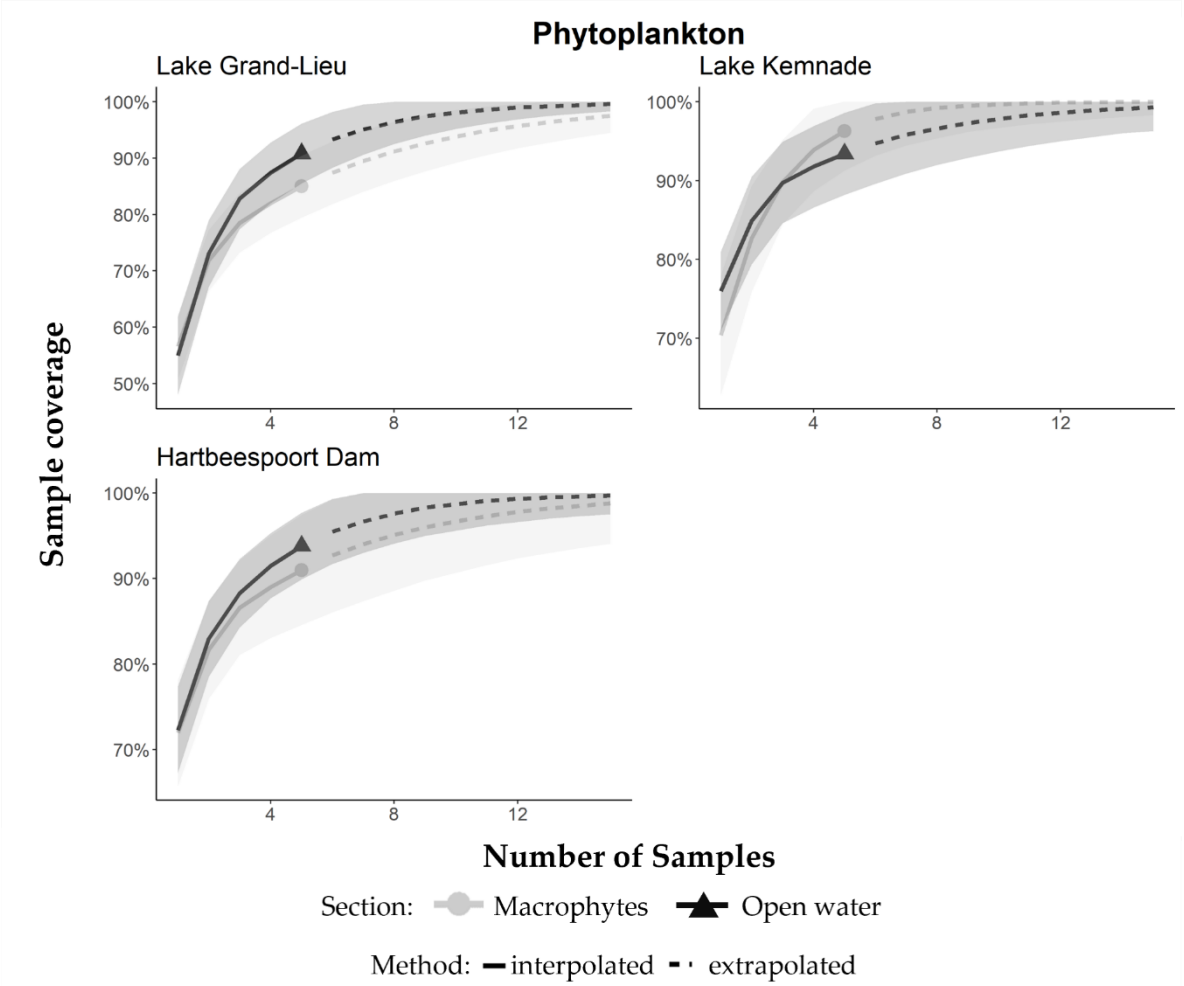

Figure 20: Rarefaction curves showing the sampling coverage as the number of samples increases. Solid lines demonstrate interpolated data and dashed lines extrapolated data. Color and symbol represent the different sections: light gray/round: Macrophytes; black/triangles: Open water

4.4. Discussion

The zooplankton communities within macrophytes differ from those in open water in all sites, but we could only partially confirm our hypotheses for the zooplankton communities. Our first hypothesis (i) of increased zooplankton density and richness within macrophytes was only confirmed in Hartbeespoort Dam (density) and Lake Grand-Lieu (richness). Thus, our results only partially correspond with former studies, which showed increased density and richness of zooplankton within macrophytes (Declerck et al., 2005). The second hypothesis (ii) of a higher

number of samples needed in macrophytes to reach a 95% sample coverage could be confirmed for Lake Grand-Lieu and Hartbeespoort Dam, where we found that more samples are needed to reach the same sampling coverage as in open water. Surprisingly, in Lake Kemnade, we found opposite results, as fewer samples were needed for the same sampling coverage in macrophytes compared to open water. This could be explained by the fact that Lake Kemnade is the only site where the water is slightly flowing, which might lead to a regular introduction of species from the macrophytes-associated community to the open water community.

Our third hypothesis of low or no differences in richness in macrophyte beds compared to open water sites for phytoplankton was confirmed. For richness and Shannon-diversity, we did not find any differences between macrophytes and open-water sites in all three sites. Even though the impact of macrophytes on the phytoplankton community seems to be low, we found that in Lake Grand-Lieu and Hartbeespoort Dam, more samples are required in macrophyte beds compared to open water to reach the same sampling coverage. Our fourth hypothesis of comparable sampling efficiency was therefore not confirmed.

Plankton, by definition, drifts with the currents. However, not all plankton taxa drift in the same way. Since water masses between open water and macrophytes are regularly mixed, phytoplankton assemblages are homogenized. In zooplankton, the ability to actively migrate can lead to the divergence of communities. Therefore it is not surprising that we found greater differences between open water and macrophytes communities in zooplankton than in phytoplankton.

For zooplankton, the difference in the communities agrees partly with former studies. Meerhoff et al. (2007) found a higher proportion of Cladocera within macrophytes, and Calanoides had a higher proportion in the open water. Cyclopoides had a higher proportion in open water compared to macrophytes in temperate sites and an equal proportion in subtropical sites. In our study, none of the sites showed the same distribution as found in Meerhoff et al. (2007). In that study, they highlighted that migration behavior might be genus-depending and can change with changing turbidity. Different turbidity in our sites and different genera in the different sites might explain

why we did not find a general pattern.

Despite the lack of active migration, the phytoplankton community composition between macrophytes and open water differed in Hartbeespoort Dam and Lake Kemnade. Both sites showed an increased proportion of cyanobacteria and a decreased number of Chlorophytes in the macrophytes. Floating cyanobacteria can migrate horizontally, driven by the wind, and get trapped in macrophytes (Xue et al., 2022). The sedimentation rate is increased in macrophytes, which can cause sinking taxa to be buried, whereas, in open water, they are kept in suspension (Madsen et al., 2001). However, for Lake Grand-Lieu, we did not find differences, which is surprising as it was shown that *Ludwigia* sp. could reduce phytoplankton growth via allelopathic chemicals (Santonja et al., 2018)

5. Chapter Discussion

These two studies showed the effect of the presence of macrophytes on macroinvertebrates, zooplankton, and phytoplankton assemblages. Results are summarized over all groups in Table 6. For the richness and density (density not analyzed for sweep samples and phytoplankton), we found either no effect of macrophytes or a positive effect. Only for phytoplankton, we found no impact on the richness, while for all other groups, we found increased richness within macrophyte stands in some study sites. All our results align with the findings of the existing studies showing generally positive impacts on zooplankton and macroinvertebrates and no impacts on phytoplankton (Hilt et al., 2017). However, all those effects are site-dependent (Hilt et al., 2017).

The analysis of the number of samples needed to reach a 95% sample coverage showed that in most cases, a higher number of samples taken within macrophytes is needed to reach the same sample coverage as in open water. However, this is also depending on the site. For a low number of replicates, as it is standard for many research projects and biomonitoring programs for all three

organism groups, the taxonomic richness within macrophytes beds might be underestimated. Our results show the need for more replicates in macrophytes to get comparable results to samples from open water. In many cases, this is impossible, for example, due to limitations in funding or time. In that case, it must be considered that the different community structures might bias the results in the community inside and outside of macrophytes. Further research is needed to better understand differences in sampling efficiency and factors leading to differences among sites.

Table 6: Density, Richness, and Sampling Coverage with macrophytes compared to open water

	Site	Density	Richness	Sampling Coverage
Macroinvertebrates Sediment Samples	Lake Grand-Lieu	→	↑	↓
	Hartbeespoort Dam	→	→	↓
	Lake Kemnade	↑	→	→
	Lake Ebolowa (*Pistia* sp.)	↑	↑	↓
	Lake Ebolowa (*Typha* sp.)	↑	↑	↑
Macroinvertebrates Sweep Samples (in comparison with open water)	Lake Grand-Lieu	**N/A**	↑	↓
	Hartbeespoort Dam	**N/A**	↑	↓
	Lake Kemnade	**N/A**	→	↓
	Lake Ebolowa (*Pistia* sp.)	**N/A**	↑	↓
	Lake Ebolowa (*Typha* sp.)	**N/A**	↑	↓
Zooplankton	Lake Grand-Lieu	→	↑	↓
	Hartbeespoort Dam	↑	→	↓
	Lake Kemnade	→	→	↑
Phytoplankton	Lake Grand-Lieu	**N/A**	→	↓
	Hartbeespoort Dam	**N/A**	→	↓
	Lake Kemnade	**N/A**	→	↑

Chapter 2. Invasive plants as habitat and refuge

1. Introduction

In Chapter 1, we analyzed the interaction of zooplankton and invasive macrophytes in a set of highly variable sites and showed a significant effect of macrophytes on zooplankton. In this chapter, we decouple the effect of different biological types of macrophytes in a mesocosm setup.

With an experimental approach, we studied the zooplankton communities in macrophytes of different biological types and the influence of fish on this zooplankton-macrophyte interaction. This analysis of biological type is combined with testing the effect of fish presence on the zooplankton-macrophyte interaction.

2. Article 2: Effects of invasive plants as habitat and refuge for zooplankton, a mesocosm approach

Effects of invasive plants as habitat and refuge for zooplankton, a mesocosm approach

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Abstract

Macrophytes play a fundamental role in structuring aquatic environments. Macrophytes have been shown to provide habitat and refuge for zooplankton. Their impact depend among others on their biological types (i.e. submerged, emergent and free-floating macrophyte). The question remains if invasive macrophytes affect the zooplankton communities in a comparable way. To tackle this question, we designed a mesocosm experiment. Every mesocosm was split into four sections. Three invasive species of different biological types were planted in one section each, whereas the fourth section was an open-water section. Egeria densa was used as a submerged plant, Ludwigia spp. as an emergent plant, and Pistia stratiotes as a free-floating macrophyte. The four sections were sampled simultaneously for four weeks by day and nighttime. In half of the mesocosms, we introduced fish as a predator. We found that the zooplankton assemblage differs between the four sections, with higher abundance and richness found in the macrophytes compared to open water. The highest density was found between the roots of the floating plant P. stratiotes. During the night, zooplankton migrates outside the macrophytes, and differences were smaller between the sections. The presence of fish only had a negligible impact on the community.

Keywords: Diel horizontal migration, cladocerans, fish, biodiversity, macrophyte architecture

Introduction

Macrophytes in shallow aquatic systems such as lakes, streams, ponds, rivers, or wetlands act as a structuring factor for aquatic communities (Thomaz & Cunha, 2010; Debastiani-Júnior, Elmoor-Loureiro & Nogueira, 2016). They directly affect biological communities from bacteria to fishes and their interactions (Jeppesen et al., 1998). The presence of macrophytes increases the spatial complexity and habitat heterogeneity, thereby providing microhabitats for zooplankton. Further, they provide direct and indirect (via attached algae and bacteria) food sources (Thomaz & Cunha, 2010) and offer a refuge area against predators (Lauridsen & Lodge, 1996; Burks et al., 2002).

In shallow lakes, zooplankton can migrate horizontally from the pelagic zone to the littoral zone to seek refuge from the predatory fish and/or predatory macroinvertebrates in the littoral zone. This process is called diel horizontal migration (DHM). During daylight, pelagic zooplankton migrates and seeks refuge from predatory fish or/and macroinvertebrates between the macrophytes in the littoral zone (Lauridsen & Buenk, 1996; Burks, Jeppesen & Lodge, 2001). Increased habitat heterogeneity in combination with DHM leads to increased zooplankton abundance, richness, and biomass within macrophytes compared to open water sites (Declerck et al., 2005). This increase in zooplankton abundance within macrophytes leads to increased grazing rates on phytoplankton which is an essential mechanism in maintaining a clear water state in lakes (Schriver et al., 1995; Scheffer, 1999).

Macrophytes exist in various forms with different biological types, complexity, biomass, and cover; differences in their impact can therefore be expected. Most studies investigating macrophyte architecture and its effects on biodiversity focus on macroinvertebrates (Bogut et al., 2007; Thomaz et al., 2008; Van de Meutter, Cottenie & De Meester, 2008) or fish communities (Chick & Mlvor, 1997; Grenouillet & Pont, 2001; Grenouillet, Pont & Seip, 2002), whereas the interaction between macrophytes and zooplankton is less well known. Choi et al. (2014) found no effect of emergent plants in comparison with strong effects of free-floating, and submerged plants, with submerged

63 macrophytes supporting the highest abundance of zooplankton. Also, submerged macrophytes
64 provide more complex zooplankton assemblages than other plant types (Choi et al., 2014). In another
65 study, free-floating and submerged macrophytes clearly increased zooplankton abundance compared
66 to open water. However, this effect was impacted by the climate and turbidity of the lake (Meerhoff
67 et al., 2007). In contrast, Zeng et al. (Zeng et al., 2017) showed that plant complexity does not seem
68 to be a driver of overall zooplankton abundance, and interactions seem to be plant-species specific.
69 Different zooplankton taxa have been shown to select different macrophyte types (Choi et al., 2014;
70 Kuczyńska-Kippen & Joniak, 2016; Celewicz-Gołdyn & Kuczyńska-Kippen, 2017).
71 In the past decades, the spread of invasive macrophytes has become a worldwide problem (Hussner
72 et al., 2017; Verhofstad et al., 2017). Like native macrophytes, invasive species also play an essential
73 role in structuring aquatic systems and their biodiversity (Hussner et al., 2017). While for
74 macroinvertebrates and fish, extensive research was conducted (Schultz & Dibble, 2012; Evangelista,
75 Thomaz & Umetsu, 2014; Tasker, Foggo & Bilton, 2022), the interaction between zooplankton and
76 invasive macrophytes was weakly studied (Evangelista et al., 2014; Stiers & Triest, 2017). The question
77 remains if invasive macrophytes play a similar role to native macrophyte species in structuring
78 zooplankton communities. Extensive knowledge of the effects of invasive macrophytes on the
79 zooplankton community is key to understanding the functioning of aquatic ecosystems and assist in
80 decision making in the context of their management. To examine the role of invasive macrophytes in
81 defining zooplankton communities, we used a multispecies mesocosm setup with three non-native
82 macrophytes of different architecture. Each mesocosm contained a submerged (Egeria densa Planch.),
83 an emergent (Ludwigia spp.), and a free-floating macrophyte with extensive underwater roots (Pistia
84 stratiotes L.), plus an open water section. We compared zooplankton assemblages in the three
85 macrophyte beds and in the open water site. We hypothesized that a higher zooplankton density and
86 diversity will be found in macrophytes compared to the open water and relatively higher values in

more complex plants. With the addition of fish as predators, we tested if our findings depended on the presence of fish and its odor. Samples were taken during the day and night to account for DHM. We hypothesized that with the presence of fish, these differences will become stronger as zooplankton seek refugia in the plants. Finally, we expect reduced differences at night as zooplankton can migrate with less predation risk in the dark.

Material and Methods

Biological models

For this study, three macrophyte species *Egeria densa*, *Ludwigia* spp., and *Pistia stratiotes*, were used. All three species are invasive to France, where the study was conducted:

- *Egeria densa* is a submerged macrophyte native to South America. It inhabits a wide range of aquatic systems, where they grow in dense mats. *E. densa* can tolerate temperatures between 3 and 35°C (Yarrow *et al.*, 2009), can survive in systems with low levels of CO_2 (Lara, Casati & Andreo, 2002), and tolerates low light levels (Rodrigues & Thomaz, 2010). *E. densa* is widely used in aquariums and as an ornamental plant in ponds. The first introduction to France dates back to 1961, when the plant was found in a reservoir of the Sélune river (Fried, 2017). Its invasion can have strong physicochemical effects on the water body (e.g., hypoxia, reduced velocity, siltation), hinder fish movement, and was shown to reduce biodiversity (Sarat *et al.*, 2015). The potential of *E. densa* for vegetative fragmentation (stems of at least two nodes can break off and grow into a new plant) and the possibility of forming free-floating mats leads to a high invasive potential (Cook & Urmi-König, 1984). The plants used for our experiment were collected from a nearby pond (48°07'08.2"N, 01°35'40.47"W).

- *Ludwigia* spp. (a mix of *Ludwigia grandiflora* subsp. *hexapetala* (Hook. & Arn.) G.L.Nesom & Kartesz and *Ludwigia peploides* subsp. *montevidensis* (Spreng.) P.H.Raven) are emerging plants with an amphibious character. This amphibious character and their high plasticity of growth form allow them to grow in different environmental conditions and efficiently invade new systems (Thouvenot, Haury & Thiebaut, 2013). From its native range in South and Central America, *Ludwigia* spp. found its way to most parts of the world, including France, where it became one of the most invasive species (Thiébaut, Dutartre & Nairne, 2009). The introduction to France dates back to 1820 when the plants were introduced as ornamental plants (Dutartre *et al.*, 2007). *Ludwigia* spp. is out-competing native plants, and its dense mats can cause anoxic conditions and decrease water flow, causing flooding and increased sedimentation (Dandelot *et al.*, 2005). The plants used for the experiment were cultivated at the university and collected from a nearby pond southwest of Rennes (48°05'43.4"N 1°44'33.2"W).
- *Pistia stratiotes*, often also referred to as water lettuce, is a free-floating macrophyte species. *P. stratiotes* is native to tropical and subtropical lakes. Its exact origin remains unclear. Nowadays, the plant can be found on all continents except Antarctica (Parsons & Cuthbertson, 2001). *P. stratiotes* can proliferate and form dense mats covering an entire lake or river within a short time (GT IMBA, 2016). By forming dense mats, water lettuce dramatically impacts the ecosystem. They reduce light availability, thus hindering submerged macrophytes and phytoplankton. Further, they can reduce water temperature, increase stratification, reduce pH and lead to anoxic conditions (Attionu, 1976). In France, *P. stratiotes* was found occasionally. With low winter temperatures, plants could not survive. However, recently the plant was found to form a permanent population in a side channel of the Rhone river in the southeast of France, where temperatures are generally higher (personal communication: SMAGE des Gardons). The plants used for our experiment were bought from a local botanical store.

134 *Mesocosm Preparation*

135 The study was conducted in late summer 2021 at the "ECOLEX" outdoor mesocosm facilities from
136 the Université de Rennes (Rennes, France; 48°07'04.3"N 1°38'23.0"W). Ten artificial ponds (see
137 Figure 1 for a schematic representation) with a size of 130cm by 150cm were used with a water depth
138 of around 35cm (± 10cm, fluctuating depending on meteorological conditions). With pool noodles,
139 every mesocosm was split into four equal sections, in which we planted one of the three macrophyte
140 species, and one section remained plant-free. For the rooted plants *E. densa* and *Ludwigia* spp., pots
141 filled with a mix of bedding plant substrate (BP Substrate, Klasmann-Deilmann GmbH, Geeste,
142 Germany) and sand were used. The same pots were introduced in the plant-free section and in the *P.*
143 *stratiotes* section to have comparable conditions in all sections. Within each mesocosm, the location
144 for each plant was randomized to avoid bias from their arrangement (see Figure S1).

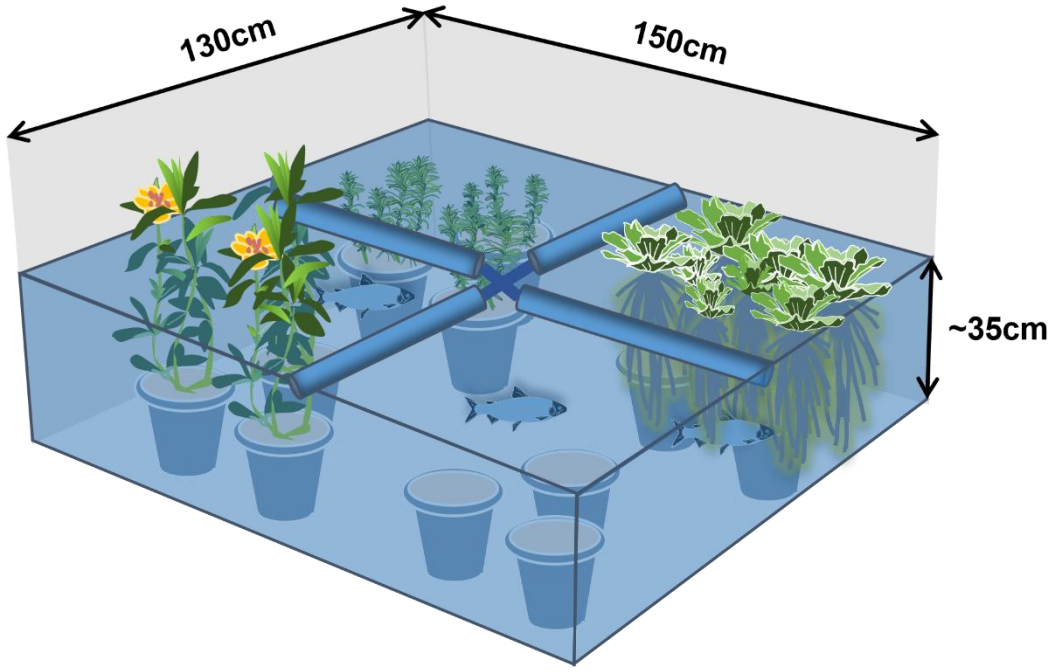

Figure 1: Schematic representation of an mesocosm with fish and the three macrophytes (from left to right) Ludwigia spp., Egeria densa and Pistia stratiotes. Measures indicate mesocosm size and water level.

145 The mesocosms were set up at the beginning of April 2021, and plants were growing in the mesocosms
146 during the summer until the start of the experiment in September 2021. Exceptionally low

147 temperatures in the earlier phase of the growing season required the addition and replacement of
148 plants to maintain a dense plant community. Until the start of the experiment, water was circulated
149 between mesocosms with a pump system big enough to exchange zooplankton between mesocosms.
150

151 *Water sampling*

152 To ensure that physical and chemical parameters remained similar between mesocosms, we measured
153 different parameters in all ponds. In every mesocosm, we installed a temperature logger (HOBO
154 Pendant® Temperature/Light 64K Data Logger) in the open water section, which measured the
155 temperature every five minutes. Further, we measured the water depth, conductivity, pH, and
156 dissolved oxygen and took water samples to analyze NO_3, NH_4, and PO_4 concentrations. This
157 sampling was performed twice per week during the experiment. Filtrations for nutrients analyses were
158 performed immediately on 0.45 μm filter (filtropur). Water samples were frozen and later analyzed
159 using a Gallery™ Plus Discrete Analyzer (ThermoFisher Scientific). Total phytoplankton biomass was
160 measured twice a week in each section using a BBE AlgaeTorch fluorescence probe BBE moldaenke).
161

162 *Plant Characteristics*

163 *E. densa* at the end of the three-week experiment had the lowest biomass of the three plants, with
164 17.97 [± 5.79] g dry biomass per mesocosm (Table S1). The plants did not reach the surface compared
165 to the two other species. *Ludwigia* spp. had, with 19.72 [± 5.33] g dry biomass, slightly higher biomass
166 than *E. densa*. *Ludwigia spp.* grew through the entire water column, reaching and emerging from the
167 surface. *P. stratiotes* is a free-floating plant. Therefore, only the plant roots are growing in the water
168 column. The root biomass of *P. stratiotes* was higher than the plant biomass measured for the other
169 species, with 20.25 [± 14.04] g dry biomass, but also showed a higher variability among mesocosm.
170 Some roots reached down to the bottom of the mesocosm.

