To Mow or Not to Mow

An ecological and societal perspective on submerged aquatic plant growth

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Cover photo shows: *Myriophyllum spicatum* (Aarvederkruid in Dutch)

Back cover shows: A beautiful underwaterworld with stylized plant management

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To Mow or Not to Mow
An ecological and societal perspective on submerged aquatic plant growth

Maaien of Niet Maaien
Een ecologisch en maatschappelijk perspectief op woekerende onderwaterplanten
(met een samenvatting in het Nederlands)

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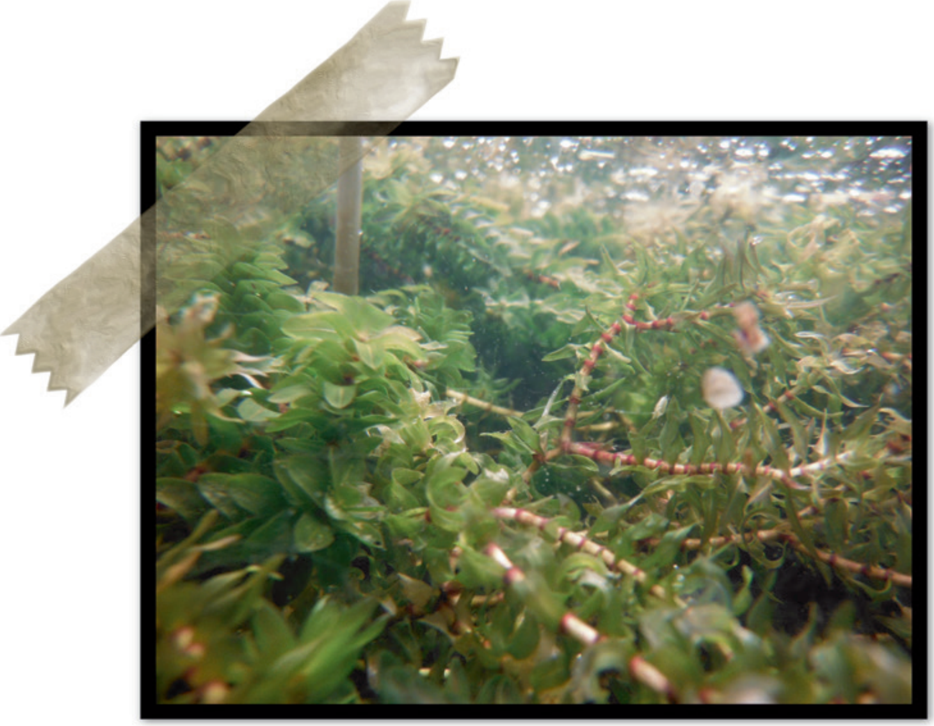

Chapter 1

General introduction

Michiel (M.J.J.M.) Verhofstad

Background

Freshwater ecosystems provide many valuable ecosystem services to humans worldwide, such as drinking water, irrigation water for crops, and food for humans (e.g. fish) (Carpenter et al., 2011). Within the shallower parts of these freshwater ecosystems (depth < ~10 m) different types of aquatic plants may grow (Middelboe & Markager, 1997; Sondergaard et al., 2013). Aquatic plants come in many shapes and sizes and can be grouped into four general growth forms: emergent species, free floating species, sediment rooted species with floating leaves, and completely submerged species (Fig. 1; Lacoul & Freedman, 2006). Emergent species root in the sediment and extend the majority of their aboveground biomass above the water surface, for example common reed (*Phragmites australis*). Free floating species have leaves that float on top of the water surface and their roots (if present) are not rooted in the sediment, for example duckweeds (*Lemna* spp.). Other species with floating leaves are rooted in the sediment and make up the third group, for example water lilies (e.g. *Nymphaea* spp.). The last group of species grows completely below the water surface, but may flower either above or below the water surface, for example *Myriophyllum spicatum* (sediment rooted submerged), *Ceratophyllum demersum* (rootless submerged), or Charophytes (i.e. freshwater macro-algae).

Benefits

Aquatic plants, and in particular submerged species, are of paramount importance for ecosystem functioning as they perform a multitude of important functions in the ecosystem (Carpenter & Lodge, 1986; Phillips et al., 2016). For example, they provide food for invertebrates, fish, and waterfowl (Fig. 2; Hargeby et al., 1994; Hansson et al., 2010). They additionally provide habitat for many invertebrate and fish species, but also for other primary producers, such as epiphyton (i.e. algae growing on top of substrates, such as plant shoots) (Fig. 2; Hargeby et al., 1994; Schriver et al., 1995; Perrow et al., 1999; Phillips et al., 2016). Furthermore, rooted aquatic plants can alter the abiotic conditions in the water and sediment and can affect the microbial community in the sediment through radial oxygen loss of their roots (Lamers et al., 2012a).

Submerged plants also provide important ecosystem services for humans, such as maintaining clear water by competing with phytoplankton for nutrients and light, but also by suppressing the phytoplankton via chemical warfare (i.e. allelopathy) (Fig. 2; Gopal & Goel, 1993; Van Donk & Van de Bund 2002; Hilt & Gross 2008; Bakker et al., 2010; Blindow et al., 2014). Rooted submerged plants can furthermore reduce sediment resuspension by stabilizing the sediment with their roots (Fig. 2; Sand-Jensen, 1998; Horppila & Nurminen, 2003; Wang et al., 2010). Next to increasing water quality, several species of aquatic plants even have direct befits to humans. Some species have been used

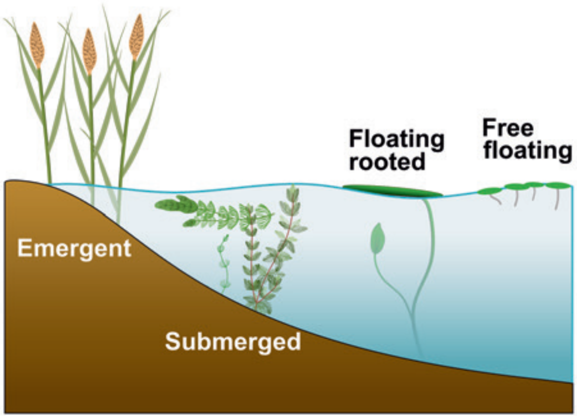

Figure 1. Scheme of aquatic plant growth forms.

for centuries as an agricultural fertilizer. For example, *Stratiotes aloides* has been mentioned frequently as an excellent fertilizer especially for potato crops in Dutch newspapers up until around 1941: *"In Drenthe, gelyk elders, met veel voordeel tot mestftof gebruikt wordende zekere Waterplant, genaamd Scheeren, Kaarden, Krabbeschaar, zynde de Stratiotes aloides van Linnæus [...]"* Vriesche Courant 26-Nov-1808. The use of aquatic plants was not unique to the Netherlands, but likely occurred all over the world where aquatic plants were available (Edwards, 1980). For example, old drawings of people harvesting aquatic plants have been found in Japan as well (personal communication, Syubei Ban, 2016; Fig. 3).

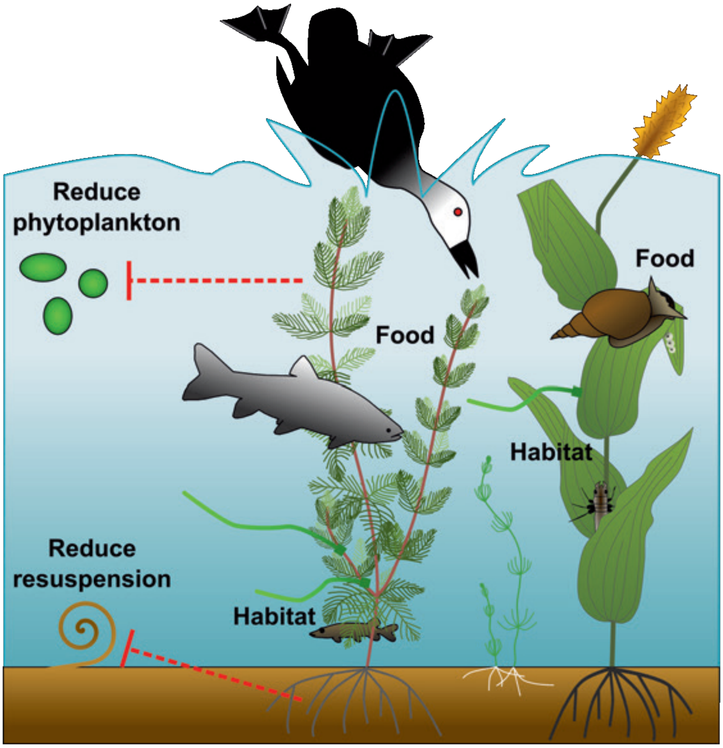

Figure 2. Main ecosystem functions of submerged aquatic plants.

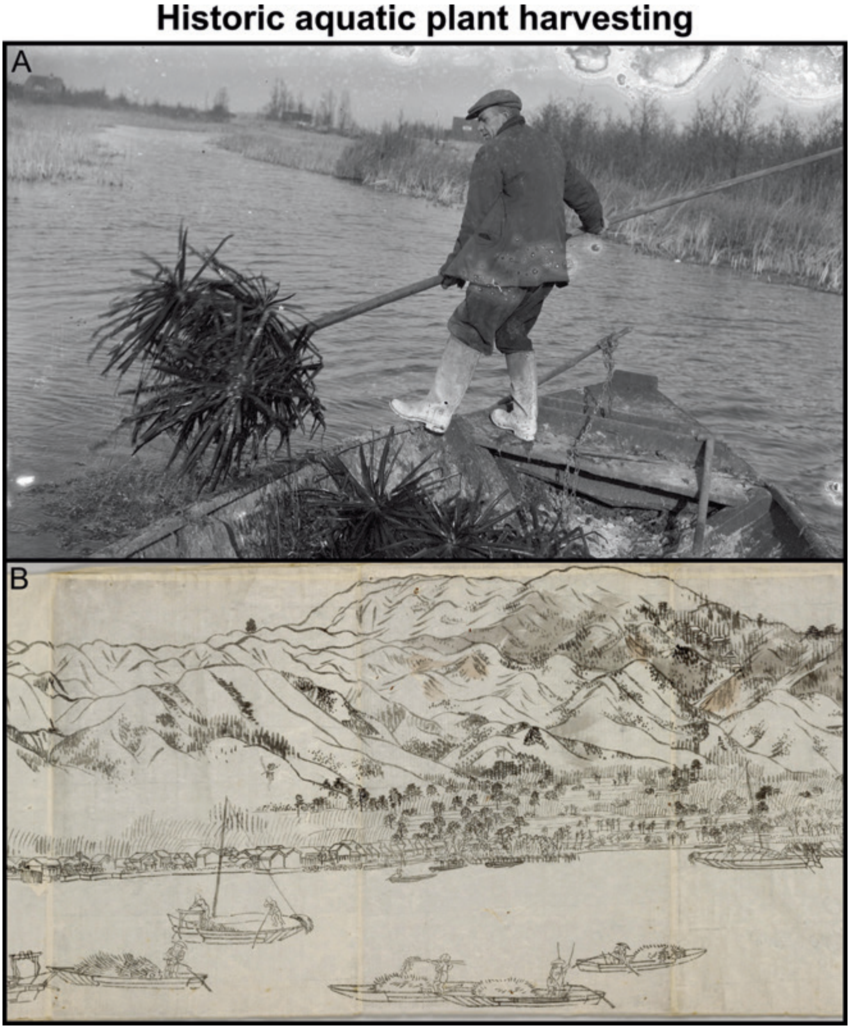

Figure 3. A. Photograph of manual aquatic plant harvesting in the Netherlands around 1941 (Stevens, 1941; courtesy of OudHilversum, the Netherlands). **B.** Ink on paper drawing of manual aquatic plant harvesting in lake Biwa, Japan around 1866 (Hakuen Hirose, 1866; courtesy of Otsu City Museum of History, Japan).

Problematic growth

Despite the many benefits of aquatic plants, they are not always deemed desirable. Sometimes aquatic plants are considered a nuisance by humans all over the world when they become overabundant (Murphy, 1988a; Nichols, 1991; Madsen, 1998; Richardson, 2008). Occasionally, aquatic plants grow so fast that they fill up the entire water column and form dense mats at the water surface (Stallings et al., 2015). This can cause large nuisance problems for humans (‘*woekerende waterplanten*’ in Dutch). These large plant stands can for example impair recreational use of the ecosystem such as swimming, boating, surfing, or fishing (Nichols, 1991; Charrudatan, 2001; Hilt et al., 2006). Large stands can also reduce water flow in rivers and canals that can lead to problems for the supply of irrigation water in irrigation channels, but can also increase the risk of flooding from clogged drainage channels (Sprecher et al., 1998; Bal et al., 2011). Dense surface mats of aquatic plants can even increase mosquito habitat and thus potentially increase the spread of mosquito-borne diseases in more tropical regions of the world (Madsen, 1998; Anderson, 2003).

Overabundance of aquatic plants is not only a problem for human use of the ecosystem, they can also negatively affect ecological functioning of the ecosystem. For example, dense surface mats can lead to periods of anoxic conditions in the water and sediment underneath these canopies, that can in turn affect aquatic fauna and water quality (Schwarz & Howard-Williams, 1993; Miranda & Hodges, 2000; Burlakova & Karatayev, 2007; Nakamura, 2008). Additionally, these dense stands are often characterized by low plant species diversity and are dominated by one or a few highly competitive species (Anderson, 2003; Richardson, 2008). Furthermore, these dense stands degrade habitat diversity and quality for a variety of animals within freshwater ecosystems (Nichols, 1991; Anderson, 2003; Stallings et al., 2015).

Often, research on nuisance growth of aquatic plants focused on problems caused by invasive plant species, but many native species can cause similar problems (Mitchell, 1996; Charrudatan, 2001; Anderson, 2003; Hilt et al., 2006; Richardson, 2008; Hussner et al., 2017).

Aims of the Thesis

During this PhD-project I focused on the problems caused by submerged aquatic plants. It is still unclear how and when nuisance growth of submerged plants actually occurs. It also remains uncertain how the problem can best be managed or halted successfully without negatively impacting other parts of the ecosystem, as management is often ineffective and its results are unpredictable. In this PhD-project I therefore aimed to (1) identify the main problems caused by nuisance aquatic plant growth and define when these problems may occur. (2) Identify why this problem is occurring at this moment in many parts of the world, including the Netherlands. (3) Identify what ecosystem managers of shallow freshwater ecosystems can do about the problems and finally (4) identify possible economical use that the nuisance aquatic plants could provide. I will explain the content of this thesis in more detail in the following sections.

(1) Defining and characterizing the nuisance problem

Surprisingly, I noticed early on that there was no clear definition of what a 'nuisance-causing' aquatic plant stand was. No general consensus was present on the dimensions (i.e. areal cover or height) at which plants started to be considered a nuisance. This is probably because there simply is no overall threshold level above which submerged plants are a nuisance and below which they are not. The threshold depends on the local services the ecosystem provides (Murphy, 1988a; Mitchell, 1996). Therefore, the first step in this PhD-project was to quantitatively define the nuisance problems, so that nuisance could be researched in a quantitative and scientific manner. In **Chapter 2** I explored how scientists, ecosystem managers, and policy makers can define the threshold level above which plants cause problems and potentially need to be managed, depending on the services provided by the ecosystem. I used a typical Dutch shallow lake as a case study to illustrate how to define the thresholds in practice.

(2) Conditions enabling nuisance

Second, I aimed to identify under what environmental conditions submerged plant stands grow to a problematic size. Scientific literature already provided a theory: high nutrient availability combined with clear water (enough light availability) would lead to fast growth of competitive submerged plants (Bloemendaal & Roelofs, 1988; Carr & Chambers, 1998; Bornette & Puijalon, 2011). Under these conditions space and light become limiting for the plants (i.e. self-shading). Several species will therefore grow very fast towards the surface to capture as much light as possible.

If nutrient availability is indeed to blame, it is logical to assume that nuisance growth of aquatic plants would also have occurred during the onset of eutrophication (e.g. Bates & Hentges, 1976), before the water became turbid by algal growth and could no longer support submerged plant life. Indeed, during the first half of the 20th century problematic aquatic plant growth was already reported in the western world (Hasler, 1945). Clear water and high nutrient availability likely also occur after restoration of eutrophicated waters. In these ecosystems, the decades of nutrient loading have made the sediment extremely nutrient rich. Improving water quality leads to high light availability combined with high sediment nutrient availability for rooted plants in these restored ecosystems, which may result in mass development of macrophytes (Bloemendaal & Roelofs, 1988; Barko et al., 1991; Carr & Chambers, 1998; Bachmann et al., 2002; Lamers et al., 2012b; Lamers et al., 2015).

However, to our knowledge, there is a lack of field data to support the theory that massive submerged plant growth is mainly governed by light and nutrient availability. In **Chapter 3** we explored how sediment nutrient availability and water clarity affected the probability of nuisance submerged plant presence in shallow Dutch lakes and ponds.

(3) Tackling the nuisance problem

Treating the cause of a problem is always better than treating the symptoms (e.g. Finlay & Vogt, 2016). However, it is not yet clear what ultimately determines whether a site will

Table 1. Main pros and cons of the main aquatic plant management methods.

Management type	Pros	Cons
Chemical (e.g. Herbicides)	Low cost; Large effect size possible; Rapid effect.	Highly destructive; Biological degradability varies; Decaying biomass causes hypoxia and nutrient release; Can negatively affect other organisms or ecosystem processes; Often illegal; Rapid regrowth possible; Risk of shifts to less desirable state of the ecosystem; Negative public perception.
Biological (e.g. Herbivores, pathogens)	Low cost; Low effort needed; Can function over multiple years; Depending on species used, can be more or less species selective.	Hard to control effect size (no effect to almost complete vegetation removal); Can negatively affect other organisms or ecosystem processes; Risk of shifts to less desirable state of the ecosystem.
Mechanical (e.g. Mowing, harvesting)	Biomass can be removed, thus removing nutrients and providing a useful product; Highly controllable; Rapid effect.	Production of plant fragments; If biomass is not removed decomposing plants deteriorate water quality; Some methods suspend the sediment; Can negatively affect other organisms or ecosystem processes; Expensive; Often only short-term effects; Risk of shifts to less desirable state of the ecosystem.

See Nichols, 1991; Madsen, 2000; Richardson, 2008; Stallings et al., 2015; Finlay & Vogt, 2016 and Hussner et al., 2017 for more extensive reviews on the topic.

harbor nuisance aquatic plant growth or not. And even if we assume for now that nutrients in the sediment are the main cause under conditions where light availability is high enough, it may not always be possible for ecosystem managers to reduce the nutrient levels in the ecosystem to such a degree that no plant can grow large enough to cause problems. This is likely why ecosystem managers, more often than not, use management methods to tackle the symptoms of the nuisance problem. In general, the main methods applied can be grouped under Chemical control (e.g. herbicide application), Biological control (e.g. introducing herbivores) and Mechanical control (e.g. mowing), all with their pros and cons (see Table 1). In this PhD-project I focused on mechanical control methods and specifically on the harvesting of nuisance submerged plants (i.e. cutting and removal of cut biomass). I focused on this method because it can be a relatively eco-friendly and highly controllable management method for reducing plant height, which simultaneously removes nutrients from the ecosystem via the harvested plant biomass (e.g. Quilliam et al., 2015; Finlay & Vogt, 2016). Another reason to focus on this method is that it is one of the most used management methods worldwide, even though the effects of harvesting are still unpredictable and often short-lived (Stallings et al., 2015; Finlay & Vogt, 2016; Hussner et al., 2017).

I performed several experiments aimed at increasing the predictability of the effects of harvesting and thereby increasing the effectiveness of the management method itself. One of the reasons why the effects of harvesting may be unpredictable could be that different submerged plant species respond differently to harvesting. Another reason could be that the effect that harvesting has on submerged plants is affected by the environmental conditions, such as light and nutrient availability (Lacoul & Freedman, 2006; Bornette & Puijalon 2011). It is well known that nutrient availability is a major driver of the growth rate of submerged aquatic plants (Barko & Smart, 1986; Best et al., 1996; Angelstein et al., 2009; Cao et al., 2012; Xie et al., 2013; Martin & Coetzee, 2014). Nutrient availability thus likely affects the plants' regrowth after cutting as well. In **Chapter 4** I explored the effects of harvesting on three different species of nuisance-causing submerged plants and how nutrient availability affected their response to harvesting, using controlled greenhouse experiments.

Intense harvesting can be very stressful for the plants. I therefore additionally needed to assess whether the submerged vegetation can handle sustained harvesting without collapsing, so that the important functions the plants perform in the ecosystem will not be lost. In **Chapter 5** I show the results of a large outdoor experiment, where the submerged vegetation was harvested with 4 different frequencies (i.e. from once a year to monthly harvests). Here, I explored how the targeted plant species responded to repeated harvesting. In **Chapter 6** a complex shallow lake ecosystem model, PCLake, was used to explore how the vegetation of an entire lake may respond to repeated harvesting over multiple years. We also tested at what intensity harvesting becomes too stressful for the submerged plants, leading to a collapse of the vegetation, and whether this is affected by the nutrient loading of the system.

On a more detailed level, I also investigated how the aquatic plant species composition and diversity might be affected by management. In **Chapter 5**, I explored how different harvesting frequencies affected aquatic plant species composition and abundance. In **Chapter 7** I zoomed out and explored how harvesting management in general may influence aquatic plant biodiversity on the landscape scale.

(4) Potential uses of nuisance aquatic plant growth

Finally, harvesting fast growing plants could potentially remove large amounts of plant biomass from ecosystems and thus large amounts of nutrients fixed within the plant biomass. Because phosphorus is a finite natural resource and is essential for agricultural food production, this plant biomass should not be treated as waste, but as a useful product (**Chapter 9**). Both in **Chapter 5** and in **Chapter 6**, I investigated how many nutrients can be removed by harvesting submerged aquatic plants and whether harvesting is a viable method to remove nutrients from the ecosystem.

There are several very promising applications for the harvested submerged plant biomass (**Chapter 9**). As mentioned at the start of this introductory chapter, aquatic plants have already been used as agricultural fertilizer or soil conditioner for centuries and this practice still has high potential today (Ho et al., 2015; Quilliam et al., 2015). If these plants are used as a fertilizer on fields close to the site where the plants were harvested, transportation is cheap and should reduce the application of external fertilizer. This in turn helps to close the local nutrient cycle (Quilliam et al., 2015). Furthermore, aquatic plants may also be used as feed for cattle (Edwards 1980; Kalita et al., 2007; Quilliam et al., 2015) or as a basis for biofuel (Abbasi et al., 1990; O'sullivan et al., 2010; Wilkie &

Evans, 2010; Quilliam et al., 2015).

The optimal use of the biomass partly depends on the characteristics of the biomass (Muñoz Escobar et al., 2011), such as water content and elemental composition (i.e. stoichiometry) (Edwards, 1980). These biomass characteristics not only differ among aquatic plant species, but can also vary greatly within a single species over space and time (Fig. 4). In the experiment reported in **Chapter 5** I explored how the C, N, P, K harvest varies over time. Furthermore, the elemental composition can be influenced by the nutrient availability in the ecosystem. I explored the elemental composition of submerged plants over a large spatial scale in **Chapter 8**.

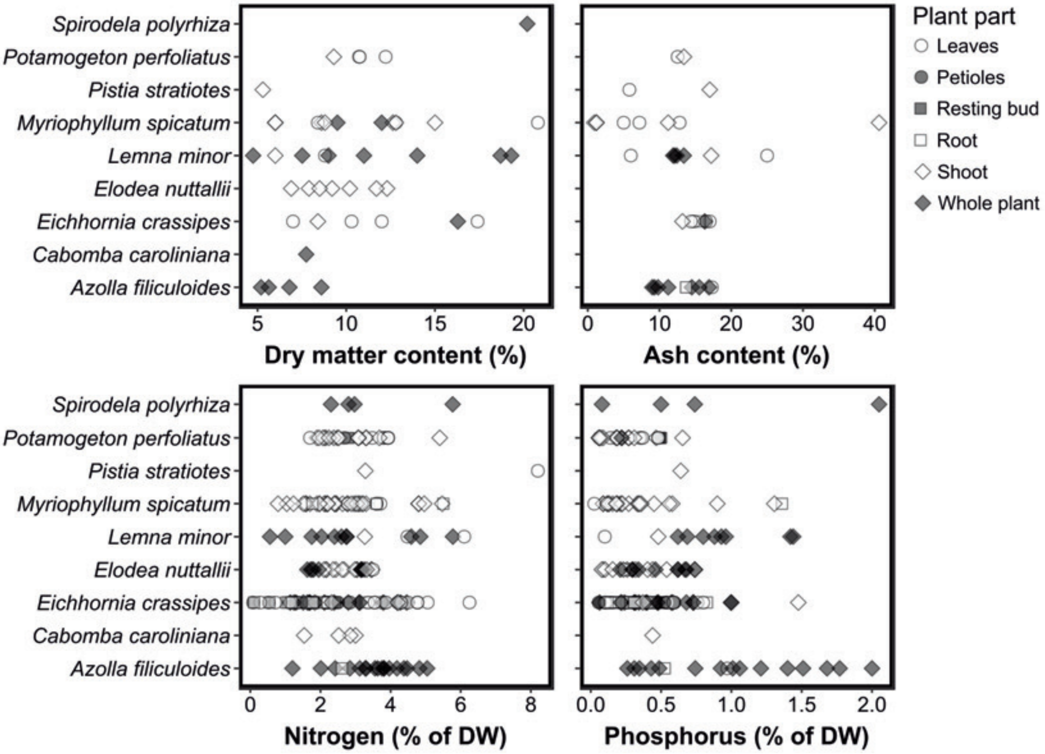

Figure 4. Variation in biomass characteristics of several free floating and submerged aquatic plant species. The plant parts that were measured are indicated by the shape and color of the points. Most often the entire plant or the entire above ground biomass (i.e. shoots) were measured (diamond shapes). Data was collected from 92 articles or reports (Verhofstad, unpublished data).

Relevance of the Thesis

In this PhD-project I gathered knowledge on 1) when aquatic plants are considered a nuisance, 2) under what environmental conditions the chance of nuisance growth is highest, 3) how submerged aquatic plants respond to harvesting, and explored 4) how the harvested biomass can be used. This knowledge will increase the predictability and effectiveness of submerged plant management. It will enable the development of sustainable submerged plant management, help to predict how many nutrients can be removed from the ecosystem via harvesting and what the most viable applications of the harvested biomass are. The combination of experimental work, literature surveys, modeling methods and extensive field surveys used in this thesis will additionally contribute to fundamental scientific knowledge on submerged vegetation dynamics and the effects of mechanical management of selected species.


MAR

Chapter 2

Classifying nuisance submerged vegetation depending on ecosystem services

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Abstract

Throughout the world, mass development of native and non-native submerged macrophytes leads to nuisance problems for humans. However, often neither the type of nuisance nor the characteristics of nuisance vegetation have been uniformly quantified, leaving nuisance vegetation as a largely unsubstantiated qualification. The lack of a synthesis of when submerged plants cause nuisance hampers comparative research on the environmental conditions leading to nuisance. Furthermore, defining and evaluating management goals to reduce nuisance caused by submerged plants is not possible when characteristics of the nuisance vegetation remain unquantified.

In this study, we performed a literature review and gathered stakeholder information to identify (1) which problems are caused by nuisance submerged macrophytes; (2) which plant characteristics underlie 'nuisance'; (3) and which species cause nuisance. We (4) synthesized this information into a framework to classify submerged vegetation as either 'nuisance' or 'non-nuisance' using a case study to illustrate the principles.

We found that most nuisance problems that affect human use of the ecosystem can be grouped into problems for boat traffic, swimming, fishing, and hydrological functioning of the system. Additionally, a multitude of ecological effects have also been reported, but these were outside of the scope of this study. Vegetation cover and canopy depth below the water surface are main determinant characters for nuisance. Therefore, both invasive and native eutrophilic species with a vertical growth strategy are particularly problematic, but other species can also cause nuisance.

Introduction

Freshwater ecosystems fulfil a wide range of ecological functions. They also provide many services for humans, including fresh water supply (e.g. for consumption and irrigation), food supply (e.g. fish), transportation routes, recreation, hydropower, and cooling water (Jackson et al., 2001; Aivazidou et al., 2016). Submerged vegetation is considered vital in shallow water ecosystems for performing a number of ecosystem functions which result in enhanced stability of a clear water state in shallow freshwater ecosystems (Carpenter & Lodge, 1986; Jeppesen et al., 1997; Van Donk & Van de Bund, 2002; Hilt & Gross, 2008). Submerged aquatic plants also provide food and habitat for other autotrophs and both vertebrate and invertebrate animal species and can thereby increase freshwater biodiversity (Hargeby et al., 1994; Schriver et al., 1995; Perrow et al., 1999; Mazzeo et al., 2003; Declerck et al., 2005; Bakker et al., 2016). An important target in restoration of degraded aquatic ecosystems is therefore the restoration of stable and diverse aquatic vegetation (Moss et al., 1996; Hilt et al., 2006; Bakker et al., 2013b). However, aquatic plants can also be perceived as problematic, in particular when they occur in large amounts (i.e. mass development) and interfere with human use of the freshwater ecosystem (Chambers et al., 1999; Hilt et al., 2006; Zehnsdorf et al., 2015).

Clear water and high availability of nutrients in the sediment are thought to facilitate this nuisance growth of macrophytes (Barko & Smart, 1986; Bini et al., 1999; Angelstein et al., 2009; Verhofstad et al., 2017). Next to nutrient effects, macrophytes generally also grow faster when temperature and light irradiance increase during the seasons (Kunii, 1984; Barrat-Segretain, 2004; Bornette & Puijalon, 2011). The annual timing of strong macrophyte development often coincides with high anthropogenic use of aquatic ecosystems, as water recreation peaks during the summer months. Nuisance growth of invasive submerged macrophytes, such as *Myriophyllum spicatum* L., *Elodea nuttallii* (Planch.) St. John, *Egeria densa* Planch. and *Cabomba caroliniana* Gray, have caused many problems for humans worldwide (Smith & Barko, 1990; Di Nino et al., 2005; Wilson et al., 2007; Tamayo & Olden, 2014). However, these effects are not limited to invasive species (Anderson, 2003; Hilt et al., 2006), as any macrophyte species that forms large stands may cause nuisance to one of the freshwater ecosystem functions or services.

Whereas nuisance growth of submerged macrophytes is regularly reported both in scientific and in popular media, it is largely undefined what ‘nuisance’ actually is. What characteristics (e.g. species composition, plant height, plant cover, etc.) does a vegetation have to be a nuisance to humans? To our knowledge, no quantitative method is available to classify whether submerged vegetation creates nuisance for specific ecosystem services or not. In this study, we developed a quantitative approach to identify and classify submerged nuisance aquatic vegetation.

We performed a review of international scientific literature to: (1) identify which problems are caused by nuisance submerged macrophytes and to which ecosystem services these are related; (2) identify macrophyte characteristics which were used to describe the vegetation as ‘nuisance’; (3) identify which species have been reported to grow to nuisance levels and whether these are native or non-native species. We used an average Dutch lake as a case study to (4) illustrate our framework classifying a vegetation as either ‘nuisance’ or ‘non-nuisance’ using selected plant characteristics and differentiating on the functions and services the ecosystem fulfils.

Methods

Literature survey

We performed a systematic search of the scientific literature, to find the most common problems reported with submerged macrophytes, the vegetation characteristics linked to these problems, and which species most often cause these problems. To find relevant scientific peer-reviewed papers, the Web of Science search engine was used with the following query:

Query: “TOPIC: (nuisance OR noxious OR problematic) AND (macrophyte* OR (aquatic AND plant*) OR (aquatic AND weed*))”.

This search yielded 346 hits on the 10th of February 2017 covering a wide range of aquatic systems and geographic regions (see Supplementary Table S 1). Based on the title and abstract, papers were selected for processing if they contained information on problems caused by submerged freshwater nuisance macrophytes and/or criteria which described macrophytes as nuisance and/or which species were considered a nuisance. Papers describing invasive non-native species were only included in the analysis if the plants were considered a nuisance because of the amount at which they occurred, not just because of the fact that they were non-native to that specific site. We found 81 papers that met our criteria from waterways and waterbodies located in North- and South-America, Europe, Africa and Australia, with the USA being the largest source of scientific studies (Table S 1). Several scientific papers cited local 'grey literature' next to peer-reviewed papers and this local knowledge is therefore indirectly also included in this review. From the selected paper, we extracted (1) problems mentioned that were caused by submerged macrophytes, (2) macrophyte vegetation characteristics linked to nuisance, and (3) nuisance macrophyte species reported.

Case study

Finally, we used the information gathered from the literature review to develop a framework classifying a macrophyte stand as either nuisance or not, depending on local conditions, such as, ecosystem service provided and water depth. To define the threshold levels at which submerged macrophytes cause nuisance that our framework requires, the information in the scientific literature was insufficient. For our case study, we therefore gathered additional information concerning the perception of nuisance in the Netherlands from recreational specialists at Alterra (Wageningen University and Research centre, The Netherlands), the Netherland's Sport Fishing Association (SVN), and the Dutch Water Sports Industry Association (HISWA). We also included information from a survey among 1269 interviewed Dutch recreationalists (for more detailed information see Goossen et al., 1997). This survey contained information on how important people value water quality and aquatic vegetation for engaging in recreational activities as fishing, swimming, and boating (Goossen et al., 1997). We used a model shallow (2 m) freshwater ecosystem as our case study, because these ecosystems are very common and typical for the Netherlands and many other countries (e.g. Gulati & Van Donk, 2002; Søndergaard et al., 2007). The framework can be tailored to specific waterbodies or waterways by surveying local users about their problems with aquatic vegetation and incorporating corresponding threshold levels of plant abundance into the classification scheme. The thresholds mentioned in our case study (see results section) are given specifically for the location that is actually used by the community, not for the entire ecosystem. We further assumed homogeneous vegetation distribution in the case study and will discuss spatial heterogeneity and implications for the whole ecosystem in the discussion.

Results

Recorded problems

A wide range of plant induced problems have been reported depending on the use of the aquatic system at hand (Table 1). The main problems can be categorized as problems for boat traffic, fishing, swimming, and hydrodynamics. Additional problems that were mentioned less frequent included problems concerning decreased aesthetics, increased sedimentation, and altered nutrient cycles (grouped under the category '*Other*' in Table 1). Many studies (n = 38) also mentioned problems that can be categorized as problems for ecological functioning, especially with regard to non-native species. Because these problems for nature do not necessarily directly impair the anthropogenic functions described, they are outside of the scope of this paper and included in the '*Other'* category, but we strongly recommend this to be taken up in future research.

Characteristics of nuisance submerged vegetation

Vegetation that was considered to be a nuisance was mainly characterised by high plant growth rate, tall height, high coverage, forming (near) monospecific vegetation, and / or high biomass (Table S 3). Plant height (n = 24) and coverage (n = 45) were the most frequently described plant parameters related to nuisance. Most striking was that only 5 of the 78 scientific papers used in this review provided a quantitative measure of nuisance

Table 1. Summary of the main services provided by the freshwater ecosystem where nuisance aquatic plants cause problems as stated in scientific literature. See Table S 2 and reference list (Table S 1) for actual references.

Main functions categorized	Category description	Number of references
Boat traffic	Impairment of physical movement of a boat or ship through the water by macrophytes, both recreational and commercial in nature.	[32]
Fishing	All problems created by macrophytes which hamper recreational or commercial fishing activities.	[18]
Swimming	All problems created by macrophytes which lead to less people entering the water for the purpose of (recreational) swimming.	[17]
Hydrodynamics	All problems caused by macrophytes which lead to problematic reduction in water flow or discharge capacity in waterways, but also congestion of waterbodies and clogging of (industrial) intake pipes.	[34]
Other *(Including 'Ecological')*	All others, ranging from decreased aesthetics and waterfront property value to increased sedimentation and altering nutrient cycling of the system. All (biotic) problems created by nuisance macrophytes which concern changes in biodiversity, in the vegetation, biotic communities, available habitat for other species and damage to the ecosystem in general. Irrespective of whether this impairs human activities or not.	[47]

vegetation, namely > 50 % plant cover, < 0.5 m plant canopy depth, or > 0.5 m plant height under 1 – 2 m of water depth (Brandrud & Roelofs, 1995; Mataraza et al. 1999; David et al. 2006; Richardson 2008; Alwin et al. 2010). In most papers a specific biomass per square metre, coverage, or plant height at which the vegetation was considered as a nuisance was lacking. Several authors did not provide a description of nuisance vegetation but only stated that macrophytes were a nuisance because they 'caused problems'. Together with the description that plants are a nuisance if they are locally very abundant, these descriptions have been categorized as 'Other' (Table S 3), as they cannot easily be attributed to any measurable plant characteristic.

Nuisance species reported

A diverse group of 33 different submerged macrophyte species were reported as being a nuisance (Table 2). Most frequently mentioned (≥ 6 references) were: *M. spicatum, Hydrilla verticillata* Royle, *Ceratophyllum demersum* L., *E. densa*, *Lagarosiphon major* Moss, *Elodea canadensis* Michaux, and *Potamogeton pectinatus* L. *M. spicatum* was reported most often as being a nuisance species, namely in 25 papers. Several macrophyte species have been reported to be a nuisance both in their native and introduced range (7), while others have mainly been reported as nuisance in either their native (17) or introduced ranges (8) (Table 2).

Determining thresholds: Case study

Following the literature (Washington et al., 1992; Table S 3), we propose that classifying nuisance for human use can best be done by combining both plant canopy depth and plant cover as factors (Fig. 1A). With plant cover we indicate the percent surface area occupied by macrophytes. Plant canopy depth is the difference between water depth and plant height, i.e. the thickness of the water column above the vegetation canopy. Submerged macrophytes become a direct nuisance for man when too many shoots physically touch humans (*swimming*) or manmade objects (*boating* and *fishing*) in both waterbodies and waterways, or when they obstruct flow in waterways (*impede hydrodynamics*). To separate nuisance from non-nuisance vegetation based on the two selected plant parameters, it is essential to first identify at what canopy depth and cover submerged macrophytes will pose a problem for the different types of ecosystem services the aquatic ecosystem may deliver (Murphy, 1988a). The first step in determining the threshold levels above which submerged macrophytes will be considered a nuisance for specific ecosystem services is thus to identify which services are provided by the ecosystem. To determine these nuisance threshold levels, we suggest surveying the local users about how many plants are perceived to impair the ecosystem service at hand. Because perception is very subjective and can be exaggerated, we suggest incorporating the available scientific knowledge on actual problems caused by submerged plant growth where possible. Additionally, quantitative metrics about the users of the ecosystem services can also help to remove perception bias. With quantitative metrics we mean for example the size and draught of the recreational boats used for the ecosystem service:

boating. In the following sections, we will illustrate in more detail how to determine the threshold values for all ecosystem services mentioned, using the Netherlands as an example.

Thresholds for Boat traffic

In many freshwater ecosystems a large portion of boat traffic will consist of recreational vehicles. Aquatic macrophytes will become a nuisance when they entangle the propellers of motorboats or hit and wrap around the keel or swards of sailing boats. This results in reduced recreational use of the location (Eiswerth et al., 2000; Dodds et al., 2009). Threshold levels thus depend on the draught of the boats present at a location. For our case study, a cover of as low as 5 % within the navigational area is suggested to cause nuisance for boating if macrophytes grow tall enough to come in contact with the boat (HISWA, personal communication). A survey of nearly the entire Dutch recreational fleet (for details see WaterrecreatieAdvies, 2005) and advice provided by HISWA made it possible to roughly group the majority of vessels into two and estimate the draught of the most common recreational boats. The report by WaterrecreatieAdvies (2005) described the amount of vessels, type and estimated size of boats present in the Dutch recreational fleet. The first group consists mainly of small boats (until 1 to 1.25 m draughts) stored on land, but which are still frequently used. The second group consists of vessels which are mainly docked at marinas or water sports associations and mainly consist of larger boats with larger draughts up until around 2 m. Because almost all boats will draw at least 0.5 m of water, all recreational boat traffic will have problems if macrophytes are present between 0 and 0.5 m below the water surface and cover at least 5%. The majority of the Dutch fleet will encounter problems when macrophyte canopy depth lies between 0 and 1 m deep. A smaller portion of the fleet in our case study (roughly half to two third) still encounters problems when macrophyte canopy depth is between 1 and 2 m below the water surface. When macrophytes are present deeper than 2 m only very few vessels will encounter problems, even when macrophyte cover is high.

Thresholds for Fishing

Lines getting entangled in vegetation pose a major problem of submerged macrophytes for fishermen. Catch is lost and/or lines can break, leading to loss of gear and pollution of the environment. High vegetation density results in reduced recreational use of affected sites (Eiswerth et al., 2000). According to the Netherland’s Sport Fishing Association (SVN) the recreational fishermen regard a macrophyte cover of between 10 and 40 % as optimal for fishing in our case study (Peters and Van Emmerik, 2013). In the USA, a similar cover of between 20 and 40 % has been suggested as optimal for a stable fish population (AERF, 2005). A cover of 50 % and higher is considered a nuisance (SVN, personal communication). Depending on the species of fish targeted, different fishing methods will be applied. When fishing for bottom dwelling species macrophytes of only a few centimetres tall can already cause problems. When fishing for species that prefer the open water macrophytes can grow much taller before becoming a nuisance. As a

Table 2. Submerged macrophytes recorded in scientific literature as nuisance species or as dominant species in nuisance vegetation and whether they were non-native in the location at hand. Number between square brackets indicates number of cases. For the geographical location of the studies, see Table S 1.

Species	**[82] Non-native$_a$**		**[57] Native$_a$**	
Butomus umbellatus L.	[1]	Madsen et al. 2016.		
Cabomba caroliniana Gray	[3]	Akhurst et al. 2012; Bickel and Schooler 2015; Hogsden et al. 2007.	[2]	Cabrera-Walsh et al. 2011; Nelson et al. 2002.
Ceratophyllum demersum L.	[3]	Clayton and Champion 2006; David et al. 2006; Wells et al. 2003.	[7]	Charudattan 2001$_a$; Cruz et al. 2015; Fulmer and Robinson 2008; Hilt et al. 2006; Nichols 1991$_a$; Poirrier et al. 2010; Zefferman and Harris 2016.
Ceratophyllum submersum L.			[1]	Hilt et al. 2006.
Chara hispida L.			[1]	Hilt et al. 2006.
Egeria densa Planch.	[8]	Anderson 2003; Clayton and Champion 2006; David et al. 2006; Madsen 1998; Santos et al. 2009; Schwarz and Howard-Williams 1993; Stalling et al. 2015; Tamayo and Olden 2014.	[2]	Charudattan 2001$_a$; Cruz et al. 2015.
Egeria najas Planch.			[2]	Charudattan 2001$_a$; Cruz et al. 2015.
Elodea canadensis Michaux	[4]	Abernethy et al. 1996; Aguiar and Ferreira 2013; Hilt et al. 2006; Schwarz and Howard-Williams 1993.	[2]	Nichols and Shaw 1984; Nichols 1991$_a$.
Elodea nuttallii (Planch.) St.John	[1]	Hilt et al. 2006.	[1]	Zefferman and Harris 2016.
Hydrilla verticillata Royle	[18]	Anderson 2003; Bacchus and Barile 2005; Clayton and Champion 2006; Cruz et al. 2015; David et al. 2006; Evans and Wilkie 2010; Everitt et al. 1999; Godfrey et al. 1994; Gu 2006; Madsen 1993; Madsen 1998; Michelan et al. 2014; Nawrocki et al. 2016; Poirrier et al. 2010; Richardson 2008; Spencer and Ksander 1999 Stalling et al.2015; Yeo and McHenry 1977.	[2]	Charudattan 2001$_a$; Nichols 1991$_a$.
Juncus bulbosus L.			[5]	Brandrud and Roelofs 1995; Brandrud 2002; Lucassen et al. 2016; Moe et al. 2013; Schneider et al. 2013.
Lagarosyphon major Moss	[7]	Bickel and Closs 2009; Caffrey et al. 2010; Clayton and Champion 2006; Hilt et al. 2006; Mangan and Baars 2013; McKee et al. 2002; Schwarz and Howard-Williams 1993.	[1]	Charudattan 2001$_a$.
Mayaca fluviatiles Aubl.	[1]	Yakandawala and Dissanayake 2010.		
Myriophyllum alterniflorum DC.			[1]	Brandrud 2002.
Myriophyllum aquaticum (Vell.) Verdc.	[7]	Aguiar and Ferreira 2013; Anderson 2003; Clayton and Champion 2006; Coetzee et al. 2011; Hofstra et al. 2006; Wersal and Madsen 2011a and b.		
Myriophyllum heterophyllum Michx.	[2]	Bailey and Calhoun 2008; Hilt et al. 2006.		

Table 2 (continued).

Species	**[82] Non-native$_a$**		**[57] Native$_a$**	
Myriophyllum spicatum L.	**[19]**	Alwin et al. 2010; Anderson 2003; Berger et al. 2015; Burlakova and Karatayev 2007; Clayton and Champion 2006; Fulmer and Robinson 2008; Goodenberger and Klaiber 2016; Harman et al. 2005; Madsen 1993; Madsen 1998; Newman et al. 1996; Nichols and Shaw 1984; Poirrier et al. 2010; Reeves et al. 2008; Richardson 2008; Stalling et al. 2015; Tamayo and Olden 2014; Trebitz et al. 1993; Zefferman and Harris 2016.	**[6]**	Abernethy et al. 1996; Cason and Roost 2011; Charudattan 2001$_a$; Hilt et al. 2006; Nichols 1991$_a$; Richter and Gross 2013.
Myriophyllum spicatum L. x *Myriophyllum sibiricum* Kom.	**[1]**	Berger et al. 2015.	**[1]**	Berger et al. 2015.
Myriophyllum sibiricum Kom.			**[1]**	Fulmer and Robinson 2008.
Myriophyllum verticillatum L.			**[1]**	Hilt et al. 2006.
Najas flexilis (Willd.) Rostk. and Schmidt			**[1]**	Jones and Cooke 1984.
Najas guadalupensis (Spreng.) Magnus			**[2]**	Charudattan 2001$_a$; Poirrier et al. 2010.
Najas marina L.			**[2]**	Fulmer and Robinson 2008; Hilt et al. 2006.
Najas minor All.	**[1]**	Stalling et al. 2015.		
Potamogeton crispus L.	**[6]**	Albright and Ode 2011; Anderson 2003; Fulmer and Robinson 2008; Nichols and Shaw 1984; Tamayo and Olden 2014; Zefferman and Harris 2016.	**[1]**	Nichols 1991$_a$.
Potamogeton foliosus Raf.			**[1]**	Zefferman and Harris 2016.
Potamogeton gramineus L.			**[1]**	Anderson 2003.
Potamogeton lucens L.			**[1]**	Hilt et al. 2006.
Potamogeton pectinatus L.			**[7]**	Fulmer and Robinson 2008; Hilt et al. 2006; Schoonbee 1991$_a$; Sisneros et al. 1998; Slade et al. 2008; Sprecher et al. 1998; Zefferman and Harris 2016.
Potamogeton perfoliatus L.			**[2]**	Hilt et al. 2006; Van Nes et al. 2002.
Ranunculus peltatus Schrank			**[1]**	Garbey et al. 2003.
Ranunculus penicillatus subsp. pseudofluitans (Syme) Webster			**[1]**	Garbey et al. 2003.
Zannichellia palustris L.			**[1]**	Zefferman and Harris 2016.

a: Species in references annotated with '$_a$' cannot be assigned as native or non-native (f.i. because the reference was a review article). They are always placed in the 'native' column (13 cases).

waterbody or waterway will most likely be used to fish for both types of fish, a macrophyte cover of 50 % or higher is classified as a nuisance for fishing independent of canopy depth in our case study. Macrophytes can also hamper fishing by causing problems for boat navigation, but these problems are described under the section concerning '*Thresholds for Boat traffic*'.

Thresholds for Swimming

When macrophytes reach very high densities they can become dangerous for swimmers, the shoots can entangle arms and legs leading to dangerous situations. However, even when macrophyte densities are not dangerously high, many people are still deterred from swimming by submerged macrophytes touching their skin. At what cover and canopy depth people will find submerged macrophytes a nuisance will depend on personality and culture. Recreational swimmers in the Netherlands generally do not mind the presence of 'some vegetation in the water' (Goossen et al., 1997). Taking the average length of humans into account, plants present at 2 m or deeper will almost never be considered a nuisance in our case study. Plants present at 1.5 to 0 m deep might be considered a nuisance as people are likely to be able to touch them while swimming. As presence of some vegetation will still be considered tolerable and not deter people to swim in waterbodies or waterways on a warm day, the cover at which macrophytes will be considered a nuisance has been arbitrarily set at 10 % in our case study, but should be adjusted to reflect local views.

Thresholds for Hydrodynamics

One of the main functions of waterways in particular, but also of hydropower reservoirs, is the transport of water (either to drain or to supply). Everything present in the water column will create resistance to the water flow and thereby reduce discharge capacity of the system. Massive development of submerged macrophytes can thereby severely reduce the amount of water which can flow through the system in a given time, increasing the chance of flooding (Bal et al., 2011). Several authors examined the hydraulic resistance of submerged macrophytes and the effects on water flow, however no clear threshold level for cover and canopy depth above which the vegetation will cause a significant problem was reported. The nuisance threshold will depend on the characteristics of the macrophyte species (e.g. flexibility), minimum flow capacity requirement of the water system at a specific time, pattern of the vegetation in the aquatic system and the bottom morphology of the waterway (Green, 2005; Vereecken et al., 2006; Bal et al., 2011). Modelling indicated increased flow resistance at vegetation blockage of a channel of more than 50 % (for details and model conditions see: Green, 2005). Empirically, more than 42 % PVI (i.e. water volume inhabited by plants) did increase flow resistance under experimental conditions (Vereecken et al., 2006). However, up to 60 % of vegetation in an artificial channel showed a flow resistance (Vereecken et al., 2006) that would not necessarily lead to flooding problems in rivers (Bal et al., 2011). Summarizing, a PVI of 50 % appears to be a good starting point threshold level at above which macrophytes will cause hydrological problems in our case study. It should be noted that the spatial pattern of the vegetation influences the resistance it will inflict on water flow (Green, 2005; Vereecken et al., 2006; Bal et al., 2011). Furthermore, plant fragments can also easily clog pipes and pumps used for cooling water or irrigation (Richardson, 2008). However, no general threshold value can be given as this will depend on the local specification of the pump (e.g. size and power), diameter of the pipes, mesh size of debris screens, etc.

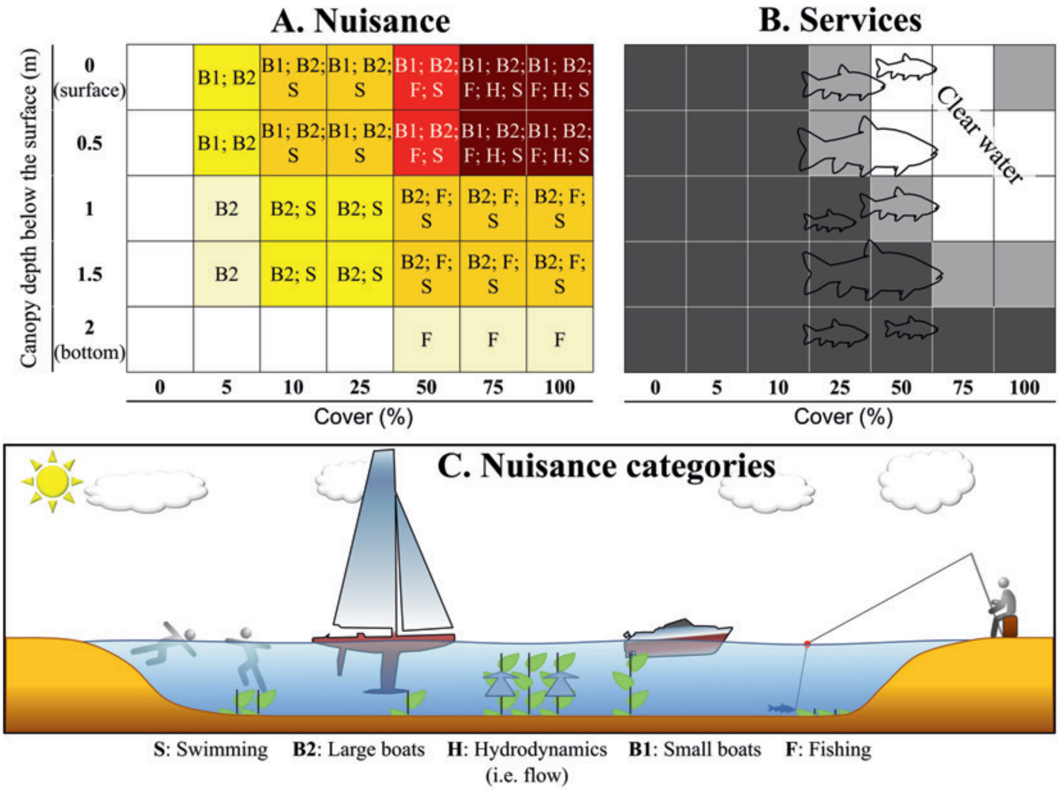
Compiling the framework

Different ecosystems may provide different ecosystem services, the first step in classifying nuisance should thus be to identify which services are provided by the ecosystem at hand. Here we will show how to develop a classification scheme for a model shallow lake in the Netherlands that provides all the ecosystem services discussed above. We combine all these threshold values for cover and canopy depth from the separate ecosystem services and create one classification scheme for nuisance submerged macrophytes for our case study. We chose an ecosystem with a water depth of 2 m as an illustrative case study, but the same method can be applied in waterbodies and waterways with different depths. Threshold nuisance submerged macrophyte levels of cover and canopy depth for boat traffic, fishing, and swimming of the case study can be used in the classification scheme without having to be transformed using our site specific water depth (B, F, S in Fig. 1A, respectively). Because our classification framework requires both canopy depth and cover as main factors, the threshold PVI value of the hydrodynamics category needs to be converted to these two parameters. The PVI can be calculated by dividing submerged macrophyte volume by water volume. Submerged macrophyte volume can be calculated by multiplying plant height with plant cover. To calculate the macrophyte canopy depth and cover at the threshold level PVI for nuisance we thus need to know the water depth. A threshold PVI of more than 50 % is reached at any combination of 75 to 100 % plant cover and canopy depth of 0.5 m below the water surface or less in our schematic (H in Fig. 1A). Even though the PVI is easy to calculate in theory, determining the PVI of plants under field conditions can be difficult. Variation in shoot densities within a plant patch can make it difficult to accurately assess true cover. Furthermore, water flow can reduce plant height by pushing the shoots downwards making actual height estimates challenging. Overall, submerged plants will not cause problems in our case study when they lie on the bottom and cover less than 50 % of the surface or when they cover less than 5 % of the area, irrespective of canopy depth at that site (Fig. 1A).

Discussion

Nuisance problems, vegetation characteristics, and nuisance species

A wide variety of problems for human use and ecological functioning of ecosystems caused by submerged macrophytes have been reported. Four main categories could be identified for human related problems: problems for boat traffic, fishing, swimming, and hydrodynamics. Each category (i.e. ecosystem service provided) has a unique threshold level at which plants become a nuisance. Threshold levels for nuisance of measurable vegetation parameters were largely absent in the scientific literature, but vegetation cover and canopy depth below the surface were most often used to describe nuisance. In our classification framework, we therefore combined plant cover with plant canopy depth as the main factors determining if a macrophyte stand will cause nuisance for a specific ecosystem service, or not.



Canopy depth below the surface (m)	0	5	10	25	50	75	100
0 (surface)		B1; B2	B1; B2; S	B1; B2; S	B1; B2; F; S	B1; B2; F; H; S	B1; B2; F; H; S
0.5		B1; B2	B1; B2; S	B1; B2; S	B1; B2; F; S	B1; B2; F; H; S	B1; B2; F; H; S
1		B2	B2; S	B2; S	B2; F; S	B2; F; S	B2; F; S
1.5		B2	B2; S	B2; S	B2; F; S	B2; F; S	B2; F; S
2 (bottom)					F	F	F

Figure 1. A: Case study (water depth = 2m) classification of nuisance macrophyte vegetation by plant canopy depth and plant cover depending on ecosystem services provided by the aquatic system, as proposed by the authors for sites in the ecosystem where the functions are performed. Letters indicate that macrophytes are classified as nuisance for each anthropogenic function: B1 (small recreational boats); B2 (large recreational boats); F (fishing); S (swimming); and H (hydrodynamics, i.e. water flow in this case). For a graphical representation, see Figure 1C. Darker (red) fills indicate more services are impaired by macrophytes.
B: Probability of maintaining two important ecosystem services (i.e. clear water and fish populations) in shallow aquatic systems considering the total area of the ecosystem. Lighter fills are generally considered more desirable for the stability of the clear water state. The school of fish indicates the plant cover that is suggested as optimal for fish populations. See discussion section: 'Aquatic plant problems versus services' for more details on how these thresholds were chosen. Fish size has no informative meaning.
C: Graphical representation of the ecosystem services impaired by submerged plant growth, as reported in Figure 1A.

Dozens of submerged macrophyte species were recorded in the scientific literature as causing problems for humans. Most of these species are known to possess a vertical growth strategy and some are notorious for forming surface canopies (e.g. *E. densa*, *Elodea* spp., *H. verticillata*, *L. major*, *M. spicatum*, *Potamogeton pectinatus*, and *P. crispus*). Species with these characteristics likely pose the biggest risk. Many of these species also require this vertical growth to reach the water surface in order to flower (see Duarte & Kalff, 1990). However, several other species reported to cause nuisance are not readily associated with this type of growth (e.g. Charophytes and *Najas flexilis*, Wingfield et al., 2006; Table 2). The list of species that can cause nuisance presented here is not exhaustive. Many more species have the potential of becoming a nuisance when environmental conditions allow for excessive growth. Environmental conditions enabling nuisance growth will most likely vary between species (reviews by Lacoul & Freedman, 2006; Bornette & Puijalon, 2011).

Applying and improving the nuisance classification framework

Integrating ecosystem morphology

We used an ecosystem with a depth of 2 m as an illustrative case study to convert nuisance threshold PVI values into canopy depth and cover. Because of the shallow depth of these ecosystems, light can often reach the bottom enabling submerged macrophyte growth throughout the ecosystem (e.g. Trebitz et al., 1993; Hilt et al., 2006; Pot & Ter Heerdt, 2014). Therefore, these shallow ecosystems may be particularly vulnerable for problems concerning nuisance growth of macrophytes (e.g. Burlakova & Karatayev, 2007; Alwin et al., 2010). The classification scheme can easily be adapted for waterbodies and waterways with different depths, as the nuisance threshold value itself remains unchanged. However, the deeper a system becomes, the less likely macrophytes are to reach the threshold value for canopy depth, and thus nuisance, due to the physiological limitations of the plants (Lacoul & Freedman, 2006).

Integrating macrophyte species characteristics

Many submerged macrophyte species have been a nuisance, but several species have been reported more often than others. This could be due to specific species' traits such as the ability to quickly grow tall and form surface canopies or show strong lateral expansion, thereby increasing the likelihood of becoming a nuisance, although species without the same traits can also become a nuisance, but perhaps not as often. Including information on specific plant species on potentially relevant plant traits like toughness (Bociag et al., 2009), flexibility or maximum shoot density could thus further improve the classification framework by including species specific threshold levels at which macrophytes are considered a nuisance. The impact species specific traits can have on the nuisance threshold levels can, for example, be included in the framework by adding a species specific correction factor (i.e. ranging from 0-1) to the general threshold levels. Here, a very mechanically strong species will have a factor of 1 and very weak species will have a lower correction factor.

The present study focussed on submerged freshwater macrophytes, but similar problems concerning nuisance growth of species with different growth forms, such as floating species have been reported (e.g. Spencer et al., 2006; Arthur et al., 2007; Wu & Wu, 2007; O'Sullivan et al., 2010; Perna et al., 2012). In contrast to submerged species, floating leaved species such as *Eichhornia crassipes* or *Trapa natans*, but also emergent species such as *Ludwigia* spp. or *Phragmites australis*, may be even more likely to cause nuisance problems, because they inherently grow on or through the water surface and thus have a high chance of causing nuisance problems to ecosystem users. Due to the general nature of the nuisance problems and the vegetation characteristics used to classify nuisance, our proposed classification can easily be adapted and extended to include macrophyte species with different growth forms. For example, the plant canopy depth factor will not be useful in the classification framework when applied to floating leaved species (i.e. canopy depth is always 0). However, the cover threshold values of the framework are still valid.

Integrating varying ecosystem services and local perception

While performing the review, it became apparent that it was impossible to create one threshold value of cover and depth above which aquatic plants will be perceived as a nuisance (e.g. Suren, 2009) and our classification will thus benefit from including site specific information on perception of nuisance. For example, Chambers et al. (1999) summarized that the tolerable upper limit of aquatic plant cover, before complaints are filed and management is triggered, lies between less than 1% and up to 50% in Canada. This is in agreement with our case study and shows the large range this threshold can have due to local views and usage requirements of the different assigned ecosystem services. We therefore propose that managers and scientist tailor the framework to the ecosystem at hand by (1) identifying the ecosystem services provided by the ecosystem, (2) determine at what percent cover and canopy depth the plants will impair each of the services provided, and (3) assess if a corresponding reduction in macrophytes is beneficial for the ecosystem or if it will lead to conflicts among different services with different plant requirements. A large reduction in submerged macrophyte PVI can potentially have detrimental effects on the ecosystem and the ecosystem services provided.

Aquatic plant problems versus services

Eliminating nuisance submerged vegetation via management may reduce anthropogenic problems for usage of aquatic systems, but many ecosystem services also rely on the ecosystem functions performed by submerged plants, such as maintaining good water quality and healthy fish populations.

Water quality is very important for recreation (Goossen et al., 1997). Fishing, hunting, and bird watching also benefit from submerged plant presence, as these plants may attract waterfowl and increase fish stocks (Noordhuis et al., 2002; AERF, 2005; O’Hare et al., 2007; Hansson et al., 2010; Peters & Van Emmerik, 2013). Removing too many plants can increase the risk of complete loss of submerged vegetation, development of potentially toxic phytoplankton blooms, and thus indirectly jeopardize several human uses of the system (Van Nes et al., 2002a; Dodds et al., 2009; Kuiper et al., 2017). However, it is unknown how much plant volume is actually needed for a stable clear water ecosystem (Hilt et al., 2006), because the minimum PVI required will differ per ecosystem and depends on factors including: macrophyte traits; nutrient status of the system; presence, type and abundance of fish, and many more (e.g. Søndergaard & Moss, 1997; Hilt & Gross, 2008; Bakker et al., 2010). Most likely, PVI in temperate freshwater ecosystems should be higher than 15 to 30 % to maintain a clear and macrophyte dominated lake (Søndergaard & Moss, 1997; Hilt et al., 2006; Nakamura et al., 2008; Søndergaard et al., 2010), but no threshold PVI level was found for waterways. However, a too high PVI of (close to) 100 % can be detrimental for water quality and ecosystem stability, especially when extensive surface canopies are formed. This can result in very low oxygen concentrations near the bottom in the macrophyte stands (Schwarz & Howard-Williams, 1993; Miranda & Hodges, 2000; Nakamura et al., 2008).

Next to increasing water clarity, submerged vegetation may additionally have a direct and positive impact on one ecosystem service in particular: fishing, likely because the aquatic plants provide food and habitat for many animals, including fish (Carpenter & Lodge, 1986), thereby maintaining a stable fish population. Similar to the provision of clear water, no single optimal value of macrophyte PVI for fish populations was found in the scientific literature. Macrophyte cover values in the region of 20-60 % have been suggested to be beneficial for fish populations and fishing (Dibble et al., 1996; AERF, 2005; Peeters & Van Emmerik, 2013). Very high macrophyte densities can result in low oxygen levels and potentially decreases fish populations (Miranda & Hodges, 2000). However, this optimal macrophyte cover may not be true for all fish species or aquatic ecosystems (e.g. stratified temperate lakes: Cheruvelil et al., 2005).

Using these suggested PVI and cover values and using our case study ecosystem with a depth of 2 m as an example, we developed an additional classification scheme showing when too few, or too many plants are present in the lake to maintain the two beneficial ecosystem functions discussed (Fig. 1B). Because these threshold values may vary among different ecosystems (Murphy, 1988a), we advise to perform an ecosystem analysis to tailor the values to specific ecosystems.

Spatial heterogeneity

It now becomes apparent that it is nearly impossible to use an entire shallow lake for the anthropogenic functions discussed while keeping the ecosystem in the plant dominated state with clear water (see Fig. 1A versus 1B). This seems only possible if abiotic factors prevent nuisance submerged plant growth all together (e.g. very low nutrient concentrations, high flow velocity, or rocky substrates). However, this does not mean that ecosystem managers will have to choose for either (1) an aquatic system in the plant dominated state, but without the possibility of unhampered anthropogenic functions, or

(2) a system without any submerged macrophytes and a high chance of poor water quality. It merely stresses the importance to incorporate whole ecosystem functioning and spatial aspects into the management plans for selected human uses of the system (Finlay & Vogt, 2016). This is especially true, because it is not always possible to assign the different ecosystem functions and services to different aquatic ecosystems.

So far, we have not addressed the spatial heterogeneity of aquatic plants and ecosystem services, but many ecosystem services do not require the whole aquatic ecosystem. Swimming, for example, will mainly be restricted to the area surrounding a beach or an easy access point and will remain close to the shoreline. So, the canopy depth and cover of the vegetation needed to prevent nuisance to the swimmers will only apply locally. Similar arguments can be made for boating and fishing. Consequently, management only needs to take place in areas where macrophyte growth actually causes problems (Finlay & Vogt, 2016). Other parts of the ecosystem can still be occupied by submerged macrophytes to maintain their functions for the ecosystem as a whole. Furthermore, submerged macrophytes themselves often show a patchy distribution in aquatic ecosystems and activities could thus be allocated towards the sites with fewer plants. If the underlying causes of nuisance submerged macrophyte growth cannot be removed, harvesting nuisance aquatic plants may be a suitable management method to temporarily alleviate local problems (Quilliam et al., 2015; Finlay & Vogt, 2016; Hussner et al., 2017). An additional advantage of this method is that it removes nutrients that are incorporated in the plant biomass from the ecosystem, which can be used for a wide variety of useful applications, for example as agricultural fertilizer or food for cattle (Edwards, 1980; Quilliam et al., 2015).

Conclusions

Our classification framework shows that the amount of plants considered to be a nuisance depends on the services the ecosystem fulfils. The framework combines vegetation cover and canopy depth as main factors for the quantitative classification of nuisance versus non-nuisance submerged vegetation. The classification framework can be used to define and evaluate lake and waterway management goals, as it is possible to quantitatively define nuisance and desirable vegetation dimensions, depending on the ecosystem service provided. The use of our classification framework in future research also enables researchers to compare individual studies where nuisance macrophyte growth is reported as well as review the underlying causes of nuisance growth.

Whereas submerged macrophytes can become nuisance vegetation for multiple ecosystem services, we stress that submerged macrophytes simultaneously provide essential services in shallow freshwater ecosystem, for example, maintaining stable clear water conditions. Therefore, we suggest that lake management should strive for spatial differentiation of human activities and plant growth if the underlying mechanisms enabling nuisance cannot be removed.

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Supplementary

Following pages contain tables S 1, S 2, and S 3.

Table S 1. List of references from the query used in the analyses with additional information on types and names of freshwater systems with reported nuisance vegetation (n=81).

Reference	Problems reported in:	Name(s) of reported ecosystem(s) with problems:	Country / region
Abernethy et al. 1996	range of freshwater systems	Not specified (lab experiment)	Europe
Aguiar and Ferreira 2013	lakes and waterways	Not specified	Iberian Peninsula / Portugal
Akhurst et al. 2012	lakes	Emigrant Creek Dam reservoir	Australia
Albright and Ode 2011	lakes	Lake Sharpe (South Dakota; US)	North America
Alwin et al. 2010	lakes and waterways	Lake Ovid, Michigan	USA
Anderson 2003	not specified: lakes and waterways in general	Examples: Olympic water-sports venue Australia; Lake Tahoe, US; Sacramento-San Joaquin Delta, US	Mainly USA & Australia
Bacchus and Barile 2005	lakes and waterways	Alexander, Wakulla, Juniper & Peacock Springs, Bullow Creek & Orange Cove, Florida	USA
Bailey and Calhoun 2008	lakes	8 lakes in Maine	USA
Berger et al. 2015	lakes and waterways	Not specified	USA
Bickel and Closs 2009	mainly lakes	Experiment in Lake Dunstan	New Zealand
Bickel and Schooler 2015	not specified, but anyways lakes	3 lakes in South-East Queensland (Lake Macdonald; Ewen Maddock Dam; Seibs Dam)	Australia
Brandrud and Roelofs 1995	mainly lakes (also general waterways)	55 lakes S and SW Norway	Norway
Brandrud 2002	lakes	SW Norway (and lit. example for Netherlands)	Norway
Burlakova and Karatayev 2007	lakes and waterways	Lake Lamar and lake Neff in Texas	USA
Cabrera-Walsh et al. 2011	lakes	Experiments in: Iberá Lake, Argentina. Mentioned from lit. in US, Australia, Japan, SE-Asia, New Guinea, China, India, Europe and S Africa.	Argentina
Caffrey et al. 2010	lakes	Lough Corrib	Ireland
Carr et al. 2003	waterways	28 rivers Ontario	Canada
Cason and Roost 2011	lakes	Wisconsin lakes	USA
Charudattan 2001	not specified: lakes and waterways in general	Examples: Brazillian hydropower reservoirs; several lakes in the US.	World (focus on USA)
Clayton and Champion 2006	lakes	Mainly Waikato hydro-lakes, New Zealand	New Zealand
Coetzee et al. 2011	lakes	Not specified	South Africa
Cruz et al. 2015	lakes and waterways	Hydroelectric reservoirs (e.g. Jupiá reservoir, Brazil)	Brazil
David et al. 2006	not specified	Not specified (experiments in Sacramento-San Joaquin River Delta, California)	USA
Evans and Wilkie 2010	not specified	Aquatic systems in South-East US	USA
Everitt et al. 1999	mainly waterway (and some lakes)	Waterways and lakes South Texas	USA
Finlay and Vogt 2016	lakes	Loch Leven	Canada
Fulmer and Robinson 2008	lakes	38 reservoirs in Arizona, US	USA
Garbey et al. 2003	waterways	Experiments in Vosges mountain streams	France
Godfrey et al. 1994	lakes	Infested pond near Mountain Ranch, California	USA
Goodenberger and Klaiber 2016	lakes	Seven county region, Minnesota	USA
Gu 2006	lakes	Florida lakes in general, US	USA
Harman et al. 2005	lakes	Lake Moraine and Hatch Lake, New York	USA
Hilt et al. 2006	lakes	Many examples in Brandenburg, Bavaria, Baden-Wuerttemberg, Lower Saxony, N Rhine-Westphalia, Saxony and Saxony-Anhalt area.	Germany
Hofstra et al. 2006	lakes and waterways	North Island and northern South Island. Fieldtest in Kaituna Wildlife Management Reserve.	New Zealand
Hogsden et al. 2007	not specified, lakes and waterways mentioned	Not specified (Experiments in lake Kasshabog, Ontaria)	Canada
Jones and Cooke 1984	lakes	Lake Rockwell, Ohio	USA
Lucassen et al. 2016	lakes	Softwater lakes in southern Norway	Europe (focus on Norway)

Reference	Problems reported in:	Name(s) of reported ecosystem(s) with problems:	Country / region
Madsen 1993	not specified, lakes and waterways mentioned	Many examples: Lake George, Buckhorn Lake; East Twin Lakes (OH); Badfish Creek; Lilly Lake (WI); Little Lake Barton.	USA
Madsen 1998	not specified	Not specified	USA
Madsen et al. 2016	lakes	Detroit Lakes	USA
Mallin et al. 2016	lakes	Greenfield Lake	USA
Mangan and Baars 2013	not specified	Not specified	Ireland, UK and parts of mainland Europe
Mataraza et al. 1999	waterways	Kings Bay, Florida	USA
McKee et al. 2002	not specified	Not specified	UK
Michelan et al. 2014	lakes and waterways	Not specified	Brazil
Mitchell 1996	not specified	Not specified	Australia
Moe et al. 2013	lakes (rivers also mentioned)	153 lakes in Southern Norway	Norway
Nawrocki et al. 2016	lakes	Shearon Harris Lake, Tar River Reservoir, Lake Tillery, Lake Gaston (North Carolina)	USA
Nelson et al. 2002	lakes and waterways	Not specified (lab experiment)	USA
Newman et al. 1996	not specified (most examples are from lakes)	North America	USA
Nichols and Shaw 1986	not specified, lakes and waterways mentioned	Not specified	World (especially NE of USA)
Nichols 1991	not specified	Not specified	not specified
Nilsson and Brittain 1996	waterways	Not specified (rivers in boreal and temperate region)	not specified
Poirrier et al. 2010	lakes and waterways	Lakes, canals, bayous, and ponds in the Jean Lafitte National Historical Park and Preserve, Louisiana	USA
Quilliam et al. 2015	lakes	Not specified	not specified
Reeves et al. 2008	lakes	29 lakes in Michigan & 1 in Wisconsin	USA
Richardson 2008	not specified, general review, most examples are on (big) lakes	Not specified	USA
Richter and Gross 2013	lakes	Not specified (mesocosm study)	Germany
Santos et al. 2009	waterways	Sacramento-San Joaquin River Delta, California, US	USA
Schneider et al. 2013	lakes and waterways	Lakes and rivers South of Norway	Norway
Schoonbee 1991	lakes and waterways	Lake Germiston and Lake Florida	South Africa
Schwarz and Howard-Williams 1993	lakes	Lake Taupo and Rotorua	New Zealand
Sisneros et al. 1998	Waterways	Not specified, Western US (Tests in canals in Idaho and Colorado)	USA
Slade et al. 2008	lakes and waterways	Not specified	not specified (USA lab. study)
Spencer and Ksander 1999	not specified	Not specified (experiments performed in Oregon House Canal, California, US)	USA
Sprecher et al. 1998	not specified, but anyways waterways	Not specified, Western US	USA
Stalling et al., 2015	not specified	Southeastern US	USA
Sukhodolov and Sukhodolova 2010	waterways	Not specified	not specified
Suren 2009	waterways	Urban streams in Christchurch, New Zealand	New Zealand
Tamayo and Olden 2014	lakes	319 lakes, Washington state	USA
Trebitz et al. 1993	lakes	Lake Wingra (Wisconsin)	USA
Van Nes et al. 1999	lakes	Not specified	not specified
Van Nes et al. 2002	lakes	Not specified	not specified
Washington et al. 1992	lakes	Not specified	not specified
Wells et al. 2003	waterways	Rangiriri and Churchill East drains, New Zealand	New Zealand
Wersal and Madsen 2011a	lakes and waterways	Not specified	USA
Wersal and Madsen 2011b	lakes and waterways	Not specified	USA
Yakandawala and Dissanayake 2010	not specified	Not specified	Sri Lanka
Yeo and McHenry 1977	lakes and waterways	Example given: Lake Ellis, California	USA
Zefferman 2014	waterways	Not specified (experiments performed in artificial streams, California, US)	not specified
Zefferman and Harris 2016	waterways	Putah Creek	USA

Table S 2. Summary of the main ecosystem services of the freshwater ecosystem where nuisance aquatic plants cause problems as stated in scientific literature. Number between square brackets indicates number of references.