171 *Zooplankton Community*

172 Zooplankton was introduced from different ponds and lakes near the Université de Rennes: Lac de
173 Grand-Lieu (47°05'40.2"N 1°40'30.9"W; harboring fish), which is a large shallow lake known for its
174 high planktonic diversity, a small pond located in an urban park (Gayeulles - 48°07'56.4"N
175 1°38'31.1"W; harboring fish), and one pond on the university campus, located a few dozen meters
176 from the mesocosms (48°07'08.8"N 1°38'21.3"W; fishless). In all sites, several dozen liters of water
177 were filtered with a 20µm mesh net to collect zooplankton and phytoplankton. The filtrate was equally
178 distributed among the mesocosms. In addition, we collected sediment in all the sites mentioned above
179 and deposited them at the bottom of the mesocosms as an additional inoculum of zooplankton
180 (through resting eggs). Zooplankton filtrate and sediment were added during the setup of the
181 mesocosm in April 2021. To minimize variation in zooplankton assemblages between mesocosms,
182 water was circulated from spring until the beginning of the experiment between mesocosms.
183
184 *Introduction of Fish*
185 After the first sampling, we introduced fish as predators into half of the mesocosms. The five
186 mesocosms containing fish were randomly chosen (Figure S1). In each mesocosm, we added three
187 individuals of the species *Leuciscus idus* L., commonly known as Ide (from a local fishery store
188 harvesting them in natural ponds), as predators. *L. idus* is widely spread in Europe and can be found
189 in the region where the experiment was performed. Juvenile fish were used with an average size of
190 10.30 ± 0.95 cm. *L. idus* feed on various aquatic animals, from zooplankton to macroinvertebrates and
191 fish, and plant material (Kottelat & Freyhof, 2007). A relatively low natural predator density was
192 chosen to avoid high predation, while zooplankton was exposed to fish odor.
193

194 *Zooplankton Sampling*

195 The first sampling was performed at the start of the experiment, just before fish were put into the
196 mesocosms. Two liters of water were taken in all four parts of the mesocosm simultaneously to avoid
197 zooplankton escaping to neighboring habitats during the sampling process. The same sampling was
198 repeated in the three following weeks, always at the solar noon (14h ± 1h). One and two weeks after
199 the start, an additional sampling by night (12 hours after the day sampling) was performed. All samples
200 were directly filtered with a 60 μm mesh before narcotization with carbonated water. Samples were
201 stored in 70% ethanol at 4° C until identification. Subsamples with a fixed volume were counted and
202 identified (Dussart, 1967; Amoros, 1984; Bledzki & Rybak, 2016). The subsampling was stopped after
203 400 individuals were reached. Otherwise, the entire sample was counted. Finally, the abundance was
204 calculated per liter of water. Only taxa found in at least 5% of all samples were included in the analysis.
205 Only one species of Calanoides was observed in samples (*Eudiaptomus gracilis*), while cyclopoids were
206 represented by three species (*Macrocyclops albidus, Macrocyclops fuscus,* and *Eucyclops serrulatus*). For
207 Copepoda, requiring dissection of each organism for identification to species level, we choose to count
208 at the genus level. Rotifers were not identified for this study.

209

210 *Statistical Analysis*

211 All statistical analyses were performed in R version 4.1.2 (R Core Team, 2020). NMDSs were created
212 using the function metaMDS from the *vegan* package (Oksanen *et al.*, 2019). Differences among groups
213 were analyzed using the function "adonis" from the same package and the function "pairwise.adonis2"
214 from the package *pariwiseAdonis* (Arbizu, 2017). The function "multipatt" from the package *indicspecies*
215 was used to identify indicator species (Cáceres & Legendre, 2009). To test for significant differences
216 in density, richness and Shannon-diversity, we used the functions "anova_test" and "pairwise_t_test"
217 from the *rstatix* package (Kassambara, 2020); for p-value correction, the Bonferroni method was used

(Bland & Altman, 1995). To test for the effect of fish, linear mixed models (LMM) were performed with the function "lmer" from the package "*lme4*" (Bates *et al.*, 2015). The "fish presence", "section" and "days since experiment start", and their interaction were used as fixed effects, and the "mesocosm" plus "days since experiment start" were included as random factors.

The correlation of the density of zooplankton to plant biomass (dry mass measured after the experiment) was tested over all plants and for each plant, respectively, using the cor.test function from the package *stats* (R Core Team, 2020). Overall, density was not correlated with the biomass of plants. Testing each plant type separately did also not reveal a correlation between density and biomass by plant type. Based on these comparisons, the plant biomass was not included in further analyses.

Results

Mesocosm charecteristics

All mesocosms showed similar values at the beginning and during the experiment period (Table S2). The water conductivity reached average values between 204.0 and 270.8 µS cm-1, and water temperatures averaged between 17.7°C and 18.3°C in the different mesocosms. The water was alkaline and with low nutrient concentrations (< 20 µg N-NO3- L-1 and < 20 µg P-PO43- L-1). Oxygen values ranged from 6.2 to 9.6 mg L-1.

The zooplankton assemblage in different plant species

Multiple aspects of the zooplankton assemblage associated with the three plant species and the open water were analyzed for the first sampling session before fish were added (Figure 2). The zooplankton assemblage differed between the four sections (PERMANOVA: $F= 7.839$, $R2= 0.395$, $p<0.005$). Pairwise comparisons revealed significant differences in the assemblages between *P. stratiotes* and the three other sections (statistical summary in Table S3). In addition, the assemblage in *Ludwigia* spp. and

242 *E. densa* differed significantly from open water samples. No differences were found between the
243 assemblage of *Ludwigia* spp. and *E. densa*. Further, we analyzed the indicator taxa for the four different
244 sections. Cyclopoides ($p<0.001$) and *Chydorus* ($p=0.005$) were found to be associated with all three
245 plants but not with open water. In addition, *Simocephalus* sp. ($p<0.001$), *Ceriodaphnia* sp. ($p<0.001$),
246 *Eurycercus* sp. ($p=0.002$), and *Alona* sp. ($p=0.040$) were associated with *P. stratiotes*.
247 Besides the zooplankton assemblage, we also compared the density of zooplankton, the taxa richness,
248 and the Shannon-diversity between the four sections (Figure 2). Significant differences between
249 sections were found for density (repeated measure ANOVA: F: 33.854, $p<0.0001$) and richness
250 ($F=9.591$, $p<0.001$), but not for Shannon-diversity ($F=2.342$, $p=0.128$). Looking at the pairwise
251 comparison, a significantly higher density than the open water site was found for *Ludwigia* spp.
252 (pairwise t-test: $p=0.0036$) and *P. stratiotes* ($p<0.0001$). Further, *P. stratiotes* showed a significantly higher
253 density than *E. densa* ($p<0.0001$) and *Ludwigia* spp. ($p<0.0001$). Taxonomic richness revealed similar
254 patterns. *Ludwigia* spp.($p=0.0247$) and *P. stratiotes* ($p<0.001$) had a significantly higher taxonomic
255 richness than the open water section. In addition, *P. stratiotes* had a significantly higher taxonomic
256 richness than *E. densa* ($p=0.0369$). All the unmentioned pairwise comparisons did not show a
257 significant difference.
258
259 *The zooplankton assemblage in different plant species by night*
260 The same analysis as for the zooplankton assemblage during the day was also done for the samples
261 from the night (Figure 3). The zooplankton assemblage in the four sections was shown to be also
262 different at night (PERMANOVA: $F= 2.525$, $R2= 0.174$, $p<0.005$). Pairwise comparisons (statical
263 summary in Table S4) showed a significant difference in the assemblage in *P. stratiotes* to all other
264 sections (pairwise PERMANOVA p-value<0.001). In addition, we found a difference between the
265 assemblage found in *Ludwigia* spp. and open water ($p=0.021$). For the assemblage in *Ludwigia* spp. and

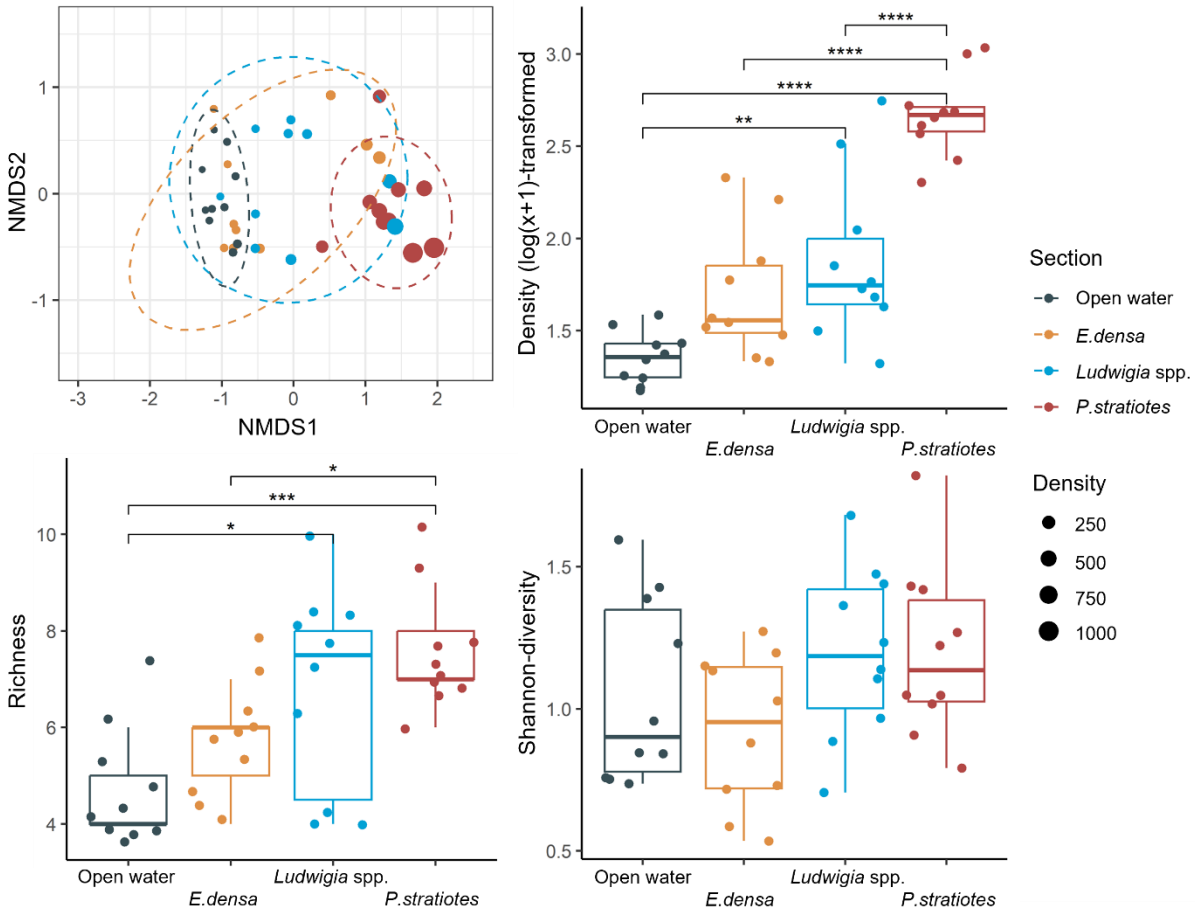

*Figure 2: Comparison of the zooplankton community in Open water (black), E. densa (orange), Ludwigia spp. (blue) and P. stratiotes (red) by day. Top left: NMDS showing the species assemblage of the four sections; Top right: Boxplot showing the Log+1 transformed density of zooplankton in the 4 sections; Bottom left: Boxplot showing the Richness of zooplankton in the 4 sections; Bottom right: Boxplot showing Shannon-diversity of zooplankton in the 4 sections. For the boxplots asterisk indicate the resulting p-values of pairwise t-tests: *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001; only significant (p<0.05) values are shown.*

266 *E. densa,* we did not find a significant difference during the night. In the night, we only found two taxa
267 associated with some of the sections. *Eurycercus* sp. was found to be an indicator species for *Ludwigia*
268 spp. and *P. stratiotes* (p<0.001), and *Simocephalus* sp. was found to be the indicator species for *Pistia* sp.
269 (p=0.002). As for the day, sampling density (repeated measure ANOVA: F: 11.142, p<0.0001) differs
270 between sections. For Richness and Shannon-diversity, we did not find a significant difference
271 between the four sections. Pairwise comparisons revealed significantly higher density in *P. stratiotes*
272 than all other sections (open water: p<0.001, *E. densa*: p<0.001, and *Ludwigia* spp. p=0.007). The other

pairwise combination showed no significant differences.

Comparing the zooplankton assemblage by night with the assemblage by day, we found differences (Figure S2; PERMANOVA: $F=3.3188$, $R2=0.23495$, $p<0.005$). A pairwise comparison (statistical summary see Table S5) of the assemblage by day and night per section revealed significant differences for *Ludwigia* spp. ($p=0.002$), *E. densa* ($p=0.022$) and open water ($p=0.016$) samples. Only the zooplankton assemblage found in *P. stratiotes* did not differ in the day and night ($p=0.236$). Indicator taxa analysis showed that *Eurycerus* sp. was associated with *P. stratiotes* by day and night, but also with *Ludwigia* spp. by night ($p<0.001$). *Ceriodaphnia* sp. was associated with *P. stratiotes* during the day but with all four sections at night ($p<0.001$). In all sections by night and only in *Ludwigia* spp. and *P. stratiotes* by day, we also found Cyclopoids ($p<0.001$) as an indicator species.

283

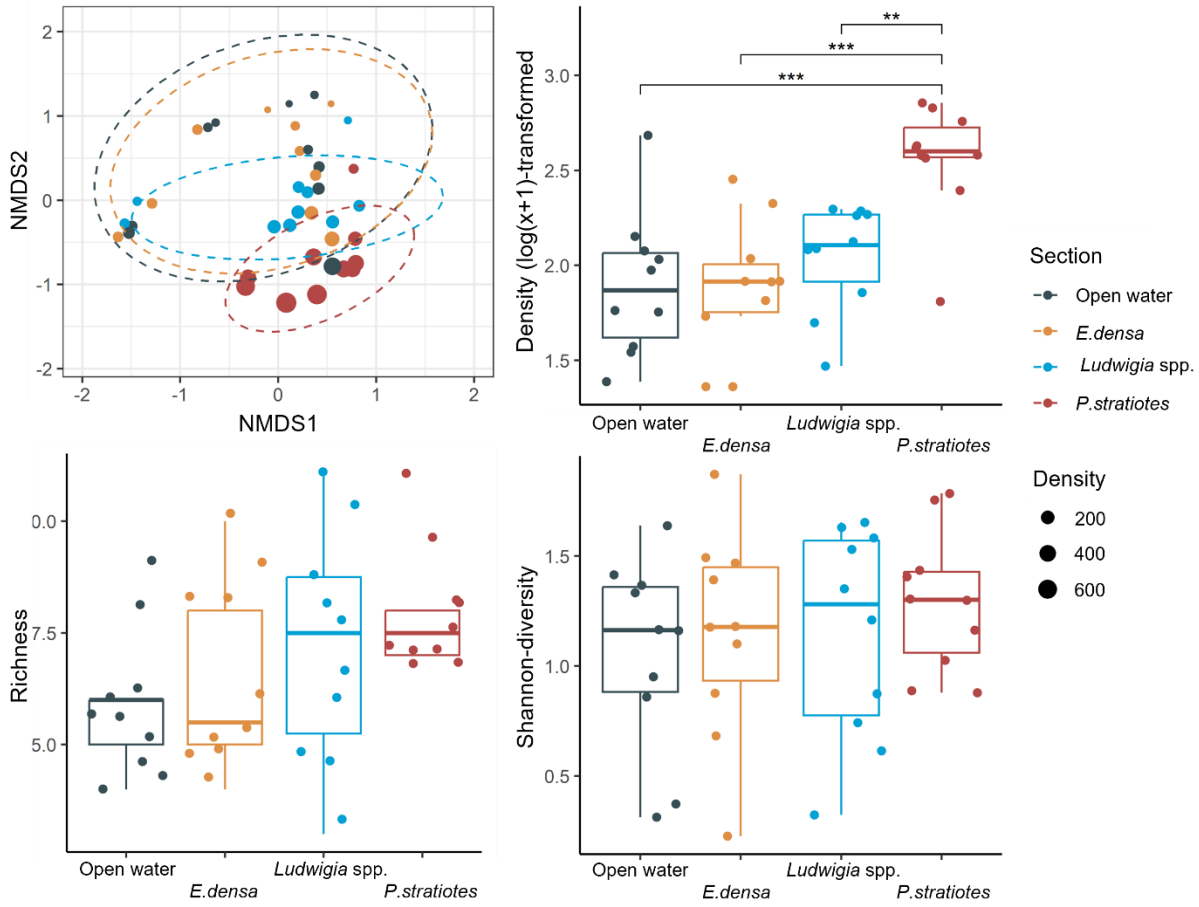

*Figure 3: Comparison of the zooplankton community in Open water (black), E. densa (orange), Ludwigia spp. (blue) and P. stratiotes (red) by night. Top left: NMDS showing the species assemblage of the four sections; Top right: Boxplot showing the Log+1 transformed density of zooplankton in the 4 sections; Bottom left: Boxplot showing the Richness of zooplankton in the 4 sections; Bottom right: Boxplot showing Shannon-diversity of zooplankton in the 4 sections. For the boxplots asterisk indicate the resulting p-values of pairwise t-tests: *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001; only significant (p<0.05) values are shown.*

284 *Habitat preference by species*

285 Ranking the habitat preference for each taxon revealed similar patterns for many taxa with some
286 exceptions (Table 1). Most taxa were most abundant in *P. stratiotes* and least abundant in open water.
287 *Eudiaptomus* sp., *Daphnia* sp., and *Scapholoberis* sp. showed the opposite pattern. *E. densa* and *Ludwigia*
288 spp. were, for most taxa, ranked second or third priority, but a clear preference for one of them cannot
289 be seen comparing day sampling with night sampling. For most taxa, *P. stratiotes* was the preferred
290 habitat. *E. densa* became the least preferred habitat for many taxa, while open water ranked second or
291 third. For *Eudiaptomus* sp. and Macrothricidae, open water was the preferred habitat during the night,
292 whereas no Macrothricidae was found in the open water during the day. *Acroperus sp*., which was not
293 present during the day, appeared at night within *P. stratiotes* and *Ludwigia* spp. Looking at the preferred
294 habitat in the with fish, we saw the effect on *Eudiaptomus* sp., which also preferred *P. stratiotes* instead
295 of open water with the presence of fish. *Ludwigia* spp. ranked second for most taxa and *E. densa* third.
296 At night, open water was the least preferred habitat for most species, while *P. stratiotes* became less
297 preferred for Copepoda Naupplii, *Eudiaptomus* sp., *Daphnia* sp., and *Scapholoberis* sp.
298
299 *The effect of fish presence*
300 The data from the three weeks of the experiment after the addition of fish were analyzed (statistical
301 information see Table S6). Overall density plus the density for every taxon was modeled depending
302 on fish presence, plant, time (number of days after the start of the experiment), and all possible
303 interactions. The models showed no direct effect of fish on the overall density or for single taxa. A
304 significant interaction between fish and time was found for *Eudiaptomus* sp. For all the other taxa and
305 the overall density, we found only a significant effect of plants or no significant effect at all.
306 As for the day samples, we ran the same model using the data from the night sampling (statistical
307 information in Table S6). By night Cyclopoides and *Chydorus* sp. showed a significant Plant:Date

interaction. For all the other taxa and the overall density, we found only a significant effect of plants, time, or no significant effect at all. Comparing the habitat preference with fish predation and without fish revealed that the proportion of most taxa declined in open water under fish predation (Table 1). At the same time, the proportion within *P. stratiotes* declined, leading to more equal distribution among the three plant types.

Table 7: Habitat preference for different taxa by day and night with the presence and absence of fish. Dark green: most preferred habitat, light green: second most preferred habitat, dark red: least preferred habitat, light red: second least preferred habitat, white: no individuals found. "%" indicate the proportion of all individuals per section, while 25% is expected if no preference.

	Daytime	**No Fish**				**Fish**			
Taxa		Open water	*E. densa*	*Ludwigia* spp.	*P. stratiotes*	Open water	*E. densa*	*Ludwigia* spp.	*P. stratiotes*
Copepoda Nauplii	Day	13%	22%	21%	44%	16%	23%	30%	30%
	Night	18%	8%	33%	41%	15%	33%	26%	26%
Eudiaptomus sp.	Day	34%	20%	32%	14%	15%	14%	35%	36%
	Night	40%	18%	31%	11%	25%	28%	30%	17%
Cyclopoides	Day	2%	7%	17%	73%	2%	8%	31%	69%
	Night	14%	6%	27%	53%	3%	21%	37%	39%
Chydorus sp.	Day	7%	9%	20%	64%	7%	16%	39%	38%
	Night	15%	6%	14%	65%	9%	22%	35%	34%
Acroperus sp.	Day	-	-	-	-	-	-	-	-
	Night	-	-	33%	67%	-	14%	36%	50%
Alona sp.	Day	6%	19%	16%	59%	1%	18%	40%	41%
	Night	26%	12%	12%	50%	15%	46%	5%	34%
Eurycercus sp.	Day	3%	3%	18%	76%	-	17%	8%	75%
	Night	4%	3%	10%	83%	4%	19%	40%	37%
Daphnia sp.	Day	11%	82%	7%	-	-	100%	-	-
	Night	33%	61%	5%	1%	65%	15%	15%	5%
Ceriodaphnia sp.	Day	5%	13%	13%	69%	<1%	1%	24%	75%
	Night	15%	7%	28%	50%	10%	8%	31%	51%
Simocephalus sp.	Day	-	20%	14%	66%	-	8%	28%	64%
	Night	-	6%	31%	63%	6%	16%	32%	46%
Scapholoberis sp.	Day	35%	33%	19%	13%	22%	12%	50%	16%
	Night	8%	42%	44%	6%	29%	22%	43%	6%
Macrothricidae	Day	-	-	7%	93%	-	-	-	100%
	Night	66%	1%	3%	30%	25%	8%	-	67%

Discussion

Correlation between macrophyte biomass and zooplankton

We did not find a significant positive correlation between macrophyte biomass and zooplankton density or diversity as was found in former studies with native macrophytes (Basu, Kalff & Pinel-Alloul, 2000; Kuczyńska-Kippen & Joniak, 2016). In our study, the used plants belonged to different biological types, and therefore, the parts of the plant that contributed most to the biomass in the water column differed. While *E. densa* grew submerged and produced a lot of leaves and stems underwater, the emergent *Ludwigia* spp. developed mainly defoliated stems underwater, and the floating *P. stratiotes* produced a dense network of roots underwater (Fig. 1). These different growth strategies can explain why we did not find a correlation between macrophyte biomass and zooplankton density. However, we also did not find any correlation between macrophyte biomass and zooplankton density when we split the analysis for the three species. The differences in plant biomass between mesocosms were probably insufficient to create a gradient and induce a correlation. The correlation with biomass could also be affected by the fact that all three species in our study are able to produce allelochemicals as defense mechanisms against algae growth (Wu *et al.*, 2013; Espinosa-Rodríguez, Sarma & Nandini, 2017; Thiébaut, Thouvenot & Rodríguez-Pérez, 2018). This trait, commonly found in many invasive plants, helps repress native competitors (Callaway & Ridenour, 2004; Kim & Lee, 2011) and also reduces phytoplankton biomass. However, zooplankton would not have migrated into the macrophytes, if the allelochemicals effect had been too strong. Phytoplankton and periphyton may also have been limited by the nutrients, whose concentrations remained low in mesocosms due to uptake and tissue storage by macrophytes. Limited resource for herbivorous zooplankton would have affected the community of all the mesocosm and limited its population.