Main services categorized	Category description		References
Boat traffic	Impairment of physical movement of a boat or ship through the water by macrophytes, both recreational and commercial in nature.	**[32]**	Aguiar & Ferreira, 2013; Alwin et al., 2010; Anderson, 2003; Bacchus & Barile, 2005; Bailey & Calhoun, 2008; Bickel & Closs, 2009; Caffrey et al., 2010; Cason & Roost, 2011; Charudattan, 2001; David et al., 2006; Evans & Wilkie, 2010; Everitt et al., 1999; Finlay & Vogt, 2016; Fulmer & Robinson, 2008; Goodenberger & Klaiber, 2016; Gu, 2006; Hilt et al., 2006; Hogsden et al., 2007; Jones & Cooke, 1984; Madsen, 1993; Madsen, 1998; Mataraza et al., 1999; Newman et al., 1996; Reeves et al., 2008; Richardson, 2008; Santos et al., 2009; Schoonbee, 1991; Stalling et al.2015; Tamayo & Olden, 2014; Van Nes et al., 2002; Van Nes et al.1999; Washington et al., 1992.
Fishing	All problems created by macrophytes which hamper recreational or commercial fishing activities.	**[18]**	Aguiar & Ferreira, 2013; Albright & Ode, 2011; Alwin et al., 2010; Anderson, 2003; Bacchus & Barile, 2005; Bickel & Closs, 2009; Caffrey et al., 2010; Charudattan, 2001; Cruz et al., 2015; Fulmer & Robinson, 2008; Jones & Cooke, 1984; Madsen, 1993; Reeves et al., 2008; Richardson, 2008; Schoonbee, 1991; Stalling et al., 2015; Tamayo & Olden, 2014; Van Nes et al.1999.
Swimming	All problems created by macrophytes which lead to less people entering the water for the purpose of (recreational) swimming.	**[17]**	Alwin et al., 2010; Bacchus & Barile, 2005; Bailey & Calhoun, 2008; Bickel & Closs, 2009; Caffrey et al., 2010; Cason & Roost, 2011; Finlay & Vogt, 2016; Fulmer & Robinson, 2008; Goodenberger & Klaiber, 2016; Hilt et al., 2006; Jones & Cooke, 1984; Madsen, 1993; Reeves et al., 2008; Stalling et al., 2015; Tamayo & Olden, 2014; Van Nes et al., 2002; Van Nes et al.1999.
Hydro-dynamics	All problems caused by macrophytes which lead to problematic reduction in water flow or discharge capacity in waterways, but also congestion of water bodies and clogging of (industrial) intake pipes.	**[34]**	Aguiar & Ferreira, 2013; Anderson, 2003; Berger et al., 2015; Bickel & Closs, 2009; Burlakova & Karatayev, 2007; Carr et al., 2003; Cason & Roost, 2011; Charudattan, 2001; Clayton & Champion, 2006; David et al., 2006; Evans & Wilkie, 2010; Everitt et al., 1999; Garbey et al., 2003; Gu, 2006; Hilt et al., 2006; Hofstra et al., 2006; Hogsden et al., 2007; Madsen, 1993; Madsen, 1998; Newman et al., 1996; Richardson, 2008; Santos et al., 2009; Schoonbee, 1991; Schwarz & Howard-Williams, 1993; Sisneros et al., 1998; Slade et al., 2008; Sprecher et al., 1998; Stalling et al., 2015; Sukhodolov & Sukhodolova, 2010; Van Nes et al.1999; Washington et al., 1992; Wells et al., 2003; Zefferman, 2014; Zefferman & Harris, 2016.
Other *(Including 'Ecological')*	All others, ranging from decreased aesthetics and waterfront property value to increased sedimentation and altering nutrient cycling of the system. All (biotic) problems created by nuisance macrophytes which concern changes in biodiversity, in the vegetation, biotic communities, available habitat for other species and damage to the ecosystem in general. Irrespective of whether this impairs human activities or not.	**[47]**	Aguiar & Ferreira, 2013; Albright & Ode, 2011; Alwin et al., 2010; Anderson, 2003; Bailey & Calhoun, 2008; Berger et al., 2015; Bickel & Closs, 2009; Brandrud, 2002; Burlakova & Karatayev, 2007; Caffrey et al., 2010; Cason & Roost, 2011; Charudattan, 2001; Clayton & Champion, 2006; Cruz et al., 2015; David et al., 2006; Evans & Wilkie, 2010; Everitt et al., 1999; Fulmer & Robinson, 2008; Garbey et al., 2003; Goodenberger & Klaiber, 2016; Gu, 2006; Harman et al., 2005; Hogsden et al., 2007; Madsen, 1993; Madsen, 1998; Mallin et al., 2016; Mangan & Baars, 2013; Mataraza et al., 1999; Michelan et al., 2014; Nelson et al., 2002; Nichols & Shaw, 1986; Nichols, 1991; Poirrier et al., 2010; Reeves et al., 2008; Richardson, 2008; Santos et al., 2009; Stalling et al., 2015; Suren, 2009; Tamayo & Olden, 2014; Trebitz et al., 1993; Washington et al., 1992; Wersal & Madsen, 2011a & b; Yakandawala & Dissanayake, 2010; Yeo & McHenry, 1977; Zefferman, 2014; Zefferman & Harris, 2016.

Table S 3. Summary of vegetation characteristics used in scientific literature to describe/classify nuisance vegetation. Combinations of categories have been used in references. Number between square brackets indicates number of references.

Main description class	Descriptions included in main class		References
High growth rate	Macrophytes exhibit nuisance growth when they grow rapidly, aggressively or any other growth rate related expression.	**[4]**	Br&rud & Roelofs, 1995; Caffrey et al., 2010; Charudattan, 2001; Reeves et al., 2008.
High height	Nuisance macrophytes are species that form surface canopies, grow to the surface (i.e. canopy depth = 0 m), form tall stands or emerge from the water column.	**[24]**	Alwin et al., 2010; Berger et al., 2015; Br&rud & Roelofs, 1995; Br&rud, 2002; Caffrey et al., 2010; Cason & Roost, 2011; Clayton & Champion, 2006; Goodenberger & Klaiber, 2016; Harman et al., 2005; Hilt et al., 2006; Lucassen et al., 2016; Madsen, 1993; Moe et al., 2013; Newman et al., 1996; Poirrier et al., 2010; Richter & Gross, 2013; Schwarz & Howard-Williams, 1993; Sisneros et al., 1998; Tamayo & Olden, 2014; Van Nes et al., 2002; Washington et al., 1992; Wersal & Madsen, 2011a; Yeo & McHenry, 1977; Zefferman & Harris, 2016.
High coverage	Nuisance vegetation consists of macrophytes that: 'completely cover a water body', 'cover large expanses', 'grow extensively', forms dense beds or dense stands and similar descriptions.	**[45]**	Albright & Ode, 2011; Alwin et al., 2010; Bacchus & Barile, 2005; Bailey & Calhoun, 2008; Berger et al., 2015; Br&rud & Roelofs, 1995; Br&rud, 2002; Burlakova & Karatayev, 2007; Cabrera-Walsh et al., 2011; Caffrey et al., 2010; Cason & Roost, 2011; Charudattan, 2001; Clayton & Champion, 2006; Cruz et al.2015; David et al., 2006; Evans & Wilkie, 2010; Finlay & Vogt, 2016; Fulmer & Robinson, 2008; Gu, 2006; Hilt et al., 2006; Hogsden et al., 2007; Jones & Cooke, 1984; Lucassen et al., 2016; Madsen, 1998; Mangan & Baars, 2013; Mataraza et al., 1999; Moe et al., 2013; Nawrocki et al., 2016; Nelson et al., 2002; Newman et al., 1996; Nichols, 1991; Poirrier et al., 2010; Reeves et al., 2008; Richardson, 2008; Schneider et al., 2013; Schoonbee, 1991; Schwarz & Howard-Williams, 1993; Sprecher et al., 1998; Stalling et al., 2015; Suren, 2009; Tamayo & Olden, 2014; Trebitz et al., 1993; Van Nes et al., 1999; Washington et al., 1992; Yeo & McHenry, 1977.
Forms (near) monospecific vegetation	Descriptions stating that nuisance plants form (almost) monospecific vegetation by either replacing or dominating the local plant community.	**[9]**	Cabrera-Walsh et al., 2011; Charudattan, 2001; Fulmer & Robinson, 2008; Hogsden et al., 2007; Madsen, 1998; Mangan & Baars, 2013; Nelson et al., 2002; Schneider et al., 2013; Tamayo & Olden, 2014.
High biomass	Descriptions stating that nuisance macrophytes form: 'too much biomass' or 'excessive biomass'.	**[12]**	Akhurst et al., 2012; Carr et al., 2003; Clayton & Champion, 2006; Fulmer & Robinson, 2008; Lucassen et al., 2016; Madsen, 1993; Newman et al., 1996; Nilsson & Brittain, 1996; Quilliam et al., 2015; Suren, 2009; Wells et al., 2003; Zefferman & Harris, 2016.
Other	I.e. where no real measurable plant trait was mentioned, think of: Macrophytes were a described as nuisance if they: 'caused problems'; 'caused (serious) nuisance'; 'became noxious'; 'were found in problematic quantities'; 'are (legally) classified as a noxious weed'; 'forms nuisance growth', were locally very abundant or if there is 'a nuisance infestation of macrophytes'.	**[49]**	Abernethy et al., 1996; Aguiar & Ferreira, 2013; Akhurst et al., 2012; Anderson, 2003; Bacchus & Barile, 2005; Bickel & Closs, 2009; Bickel & Schooler, 2015; Br&rud & Roelofs, 1995; Br&rud, 2002; Charudattan, 2001; Coetzee et al., 2011; Cruz et al., 2015; David et al., 2006; Evans & Wilkie, 2010; Everitt et al., 1999; Garbey et al., 2003; Godfrey et al., 1994; Gu, 2006; Harman et al., 2005; Hilt et al., 2006; Hofstra et al., 2006; Hogsden et al., 2007; Madsen, 1998; Madsen et al., 2016; Mallin et al., 2016; McKee et al., 2002; Michelan et al., 2014; Mitchell, 1996; Nelson et al., 2002; Nichols & Shaw, 1986; Nichols, 1991; Nilsson & Brittain, 1996; Poirrier et al., 2010; Reeves et al., 2008; Richardson, 2008; Richter & Gross, 2013; Schneider et al., 2013; Schoonbee, 1991; Slade et al., 2008; Sprecher et al., 1998; Stalling et al., 2015; Sukhodolov & Sukhodolova, 2010; Suren, 2009; Tamayo & Olden, 2014; Washington et al., 1992; Wersal & Madsen, 2011b; Yak&awala & Dissanayake, 2010; Zefferman, 2014; Zefferman & Harris, 2016.


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Chapter 3

Mass development of monospecific submerged macrophyte vegetation after the restoration of shallow lakes:

roles of light, sediment nutrient levels, and propagule density

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Abstract

After restoration, eutrophicated shallow freshwaters may show mass development of only one or two submerged macrophyte species, lowering biodiversity and hampering recreation. It is unclear which environmental factors govern this high percentage of the volume inhabited (PVI) by submerged macrophytes, and whether the development of a more diverse, low canopy vegetation is likely to occur if dominant species decline in abundance.

We hypothesized that (1) adequate light and high sediment nutrient availability leads to massive development of submerged macrophytes, and (2) that macrophyte species richness is low at high PVI, but that this is not caused by a lack of viable propagules of non-dominant species (especially charophytes).

To test these hypotheses, fifteen shallow waters in the Netherlands were studied with respect to submerged vegetation (including propagules), water, and sediment characteristics.

The probability of high submerged macrophyte PVI is highest in shallow waters where light availability in the water layer and phosphorus availability in the sediment are abundant. These conditions typically occur upon restoration of eutrophic waterbodies by reducing water nutrient loading or applying biomanipulation. Other factors, as top-down control, can additionally influence realised PVI.

Viable propagules of species other than the dominant ones, including charophytes, were found in most of the sediments, indicating that once the dominant species declines, there is local potential for a diverse submerged vegetation to develop. Results can be used to predict when mass development occurs and to tackle the factors causing mass development.

Introduction

Shallow waters worldwide suffer from high anthropogenic nutrient input leading to loss of submerged macrophytes by dominance of floating macrophytes, algae or cyanobacteria. Submerged macrophytes are key players in these ecosystems, because they provide a positive feedback for a clear water state and enhance biodiversity (Carpenter & Lodge, 1986). A wide variety of restoration measures have been taken to restore water transparency and submerged macrophyte vegetation in eutrophicated lakes, in particular through the reduction of external nutrient input and the removal of zooplanktivorous and sediment disturbing fish (i.e. biomanipulation) (Gulati & Van Donk, 2002; Jeppesen et al., 2007). After successful restoration of water transparency, a diverse vegetation of submerged macrophytes can reappear (Bakker et al., 2013; Pot & Ter Heerdt, 2014).

The restoration of clear water in eutrophicated lakes may also lead to massive development of submerged macrophytes, which is often characterised by monospecific stands of eutrophic vascular species with a vertical growth strategy and surface canopy formation, leading to a high percentage of volume inhabited (PVI) in the water column (Hilt et al., 2006; Lamers et al., 2012). These massive stands of tall submerged macrophytes can prevent the development of a more diverse vegetation by being superior competitors for light and space over slower growing species, especially isoetid and charophyte species. Additionally, mass development of submerged macrophytes can cause problems for human use of lakes, for example for recreation and navigation (Zehnsdorf et al., 2015). It is, however, unclear whether these large macrophyte stands are always species poor, or whether other species may still be present below the canopy of the dominant species. It is also unclear whether there is local potential for a more diverse and low-growing vegetation to develop in these ecosystems. In particular the development of charophytes is of interest in this respect, because they maintain low canopies that cause less interference with human use of lakes (e.g. Van Nes et al., 2002a). Charophytes are additionally favoured by water managers because they are promotors of good water quality (Bakker et al., 2010; Blindow et al., 2014), they can maintain large and long-lived propagule banks (Bakker et al., 2013), and they are rapid colonizers of new or restored water bodies (Noordhuis et al., 2002; Pot & Ter Heerdt, 2014). Charophyte species can in principle be a dominant component of a stable clear water state in eutrophic shallow lakes (Van Nes et al., 2002b).

The exact size of the macrophyte stand at which it causes problems depends on the specific ecosystem service provided by the lake (Mitchell, 1996). We will therefore not use a single threshold level to describe problematic stands, but will investigate which factors influence submerged macrophyte PVI in general under field conditions. Both light energy (photosynthetically active radiation, PAR) and nutrient availability highly influence the growth and abundance of autotrophs, including submerged macrophytes (Bornette & Puijalon, 2011). Light availability for the plants can be reduced for example by phytoplankton growth in the water column or by periphyton growth on the macrophytes (Hilt et al., 2006; Bornette & Puijalon, 2011; Phillips et al., 2016). Restoration measures are often aimed at improving light availability (Bakker et al., 2013). An often-overlooked component that may determine whether mass development of macrophytes occurs after water clarity has been restored is sediment nutrient availability (e.g. Bachmann et al., 2002; Eigemann et al., 2016). Rooted submerged macrophytes are able to acquire nutrients from the sediment (Carignan & Kalff, 1980; Halbedel, 2016). Generally, high abundance of macrophytes in the water column, expressed as PVI, may thus occur more frequently at high sediment nutrient conditions (Barko et al., 1991; Carr & Chambers, 1998; Figure 1). Indeed, laboratory growth experiments have shown that submerged macrophyte species grow faster or taller at increasing sediment nutrient concentrations (e.g. Barko & Smart, 1986; Angelstein et al., 2009; Martin & Coetzee, 2014). However, to our knowledge, field evidence is still largely lacking (Bachmann et al., 2002).

In this study, we hypothesised that: (1) high submerged macrophyte PVI will occur when sufficient light is available for submerged macrophytes to germinate and grow, and sediment nutrient availability supports high growth rates. (2) Massive stands of submerged macrophytes will consist of a lower number of plant species than stands with lower PVI, but viable propagules of species other than the dominant species will be present in the sediment top layer below massive stands, especially from charophyte species.

To test these hypotheses, we measured vegetation and environmental parameters and sampled the propagule bank in shallow lakes and ponds in the Netherlands, varying in submerged macrophyte abundance, throughout the growing season. We focused on both N and P in the nutrient analyses, because they are both considered to be key nutrients in determining the growth of photoautotrophs in shallow lakes (Moss et al., 2013).

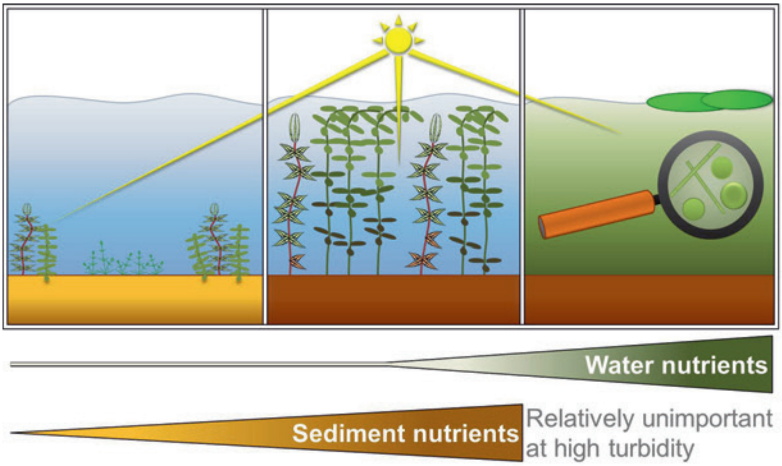

Figure 1. Theoretical relashionship between submerged plant PVI and sediment nutrient levels. At increasing nutrient availability, submerged macrophyte PVI increases, but diversity decreases. At high water turbidity, for example by high water nutrient load, submerged macrophytes are inhibited irrispective of sediment nutrient levels. Figure is adapted from Lamers et al., 2012: Fig. 1.

Methods

Study sites

We selected 15 shallow lakes and ponds throughout the Netherlands that were eutrophicated and have undergone restoration management and/or experienced problems with massive stands of submerged vegetation (see Table 1 & Supplementary Table S 1 for restoration methods applied and study site characteristics). Most of the intensive restoration measures have taken place many years ago and will therefore not have influenced the amount of submerged plants present directly, but only indirectly via the abiotic conditions as a result of the management. Most of these abiotic conditions are measured in this study. In several lakes, submerged plants are still harvested locally, but these harvested sites were avoided in our study. The surveyed aquatic ecosystems can be characterized as meso- to eutrophic (based on surface water nutrient concentrations) water with moderate to high surface water alkalinity and pH (lake average alkalinities: 1.4 - 4.6 meq.L^{-1} and daytime pH: 8.3 – 9.6). Total P in the surface water averaged (±SE) 0.13±0.03 mg P.L^{-1}, whereas total N averaged 0.31±0.03 mg N.L^{-1} in sites with submerged macrophytes. In sites without submerged macrophytes, total P and N in the surface water averaged 0.09±0.01 and 0.59±0.05 mg.L^{-1}, respectively.

We selected four sites per ecosystem using the following two criteria: (1) they should be situated in open water, where water depth is between 1 and 1.5 metres and (2) their position in the waterbody is most northern (N), eastern (E), southern (S) or western (W), respectively for each site. We avoided areas with apparent direct anthropogenic disturbance including: macrophyte mowing sites, harbours, navigation channels, and areas close to beaches or fishing locations. Sites heavily shaded by large shoreline trees were also avoided.

Table 1. Lake description and recorded management measures.

				Recorded management measures taken								
Waterbody	**Coordinates (decimal)**	**Longest Length (km)**	**Mean depth (m)**	Reduced external P-loading	Biomanipulation (manipulation fish stock)	Dredging	Sand addition	Introducing macrophytes	Mowing (●) and harvesting	Internal nutrient binding with addition of chemical(s)	Other$^{\#}$	**References management***
Zwemlust	52.194°N : 5.006°E	0.17	1.50		■	■					■	4; 5; 6
Loosdrecht	52.198°N : 5.056°E	4.97	2.20	■							■	4; 5; 11
De Waay	51.922°N : 5.995°E	0.48	2.50		■				■			5; 6; 11
Noorderplassen	52.402°N : 5.226°E	2.69	N/A						●			7
Bleiswijkse Zoom	52.014°N : 4.554°E	1.70	1.10		■							5; 6
Bergse Achterplas	51.955°N : 4.486°E	0.95	2.00	■	■	■	■					2
NIOO pond	51.987°N : 5.671°E	0.12	1.00					■				8
Zevenhuizerplas	51.986°N : 4.574°E	1.98	N/A						●			9
Zuidlaardermeer	53.144°N : 6.698°E	4.04	1.00	■	■			■				6; 11
Nannewijd	52.951°N : 5.864°E	1.72	1.00	■	■	■				■		3; 6
Sondeler Leien	52.865°N : 5.624°E	1.03	1.00		■							6
Oldambtmeer	53.198°N : 7.039°E	2.40	1.70					■	■			10
Duinigermeer	52.724°N : 6.003°E	0.87	1.00		■							5; 6
Het Bovenwater	52.496°N : 5.407°E	1.73	1.30						■			7
Loenderveenseplas Oost	52.219°N : 5.058°E	2.22	2.00	■	■							1

* References: [1] Pot & Ter Heerdt (2014); [2] Hosper et al. (2011); [3] Søndergaard et al. (2007); [4] Dionisio Pires et al. (2004); [5] Gulati & Van Donk (2002); [6] Meijer et al. (1999); [7] Pers. com. Waterboard Zuiderzeeland; [8] Unpubl. data NIOO; [9] Pers. com. Grontmij; [10] Pers. com. Waterboard Hunze en Aa's; [11] Report: Onderzoek meren (Helpdeskwater, NL). # Measures grouped under the 'Other' category were less common and include: herbicide application (Zwemlust in 1968, with no long-term inhibition of plants) and hydraulic isolation of the lake (Loosdrecht Lakes).

Because vegetation was expected to vary not only spatially, but also temporally within an ecosystem, sites were visited three times throughout the growing season, using a small flat-bottomed boat. All sites were visited in three rounds: from May 13 until June 26, from July 8 until August 15, and from August 21 to October 4, using a high-sensitivity GPS device to determine each location (eTrex® H., Garmin Ltd., Southampton, UK).

Macrophyte survey

At each site we measured water depth and depth of the submerged macrophyte canopy below the water surface (hereafter referred to as 'canopy depth'), from which submerged macrophyte height was calculated (water depth – canopy depth). We visually estimated total cover (%) and relative abundance per species (%) at four spots around the perimeter of the boat using an aquascope (also known as a bathyscope). This resulted in a survey area of approximately 10-15 m^2 per site. We used submerged macrophyte height and cover, together with water depth, to calculate PVI. To account for possible rare species present underneath the dominant vegetation, we additionally used a rake to sample the vegetation (30.4 cm wide rake with: large, 6.6 cm, teeth 2.7 cm apart; and small, 0.6 cm, teeth 0.6 cm apart). The rake was thrown four times, once at each corner of the boat, and was dragged one metre across the sediment. If a species was found using the rake that was not observed by the visual inspection from the boat, the macrophyte was assumed to lie on the bottom (i.e. recorded plant height = 1 cm). All raked macrophytes were collected and abundance per species was estimated as well as total coverage using a conversion table provided by Immers et al. (2015), adapted by our own field observations (see Table S 2). If visibility was too low for visual cover estimation, cover was only estimated using the rake (115 cases). In 68 % of the 115 cases no submerged macrophytes were found at all. Furthermore, in 96 % of the 115 cases with too low visibility the estimated submerged macrophyte PVI was less than 5 %. Therefore possible bias in submerged vegetation measurements due to the different estimation methods is likely very small.

Water and sediment sampling and field measurements

We took four 2 L surface water samples, one from each corner of the plot, and mixed them in a bucket. This mixed sample was used for on the spot turbidity measurements (Turb430IR, WTW GmbH, Weilheim, Germany). Water was filtered using Whatman GF/F glass microfiber filters (GE Healthcare GmbH, Germany) and stored at -20 °C upon arrival at the lab for later inorganic nutrient (N and P) analyses. The filters were dried (60°C) and stored for suspended solid nutrient analysis. Light (Photosynthetically Active Radiation: PAR) was measured at regular depth intervals in the ecosystem (LI-250 light meter and underwater quantum sensor, LI-COR inc., Lincoln, NE, USA). The light extinction coefficient of the water was calculated using PAR measurements at 31 and 56 cm depth. The coefficient was not calculated if the aquatic vegetation was too dense, i.e. when vegetation severely shaded the sensor and no open space could be made.

The top 10-15 cm of sediment was collected during the first visit using a core sampler (inner diameter 5 cm). Four cores were taken at each site, one at each corner of the boat, and immediately put into a single airtight bag per site to limit exposure to oxygen. If no sediment sample could be taken during the first round due to equipment failure by hard substrate, the sample was taken during subsequent visits (18/60 cases). On the same day, sediment was homogenized inside the closed bag and porewater was extracted overnight in the dark at 4 °C. To extract porewater, we pierced the bag and inserted a rhizon (Rhizon SMS, RRP B.V., Wageningen, the Netherlands) through the hole. The hole was sealed with tape. Porewater was stored at -20 °C until nutrient analyses. Three subsamples (approx. 45 mL each) of the homogenized sediment were taken from the bag and dried at 60 °C for determination of moisture content and for analyses of total and extractable nutrients.

Chemical analyses

We measured inorganic nitrogen (NO_2-N; NO_3-N; NH_4-N) and phosphorus (PO_4-P) concentrations in filtered surface water and sediment porewater colourimetrically with an autoanalyser system (QuAAtro SFA, Seal Analytical, Germany). Total carbon and nitrogen of sediment and surface water suspended solids were analysed using a CN analyser (FlashEA 1112 Series, Thermo Scientific, MA, USA). The sediment samples and surface water suspended solids were ashed to determine total P concentration (30 min. at 550 °C). We subsequently digested the ashed solids with a 2.5 % persulphate solution in an autoclave at 121 °C for 30 minutes and analysed the solution colourimetrically on the autoanalyser. We calculated total N and P of the surface water by adding the amount of inorganic N or P to the amount of N or P in the suspended solids, respectively.

To estimate the amount of plant available nitrogen in the sediment, a KCl-extraction was performed on the dried sediment as applied by Tang et al. (2017). 12.5 mL 1 M KCl was added to 2.5 g of dried sediment and subsequently shaken for 2.5 h at 250 rpm. Two subsamples (2 ml each) were centrifuged for 10 min at 10,000 rpm and the supernatant was stored at -20 °C for nitrogen analysis on the autoanalyser. We estimated plant available P in the sediment using an adapted P-Olsen protocol, as applied by Tang et al. (2017). 50 mL 0.5 M $NaHCO_3$ (at pH 8.5) was added to 2.5 g of dried sediment and subsequently shaken for 30 min, after which the solution was immediately filtered (Whatman Grade 42, GE Healthcare Europe GmbH, Eindhoven, the Netherlands). Sulphuric acid (1.04 mL, 2.5 M) was added to 10 mL of the filtrate in an Erlemeyer flask. The flask was shaken until no more gas development was visible. The filtrate was filtered once more (Whatman Grade 42) and stored at -20 °C until analysis for phosphate on the autoanalyser system. When insufficient sediment material was available for both N and P extractions (n = 13 sites with submerged macrophytes), P-extraction was prioritized and when insufficient sample was present, a corresponding reduction in reagent volume was applied to keep sediment:reagent-ratio equal (n = 2 and 5 sites with submerged macrophytes for N and P, respectively).

We converted sediment nutrient concentration to mmol per litre of sediment (mmol.$L_{sediment}^{-1}$) by using the sediment moisture content (grams / grams wet weight) and the sediment's specific density (grams wet weight.$L_{sediment}^{-1}$).

Germination experiment

To identify viable propagules in the sediment, four samples of the sediment top-layer (approx. 3-5 cm thick) were taken at each field site during the first visit using an Ekman type bottom grab sampler of 15 x 15 cm (l x w). These four samples were pooled on site and stored in the dark at 4 °C upon arrival at the lab that same day. Samples were kept at 4 °C (cold stratification) for at least 1 week before using them in the germination experiment. We used 1 L of homogenized sediment per site, divided over four containers (1.5 L white PP), each containing 0.25 L of sediment. This created a layer of approximately 1.5 cm, which is thin enough for many submerged species to germinate (Van Zuidam et al., 2014 and literature therein). This sediment was spread on top of a 3 cm thick layer of clean sand (grain size: 0.4 to 0.8 mm) and the container was filled with 5 cm of tap water. Four aquaria containing only clean sand and tap water were used as negative controls. We gently refilled the water twice a week to compensate for evaporation. Macrophyte germination was followed for at least 2 months in a climate controlled greenhouse between June and August 2013, after which no further germination was observed (air temperature set at 21°C and 16 °C during the day and night, respectively at natural light). No macrophytes developed in any of the controls.

Statistical analysis

We performed a logistic regression analysis to test our hypothesis that submerged macrophytes will only be present at a location with adequate light availability ('glm' function in R). For this analysis, we used the average turbidity from all three visits and binary data on whether submerged macrophytes were encountered at any of the sites, during any visit ('1') or not at all ('0'). We used data from the whole ecosystem to test this, as water quality (incl. turbidity) was often highly correlated within an ecosystem. This is likely caused by to the relatively small size of the sampled waterbodies (0.1 to 4.97 km longest length; Table 1).

To test whether the chance of mass development of submerged macrophytes will increase with increasing sediment nutrient availability, we included only waterbodies where more than 1% submerged macrophyte cover was found in at least one of the three visits in the following analyses. We set this limit at 1 % because sites with a lower cover often only had loose fragments of submerged plants, making up this 0-1 % cover. We therefore believe that this fragment was probably transported into the system via connected ecosystems with more abundant submerged macrophyte vegetation. Another reason for setting the threshold at 1 % is that in all of the sites with a year maximum cover of 0-1 %, no submerged macrophytes were found at all in at least 1 of the visits. We used the following variables to describe the vegetation: maximum submerged macrophyte cover (%), maximum submerged macrophyte canopy height (%), and maximum PVI (%). The ‘maximum’ in these parameters refers to the highest value recorded for each individual site over all three visits. Because cover and height were both highly correlated with PVI (Spearman rank correlation: $\rho=0.96$ and 0.70, respectively, $p<0.001$), we only present results on PVI.

To test whether the maximum PVI of submerged macrophytes related positively with the sediment nutrient parameters, we used mixed models with a sediment nutrient parameter as fixed factor and ‘Waterbody ID’ as random factor. The ‘Waterbody ID’ factor is a character variable stating the name of the waterbody. For several sediment parameters, 1 – 3 waterbodies had to be excluded from this analysis due to missing data. We used the ‘lme’-function in R for the analyses (‘nlme’ package version 3.1-118) and p-values were conservatively adjusted to correct for multiple tests by Bonferroni’s method.

The restoration potential with regard to local development of diverse submerged vegetation with charophytes was assessed by comparing species richness found in the field with species richness of the plants germinated in the greenhouse from gathered sediment from the waterbodies, using paired-sample t-tests (‘t.test’ function). There is local potential for creating a more species diverse vegetation with charophytes if the amount of species found in the field is lower than the amount of species germinating from viable propagules in the sediment collected from the same site.

To explore possible correlations between variables, Spearman rank correlations between all measured environmental parameters and maximum PVI can be found in table A.3. We used the ‘rcorr’ and ‘corrplot’ R functions from ‘Hmisc’ version 3.17-0 and ‘corrplot’ version 0.73 packages, respectively. For these correlations, the critical p-value (α) was adjusted for multiple comparison with the number of other parameters tested against PVI ($\alpha = 0.05/29$).
All statistical analyses were performed using the R programme (version 3.1.2).

Results

Submerged macrophyte PVI and light

The probability that submerged macrophytes were present at a location was inversely related to waterbody turbidity, with almost no submerged macrophyte presence above a turbidity of 14 NTU (Fig. 2). This corresponds to 1 to 4 % light reaching the bottom, calculated from linear regression between the measured light extinction coefficient (LEC) and turbidity (Trb) values ($LEC = 0.59*Trb + 0.598$; $R^2 = 0.76$; $p < 0.001$, $n = 132$). Below this critical turbidity level the PVI varied extensively, with no significant correlation between turbidity and submerged macrophyte PVI ($R^2 = 0.06$, $p = 0.11$).

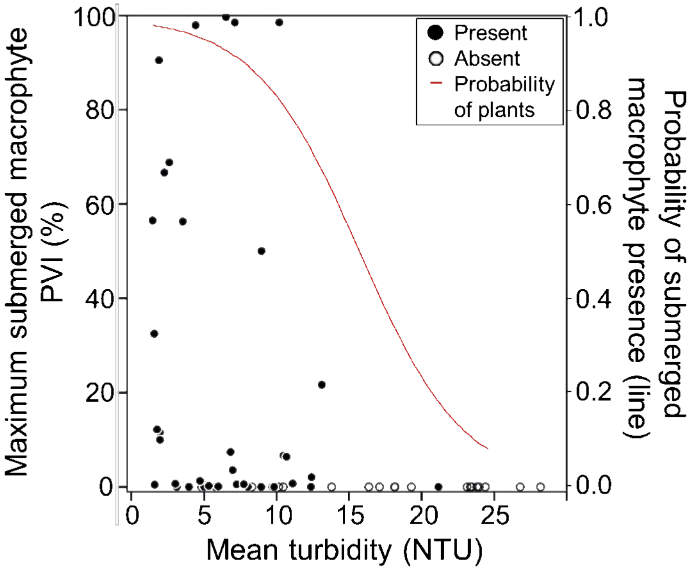

Figure 2. Maximum PVI of submerged macrophytes per site (n = 60) in relation to the average turbidity of the surface water of the entire lake (left y-axis). The maximum of the 3 PVI's measured in one site during 3 visits is taken as 'Maximum PVI'. Site with and without submerged vegetation are represented by closed and open circles represent, respectively. Line: Logistic regression on average turbidity per waterbody in relation to the presence or absence of submerged macrophytes at any of the 4 sampled sites in the waterbody (p = 0.04; right y-axis).

Submerged macrophyte PVI and sediment nutrients

When the light availability did not prevent submerged plants from growing, no significant relation between sediment nutrient concentrations and submerged macrophyte PVI was found using the mixed model analyses (Table 2). However, non-parametric correlation analyses between PVI and environmental variables of sites where submerged macrophytes were found did show a significant positive correlation between plant-available P in the sediment and yearly maximum PVI ($\rho = 0.53$; $p_{adj.} = 0.045$; Fig. 3A), but not for any of the other sediment nutrient parameters (Fig. 3B-F; Table S 3). A high submerged macrophyte PVI was possible over a wide range of sediment nutrient levels (Fig. 3).

Local restoration potential: Species richness and viable propagules

Submerged macrophyte species were found in 11 of the visited ecosystems and species richness in the field was positively correlated with submerged macrophyte PVI ($R^2_{adj.} = 0.32$, $p < 0.001$ on log transformed data, Fig. S 1). However, this positive correlation is driven by a sharp increase in species richness when PVI increases from < 1% to 5 %, whereas at > 5% PVI species richness appears unrelated to macrophyte PVI (Fig. S 1).

In the 11 ecosystems where submerged macrophytes were encountered in the field, submerged macrophytes also emerged from the incubated sediment (Table 3).

Table 2. Correlation between environmental nutrient parameters (predictor variables) and maximum submerged plant PVI (dependent variable) with Waterbody ID (i.e. ecosystem name) as random factor. The maximum of the 3 PVI's measured in one site during 3 visits is taken as 'Max submerged plant PVI'. Predictor variables were expressed in mmol.L-1. P-values were adjusted for multiple tests using the Bonferroni method for multiple comparisons. Unadjusted P-values are also provided.

Dependent variable	Predictor variable	df (num,den)	F	P	$P_{adjusted}$
Max. submerged plant PVI	Olsen Extracted P	1,17	0.43	0.52	1
	KCl Extracted N	1,17	2.16	0.16	1
	Porewater free P	1,23	0.16	0.69	1
	Porewater free N	1,23	5.14	0.03	0.26
	Sediment total P	1,23	1.78	0.20	1
	Sediment total N	1,23	8.34	0.008	0.07
	Mean surface water total P	1,26	0.52	0.48	1
	Mean surface water total N	1,26	1.25	0.27	1

Charophyte species emerged from the sediments from 8 of these waterbodies. Significantly more charophyte species emerged from the sediment compared to the number of charophyte species found in the corresponding field site, on average 1.0 and 0.5 species from the sediment and the field, respectively (paired-sample t-test on sites with submerged macrophytes: t = 2.55; df = 39; p = 0.015). No submerged macrophytes emerged from the sediments from the 4 waterbodies where no submerged macrophytes were found at any of the four sampled sites in the field (Table 3).

Discussion

We found that submerged macrophytes almost exclusively occurred (i.e. cover > 1 %) at turbidity levels below 14 NTU, confirming the first part of our hypothesis. When turbidity was below 14 NTU, a weak correlation between PVI and plant-available P in the sediment was found (also see Sterner et al., 1997), with high PVI mainly occurring at Olsen P levels above 0.35 $\text{mmol.L}_{\text{sediment}}^{-1}$. Interestingly, a high PVI was observed over a wide range of sediment nutrient levels. The local propagule bank in sites with submerged vegetation contained additional species to the ones present in the vegetation itself, often including charophyte species. This confirms our hypothesis that most sites had the potential to develop submerged vegetation of higher macrophyte diversity.

Submerged vegetation, light and nutrients

In our study, submerged macrophytes were almost exclusively present at sites where light (PAR) at the bottom was higher than 1 to 4 % of the irradiance at the water surface. This threshold is also applicable for many other submerged macrophyte species than the ones found in our study (Bornette & Puijalon, 2011). Mixed model analyses of our field data did not support the hypothesis that this variation was primarily caused by sediment

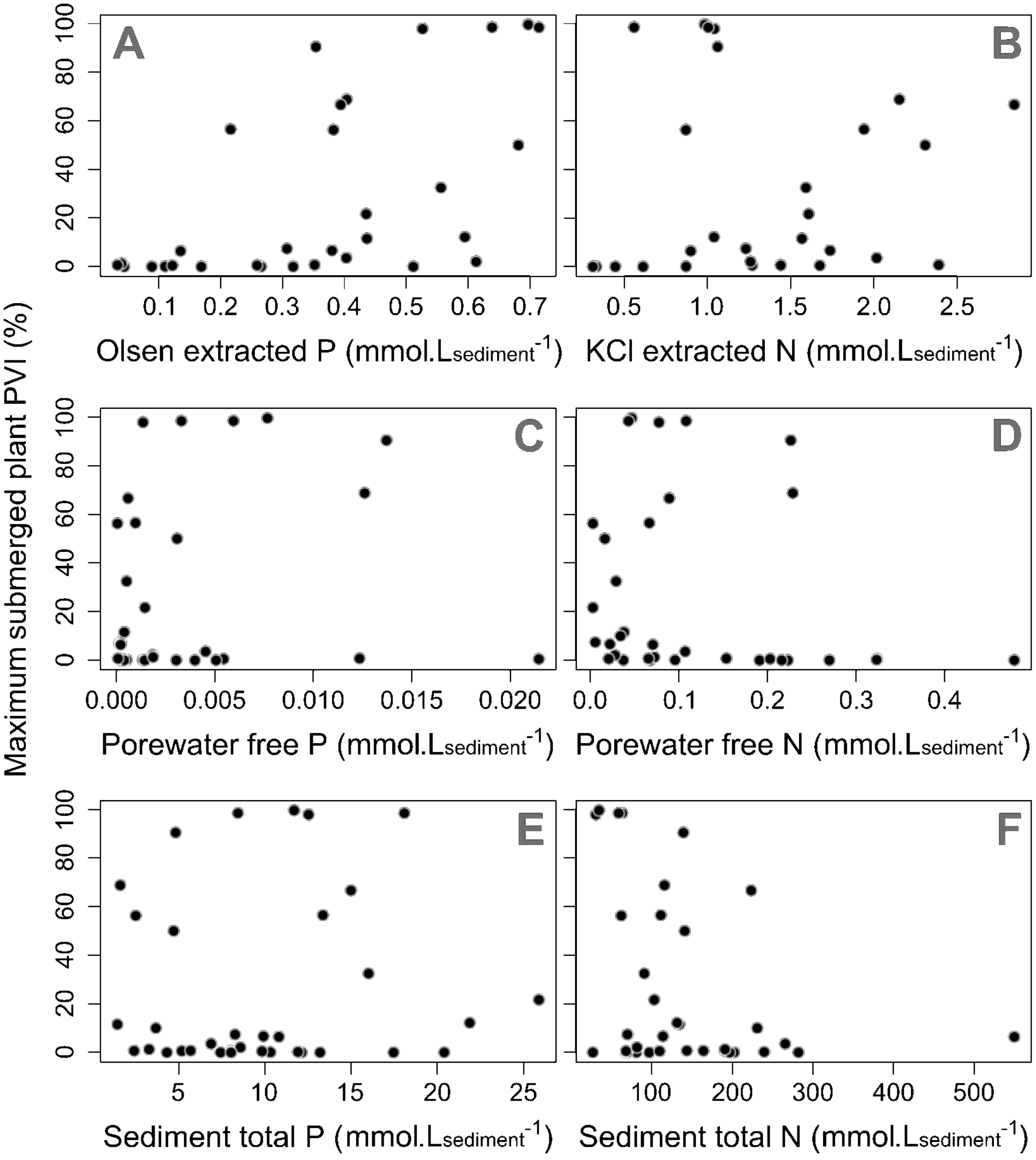

Figure 3. The relationship between sediment nutrient parameters and submerged plant volume (PVI) in sites with submerged macrophytes (i.e. > 1% cover). The maximum of the 3 PVI's measured in one site during 3 visits is taken as 'Maximum submerged plant PVI'. For all PVI values, see Table S 4.

Table 3. List of all macrophyte species found growing in the waterbody and/or that emerged from the incubated sediment samples in the greenhouse germination experiment. Only submerged species are used in the analyses.

Species found in:
■ Lake & sediment
▪ Lake only
□ Sediment only

Waterbody	Location	*Callitriche* spp.	*Ceratophyllum demersum*	*Chara* sp.	*Chara aspera*	*Chara connivens*	*Chara contraria*	*Chara globularis*	*Chara vulgaris*	*Elodea canadensis*	*Elodea nuttallii*	*Fontinalis antipyretica*	*Lemna minor*	*Lemna minuta*	*Lemna trisulca*	*Myriophyllum spicatum*	*Najas marina*	*Nitella* sp.	*Nitellopsis obtusa*	*Nuphar lutea*	*Nymphaea alba*	*Nymphoides peltata*	*Potamogeton berchtoldii*	*Potamogeton crispus*	*Potamogeton lucens*	*Potamogeton mucronatus*	*Potamogeton obtusifolius*	*Potamogeton pectinatus*	*Potamogeton perfoliatus*	*Potamogeton pusillus*	*Ranunculus circinatus*	*Spirodela polyrhiza*	*Stratiotes aloides*	*Tolypella intricata*	*Tolypella prolifera*	*Zannichellia palustris*	Additional emergent and wetland species found
Zwemlust	N							□						▪				□		▪								▪								□	0
	S							□																				▪								■	0
	E			▪																																▪	0
	W							□					▪	▪						▪								▪		□		▪				□	0
Loosdrecht	N																																				0
	S																																				0
	E																			▪																	3
	W																			▪	▪	■															1
De Waay	N						□		□		■				■					▪										□							3
	S						□		□		▪	▪			▪					▪					▪			▪		□		▪					7
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	W						□	□	□		▪	□			■													▪		□		▪				□	4
Noorderplas-sen	N	▪	▪							▪	■					▪																					1
	S	▪	▪							▪	■		▪			▪							□	□				▪			■						0
	E	▪	▪							▪	▪					▪													▪		▪					▪	0
	W	▪					□				▪		□			▪												■			■					□	0
Bleiswijkse Zoom	N							□					□																			□					0
	S		▪										▪	▪										▪								▪					0
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	W												■																			▪					1
Bergse Achterplas	N												▪							▪								▪									1
	S																			▪																	1
	E								□																												0
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NIOO Pond	N									▪	■		■		▪															□		▪					0
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Zevenhuizer plas	N		▪				■		□		▪					▪												■		▪	▪						0
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	E						■	□	■		▪					▪												▪	▪		▪					▪	0
	W						■	■	□		▪					▪												■	▪		▪				□	▪	0
Zuidlaarder meer	N																																				0
	S																																				0
	E																																				0
	W																																				0
Nannewiid	N																																				1
	S																																				0
	E																																				0
	W																																				0
Sondeler Leien	N																																				2
	S																																				0
	E																																				1
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Oldambt-meer	N							▪			■				▪	▪													▪	▪							1
	S										▪				▪	▪								□					▪							▪	2
	E										▪																		■								0
	W							▪			▪				▪	▪													▪								3
Duiniger-meer	N							□							▪				▪														▪				0
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	E			□				■											□							▪							▪		▪		1
	W						□	□																									▪	□		▪	0
Het Bovenwater	N								□																			▪		■						■	0
	S																							■				▪		■						■	1
	E				▪		□		□															■				▪		■						■	1
	W																											▪		□							1
Loender-veenseplas Oost	N							▪			▪					▪																					1
	S							▪			▪						▪													▪							0
	E							■			▪					▪								▪					▪	■							0
	W				■	▪	▪	▪			▪					▪			▪							▪				■							1

nutrient availability. Using Spearman rank correlation, we did demonstrate that the chance of mass development of submerged macrophytes increased with increasing sediment P availability for plants (Fig. 3; Table S 3). These conflicting statistical results are partly caused by the limited number of sites sampled within one waterbody on which the mixed model regression is based (n = 4). The significant Spearman rank correlation can thus be the result of the higher number of data points available for this test. Whereas controlled experiments do show positive effects of sediment nutrients on submerged plant growth (e.g. Barko & Smart, 1986; Angelstein et al., 2009; Martin & Coetzee, 2014), the relationship between nutrient availability and submerged plant PVI under field conditions is weak (this study; Backmann et al., 2002; Demars & Edwards, 2007), pointing at either nutrients being non-limiting for PVI in our sites or at additional controlling factors.

Other factors impacting PVI

Here we will discuss these possible reasons for the lack of a strong relationship between PVI and sediment nutrient availability under field conditions. First, shallow freshwater ecosystems are particularly vulnerable for submerged macrophyte reaching high PVI. Light availability will generally be higher in shallower water due to the limited depth of the water column. This enables submerged macrophytes to germinate and meet their light requirements for growth in shallow waters, even when the actual light attenuation in the water is high (i.e. high turbidity; Søndergaard et al., 2013). Additionally, several fast-growing species may still grow 1 to 2 m tall, and thus reach the water surface, even if environmental conditions limit their growth rates (e.g. Rattray et al., 1991). Our data substantiated this, as macrophyte stands with a high PVI occurred over a wide range of sediment nutrient levels.

Second, whereas the probability of the occurrence of high submerged macrophyte PVI may increase with sediment nutrient level, the realised PVI may not reach its full potential due to inhibition by other factors. Abiotic factors, such as carbon limitation (e.g. low CO_2 levels due to higher pH) and high sediment organic matter content, can reduce macrophyte abundance (e.g. Barko & Smart, 1986; Raun et al., 2010). Strong effects of carbon limitation on PVI do not seem likely in our ecosystems, as surface water pH did not significantly correlate with submerged macrophyte PVI during the first two sampling dates (Table S 3). During the last sampling date, surface water pH correlated positively with maximum PVI, indicating that the high pH (and potentially low CO_2) was more likely a consequence of high plant growth than a factor that severely limited macrophyte growth. In addition, most of the dominant species in our ecosystems, for example *E. nuttallii* and *M. spicatum*, are also able to take up and use HCO_3^- for growth when CO_2 concentrations are low (Eighmy et al., 1992; Hussner & Jahns, 2015). Strong growth limitation by high sediment organic matter content is also not probable in our sites with submerged plants, as organic matter content did not significantly correlate to PVI during our visits and only few sites (8) had more than 20 % sediment organic matter (data not shown; Barko & Smart, 1986). However, even when sediment and water properties are optimal for macrophyte growth, the realised macrophyte abundance can still be regulated top-down. Herbivory by invertebrates, fish, or waterfowl can strongly regulate submerged vegetation composition and abundance (Van Donk & Otte, 1996; Gross, Feldbaum & Choi, 2002; Bakker et al., 2016). This has also been observed in one of our sampled ecosystems, Lake Zwemlust, where coots (*Fulica altra*) and rudd (*Scardinius erythrophthalmus*) substantially decreased macrophyte abundance (Van Donk & Otte, 1996). Additionally, periphyton can also regulate submerged macrophyte growth by reducing light availability to the plants (Phillips et al., 2016) , which may severely limit plant standing crop, even more so in combination with grazing (Hidding et al., 2016). We therefore propose that these additional top-down factors severely limit submerged macrophyte PVI under field conditions, which can thus obscure bottom-up mechanisms underlying the mass development of submerged macrophytes.

Development of macrophytes during eutrophication and after restoration of clear water

Here we will discuss when massive stands of submerged macrophytes are likely to develop and we outline these concepts in Figure 4. The chance of high macrophyte PVI will increase with eutrophication of oligo- to mesotrophic waterbodies (Fig. 4: panel 1 to panel 2). Indeed, it has been observed that macrophyte abundance can be enhanced during eutrophication when water is still clear, leading to mass development (e.g. Hasler, 1947), before the system is dominated by floating macrophytes or algae after continued eutrophication (Sand-Jensen & Borum, 1991; Sayer et al., 2010; Fig. 4: panel 2 to panel 4).

When lakes are restored by gradually reducing the nutritional status of the water, the potential for high submerged macrophytes PVI will most likely increase when water transparency improves, while the sediment is still high in historically loaded nutrients (Fig. 4: panel 4 to panel 2), as high water nutrient levels lead to sediment storage (Tang et al., 2017). One condition first needs to be met, however: viable propagules need to be present for the vegetation to develop at all (this study; Hilt et al., 2006). When propagules are present, the reduction in nutrient loading and the improvement of water transparency in temperate lakes has enabled macrophytes to return (Jeppesen et al., 2005), and has facilitated mass development of macrophytes (Hilt et al., 2006; Zehnsdorf et al., 2015; Fig. 4: panel 4 to panel 2a).

A similar effect can be expected after the removal of sediment disturbing or zooplanktivorous fish (i.e. biomanipulation). Below a certain threshold of nutrient concentrations, biomanipulation can instantly improve water transparency (e.g. Meijer et al., 1999; Bernes et al., 2015), while the concentration of nutrients in the ecosystem remains similar (Fig. 4: panel 4 to panel 2a). Indeed, many lakes initially show a rapid increase in water transparency after biomanipulation (Bernes et al., 2015), which may

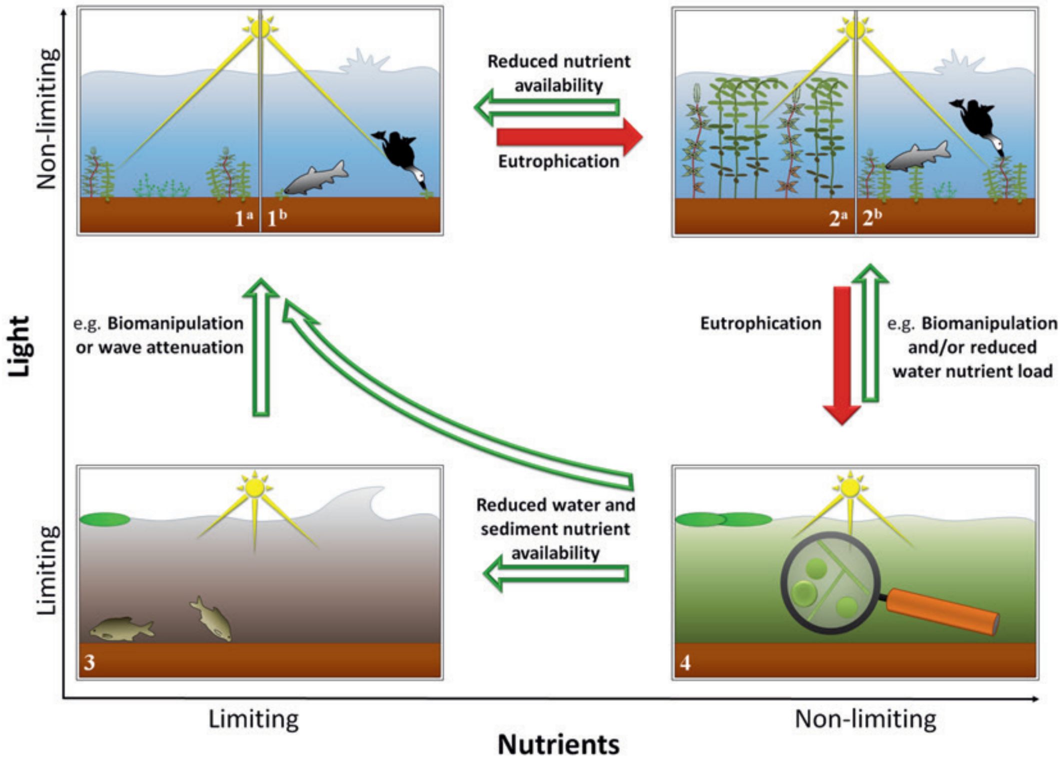

Figure 4. Graphical representation of factors impacting macrophyte PVI. Main abiotic factors are different nutrient levels (x-axis) and light availability (y-axis), which can be limiting or non-limiting for submerged macrophyte growth. Standing submerged macrophyte PVI is further impacted by top-down pressures, e.g. herbivores (represented as fish or waterfowl), but also shading by periphyton. Red (filled) arrows indicate eutrophication first leading to increased submerged macrophytes production, but eventually leading to disappearance of submerged macrophytes. Green (open) arrows indicate restoration measures to regain submerged macrophytes either through reducing nutrient availability or additional measures such as biomanipulation. **Panel 1:** Low growing diverse vegetation **(1a)** or almost no submerged vegetation due to high top-down pressures **(1b)**. **Panel 2:** Massive development of tall growing submerged macrophytes **(2a)** or less vegetation and no mass development due to high top-down pressures **(2b)**. **Panel 3:** No submerged plants due to low light, mainly caused by suspended particles other than algae (e.g. suspended sediment). **Panel 4:** No submerged plants due to low light, mainly caused by algae, cyanobacteria and possibly also by floating plants. Part of the information presented in this figure is based on literature instead of our own data, including the effects of herbivory, wave-action and extreme nutrient limitation. See discussion for more details.

well result in mass development of submerged macrophytes (Strand & Weisner, 2001; Van de Bund & Van Donk, 2002; Pot & Ter Heerdt, 2014).

Restoring target vegetation

When submerged macrophytes finally reappear after successful restoration of eutrophicated ecosystems, species with a vertical growth strategy (for example several *Potamogeton*, *Myriophyllum*, or *Elodea* spp.) are most likely able to benefit from this 'new' situation with clear water and high sediment nutrient availability (Meijer et al., 1991; Hilt et al., 2013). In our study, *E. nuttallii* was often dominant and showed the highest PVI, but seven other species also became dominant in macrophyte stands, including several *Potamogeton* species. All these dominant species are known to possess a vertical growth strategy and can form canopies at the water surface.

When management efforts further decrease nutrient availability by either physically removing (e,g, dredging) or chemically binding them, other species, in particular charophytes, can potentially outcompete these dominant vascular plants (Hidding et al., 2010; Richter & Gross, 2013), provided that viable propagules are present. In our study, there was a local species pool (including charophytes) from which other species could take over once the dominant species declines in abundance. The period of mass development of tall growing species may thus be a transient phase that can give way to the development of a less dense diverse submerged vegetation including charophytes, as recently observed in one of the water bodies studied here: Loenderveense Plas Oost (Pot & Ter Heerdt, 2014). Such a shift from vegetation dominated by species with a vertical growth strategy to vegetation with shorter species has also been observed in several other lakes during oligotrophication. In Lake Krankesjön in Sweden for example, submerged vegetation redeveloped after a period of high turbidity (Hargeby et al., 1994). *Potamogeton pectinatus* first expanded in Krankesjön, but was largely replaced by *Chara tomentosa* within 6 years, which coincided with a decrease in total P levels in the lake (Hargeby et al., 1994). Similarly, in Lake Veluwemeer in the Netherlands, the P load of the surface water was reduced leading to recovery of submerged macrophytes. In this lake *Potamogeton perfoliatus* expanded first, while a subsequent transition towards charophytes took place (Noordhuis et al., 2002).

Reducing external nutrient input alone, however, does not guarantee a rapid transition towards a diverse vegetation that will not cause nuisance to people, as macrophyte recovery may potentially take decades (Eigemann et al., 2016). Additional reduction of the availability of nutrients stored in the sediment by, for example dredging or chemical P-binding, is likely required and has promoted the development of a more desired vegetation in several ecosystems (Immers et al., 2015; Spears et al., 2016). However, the extent of nutrient reduction required may not be achievable for all ecosystems (Zehnsdorf et al., 2015), as mass development can already occur under low nutrient concentrations and even charophytes can occasionally grow to problematic proportions (personal observation on *Nitellopsis obtusa* in lake Duinigermeer; Sidorkewicj et al., 1998; Schneider et al., 2013). When substantial nutrient reduction is not feasible, or when macrophyte species remain to cause nuisance after nutrient reduction, other management techniques can be applied to reduce nuisance locally. For example, mowing and removing macrophyte biomass (i.e. mimicking high grazing pressure) could directly reduce localized nuisance problems and simultaneously remove nutrients from the system, which can then be reused as fertilizer for example (e.g. Quilliam et al., 2015; Kuiper et al., 2017).

Conclusions

Light availability and propagule presence determine if submerged macrophytes are encountered or not. Under adequate light levels, sediment nutrient availability was not the major driver in determining rooted submerged macrophytes PVI. We found that high submerged macrophyte PVI was possible over a large range of sediment nutrient levels. The presence of viable propagules, including charophytes, in most of our ecosystem's sediments indicates that once the dominant species diminish, either as a result of oligotrophication over time or after active management, there is local potential for a more diverse submerged vegetation to develop. We propose that the enhanced risk of mass development of submerged macrophytes may be a typical phase when restoring eutrophic shallow ecosystems to a more oligotrophic state. During this transition, water becomes clear, but the sediment still holds ample nutrients. This increases the risk of mass development of submerged macrophytes, but whether mass development is realised depends on other limiting factors, in particular top-down control by herbivores, which can be mimicked by active mowing and removal of aquatic macrophytes.

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Supplementary

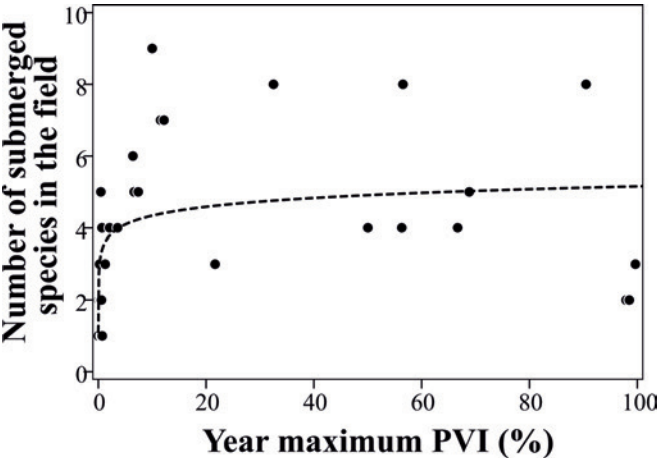


Figure S 1. Yearly maximum submerged plant PVI (of 3 visits) versus mean number of species found in 2013 at a site in the field.

Table S 1. A. Descriptive statistics of lake morphological and water characteristics of each lake per visit. **B.** Descriptive statistics of lake sediment characteristics. “E” represents “10^”.

A

Lake	Bergse Achterplas											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	3	1.23	0.03	4	1.31	0.03	4	1.18	0.03	11	1.24	0.02
pH	3	8.40	0.02	4	8.49	0.05	4	8.21	0.03	11	8.36	0.04
Water temperature (°C)	3	15.7	0.1	4	25.8	0.5	4	19.8	0.0	11	20.9	1.3
Conductivity (µS/cm)	3	929	3	4	989	7	4	969	4	11	965	8
Turbidity (NTU)	3	3.0	0.7	4	5.1	0.4	3	7.4	0.5	10	5.2	0.6

Lake	Bleiswijkse Zoom											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	4	1.24	0.05	4	1.11	0.06	4	1.18	0.05	12	1.18	0.03
pH	4	8.42	0.16	4	8.47	0.03	4	8.49	0.06	12	8.46	0.05
Water temperature (°C)	4	16.8	0.1	4	23.2	0.4	4	15.6	0.1	12	18.5	1.0
Conductivity (µS/cm)	4	781	4	4	820	5	4	772	2	12	791	7
Turbidity (NTU)	4	17.3	3.4	4	24.5	3.0	4	15.9	0.6	12	19.2	1.8

Lake	De Waay											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	4	1.42	0.02	4	1.53	0.08	4	0.69	0.08	12	1.21	0.12
pH	4	8.38	0.07	4	8.79	0.21	4	7.80	0.05	12	8.32	0.14
Water temperature (°C)	4	14.1	0.3	4	21.1	0.1	4	18.7	0.0	12	18.0	0.9
Conductivity (µS/cm)	4	623	2	4	536	3	4	549	35	12	569	16
Turbidity (NTU)	4	5.9	0.4	4	4.8	0.4	4	8.2	1.1	12	6.3	0.6

Lake	Duinigermeer											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	4	1.04	0.04	4	1.06	0.05	4	1.10	0.04	12	1.07	0.02
pH	4	8.54	0.04	4	8.75	0.26	4	8.12	0.05	12	8.47	0.11
Water temperature (°C)	4	22.7	0.2	4	20.2	0.4	4	11.9	0.2	12	18.3	1.4
Conductivity (µS/cm)	4	436	1	4	316	6	4	411	1	12	387	16
Turbidity (NTU)	4	6.2	1.4	4	1.6	0.6	4	10.8	6.2	12	6.2	2.2

Lake	Het Bovenwater											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	4	1.28	0.07	4	1.20	0.07	4	1.16	0.09	12	1.21	0.04
pH	4	8.28	0.21	4	9.26	0.09	4	8.46	0.04	12	8.66	0.15
Water temperature (°C)	4	18.7	1.1	4	19.1	0.7	4	14.4	0.1	12	17.4	0.7
Conductivity (µS/cm)	4	575	9	4	585	1	4	705	0	12	622	18
Turbidity (NTU)	4	3.9	0.6	4	23.8	2.1	4	5.1	0.2	12	10.9	2.8

Lake	Loenderveenseplas Oost											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	4	1.28	0.02	4	1.21	0.01	4	1.24	0.03	12	1.24	0.01
pH	4	8.80	0.12	4	9.31	0.22	4	8.06	0.05	12	8.72	0.17
Water temperature (°C)	4	17.5	0.3	4	19.8	0.1	4	12.7	0.2	12	16.7	0.9
Conductivity (µS/cm)	4	310	1	4	276	1	4	295	1	12	294	4
Turbidity (NTU)	4	9.2	6.3	4	4.3	1.4	4	1.8	0.6	12	5.1	2.2

Lake	Loosdrecht											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	4	1.26	0.04	4	1.26	0.04	4	1.26	0.03	12	1.26	0.02
pH	4	8.05	0.08	4	8.77	0.13	4	8.48	0.10	12	8.43	0.10
Water temperature (°C)	4	13.2	0.0	4	21.9	0.1	4	22.4	0.6	12	19.1	1.3
Conductivity (µS/cm)	4	393	2	4	417	0	4	468	8	12	426	10
Turbidity (NTU)	4	11.4	1.0	4	10.7	0.4	4	7.0	0.7	12	9.7	0.7

Lake	Nannewiid											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	4	0.98	0.00	4	0.99	0.01	4	1.01	0.00	12	0.99	0.01
pH	4	9.80	0.04	4	9.64	0.03	4	9.29	0.03	12	9.58	0.07
Water temperature (°C)	4	15.1	1.3	4	24.3	0.2	4	14.3	0.1	12	17.9	1.4
Conductivity (µS/cm)	4	315	5	4	340	0	4	391	1	12	349	10
Turbidity (NTU)	4	18.1	0.6	4	45.3	3.2	4	10.5	0.4	12	24.6	4.6

Lake	NIOO pond											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	4	0.98	0.08	4	0.86	0.05	4	0.76	0.05	12	0.86	0.04
pH	4	9.05	0.29	4	9.92	0.26	4	9.17	0.11	12	9.38	0.17
Water temperature (°C)	4	14.8	1.3	4	23.6	0.4	4	14.9	0.3	12	17.8	1.3
Conductivity (µS/cm)	4	435	18	4	383	9	4	336	49	12	384	20
Turbidity (NTU)	4	11.1	1.1	4	5.4	1.1	4	4.7	1.9	12	7.1	1.1

Lake	Noorderplassen											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	4	1.18	0.04	4	1.21	0.05	4	1.07	0.08	12	1.15	0.04
pH	4	8.26	0.05	4	8.34	0.03	4	8.49	0.14	12	8.37	0.05
Water temperature (°C)	4	14.3	0.4	4	23.5	0.4	4	20.7	0.2	12	19.5	1.2
Conductivity (µS/cm)	4	1959	18	4	1889	73	4	1799	82	12	1882	39
Turbidity (NTU)	4	1.7	0.3	4	3.6	0.4	4	0.8	0.1	12	2.0	0.4

Lake	Oldambtmeer											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	4	1.31	0.03	4	1.26	0.04	4	1.24	0.04	12	1.27	0.02
pH	4	8.57	0.18	4	8.35	0.15	4	7.91	0.03	12	8.28	0.11
Water temperature (°C)	4	19.1	0.6	4	19.7	0.0	4	12.6	0.1	12	17.1	1.0
Conductivity (µS/cm)	4	490	4	4	455	4	4	449	0	12	465	6
Turbidity (NTU)	4	6.6	0.8	4	8.2	4.4	4	8.1	2.5	12	7.6	1.6

Lake	Sondeler Leien											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	4	1.11	0.05	4	1.13	0.07	4	1.13	0.06	12	1.12	0.03
pH	4	8.90	0.11	4	8.50	0.05	4	8.61	0.03	12	8.67	0.06
Water temperature (°C)	4	18.6	0.1	4	22.8	0.1	4	14.8	0.2	12	18.7	1.0
Conductivity (µS/cm)	4	609	1	4	586	1	4	624	1	12	606	5
Turbidity (NTU)	4	27.2	4.3	4	33.7	1.5	4	13.1	1.2	12	24.7	2.9

Lake	Zevenhuizerplas											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	4	1.21	0.04	4	1.19	0.01	4	1.28	0.05	12	1.22	0.02
pH	4	8.72	0.01	4	8.51	0.04	4	8.28	0.06	12	8.50	0.06
Water temperature (°C)	4	17.6	0.7	4	24.5	0.4	4	17.8	0.2	12	20.0	1.0
Conductivity (µS/cm)	4	1244	2	4	1214	2	4	1189	2	12	1216	7
Turbidity (NTU)	4	4.0	0.7	4	1.2	0.3	4	0.1	0.1	12	1.8	0.5

Lake	Zuidlaardermeer											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	4	1.01	0.01	4	1.11	0.13	4	0.99	0.00	12	1.04	0.04
pH	4	8.87	0.26	4	8.46	0.05	4	8.17	0.11	12	8.50	0.12
Water temperature (°C)	4	19.1	0.6	4	21.9	0.1	4	15.5	0.1	12	18.8	0.8
Conductivity (µS/cm)	4	389	5	4	403	0	4	370	3	12	387	5
Turbidity (NTU)	4	18.0	1.3	4	21.4	2.5	4	9.6	1.2	12	16.4	1.8

Lake	Zwemlust											
Visit	1			2			3			Total		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Site water depth (m)	4	1.28	0.04	4	1.28	0.05	4	1.37	0.02	12	1.31	0.02
pH	4	8.48	0.06	4	8.16	0.03	4	8.03	0.10	12	8.22	0.07
Water temperature (°C)	4	15.8	0.0	4	24.4	0.2	4	22.6	0.5	12	20.9	1.1
Conductivity (µS/cm)	4	452	0	4	468	1	4	516	1	12	478	8
Turbidity (NTU)	4	2.8	0.5	4	6.8	1.2	4	17.9	1.2	12	9.1	2.0

Table S 1 (continued). B. Descriptive statistics of lake sediment characteristics. “E” represents “10^”.

B

Lake	Bergse Achterplas			Bleiswijkse Zoom			De Waay			Duinigermeer			Het Bovenwater		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Sediment total N (mmol/l sed)	4	184.2	50.9	4	94.4	35.8	3	148.4	59.7	4	230.2	19.8	4	125.4	10.0
Sediment total P (mmol/l sed)	4	21.6	6.9	4	11.1	3.6	3	8.4	1.0	4	7.9	1.6	4	11.6	4.9
Porewater N (mmol/l sed)	4	3.05E-01	9.75E-02	4	3.14E-01	2.17E-01	3	1.35E-01	3.40E-02	4	3.22E-01	5.66E-02	4	4.87E-02	3.51E-02
Porewater P (mmol/l sed)	4	2.56E-02	1.49E-02	4	2.31E-02	6.50E-03	3	3.50E-03	1.51E-03	4	2.21E-03	1.14E-03	4	4.26E-03	2.76E-03
Extracted N (mmol/l sed)	3	0.860	0.126	4	2.266	1.437	3	1.386	0.336				4	2.012	0.198
Extracted P (mmol/l sed)	3	0.691	0.129	4	1.085	0.274	3	0.257	0.085	2	0.066	0.022	4	0.462	0.075
Sediment ash (% DW)	4	87.7	3.0	4	96.9	0.7	4	96.5	1.0	4	60.1	4.4	4	92.8	1.0
Sediment moisture content (g/g FW)	4	0.511	0.056	4	0.328	0.025	3	0.374	0.037	4	0.814	0.015	4	0.442	0.053

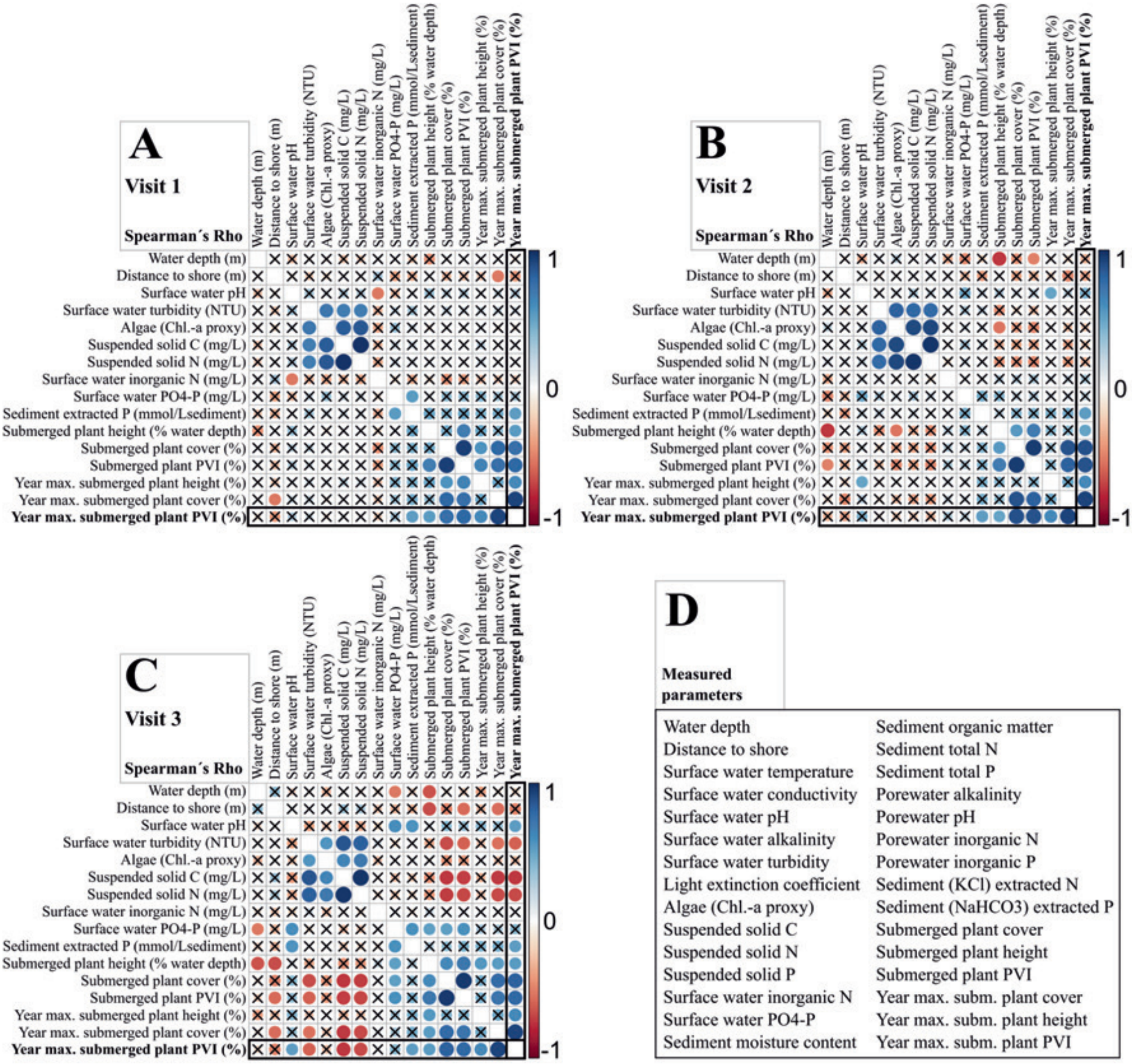
Lake	Loenderveenseplas Oost			Loosdrecht			Nannewiid			NIOO pond			Noorderplassen		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Sediment total N (mmol/l sed)	4	284.0	89.7	4	187.2	101.5	4	168.4	45.7	4	46.9	8.3	4	145.1	16.1
Sediment total P (mmol/l sed)	4	5.0	1.9	4	15.8	7.1	4	11.8	7.0	4	12.7	2.0	4	4.4	2.0
Porewater N (mmol/l sed)	4	6.06E-02	8.98E-03	4	6.15E-02	2.20E-02	4	1.42E-01	7.65E-02	4	6.88E-02	1.52E-02	4	2.04E-01	5.98E-02
Porewater P (mmol/l sed)	3	7.20E-04	5.73E-04	3	6.16E-04	2.73E-04	3	1.08E-03	3.38E-04	4	4.57E-03	1.40E-03	4	1.20E-02	4.35E-03
Extracted N (mmol/l sed)	1	0.900		3	0.612	0.072	3	0.508	0.123	4	0.897	0.113	4	1.615	0.224
Extracted P (mmol/l sed)	3	0.069	0.033	4	0.140	0.061	4	0.315	0.044	4	0.644	0.043	4	0.329	0.071
Sediment ash (% DW)	4	52.9	8.6	4	81.2	6.9	4	85.2	3.9	4	98.3	0.2	4	88.9	2.5
Sediment moisture content (g/g FW)	4	0.727	0.072	4	0.483	0.079	4	0.519	0.078	4	0.250	0.008	4	0.514	0.038

Lake	Oldambtmeer			Sondeler Leien			Zevenhuizerplas			Zuidlaardermeer			Zwemlust		
	n	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*
Sediment total N (mmol/l sed)	4	70.4	4.1	4	235.7	30.9	4	139.2	29.3	2	206.0	160.3	4	68.3	15.0
Sediment total P (mmol/l sed)	4	6.1	1.4	4	15.9	4.8	4	16.6	1.9	2	14.2	7.7	4	15.8	1.9
Porewater N (mmol/l sed)	4	1.42E-02	5.98E-03	4	1.35E-01	1.78E-02	3	6.16E-02	1.75E-02	2	5.70E-03	3.55E-03	4	1.30E-01	4.55E-02
Porewater P (mmol/l sed)	3	7.00E-04	5.73E-04	4	2.44E-02	8.27E-03	3	6.92E-04	1.39E-04	2	8.82E-04	6.59E-04	4	1.74E-03	8.00E-04
Extracted N (mmol/l sed)	4	1.200	0.119	2	0.856	0.244	4	1.855	0.379	2	1.026	0.508	4	0.426	0.069
Extracted P (mmol/l sed)	4	0.414	0.068	3	0.455	0.107	4	0.440	0.087	2	0.101	0.047	4	0.316	0.072
Sediment ash (% DW)	4	93.8	0.4	4	78.2	4.1	4	94.1	0.8	4	94.7	1.7	4	97.9	0.4
Sediment moisture content (g/g FW)	4	0.301	0.021	4	0.668	0.042	4	0.418	0.038	2	0.424	0.105	4	0.332	0.026

Table S 2. Submerged plant cover estimation by using a rake. A full bucket represents approx. 25% of cover, if more material was collected than that fits in the bucket, the cover was estimated by the density of plants on the rake itself. Adapted from Immers et al. (2015) using own observations.