342 *Habitat preference of zooplankton*

343 Our results showed that the zooplankton community in invasive macrophytes was more diverse and
344 abundant than in open water. Small patches of macrophytes, as used in our study (<0.5m^2 per plant),
345 offered already enough structure to change the zooplankton assemblage. Even if the size of the
346 mesocosms was too small to allow the establishment of horizontal physical (temperature) and chemical
347 (oxygen, nutrients) gradients, the spatial functional heterogeneity of zooplankton could be observed
348 at a scale of less than one meter. Simultaneous sampling of all four habitats was crucial in the protocol
349 to prevent zooplankton from escaping to adjacent sections. Such patchiness at a fine scale is not new
350 in natural habitats and mainly driven by biotic processes (Pinel-Alloul 1998). Earlier work has shown
351 that macrophyte bed size is an important factor defining the zooplankton community and that
352 especially the edge areas of macrophytes are used as a refuge, leading to higher zooplankton densities
353 in smaller beds compared to bigger macrophyte beds (Lauridsen *et al.*, 1996). Bigger mats of
354 macrophytes can be expected to have stronger impacts on the environment than the small patches of
355 plants used in our study.
356 Our first hypothesis of increased density and diversity in invasive macrophytes compared to the open
357 water and increasing values with higher plant complexity is confirmed. The zooplankton community
358 found around the floating plant *P. stratiotes* was the most diverse compared to the other three sections.
359 The overall abundance associated with *P. stratiotes* was also the highest. Roots from *P. stratiotes* offered
360 more complex habitats than leaves and stems provided by the two other plant species, which might
361 explain the higher diversity and abundance detected (Padial, Thomaz & Agostinho, 2009; Huang *et al.*,
362 2018). However, our results only partially align with the findings of former studies. Brendonck et al.
363 (2003) found that the presence of *Pontederia crassipes* (former *Eichhornia crassipes;* a free-floating
364 macrophyte) in a subtropical impoundment, increased zooplankton abundance compared to open
365 water as in our study, but that the diversity decreased under *P. crassipes* mats. Meanwhile, Choi *et al.*

366 (2014) found the highest zooplankton density within submerged macrophytes. Taxa found being
367 associated with *P. stratiotes* in our study (*Simocephalus* sp., *Ceriodaphnia* sp., *Eurycercus* sp., and *Alona* sp.)
368 were found to be more associated with submerged macrophytes, as shown in earlier studies (Lauridsen
369 *et al.*, 1996; Meerhoff *et al.*, 2007; Choi *et al.*, 2014). The taxa *Alona* sp. and *Eurycercus* are benthic taxa
370 and generally more common at the interface between water and sediment than in the water column
371 (Balcer, Korda & Dodson, 1984). The two taxa *Chydorus* sp. and Cyclopoides were associated with all
372 three plant species. Plant association of *Chydorus* sp. was already shown in other studies (Geraldes &
373 Boavida, 2004; Meerhoff *et al.*, 2007). This is in accordance with their periphyton scraper diet (Barnett,
374 Finlay & Beisner, 2007), even if they can also feed on small algae and detritus (Balcer *et al.*, 1984).
375 Cyclopoides predate on small zooplankton species such as *Chydorus* sp., similar distribution as their
376 prey can therefore be expected and was shown in other studies (Geraldes & Boavida, 2004). Other
377 studies showed no plant association with Cyclopoides (Meerhoff *et al.*, 2007). The effects of
378 macrophytes on zooplankton seem to be taxa-specific, depending on their diet, preferred habitat and
379 migratory capabilities. While some taxa (*Simocephalus* sp., *Ceriodaphnia* sp., *Eurycercus* sp., and *Alona* sp.)
380 preferred here the densest habitat in the roots of *P. stratiotes*, other taxa preferred the presence of plants
381 in general (*Chydorus* sp., Cyclopoides) or no habitat association (the Calanoide *Eudiaptomus gracilis*,
382 *Acroperus* sp., *Daphnia* sp., *Scapholoberis* sp., Macrothricidiae). In real-life situations, invasive
383 macrophytes often replace more diverse native vegetation (Stiers *et al.*, 2011; Gallardo *et al.*, 2016). In
384 such situations, the provided structure by the invasive species might not be high enough to replace
385 the structure of the native vegetation leading to a decreased abundance or diversity after invasion by
386 the invasive macrophyte (Gallardo *et al.*, 2016).
387
388
389

390 *The effect of fish*

391 The second hypothesis of stronger differences among sections after adding fish as predators could
392 only be partially confirmed. The density of fish used in this study was low, so a significantly decreasing
393 zooplankton abundance could be avoided over time. Fish odor, chemicals indicating its presence, has
394 been shown to change the behavior of zooplankton and even trigger morphological adaption
395 (Lauridsen & Lodge, 1996; Gilbert, 2013). However, we found only weak effects of fish predation in
396 our experiment. During the day only the Calanoid *Eudiaptomus gracilis* showed a significant interaction
397 with fish over the time of the experiment. In the night, only Cyclopoides and *Chydorus* sp. showed an
398 impact of fish presence over time. These latter taxa avoided open water at night in the presence of
399 fish. We expected to find an increase in differences between sections with the addition of macrophytes.
400 However, already without the presence of fish, we found substantial differences between the different
401 sections, either caused by the provision of habitat and food by the macrophytes or by already present
402 predator pressure caused by macroinvertebrate predators, such as Zygoptera larvae, Notonectidae or
403 Corixidae, which were indeed present in the mesocosms and have been shown to impact zooplankton
404 (Sagrario et al., 2009; Deosti et al., 2021). We showed here that fish odor was not a prerequisite here
405 for DHM. Further study should include the analysis of the other predators, such as macro-
406 invertebrates and frogs larvae.

407

408 *Differences between night and day sampling*

409 The zooplankton community captured during the night sampling differed from the one from the day
410 sampling. While the zooplankton density was still highest for *P. stratiotes*, the other three sections did
411 not show any differences. The high variation in richness found during the day was not found in the
412 night samples. Considering the zooplankton assemblages at night, we found that the assemblage in *P.*
413 *stratiotes* differs from the other sections, and a difference between *Ludwigia* spp. and open water was

414 found. *Simocephalus* sp. was, as during the day, an indicator species for *P. stratiotes*. *Eurycerus* sp. was
415 now found to be associated with *Pistia* and *Ludwigia spp.*. *Ceriodaphnia*, *Alona*, *Chydorus,* and *Cyclopoides*
416 were no longer associated with any section. We found a higher diversity and abundance at night than
417 during day in open water. These findings confirm the DHM of individuals seeking refuge in the plants
418 during daylight and moving freely during the night hours. Higher density in *P. stratiotes* might be
419 explained by the dense network of roots, hindering zooplankton from migrating quickly, which also
420 explains that *Simocephalus* sp., one of the bigger taxa, was still associated with *P. stratiotes*. *Simocephalus*
421 taxa are reported between vegetation, either submerged or with floating leaves (Bledzki & Rybak,
422 2016). Meerhof et al. (2007) found no sign of migration for *Simocephalus* sp. in temperate lakes with
423 low turbidity. In the same study, *Ceriodaphnia* sp. showed no sign of migration which in our study was
424 found to do DHM. Physical and chemical parameters inside macrophytes beds, such as low oxygen
425 and high pH, may influence DHM, while the small mesocosm size limits here the horizontal gradient
426 (Burks *et al.*, 2002).
427

428 ## Conclusion

429 Invasive macrophytes can increase structural complexity in the water column, increasing zooplankton
430 abundance and richness. All three types of macrophytes showed a positive impact in our study, but
431 the effects will depend on the relative scales of the beds and the water body, and the maintenance of
432 habitats heterogeneity. The extent of their impact might change according to the specific architecture
433 of the chosen species with stronger impact of more complex species. In our study, we compared
434 invasive macrophytes with open water. Future research is needed to better understand the impact of
435 invasive macrophytes on zooplankton and inform stakeholders as a basis for developing well-
436 informed and balanced management strategies.
437

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Data availability statement

The data is available upon request.

Declaration of conflicts of interest

All involved authors have no conflicts of interest to declare.

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665 **Supplementary Information:**

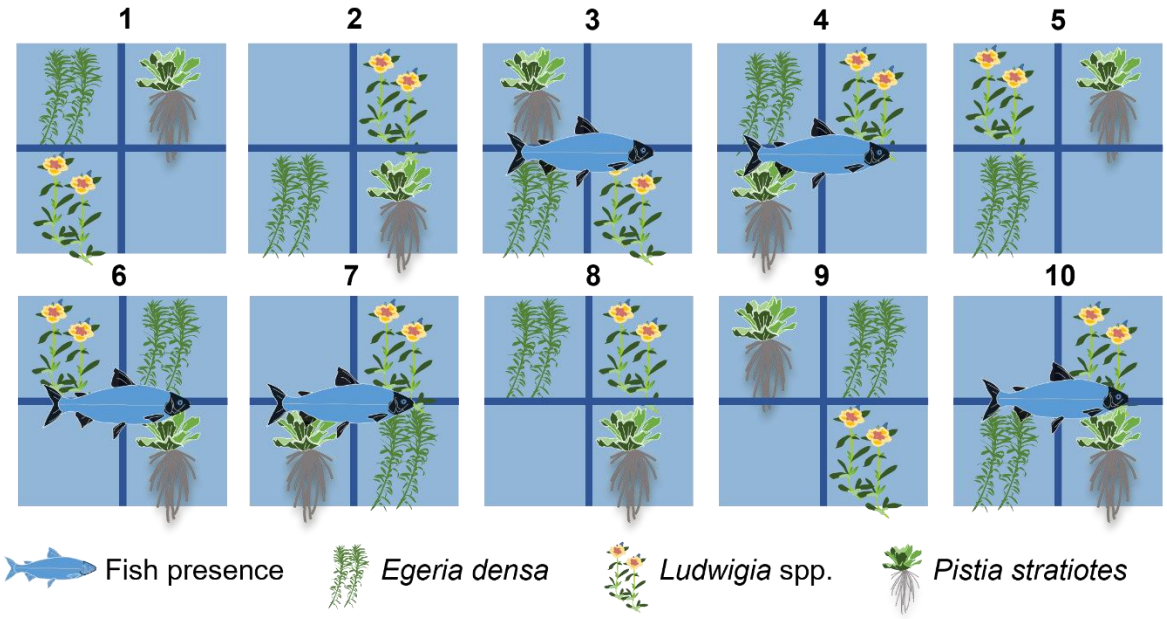

Figure S1: Arrangement of the mesocosm from Mesocosm 1 (top left) to Mesocosm 10 (bottom right). The location of each plant and the presence of fish are indicated by the corresponding icon.

666

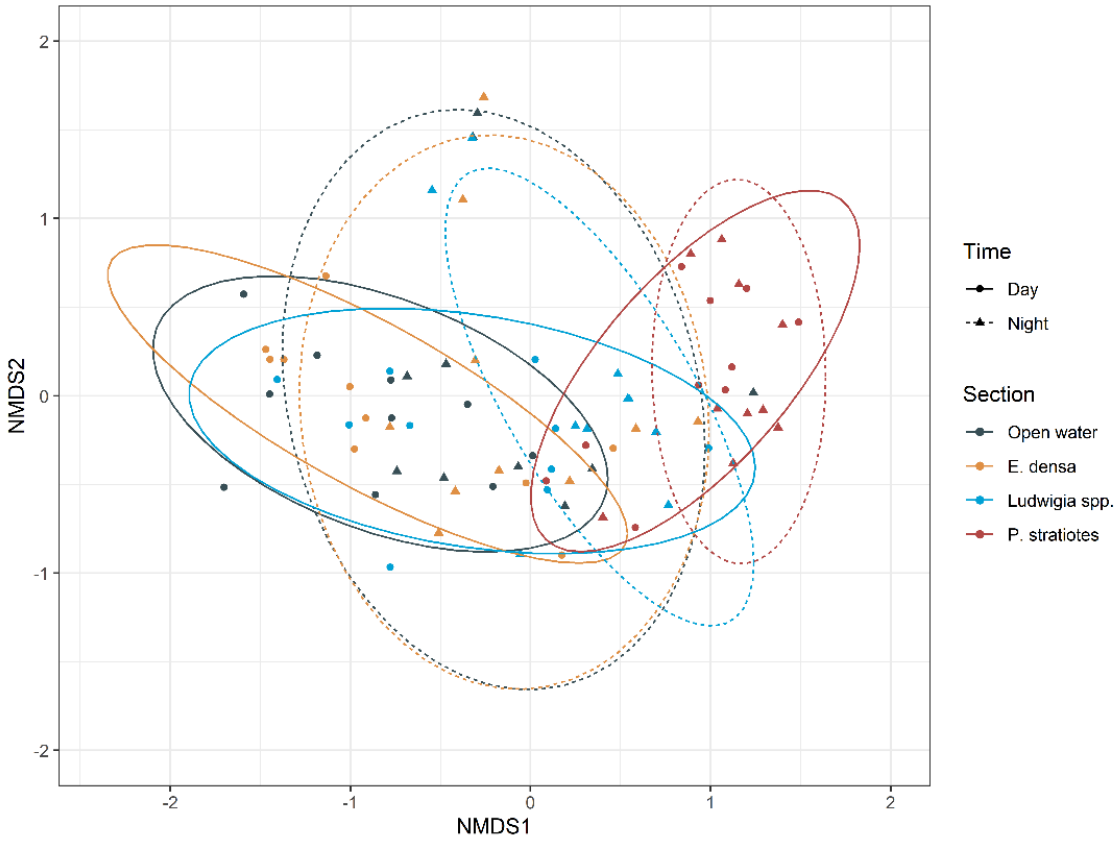

Figure S2: NMDS-showing the zooplankton community composition of Open water (black), E. densa (orange), Ludwigia spp. (blue) and P. stratiotes (red) by day (circles, solid lines) and night (triangles, dashed lines).

667

Table S1: Biomass of macrophytes per mesocosm: Stem and leaves are measured for Ludwigia spp. and Egeria densa. Roots are measured for Pistia stratiotes.

Mesocosm	*Ludwigia* spp. (g dry biomass)	*Egeria densa* (g dry biomass)	*Pistia stratiotes* (g dry biomass)
1	30.80	19.09	20.87
2	24.19	27.09	30.70
3	19.61	23.98	57.98
4	20.22	21.57	11.89
5	20.63	17.08	10.77
6	12.90	7.86	12.33
7	24.34	13.08	14.38
8	14.24	10.04	11.29
9	15.67	18.66	21.89
10	14.61	21.27	10.36

(Table S2 can be found on the next page due to page formatting)

Table S1: Statistical information for pairwise comparison corresponding to Figure 2 (top left). Significant p-values are highlighted in bolt.

Sections	Degree of freedom	Sum of squares	R2	F-value	p-value
***E. densa* vs. Open water**	1	0.4251	0.12704	2.6196	**0.026**
***E. densa* vs. *Ludwigia* spp.**	1	0.1425	0.03275	0.6095	0.713
E. densa* vs. *P. stratiotes	1	1.7958	0.35282	9.813	**0.002**
Open water vs. *Ludwigia* spp.	1	0.8748	0.23891	5.6504	**0.006**
Open water vs. *P. stratiotes*	1	3.3142	0.63916	31.884	**0.002**
Ludwigia* spp. vs. *P. stratiotes	1	1.393	0.30596	7.9352	**0.004**

682 *Table S2: Mesocosm characteristics: Temperature are averaged logger measurements taken every 5 minutes. All other values are average values of two samples*
683 *per week during the sampling period.*

Mesocosm	Depth (cm) (SD)	Conductivity (µS/cm) (SD)	pH (SD)	Oxygen (mg/l) (SD)	Temperature (°C) (SD)	NO_3 (mg N/l)	NH_4 (mg N/l)	PO_4 (mg P/l)	Chl-a (µg/l) (SD)
1	33.2 (5.4)	245 (34.8)	9.15 (0.13)	8.12 (1.68)	18.16 (1.82)	<0.02	<0.05	<0.02	13.56 (10.47)
2	32.5 (4.8)	270.8 (35.4)	8.35 (0.21)	6.98 (1.43)	17.7 (1.92)	<0.02	<0.05	<0.02	15.32 (16.79)
3	35.5 (2)	234.5 (25.8)	8.47 (0.26)	8.09 (2.06)	18.07 (1.79)	<0.02	<0.05	<0.02	8.24 (5.68)
4	35.8 (4.2)	206.7 (21.9)	9.62 (0.16)	9.35 (1.33)	18.24 (1.77)	<0.02	<0.05	<0.02	10.25 (11.31)
5	32.2 (3.8)	206 (26.2)	9.29 (0.14)	8.27 (1.1)	17.99 (1.91)	<0.02	<0.05	<0.02	10.10 (13.02)
6	30.2 (2.7)	232.8 (32.3)	9.5 (0.13)	8.62 (0.99)	18.04 (1.91)	<0.02	<0.05	<0.02	8.88 (4.47)
7	38.2 (1.3)	236 (25)	9.61 (0.15)	9.23 (0.87)	18.33 (2.14)	<0.02	<0.05	<0.02	5.30 (2.87)
8	33.8 (4.7)	210.7 (30)	9.13 (0.23)	8.2 (0.92)	18.27 (1.94)	<0.02	<0.05	<0.02	13.90 (15.39)
9	36.2 (3.2)	240 (30)	8.26 (0.28)	6.24 (0.68)	18.33 (1.95)	<0.02	<0.05	<0.02	7.38 (3.07)
10	34.7 (4.4)	204 (28.7)	8.94 (0.31)	7.93 (1.31)	17.9 (1.8)	<0.02	<0.05	<0.02	5.98 (2.14)

684

Table S2: Statistical information for pairwise comparison corresponding to Figure 3 (top left). Significant p-values are highlighted in bolt.

Sections	Degree of freedom	Sum of squares	R2	F-value	p-value
***E. densa* vs. Open water**	1	0.0221	0.0042	0.076	0.983
***E. densa* vs. *Ludwigia* spp.**	1	0.2509	0.05114	0.9702	0.072
E. densa* vs. *P. stratiotes	1	1.1986	0.22343	5.1787	**0.001**
Open water vs. *Ludwigia* spp.	1	0.3381	0.0654	1.2596	**0.021**
Open water vs. *P. stratiotes*	1	1.2510	0.22363	5.1847	**0.002**
Ludwigia* spp. vs. *P. stratiotes	1	0.7266	0.16206	3.4812	**0.001**

Table S3: Statistical information for pairwise comparison corresponding to Figure S2. Significant p-values are highlighted in bolt.

Section	Degree of freedom	Sum of squares	R2	F-value	p-value
Open Water	1	0.5449	0.10197	2.0439	**0.016**
E. densa	1	0.5007	0.09462	1.8812	**0.022**
***Ludwigia* spp.**	1	0.5050	0.10592	2.1323	**0.002**
P. stratiotes	1	0.1380	0.03701	0.6918	0.263

Table S6: Model output, p-values. Significant values (p<0.05) are marked in bolt. Fish related parameters are shadowed.

		(Intercept)	Fish	Plant	Date	Fish:Plant	Fish:Date	Plant:Date	Fish:Plant: Date
Overall Density	Day	**0.010**	0.936	**0.030**	0.771	0.931	0.386	0.226	0.601
	Night	**<0.001**	0.272	0.086	0.395	0.850	0.079	0.152	0.465
Copepoda Nauplii	Day	0.362	0.658	0.570	0.707	0.552	0.727	0.578	0.843
	Night	0.186	0.773	0.942	0.441	0.909	0.326	0.537	0.534
Eudiaptomus sp.	Day	0.092	0.237	0.627	0.500	0.390	**0.045**	0.255	0.311
	Night	**<0.001**	0.442	0.188	0.347	0.630	0.207	0.182	0.524
Cyclopoides	Day	0.308	0.388	**0.016**	0.937	0.925	0.444	0.523	0.935
	Night	**<0.001**	0.371	**0.042**	**0.028**	0.618	**0.046**	0.066	0.192
Chydorus sp.	Day	0.462	0.211	0.010	0.567	0.914	0.801	0.943	0.926
	Night	**<0.001**	0.192	0.069	0.094	0.492	**0.024**	**0.041**	0.156
Acroperus sp.	Day	0.710	0.774	0.522	0.758	0.381	0.812	0.122	0.125
	Night	0.059	0.173	0.438	0.121	0.695	0.194	0.574	0.857
Alona sp.	Day	0.719	0.979	0.750	0.880	0.946	0.909	0.132	0.214
	Night	0.256	0.139	0.232	0.899	0.364	0.227	0.465	0.376
Eurycercus sp.	Day	0.970	0.701	0.202	0.661	0.384	0.642	0.066	0.919
	Night	0.057	0.241	0.034	0.302	0.293	0.167	0.074	0.360
Daphnia sp.	Day	0.925	0.677	0.073	0.177	0.239	0.339	0.098	0.370
	Night	0.642	0.841	0.538	0.312	0.705	0.649	0.549	0.547
Ceriodaphnia sp.	Day	0.758	0.588	**0.049**	0.880	0.814	0.492	0.907	0.843
	Night	0.034	0.775	0.470	0.323	0.903	0.971	0.060	0.996
Simocephalus sp.	Day	0.765	0.941	**0.004**	0.860	0.953	0.901	0.934	0.853
	Night	0.287	0.839	0.069	0.515	0.276	0.725	0.797	0.616
Scapholoberis sp.	Day	0.193	0.516	0.216	0.639	0.379	0.531	0.361	0.502
	Night	0.822	0.926	0.734	0.391	0.991	0.480	0.453	0.889
Macrothricidae	Day	1.000	1.000	0.683	1.000	0.823	1.000	0.771	0.986
	Night	0.728	0.752	0.490	0.840	0.815	0.797	0.456	0.724

Chapter 3. Impact of macrophyte removal on biodiversity

1. Introduction

In the first two chapters, the influence of dense mats of macrophytes on phytoplankton, zooplankton, and macroinvertebrates was shown. From a human perspective, dense mats of macrophytes are often considered problematic, and macrophytes are removed without knowing the consequences for the ecosystem.

This chapter aims to study the impact of mechanical macrophyte removal on phytoplankton, zooplankton, and macroinvertebrate communities. The chapter is split into two main parts; a published article summarizing the short-term effects (one and six weeks after removal) of macrophyte removal on aquatic biodiversity in rivers and lakes and a second part studying the long-term effects (one year after removal) on the communities and their resilience.

As macrophytes showed positive effects for zooplankton and macroinvertebrates, we can expect negative effects of plant removal for those two organism groups. On the opposite, the phytoplankton community was not strongly affected by the presence of macrophytes; therefore, the impact of removal can be expected to be low.

2. Methods

2.1. Study Sites

This chapter covers Lake Grand-Lieu (France), Lake Kemnade (Germany), and Hartbeespoort Dam (South Africa), already described in Chapter 1. These study sites were combined with two rivers; the Otra in Norway and the Spree in Germany (Figure 21). The sites vary in their characteristics (e.g., climate, trophic state, ecosystem type), but they share one common

characteristic: they are all invaded by dense mats of macrophytes. As part of the local management strategy, macrophytes were removed, and the phytoplankton, zooplankton, and macroinvertebrate community were monitored to assess the impact of macrophyte removal on biodiversity.