Amount of vegetation in an 11 litre bucket after throwing rake 4x	Estimated cover (%)
Nothing	0
One branch	0.5
2 - 3 branches	1
Small clump	3
Bottom of bucket covered	5
Small layer present in bucket	10
Bucket 50-75% full	20
Full bucket	25
Rakes reasonably full	25-50
Thick wigs on rake	50-100

Table S 3. Non-parametric correlations between environmental factors and yearly maximum PVI from sites with submerged vegetation present measured during the first (A), second (B) and/or third (C) visit. From all the measured parameters (D), only parameters are plotted here that correlated significantly with submerged plant parameters during at least one visit. ‘X’ indicates non-significant correlations and colour and size of the circles represent Spearman’s Rho. Critical p-value is adjusted to the number of comparisons with maximum PVI: critical p = 0.05/29 = 0.0017. (Sediment was measured only once per location during the 3 visits, see Methods section of the main text).



Measured parameters

Water depth
Distance to shore
Surface water temperature
Surface water conductivity
Surface water pH
Surface water alkalinity
Surface water turbidity
Light extinction coefficient
Algae (Chl.-a proxy)
Suspended solid C
Suspended solid N
Suspended solid P
Surface water inorganic N
Surface water PO4-P
Sediment moisture content
Sediment organic matter
Sediment total N
Sediment total P
Porewater alkalinity
Porewater pH
Porewater inorganic N
Porewater inorganic P
Sediment (KCl) extracted N
Sediment (NaHCO3) extracted P
Submerged plant cover
Submerged plant height
Submerged plant PVI
Year max. subm. plant cover
Year max. subm. plant height
Year max. subm. plant PVI

Table S.4. Vegetation cover, canopy depth below the surface, and PVI per lake, site and sampling round. If the visibility was too low to visually see submerged plants, the secchi depth is given indicated by '>'.

		Submerged plant cover (%)				*Average canopy depth (m)*				*Submerged plant PVI (%)*			
Visit	**Waterbody**	**N**	**S**	**E**	**W**	**N**	**S**	**E**	**W**	**N**	**S**	**E**	**W**
1	Bergse Achterplas	0	n/a	0	0	-	n/a	-	-	0	n/a	0	0
	Bleiswijkse Zoom	0	0	0	0	-	-	-	-	0	0	0	0
	De Waay	74	3	3	15	>0.80	1.4	>0.85	>0.70	<1	<1	<1	<1
	Duinigermeer	0	0	1	0	-	-	>0.75	-	0	0	<1	0
	Het Bovenwater	23	50	18	1	0.05	0	0.78	0.3	22	50	7	<1
	Loenderveenseplas Oost	2	0	0.3	4	1.2	-	>1.00	0.8	<1	0	<1	2
	Loosdrecht	0	0	0	0	-	-	-	-	0	0	0	0
	Nannewiid	0	0	0	0	-	-	-	-	0	0	0	0
	NIOO pond	98	99	98	80	0	0.05	0	0	98	92	98	80
	Noorderplassen	1	1	2	3	1.1	1.3	1.2	1.1	<1	<1	<1	<1
	Oldambtmeer	0.4	0.3	5	29	0.25	>0.90	>0.90	0	<1	<1	<1	29
	Sondeler Leien	0	0	0	0	-	-	-	-	0	0	0	0
	Zevenhuizerplas	20	90	32	100	0.95	1.1	1	1	4	11	3	20
	Zuidlaardermeer	0	0	0	0	-	-	-	-	0	0	0	0
	Zwemlust	0.01	0.2	0	0.01	0.95	0.95	-	0.85	<1	<1	0	<1
2	Bergse Achterplas	0	0	0	0	-	-	-	-	0	0	0	0
	Bleiswijkse Zoom	0	1	0	0	-	>0.45	-	-	0	<1	0	0
	De Waay	1	0.1	0	0	>1.10	>0.80	-	-	<1	<1	0	0
	Duinigermeer	0	1	0	0.01	-	0.75	-	0.1	0	<1	0	<1
	Het Bovenwater	8	2	3	3	>0.33	>0.40	>0.40	>0.33	<1	<1	<1	<1
	Loenderveenseplas Oost	10	4	4	10	>1.10	1.1	1.1	0	<1	<1	<1	10
	Loosdrecht	0	0	0	0	-	-	-	-	0	0	0	0
	Nannewiid	0	0	0	0	-	-	-	-	0	0	0	0
	NIOO pond	69	100	99	98	0	0	0	0	69	100	99	98
	Noorderplassen	60	25	100	1	0.6	0.7	0.6	0.5	27	12	51	<1
	Oldambtmeer	40	47	20	97	1.1	>0.8	>0.75	0.5	7	<1	<1	56
	Sondeler Leien	0	0	0	0	-	-	-	-	0	0	0	0
	Zevenhuizerplas	40	100	60	100	0.95	0.4	0.55	0.5	8	67	33	57
	Zuidlaardermeer	0	0	0	0	-	-	-	-	0	0	0	0
	Zwemlust	0	1	3	0	-	>1.00	>1.12	-	0	<1	<1	0
3	Bergse Achterplas	1	0	0	0	>0.90	-	-	-	<1	0	0	0
	Bleiswijkse Zoom	0	0	0	0	-	-	-	-	0	0	0	0
	De Waay	0	4	0.4	0	-	0.05	0	-	0	4	<1	0
	Duinigermeer	0.01	0	0	0	1.2	-	-	-	<1	0	0	0
	Het Bovenwater	0	1	0	0	-	>1.00	-	-	0	<1	0	0
	Loenderveenseplas Oost	10	3	20	15	1.03	0.93	0.9	1.1	1	<1	6	<1
	Loosdrecht	0	0	0	0	-	-	-	-	0	0	0	0
	Nannewiid	0	0	0	0	-	-	-	-	0	0	0	0
	NIOO pond	94	98	97	99	0	0	0	0	94	98	97	99
	Noorderplassen	90	10	100	2	0.2	0.2	0.1	0.9	69	8	90	<1
	Oldambtmeer	4	4	3	10	1.2	0.57	0.97	0.7	<1	2	<1	4
	Sondeler Leien	0	0	0	0	-	-	-	-	0	0	0	0
	Zevenhuizerplas	20	35	30	23	0.45	1.3	1.03	1.28	12	3	5	<1
	Zuidlaardermeer	0	0	0	0	-	-	-	-	0	0	0	0
	Zwemlust	0	0	0	0	-	-	-	-	0	0	0	0

Chapter 4

High tolerance to harvesting management of three major nuisance aquatic plant species over a wide eutrophication gradient

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D. Waasdorp & E.S. Bakker

Abstract

Nuisance growth of submerged aquatic plants is a worldwide problem. A widely used management technique is the mechanical cutting of the plants, but the effects remain unpredictable and cutting is often unsuccessful to effectively reduce the problems associated with nuisance growth.

To better understand and predict the effects harvesting has on nuisance submerged plants, we asked: (1) How does cutting affect the growth, biomass composition (i.e. nutrient and water content) and biomass allocation (i.e. roots-shoots) of common submerged aquatic plants that can cause nuisance? (2) Does the effect of cutting depend on sediment nutrient availability?

To answer these questions, we performed a full-factorial greenhouse experiment applying three sediment nutrient levels and a cutting treatment to three submerged plant species. We measured the effects of the treatments on plant height and biomass production.

Cut Potamogeton perfoliatus shoots remained shorter than uncut shoots four weeks after cutting, but had no significant effect on Myriophyllum spicatum and Elodea nuttallii. Cutting did not affect total biomass production, composition, or allocation. Nutrient availability affected M. spicatum's response to cutting, but not for the other species. Reducing nutrient availability had a much stronger negative effect on the plants than cutting in our experiment and is arguably a better long-term management strategy against nuisance plant growth.

Introduction

Shallow freshwater ecosystems provide many services to humans, including supplying drinking and irrigation water, providing food and fiber, and degrading pollutants (Carpenter et al., 2011). Submerged plants play a major role in maintaining these ecosystem services in shallow freshwater lakes and waterways through their ecosystem functions (Carpenter & Lodge, 1986), including increasing water transparency by competing with algae and reducing sediment resuspension with their roots. Additionally, submerged plants provide food and habitat for a variety of animal species, including fish and water birds (Bakker et al., 2016; Wood et al., 2016).

However, in some cases submerged aquatic plants can grow so massively that they cause problems for human use of the ecosystem (Hilt et al., 2006; Verhofstad & Bakker, 2017). Large expanses of tall plants can, for instance, impair recreation and reduce water flow, causing flooding (Verhofstad & Bakker, 2017; Nichols, 1991; Vereecken et al., 2006). To counteract these problems, water managers apply various management techniques, costing large sums of money each year (Hilt et al., 2006; Hussner et al., 2017). In this study, we focused on mechanical control (e.g. cutting and harvesting) as it is widely used worldwide and has several advantages over other methods. First, mechanical cutting directly reduces plant length and thus the plant's potential to cause nuisance. Second, mechanical cutting depth and cutting area are highly controllable. Cutting can therefore reduce localized nuisance problems while maintaining a viable plant population in the rest of the ecosystem that can keep performing its important ecosystem functions (Finlay & Vogt, 2016). Harvesting the cut biomass furthermore prevents the release of inorganic nutrients from the decaying plant material into the water and also allows for the biomass to be used as a valuable resource as, for example, agricultural fertilizer or as a green energy source (Edwards, 1980; O'Sullivan et al., 2010; Quilliam et al., 2015). This helps to close the local nutrient cycle (Chowdhury et al., 2017). The nutrient concentration and water content of the biomass are important quality variables for these applications of the harvested biomass (Edwards, 1980; O'Sullivan et al., 2010; Quilliam et al., 2015). Environmental nutrient availability and the cutting regime itself may affect these biomass characteristics, but also may affect the plant's investment in various parts of the plant, such as roots or shoots (e.g. Cronin & Lodge, 2003; Miler & Straile, 2010; Velthuis et al., 2017).

Even though mechanical cutting is widely used and has several advantages over other removal methods, it's effectiveness in reducing the nuisance caused by excessive submerged plant growth varies considerably (Hussner et al., 2017). This variation in success rate may be caused by several mechanisms: First, some aquatic plants might generally be very tolerant to cutting and show rapid regrowth in length and biomass causing low success rates of cutting management (Painter, 1988; Abernethy et al., 1996; Richardson, 2008). Second, management success rates may also vary because different submerged plant species respond differently to cutting, for example due to differences in biomass allocation towards shoots versus roots when cut. Third, the impact of cutting may depend on environmental conditions. Aquatic plant growth rates generally increase with nutrient availability up until their maximum growth rate (Barko & Smart, 1986; Bornette & Puijalon, 2011) or until the plants become light limited due to growth of periphyton and phytoplankton (Bakker et al., 2013a; Hidding et al., 2016). Therefore, the plant's resilience to cutting and their regrowth potential likely increases with environmental nutrient availability, until phytoplankton growth prevents their regrowth (e.g. Kuiper et al., 2017), but empirical proof is still largely absent.

To improve our understanding and predictions of the effects of harvesting on nuisance submerged plants, we focused on the following questions: How does cutting affect the growth, biomass composition (i.e. nutrient and water content) and biomass allocation (i.e. roots versus shoots) of common submerged aquatic plants that can cause nuisance? Does the effect of cutting depend on sediment nutrient availability? We hypothesized that (1) cutting will reduce plant length, plant biomass, plant tissue nutrient concentration and will increase root:shoot-ratios during several weeks after cutting. Furthermore, we hypothesized that (2) cutting will have a larger impact on the plants at lower nutrient availability, because of reduced regrowth potential due to resource limitation.

To test these hypotheses, we performed a controlled greenhouse experiment, where the plants were grown for two months. We used a full-factorial design with three nutrient levels and one cutting treatment (i.e. cut – uncut) and measured plant shoot and root growth to assess how nutrient availability affected the plants' response to cutting. *Elodea nuttallii*, *Myriophyllum spicatum* and *Potamogeton perfoliatus* were chosen as study species, because they represent major genera of rooted submerged plants and are all common and reported to cause nuisance worldwide (Hilt et al., 2006; Zefferman & Harris, 2016; Verhofstad & Bakker, 2017). Hence, choosing these species will enable us to assess the generality of the response to cutting of nuisance causing submerged macrophytes. Furthermore, they are all able to take up nutrients from the sediment (i.e. the primary source in our study) (Smith & Barko, 1990; Christiansen et al., 2016; Halbedel, 2016).

Methods

Plant collection and acclimation

We collected shoots of *E. nuttallii* from a culturing pond of the Netherlands Institute of Ecology (NIOO-KNAW, 51°59'17.2"N-5°40'25.0"E) and shoots of *M. spicatum* from a constructed wetland near Elst in the Netherlands (51°55'35.6"N-5°53'23.6"E). Shoots of *P. perfoliates* were ordered from a local supplier (WaterplantGigant, De Mortel, the Netherlands). After collection, the plants were gently cleaned with tap water to remove algae and fauna. Only healthy looking shoots were selected and the upper 20 cm was cut off and used for acclimation to greenhouse conditions. These rootless shoots were planted in 200 L cattle tanks (diameter = 66 cm, height = 60 cm) for the acclimation. The cattle tanks were first filled with approximately 30 L of clean sand (grain size = 0.4-0.8 mm) and 50 grams of slow release fertilizer (Basacote 6M 16-8-12 PLUS; referred to as 'SRF') were mixed into this layer of sand. Approximately 10 L of clean sand was added on top to reduce nutrient leakage from the sediment into the overlying water layer. The cattle tanks were subsequently filled with tap water up until the 200 L mark. Shoots were planted 5 cm into the sediment using tweezers. Each of the three species was planted in a separate tank. The acclimation lasted for 2 weeks. At the end of the acclimation period, most shoots developed roots, appeared healthy, and had grown. At this time, the daytime pH of the surface water in the acclimation tanks ranged from 9.0 to 9.9 with an alkalinity of 0.6 to 0.9 meq L^{-1}.

Experimental setup

Because we aimed to add a similar amount of dry weight (DW) of each plant species in each experimental aquarium of the main experiment (see Fig. 1 for the dimensions and specifications), we harvested six 15 cm long shoot samples of all three species one day before starting the main experiment to determine their fresh weight to dry weight ratio (FW:DW ratio). The following day we cut the top 15 cm of all healthy shoots from the

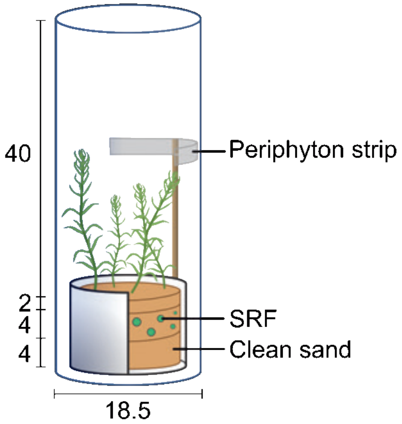

Figure 1. Schematic of the cylindrical glass experimental aquarium. Values represent the dimensions in cm. The sediment was put in a white PP container with a diameter of 11 cm. Total sediment volume was 1 L and total aquarium volume was 13.4 L. The periphyton strip consisted of a 21*2 cm strip of mildly textured GBC Polyclearview PP.

acclimation tanks and cleaned them in the same way as after collection. Using the measured FW:DW ratio, we divided them into equal treatment portions of 4.0 g FW, 1.7 g FW, and 3.5 g FW, for *E. nuttallii*, *M. spicatum*, *P. perfoliatus*, respectively. This resulted in 7 - 11 shoots, 3 - 4 shoots, and exactly 3 shoots per portion for *E. nuttallii*, *M. spicatum*, *P. perfoliatus*, respectively. We planted the pre-weighed shoots 5 cm into the sediment of the experimental aquaria, using tweezers. This ensured that the shoot was firmly fixed in the sediment and could easily access the nutrients released by the SRF. We planted the individual shoots evenly over the sediment surface area. Because one portion of *E. nuttallii* shoots consisted of around 10 shoots, we bundled two shoots together during planting. After the shoots were planted, we filled the aquaria to the brim with tap water (total oxidized nitrogen (TON) < 0.05 mg-N L^{-1}; NH4 < 0.5 mg-N L^{-1}; PO4 < 0.05 mg-P L^{-1}). After filling, we added a periphyton strip to measure epiphyte growth (Fig. 1). The aquaria were placed in three 2x18 aquaria rows with 50 cm in between the rows. Daily light regime was set to 16 hours of light and 8 hours of dark and the water temperature averaged (± SD) 24.4 ± 1.8 °C during the day and 22.7 ± 1.5 °C during the night.

Nutrient and cutting treatments

For all three species, we setup a full-factorial experiment with three sediment nutrient levels (low, medium and high) and a cutting treatment (cut and uncut). For the low nutrient treatment, we added 0.25 ± 0.01 g SRF L^{-1} sand, for the medium treatment we added 1.0 ± 0.01 g SRF L^{-1} sand, and for the high treatment we added 2.0 ± 0.01 g SRF L^{-1} of sand (mean ± SD; after Bakker et al. (2013a)). These nutrient treatments were earlier tested to cover the gradient from nutrient limited to excess nutrient availability, for *P. perfoliatus* (Bakker et al., 2013a). We separately tested the amount of inorganic nutrient released by the SRF for each of the nutrient treatments in unvegetated control aquaria (see Supplementary fig. 1 and 2 online for methods and results).

In the cut treatment, plants were cut 10 cm above the sediment (i.e. reset to initial height, see following section) halfway through the experiment (i.e. after four weeks) to simulate a harvesting event. The cut parts were removed from the aquarium. All treatments were replicated six times, resulting in 108 experimental units, excluding controls without plants.

Harvest

At the end of the experiment we first removed the periphyton strip and inserted each strip in a 50 ml tube and stored this in the dark at 4 °C for biomass measurements. We then removed the container holding the sediment and plants from the aquarium. All the shoot material was removed and gently washed to remove algae. If a substantial amount of filamentous algae was present (i.e. enough to be able to collect it), we stored and analyzed the algae as well (two cases: 0.15 and 0.04 g DW). Subsequently, the roots were washed. The fresh shoot, root, and filamentous algae biomass was weighed, dried (60 °C) and stored for DW and C:N:P analyses.

Pest control

Even though plants were thoroughly washed before planting some herbivorous caterpillars still hatched in our experimental aquaria. We removed them immediately from the aquaria to prevent severe damage to the plants. If we could not remove the animals, for example when they were holding on tight to the plant, we killed those animals using tweezers to avoid damaging or uprooting the plant.

Plant measurements

To follow plant growth and the effectiveness of cutting, the length of all shoots in an aquarium was recorded twice a week by measuring the distance between the top of the shoots and the brim of the aquarium. We subtracted this number from the distance between the sediment surface and the top of the aquarium, giving the plant length. We averaged the plant length per aquarium for the statistical analyses on plant height.

The C, N, and P concentration of all shoot and root biomass samples was determined as follows. Samples were first ground to a powder using either a mill grinder or a ball grinder, depending on the amount of material available. For C and N analysis, approx. 0.5 mg DW was folded into a tin cup and subsequently analyzed using a CN-analyzer (FlashEA 1112 Series, Thermo Scientific, MA, USA). P content was determined by ashing 0.5-1 mg dry biomass for 30 minutes at 550°C. We then digested the samples with a 2.5 % potassium persulphate solution in an autoclave at 121 °C for 30 minutes and analyzed the supernatant colorimetrically with an autoanalyzer system (QuAAtro SFA, Seal Analytical, Germany).

Periphyton measurements

Within one week after the experiment ended, the periphyton was scraped of both sides of the periphyton strip with a toothbrush into a filter cup filled with demiwater. The demiwater with periphyton was then pulled over a prewashed and pre-weighed Whatman GF/F (GE Healthcare GmbH, Germany) glass microfiber filter under a vacuum. Finally, we rinsed the sides of the filter cup with demiwater to ensure no significant amounts of periphyton remained in the setup. The filter was then dried at 60 °C to determine periphyton DW.

Water measurements

Free NO_2, TON, NH_4 and PO_4 in the surface water was measured at the start, one day before cutting, one day after cutting (to assess nutrient leakage), and one day before the end of the experiment. On these days, we took a water sample using a 15 ml tube and stored it at -20 °C until analysis. One day before the analysis, samples were thawed overnight at 4 °C. Inorganic nutrients were measured colorimetricaly on the auto-analyzer system mentioned above. No indications for nutrient leakage were found (Supplementary Results S 1; Supplementary Figure S 2).

pH and turbidity were measured twice a week in the afternoon using a multimeter and turbidity meter, respectively (Multi350i WTW GmbH, Germany, and Turb430IR, WTW GmbH, Weilheim, Germany, respectively). For water quality results see Supplementary results S 1.

Water temperature was measured every half hour using data loggers, placed around 10 cm above the sediment surface in six of the aquaria (iButton, HomeChip, Milton Keynes, England). Four were placed in aquaria in the corners of the experimental setup and two in the middle.

Surface water alkalinity was measured once a week. Fifty ml samples were collected around 6 PM on the day before and stored in the dark at 4 °C overnight in closed, airtight tubes. Alkalinity of a 20 ml subsample was determined via titration to pH 4.2 with a 0.01 M HCl solution (TitraLab 840® Radiometer Analytical SAS, France).

Sediment measurements

A sediment porewater sample (10 ml) was extracted weekly using rhizons and a 50 ml syringe. The porewater was stored in 15 ml tubes at -20°C for free NO_2, TON, NH_4 and PO_4 analysis. Samples were diluted five or 10 times if necessary and analyzed as described under the section 'water measurements'.

Statistical analyses

We used ANOVA-tests to analyze the biomass data, final plant length, and water characteristics at the end of the experiment using 'Species name', 'Nutrient level', and 'Cutting treatment' as fixed factors, including all interactions between the factors. Test assumptions for normality of residuals and equal variance among groups were visually assessed using QQ and variance plots. If the data distribution did not meet the requirements for the test the data was transformed. If we had to transform data with zeros, we added the lowest value + 0.001 to all values to allow transformation. Differences between the ANOVA results from the untransformed and from the transformed data were very similar. If a factor explained a significant amount of variation in the ANOVA model, a post-hoc test was performed with Bonferroni p-adjustments to

Table 1. Effect of the treatments on vegetation parameters. Sp (Initial): differences among species at the start of the experiment (grey fill). Sp: effect of species, Nut: effect of nutrient availability, Cut: effect of cutting. When interactions are present, main effects are disregarded (grey text). Bold faced p–values are statistically significant at 0.05.

	Sp (Initial)	Sp	Nut	Cut	Sp:Nut	Sp:Cut	Nut:Cut	Sp:Nut:Cut
Removed biomass during cutting $(g_{DW}.g_{DW}^{-1})$	-	$F_{2,45} = 40.9$ **p < 0.001**	$F_{2,45} = 21.6$ **p < 0.001**	-	$F_{4,45} = 1.1$ $p = 0.36$	-	-	-
Standing biomass at end $(g_{DW}.g_{DW}^{-1})$	-	$F_{2,90} = 34.9$ $p < 0.001$	$F_{2,90} = 20.2$ $p < 0.001$	$F_{1,90} = 30.2$ $p < 0.001$	$F_{4,90} = 8.8$ **p < 0.001**	$F_{2,90} = 2.4$ $p = 0.098$	$F_{2,90} = 3.7$ **p = 0.028**	$F_{4,90} = 0.7$ $p = 0.57$
Total biomass produced $(g_{DW}.g_{DW}^{-1})$	-	$F_{2,90} = 46.8$ $p < 0.001$	$F_{2,90} = 27.2$ $p < 0.001$	$F_{1,90} = 0.6$ $p = 0.42$	$F_{4,90} = 6.9$ **p < 0.001**	$F_{2,90} = 0.0$ $p = 0.98$	$F_{2,90} = 1.6$ $p = 0.20$	$F_{4,90} = 0.8$ $p = 0.53$
Shoot FW:DW ratio $(g.g^{-1})$	$F_{2,15} = 19.0$ **p < 0.001**	$F_{2,90} = 61.9$ $p < 0.001$	$F_{2,90} = 152.9$ $p < 0.001$	$F_{1,90} = 0.5$ $p = 0.50$	$F_{4,90} = 7.2$ **p < 0.001**	$F_{2,90} = 0.4$ $p = 0.66$	$F_{2,90} = 0.1$ $p = 0.89$	$F_{4,90} = 1.0$ $p = 0.43$
Root:Shoot ratio at end $(g_{DW}.g_{DW}^{-1})$	-	$F_{2,90} = 47.2$ $p < 0.001$	$F_{2,90} = 45.6$ $p < 0.001$	$F_{1,90} = 9.0$ **p = 0.004**	$F_{4,90} = 20.1$ **p < 0.001**	$F_{2,90} = 1.6$ $p = 0.20$	$F_{2,90} = 0.2$ $p = 0.85$	$F_{4,90} = 0.7$ $p = 0.57$
Total root biomass produced $(g_{DW}.g_{DW}^{-1})$	-	$F_{2,90} = 49.1$ $p < 0.001$	$F_{2,90} = 16.7$ $p < 0.001$	$F_{1,90} = 0.4$ $p = 0.55$	$F_{4,90} = 14.2$ **p < 0.001**	$F_{2,90} = 1.8$ $p = 0.18$	$F_{2,90} = 0.7$ $p = 0.52$	$F_{4,90} = 1.9$ $p = 0.12$
Root FW:DW ratio $(g.g^{-1})$	-	$F_{2,90} = 140.7$ **p < 0.001**	$F_{2,90} = 16.8$ **p < 0.001**	$F_{1,90} = 0.8$ $p = 0.38$	$F_{4,90} = 1.6$ $p = 0.18$	$F_{2,90} = 0.8$ $p = 0.46$	$F_{2,90} = 2.7$ $p = 0.072$	$F_{4,90} = 0.6$ $p = 0.69$
Average plant height at end (cm)	-	$F_{2,90} = 171.8$ $p < 0.001$	$F_{2,90} = 57.5$ $p < 0.001$	$F_{1,90} = 58.4$ $p < 0.001$	$F_{4,90} = 2.6$ **p = 0.040**	$F_{2,90} = 9.3$ **p < 0.001**	$F_{2,90} = 0.3$ $p = 0.75$	$F_{4,90} = 1.2$ $p = 0.30$
Biomass C $(mmol.g_{DW}^{-1})$	$F_{2,15} = 8.8$ **p = 0.003**	$F_{2,89} = 61.4$ $p < 0.001$	$F_{2,89} = 21.6$ $p < 0.001$	$F_{1,89} = 0.7$ $p = 0.41$	$F_{4,89} = 2.8$ **p = 0.032**	$F_{2,89} = 0.2$ $p = 0.84$	$F_{2,89} = 1.8$ $p = 0.18$	$F_{4,89} = 1.3$ $p = 0.28$
Biomass N $(mmol.g_{DW}^{-1})$	$F_{2,14} = 52.8$ **p < 0.001**	$F_{2,90} = 6.1$ $p = 0.003$	$F_{2,90} = 339.9$ $p < 0.001$	$F_{1,90} = 0.0$ $p = 0.83$	$F_{4,90} = 5.4$ **p < 0.001**	$F_{2,90} = 0.3$ $p = 0.71$	$F_{2,90} = 0.0$ $p = 0.99$	$F_{4,90} = 0.3$ $p = 0.86$
Biomass P $(mmol.g_{DW}^{-1})$	$F_{2,15} = 1.5$ $p = 0.26$	$F_{2,90} = 0.3$ $p = 0.73$	$F_{2,90} = 1.1$ $p = 0.33$	$F_{1,90} = 0.1$ $p = 0.76$	$F_{4,90} = 4.0$ **p = 0.005**	$F_{2,90} = 0.1$ $p = 0.95$	$F_{2,90} = 0.8$ $p = 0.43$	$F_{4,90} = 0.9$ $p = 0.45$

identify the differences among individual treatments. All tests were performed and all graphs were made in R (version 3.1.2, R Core Team).

Data availability

The raw data generated during the current study will be placed in Data Dryad upon publication of the paper.

Results

Effects of cutting on plants

Plant height

The effect of cutting on plant height differed among the plant species (Table 1). Cutting significantly reduced the average length of *P. perfoliatus* shoots at low and medium nutrient levels (Fig. 2: panel C). There were no significant effects of cutting on the other species (Fig. 2).

Biomass production, dry matter content and nutrient concentration

The effect of cutting depended on plant species and nutrient level (Table 1). Cutting significantly reduced standing biomass at the end of the experiment for *M. spicatum* at the medium nutrient level (Fig. 3: panel D) but not for the other species.

Cutting significantly increased the final root:shoot ratio (Table 1: significant main effect). This was mainly caused by the lower shoot mass of the cut plants, as the cut plants did not produce significantly more roots than uncut plants (Table 1; Supplementary Fig. S 3).

Cutting did not significantly affect the total amount of biomass produced during the entire experimental period for any species (Table 1; Supplementary Fig. S 4). Cutting also did not significantly affect the final fresh- to dry-weight ratio (FW:DW) of the shoots (Supplementary Fig. S 5) nor the N and P concentration of the shoots and roots (Table 1; Fig. 4).

Effects of nutrient availability on cutting plants

Plant height

The effect of nutrient availability on the growth of the plants differed per species, but no significant interaction of nutrient availability with cutting was observed (Table 1). The uncut shoots of *P. perfoliatus* and *M. spicatum* were taller in the high nutrient treatment than in the low nutrient treatment. For *P. perfoliatus*, this was also true for the cut shoots (Fig. 2). Overall, *E. nuttallii* had the shortest shoots of all species and its height was not significantly affected by sediment nutrient level (Fig. 2).

Biomass production

We found a significant interaction between cutting and nutrient availability on the amount of standing biomass (DW) at the end of the experiment (Table 1). Here, cut plants of *M. spicatum* had significantly less biomass (DW) than uncut plants in the

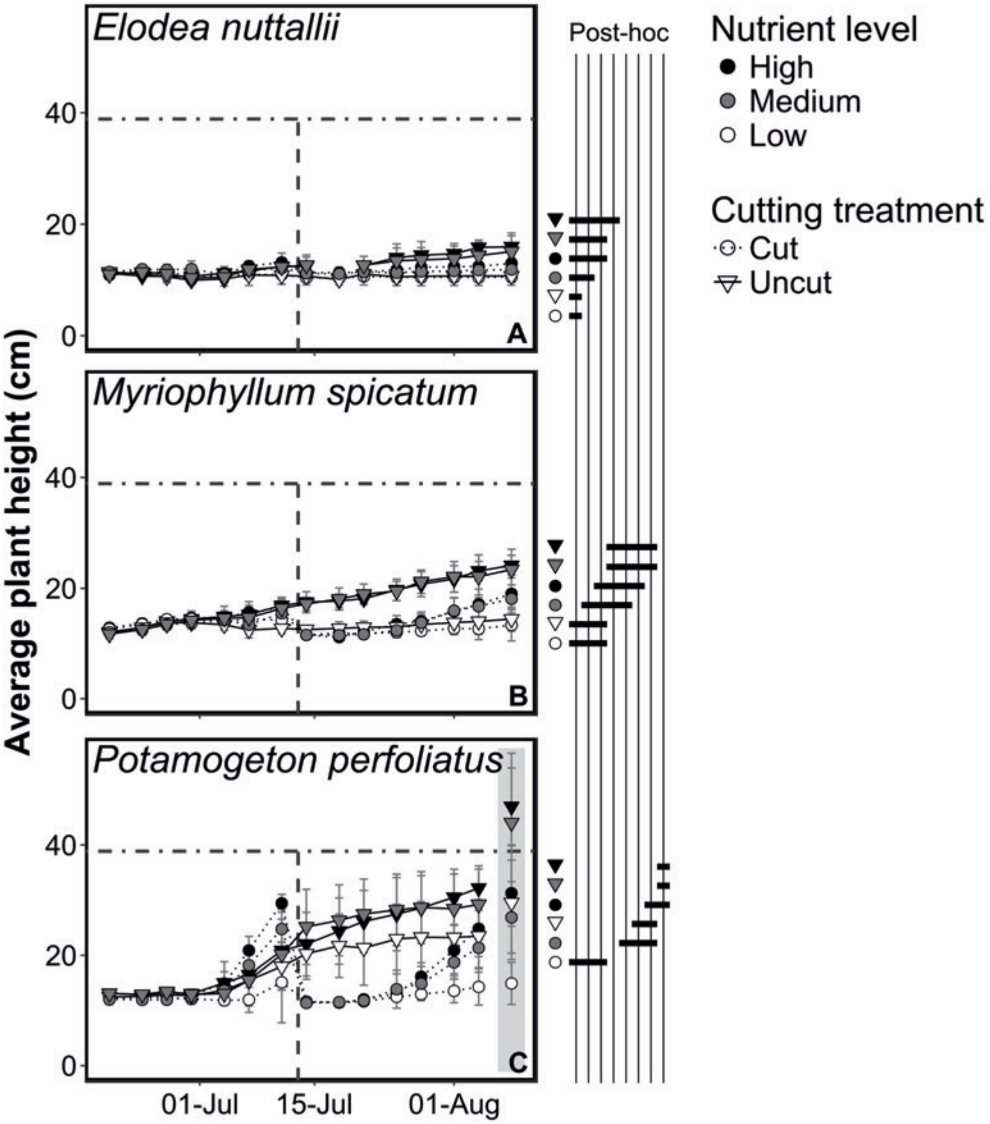

Figure 2. Mean height of the shoots of the different plant species in the different treatments. Darker fill represents higher nutrient availability, circles connected by dotted lines represent the cut plants and triangles connected by solid lines represent uncut plants. The vertical dashed line shows the cutting date. The horizontal dot-dashed line shows the water depth. Error bars represent the standard deviation of the mean. P. perfoliatus (**C**) grew to the water surface in several aquaria and the total length of the shoots was only measured during the last measurement (grey area, **panel C**), the maximum possible length was equal to the water depth during the earlier dates. Differences among treatments are statistically significant at the end of the experiment if horizontal lines under 'Post-hoc' do not overlap. For ANOVA results, see Table 1.

medium nutrient treatment, but not in the other nutrient treatments (Fig. 3). No significant interactions were identified in the post-hoc analyses for the other two species.

Nutrient availability significantly increased most of the measured plant biomass parameters, often with significant interactions with the species (Table 1). During the mid-experimental cutting, more biomass (DW) was removed at higher nutrient levels compared to the low nutrient level for all species (Fig. 3).
Over the whole experimental period, all species except *P. perfoliatus* produced significantly more biomass (DW), at high than at low nutrient availability (Supplementary Fig. S 4). *E. nuttallii* produced on average around two times more biomass at medium and high nutrient treatments than at the low nutrient treatment. At medium and high nutrient treatments *M. spicatum* produced most DW of all species during the experiment. *P. perfoliatus* and *M. spicatum* also produced significantly more root biomass (DW) at low than at high nutrient availability, which was not the case for *E. nuttallii*. Furthermore, uncut plants of *M. spicatum* had significantly higher root:shoot ratios at low nutrient levels than at high nutrient levels at the end of the experiment, indicating a higher investment towards root biomass (Supplementary Fig. S 3).

Plant dry matter content and nutrient concentration
The shoot FW:DW ratio was higher at higher nutrient availability for all species at the end of the experiment, with no significant interaction with cutting (Table 1; Supplementary Fig. S 5).
At the start of the experiment, *M. spicatum* had the lowest shoot FW:DW ratio among the three species (9.3 ± 0.4, mean ± SE, ANOVA: $F_{2,15} = 19.0$, $p<0.001$), whilst it had the highest ratio at the end of the experiment at medium and high nutrient treatments (Supplementary Fig. S 5). The FW:DW ratios of *E. nuttallii* and *P. perfoliatus* at the start were 12.5 ± 0.2 and 11.2 ± 0.5 (mean ± SE), respectively.

Increasing nutrient availability significantly increased plant N concentrations, but no significant interaction was found between nutrient availability and cutting on any biomass N or P parameter (Fig. 4; Table 1). Root, shoot and whole plant (i.e. roots + shoots) N concentration of all three species was more than 2 times higher in the high nutrient treatment than in the low nutrient treatment (Fig. 4A-F, Table 1), which was also reflected in the lower tissue C:N ratio (Supplementary Table S 1). Nutrient availability did not significantly affect plant (i.e. shoot + root) P concentrations (Fig. 4G-L, Table 1).
At the start of the experiment, shoot P concentrations were similar among the three species (Table 1). *M. spicatum* had a significantly lower shoot N concentration at the start compared to the other two species (around 0.2 mmol N g^{-1} DW lower, Fig. 4A,C,E; Table 1). At the end of the experiment differences were larger. At the high nutrient treatment, *E. nuttallii* had a significantly higher shoot N concentration than the other two species at the end of the experiment, while *M. spicatum* had a significantly higher root N concentration than the other two species.