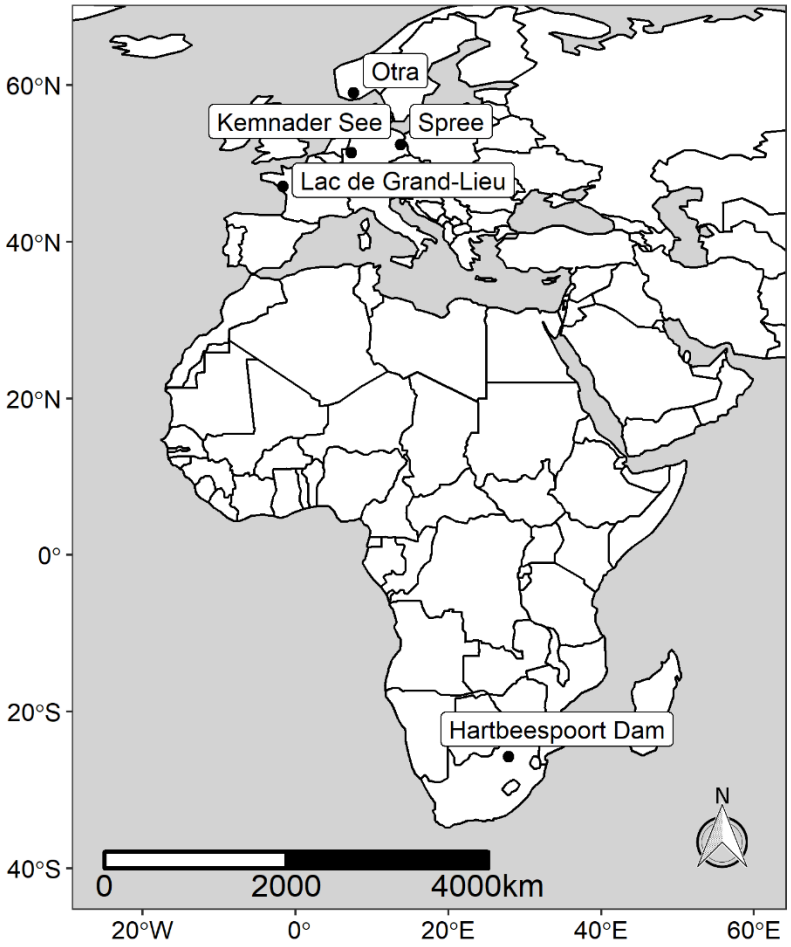

Figure 21: Map of the five study sites involved in Chapter 3.

2.1.1. Otra River

The Otra is a 245 km long river flowing through southern Norway with a watershed of around 4000 km^2 (Wright et al., 2017). Since 1964, the river has been intensely used for hydropower production. Our study site, the Rysstad basin (59°05'19.1"N 7°33'00.5"E), is located between two hydropower plants. Due to the hydropower plants, the water flow in this part of the river is relatively slow (Schneider & Demars, 2020). Water levels fluctuate with the output and input of the hydropower plants up- and downstream (Aasland, 2021). The study site has a high recreational value and offers several activities, such as boating, fishing, and swimming.

Figure 22: Pictures of the study site: Top left: Surface view on our study site; Bottom left: Underwater view on our study site; Right row: Mowing boat during the macrophyte removal.

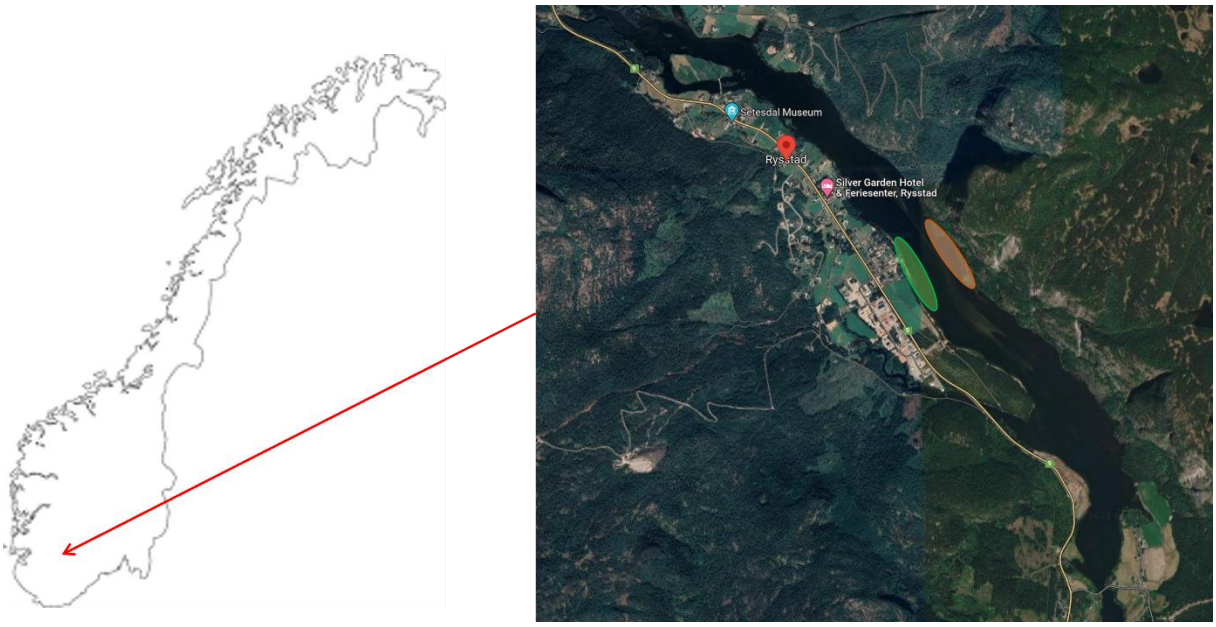

Figure 23: Map showing the location of study site in the Otra river within Norway (left) and the location of our study sites (green: impact site, orange: control site) within the river.

Juncus bulbosus L. is part of the Otra's natural vegetation, but increased growth after building the hydropower plants led to mass development. Nowadays, *J. bulbosus* covers a large area (~80%; Velle et al., 2019) and is viewed as a nuisance hindering recreational activities. Therefore, macrophytes are removed regularly, costing around 250 000 € per year (personal communication, local stakeholder). Due to plant regrowth, this needs to be repeated every two to three years. Macrophytes are removed over an area of more than 1000m^2. In our study, we defined two sections of 500 m by 60 m, the control site on the right shore and the impact site on the left shore of the river. Macrophytes were first shortened with a tool resembling an underwater saw and then removed from the sediment with a molding cutter. The removal occurred from the 15th to the 23rd of June 2020 and was organized by the "Krypsivprosjektet på Sørlandet", a consortium of hydroelectric power companies, Energy Norway, the Norwegian Water Resources and Energy Directorate (NVE), and representatives from Norwegian environmental authorities.

2.1.2. Spree River

The Spree river is a 400 km long river flowing through eastern Germany. The Spree is known as the main river of Berlin, where it flows through the city before flowing into the Havel river. Our study site lies before Berlin, where the river forms a slow-flowing river-lake network. This part of the river is also known as Lower Spree (52°23'35.3"N 13°55'44.2"E) and is intensively used for recreation and is an essential source of drinking water for Berlin.

Since the mid-1990s, native macrophyte vegetation has been gradually increasing. In our study site, the dominating species were the emergent *Sagittaria sagittifolia L.*, the submerged *Stuckenia pectinata* (L.) Börner, and the floating-leafed *Nuphar lutea* (L.) Sm.. In the past years, macrophyte biomass of 700-800 tons was estimated for a 30 km river section. Due to the development of the macrophytes, the water level has risen by 50 cm, causing problems for farmers and residents near the river.

Figure 24: Pictures of the study site showing different parts of the Spree with macrophytes and their removal.

Every year 250 – 600 tons of macrophyte biomass is removed by the "Wasser- und Landschaftspflegeverband Untere Spree" (https://wlv-untere-spree.de/), a local water management consortium under public law. The plants are cut and then removed with excavators to remove the plant material and reduce water levels. Since the mowing of the aquatic vegetation was conducted, the water quality in the downstream river sections and lakes decreased (personal communication, Wasser- und Landschaftspflegeverband Untere Spree).

Figure 25: 16Study site in the river Spree (right) and its location in Germany (left). Blue arrow indicates from direction, orange marking=control site, green marking=impact site.

2.2. Biodiversity Sampling

The sampling for this chapter followed a BACI design (Before-After-Control-Impact design). In each site, we defined a section where plants are removed and a nearby section without plant removal as a control site. Both sections were sampled before the plant removal, one week after the plant removal, and six weeks after the plant removal for the monitoring of the short-term effects. For the long-term effects, additional sampling one year after the removal was performed at Lake Grand-Lieu and Hartbeespoort Dam. At each sampling session, five phytoplankton samples, five zooplankton samples, five sweep samples for macroinvertebrates, and five grab samples for macroinvertebrates were taken, following the methods described in Chapter 1.

3. Article 3: Short-term effects of macrophyte removal on aquatic biodiversity in rivers and lakes

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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.

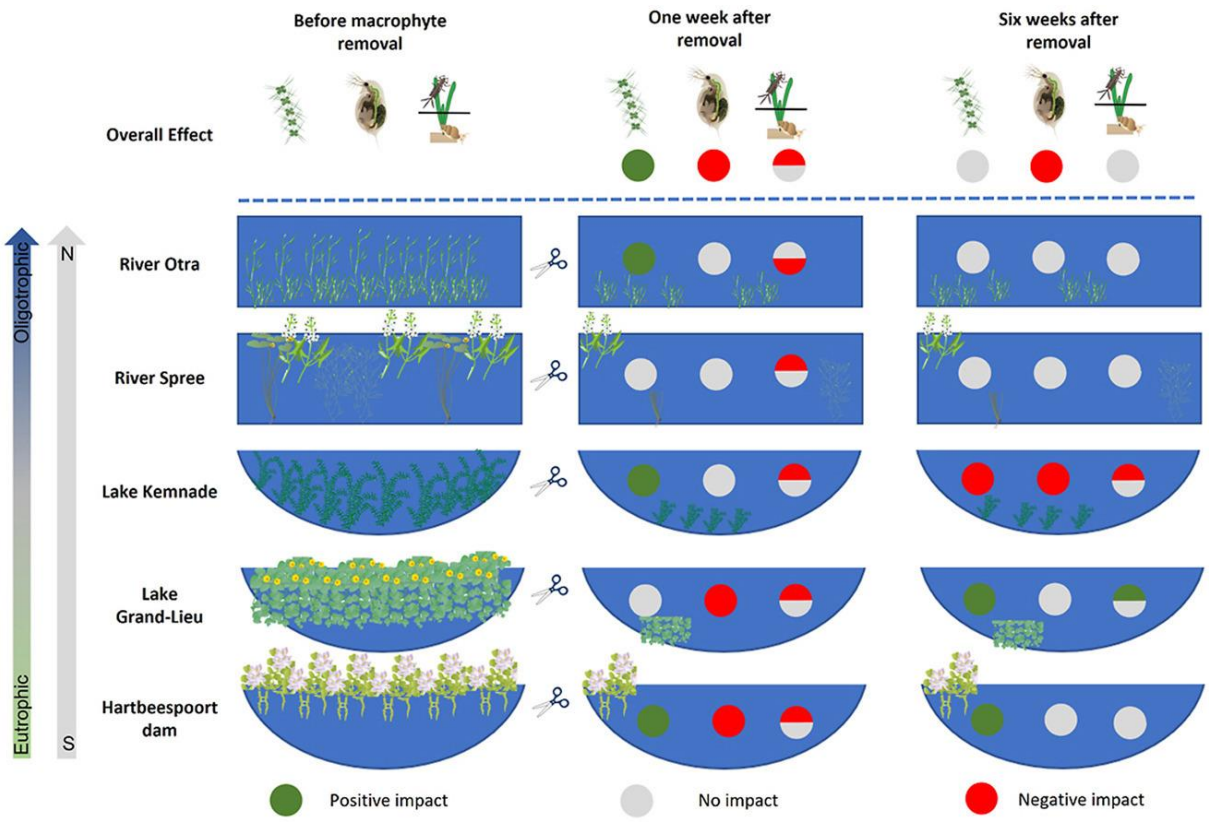
• Overall biodiversity was negatively impacted by plant removal.

• 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.

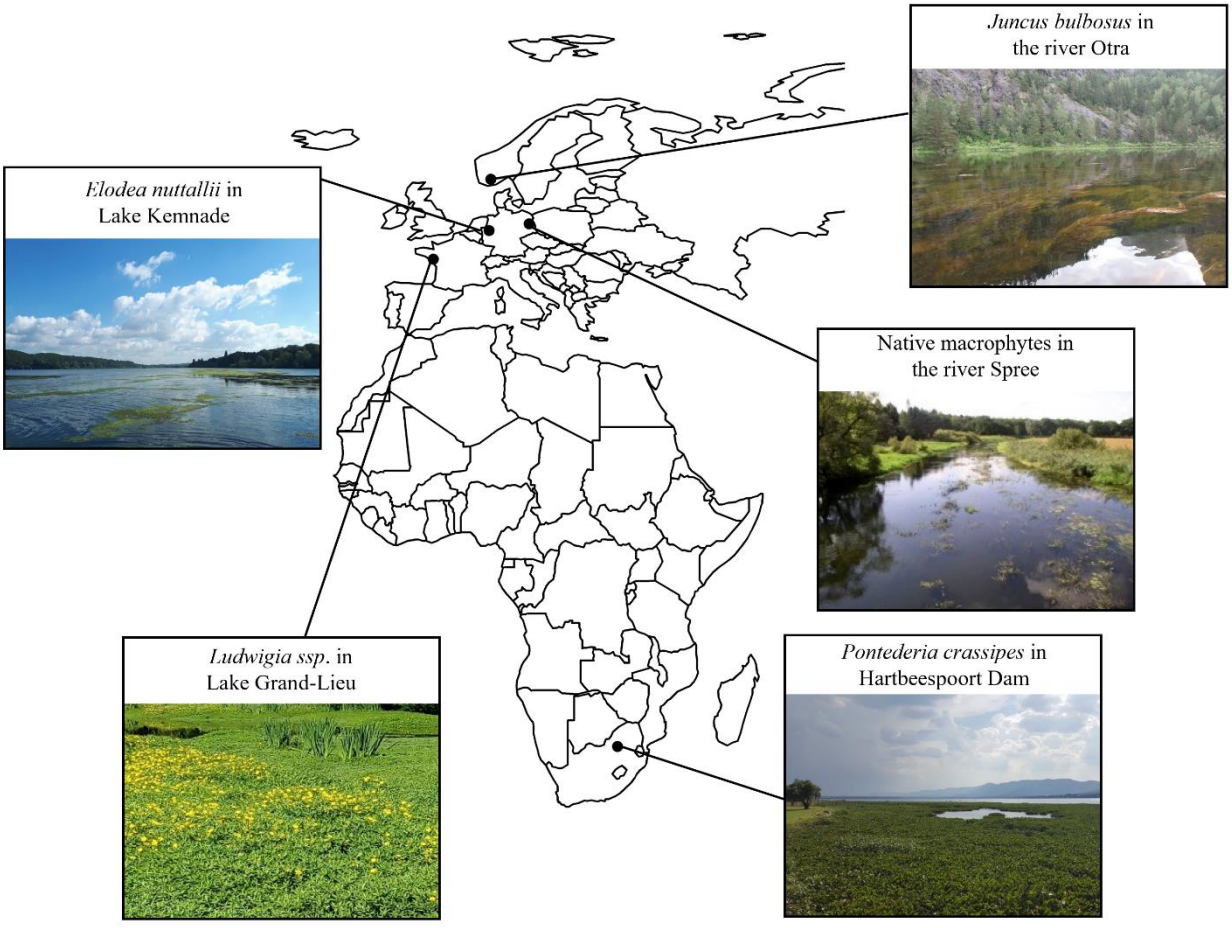


Figure 1: Location of the five study systems with mass macrophyte developments.

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).

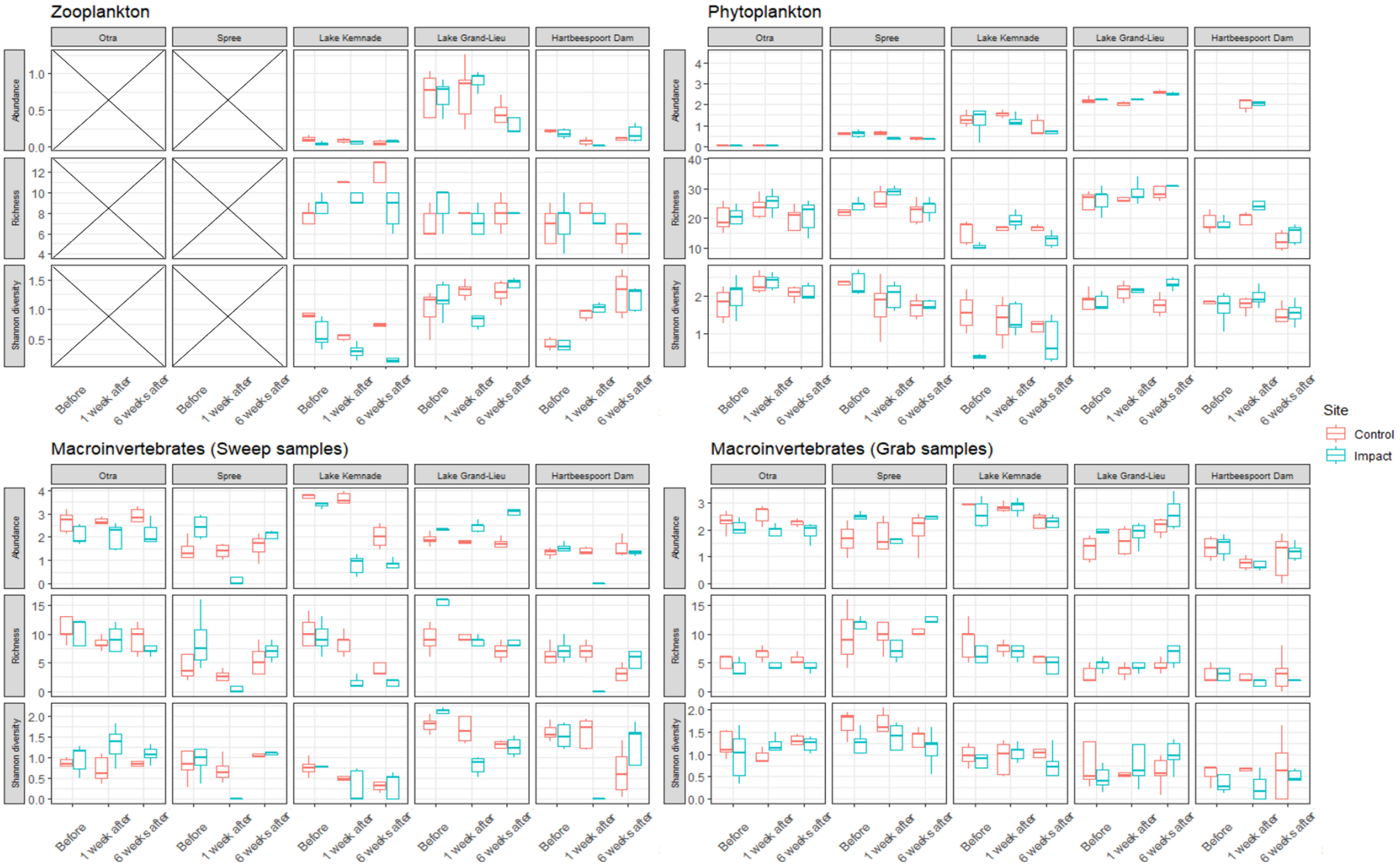


Figure 2: Abundance (log+1 transformed), species richness and Shannon diversity of zooplankton, phytoplankton, and macroinvertebrate assemblages from five sites before, one week after, and six weeks after macrophyte removal. Horizontal bold lines represent the median, boxes the 25% and 75% percentiles, and whiskers the minimum and maximum. n = 5 for each sampling time/ session.

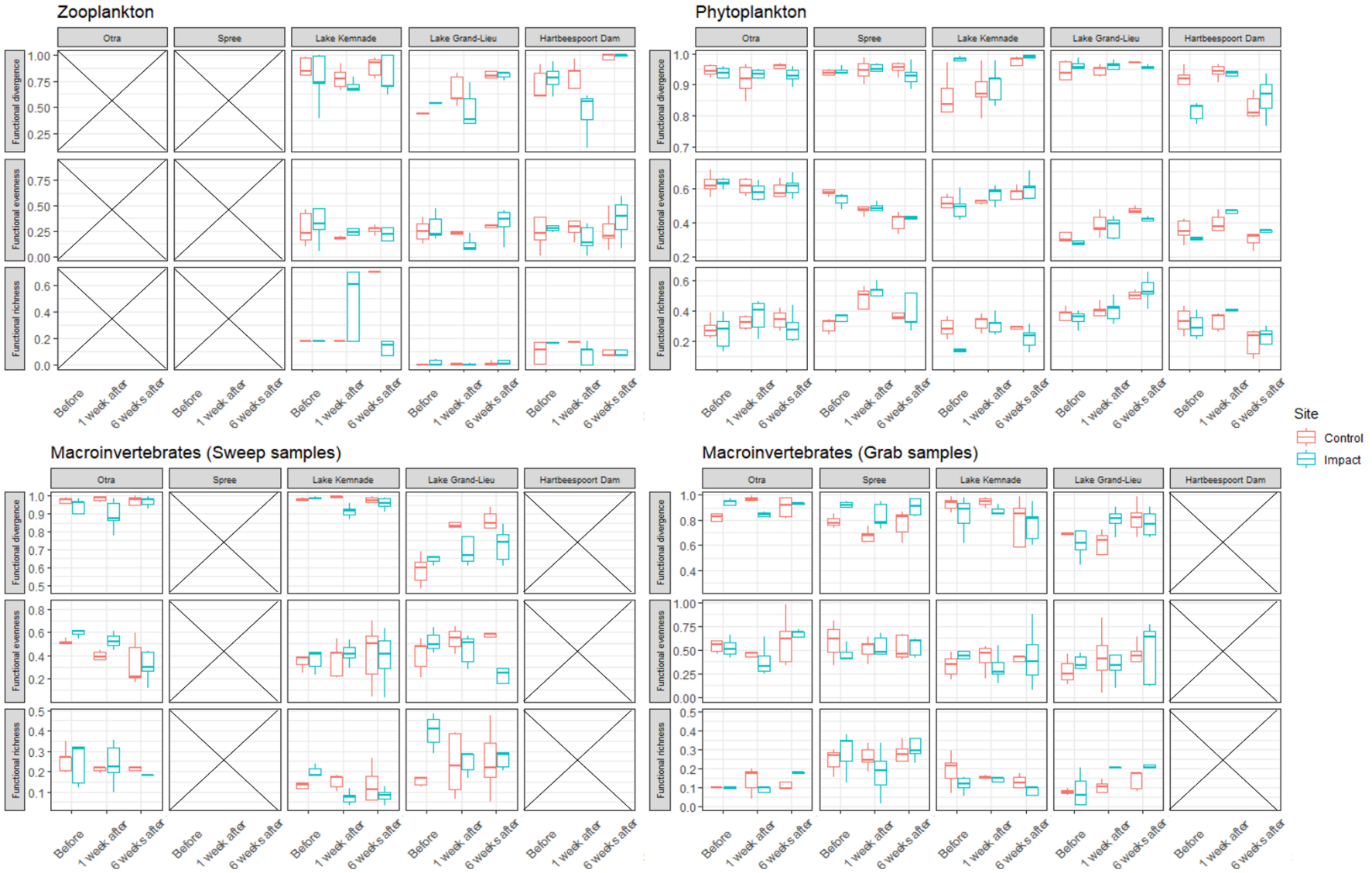


Figure 3: Functional divergence, functional evenness and functional of zooplankton, phytoplankton, and macroinvertebrate assemblages from five sites before, one week after, and six weeks after macrophyte removal. Horizontal bold lines represent the median, boxes the 25% and 75% percentiles, and whiskers 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 Sweep	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 Grab	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.

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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**		
	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**	**-2**	**-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**		
	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**	**5**	**1**
	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 Sweep	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**		
	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**	**-11**	**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 Grab	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**		
	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**	**-1**	**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**		

		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
	F- rgence	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 vise)	**-3**	**-1**	**0**	**0**	**-1**	**0**	**-1**	**-3**	**-5**	**2**	**-2**	**1**				

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

4. Long-term effects of macrophyte removal on aquatic biodiversity in rivers and lakes

4.1. Introduction

In Article 3, we showed that removing macrophytes has positive short-time effects on phytoplankton and adverse effects on zooplankton and macroinvertebrates (Misteli et al., 2023). We found a variation in the impact over time, with generally declining impacts from one week to six weeks after the removal. Following these studies, the question arises if plant removal will still impact biodiversity after a more extended period.

The long-term outcome of a disturbance, such as the removal of macrophytes, depends on the resilience capacity of the ecosystem. The term resilience has different interpretations; for this study, we define ecosystem resilience following the definition of Holling (1973; 2003) as: "Resilience is the potential of a particular configuration of a system to maintain its structure/function in the face of disturbance, and the ability of the system to re-organize following disturbance-driven change". In the case of our study, the question is if the ecosystem has a high enough resilience to maintain its structure and function despite the disturbance following macrophyte removal or if the removal will lead to a new stable ecosystem state after removal.

We compared the structure and composition of phytoplankton, zooplankton, and macroinvertebrate communities before and one year after plant removal following a BACI study design. We hypothesize that (i) the impact is further declining over time, and we will not see differences in density, richness, or Shannon-diversity after one year. Further, we hypothesize (ii) to find long-lasting differences in the community composition after plant removal.

4.2. Methods

For this study, we analyzed phytoplankton, zooplankton, and macroinvertebrate samples sampled in Hartbeespoort Dam (South Africa) and Lake Grand-Lieu (France). The study is an extension of Article 3 (Misteli et al., 2023). Samples used for Article 3 were combined with additional samples taken one year after the plant removal. The samples were taken in the same way described in Article 3 (Misteli et al., 2023).

All statistical analyses for this section were performed in R version 3.6.3 (R Core Team, 2021). Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity was used to compare the community composition. NMDSs were performed with the metaMDS function from the *vegan* package. This was combined with pairwise permutational multivariate ANOVA (PERMANOVA) computed with the pairwise.adonis2 function from the *pairwiseAdonis* package (Arbizu, 2017). The impact on density, taxa richness, and Shannon-diversity was analyzed the same way as in Article 3 (Misteli et al., 2023). Plots were generated using ggplot2 (Wickham, 2016) and ggpubr for boxplots (Kassambara, 2020).

4.3. Results

4.3.1. Phytoplankton

The phytoplankton communities identified after one year were significantly different from those found before the macrophyte removal in both Lake Grand-Lieu (Figure 26; pairwise PERMANOVA: p-value=0.008) and Hartbeespoort Dam (p=0.012). A comparable change was also found in the control site without macrophyte removal (Lake Grand-Lieu: p=0.009; Hartbeespoort Dam: p=0.009). In Lake Grand-Lieu, the impact and control site communities were significantly different (p=0.005); in Hartbeespoort Dam, no difference between the two sites was found after one year (p=0.313). The richness and Shannon-diversity showed no impact of plant

removal after one year. For density, no data was available for the one-year after samples (Figure 26).

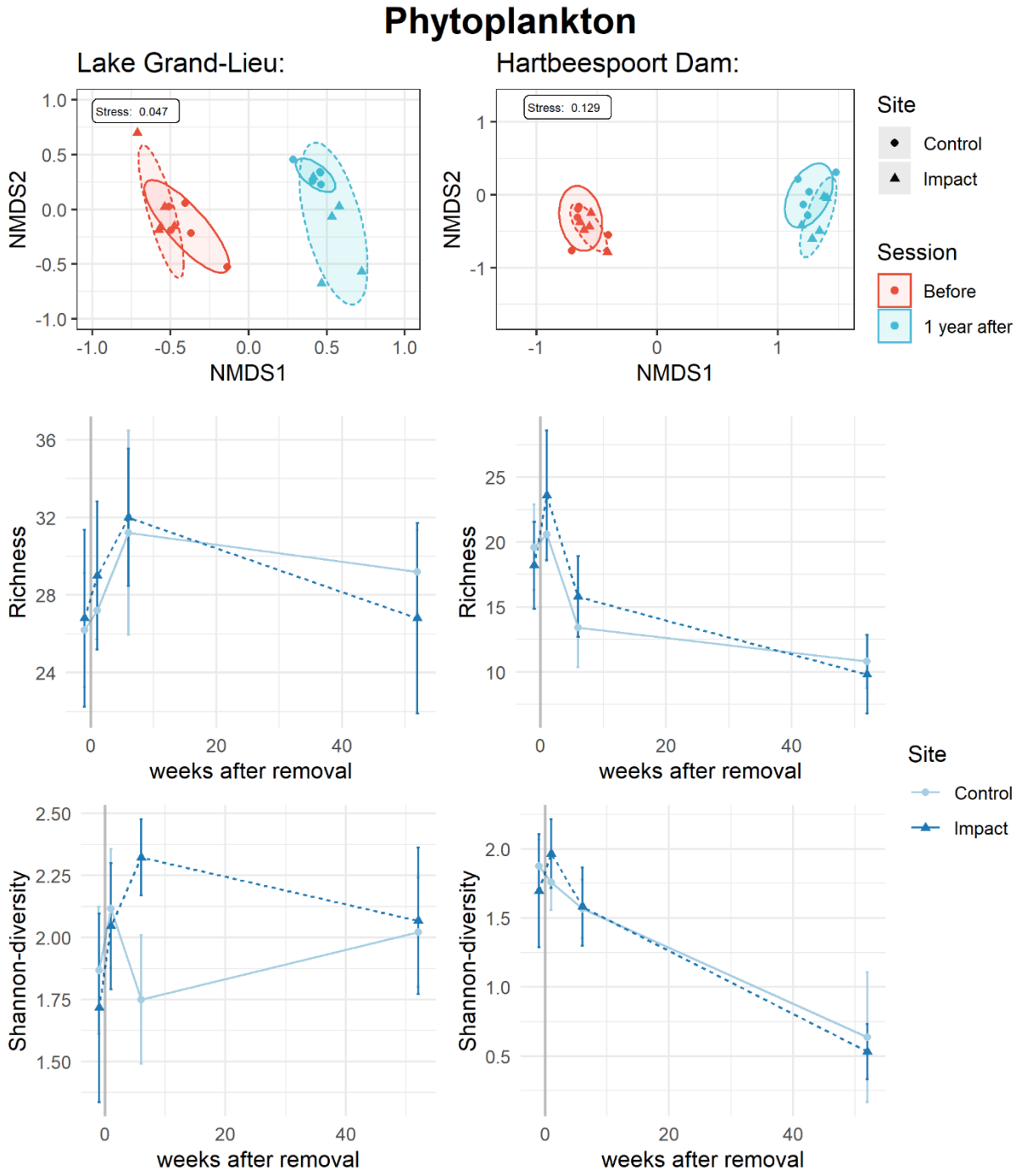

Figure 26: Comparison between the phytoplankton community in the impact site and control site before and one year after macrophyte removal for Lake Grand-Lieu (left) and Hartbeespoort Dam (right). Top: NMDS showing the community composition before and one year after plant removal. Bottom: Line plots showing the change of richness and Shannon-diversity over time in the two sections.

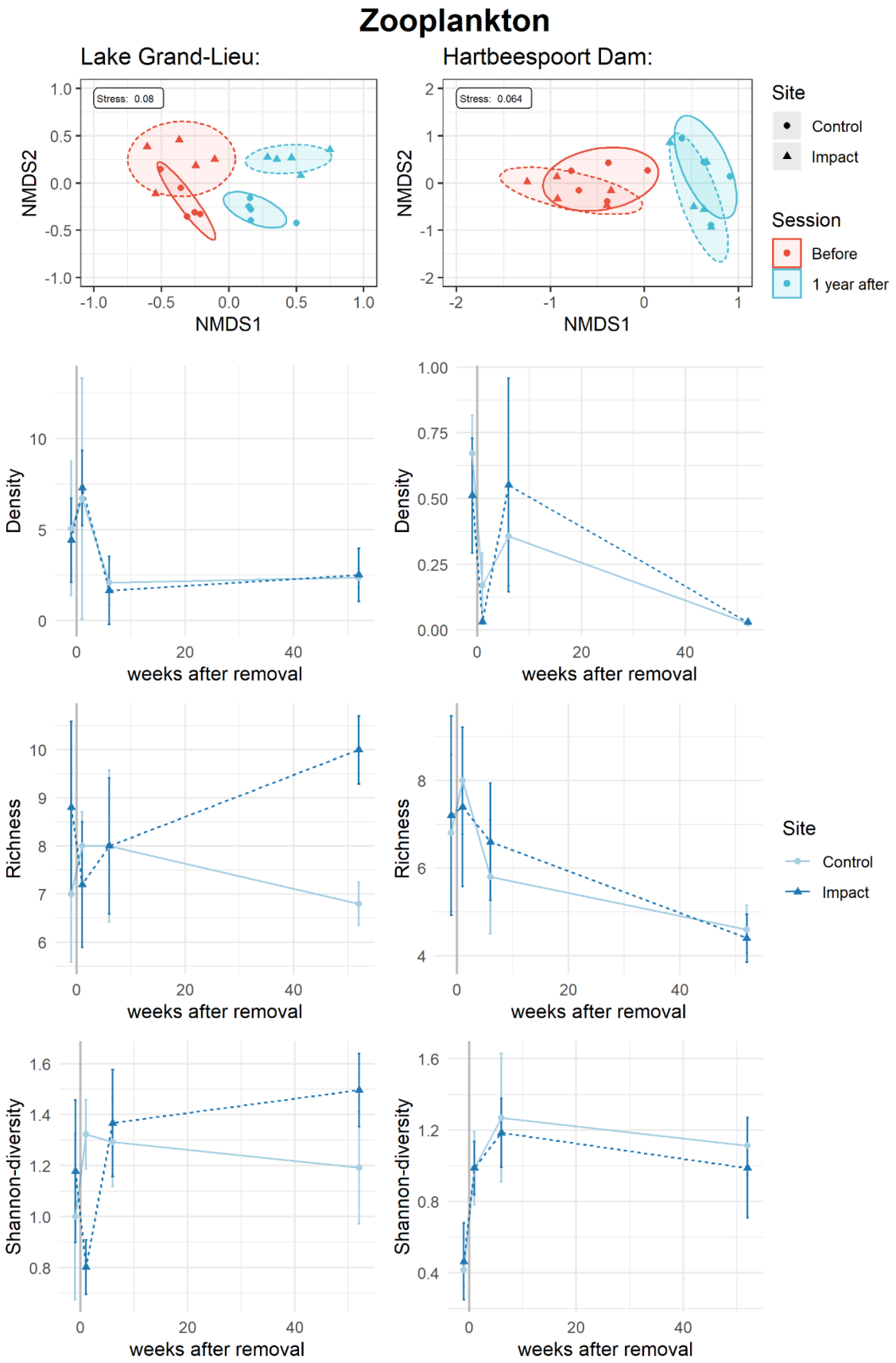

Figure 27: Comparison between the zooplankton community in the impact site and control site before and one year after macrophyte removal for Lake Grand-Lieu (left) and Hartbeespoort Dam (right). Top: NMDS showing the community composition before and one year after plant removal. Bottom: Line plots showing the change of density, richness and Shannon-diversity over time in the two sections.

4.3.2. Zooplankton

In both sites, we found a significant change in the zooplankton communities one year after plant removal compared to the "before" sampling (Figure 27). In Lake Grand-Lieu, the communities differed in the impact site before and one year after removal (Figure 27; pairwise PERMANOVA: p-value=0.005) and in the control site (p=0.014). Also, in Hartbeespoort Dam, the communities differed in the impact (p=0.008) and the control site (p=0.007). One year after macrophyte removal, the community composition between the control and impact site was not significantly different in Lake Grand-Lieu (p=0.32) and Hartbeespoort Dam (p=0.245). The richness and Shannon-diversity showed no impact on plant removal after one year (Figure 27).

4.3.3. Macroinvertebrates

The macroinvertebrate communities sampled with grab samples (Figure 28) and sweep samples (Figure 29) showed significant differences between the communities before and one year after removal. For grab samples, we found significant differences before and one year after removal in Lake Grand-Lieu for the impact (pairwise PERMANOVA: p-value=0.009) and control site (p=0.01). Before the macrophyte removal, the communities in the impact and control site were different (p=0.031) in Lake Grand-Lieu, but no difference was found after the removal (p=0.313). Also, in Hartbeespoort Dam, we did not find a difference in control and impact site after the macrophyte removal p= (0.462). For sweep samples, we found the highest variability of all four groups. Before the macrophyte removal, the community composition was already different between control and impact sites (Lake Grand-Lieu: p=0.03, Hartbeespoort Dam: p=0.022). The same was found one year after the removal (Lake Grand-Lieu: p=0.029, Hartbeespoort Dam: p=0.023). Also, the community composition in the control site before and after removal was different (Lake Grand-Lieu: p=0.013, Hartbeespoort Dam: p=0.013), and the same for the impact site (Lake Grand-Lieu: p=0.01, Hartbeespoort Dam: p=0.014). The macrophyte removal did not

impact density, richness, and Shannon-diversity after one year for either grab or sweep samples.

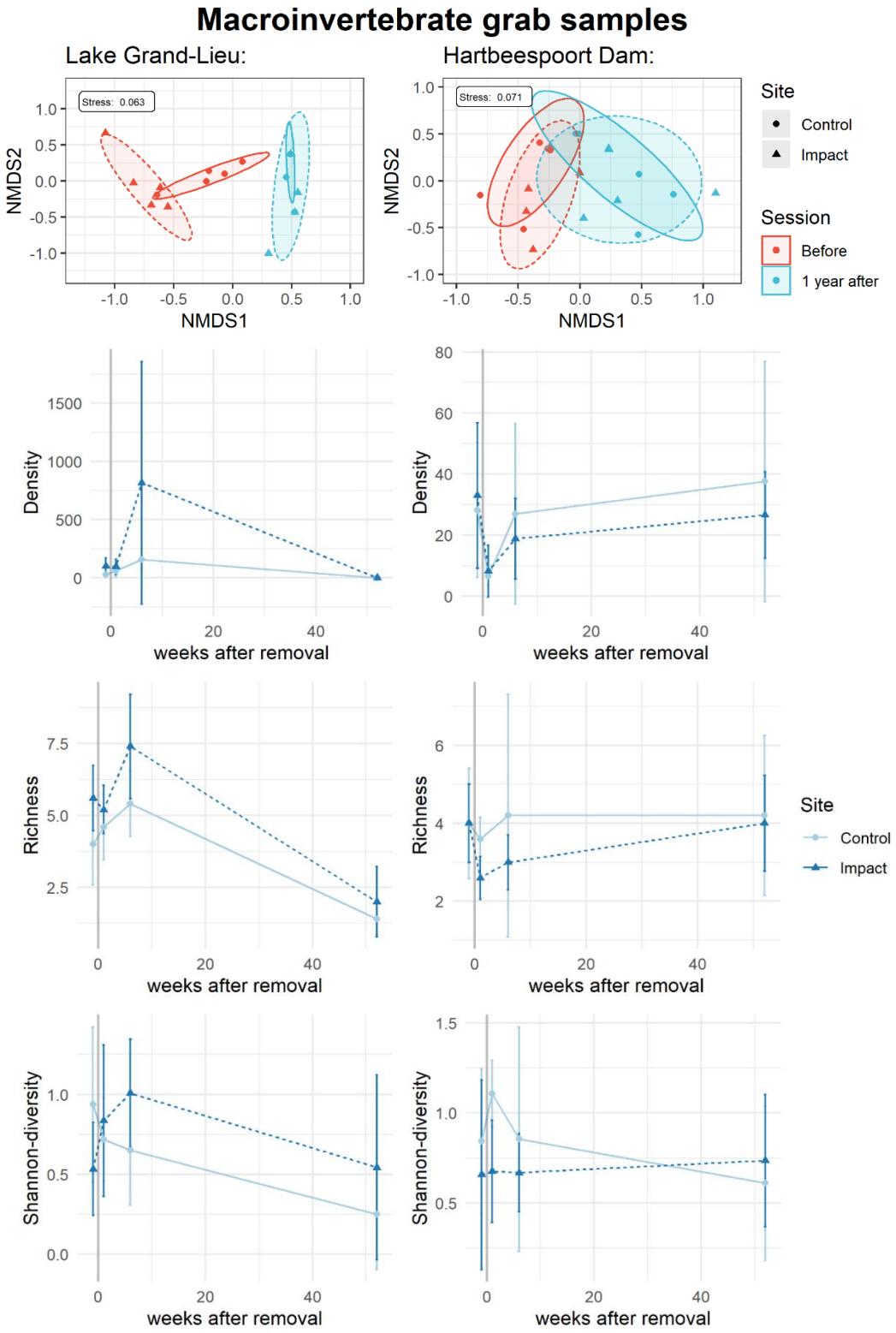

Figure 28: Comparison between the macroinvertebrate community found in grab samples in the impact site and control site before and one year after macrophyte removal for Lake Grand-Lieu (left) and Hartbeespoort Dam (right). Top: NMDS showing the community composition before and one year after plant removal. Bottom: Line plots showing the change of density, richness and Shannon-diversity over time in the two sections.

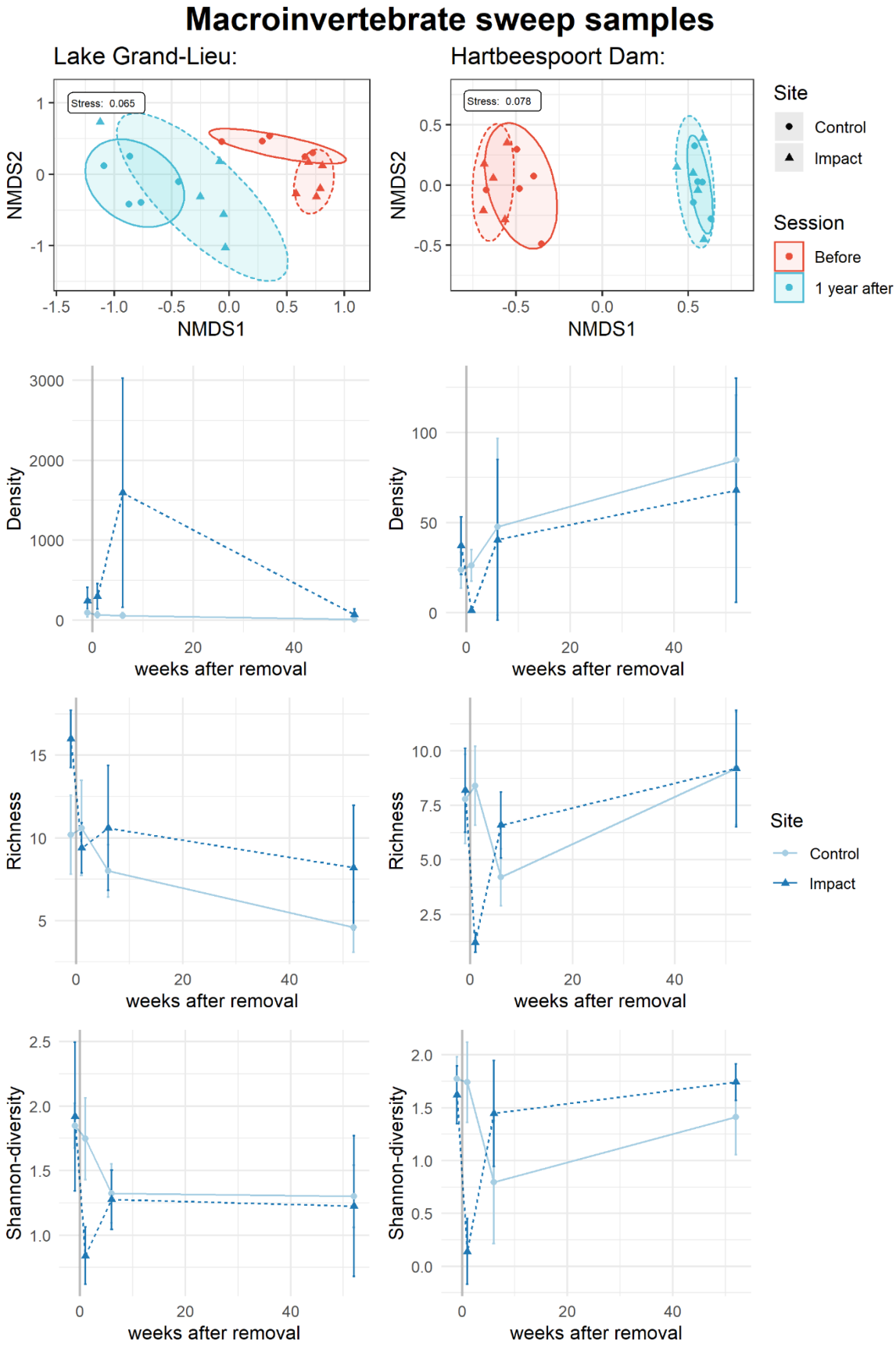

Figure 29: Comparison between the macroinvertebrate community found in sweep samples in the impact site and control site before and one year after macrophyte removal for Lake Grand-Lieu (left) and Hartbeespoort Dam (right). Top: NMDS showing the community composition before and one year after plant removal. Bottom: Line plots showing the change of density, richness and Shannon-diversity over time in the two sections.

4.4. Discussion

Our first hypothesis of a decline in the impact of macrophyte removal after one year was confirmed for all three groups in both sites. Neither the density, richness, nor Shannon-diversity was impacted after one year. In Article 3 (Misteli et al., 2023), we showed a negative impact of macrophyte removal on zooplankton and macroinvertebrates from sweep samples in Lake Grand-Lieu one week after the removal and positive effects on phytoplankton and macroinvertebrates in sweep samples six weeks after the removal. One year after the removal, none of these effects remained. For Hartbeespoort Dam, we found negative effects one week after the macrophyte removal and no effects on diversity, richness, or Shannon-diversity after six weeks. Also, we did not find any impact on the measured parameters after one year.

To our knowledge, this is the first multi-group assessment of the long-term effect of macrophyte removal for aquatic biodiversity, as known studies only cover a short-term impact or only study a single organism group (for example: Känel et al., 1998; Wojciechowski et al., 2018). Thiemer et al. (2021) established in a review that reduced abundance and potential changes in macroinvertebrate diversity post-removal might only be temporary. Invertebrate abundance has been reported to recover within 1-10 months, depending on the time for plants to regrow and colonization from upstream or nearby areas (Habib & Yousuf, 2014; Känel et al., 1998; Monahan & Caffrey, 1996). Long-term studies on plankton community response to macrophyte removal are scarce. To our knowledge, only one study exists covering the impact of the removal of floating macrophytes on the density and richness of zooplankton over two years after removal (Choi, Jeong, La, et al., 2014). In their study only free-floating macrophytes were removed, while submerged macrophytes were left in the system. Increased diversity of submerged macrophytes after removing floating macrophytes favored the density and richness of zooplankton.

Our second hypothesis of long-lasting differences in the community composition after plant removal could be partly confirmed. In addition to the descriptive parameters (density, richness,

and Shannon-diversity), we also compared the species composition before and one year after macrophyte removal. This comparison allows us to see if the disturbance of macrophyte removal leads to a shift in the community composition between the impact and control site or if the communities are resilient and not affected by the removal. One year after the macrophyte removal, both sites still showed strong differences. Free-floating *E. crassipes* were completely removed and kept away from the impact site (use of an artificial floating barrier), and emergent *Ludwigia* spp. only showed little regrowth after one year (personal observation). On the opposite, the control sites in both lakes were still covered by a dense mat of macrophytes. We showed (see Chapter 1, Chapter 2) that the communities in open-water sites and macrophytes are different for all studied groups. Based on this result, we could expect two outcomes: either the resilience of the ecosystem is so high that the community recovers back to a community similar to the one in the "macrophytes", or the disturbance is too high and a different stable community develops, the "open-water" community. The communities of zooplankton and macroinvertebrates showed no difference after macrophyte removal compared to the control site, showing high resilience of these two groups to macrophyte removal, although the macrophytes did not regrow one year after. Only the phytoplankton community of Lake Grand-Lieu differed after the plant removal. This comes as a surprise as Lake Grand-Lieu did not show a difference between open water and macrophytes (Misteli et al., 2022). The summer when macrophyte removal took place was extraordinarily hot and wet in this region, especially from the time of the removal till six weeks after sampling. High water temperature and low water level might have limited the water mixing in the section where macrophytes were removed, especially as the study took place in a side arm of the lake. Declining water levels (caused by high temperatures) in the six weeks after macrophyte removal might have contradicted the short-term effects of the removal as phytoplankton was concentrated. Macroinvertebrates in sweep samples were the group with the highest variability. The community composition in all four samplings (before/1 year after, control/impact) differed, therefore not allowing a clear assessment of the resilience of the community.