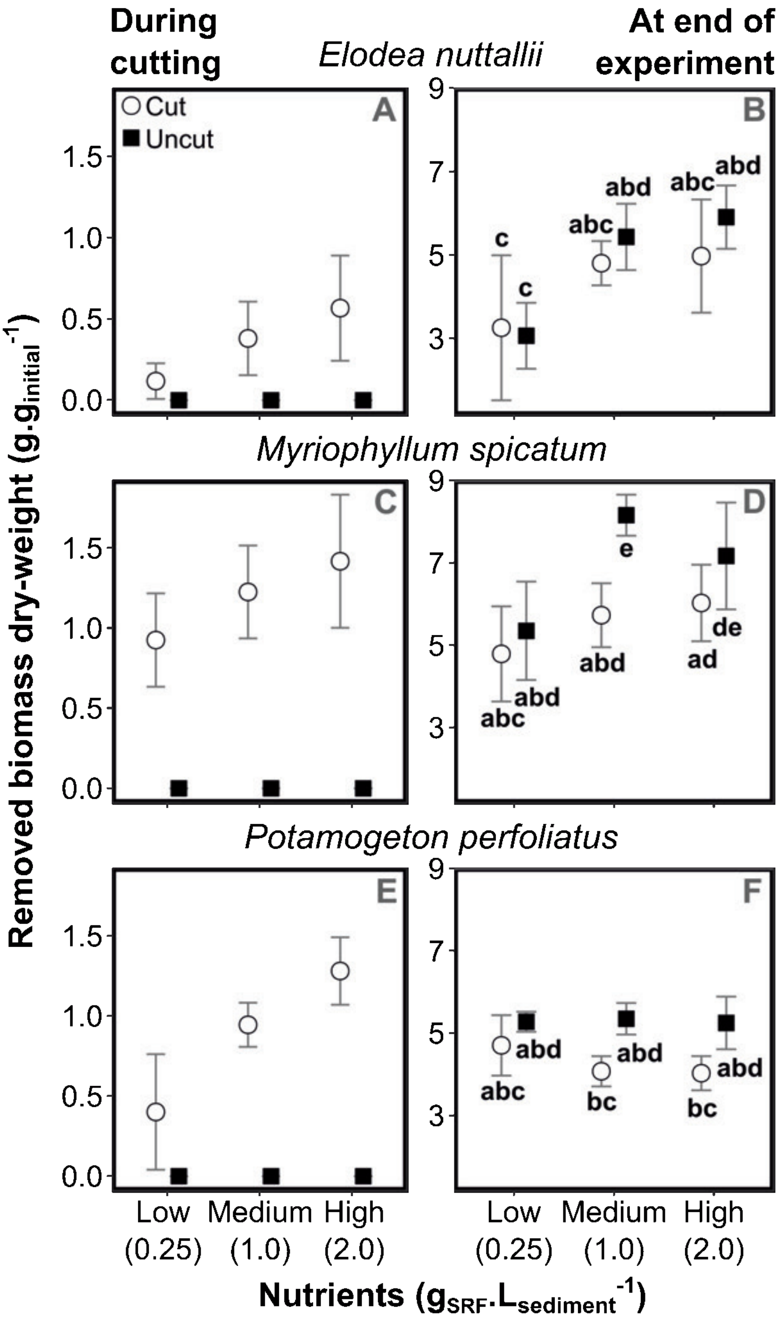

Figure 3. Amount of dry mass removed per gram of initial biomass for each of the three species over the experimental period under the different cutting and nutrient treatments. Error bars show the standard deviation of the mean. The amount of biomass removed during cutting differed significantly among all species ($F_{2,45} = 40.9$; $p < 0.001$) and more biomass was removed from the high compared to the low nutrient treatment ($F_{2,45} = 21.6$; $p < 0.001$). Lower case letters represent significant differences in removed biomass at the end of the experiment among the treatments (**panels B,D,F**). For ANOVA results, see Table 1.

Discussion

In this study, we tested how three major nuisance species of submerged macrophytes responded to a single cutting event and whether this response was affected by nutrient availability. We found that all species were very tolerant to cutting and that nutrient availability had a much larger impact on the plant's growth and biomass characteristics than our cutting regime. We found partial support for our hypotheses as cutting reduced the standing biomass of *M. spicatum* during four weeks (hypothesis 1) and this effect was only present at medium nutrient availability (significant interaction between cutting and nutrient availability: hypothesis 2). Furthermore, cutting significantly reduced the height of *P. perfoliatus* at the low and medium nutrient treatments, however no significant interaction between cutting and nutrient availability was found for plant height. We found limited effects of cutting on the other plant species and other measured plant parameters, including total biomass production and biomass characteristics, over the tested nutrient gradient. We thus conclude that these species are rather tolerant to cutting over a very broad environmental nutrient gradient.

Effects of cutting

We will discuss three possible reasons for the lack of a strong effect of cutting on total macrophyte biomass production and tissue nutrient concentrations in our experiments. First, perhaps the plants became nutrient limited in all nutrient treatments around the time of cutting due to nutrient depletion. This is unlikely however, as the slow release fertilizer (SRF) constantly released nutrients over the experimental period (as demonstrated by the unvegetated control aquaria, see Supplementary Figure S 1 & S 2) and the plants continued to grow.

Second, perhaps low carbon availability limited the growth of the plants (Bornette & Puijalon, 2011). In our experiment, surface water alkalinity and pH levelled off around the time of cutting (0.6-0.8 meq.L^{-1} and 9-10, respectively; Supplementary Figure S 6 and S 7). This could indicate low carbon availability for the plants, and could have limited growth of all plants. However, this is also not likely because the shoot length of the plants still increased after the cutting took place. Of course, plants can also elongate their stems by increasing water content instead of producing new cells and thus biomass (e.g. Dixon et al., 2006). But this also was not the case in our study, as we did not observe that the distance between leaves (i.e. internode length) increased (data not shown). This would be the case if the plant had stretched instead of grown. Furthermore, shoot FW:DW ratio was also similar at the time of cutting and at the end of the experiment. If carbon would have severely limited macrophyte growth, we would expect a lower tissue carbon concentration in the more productive treatment (i.e. high nutrient treatment). This was not the case (Supplementary Table S 1). All three species have specific traits that allow them to grow under low CO_2 concentrations. They can use HCO_3^- when CO_2 availability is low (Eighmy et al., 1991; Raun et al., 2010; Hussner & Jahns, 2015). The alkalinity during the acclimation phase was also relatively low (i.e. similar to the experiment itself).

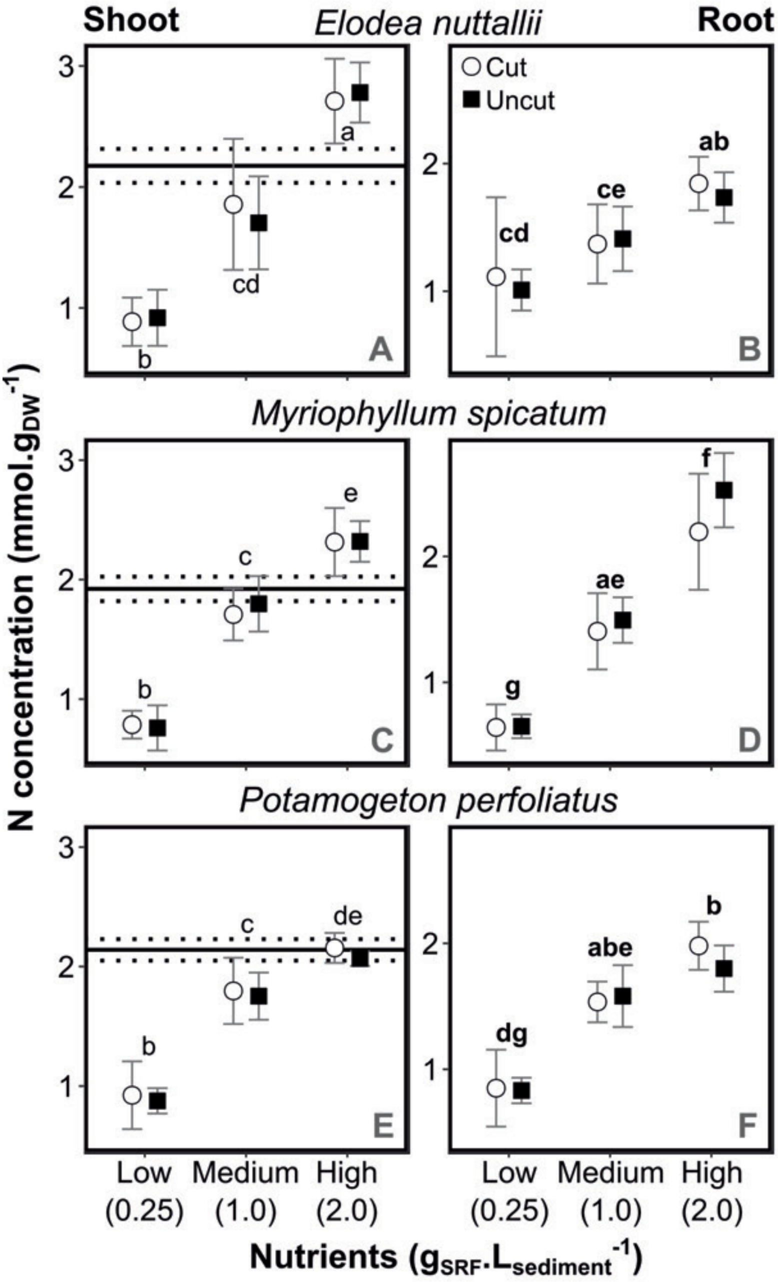

Figure 4. Mean nitrogen (**A-F**) and phosphorus (**G-L**) concentrations in shoots and roots at the end of the experiment for the different treatments. Error bars represent the standard deviation of the mean. Black horizontal and dotted lines (**panels A,C,E,G,I,K**) show the mean and standard deviation, respectively of the initial N or P concentration of the planted shoots. Different lowercase letters show statistically significant differences in shoot (normal face) or root (bold face) nitrogen or phosphorus concentrations among species and nutrient treatment (ANOVA post-hoc: $p < 0.05$).

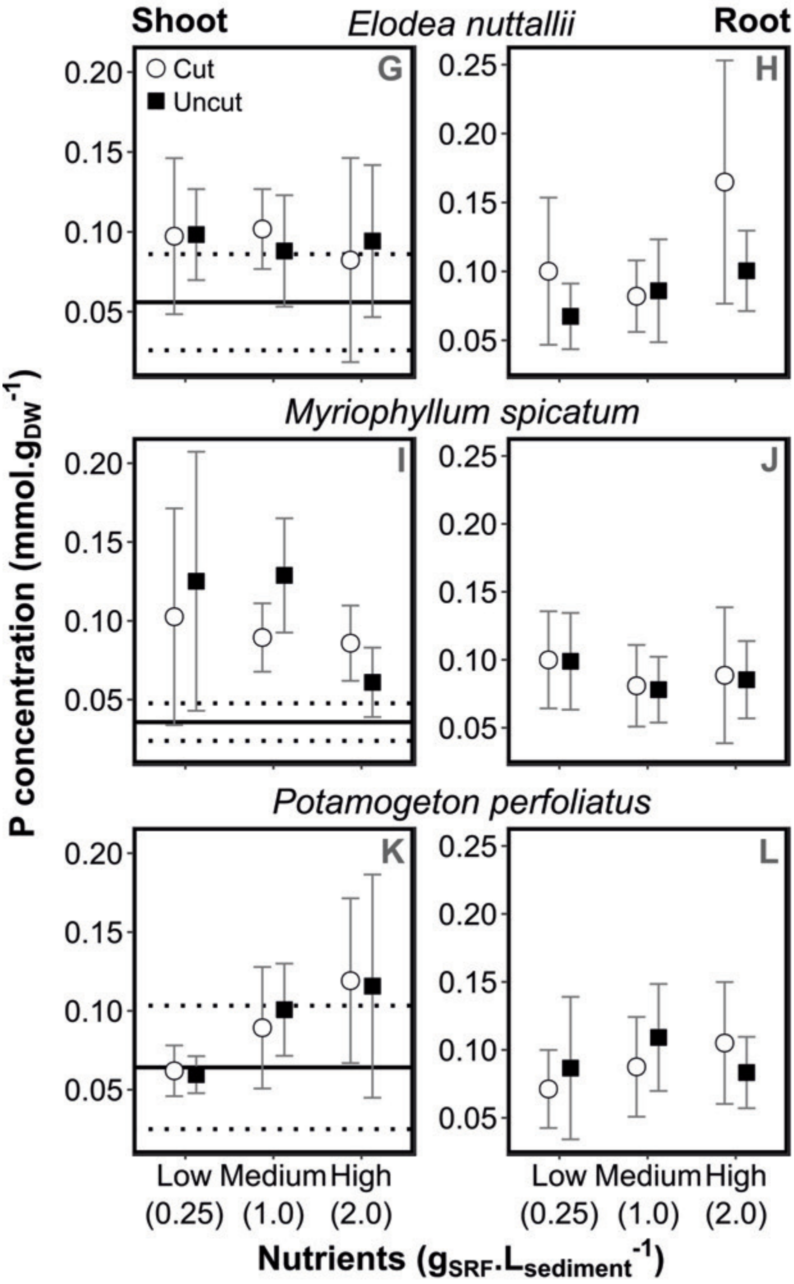

Figure 4 (continued).

Therefore, the plants were already acclimated to low CO_2 concentrations, which could have increased their HCO_3^- uptake in the experiment even further (Hussner & Jahns, 2015).

We thus conclude that the most likely reason why growth was not affected by cutting is that the tested species are very tolerant to cutting and that their growth rate is not severely impacted by harvesting part of their aboveground biomass.

Cutting did not affect all species and plant parameters tested in our study, indicating that cutting may have variable effects on submerged plants. Indeed, cutting has been reported to decrease standing biomass, plant height, or growth across studies (Table 2). In other cases, it had no effector even stimulated plant growth (e.g. Li et al., 2010). The large variability in effects of cutting is likely due to different environmental conditions, species characteristics and the cutting method applied (i.e. timing, frequency, and intensity) (Table 2; Van Zuidam & Peeters, 2012).

Effect of environmental conditions on cutting response

Next to the effect of cutting on submerged macrophytes, we also investigated the interactive effect that environmental nutrient availability could have on the plant's response to cutting. Increased nutrient availability significantly increased plant growth and tissue N concentrations, but not P concentrations, in our study. We also found a significant interaction between the response to cutting of the species and sediment nutrient availability was the decreased growth (biomass DW) of cut *M. spicatum* at medium nutrient availability, but not in the other nutrient treatments or for any other measured plant parameters. Even though we did not find as strong interactions between the effects of cutting and nutrient availability as we hypothesized, the overall lower macrophyte growth in the low nutrient treatments indicates that repeated cutting may have a longer lasting effect in more nutrient deprived sites, as regeneration of the tissue will take longer and the plant's resources may become exhausted. Nutrient availability has been shown to significantly increase the regeneration rate of *V. spiralis* after cutting (Li et al., 2010; Table 2), suggesting that the effect of cutting may be affected by nutrient availability, at least under some conditions. The reported positive effect of nutrient availability on *V. spiralis* regrowth may be due to more favorable abiotic conditions, as Li et al. (2010) used higher temperatures, higher nutrient levels and higher sediment organic matter than we did. It is therefore possible that the tested species in our study might also show higher compensatory growth after cutting under these conditions.

Another important abiotic factor is light availability, as cutting may induce a stronger reduction in biomass under low light conditions compared to higher light availability, as shown for *M. spicatum* (Abernethy et al., 1996). In our study, there was no indication that plants became light limited, as the plant received 16 h of light per day (mainly direct sunlight), the water remained very transparent (Supplementary Figure S 8) and the observed periphyton growth was very low across all treatments.

Species characteristics

There are inherent differences among species in their response to cutting, as Abernethy et al. (1996) showed that *Elodea canadensis* was less affected by cutting than *M. spicatum* when subjected to the same treatments (Table 2). In our study, the different species often responded differently to environmental nutrient availability as shown by many significant interactions between species and nutrient treatment (Table 1). However, because we found only very few significant interactions between species and cutting, *E. nuttallii*, *P. perfoliatus* and *M. spicatum* responded very similarly to cutting stress under the prevailing conditions.

M. spicatum and *P. perfoliatus* grew better than *E. nuttallii* at low sediment nutrient availability in our experiment. *M. spicatum* and *P. perfoliatus* also produced more root biomass per gram of initial biomass in the low nutrient treatment, while *E. nuttallii* did not, which presumably helped *M. spicatum* and *P. perfoliatus* to achieve their higher growth at low nutrient availability.

In our study, all three plant species were tolerant to mechanical cutting. However, cutting may have a larger impact on other plant species (Sabbatini & Murphy, 1996; Zhang et al., 2014), which could lead to shifts in plant community composition in response to cutting (Zhang et al., 2014). Cutting vegetation in natural systems can consequently promote the dominance of stress tolerant species through several mechanisms. First, stress tolerant species are often able to reproduce by fragmentation (Nichols, 1991) and fragmentation is stimulated by cutting, thus promoting the spread of the nuisance species (Hussner et al., 2017). Second, the impact of cutting on the growth of stress tolerant species will be lower than the impact on more sensitive species, increasing the competitiveness of the tolerant species. Field studies have indeed indicated that cutting a submerged plant community can enable stress tolerant species, such as *Elodea nuttallii*, *Ceratophyllum demersum* and several *Myriophyllum* species to establish (Engel, 1990; Garbey et al., 2003). Cutting tolerant species often only has short term effects on the tolerant species in the field (Johnson & Bagwell, 1979; Engel 1990; Serafy et al., 1994), but can have longer effects on other species (Baattrup-Pedersen & Riis, 2004; Van Zuidam & Peeters, 2012), thus leading to selective pressures. Contrarily, examples are also available where cutting and harvesting has increased submerged vegetation diversity and stimulated charophyte abundance by reducing the abundance of the dominant plant species (Engel 1990; Howard-Williams et al., 1996; Zhang et al., 2014; Verhofstad et al., 2017b). The response the vegetation shows to cutting (i.e. either reduced or increased macrophyte diversity) may depend on the combination of cutting stress intensity with productivity of the system. The dominant nuisance species may have a competitive advantage in highly productive systems, but may be outcompeted under low nutrient availability (Hidding et al., 2010; Richter & Gross, 2013).

Table 2. Effects of mowing on plant biomass and length for different submerged plant species, measured in controlled experiments. 'MIN' indicates a decrease and 'PLUS' indicates an increase in the measured parameters due to cutting and NS that the effect was not statistically significant. Measured duration after cutting indicates the observed duration of the effect and thus represent the minimum time that the effect was visible.

Species	Cutting frequency	Cutting timing	Cutting intensity	Effects on biomass	Effects on length	Measured duration after cutting	Measure	Comments	Ref.
Elodea canadensis	1x	After 35 days	5cm above sediment	MIN 41%	NS(±MIN 14%)	13 weeks+	Standing stock		19
E. canadensis	2x	After 35 and 66 days		MIN 59%	MIN 44%	8 weeks+			
M. spicatum	1x	After 35 days		MIN 45%	MIN 22%	13 weeks+			
M. spicatum	2x	After 35 and 66 days		MIN 90%	MIN 70%	8 weeks+			
M. spicatum	1x	After 21 days	50% of shoot length	NS (MIN ±11%)	NA	11 weeks+	Growth rate	Length is stolon length in this case.	53
M. spicatum	4x	After 21 days and every 15 days	First cut: 50%, rest: 25% of shoot	NS (MIN ±10%)	NA	11 weeks+			
Myriophyllum verticillatum	1x	After 21 days	50% of shoot	MIN ±15%	NS (PLUS ±2%)	11 weeks+			
M. verticillatum	4x	After 21 days and every 15 days	First cut: 50%, rest: 25% of shoot	MIN ±17%	NS (PLUS ±3%)	11 weeks+			
Potamogeton coloratus	1x	After 21 days	50% of shoot	MIN ±19%	NS (PLUS ±2%)	11 weeks+			
P. coloratus	4x	After 21 days and every 15 days	First cut: 50%, rest: 25% of shoot	MIN ±31%	NS (PLUS ±3%)	11 weeks+			
Potamogeton lucens	2.50x	When plants reach surface (i.e. 35cm)	17.5cm above sediment	NS (0)	NS (MIN 9%)	23 weeks+	Growth rate	Cut throughout the 160 day experiment when plants reached surface. First cut at around 85 days. Cutting frequency given is the mean of the treatment.	35
P. lucens	2.00x		9cm above sediment	NS (MIN 24%)	NS (MIN 25%)	23 weeks+			
P. lucens	1.25x		0cm above sediment	NS (MIN 56%)	NS (MIN 54%)	23 weeks+			
Potamogeton compressus	4.75x		17.5cm above sediment	MIN 31%	NS (PLUS 4%)	27 weeks+		Cut throughout the 187 day experiment when plants reached surface. First cut at around 31 days. Cutting frequency given is the mean of the treatment.	
P. compressus	4.25x		9cm above sediment	MIN 33%	NS (0)	27 weeks+			
P. compressus	1.50x		0cm above sediment	MIN 87%	MIN 77%	27 weeks+			

Table 2 (continued).

Species	Cutting frequency	Cutting timing	Cutting intensity	Effects on biomass	Effects on length	Measured duration after cutting	Measure	Comments	Ref.
Potamogeton pectinatus	1x	60 days after acclimation	2cm above sediment	PLUS 23%	NS (±PLUS 12%)	12 weeks+	Standing stock		54
P. pectinatus	2x	60 & 90 days after acclimation		MIN 53%	NS (±MIN 26%)	8 weeks+			
V. spiralis	1x	At start	28 to 30% of biomass	PLUS 108 to 179%	NA	4 weeks+	Standing stock		34
				PLUS 17.9 to 64.1%	NA	4 weeks+	Growth rate		
Hydrilla verticillata	1x	After 15 days	Slashed four times per cutting	±MIN 29%	NS (±MIN 28%)	7 weeks+	Standing stock	Plants were growing together as a community	37
H. verticillata	2x	After 15 and 35 days		±MIN 54%	±MIN 64%	4 weeks+			
E. canadensis	1x	After 15 days		NS (±PLUS 4%)	NS (±PLUS 6%)	7 weeks+			
E. canadensis	2x	After 15 and 35 days		NS (±MIN 15%)	NS (±MIN 24%)	4 weeks+			
Ceratophyllum demersum	1x	After 15 days		NS (±MIN 17%)	NS (±MIN 18%)	7 weeks+			
C. demersum	2x	After 15 and 35 days		NS (±PLUS 8%)	NS (±MIN 24%)	4 weeks+			
Chara fragilis	1x	After 15 days		NS (±PLUS 13%)	NS (±PLUS 46%)	7 weeks+			
C. fragilis	2x	After 15 and 35 days		NS (±PLUS 9%)	NS (±PLUS 16%)	4 weeks+			
M. spicatum	1x	After 15 days		NS (±PLUS 13%)	NS (±PLUS 6%)	7 weeks+			
M. spicatum	2x	After 15 and 35 days		NS (±MIN 31%)	NS (±MIN 39%)	4 weeks+			

NOTE: percentage calculated as: (cut - uncut) / uncut * 100%. Lighter values between brackets indicate that the difference between cut and uncut was not statistically significant, but differed this amount. ± indicates that this value was read from a graph using the image prosessing and analyses software: Image-J.

References:19 (Abernethy et al., 1996); 34 (Li et al., 2010a); 35(Van Zuidam & Peeters, 2012); 37 (Zhang et al., 2014); 53 (Mony et al., 2011); 54 (Filizadeh & Murphy, 2002)

Timing, frequency and intensity of cutting

We applied a cutting treatment once and measured its short-term effects. Increasing the cutting intensity (Wile, 1978; Madsen et al., 1988; Engel, 1990), cutting closer to the sediment (Van Zuidam & Peeters, 2012), removing both roots and shoots, and timing cutting when the plants are most vulnerable (i.e. taking life-history into account) will all increase the impact of cutting (Garbey et al., 2003; Bal et al., 2006; Zhang et al., 2014).

Cutting as a management strategy

It is clear that cutting stress tolerant macrophyte species is not always effective as a management method to control their nuisance growth. Cutting can be used successfully if the goal is a rapid, but temporarily, relief from local nuisance problems by reducing the plant height or biomass (Finlay & Vogt, 2016; Hussner et al., 2017). If parts of the shoots remain intact, this management method simultaneously preserves the ecosystem functions the plants provide (Finlay & Vogt, 2016), such as preventing algal blooms (e.g. Kuiper et al., 2017). Sustainable harvesting by cutting can also be used successfully if the goal is to use the submerged plants to extract nutrients from the water column in constructed wetlands (i.e. nutrient polishing, Tang et al., 2017).

Cutting is not effective if the goal is to drastically reduce the dimensions of submerged macrophyte stands for a long period of time, as effects of cutting are often short-lived (weeks to months) and cutting may even stimulate the growth of stress tolerant species. When the goal is to achieve a long-term reduction in plant height and biomass, altering the growth conditions (i.e. bottom-up management) is a more sustainable method (Finlay & Vogt, 2016). Our study showed that reducing the nutrient availability has much larger effects on the plants than cutting for all three species. Furthermore, a reduction in nutrient loading may instill a shift in plant species community composition towards shorter species, such as charophytes (Richter & Gross, 2013) that are less likely to cause problems. However, even though reducing nutrient availability may reduce the growth of aquatic plant species, it does not guarantee that no species will grow tall at all, as nuisance growth of some species may even be possible under relatively low nutrient levels (Schneider et al., 2013; Verhofstad et al., 2017a). The reduction in nutrient loading required to prevent nuisance all together is arguably very difficult to achieve, but nutrient reduction in general will already reduce the scale of the nuisance problems and its effects will be long-lasting.

Conclusions

Cutting once at 10 cm above the sediment had short-term effects on common nuisance plants and the plant’s response to cutting partially depended on nutrient availability. All species grew faster at increasing nutrient availability. Cutting stress tolerant species will therefore only have short-term effects on the macrophyte vegetation in many nuisance-stricken ecosystems, as these are typically characterized by high nutrient availability. Cutting and harvesting the biomass can in general best be used to rapidly reduce localized nuisance problems associated with high submerged macrophyte growth. However, achieving long-term effects through harvesting management is either unlikely or very expensive and destructive. Reducing nutrient availability will have larger and long-lasting effects on the productivity of the submerged macrophytes and their growth rate.

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Supplementary

Results S 1. Effects cutting on water quality

Water Nutrients

We measured the highest total oxidized nitrogen (TON) values in aquaria with cut *E. nuttallii*. Cutting had no significant effect on surface water TON content at the end of the experiment (Supplementary Fig. S 2; Supplementary Table S 2). We furthermore did not observe a clear nutrient peak after cutting that would have indicated nutrients leaking from the cut shoots. In general, free inorganic N and P concentrations in the surface water were very low throughout the experiment; close or below the lowest analytical standard (see Supplementary Fig. S 2).

pH and Alkalinity

The pH of the water increased during the experiment in all aquaria to around 10, except in the controls where it remained stable. (Supplementary Fig. S 6; Supplementary Table S 2). Cutting generally lowered the pH slightly, but no significant differences between cut and uncut aquaria were found within the same species and nutrient treatment in post-hoc analyses.

Alkalinity decreased over time in all experimental aquaria, but remained relatively stable in the controls (Supplementary Fig. S 7). The alkalinity at the end of the experiment was lower at low nutrient level than at high nutrient level in experimental aquaria with *P. perfoliatus* and *M. spicatum*, however, no effect of nutrient availability was observed for *E. nuttallii* (Supplementary Table S 2). Nutrient level also affected alkalinity in the controls with higher nutrient levels leading to lower alkalinity.

Turbidity and Periphyton

The water remained very clear in all experimental aquaria and was around 1 NTU at the end of the experiment (Supplementary Fig. S 8). Turbidity was generally highest at high nutrient levels. We furthermore found a main effect of cutting, where cutting generally decreased turbidity (i.e. increased water clarity; Supplementary Table S 2) however, these differences were not statistically significant in post-hoc analyses. Periphyton growth was very limited in all aquaria, with average periphyton biomass of 0.2 g DW m^{-2} at the end of the experiment. This low amount could not be measured accurately on the micro-balans, therefore no statistical analyses was performed.

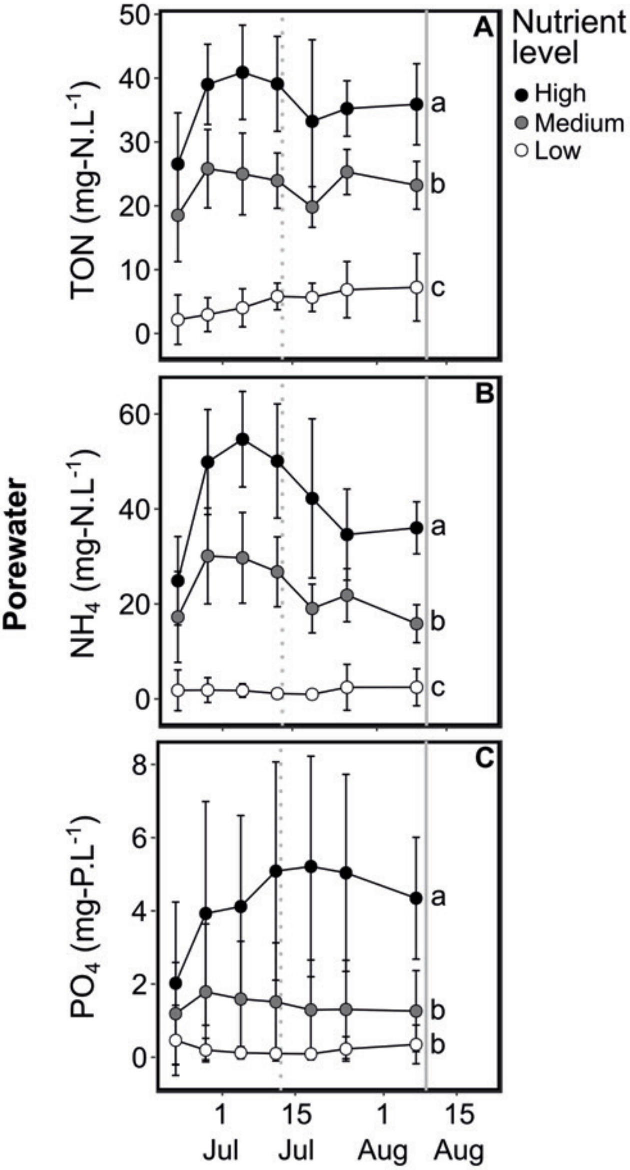


Figure S 1. Mean inorganic nutrient concentrations in sediment porewater of unvegetated control aquaria over the experimental period. Point fill indicates the nutrient availability, dotted vertical line indicates the cutting date (13 July 2016), solid vertical line indicates the end of the experiment (11 August 2016) and the error bars represent the standard deviation of the mean. Lowercase letters show statistically significant differences in porewater nutrients on the last sampling before the end (Fertilizer level: $p < 0.001$).

To accurately measure nutrient release, we added six additional aquaria without plants for all three nutrient treatments as controls to measure actual nutrient availability over time. We covered the control aquaria with a cardboard box to eliminate light, thus preventing photoautotrophic growth. We inserted rhizons (Rhizon SMS, RRP B.V., Wageningen, the Netherlands) through the entire sediment layer of these control aquaria to sample the porewater. Nutrient availability in the control aquaria differed significantly among the nutrient treatments, with the lowest amounts of porewater nutrient availability present in the low nutrient aquaria and the highest amounts present in the high nutrient aquaria (Supplementary Fig. S 1). Part of the total oxidized nitrogen (TON, i.e. primarily nitrate in our case) released by the SRF leaked to the surface water. TON in the surface water of the control aquaria increased with increasing sediment SRF concentration (Supplementary Fig. S 2: Panel D).

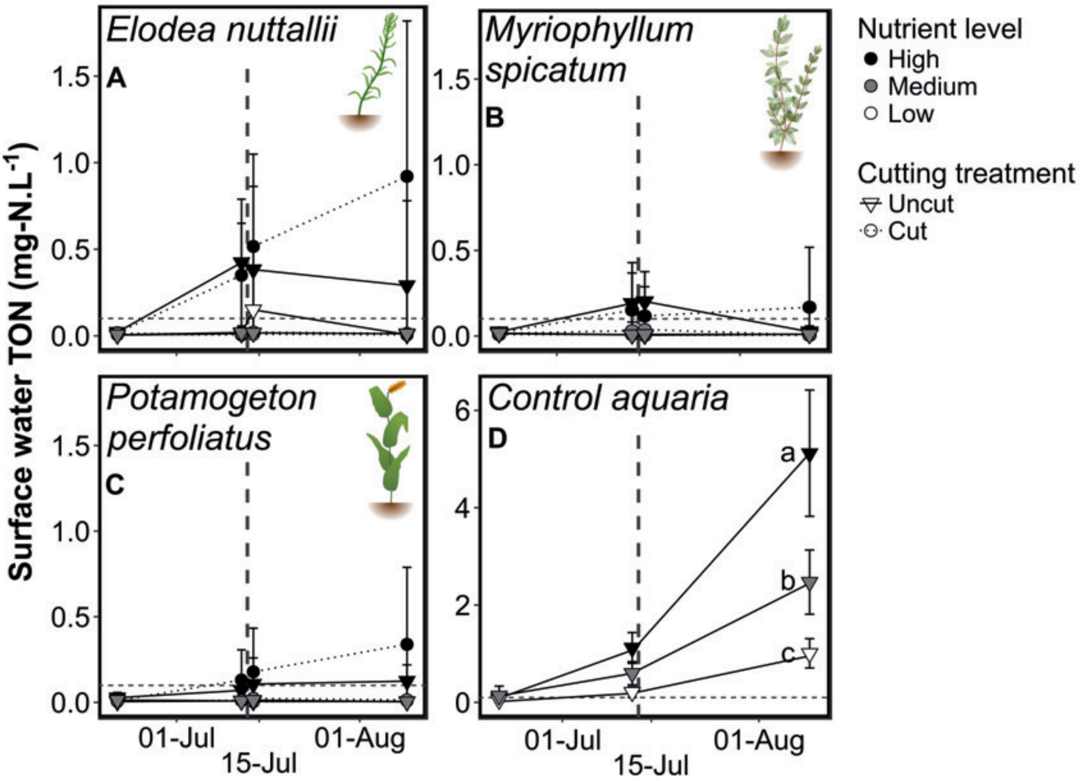


Figure S 2. Mean total oxidized nitrogen (TON) concentration in the surface water. Point fill indicates the fertilizer level, dashed horizontal line indicates the lowest standard used, dashed vertical line indicates the cutting date and the error bars represent the standard deviation of the mean. Lowercase letters in **panel D** show statistically significant differences in surface water TON on the last sampling before the end in the unvegetated control aquaria (Fertilizer level: $p < 0.001$). For ANOVA results for the vegetated aquaria, see Supplementary Table 2 online. Here, surface water TON was significantly higher in the high nutrient treatment than in the other treatments for E. nuttallii and P. perfolatius ($p > 0.05$). No other differences were statistically significant in post-hoc analyses.

The nutrient levels in the surface water in the vegetated experimental aquaria were very low throughout the experiment, often more than two times below the lowest calibration standard (i.e. 0.1 mg.L-1, TON-N, NO2-N, PO4-P, 1 mg.L-1 for NH4-N). Only TON was above the lowest standard in most of the high nutrient aquaria at the end of the experiment. More TON was available in the surface water in the high nutrient treatments compared to the lower nutrient treatments at the end of the experiment (**panels A-C**).

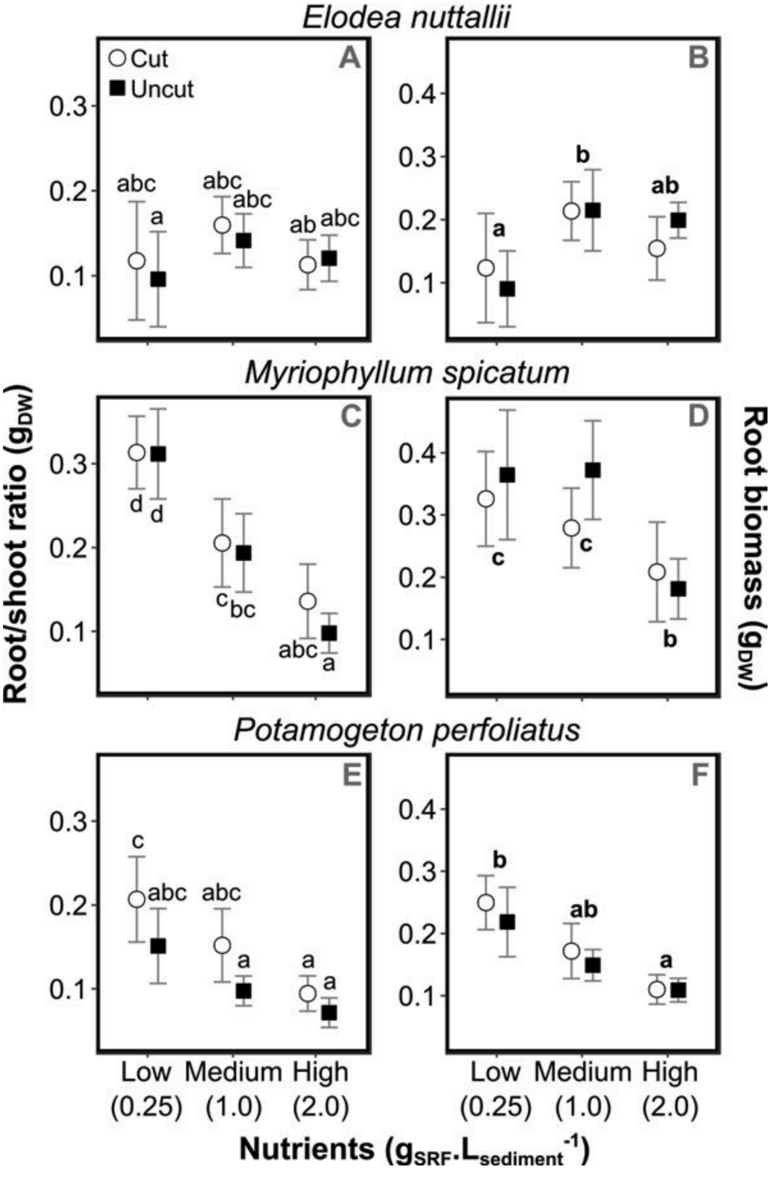


Figure S 3. Mean root/shoot ratio at the end of the experiment (**A,C,E**) and amount of root biomass dry weight produced during the two-month experimental period for the different treatments (**B,D,F**). Error bars show standard deviation of the mean. Different lowercase letters show statistically significant differences, for ANOVA results, see Table 1.