5. Chapter Discussion

Our studies showed the effect of macrophyte removal on macroinvertebrates, zooplankton, and phytoplankton assemblages. We found that the effect of macrophyte removal had only short-term effects on these assemblages considering abundance, richness, Shannon-diversity, and functional diversity. Aquatic biodiversity seems to be disturbed directly after the removal but shows resilience against the removal. Based on our results, macrophyte removal has no long-lasting impact on biodiversity. The fast recovery of the communities could be explained by the short life cycle of the studied organisms. All systems in our study were already managed, in one way or another, before our experiment. Disturbance-sensitive taxa might already have disappeared after earlier management efforts, and only more robust taxa remain. In macroinvertebrates, for example, the community was dominated by Diptera, Oligochaeta, and Acari, three groups that are known to be tolerant taxa (Goldschmidt, 2016; Tampo et al., 2021). The invasion of macrophytes, as well as the mass development of native macrophytes, is more likely to happen in disturbed systems, such as after dam construction as in the river Otra (Velle et al., 2019) or after eutrophication such as in Hartbeespoort Dam (Harding, 2008). Such disturbed systems might come with a disturbance-resistant community.

Besides negative short-term impacts, we also showed high variability in response to macrophyte removal. Such high variability in response to macrophyte removal corresponds with earlier studies which showed contrasting findings in different studies (Thiemer et al., 2021). The drivers leading to such variability remain unknown. Using different removal methods might impact the community differently (Greer et al., 2012). Further, different environmental characteristics of the systems (e.g., climate, trophic level, season) or different biological types of macrophytes can be expected to change the interaction between macrophytes and biodiversity and, might alter the effects of removal (Meerhoff et al., 2007, Article 2). Given this high variability, the outcome of our long-term analysis needs to be confirmed with further studies covering more variable systems.

General Discussion

One of the greatest challenges of this century is to stop the decline in biodiversity, especially for freshwater biodiversity, which is experiencing a stronger reduction than terrestrial or marine biodiversity (WWF, 2022). While the spread of invasive species, land use change, eutrophication, and climate change are further threatening biodiversity (Sala et al., 2000), the available fundings for conservation are limited (Gerber, 2016). Sustainable and evidence-based management strategies are keys to investing these limited funds in the most effective way possible. Dense mats of macrophytes are often removed as they interfere with human activities. The effect of dense mats of macrophytes on biodiversity remains unclear, let alone the consequences of their removal. To fill in this knowledge gap, I studied the effects of dense mats of macrophytes and their removal on biodiversity (phytoplankton, zooplankton, and macroinvertebrates) in this thesis. All results obtained in this thesis are summarized in Figure 30.

Role of dense mats of macrophytes on biodiversity

Macrophytes play an important role in many aquatic systems (Thomaz & Cunha, 2010). Their impact on other organisms, such as phytoplankton, zooplankton, or macroinvertebrates, is already well known. Macrophytes are in direct concurrence with phytoplankton for nutrients and light. The presence of macrophytes in shallow lakes leads to a shift from phytoplankton-dominated lakes to macrophyte-dominated lakes with lower phytoplankton biomass (Scheffer et al., 1993), but phytoplankton diversity was shown to be higher within macrophytes (Declerck et al., 2007). Increased structural heterogeneity within macrophytes has been linked to increased density and diversity of zooplankton and macroinvertebrates (Burks et al., 2002; Thomaz & Cunha, 2010). However, most of these studies focus on stable natural macrophyte communities. Dense mats of macrophytes differ in multiple aspects from stable, diversified plant communities (Thiemer, 2022), and the findings of studies on these communities have limitations when generalized to systems with dense mats of macrophytes. Nonetheless, extensive research on the effect of such dense mats of macrophytes on biodiversity remains scarce.

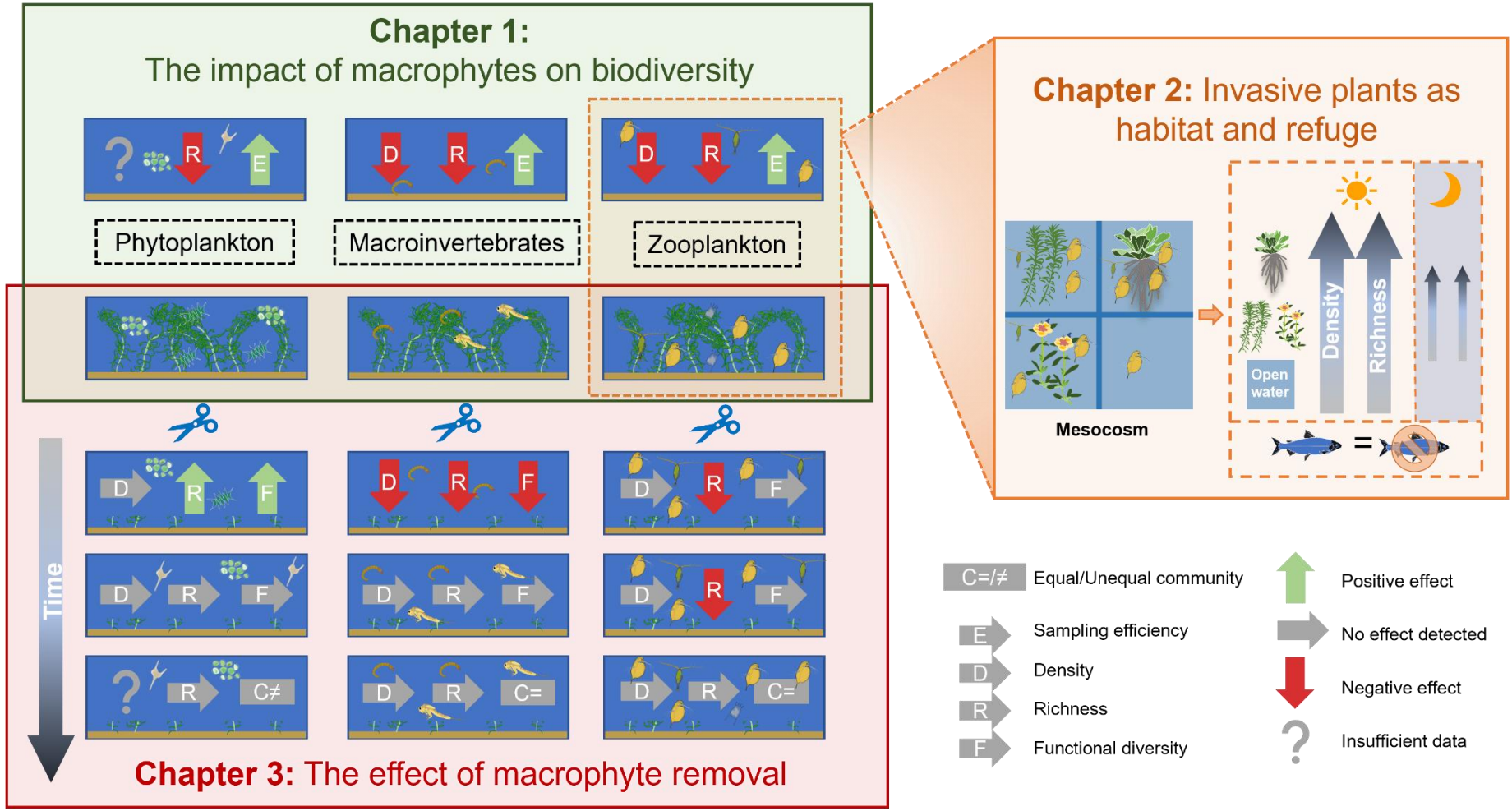

Figure 30: Summary of all results obtained in this thesis

Under favorable conditions (light, nutrients, and temperature), macrophytes can form dense plant mats covering large parts of a system (Riis & Biggs, 2001; Verhofstad et al., 2017). They are usually formed by a single plant species, growing at a high density and covering a huge area (Hussner et al., 2017; Verhofstad et al., 2017). Structural heterogeneity, shown as a key determinant of the associated biodiversity (Thomaz & Cunha, 2010), is reduced in monospecific mats compared to multispecies macrophytes beds, which leads to reduced biodiversity (Deosti et al., 2021). The density (Ding et al., 2020) and size (Lauridsen et al., 1996) of macrophyte mats have also been shown to influence surrounding biodiversity. Furthermore, dense mats of macrophytes are often formed by invasive alien species. Invasive alien macrophytes have been shown to negatively affect biodiversity, for example, by the production of allelopathic chemicals impacting phytoplankton (Santonja et al., 2018) and epiphytic algae (Lv et al., 2022) or by providing unsuitable detritus for colonization by native detritivores (Stiers et al., 2011).

In Chapter 1, we analyzed the effect of dense mats of macrophytes in comparison with open-water sections for phytoplankton, zooplankton, and macroinvertebrates. Dense monospecific mats of macrophytes colonized all studied sites. We showed that overall the taxa richness and density of macroinvertebrates were higher under dense mats of macrophytes in comparison to open water sections. These findings confirm results from studies of macroinvertebrates within native macrophytes. Also, for zooplankton, we found an increased density and taxa richness in macrophyte beds compared to open-water sections. We did not find an increased taxa richness for phytoplankton in either of the sites (density was not measured for the phytoplankton). Compared to zooplankton and macroinvertebrates, phytoplankton has no possibility for active horizontal migration, and on this small scale, water movement can be expected to homogenize the communities. However, earlier studies showed increased taxa richness also for phytoplankton (Declerck et al., 2007).

We found differences in the communities within macrophyte stands and open water for all three groups. We found unique taxa of macroinvertebrates for the macrophytes and the open water

section, respectively. We also showed that different macroinvertebrate taxa inhabit the two macrophyte species in Lake Ebolow (Misteli et al., 2022). Further, we showed that the community composition differs between the macrophyte and open water sections for zooplankton and phytoplankton.

Overall our results show high variability between sites. We only found effects on density and taxa richness for macroinvertebrates in three out of five sites. In contrast, for zooplankton, only one site showed a positive impact on density and one on diversity. Considering the high diversity of the sites and the macrophyte species, this variability in outcome is not surprising. Despite the relatively small number of study sites, we can still find global patterns. However, further research is needed to define the drivers of site-specific effects of macrophytes on biodiversity.

One possibility to study such drivers in an isolated way is using aquatic mesocosms. Aquatic mesocosms are self-sufficient experimental ecosystems designed to test multiple scenarios under controlled conditions (Caquet, 2013; Stewart et al., 2013). Besides that, we investigated the role of dense mats of invasive macrophytes on zooplankton in natural systems in Chapter 1, and we used a semi-controlled mesocosm approach to further study this macrophyte-zooplankton interaction in Chapter 2. We analyzed the impact of three invasive macrophyte species of three different biological types on zooplankton. Further, we studied how this interaction changes in the presence of fish. We showed that small patches of invasive macrophytes have a substantial impact on structuring the zooplankton community compared to open water. During the day, the three macrophyte sections were preferred by most zooplankton taxa. This aligns with former studies showing that macrophytes are an essential refuge for zooplankton during the day (Burks et al., 2002). The daytime migration of zooplankton to macrophytes (DHM; Burks et al., 2002) was also confirmed by the comparison between daytime sampling and night sampling, which showed less macrophyte association of zooplankton. Zooplankton density and diversity increased with the structural complexity of the macrophytes. The complex net of roots of the floating *Pistia stratiotes* attracts more zooplankton than the less complex leaves and stems of the submerged *Egeria densa*

and the emergent *Ludwigia* spp. As stems and leaves, roots offer a substrate for the growth of a well-developed biofilm, providing food for zooplankton (Han et al., 2018). This result shows that the effect of invasive macrophytes on zooplankton mainly depends on the structural complexity, which is similar to the effects of native macrophytes on zooplankton (Bolduc et al., 2020). The presence of fish did not change the results in a significant way. The macroinvertebrate predators that colonized the mesocosms may already be sufficient to cause similar effects as expected for fish, as zooplankton showed DHM even without fish. Our result agrees with a former study showing that the role of macrophytes in zooplankton (refuge vs. risk area) depends on the macroinvertebrate community (Sagrario et al., 2009). This result indicates that our findings are not only valid in systems such as shallow lakes or rivers, which are usually inhabited by fish, but also in smaller ecosystems such as ponds which are often free of fish. In a future study, macroinvertebrate communities should also be controlled to properly assess the role of predator presence on the habitat choice of zooplankton.

Dense mats of macrophytes and their impact on sampling efficiency

As we expected to find a different community structure within macrophytes and open water, we also analyzed the sampling efficiency of our respective methods for the three groups in Chapter 2. We hypothesized that due to the higher number of taxa and the increased structural heterogeneity within macrophytes, the required number of samples is higher in macrophytes to reach the same sampling coverage (% of taxa of the overall community collected) as in open water. This hypothesis was confirmed in most sites for all three groups. With five samples per section, we coverd a smaller proportion of the community within macrophytes than open water, indicating to an underestimation of the actual community found within macrophytes. This gap in sampling efficiency is bigger than the differences in density and richness between the two sections and showed less variability. To our knowledge, this was the first analysis of sampling efficiency in and outside of macrophytes on three different organism groups. Our findings should be considered

when planning biomonitoring and research with macrophytes. In both environmental management and research, available funding (and thereby working hours) are limited. Therefore the number of samples taken cannot be increased to lower such differences in sampling efficiency. Other ways to account for these differences need to be considered, such as using estimated species richness (Chao et al., 2014) or pooling subsamples (Rodríguez-Ramos et al., 2014).

The management of dense mats of macrophytes and its consequences for biodiversity

While macrophytes are declining globally (Phillips et al., 2016), invasive macrophytes are becoming increasingly a problem worldwide (Hussner et al., 2017). Not only invasive macrophytes but also native macrophytes can show periods of mass development and form dense mats which cover an entire lake or river (Hussner et al., 2017; Verhofstad et al., 2017). These dense mats are often considered problematic in different aspects (See Thiemer et al., 2021, and citations therein). Our set of study sites offers good real-world examples of how dense mats of macrophytes can be problematic. In the case of Lake Grand-Lieu, Lake Kemnade, and Hartbeespoort Dam, invasive species form the dense mats. Removal of invasive macrophytes is an often-used measure to restrict their further spread (Hussner et al., 2017). In all studied sites, dense mats of macrophytes hinder economic and recreational uses such as fishing, boating, sailing, or swimming. In the river Otra and Lake Kemnade, the study site lies upstream of a hydropower plant for which floating macrophyte materials are problematic as they clog the water input to the power plant. Further, the dense growth of macrophytes is conceived negatively by the residents and visitors to the lake/river (Thiemer, 2022). In the river Spree, dense mats of macrophytes slow down the water flow and thereby increase the water level leading to increased flood risk to adjacent land. In all sites, the local stakeholders responsible for managing the systems are performing mechanical removal measures as a quick decision to restore the system for human use. The decision for removal is often based on the negative human perception of dense mats of macrophytes, and possible positive ecosystem

services provided by dense mats of macrophytes are overlooked (Thiemer et al., 2021). Such possible ecosystem services could be the provision of habitat for different organism groups covered in this thesis, but also, for example, the retention of climate-relevant gases such as CO_2 and CH_4 (Harpenslager et al., 2022) or nutrient retention in the plant tissues (Schulz et al., 2003).

As we have shown in Chapter 1 and Chapter 2, dense mats play an important role in structuring the communities of phytoplankton, zooplankton, and macroinvertebrates. Based on our results, we hypothesize that the removal of macrophytes negatively impacted the biodiversity in the system, especially zooplankton and macroinvertebrates.

The effect of macrophyte removal has been studied before (see, for example: Choi, Jeong, La, et al., 2014; Greer et al., 2012; Känel et al., 1998; Mangas-Ramírez & Elías-Gutiérrez, 2004). However, existing research focuses either on a single group, a single study site, only a single time point, or used a weak study design (missing control section or missing "before" sampling). In this thesis, we provide an international study on the short and long-term effects of macrophyte removal on a set of study sites spanning a high variability (Chapter 3). The studies were based on a BACI design using "before" and "after" sampling in a "control" and "impact" section. The choice of this sampling design has turned out to be necessary, as we found a high temporal variation in our sampled community which we would not have detected without using a control section. Further, we also found differences in the two sampling sections before the removal of the macrophytes showing the importance of sampling before the impact, in our case, the removal of macrophytes.

Our hypothesis was confirmed: we found negative effects of macrophyte removal on zooplankton and macroinvertebrates one week after the removal, while positive effects on phytoplankton were found. Six weeks after the removal, the impact consequences were only weak; one year after the removal, we did not find an effect of the impact anymore. This is an important finding as most published research on the effects of macrophyte removal only covers the effects of a few days to a few months after the macrophyte removal and often uses a single time point only. They only capture the short-term effects of macrophyte removal and overlook possible temporal variability.

Based on our findings on the effects of macrophytes on the community structure, we hypothesize that the communities after the plant removal might differ and they resemble more an open water than a macrophyte community. This hypothesis can only be confirmed for phytoplankton in one site, while the communities of zooplankton and macroinvertebrates showed a high resilience and returned to the state before the removal. The incomplete removal of macrophytes (remaining roots, plant fragments, and plant litter in the system) and fast regrowth might help the community stay resilient.

Removing dense mats of macrophytes might be a suitable tool to improve multiple ecosystem services affected by dense mats of macrophytes without harming the local biodiversity on a long-term basis. However, our results also showed a high variation between systems which shows the need for a local assessment of the effects on biodiversity before macrophytes are removed.

Solving the management dilemma: is there a way out?

The partial removal of macrophytes in a lake and their fast regrowth is an important issue for the local stakeholders managing the macrophytes. The commonly used mechanical removal does, in many cases, not offer a sustainable solution as the measures must be repeated regularly and are expensive. For example, in Lake Kemnade, the mowing boat has been active daily during the growing phase of the plants for several years. For the management of *E. nuttallii* in Lake Kemnade, a list of possible measures to control mass development has been developed (Podraza, 2017). Besides the current mechanical removal with a mowing boat, the following control measures are proposed: use of pesticides, deepening of the lake via sediment removal, emptying the lake and destroying the seed bank with freezing/drying conditions, black foil covering the plants to repress light, the introduction of herbivorous fish for biocontrol, or the increase of turbidity via increased phytoplankton growth induced by the addition of phosphorus. The use of pesticides, the introduction of herbivorous fish, and the addition of phosphorus are forbidden by German law. The deepening of the lake would not be successful as *E. nuttallii* has been shown to grow to a depth

of 10 m (current mean depth of the lake: 2.4 m). For black foil, the affected lake area is too big (1.25 km^2, which is mostly covered with macrophytes) and emptying the complete system is technically impossible for reservoirs.

All these regulations and restrictions leave one measure on the list: mechanical removal using a mowing boat. This causes a dilemma, especially in Europe, where invasive mechanical measures and the use of herbicide and biocontrol are forbidden by European and/or national law (Hussner et al., 2017). While mechanical removal is a handy tool to reach short-term success without harming the ecosystem strongly, it is not a sustainable management method. To improve the management of dense mats of macrophytes in the future, available management methods must be improved, and regulations also need to be revised. As shown in Hartbeespoort Dam, the introduction of biocontrol agents from the native range of the macrophytes, in this case, the introduction of *Megamelus scutellarin*, *Neochetina eichhorniae*, *Neochetina bruchi,* and *Eccritotarsus catarinensis* for the control of *P. crassipes*, could be a sustainable method to manage invasive macrophytes (Coetzee et al., 2021). Also, chemical control has been shown to be an option to eradicate macrophytes (Champion & Wells, 2014). However, biological and chemical control requires extensive research on their environmental impact before they can be applied. Using different management methods than covered in our studies, such as biological or chemical removal or mechanical control measures with a more substantial impact discussed above, might impact the biodiversity in different ways than we found in our study, and a reassessment of the effects of removal is required.

In this thesis, we investigated the effect of macrophyte removal by mechanical removal in only a fraction of the lake/river. More sustainable management would include the removal of the macrophyte from the entire system. Such a removal might lead to a more substantial impact on the system than partial removal. Considering that a macrophyte species is successfully removed, we expect three possible outcomes. Firstly, the whole system could switch from a macrophyte to a phytoplankton-dominated system (Scheffer et al., 1993). Secondly, a new invasive macrophyte species could invade the empty niche. In the river Ruhr and its reservoirs (Lake Kemnade and older

reservoirs), the first problem with the mass development of plants was recorded in 1930. Since then, management measures have been applied, leading to a succession of different macrophyte species until the current *E. nuttallii* invasion (Podraza et al., 2008). The third possible outcome would be the colonization of a mix of native macrophyte species replacing the dense monospecific mats. Option one, a phytoplankton-dominated system, removes the problematic dense mats of macrophytes, but it often comes with algal fouling and toxic cyanobacteria blooms, which is also not desired as it impairs the provision of drinking water and recreation (Hilt et al., 2017). The second option would replace one problematic macrophyte with another, which will cause similar problems as soon the macrophytes start to grow in dense mats again. Option three, maintaining a macrophyte-dominated state with a mix of native macrophyte species, might, in most cases, be the desired outcome of a removal. In the case of invasive macrophytes, the invasive species might be removed. However, depending on the system, native macrophyte communities can also cause problems, as seen in the river Spree.

Eutrophication, human modification of the systems (e.g., due to building dams for hydropower production), and the spread of invasive species have led to the problematic spread of invasive macrophytes and the increase of dense mats of macrophytes. While sustainable management of a system would be desired, it is nearly impossible without tackling the aforementioned causes of the mass development of macrophytes. While it is the job of the local stakeholders to control the dense mats of macrophytes, changes on a larger scale are necessary to solve this problem. Regional, national, or even international regulations are needed to stop those increasing impacts and provide a framework for effective macrophyte control measures. Until then, local stakeholders have to balance the benefits and disbenefits of macrophyte removal for all ecosystem services and, based on that, decide if it is worth removing macrophytes again and again.

Conclusion

Macrophytes play an important role in aquatic ecosystems. Besides coming with many negative impacts for humans, even dense monospecific mats and invasive macrophyte species can affect biodiversity positively. Assessing the diversity in dense mats of macrophytes, though a straightforward idea, is not an easy task: the biodiversity in macrophyte beds is often underestimated with the current sampling effort, as sampling efficiency within macrophytes is lower compared to open water sites.

The mass development of dense mats of macrophytes in freshwater ecosystems induces nuisances for humans, and macrophytes are often removed. However, removing macrophytes might reduce biodiversity, given that the presence of macrophytes promotes biodiversity. Our findings confirmed that this holds true for zooplankton and macroinvertebrates, while phytoplankton profits from macrophyte removal. Nonetheless, our results also showed that these effects were strongest directly after the removal and that effects were no longer detected after one year.

We found high variability in our results, suggesting that more research and local studies are needed for an evidence-based development of management strategies. Furthermore, not only biodiversity but also many other ecosystem functions, such as nutrient retention, carbon burial, and greenhouse gas emissions, might be affected by the removal of macrophytes. Developing a sustainable management strategy requires an assessment of all possible positive and negative effects of macrophyte removal.

Perspectives

The world's ecosystems, especially freshwater systems, are under pressure, and biodiversity is declining. We might even be at the beginning or in the midst of the sixth Mass Extinction event. Unlike the five earlier Mass Extinction events, this one is caused entirely by humans (Cowie et al., 2022). Global climate change, increased nutrient deposition, land use change, and the spread of invasive species are some but not all, causes of biodiversity decline.

All these causes act on a global scale, and biodiversity is declining in all regions. Meanwhile, ecological research is mainly carried out in Europe, North America, and Oceania (Nuñez et al., 2019). International and collaborative projects such as MadMacs are essential to finding globally applicable solutions. The integration of five countries on three continents (Brazil was unfortunately unable to collect samples on time due to Covid restrictions) and experts in different subfields of aquatic ecology offers excellent benefits. While local knowledge is important to understand the systems as well as local regulations and to collaborate with stakeholders, the international network within the project allows us to find global patterns and summarise findings useful to a big community. In my opinion, we need more projects of this kind in the future to provide a profound knowledge of environmental management for regions where less funding is available. The global challenges of the 21st century can only be solved globally by joint efforts. Working within the MadMacs framework helped me personally to extend my understanding of aquatic ecology which will be beneficial in my future.