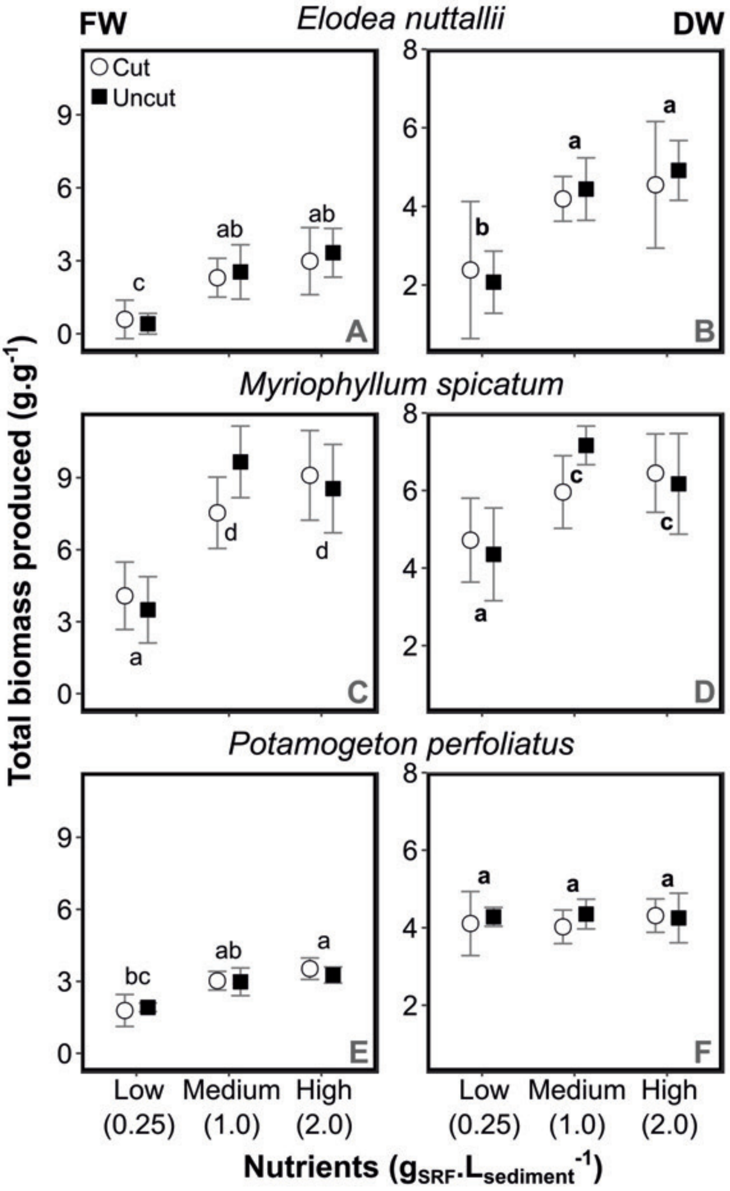


Figure S 4. Total amount of fresh (FW) and dry mass (DW) produced per gram of initial biomass by the three species over the entire experimental period under the different treatments. Error bars show the standard deviation of the mean. Lower case letters represent significant differences between produced fresh weight (**panels A,C,E**) or dry weight (**panels B,D,F**). For ANOVA results, see Table 1.

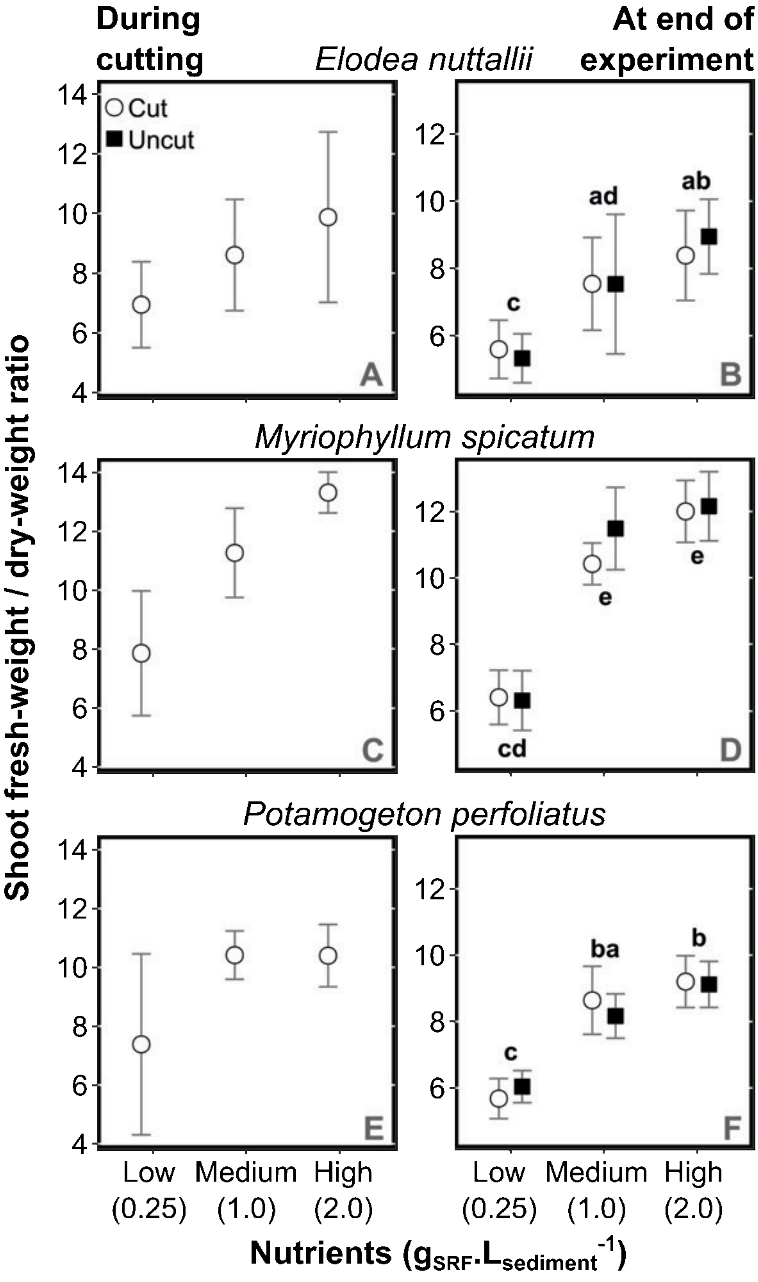


Figure S 5. Shoot of the three species over the entire experimental period under the different treatments. Error bars show the standard deviation of the mean. The fresh to dry weight ratio of shoots removed during cutting differed significantly between E. nuttalli and M. spicatum (F2,43 = 7.2; p = 0.002) and overall the shoots had lower in the low nutrient treatment compared to the other nutrient treatments (F2,43 = 18.9; p < 0.001). Lower case letters represent significant differences in shoot FW:DW ratios of the biomass at the end of the experiment (**panels B,D,F**). For ANOVA results, see Table 1.

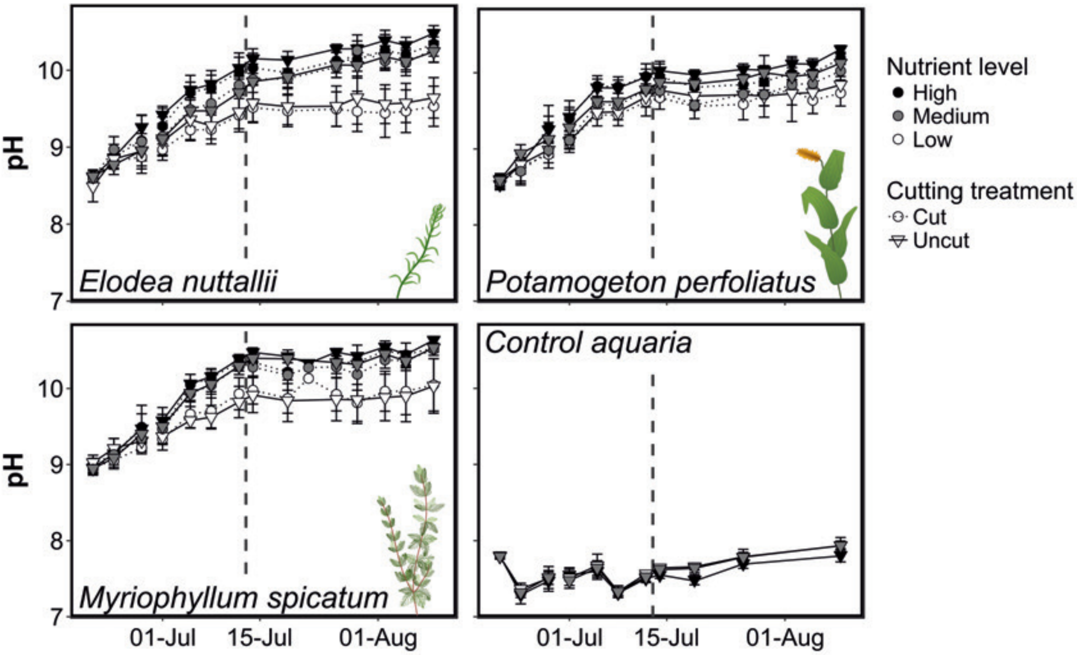


Figure S 6. Mean surface water pH. Point fill indicates the fertilizer level, dashed vertical line indicates the cutting date, solid vertical line indicates the end of the experiment and the error bars represent the standard deviation of the mean. Unvegetated aquaria: Fertilizer level p > 0.05. For ANOVA results for the vegetated aquaria, see Supplementary Table S 2.

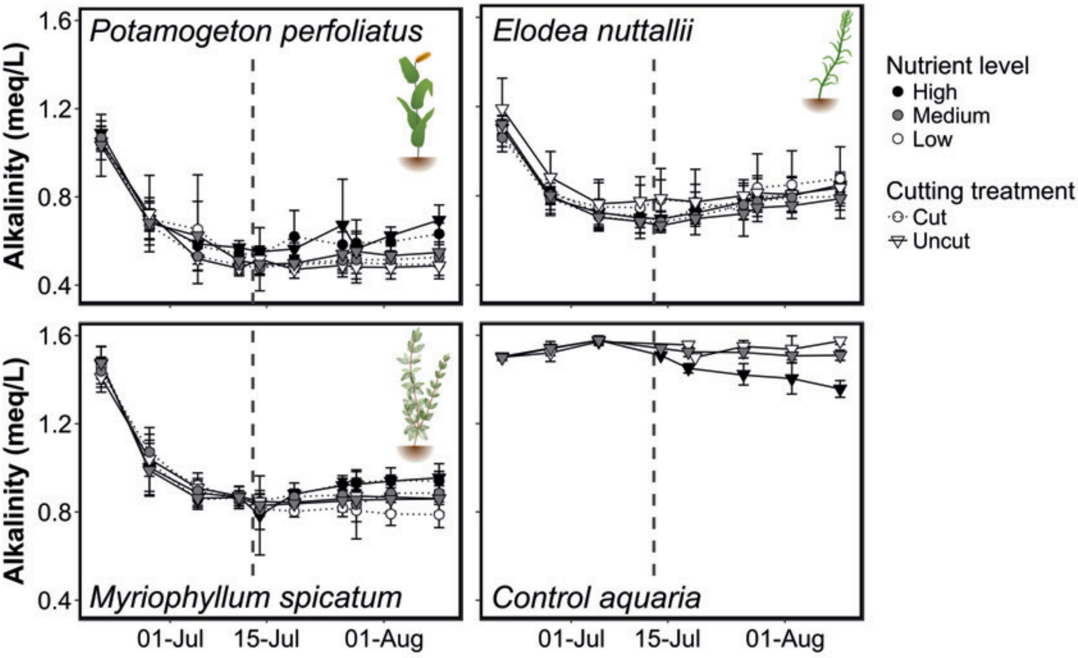


Figure S 7. Mean surface water alkalinity. Point fill indicates the fertilizer level, dashed vertical line indicates the cutting date, solid vertical line indicates the end of the experiment and the error bars represent the standard deviation of the mean. For ANOVA results for the vegetated aquaria, see Supplementary Table S 2. In the control aquaria, Alkalinity was significantly different among all three treatments (p < 0.001).

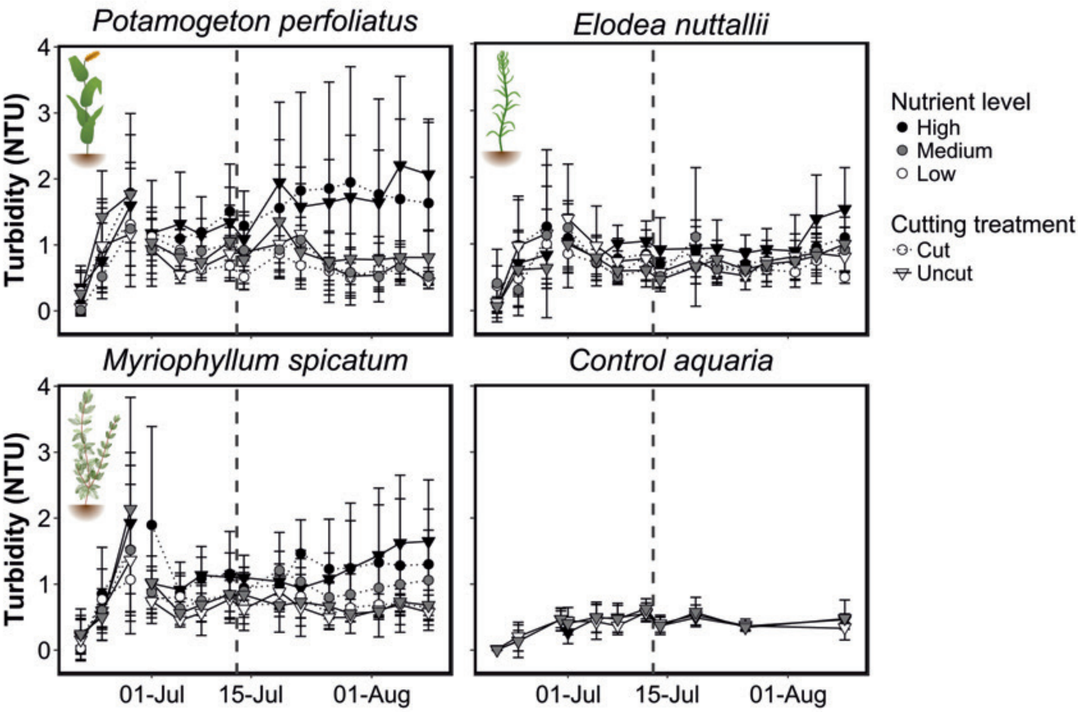


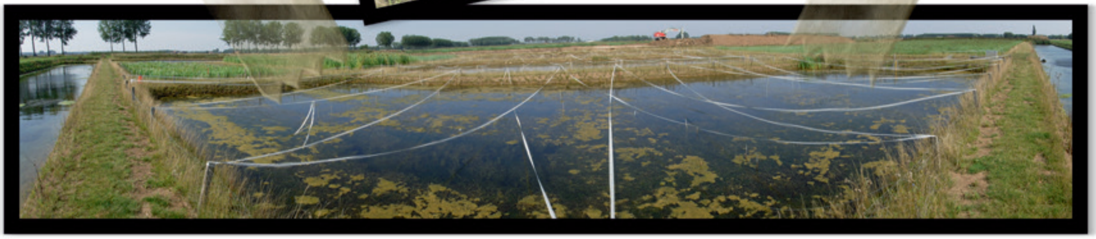
Figure S 8. Mean surface water turbidity. Point fill indicates the fertilizer level, dashed vertical line indicates the cutting date, solid vertical line indicates the end of the experiment and the error bars represent the standard deviation of the mean. No statistics could be performed on the unvegetated aquaria, due to too many zeros. For ANOVA results for the vegetated aquaria, see Supplementary Table S 2.

Table S 1. Mean ± SD of the initial (t = 0) and final (t = 8 weeks) shoot and root carbon concentrations, C:N ratios, C:P ratios, and N:P ratios per species and treatment (n = 6). Species are abbreviated as: Enut (E. nuttallii), Mspi (M. spicatum), and Pper (P. perfoliatus). Ratios are given in mol:mol.

				Shoot				**Root**			
Time	**Species**	**Nutrient**	**Cutting**	**C**	**C:N**	**C:P**	**N:P**	**C**	**C:N**	**C:P**	**N:P**
Initial (t = 0)	Enut	-	-	31.2 ± 0.4	14.4 ± 0.9	673 ± 272	46.6 ± 17.9	-	-	-	-
	Mspi	-	-	32.4 ± 1.4	16.9 ± 1	973 ± 257	58.4 ± 17.3	-	-	-	-
	Pper	-	-	30.7 ± 0.3	14.3 ± 0.5	703 ± 485	48.3 ± 32	-	-	-	-
Final (8 wks)	Enut	High	Cut	30.8 ± 0.8	11.5 ± 1.3	491 ± 195	43.3 ± 18.5	22.6 ± 1.8	12.3 ± 0.5	169 ± 84	13.8 ± 6.8
			Uncut	31.3 ± 1	11.3 ± 0.8	425 ± 230	37.5 ± 20.4	23.2 ± 1.6	13.5 ± 1.2	254 ± 101	19.1 ± 8.6
		Medium	Cut	29.9 ± 1.1	17.4 ± 5.6	309 ± 73	19.5 ± 7.4	23.5 ± 2.7	17.8 ± 4	320 ± 141	17.5 ± 3.2
			Uncut	29.4 ± 1.3	17.8 ± 3.3	382 ± 148	22.8 ± 11.8	26.1 ± 2.3	19 ± 4	354 ± 153	18.5 ± 6.5
		Low	Cut	28.2 ± 0.7	33.1 ± 7.7	360 ± 202	11.3 ± 5.6	22.9 ± 6.1	24.3 ± 8.6	293 ± 175	13.5 ± 9.3
			Uncut	26.6 ± 4.1	29.8 ± 4.6	300 ± 136	10.4 ± 5	25.7 ± 2.4	25.9 ± 3.8	448 ± 241	17.9 ± 10.3
	Mspi	High	Cut	31.3 ± 0.5	13.7 ± 1.8	390 ± 115	28.8 ± 9.4	32.5 ± 0.7	15.5 ± 3.9	526 ± 364	33.9 ± 21.2
			Uncut	31.1 ± 0.3	13.5 ± 1.1	570 ± 211	42.1 ± 14.8	32.6 ± 0.7	13.1 ± 2	418 ± 133	33.2 ± 13.5
		Medium	Cut	30.1 ± 1.5	17.8 ± 2.3	357 ± 105	20.3 ± 6.8	31.7 ± 0.7	23.6 ± 5.6	450 ± 196	19.3 ± 7
			Uncut	31.2 ± 0.3	17.6 ± 2.1	265 ± 100	15.1 ± 5	31.7 ± 0.8	21.5 ± 2.5	458 ± 206	21.5 ± 9.9
		Low	Cut	29.6 ± 1.3	38.4 ± 5.6	398 ± 215	10.8 ± 6.6	32.5 ± 0.8	54.1 ± 15.4	365 ± 136	6.9 ± 2.5
			Uncut	29 ± 2.3	39.9 ± 8.4	340 ± 214	8.5 ± 5.1	32.3 ± 0.7	50.5 ± 8.1	367 ± 143	7.3 ± 2.8
	Pper	High	Cut	29.3 ± 0.6	13.6 ± 0.7	278 ± 98	20.3 ± 6.9	25.1 ± 2.7	12.7 ± 1.5	289 ± 152	22.8 ± 11.3
			Uncut	28.9 ± 0.9	14 ± 0.4	330 ± 175	23.5 ± 12.1	23.3 ± 3.8	12.9 ± 1.1	312 ± 133	23.9 ± 9
		Medium	Cut	28.2 ± 0.8	15.9 ± 2	392 ± 219	24.4 ± 13.3	25.3 ± 2	16.7 ± 2.5	332 ± 138	19.4 ± 5.7
			Uncut	29 ± 0.6	16.7 ± 1.9	309 ± 91	19.1 ± 7.1	26 ± 2.6	16.8 ± 3.1	260 ± 85	16.1 ± 6.2
		Low	Cut	28.4 ± 1.1	33.2 ± 9.9	485 ± 135	16.2 ± 8	27.6 ± 1.6	35.4 ± 10.1	441 ± 167	12.7 ± 4.2
			Uncut	28 ± 0.9	32.2 ± 3.3	482 ± 74	15.1 ± 3	27.4 ± 2.7	33.2 ± 4.2	397 ± 170	12 ± 5.1
			Unit:	(mmol.g_{DW}^{-1})	(mol:mol)	(mol:mol)	(mol:mol)	(mmol.g_{DW}^{-1})	(mol:mol)	(mol:mol)	(mol:mol)

Table S 2. Effect of the treatments on surface water parameters at the last date of the experiment. Sp: effect of species, Nut: effect of nutrient availability, Cut: effect of cutting. When interactions are present, main effects are disregarded (grey text). Bold faced p–values are statistically significant at 0.05.

	Sp	**Nut**	**Cut**	**Sp:Nut**	**Sp:Cut**	**Nut:Cut**	**Sp:Nut:Cut**
Surface water TON (mg.L-1)	$F_{2,90} = 4.0$ $p = 0.021$	$F_{2,90} = 22.6$ $p < 0.001$	$F_{1,90} = 3.9$ $p = 0.052$	$F_{4,90} = 3.5$ **$p = 0.011$**	$F_{2,90} = 0.2$ $p = 0.82$	$F_{2,90} = 3.0$ $p = 0.057$	$F_{4,90} = 1.0$ $p = 0.42$
Surface water pH	$F_{2,90} = 82.6$ $p < 0.001$	$F_{2,90} = 150.9$ $p < 0.001$	$F_{1,90} = 7.4$ **$p = 0.008$**	$F_{4,90} = 5.9$ **$p < 0.001$**	$F_{2,90} = 0.1$ $p = 0.89$	$F_{2,90} = 2.3$ $p = 0.11$	$F_{4,90} = 0.3$ $p = 0.85$
Surface water alkalinity (meq.L-1)	$F_{2,90} = 237.2$ $p < 0.001$	$F_{2,90} = 22.2$ $p < 0.001$	$F_{1,90} = 0.7$ $p = 0.41$	$F_{4,90} = 6.7$ **$p < 0.001$**	$F_{2,90} = 0.9$ $p = 0.39$	$F_{2,90} = 0.6$ $p = 0.57$	$F_{4,90} = 1.2$ $p = 0.32$
Surface water turbidity (NTU)	$F_{2,90} = 1.4$ $p = 0.25$	$F_{2,90} = 48.3$ **$p < 0.001$**	$F_{1,90} = 4.6$ **$p = 0.035$**	$F_{4,90} = 2.3$ $p = 0.068$	$F_{2,90} = 1.7$ $p = 0.18$	$F_{2,90} = 0.9$ $p = 0.40$	$F_{4,90} = 1.0$ $p = 0.39$



Chapter 5

Finding the harvesting frequency to maximize nutrient removal in a constructed wetland dominated by submerged aquatic plants

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Abstract

Water quality is still poor in many freshwater ecosystems around the world as a result of anthropogenic nutrient loading. Constructed wetlands can be used to remove excess nutrients. In these wetlands, helophytes or free floating aquatic plants are traditionally used to absorb the nutrients. The nutrients are subsequently exported upon harvesting of the plants. However, rooted submerged plants may be more effective to extract nutrients from moderately eutrophicated ecosystems than helophytes or floating species.

Here, we tested how the frequency of harvesting affected submerged biomass production, biomass nutrient content and the resulting amount of nutrients removed, as well as the vegetation composition and structure. Two Myriophyllum spicatum dominated shallow ponds, with moderately low surface water nutrient loading (~5.6 mg N.m^{-2}.d^{-1} and ~1.32 mg P.m^{-2}.d^{-1}) were used. Each pond was subjected to four harvesting treatments: mowing 1x, 2x, 3x or 5x between May and September 2015.

Harvesting 2x or 3x removed most biomass and nutrients, while mowing either 5x or only once at the end of the growing season removed the lowest amount of nutrients from the system. Furthermore, the dominance of M. spicatum in the vegetation was best maintained in plots mown 2x, while its cover declined in plots mown more frequently, resulting in an increase of charophyte abundance.

We conclude that harvesting at an intermediate frequency is best when aiming to remove the maximum amount of nutrients under a moderately low nutrient loading. Harvesting more frequently may be a suitable management method to reduce dominance of M. spicatum in situations where it causes nuisance problems due to massive growth.

Introduction

Many aquatic ecosystems worldwide have been, and still are, impacted by human-induced eutrophication (Meuleman et al., 2004; Bernhardt et al., 2008; Cusell et al., 2014; Chowdhury et al., 2017). Eutrophication leads to severe problems in freshwater ecosystems, including the development of harmful phytoplankton blooms and hypoxia (Hasler et al., 1947; Smith, 2003; Dodds et al., 2009; Chowdhury et al., 2017). To reduce external nutrient loading, several measures have been taken, including the construction of wastewater treatment plants and fertilizer application quota (e.g. Lewis et al., 2011; European Union, 1991a,b; European Union, 2000).

New techniques are currently being tested to further improve water quality such as on site chemical nutrient immobilization (Immers et al., 2015; Spears et al., 2015), the use of phytoplankton in waste water treatment (Fernandes et al., 2015), but also the clever use of aquatic plants (i.e. macrophytes) for water nutrient polishing (Vymazal, 2007;

Kwakernaak et al., 2015; Smolders & Van Kempen, 2015; Tang et al., 2017). The concept behind nutrient polishing with plants is that plants incorporate the nutrients into their tissue during the growing season and can subsequently be harvested. The harvested biomass can be used again for a variety of applications, for example as fertilizer, soil conditioner or animal feed (Shilton et al., 2012; Ho et al., 2015; Quilliam et al., 2015), potentially in combination with biogas production (Verma et al., 2007; O'Sullivan et al., 2010).

Constructed wetlands

Traditionally, emergent and free floating macrophyte species have mainly been used for nutrient removal in constructed wetlands and can remove around 250 to 630 g $N.m^{-2}.y^{-1}$ and 45 to 70 g $P.m^{-2}.y^{-1}$ under high nutrient loading (Vymazal, 2007). Generally, free floating species can remove more nutrients than species with other growth forms, providing that the harvesting regime permits their maximum growth rate (Vymazal, 2007; Tang et al., 2017). At high nutrient loading, the actual nutrient uptake by macrophytes in general is far from 100% of the load (Vymazal, 2007; Tang et al., 2017). Removing a majority of the N and P load by harvesting macrophytes is thus only viable under moderately low environmental loadings, i.e. a load of < 10-100 g $N.m^{-2}.y^{-1}$ and < 2-10 g $P.m^{-2}.y^{-1}$ depending on the macrophyte species and growth conditions (Vymazal, 2007; Tang et al., 2017; Kuiper et al., 2017).

In this study, we focus on temperate freshwater ecosystems with these moderately low surface water nutrient levels. In these cases, using submerged macrophytes instead of other growth forms may be especially beneficial for several reasons. First, submerged macrophytes can potentially take up nutrients from the entire water column and are better able to take up nutrients and grow in water with lower nutrient concentrations than free floating species (Bornette & Puijalon, 2009; Van Gerven et al., 2015). Second, submerged macrophytes have higher tissue nutrient concentrations than emergent species (Demars & Edwards, 2008), due to a lower need for carbon-rich structural tissue for vertical growth. Third, many fast growing submerged macrophyte species exist and they can reach high biomass of up to 1 kg dry mass m^{-2} (e.g. Schwarz & Howard-Williams 1993; Di Nino et al., 2005; Morris et al., 2006; Van Zuidam & Peeters 2013). Additionally, several submerged macrophytes, such as *Elodea canadensis* and *Myriophyllum spicatum*, have a high tolerance to cutting (Painter 1988; Abernethy et al., 1996; Richardson 2008). All these plant characteristics may allow managers to frequently harvest the nutrients fixed in macrophyte tissue. We propose that these types of fast growing and stress-tolerant submerged macrophytes are therefore ideal to polish nutrients from the surface water with moderately low nutrient loading.

Optimal macrophyte harvesting regime

To remove as many nutrients from the ecosystem as possible, harvesting should be done such as to optimize macrophyte growth, nutrient content and regrowth potential after cutting. If the regrowth potential of a submerged macrophyte species is too low compared to its harvesting frequency, then there is a risk that the entire vegetation could collapse (Kuiper et al., 2017). Furthermore, harvesting may alter macrophyte species composition and abundance. Depending on the harvesting frequency, the dominant fast-growing species may potentially strengthen its dominance, if it is tolerant to cutting (e.g. Johnson & Bagwell, 1979; Engel, 1990; Serafy et al., 1994) or alternatively harvesting may reduce its competitive strength and stimulate the growth of subordinate species by creating open space (e.g. Engel 1990; Howard-Williams et al., 1996; Zhang et al., 2014). The change in species composition and abundance may also alter the nutrient removal efficiency.

Maximum nutrient removal and impact on submerged vegetation

Current scientific knowledge on submerged macrophyte growth and their tolerance to harvesting is insufficient to design a sustainable harvesting plan aimed at maximizing the removal of nutrients from the ecosystem while maintaining a stable submerged macrophyte vegetation. In this study, we aimed to define a harvesting strategy which will remove most nutrients from an ecosystem with the least amount of effort, without impacting the submerged vegetation to the point of collapse. We designed an experiment where we applied different harvesting frequencies to shallow constructed wetlands which were planted with the submerged angiosperm *M. spicatum*. We measured water and sediment nutrient concentrations, harvested macrophyte biomass, harvested nutrients, macrophyte species composition, macrophyte cover and macrophyte height.

Methods

Study system

We used two shallow ponds of 30 x 15 metres and a water depth of approximately 75 cm as experimental ecosystems, located near Bemmel, the Netherlands. The ponds were dug in early 2014 and were subsequently planted with *M. spicatum* in April of the same year (Fig. 1). The two ponds are part of a larger constructed wetland designed to remove nutrients and increase water quality. This wetland consists of three consecutive sections: a settling basin, a wetland with helophytes, and our *M. spicatum* ponds. The ponds serve as the final step in the water purification process. The mean residence time of the surface water in the ponds was approximately 8.1 days during the experimental period.

Nutrients and other environmental parameters

Water samples were collected weekly near the inflow of the *M. spicatum* ponds. At five sub-sites within each pond, sediment porewater samples were collected from the upper sediment layer with ceramic cups (Eijkelkamp, Giesbeek) in April and September of 2015. These values were averaged per site to estimate average porewater nutrient concentrations during the experiment. NaCl-extractions and Olsen-P extractions of sediment samples were carried out as described in Tang et al. (2016 & 2017).

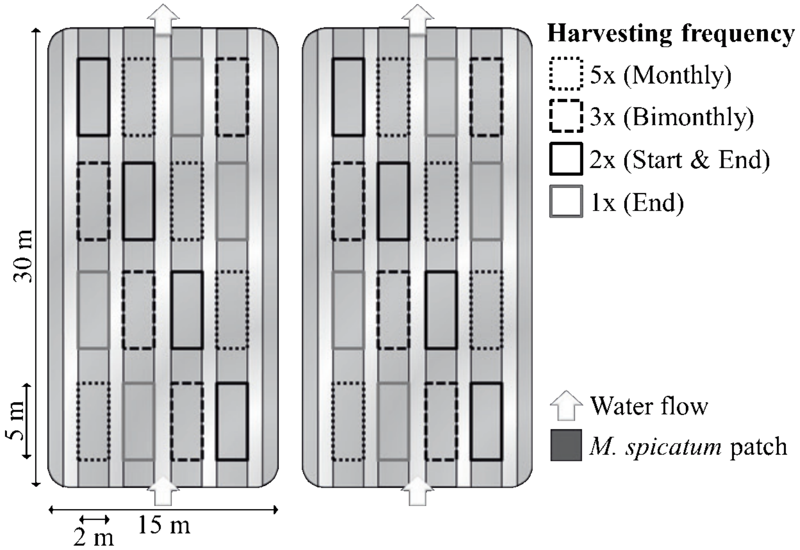

Figure 1. Schematic of the experimental setup in the two ponds of the constructed wetland.

The pH of surface water and porewater samples was measured using a pH electrode with a Ag/AgCl internal reference (Orion Research, Beverly, CA, USA) and a TIM800 pH meter. Total dissolved inorganic carbon concentrations were measured using infrared gas analysis (IRGA, ABB Advance Optima, Zürich, Switzerland). To prevent metal precipitation in the water samples, 0.1 ml (65 %) HNO_3^- was added to each 10 ml sample. The samples were stored at 4°C until analyses. For the analyses of P, Ca, Mg, Fe, S, K, Si and Al, inductively coupled plasma spectrophotometry (ICP-Optical Emission Spectrometer, Thermo Scientific iCAP 6000 Series ICP) was used, unless specified otherwise. To determine nitrate (Kamphake et al., 1967), ammonium (Grasshoff & Johanssen, 1972), ortho-phosphate (Henriksen, 1965), sodium, and chloride concentrations, a 20 ml water sample was stored at -20°C and analyzed colorimetrically with an Auto Analyzer 3 system (Bran and Luebbe). Sodium and potassium were determined with a Technicon Flame Photometer IV Control (Technicon Corporation).

Overall the two ponds were similar to each other with regard to the nutrient concentrations in the inflow surface water and porewater, but some differences were present (see Table 1). The estimated inorganic N and total P load of the surface water of the ponds was considered moderately low and averaged around 3.38-7.91 and 1.16-1.48 $mg.m^{-2}.d^{-1}$, respectively for N and P during the experimental period.

Harvesting treatments

We applied four different harvesting frequencies in the experimental ponds from May up to September 2015: **5x** (i.e. monthly), **3x** (i.e. bimonthly), **2x** (i.e. in May and September) and **1x** (i.e. in September). Each harvesting treatment was replicated eight times, with

Table 1. Average nutrient concentrations of the surface water flowing into the ponds during the experimental period (1 May – 1 Oct 2015) and of the porewater and sediment (8 April and 23 September). Free N is calculated as NO3-N + NH4-N and TIC as CO2-C + HCO3-C. ‘n’ indicates the number of data points for pond 1 and pond 2 separate over which the mean and SD are calculated. Grey values for PO4 indicate the presence of too many identical values near the detection limit; therefore no statistics could be performed. See Supplementary Table S 1 for additional variables.

		Pond 1		*Pond 2*						
Sample type	**Variable**	**mean**	**SD**	**mean**	**SD**	**n**	**Df**	**Test-stat.**	**p-value**	**Test**
Surface water input (μmol.L^{-1})	TIC	**2239**	**449**	**1593**	**344**	19	1;36	24.67	**<0.001**	T-test
	CO_2	**130.4**	**90.6**	**39.1**	**24.7**	19	-	2.86	**0.004**	Mann-Whitney
	HCO_3	**2108**	**387**	**1554**	**346**	19	1;36	21.68	**<0.001**	T-test
	Free N	**3.47**	**1.63**	**5.38**	**3.65**	19	1;35	4.29	**0.046**	T-test*
	NO_3	**1.26**	**1.01**	**3.24**	**3.38**	19	1;35	5.47	**0.025**	T-test*
	NH_4	2.21	1.11	2.13	1.08	19	1;36	0.06	0.814	T-test
	Total P	0.626	0.218	0.539	0.287	19	1;35	1.09	0.303	T-test*
	PO_4	*0.40*	*0.50*	*0.45*	*0.56*	19			NA#	
Porewater (μmol.L^{-1})	Free N	10.20	7.94	12.08	4.02	4;5	1;7	0.22	0.656	T-test
	Total P	6.02	5.38	5.85	3.15	4;5	1;7	0.13	0.732	T-test
	K	58.08	12.06	74.08	23.21	4;5	1;7	1.54	0.255	T-test
Sediment extraction (μmol.L^{-1})	NaCl-N	144.7	20.8	149.6	22.2	5	1;8	0.13	0.729	T-test
	Olsen-P	**308.9**	**56.4**	**450.1**	**105.9**	5	1;8	6.93	**0.026**	T-test
	NaCl-K	1555.2	386.1	1931.3	449.9	5	1;8	2.01	0.194	T-test

* Removed one outlier from pond 2 for statistical test. # Test could not be performed.

four replicates in each of the two ponds (Fig. 1). The spatial position of replicates was chosen to account for a possible gradient in water nutrient availability that might develop within each pond, because of the unidirectional flow of the water through each pond. Experimental units consisted of 5 x 2 metre plots within the vegetated sections of the ponds. The plots were separated from each other by 2 metres of uncut vegetation on the longitudinal sides and by a 1 metre wide unvegetated path on the latitudinal sides. Upon harvesting, the submerged vegetation was manually cut at ~20 cm above the sediment using hedge trimmers to maintain viable shoots. Directly after cutting, the cut material from a single plot was collected and put in plastic bags. The bags were stored in the dark at 4 °C upon arrival at the lab and were processed the next day.

Harvested biomass

The collected biomass samples were weighted, dried (48 h at 60 °C) and re-weighted to determine biomass water content and harvested dry weight. Because the harvested biomass can potentially be used as agricultural fertilizer, and N, P and K are the most important macro-nutrients, we focus on these elements in the macrophyte biomass nutrient analyses. Dried macrophyte samples were ground to a powder and homogenized after which 200 mg of dry macrophyte material was digested in a microwave oven (MLS-1200 Mega, Milestone Inc., Sorisole, Italy) using 4 ml 65% HNO_3 and 1ml 30% H_2O_2 to determine total P and K concentrations. The digested solution was analyzed with inductively coupled plasma-optical emission spectrometry (ICP-OES; IRIS Intrepid II, Thermo Electron Corporation, Franklin, MA, USA). Dry macrophyte material (3 mg) was combusted to determine C and N content with an elemental analyzer (Carlo Erba NA 1500, Thermo Fisher Scientific, Waltham, MA, USA).