The end of my three years of PhD does not mean that all the open questions about the impact of dense mats of macrophytes and their removal on biodiversity are solved. In our studies, we covered phytoplankton, zooplankton, and macroinvertebrates. These are three important groups living within macrophyte beds, but they are not the only ones. Some vertebrates such as fish, waterfowl, amphibians, or mammals might also use dense mats of macrophytes as a habitat, and also, on a smaller scale, we find organisms such as bacteria or fungi which might be affected by the macrophytes. Dense mats of macrophytes and their removal might impact those groups in similar ways to our studied groups. This offers plenty of research opportunities for further studies.

Especially the effect of dense mats of macrophytes and their removal on fish might be a relevant field of study as fish are often of economic importance for the people living nearby, especially in countries of lower income. Further, the analysis of functional diversity offers a lot of possibilities to better understand the impact of macrophytes and their removal on biodiversity. A proper assessment of functional diversity requires a good knowledge of functional traits. On an international scale, this is a difficult task due to the lack of global databases and the lack of knowledge on functional traits for many species, especially in regions of the world where research fundings are low.

In Chapter 1, we were able to find the global effects of dense mats of macrophytes on biodiversity. However, we also found high variability among study sites in the results. As our study sites were highly variable, this was no big surprise. The goal of our study was to reveal global patterns, while the question of how different ecosystem and plant characteristics affect these findings remain open questions for further research. Meerhoff et al. (2007) showed that habitat complexity affected zooplankton communities and their predator avoidance behavior differently in temperate and subtropical shallow lakes. Such questions could also be interesting for the other organism groups. Further drivers which might lead to differences in the biodiversity-macrophyte interaction could be trophic levels, the biological type of the macrophytes, the size of the system (ponds vs. lakes), the size of the macrophyte mats or the type of system (lentic vs. lotic). A large-scale project such as MadMacs containing more study sites with less variability might be a better solution to study this in a real-world situation. However, such a project is hardly feasible. Instead, mesocosm-based studies are a cheaper way in which such drivers can be studied isolated in near natural conditions.

Sustainable management of macrophytes must be the aim for the future, but there is still a long way to go. Current management techniques are often only of short time success, especially in Europe, where regulations strongly limit the available management methods. Developing evidence-based sustainable management requires a broad knowledge of the effects of macrophyte removal. This study provides a good start for a better understanding of the effects of macrophyte removal

on biodiversity. The MadMacs project extends this study by further analyzing the effects of dense mats of macrophytes and their removal on biogeochemistry and other ecosystem services. However, many questions remain open. Our long-term study only covered two study sites. Considering the generally high variability in our results, this needs to be studied further. All our sites were already managed one way or another before our study. Using entirely new sites for such a study might provide different insights into the beginning of macrophyte removal. Successfully fighting the mass development of macrophytes will require more robust measures (drying of ponds, removal of sediment from lakes and rivers), new approaches (evidence-based biological or chemical control) or a combination of multiple measures to remove macrophytes and avoid a regrowth or reinvasion. Using new approaches to control macrophytes requires new studies to assess their impact on biodiversity and other ecosystem services. A BACI design used in the MadMacs project with an extension of the six week time limit showed to be an appropriate method to monitor such impacts even in systems with high temporal variability and high spatial heterogeneity.

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Résumé

Introduction :

Biodiversité aquatique

L'eau douce ne représente que 0,01% de l'eau de la planète et couvre moins de 1% de la surface du globe (Gleick, 1996). Malgré cette faible proportion, les écosystèmes d'eau douce abritent une grande biodiversité avec un total de 125 530 espèces animales connues. En d'autres termes, les écosystèmes d'eau douce fournissent un habitat à une espèce connue sur dix dans le monde (1 324 000 espèces: United Nations Environment Programme, 2002). Aujourd'hui, cette biodiversité des eaux douces est menacée par des changements globaux tels que l'eutrophisation, la pollution, les changements climatiques et les espèces invasives. L'indice Planète vivante 2022 a fait état d'un déclin de 83 % des populations d'espèces d'eau douce, un déclin bien plus important que pour les espèces terrestres ou marines (WWF, 2022).

En tant que producteurs primaires, le phytoplancton et les macrophytes (organismes photosynthétiques aquatiques, suffisamment grands pour être vus à l'œil nu) constituent la base de tous les réseaux trophiques. Les consommateurs primaires comme le zooplancton, les macro-invertébrés, les poissons planctonivores et les poissons herbivores dépendent directement des producteurs primaires comme source de nourriture. Les consommateurs secondaires, comme les espèces plus grandes de zooplancton, les macroinvertébrés prédateurs et les poissons prédateurs, consomment les consommateurs primaires et sont ensuite consommés par des prédateurs plus grands, généralement de gros poissons prédateurs. La matière organique morte constitue une autre source de nourriture. Les décomposeurs tels que les macroinvertébrés détritivores, le zooplancton et les poissons fragmentent et décomposent en partie la matière organique, la rendant accessible à des décomposeurs plus petits tels que les bactéries et les champignons, renvoyant l'énergie et les

nutriments dans le réseau alimentaire. Outre ces organismes strictement aquatiques, il existe divers organismes qui relient les systèmes terrestres et aquatiques, tels que les insectes semi-aquatiques, les oiseaux, les grands mammifères et les humains.

Macrophytes dans les écosystèmes aquatiques

Les macrophytes peuvent être classés en quatre types biologiques selon Arber (1920) et Sculthorpe (1967). Les macrophytes émergents sont des plantes enracinées dont les feuilles sortent au-dessus de la surface de l'eau. On distingue aussi les macrophytes enracinés à feuilles flottantes. Les macrophytes immergés sont également enracinés, mais poussent entièrement sous l'eau. Enfin, les macrophytes flottants circulent librement à la surface de l'eau et leurs racines ne sont pas fixées dans le sédiment.

Rôle des macrophytes sur le fonctionnement des écosystèmes et la biodiversité

Les macrophytes jouent un rôle fondamental dans la structuration des écosystèmes aquatiques (Jeppesen et al., 1998). Ils ont une influence directe et indirecte sur les caractéristiques biotiques et abiotiques des systèmes aquatiques; ils sont souvent qualifiés d'ingénieurs de l'écosystème. Ils offrent un habitat diversifié (Choi, Jeong, Kim, et al., 2014 ; Scheffer, 2004 ; Thomaz et al., 2008). Les macrophytes servent d'habitat, de refuge ou de source de nourriture pour divers organismes, notamment le zooplancton (Choi, Jeong, Kim, et al., 2014 ; Sagrario et al., 2009), les macroinvertébrés (Ferreiro et al., 2013 ; Smock & Stoneburner, 1980 ; Taniguchi et al., 2003), les poissons (Petry et al., 2003 ; Vono & Barbosa, 2001) et les oiseaux d'eau (Hansson et al., 2010 ; Klaassen & Nolet, 2007 ; van Altena et al., 2016) et de support pour les algues épiphytes, et les algues de surface (Cattaneo et al., 2008 ; Ferreiro et al., 2013 ; Kiss et al., 2003 ; Wijewardene et al., 2022). Hilt et al. (2017) ont analysé des études comparant la biodiversité de différents groupes d'organismes (oiseaux aquatiques, poissons, macrozoobenthos, zooplancton, macrophytes,

phytoplancton ou periphyton, et bactéries) dans des lacs peu profonds dominés par les macrophytes et le phytoplancton. L'abondance et/ou la diversité étaient plus élevées dans la plupart des études, sauf pour les bactéries. Cependant, pour tous les groupes d'organismes, des résultats mitigés ont été trouvés.

L'étude des effets des macrophytes sur le phytoplancton, le zooplancton et les macroinvertébrés est l'objectif de cette thèse. Parmi ces trois groupes, le phytoplancton pourrait être le groupe le plus fortement impacté par les macrophytes. En effet, les macrophytes et le phytoplancton seraient en compétition pour la lumière et les nutriments dans la colonne d'eau. Un autre effet négatif de la présence des macrophytes, sur le phytoplancton, serait l'augmentation de la prédation par le zooplancton, (Timms & Moss, 1984 ; Van Donk & van de Bund, 2002). En outre, la production de substances chimiques allélopathiques chez certaines plantes pourrait également limiter la croissance du phytoplancton (Hilt & Gross, 2008 ; Körner & Nicklisch, 2002 ; Švanys et al., 2014). Les macrophytes auraient des effets plus positifs sur le zooplancton. Ils offrent des habitats et fournissent de la nourriture, de manière directe ou indirecte, via le biofilm (Thomaz & Cunha, 2010). De plus, ils servent de refuge contre les prédateurs comme les poissons ou les macroinvertébrés (Burks et al., 2002 ; Lauridsen & Lodge, 1996).

De même que pour le zooplancton, les macrophytes influenceraient positivement les macroinvertébrés. La création de nouvelles niches pour les petits macroinvertébrés (Labat et al., 2022 ; Taniguchi et al., 2003 ; Thomaz et al., 2008) ainsi que la réduction de la pression de prédation au sein des macrophytes (Balci & Kennedy, 2003 ; Sato et al., 2014 ; Warfe & Barmuta, 2004) sont liées à l'augmentation de l'abondance des macroinvertébrés parmi les macrophytes. Pour les macroinvertébrés herbivores, perceurs et omnivores, les macrophytes constituent une source de nourriture directe (Cyr & Face, 1993 ; Wood et al., 2017), tandis que les brouteurs consomment le biofilm qui y est fixé (Cyr & Face, 1993 ; Wolters et al., 2019) et les broyeurs et les collecteurs consomment la litière végétale en décomposition (Casagranda et al., 2006 ; Saulino & Trivinho-Strixino, 2018).

Proliférations végétales

Bien que les macrophytes jouent un rôle positif sur le fonctionnement des écosystèmes aquatiques, ils peuvent induire des nuisances lorsqu'ils forment des herbiers denses mono ou paucispécifiques. Ces proliférations s'expliquent par une disponibilité élevée des ressources (lumière et nutriment), une absence de perturbation et de prédation (Hussner et al., 2017 ; Verhofstad & Bakker, 2019). . (Riis & Biggs, 2001 ; Verhofstad et al., 2017).

Les herbiers denses de macrophytes peuvent entraver la circulation et les activités récréatives telles que la pêche, la natation ou d'autres sports aquatiques (Dugdale et al., 2013 ; Güereña et al., 2015 ; Verhofstad & Bakker, 2019). Ils peuvent également entraîner des conditions anoxiques dans l'eau (Janse & Van Puijenbroek, 1998 ; Verhofstad et al., 2017) et favoriser des mortalités piscicoles par exemple. La formation d'herbiers monospécifique ou paucispécifique se fait au détriment d'une végétation plus diversifiée (Stiers et al., 2011). La modification de l'écoulement de l'eau due aux herbiers peut entraîner une augmentation du niveau de l'eau et un risque d'inondation pour les terres adjacentes (Dugdale et al., 2013 ; Riis & Biggs, 2001). Les fragments végétaux peuvent obstruer les centrales hydroélectriques (Clayton & Champion, 2006 ; Dugdale et al., 2013). On s'attend à ce que les proliférations végétales augmentent suite aux changements globaux et que ces problèmes deviennent de plus en plus critiques (Hussner et al., 2017 ; Thiemer et al., 2021).

Gestion des proliférations de macrophytes

Trois grands types de gestion des proliférations végétales existent : la lutte chimique, la lutte biologique et la lutte mécanique/arrachage manuel. La lutte chimique consiste à appliquer un ou des herbicides sur les macrophytes. Elle permet des traitements à grande échelle, est rentable et a un impact environnemental moindre sur l'écosystème lorsqu'elle est utilisée correctement par rapport aux autres méthodes. L'utilisation de produits chimiques n'est pas spécifique à une cible et peut avoir des effets secondaires importants sur les autres producteurs primaires, mais elle entraîne

également des changements dans la clarté de l'eau et nuit aux poissons et aux macro-invertébrés (Hussner et al., 2017). En Europe, l'utilisation de la lutte chimique est fortement limitée, et son application est régie par la loi (Hussner et al., 2017). La lutte biologique décrit l'utilisation d'un ou de plusieurs organismes pour gérer les macrophytes. Pour les plantes exotiques, il est possible d'importer des agents de biocontrôle, souvent des insectes, depuis leur aire de répartition d'origine. En outre, l'importation d'agents pathogènes ou d'herbivores ou l'introduction d'herbivores indigènes peut être une autre option. La lutte biologique est peu coûteuse et peut durer, lorsqu'elle est efficace, pendant des décennies. Cependant, il faut trouver un agent de contrôle spécifique à l'espèce pour éviter un impact environnemental incontrôlable de l'espèce importée, ce qui nécessite des recherches et des recherches approfondies avant de pouvoir utiliser ces agents (Coetzee et al., 2022). C'est pourquoi la lutte biologique est fortement réglementée par la loi en Europe. L'arrachage mécanique ou manuel consiste à arracher ou couper les végétaux et les récolter. Cette technique est largement utilisée pour la gestion des proliférations végétales. De plus, l'arrachage des macrophytes est couteux et le succès n'est souvent que de courte durée, car les plantes ne sont pas entièrement éliminées et repoussent rapidement.

Dans cette thèse, les effets de l'arrachage mécanique des macrophytes sur la biodiversité seront recherchées, car cette méthode utilisée dans le monde entier s'applique à tous les écosystèmes aquatiques et à tous les types de plantes.

Objectifs et hypothèses

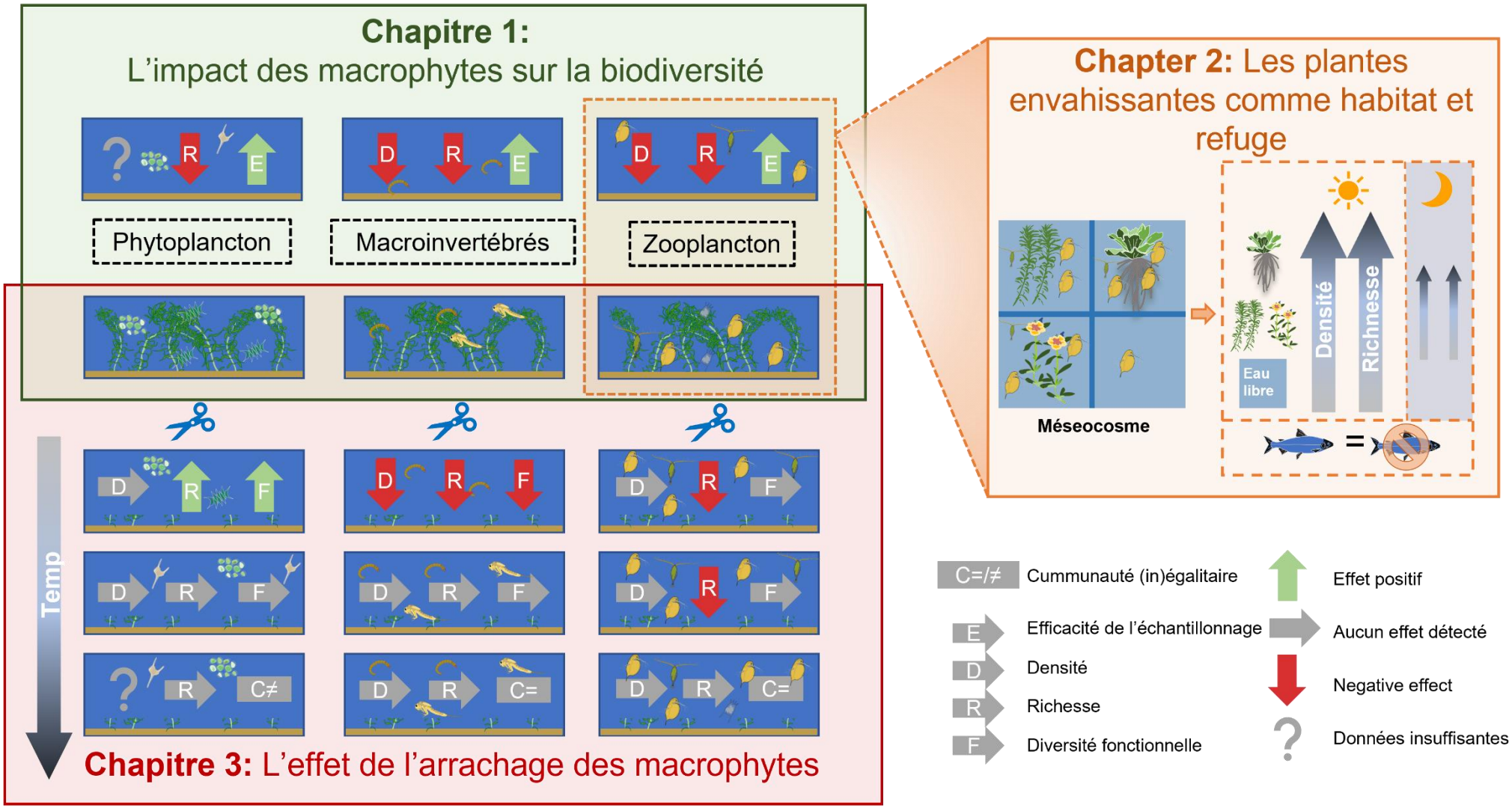
Les objectifs généraux de cette thèse sont d'évaluer le rôle des macrophytes sur la biodiversité de plusieurs groupes taxonomiques (c'est-à-dire le phytoplancton, le zooplancton et les macroinvertébrés) et l'effet de l'arrachage des herbiers de macrophytes sur la biodiversité environnante de plusieurs lacs et rivières en Europe et en Afrique.

Le **chapitre 1** traite des impacts des herbiers denses de macrophytes sur le phytoplancton, le zooplancton et les macroinvertébrés. Nous nous attendons à voir des effets positifs des macrophytes sur l'abondance et la diversité des macroinvertébrés et du zooplancton, mais des effets négatifs pour le phytoplancton. De plus, l'efficacité de l'échantillonnage des macroinvertébrés à l'intérieur et à l'extérieur des peuplements de macrophytes a été analysée. Notre hypothèse est que la présence de macrophytes affecte l'efficacité de l'échantillonnage et qu'un plus grand nombre d'échantillons dans les macrophytes est nécessaire pour obtenir des résultats comparables à ceux obtenus en eau libre.

Le **chapitre 2** traite du rôle des plantes envahissantes en tant qu'habitat et refuge pour le zooplancton. En réalisant une étude en mesocosmes, nous étudions le rôle d'habitat et de refuge contre la prédation, par les poissons, des plantes envahissantes. L'utilisation d'une approche en conditions semi-contrôlées permet de tester l'effet de plusieurs types biologiques de plantes aquatiques et de la présence de poissons. Nous supposons que les peuplements de macrophytes jouent un rôle essentiel pour le zooplancton en tant qu'habitat et refuge contre la prédation pendant le jour.

Dans le **chapitre 3,** nous analysons l'effet de l'arrachage des macrophytes sur la biodiversité environnante. Nous prévoyons un effet négatif de l'arrachage des macrophytes sur les communautés de zooplancton et de macroinvertébrés et un effet positif sur le phytoplancton, par suppression respectivement de l'habitat et du compétiteur. Les résultats de cette étude à court terme sont combinés aux résultats d'une étude à long terme (une année).

Résumé graphique Chapitre 1-3 :



Chapitre 1: Impact des macrophytes sur la biodiversité :

Dans ce chapitre, nous avons analysé l'impact des herbiers denses de macrophytes sur les macroinvertébrés, le zooplancton et le phytoplancton ainsi que l'efficacité de l'échantillonnage à l'intérieur et à l'extérieur des macrophytes. Cinq lacs ont été sélectionnés : le lac Grand-Lieu (France, envahi par *Ludwigia* spp.) et le lac Kemnade (Allemagne, envahi par *Elodea nuttallii*) en Europe, le barrage Hartbeespoort (Afrique du Sud, envahi par *Pontederia crassipes*) et le lac Ebolowa (Cameroun, envahi par *Typha australis* et *Pistia stratiotes*, utilisé uniquement pour l'analyse des macroinvertébrés) en Afrique. Ces sites diffèrent en terme d'état trophique, de surface et de profondeur, et de type de macrophytes. Sur chaque lac, un site dans les herbiers de macrophytes et un autre dans l'eau libre ont été retenus. Le phytoplancton, le zooplancton et les macroinvertébrés ont été échantillonnés.

Nous avons mis en évidence une densité et une richesse plus élevées en macroinvertébrés dans les peuplements de macrophytes par rapport à l'eau libre. Les résultats dépendent du site, et tous les sites n'ont pas montré un impact positif des macrophytes sur les macroinvertébrés. L'efficacité d'échantillonnage (% de la communauté échantillonnée avec cinq répétitions) est plus faible au sein des macrophytes dans la plupart des sites. Des résultats similaires ont été trouvés pour le zooplancton. Cependant, nous n'avons trouvé des différences de densité ou de richesse que dans un site sur trois. Nous n'avons pas trouvé de différences de richesse en phytoplancton entre les herbiers et l'eau libre, mais l'efficacité d'échantillonnage était généralement plus faible dans les macrophytes. De plus, nous avons montré pour les trois groupes que la composition de la communauté diffère entre les macrophytes et les eaux libres, avec des taxons spécifiques à ces habitats.

Nos résultats montrent que les herbiers denses de macrophytes ont des impacts sur la biodiversité environnante. En outre, nos résultats soulignent la nécessité de développer une méthode permettant un échantillonnage comparable à l'intérieur et à l'extérieur des macrophytes. Une telle méthode servira de base aux recherches futures et à la gestion des systèmes aquatiques.

Chapitre 2: Les plantes envahissantes comme habitat et refuge :

Les macrophytes indigènes fournissent un habitat et un refuge au zooplancton. Cependant, la question demeure de savoir si tous les types biologiques affectent les communautés de zooplancton de manière similaire. Pour répondre à cette question, nous avons conçu une expérience en mésocosme. Chaque mésocosme a été divisé en quatre unités expérimentales UE. *Egeria densa* a été utilisée comme plante submergée, *Ludwigia* spp. comme plante émergente et *Pistia stratiotes* comme macrophyte flottant librement, la dernière unité expérimentale était dépourvue de végétation. Dans la moitié des mésocosmes, nous avons introduit des poissons comme prédateurs pour analyser le rôle des macrophytes comme refuge. Les quatre sections ont été échantillonnées simultanément pendant quatre semaines, de jour comme de nuit. Nous avons comparé l'assemblage de zooplancton dans les différentes espèces de plantes et comparé la densité, la richesse et la diversité de Shannon dans les échantillonnages de jour et de nuit. De plus, nous avons comparé la préférence des différentes espèces pour les quatre habitats. En outre, nous avons testé l'effet des poissons.

Nous avons constaté que l'assemblage de zooplancton diffère entre les quatre UE, avec une abondance et une richesse plus élevée dans les macrophytes par rapport à l'eau libre. La densité la plus élevée a été trouvée entre les racines de *P. stratiotes*. Alors que la plupart des taxons montre ce schéma, certains ont montré des effets opposés. La nuit, le zooplancton migre en dehors des macrophytes, et les différences étaient plus faibles entre les UEs. La présence de poissons n'a pas eu un effet significatif sur le zooplancton.

Chapitre 3: Impact de l'arrachage des macrophytes sur la biodiversité :

Les herbiers denses de macrophytes peuvent entraver des activités comme la navigation de plaisance, la pêche ou la production d'énergie hydroélectrique. La méthode de gestion privilégiée de ces proliférations végétales est l'arrachage mécanique/manuel. Dans cette étude, nous avons analysé l'effet de l'arrachage mécanique/manuel des macrophytes sur les assemblages de phytoplancton, zooplancton et macroinvertébrés (échantillons pélagiques et benthiques). Notre étude a porté sur cinq sites dans trois pays d'Europe (Norvège, Allemagne, France) et en Afrique du Sud et sur cinq modèles biologiques *Juncus bulbosus* dans une rivière en Norvège ; un mélange de macrophytes indigènes dans une rivière allemande ; *Elodea nuttallii* dans un lac en Allemagne ; *Ludwigia* spp. dans le lac français ; *Pontederia crassipes* dans le lac sud-africain. Un protocole BACI (Before-After-Control-Impact) a été appliqué sur chaque site, comprenant un échantillonnage " avant ", " une semaine après ", " six semaines après " et " un an après " (uniquement dans le lac français et le barrage sud-africain) dans une zone témoin (sans gestion) et une zone gérée par arrachage. Nous avons testé l'effet de cette gestion sur le phytoplancton, le zooplancton et les macroinvertébrés.

Dans la première partie, nous avons étudié les effets à court terme de la gestion des macrophytes. Nous avons analysé trois paramètres structurels et trois paramètres fonctionnels pour chaque groupe taxonomique, que nous avons combinés dans un système de notation. Dans l'ensemble, l'arrachage des macrophytes a eu un effet négatif sur la biodiversité, en particulier sur les assemblages de zooplancton et de macroinvertébrés. En revanche, l'arrachage des plantes a eu des effets positifs sur le phytoplancton. Les effets étaient plus prononcés une semaine après l'enlèvement que six semaines après. Nous avons également montré un effet site marqué.

Dans la deuxième partie, nous avons étudié la capacité de résilience des communautés un an après l'arrachage des macrophytes. Tous les effets à court terme n'étaient plus visibles après un an. De plus, nous avons montré que les communautés de zooplancton et de macroinvertébrés ne présentaient aucune différence après l'arrachage des macrophytes par rapport au site témoin, ce qui montre une

grande résilience de ces deux groupes à l'enlèvement des macrophytes, bien que les macrophytes n'aient pas encore repoussé un an après. Seule la communauté phytoplanctonique du lac français a différé après l'arrachage des plantes.

Notre étude a montré que les effets d'une gestion partielle des herbiers affectent négativement la biodiversité, en particulier les macroinvertébrés et le zooplancton. Cependant, les effets ne sont que de courte durée, et les communautés montrent une forte résilience. Par conséquent, la gestion par arrachage des macrophytes pourrait être une solution pour maintenir des services écosystémiques multiples sans impacter la biodiversité. Il est nécessaire cependant de mener des études préalables détaillées avant de prendre une décision de gestion visant à éliminer les macrophytes.

Discussion générale :

L'un des plus grands défis de ce siècle est de ralentir le déclin de la biodiversité, en particulier la biodiversité d'eau douce, qui connaît une réduction plus forte que la biodiversité terrestre ou marine (WWF, 2022). Les proliférations végétales sont souvent considérées comme responsables d'une perte de biodiversité dans les écosystèmes aquatiques. Les herbiers denses de macrophytes sont souvent gérés, car ils entravent les activités humaines. Cependant, leurs effets sur la biodiversité restent peu clair, sans parler des conséquences de leur arrachage. Pour combler ce manque de connaissances, les effets des herbiers denses de macrophytes et de leur arrachage sur la biodiversité (phytoplancton, zooplancton et macroinvertébrés) ont été étudiés dans le cadre de cette thèse.

Rôle des herbiers denses de macrophytes sur la biodiversité

Les macrophytes jouent un rôle important dans de nombreux systèmes aquatiques (Thomaz & Cunha, 2010). Leur impact sur d'autres organismes, tels que le phytoplancton, le zooplancton ou les macroinvertébrés, est déjà bien connu. Cependant, la plupart de ces études portent sur des communautés naturelles stables de macrophytes. Les herbiers denses de macrophytes diffèrent à

de nombreux égards des communautés végétales stables et diversifiées (Thiemer, 2022).

Dans le chapitre 1, nous avons analysé l'effet des herbiers denses de macrophytes par rapport aux zones d'eau libre pour le phytoplancton, le zooplancton et les macroinvertébrés. Bien que nous ayons trouvé une densité et une richesse taxonomique accrues du zooplancton et des macroinvertébrés dans les herbiers denses de macrophytes par rapport à l'eau libre, nous n'avons pas trouvé de différences pour le phytoplancton. Comparé au zooplancton et aux macroinvertébrés, le phytoplancton n'a aucune possibilité de migration horizontale active, et à cette petite échelle, on peut s'attendre à ce que le mouvement de l'eau homogénéise les communautés. Cependant, des études antérieures ont montré une augmentation de la richesse en taxons également pour le phytoplancton (Declerck et al., 2007).

En termes de structure des communautés, nous avons trouvé des différences dans les assemblages entre macrophytes et eau libre pour les trois groupes. Nous avons trouvé des taxons de macroinvertébrés spécifiques dans les macrophytes et d'autres dans l'eau libre. Nous avons également montré que différents taxons de macroinvertébrés habitent dans les deux espèces de macrophytes dans le même lac, le Lac Ebolow (Misteli et al., 2022). De plus, nous avons montré que la composition de la communauté diffère entre les zones de macrophytes et d'eau libre pour le zooplancton et le phytoplancton. Dans l'ensemble, nos résultats montrent cependant une grande variabilité entre les sites. Compte tenu de la grande diversité des sites et des espèces de macrophytes, cette variabilité des résultats n'est pas surprenante. Malgré le nombre relativement faible de sites étudiés, nous pouvons tout de même mettre en évidence une tendance générale. Cependant, d'autres recherches sur un plus large panel de sites sont nécessaires pour mieux caractériser les effets des macrophytes sur la biodiversité.

Par ailleurs, nous avons étudié le rôle de trois types biologiques de macrophytes (flotttant : *Pistia stratiotes*, submergé : *Egeria densa* , émergent : *Ludwigia* spp.) et des poissons sur le zooplancton par une approche en conditions semi-contrôlées en utilisant des mésocosmes (chapitre 2). Nous avons montré que les macrophytes ont un impact sur la structuration de la communauté zooplanctonique

par rapport à l'eau libre, même lorsque la taille de l'herbier est réduite à quelques dizaines de cm. Pendant la journée, les trois espèces de macrophytes étaient privilégiées par la plupart des taxons de zooplancton. Ceci est en accord avec des études antérieures montrant que les macrophytes sont un refuge essentiel pour le zooplancton pendant la journée (Burks et al., 2002). La migration diurne du zooplancton vers les macrophytes (DHM ; Burks et al., 2002) a également été confirmée par la comparaison entre l'échantillonnage de jour et l'échantillonnage de nuit. La densité et la diversité du zooplancton augmentaient avec la complexité architecturale de l'espèce de macrophytes. Le réseau complexe de racines du *Pistia stratiotes* flottant a en effet attiré plus de zooplancton que les feuilles et les tiges simples de l'*Egeria densa* et de *Ludwigia* spp. Comme les tiges et les feuilles, les racines offrent un substrat pour la croissance d'un biofilm bien développé, fournissant de la nourriture au zooplancton (Han et al., 2018). Ce résultat montre que l'effet des macrophytes sur le zooplancton dépend principalement de la complexité de l'architecture de la plante, ce qui est similaire aux effets des macrophytes indigènes sur le zooplancton (Bolduc et al., 2020). La présence de poissons n'a pas changé les résultats de manière significative. Les prédateurs macroinvertébrés qui ont colonisé les mésocosmes peuvent déjà être suffisants pour provoquer des effets similaires à ceux attendus pour les poissons, puisque le zooplancton a montré des DHM même sans poissons. Notre résultat est en accord avec une étude antérieure montrant que le rôle des macrophytes sur le zooplancton (zone refuge vs. zone à risque) dépend de la communauté de macroinvertébrés (Sagrario et al., 2009). Ce résultat indique que nos conclusions ne sont pas seulement valables dans des systèmes comme les lacs ou les rivières peu profondes, qui sont généralement habités par des poissons, mais aussi dans des écosystèmes plus petits comme les étangs et mares qui sont souvent exempts de poissons. Afin d'évaluer le rôle de la présence de prédateurs sur le choix de l'habitat du zooplancton, une étude ultérieure sans macroinvertébrés s'avère indispensable.

Les herbiers denses de macrophytes et leur impact sur l'efficacité de l'échantillonnage

Comme nous nous attendions à trouver une structure de communauté différente au sein des macrophytes et de l'eau libre, nous avons également analysé l'efficacité d'échantillonnage de nos méthodes respectives pour les trois groupes dans le chapitre 2. Nous avons émis l'hypothèse qu'en raison du nombre plus élevé de taxons et de l'hétérogénéité structurelle accrue au sein des macrophytes, le nombre d'échantillons requis est plus élevé dans les macrophytes pour atteindre la même efficacité d'échantillonnage (% de taxons de la communauté globale collectés) que dans les eaux libres. Cette hypothèse a été confirmée dans la plupart des sites pour les trois groupes. Avec cinq échantillons par section, nous avons couvert une plus petite proportion de la communauté dans les macrophytes que dans les eaux libres, ce qui indique une sous-estimation de la communauté réelle trouvée dans les macrophytes. Cet écart dans l'efficacité de l'échantillonnage est plus important que les différences de densité et de richesse entre les deux sections et a montré moins de variabilité. À notre connaissance, il s'agit de la première analyse de l'efficacité de l'échantillonnage à l'intérieur et à l'extérieur des macrophytes sur trois groupes d'organismes différents. Nos résultats devraient être pris en compte lors de la planification de suivis biodiversité et dans le cadre de la recherche sur les macrophytes. Déplus, le nombre d'échantillons prélevés ne peut pas être augmenté pour réduire ces différences d'efficacité d'échantillonnage. D'autres moyens de tenir compte de ces différences doivent être envisagés, comme l'utilisation de la richesse estimée des espèces (Chao et al., 2014) ou le mélange de sous-échantillons (Rodríguez-Ramos et al., 2014).

La gestion des proliférations de macrophytes et ses conséquences sur la biodiversité

Le développement d'herbiers de macrophytes est souvent considéré comme une nuisance et les macrophytes sont donc gérés. À la lumière de nos résultats, nous émettons l'hypothèse que l'arrachage des macrophytes a un impact négatif sur la biodiversité du système, en particulier sur le zooplancton et les macroinvertébrés.

L'effet de l'arrachage des macrophytes a déjà été étudié auparavant (voir par example: Choi, Jeong,

La, et al., 2014; Greer et al., 2012; Känel et al., 1998; Mangas-Ramírez & Elías-Gutiérrez, 2004). Cependant, les recherches existantes se concentrent soit sur un seul groupe, un seul site d'étude, une seule date, ou ont utilisé un design d'étude faible (section témoin manquante ou échantillonnage " avant " manquant). Dans le cadre de cette thèse, les effets à court et à long terme de l'arrachage des macrophytes ont été recherchés sur le phytoplancton, le zooplancton et les macroinvertébrés de plusieurs sites en utilisant le même protocole BACI. Le choix de ce plan d'échantillonnage s'est avéré nécessaire, car nous avons constaté une forte variation temporelle dans nos communautés échantillonnées, que nous n'aurions pas détectée sans l'utilisation d'une zone témoin. De plus, nous avons également trouvé des différences dans les deux zones d'échantillonnage avant l'arrachage des macrophytes, ce qui montre l'importance de l'échantillonnage avant l'impact, dans notre cas, l'arrachage des macrophytes.

Notre hypothèse a été confirmée : nous avons trouvé des effets négatifs de l'arrachage des macrophytes sur le zooplancton et les macroinvertébrés une semaine après l'enlèvement, alors que des effets positifs sur le phytoplancton ont été trouvés. Six semaines après l'arrachage, les conséquences de l'impact n'étaient plus que faibles ; un an après la gestion, nous n'avons plus trouvé d'effet de l'impact. Il s'agit d'un résultat important, car la plupart des recherches publiées sur les effets de l'arrachage des macrophytes ne couvrent que les effets à quelques jours (voire quelques mois après l'enlèvement des macrophytes) et n'utilisent souvent qu'un seul point dans le temps. Elles ne rendent généralement compte que des effets à court terme de l'arrachage des macrophytes et négligent une éventuelle variabilité temporelle.

Sur la base de nos résultats concernant les effets des macrophytes sur la structure de la communauté (cf. Chap. 1), nous supposons que les communautés de la zone impactée par l'arrachage des plantes pourraient être différentes de la zone témoin, et qu'elles ressemblent plus à une communauté d'eau libre qu'à une communauté de macrophytes. Cette hypothèse ne peut être confirmée que pour le phytoplancton dans un site, tandis que les communautés de zooplancton et de macroinvertébrés ont montré une grande résilience et sont revenues à l'état antérieur à l'arrachage. L'arrachage

incomplet des macrophytes (racines restantes, fragments de plantes et litière végétale dans le système) et la repousse rapide des plantes pourraient aider la communauté à rester résiliente, en particulier compte tenu de la proximité d'herbiers restants.

L'arrachage des herbiers denses de macrophytes pourrait donc être un outil approprié pour maintenir les multiples services écosystémiques sans nuire à la biodiversité locale à long terme. Cependant, nos résultats ont également montré un effet site important, ce qui montre la nécessité d'une évaluation locale des effets sur la biodiversité avant l'arrachage des macrophytes.

Résoudre le dilemme de la gestion : y a-t-il une issue ?

L'arrachage partiel des macrophytes dans un lac et leur repousse rapide est une question importante pour les acteurs locaux qui gèrent les macrophytes. L'arrachage couramment utilisé n'offre généralement pas une solution durable, car les mesures doivent être répétées régulièrement et sont coûteuses. Par exemple, dans le lac Kemnade, l'un de nos sites d'étude, le bateau de fauchage a été actif quotidiennement pendant la phase de croissance des plantes pendant plusieurs années. Pour la gestion d'E. *nuttallii* dans le lac de Kemnade, une liste de mesures possibles pour contrôler le développement des herbiers a été développée (Podraza, 2017). En plus de l'enlèvement mécanique actuel avec le bateau faucardeur, les mesures de contrôle suivantes ont été proposées : l'utilisation de pesticides, l'approfondissement du lac par l'enlèvement des sédiments, la vidange du lac et la destruction de la banque de graines dans des conditions de congélation/séchage du sédiment, une bâche noire recouvrant les plantes pour réduire la lumière, l'introduction de poissons herbivores pour le biocontrôle, ou l'augmentation de la turbidité par une croissance accrue du phytoplancton induite par l'ajout de phosphore. L'utilisation de pesticides, l'introduction de poissons herbivores et l'ajout de phosphore sont cependant interdits par la loi allemande. L'approfondissement du lac ne serait pas une réussite, car il a été démontré que *E. nuttallii* peut se développer jusqu'à une profondeur de 10 m (profondeur moyenne actuelle du lac : 2,4 m). Pour la bâche noire, la zone du lac à traiter est souvent trop grande (ici 1,25 km^2, qui est principalement couverte de macrophytes)

et la vidange complète du système est techniquement impossible pour beaucoup de réservoirs. Toutes ces réglementations et restrictions laissent l'enlèvement mécanique à l'aide d'un bateau faucardeur comme seule solution. Cela pose un dilemme, surtout en Europe, où l'utilisation d'herbicides et de biocontrôle sont interdites par la loi européenne et/ou nationale (Hussner et al., 2017). Si l'arrachage mécanique est un outil pratique pour obtenir un succès à court terme sans nuire fortement à l'écosystème, ce n'est pas une méthode de gestion durable. Pour améliorer la gestion des proliférations de macrophytes à l'avenir, les méthodes de gestion disponibles doivent être améliorées, et les réglementations doivent également être révisées. L'utilisation de la lutte biologique ou chimique pourrait être une méthode durable pour gérer les développements de macrophytes (Champion & Wells, 2014 ; Coetzee et al., 2021). Cependant, ces deux méthodes nécessitent des recherches approfondies sur leur impact environnemental avant de pouvoir être appliquées. L'utilisation de méthodes de gestion différentes de celles couvertes par nos études, telles que la suppression biologique ou chimique ou les mesures de contrôle mécanique ayant un impact plus substantiel discuté ci-dessus, pourrait avoir un impact sur la biodiversité différent de celui que nous avons trouvé dans notre étude, et une réévaluation des effets de l'enlèvement est nécessaire. Dans cette thèse, nous avons étudié l'effet de l'arrachage des macrophytes par enlèvement mécanique dans seulement une fraction du lac/rivière. Une gestion plus durable inclurait l'arrachage du macrophyte dans l'ensemble du système. Un tel arrachage pourrait avoir un impact plus important et durable sur le système qu'un arrachage partiel. En considérant qu'une espèce de macrophyte est éliminée avec succès, nous nous attendons à trois résultats possibles. Premièrement, l'ensemble du système pourrait basculer d'un système dominé par les macrophytes à un système dominé par le phytoplancton (Scheffer et al., 1993). Deuxièmement, une nouvelle espèce de macrophyte invasive pourrait envahir la niche vide. Dans la rivière Ruhr et ses réservoirs (lac Kemnade et réservoirs plus anciens), le premier développement massif de macrophytes a été enregistré en 1930. Depuis lors, des mesures de gestion ont été appliquées, entraînant une succession de différentes espèces de macrophytes jusqu'à l'invasion actuelle d'*E. nuttallii*. (Podraza

et al., 2008). Le troisième résultat possible serait la colonisation d'un mélange d'espèces de macrophytes indigènes remplaçant les herbiers denses monospécifiques. La première option, un système dominé par le phytoplancton, élimine les herbiers denses de macrophytes qui posent unproblème s'ils sont immergés, mais elle s'accompagne souvent d'une augmentation de la turbidité de l'eau induit par les algues et des proliférations de cyanobactéries toxiques, qui nuisent à l'approvisionnement en eau potable et aux activités récréatives (Hilt et al., 2017). La deuxième option consiste à remplacer un macrophyte problématique par un autre, ce qui entraînera des problèmes similaires dès que les macrophytes recommenceront à pousser en tapis denses. La troisième option, qui consiste à maintenir un état dominé par les macrophytes avec un mélange d'espèces de macrophytes indigènes, pourrait, dans la plupart des cas, être le résultat souhaité. Dans le cas de macrophytes envahissants, l'espèce envahissante pourrait être éliminée. Cependant, selon le système, les communautés de macrophytes indigènes peuvent également poser des problèmes, comme on l'a vu dans la rivière Spree.

L'eutrophisation, la modification des systèmes hydrographiques par l'homme (par exemple, en raison de la construction de barrages pour la production d'énergie hydroélectrique) et la propagation d'espèces envahissantes ont conduit à la propagation problématique des macrophytes envahissants et à l'augmentation des macrophytes. Si la gestion durable des systèmes est souhaitée, elle est presque impossible sans s'attaquer aux causes susmentionnées du développement des macrophytes. Bien qu'il incombe aux acteurs locaux de contrôler les herbiers denses de macrophytes, des changements à plus grande échelle sont nécessaires pour résoudre ce problème. Des réglementations régionales, nationales, voire internationales, sont nécessaires pour mettre fin à ces impacts croissants et fournir un cadre pour des mesures efficaces de contrôle des macrophytes. D'ici là, les acteurs locaux doivent mettre en balance les avantages et les inconvénients de l'arrachage des macrophytes pour tous les services écosystémiques et, sur cette base, décider s'il vaut la peine de gérer les macrophytes de manière répétée.

Conclusion :

Les macrophytes jouent un rôle important dans les écosystèmes aquatiques. Même les herbiers denses monospécifiques et les espèces de macrophytes envahissantes peuvent avoir un effet positif sur la biodiversité. L'évaluation de la diversité dans les herbiers denses de macrophytes est souvent sous-estimée avec un effort d'échantillonnage peu adapté, car calé sur celui utilisé dans l'eau libre.

Le développement d'herbiers denses de macrophytes dans les écosystèmes d'eau douce entraîne des nuisances pour l'homme, et les macrophytes sont souvent gérés. Cependant, l'arrachage des macrophytes pourrait réduire la biodiversité, étant donné que la présence de macrophytes favorise la biodiversité. Nos résultats ont confirmé que cela est vrai pour le zooplancton et les macroinvertébrés, tandis que le phytoplancton profite de l'arrachage des macrophytes. Néanmoins, nos résultats ont également montré que ces effets étaient temporaires avec un optimum directement après la gestion et sans effet un an après.

Nous avons constaté une grande variabilité de réponses entre les sites dans nos résultats, ce qui suggère que davantage de recherches et d'études locales soient nécessaires pour élaborer des stratégies de gestion fondées sur des connaissances scientifiques. En outre, non seulement la biodiversité, mais aussi de nombreuses autres fonctions de l'écosystème, telles que la rétention des nutriments, l'enfouissement du carbone et les émissions de gaz à effet de serre, pourraient être affectées par l'arrachage des macrophytes. Le développement d'une stratégie de gestion durable nécessite une évaluation conjointe de tous les effets positifs et négatifs possibles de l'arrachage des macrophytes.

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UNIVERSITÉ DE RENNES 1

Titre : Le rôle structurant des macrophytes sur les communautés aquatiques: une approche internationale et multi groupes

Mots clés : gestion, type biologique, biodiversité, macroinvertébrés, zooplancton, phytoplancton

Résumé : Les proliférations végétales sont une source majeure de préoccupations pour les gestionnaires. Les herbiers denses de macrophytes peuvent entraver les activités humaines telles que la navigation de plaisance, la pêche, etc. Afin de limiter ces nuisances, l'arrachage mécanique est la mesure de gestion généralement utilisée. Néanmoins, ces herbiers aquatiques constituent des habitats pour la faune et un potentiel support de biodiversité. Les effets des herbiers denses de macrophytes et de leur arrachage sur la biodiversité ne sont pas clairement connus. Nous supposons que les herbiers denses de macrophytes ont un impact positif sur la richesse et l'abondance du zooplancton, du phytoplancton et des macroinvertébrés. Ainsi, notre hypothèse est que l'arrachage réduise la biodiversité dans les sites gérés. Nos résultats ont démontré que la présence des herbiers de macrophytes est favorable à la biodiversité et ce pour les trois groupes étudiés. Toutefois l'effet est site dépendant. Nous avons mis en évidence par ailleurs qu'une pression d'échantillonnage plus importante est nécessaire au sein des herbiers par comparaison avec l'eau libre. Nous avons également établi que le zooplancton est influencé par la présence de plantes mais que l'effet varie en fonction du type biologique de l'espèce de macrophyte. L' arrachage mécanique des macrophytes affecte négativement la biodiversité globale, en particulier le zooplancton et les assemblages de macroinvertébrés. En revanche, il a un effet positif sur le phytoplancton. L'impact de l'arrachage sur les communautés est plus marqué une semaine après mais diminue avec le temps. Il est également site dépendant. Cette thèse souligne l'importance de considérer les impacts de la gestion sur les services écosystémiques (rôle d'habitat) et sur la biodiversité dans le cadre du développement durable.

Title : The structuring role of macrophytes on aquatic communities: an international and multigroup approach

Keywords : macrophyte removal, plant morphotype, biodiversity, macroinvertebrates, zooplankton, phytoplankton

Abstract :

Rapid mass development of native and invasive macrophytes is causing global concerns. Dense mats of macrophytes can negatively affect activities like boating, fishing, etc., and one of the measures often applied is mechanical removal. Nonetheless, they provide habitats and thereby potentially support biodiversity. The effects of dense mats of macrophytes and their removal on biodiversity remain unclear. We hypothesize that dense mats of macrophytes have a positive impact on the richness and abundance of zooplankton, phytoplankton, and macroinvertebrates. Thus, the removal of macrophytes is expected to reduce biodiversity in comparison to sites where macrophytes remain. We find that dense mats of macrophytes are overall beneficial for the three studied groups, but results are site-dependent. Different community structures inside and outside of macrophytes affect the sampling efficiency. A greater sampling effort is needed within macrophytes to reach comparable sampling coverage as in the open water. We also show that different plant types affect zooplankton to a different extent though in a similar way. The mechanical removal of macrophytes negatively affects the overall biodiversity, especially of zooplankton and macroinvertebrate assemblages. In contrast, plant removal has positive effects on the phytoplankton assemblages. Effects are most pronounced one week after removal and decline over time, and they differ among sites. This thesis highlights the importance of considering impacts on ecosystem services like the provision of habitats for biodiversity in sustainable management practices of macrophytes in the future.