Vegetation survey

The vegetation cover and species composition was determined in all experimental plots 0 to 3 days before a harvesting event took place. Macrophyte and filamentous algae cover was visually estimated separately for all species that were visible from above. Additionally, we estimated the average height of the vegetation per plot by measuring the distance from the vegetation canopy to the water surface and the water depth. The cover and height estimates were always made by the same researcher to obtain consistent results throughout the experiment. Macrophyte species were identified using identification keys from Pot (2004) and Bruinsma et al. (1998).

Statistical analyses

A 2-way-ANOVA was used to analyse whether the total amount of harvested biomass, harvested nutrients, and biomass nutrient concentration were significantly affected by the harvesting frequency. Because two ponds with slightly different nutrient concentrations were used in the experiment, ‘pond’ was a relevant parameter and thus included as a fixed factor in the model (Dependent Variable ~ Harvesting frequency + Pond). Test assumptions on data distribution (variance and normality) were visually assessed before continuing to the model output. If the ANOVA test showed a significant effect of harvesting frequency, Tukey post-hoc multiple comparison tests were used to identify which frequencies differed from one another.

Because some of our data did not meet the assumptions of the ANOVA tests, we used Kruskal-Wallis tests to analyse whether macrophyte cover, and submerged vegetation canopy height was significantly different between harvesting frequencies. If the Kruskal-

Wallis test (*kruskal.tes*t function from *stat* package) indicated that the dependent variable was significantly different among harvesting frequencies, Mann-Whitney tests (*wilcox.test* function from *stat* package) were used to identify which treatments different from each other. We adjusted the critical p-value (α) in these Kruskal-Wallis tests, because individual tests were used for all four harvesting dates instead of one overall test (significant effect of treatment at $p < 0.0125$; i.e.). T-tests were used to test whether the average nutrient availability of the surface water and sediment differed between the two experimental ponds; if the data violated the assumptions of the t-test, a Mann-Whitney rank test was used to identify statistically significant differences.

We performed all statistical calculations in R version 3.1.2 (R Core Team, 2014) using the *lm*, *Anova* (with type II errors) and *glht* functions from the *stats* (R Core Team, 2014), *car* (Fox and Weisberg, 2011) and *multcomp* (Hothorn et al., 2008) packages, respectively.

Results

Harvested biomass and nutrients

Harvesting frequency strongly affected the amount of biomass harvested during one growing season. Most biomass was harvested at harvesting frequencies of 2x or 3x per season (Fig. 2). Harvesting with a frequency of either 1x or 5x per season removed around 32 % and 27 % less biomass than when harvesting 2x, respectively. Similar effects of harvesting frequency are found on total harvested nitrogen, phosphorous, and potassium, which are removed as component of the macrophyte biomass (Table 2). On average, a little over 6 grams of N, 1 gram of P, and 3 grams of K per square metre was removed from the ecosystem, when harvesting 2x per season.

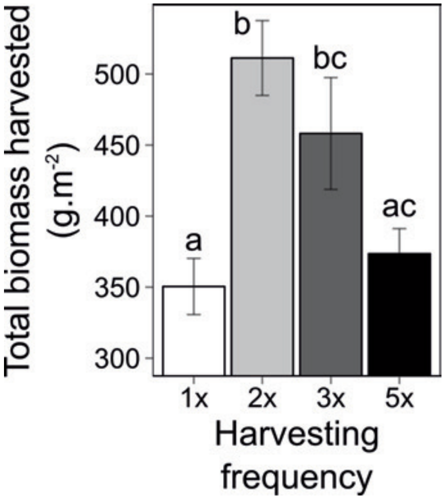

Figure 2. Cumulative amount of biomass (mean ± SE of dry mass) harvested per m^2 per harvesting frequency. Different letters indicate statistically significant differences in the amounts of harvested biomass among harvesting frequencies at $p < 0.05$ (Tukey post-hoc; ANOVA: Df = 3, F = 12.14, $p < 0.001$).

Table 2. Total amount (g) of carbon, nitrogen, phosphorous and potassium harvested per m² for each harvesting frequency and pond. Different letters indicate statistically significant differences in harvested nutrients per harvesting frequency and pond (ANOVA with Tukey post-hoc tests).

	Total amount harvested (mean ± SE g.m^{-2})			
	C	**N**	**P**	**K**
Frequency	Df=3, F=12.38, p<0.001	Df=3, F=6.66, p=0.002	Df=3, F=6.35, p=0.003	Df=3, F=11.57, p<0.001
1x (end)	103 ± 5 $^{\mathbf{AC}}$	5.01 ± 0.42 $^{\mathbf{AC}}$	0.79 ± 0.06 $^{\mathbf{A}}$	2.09 ± 0.11 $^{\mathbf{A}}$
2x (start & end)	138 ± 8 $^{\mathbf{B}}$	6.17 ± 0.26 $^{\mathbf{B}}$	1.02 ± 0.05 $^{\mathbf{B}}$	3.04 ± 0.14 $^{\mathbf{B}}$
3x (bimonthly)	119 ± 11 $^{\mathbf{AB}}$	5.72 ± 0.35 $^{\mathbf{AB}}$	0.89 ± 0.08 $^{\mathbf{AB}}$	2.85 ± 0.25 $^{\mathbf{B}}$
5x (monthly)	95 ± 4 $^{\mathbf{C}}$	4.59 ± 0.23 $^{\mathbf{C}}$	0.74 ± 0.04 $^{\mathbf{A}}$	2.29 ± 0.12 $^{\mathbf{A}}$
Pond	Df=1, F=25.37, p<0.001	Df=1, F=11.31, p=0.003	Df=1, F=15.24, p<0.001	Df=1, F=16.57, p<0.001
Pond 1	125 ± 6 $^{\mathbf{A}}$	5.77 ± 0.24 $^{\mathbf{A}}$	0.94 ± 0.04 $^{\mathbf{A}}$	2.80 ± 0.14 $^{\mathbf{A}}$
Pond 2	98 ± 5 $^{\mathbf{B}}$	4.81 ± 0.23 $^{\mathbf{B}}$	0.75 ± 0.04 $^{\mathbf{B}}$	2.26 ± 0.12 $^{\mathbf{B}}$

Significantly more nutrients (NPK) were harvested from pond 1 compared to pond 2 (around 20% more, Table 2). Small differences between ponds were already present during the first harvest, as harvested N already differed between ponds with slightly higher amounts harvested in pond 2 during this first harvest ($F = 4.60$, $p = 0.044$; Supplementary Fig. S 1). We also expected a nutrient gradient to develop in the ponds, with the highest concentrations near the inflow and the lower concentrations near the outflow. A small gradient in biomass C, N and P could be observed at the end of the experiment in pond 2, but not in pond 1 (Supplementary Fig. S 2). This gradient was similar for all treatments, owing to our experimental design.

Macrophyte abundance and species composition

Five submerged macrophyte species grew to the top of the vegetation in the experimental plots during the experiment: *M. spicatum*, *Elodea nuttallii*, *Potamogeton pusillus*, *Chara globularis*, and *Chara vulgaris*. *M. spicatum* remained the most dominant species in all plots, after having being planted there one year earlier (Fig. 3A). The other species spontaneously colonized the ponds from the connected waters. Harvesting significantly impacted the submerged vegetation during the experiment. *M. spicatum* cover was lower in the plots harvested 5x per season (i.e. monthly) than in plots harvested 2x or 1x per season at 10, 15, and 20 weeks after the first harvest (Fig. 3A). After 15 and 20 weeks, charophyte cover was higher in plots harvested 5x or 3x than in plots harvested only 1x at the end (i.e. harvested after the last vegetation survey; Fig. 3A). We found significantly more filamentous algae in plots harvested only 1x in September than in all other plots 10 weeks after the first harvest. Fifteen weeks after the first harvest, significantly more filamentous algae were also present in the plots harvested only 1x than in plots harvested 2x or 3x (Fig. 3B), possibly because they became trapped in the tall vegetation in the plots harvested 1x. The canopy height, depicted as the distance of plants to the water surface, was significantly lower in plots harvested 5x compared to plots harvested 2x or less during all surveys except the first one, which was performed before the first harvest took place (Fig. 3C).

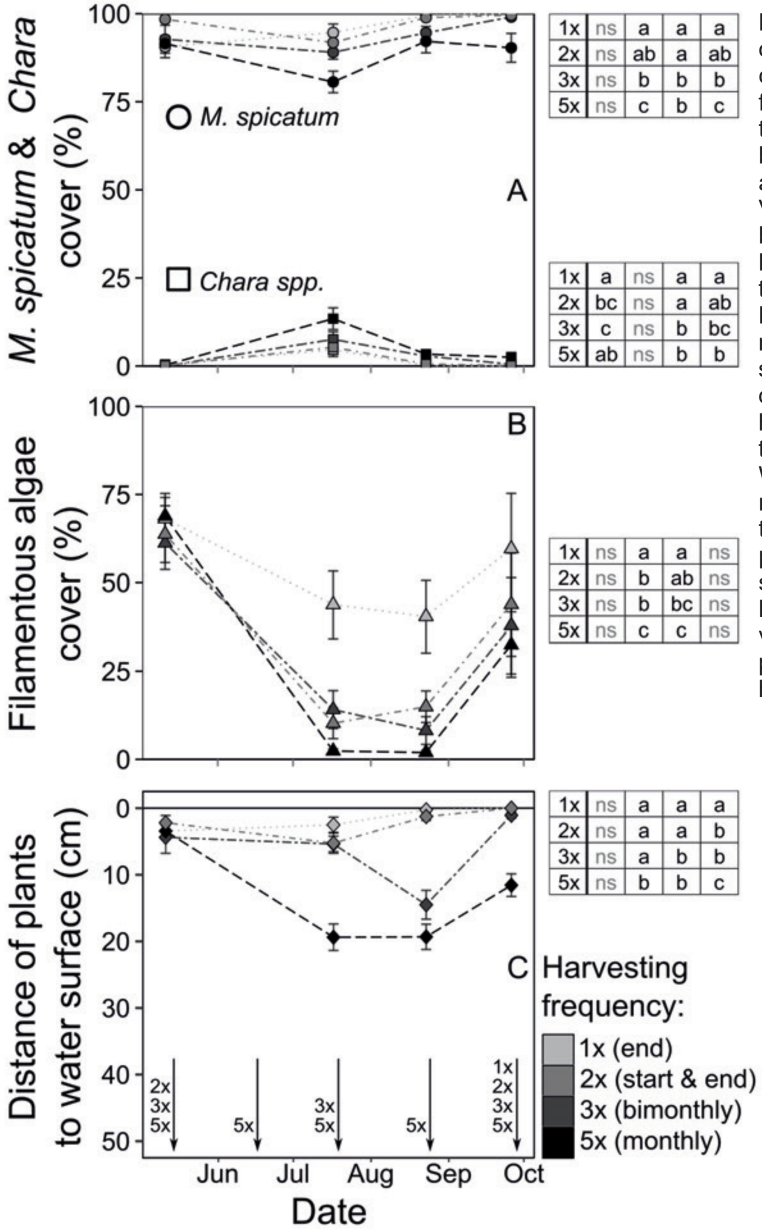

Figure 3. Mean ± SE cover of M. spicatum and charophytes (**A**), filamentous algae (**B**) and the average distance between the plant canopy and the water surface (**C**). Vertical arrows at the bottom of panel C indicate harvesting dates for each of the harvesting frequencies. Different letters in tables next to the graphs indicate statistically significant differences between harvesting frequencies on that one date (Kruskal-Wallis tests (α = 0.0125 for multiple comparisons over time) with Mann-Whitney post-hoc), 'ns' indicate non-significant overall effect of harvesting frequency. All vegetation surveys were performed right before the harvesting event.

Discussion

In this study, we successfully used submerged macrophytes to remove nutrients from a constructed wetland with a moderately low surface water nutrient loading. Harvesting frequency significantly influenced the amount of nutrients that could be recovered with a clear optimum at the intermediate harvesting frequency of 2x or 3x per year. Furthermore we found that increasing harvesting frequency had a large effect on macrophyte cover, height and species composition and abundance.

Nutrient removal

During this period a total average of 17.0 mg N.m^2.d^{-1} and 2.7 mg P.m^2.d^{-1} was sequestered via plant biomass from our ponds, excluding the first harvest and assuming that the unharvested plants (see Fig. 1) sequestered the same amount of nutrients as in the 1x harvested plots. Because the amount of N and P removed with the biomass was higher than the estimated surface water load during the same period (see M&M section 2.2.), the plants must have taken up a significant amount of nutrients from the sediment where nutrient availability was higher.

We removed most nutrients from the ecosystem by harvesting macrophytes at intermediate frequencies, i.e. 2x or 3x during the growing season. These harvesting frequencies removed 6 g N, 1 g P, and 3 g K per m^2 over the experimental period, which translates to an average of 42.9 mg N and 7.1 mg P.m^{-2}.d^{-1} over the experimental period. Other studies on nutrient recovery by submerged macrophytes are scarce (Vymazal, 2007); the nutrient uptake or recovery rates reported range from < 0.1 to 125 mg N.m^{-2}.d^{-1} and from < 0.1 to 48 mg P.m^{-2}.d^{-1} (Peterson et al., 1974; Reddy & De Busk 1985; Gumbricht, 1993b; Pietro et al., 2006; Tang et al., 2017). Our results are in the lower range of these values, likely due to the moderately low surface water nutrient loading of our ponds, as increased loading will increase nutrient sequestration up to a certain point (Li et al., 2015a; Tang et al., 2017). Additionally, some of the studies used short-term uptake experiments to calculate the nutrient removal rates. This short-term method can give valuable insights into the nutrient uptake kinetics of the submerged macrophytes, but may potentially overestimate long-term nutrient uptake in semi-natural and natural ecosystems.

Furthermore, the actual variation in nutrient removal not only depends on harvesting method, as our study shows, but also on other factors including the macrophyte species present in the vegetation. Different macrophyte species can show different growth rates, differ in their nutrient uptake capacity, and respond differently to harvesting (e.g. Gumbricht, 1993a; Abernethy et al., 1996; Barrat-Segratain, 2004; Angelstein et al., 2009), leading to different amounts of nutrients being removed from the ecosystem under identical conditions (Vymazal, 2007; Li et al., 2010a,b; Tang et al., 2017).
Plant growth conditions are additional factors influencing the amount of nutrients

removed via macrophyte harvesting. Nutrient availability in the environment is a very important factor that affects macrophyte growth and macrophyte stoichiometry (e.g. Barko & Smart 1986; Xie et al., 2013), and thus subsequently influences the amount of nutrients removed from an ecosystem by harvesting macrophyte biomass. *M. spicatum* can grow taller and produce heavier shoots on more nutrient rich sediment (Xie et al., 2013). However, in our study, water and sediment N and P concentrations were typically higher in pond 2 compared to pond 1 or similar in both ponds, while biomass production was significantly higher in pond 1 (Table 2). The higher biomass production in pond 1 is most likely caused by the higher availability of inorganic carbon in this pond, as carbon is an important nutrient for macrophytes growth (Maberly & Madsen, 1998; Hussner et al., 2016). CO_2 is the most beneficial form of carbon for the plants to take up (Maberly & Madsen, 1998; Hussner et al., 2016). In both our ponds, output concentrations of CO_2 were almost zero, indicating total CO_2 removal by primary producers. As the CO_2 concentration in the input surface water of pond 1 was much higher than in pond 2 (Table 1), this likely enabled the higher growth in pond 1.

Furthermore, microbial processes in the water and sediment can influence the amount and the chemical form of the nutrient available for plants to take up (Vymazal, 2007; Lamers et al., 2012). Additionally, microbes themselves can also directly remove nitrogen from the ecosystem via denitrification and many microbial processes in the sediment can be affected by the plant, for example via radial oxygen loss (ROL) of the roots (Lamers et al., 2012). These microbial processes and microbe-plant interactions may thus affect the amount of nutrients that are removed from the ecosystem by harvesting submerged plants (Vymazal, 2007; Tang et al., 2017). Increased harvesting might, for instance, decrease the ROL of the roots potentially increasing denitrification rates in the sediment.

The availability of essential elements can also effect the elemental composition of the biomass. Theoretically, harvesting macrophytes using the same method could thus remove more nutrients from an ecosystem in absolute terms when the ecosystem is more nutrient rich, providing that the macrophytes can maintain their growth rate. For example, *M. spicatum* plants growing on more nutrient rich sediment stored less non-structural carbon in their tissue (e.g. starch) (Xie et al., 2013), likely lowering biomass C:nutrient ratios (i.e. increasing relative nutrient concentration) and thus leading to more nutrients being removed when harvesting the same amount of biomass. In our experiment, however, the external nutrient loading was relatively low and the plants must have taken up nutrients from the sediment. It is well known that rooted macrophytes are able to obtain a large part of the required nutrients from the sediment (Carignan & Kalff, 1980; Halbedel, 2016). Our results further indicate that the inorganic carbon availability can affect biomass production and as a result the total nutrient removal from the system.

In carbon-limited systems, the use of floating plant species could be beneficial, providing water nutrient levels are high enough, because floating species can directly access atmospheric CO_2. Under these conditions, floating species, such as *Eichhornia crassipes*, may be especially useful for removing nutrients from eutrophic water in warmer climates (e.g. Chunkao et al., 2012). Furthermore, in sites with low water load, or with occasional water drawdowns, emergent species may be better suited than submerged or floating plants, due to their higher drought tolerance. We propose that submerged plants in particular are most suitable for nutrient polishing when water nutrient concentrations are moderate to low, but carbon availability and water supply are high. Submerged species are also highly suited for use in relatively deep water (up to a few meters) and can take up nutrients from the entire water column (Bornette & Puijalon, 2009). In temperate areas with strong seasonality, nutrient removal by harvesting any type of aquatic plant will vary throughout the year, and will only be possible during the growing season (Vymazal, 2007), unless the temperature and light availability are increased artificially.

Additional to the multitude of harvesting methods applied, the influence of all the factors described above may potentially explain the wide range of nutrient removal rates found in literature.

Additional impacts of harvesting and additional applications

In addition to removing nutrients from the water, submerged macrophytes can simultaneously provide more services, such as providing food for herbivores and creating habitats for many aquatic species (Carpenter & Lodge, 1986; Hargeby et al., 1994; Schriver et al., 1995; Perrow et al., 1999; Mazzeo et al., 2003; Declerck et al., 2005). Constructed wetlands with submerged macrophytes can thus increase biodiversity compared to traditional water treatment plants and polish moderately eutrophic surface water so that it can be used as inlet water for more oligotrophic nature areas, for example. It is important to realize that harvesting too many macrophytes can result in a complete loss of submerged vegetation and turbid water, under nutrient rich conditions (Kuiper et al., 2017), similar to effects caused by high herbivore pressures (Hidding et al., 2016). Overall, we suggest adjusting the cutting depth to the macrophytes height, but to always cut at some distance (e.g. 20cm) above the sediment to maintain enough viable macrophyte biomass and reduce the risk of losing the entire vegetation.

In our study, we did not expect a large impact of harvesting at low frequencies on the vegetation, because *M. spicatum* is known to tolerate stress well (Painter, 1988; Abernethy et al., 1996). Indeed, harvesting the vegetation once, at the beginning of the growing season, did not severely impact the vegetation in our ponds, as vegetation height and cover was similar in plots harvested once compared to previously unmown plots. Also in larger ecosystems, stress tolerant macrophytes, for example *M. spicatum*, *Egeria densa* and *Ceratophyllum demersum*, are able to recover within several weeks from a harvesting event (e.g. Crowell et al., 1994; Howard-Williams et al., 1996). However, less tolerant macrophyte species may decrease in abundance under a harvesting regime (e.g. Van Zuidam & Peeters, 2012). Overall, even tolerant species may be stressed more when harvesting frequency increases (e.g. Madsen et al., 1988). Our study also shows this as high harvesting frequencies negatively impacted *M. spicatum* cover and height, under the prevailing nutrient loading. Simultaneously, this negative impact on the dominant species creates open patches in which other species can grow (e.g. Zhang et al., 2014; Bakker et al., 2016). In our ponds, significantly more charophytes occurred and vegetation was less dense in plots harvested 5x (i.e. monthly) than in plots harvested only at the start of the experiment. In one of the monthly harvested plots, charophytes even made up 27 % of the vegetation canopy in July.

The traits that make submerged macrophyte species, such as *M. spicatum*, ideal for nutrient removal from constructed wetlands can also cause these species to become a nuisance in other ecosystems. These fast growing submerged macrophyte species occasionally grow so fast that they severely impair recreation, fishing, and hydrological functioning of the system (Hasler et al., 1947; Nichols, 1991; Stallings et al., 2015; Hussner et al., 2017). These plants then have to be managed to reduce these problems. Because harvesting can impact macrophyte species composition and abundance, harvesting can be applied to locally reduce nuisance problems caused by high cover of a tall growing macrophyte species, such as *M. spicatum*, and simultaneously increase the abundance of other macrophyte species in some cases (e.g. Engel, 1990; Howard-Williams et al., 1996; this study, but see: Johnson & Bagwell, 1979; Engel, 1990; Serafy et al., 1994). Long-term harvesting schemes may lead to additional changes in vegetation structure (e.g. Painter, 1988) that are not visible in single-year experiments. Continued monitoring of the vegetation in the constructed wetland is therefore advised.

Scale and costs of harvesting

The costs of harvesting submerged plants and the most suitable harvesting method will depend on the size of the constructed wetland or ecosystem that requirs harvesting. Several methods are available to cut submerged plants and remove the biomass from aquatic ecosystems, such as manual cutting or using harvester boats (Murphy, 1988b; Hussner et al., 2017). Manual harvesting, as we did, is a very controllable and precise method to remove submerged plant biomass. However, it is only viable at a small scale because it is very labor intensive, leading to high costs. Another widely used method to harvest submerged plant biomass is using a harvester boat, which cuts the shoots at a pre-set depth and transports the cuttings into a hold on the boat, using a conveyor belt. This method can be used when large scale harvesting is required. This method can cut a larger area faster than manual harvesting, but is generally less precise and only suitable for larger ecosystems. Using a harvester may cost around €100 per hour or around €350 per metric ton of harvested biomass, depending on the density of submerged vegetation (costs estimated using data from a Dutch shallow lake; Schollema, personal communication, 2014). Because harvesting the vegetation is costly, identifying the optimal harvesting frequency for the goal at hand (e.g. nutrient removal) is a very good way to keep the costs as low as possible, without compromising on removal efficiency. Using the harvested biomass for useful applications, such as bioenergy production and agricultural fertilizer, may reduce the net cost of harvesting management even further (Evans & Wilkie, 2010; Quilliam et al., 2015).

Conclusions

We conclude that nutrient removal can be highly optimized by altering the harvesting frequency. In general, we suggest that harvesting *M. spicatum* should be done twice per growing season under a moderately low nutrient loading, if the goal is to remove as many nutrients as possible with the least amount of effort. However, if the goal of management is to reduce the abundance of a dominant, nuisance causing species and to stimulate charophytes for example, we suggest harvesting the macrophytes more frequently.

As the growth and nutrient sequestration of rooted macrophytes strongly depends on external nutrient loading, sediment nutrient concentrations and inorganic carbon availability; we propose that varying the mowing frequency in experimental subplots can help to determine the optimal mowing regime in newly constructed wetlands with different nutrient availabilities.

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Supplementary

Supplementary materials on following pages.

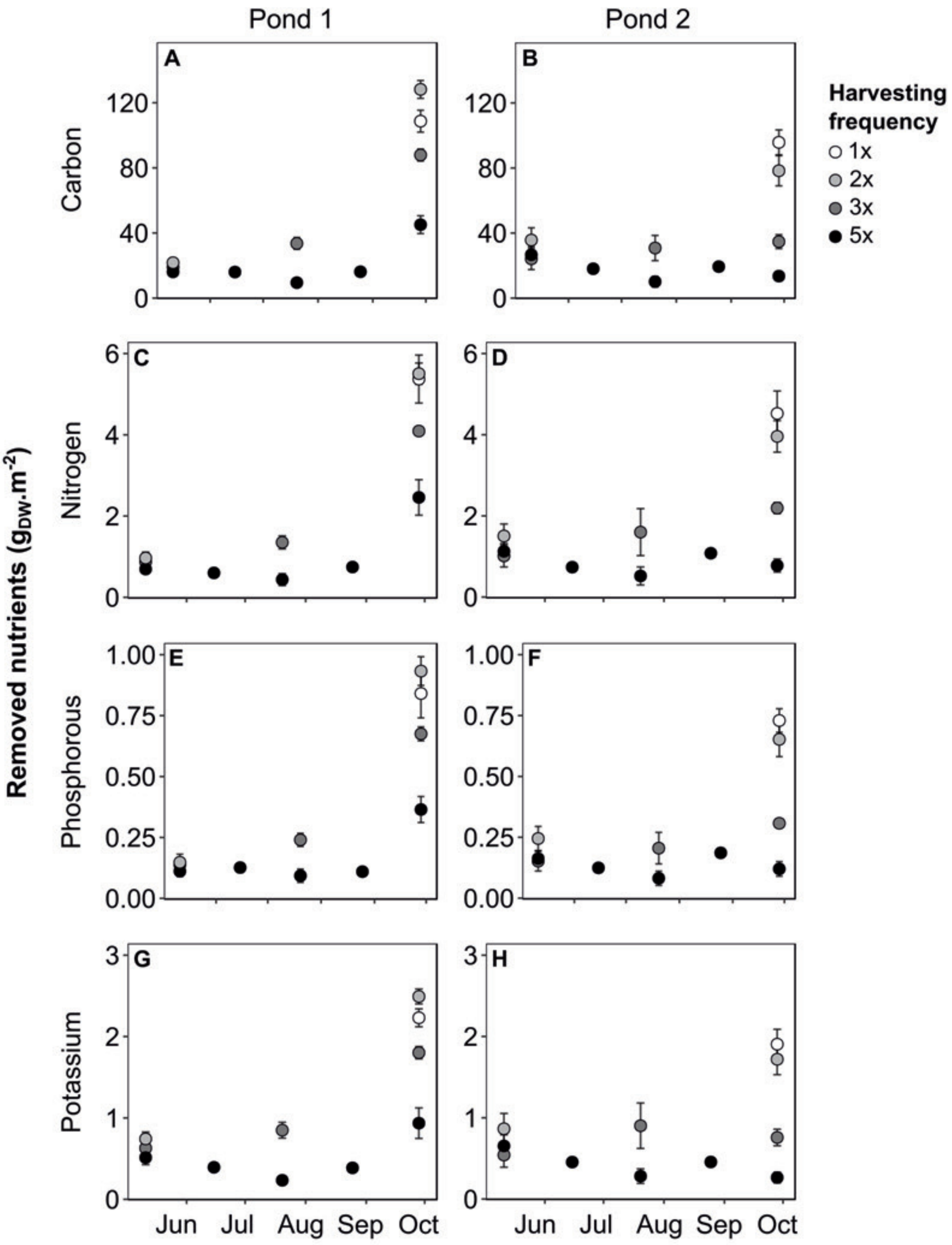

Figure S 1. Harvested amount (mean ± SE, n=4) of carbon (**A,B**), nitrogen (**C,D**), phosphorous (**E,F**) and potassium (**G,H**) per harvest for pond 1 (left) and pond 2 (right) separately.

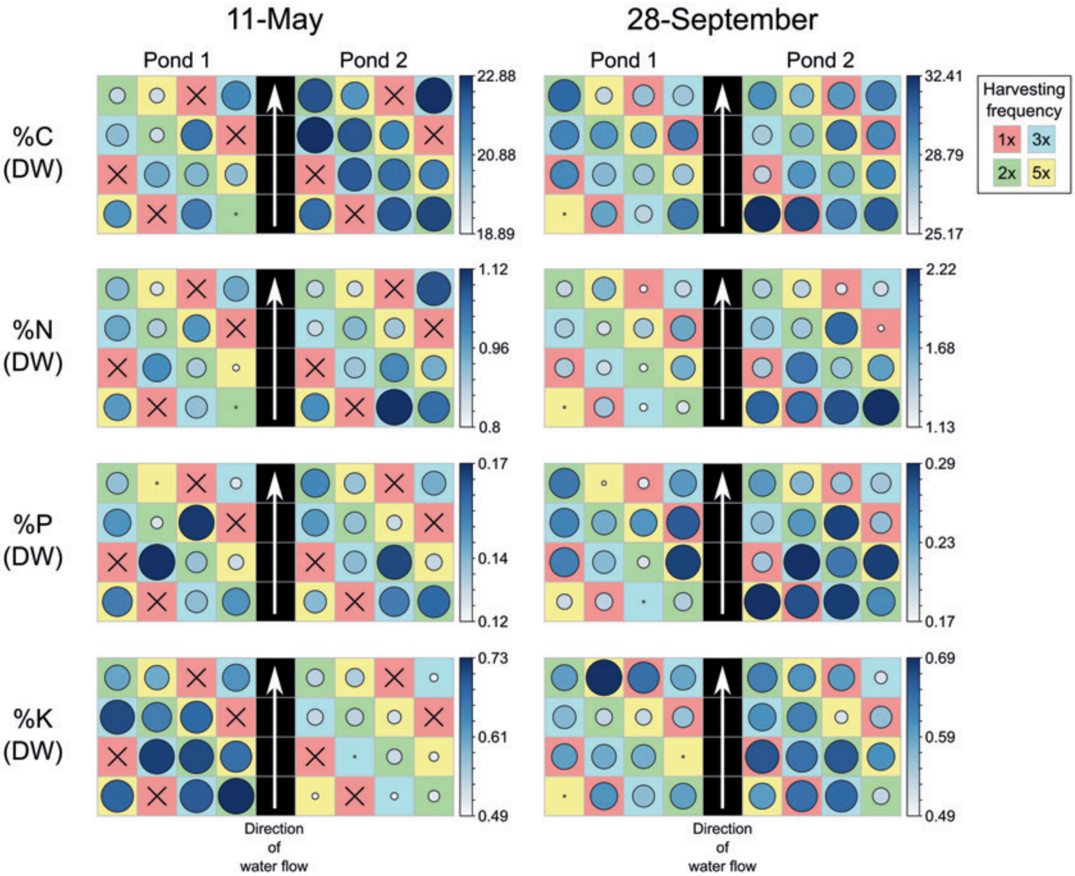

Figure S 2. Temporal and spatial variation in harvested biomass C, N, P and K concentrations within an experimental plot (i.e. squares) during the first (11-May) and last (28-September) harvest of the experiment. The size and fill of the circles represent the biomass nutrient concentration; bigger and darker circles indicate higher concentrations. The fill of the squares behind the circles shows the treatment applied to the plot. Black crosses indicate that these plots were not harvested during this harvesting date. The white arrow shows the direction of the water flow through the experimental ponds. Due to the experimental design, all treatments were equally distributed along this flow path.

Table S 1. Average concentrations of additional nutrients of the surface water flowing into the ponds during the experimental period (1 May – 1 Oct 2015) and of the sediment porewater (8 April and 23 September 2015). 'n' indicates the number of data points for pond 1 and pond 2 separately, over which the mean and SD are calculated. t-test results are also given for the difference between ponds.

		Pond 1		*Pond 2*					
Sample type	**Variable**	**mean**	**SD**	**mean**	**SD**	**n**	**Df**	**Test-stat.**	**p-value**
Surface water input (μmol.L^{-1})	Si	**30.4**	**14.7**	**15.1**	**14.1**	19	1;34	9.85	**<0.001****
Porewater (μmol.L^{-1})	NO_3	1.08	0.82	1.07	0.92	4;5	1;7	<0.01	1
	NH_4	9.12	7.48	11.01	4.48	4;5	1;7	0.22	0.651
	Fe	48.10	34.12	36.94	27.65	4;5	1;7	0.30	0.603
	Al	**0.40**	**0.44**	**1.56**	**1.22**	4;5	1;6	6.15	**0.048***
	S	185.29	59.70	206.29	61.94	4;5	1;7	0.26	0.624
	Si	280.68	12.00	299.17	18.43	4;5	1;7	2.97	0.128

[*] Removed one[*] or two[**] outlier from pond 2 for statistical test.

Chapter 6

Mowing submerged macrophytes in shallow lakes with alternative stable states: battling the good guys?

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Abstract

Submerged macrophytes play an important role in maintaining good water quality in shallow lakes. Yet extensive stands easily interfere with various services provided by these lakes, and harvesting is increasingly applied as a management measure. Because shallow lakes may possess alternative stable states over a wide range of environmental conditions, designing a successful mowing strategy is challenging, given the important role of macrophytes in stabilizing the clear water state. In this study, the integrated ecosystem model PCLake is used to explore the consequences of mowing, in terms of reducing nuisance and ecosystem stability, for a wide range of external nutrient loadings, mowing intensities and timings. Elodea is used as a model species. Additionally, we use PCLake to estimate how much phosphorus is removed with the harvested biomass, and evaluate the long-term effect of harvesting. Our model indicates that mowing can temporarily reduce nuisance caused by submerged plants in the first weeks after cutting, particularly when external nutrient loading is fairly low. The risk of instigating a regime shift can be tempered by mowing halfway the growing season when the resilience of the system is highest, as our model showed. Up to half of the phosphorus entering the system can potentially be removed along with the harvested biomass. As a result, prolonged mowing can prevent an oligo- to mesotrophic lake from becoming eutrophic to a certain extent, as our model shows that the critical nutrient loading, where the lake shifts to the turbid phytoplankton-dominated state, can be slightly increased.

Introduction

Shallow lake ecosystems depend on the presence of submerged aquatic plants (macrophytes) for good water quality and high biodiversity (Heimans & Thijsse, 1895; Carpenter & Lodge, 1986; Jeppesen et al., 1998). There is a positive feedback between aquatic plants and water clarity, through which the plants enhance their own growing conditions (Van Donk & Van de Bund, 2002; Scheffer, 2004). Such self-stabilizing mechanism causes a tendency of the system to resist changes in external environmental conditions, i.e. it promotes a clear water state within the context of alternative stable states in lakes (Scheffer, 2004).

During the second half of the twentieth century, submerged macrophytes disappeared from many shallow lakes in temperate regions because of external nutrient loading from mainly anthropogenic sources (Gulati & Van Donk, 2002; Körner, 2002). Lakes switched from a clear-water state, dominated by macrophytes, to a turbid-water state with few plants, prone to harmful cyanobacterial blooms (Scheffer et al., 1993; Carpenter et al., 1999). For many years since, tremendous management effort has been devoted to the restoration of aquatic plant communities, mainly through the reduction of external nutrient loading, especially phosphorus (P) (Cullen & Forsberg, 1988; Jeppesen et al., 2005; Hilt et al., 2006). Although lakes in the turbid state may also be resilient to changes in external environmental conditions (Hosper, 1998), reduction of external nutrient loading is effective in the long run (Jeppesen et al., 2005), and many of the impacted lakes have recovered or are now recovering to a clear-water state with submerged macrophytes (Sondergaard & Moss, 1998; Gulati & Van Donk, 2002).

Almost inevitable, the return of aquatic plants is accompanied by nuisance caused by these plants (e.g. Van Donk, 1990). The nutrient availability in restored lakes is generally still rather high, which in combination with improved light conditions allows for rampant growth of rooted macrophytes (Lamers et al., 2012). These dense stands of aquatic plants cause nuisance to bathers and swimmers, which generally dislike the touch of plants and because invertebrates living on the macrophytes may cause itches and rash of the human skin (Van Donk, 1990). Dense stands can also cause nuisance for fisherman as lines easily get stuck and because a high macrophyte cover can have a negative effect on fish abundance (Bickel & Closs 2009). Moreover, dense stands can impair (recreational) boat traffic and can decrease lakefront property values. In fact, many functions and ecosystem services may be impacted by the presence of plants (e.g. Van Nes et al., 1999; Anderson, 2003). As a result, current management practices are more and more focusing on the reduction of aquatic plants, even though the re-establishment of an aquatic plant community is still considered a prerequisite for the long-term success of lake restoration measures (Van Nes et al., 2002a). In many rapidly developing countries nuisance growth of aquatic plants is also readily apparent (Van Ginkel, 2011). There, the increased availability of nutrients stimulates plant growth in precedence of a regime shift to a phytoplankton dominated state – a part of eutrophication which also occurred in the temperate lakes before the submerged macrophytes disappeared en mass during the last century (Hasler, 1947).

A common human response to excessive growth of submerged macrophytes is mechanical cutting and harvesting (Hilt et al., 2006; Hussner et al., 2016). However, when lakes have alternative stable states, defining a sustainable mowing regime is challenging, given the important role of macrophytes in stabilizing the clear water state. Theory predicts that when a critical, in practice unknown, amount of vegetation is removed, positive feedbacks propel the system to the turbid state with phytoplankton dominance (Scheffer et al., 1993; Van Nes et al., 2002a). When less vegetation is removed, on the other hand, the system may show a swift recovery back to the vegetated equilibrium state, undoing the impact of mowing. Van Nes et al. (2002a) applied two dynamic aquatic plant models of different complexity to analyze the response of aquatic plant populations to harvesting and concluded that it may be almost impossible to maintain vegetation biomass at any desired intermediate level. Consequently, Van Nes et al. (1999 & 2002a) suggest it may be more fruitful to assign just a few key functions to entire lakes, than to pursue a compromise between conflicting destinations. In most cases however, lake managers do not have the luxury to divide functions over different lakes, for example due to legal obligations, such as the Water Framework Directive (European Union, 2000).

A potentially viable option is to aim for a temporal relief of nuisance following a discrete mowing event. When this period of relief coincides with the moment users are relying on the services provided by the lake, mowing can be convenient despite eventual recovery to the vegetated equilibrium state. Van Nes et al. (2002a) did not consider the temporal aspects of mowing in their plant modelling study, as they assumed continuous cutting strategies for simplicity. Yet it remains a tall order for water quality managers to estimate the amount of plant volume that can be safely removed, and predict the period of relief of nuisance after mowing. The numerous field and laboratory studies that have investigated the response of macrophytes and phytoplankton to harvesting (e.g. Engel, 1990; Nichols & Lathrop, 1994; Barrat-Segretain & Amoros, 1996; Morris et al., 2003; Bal et al., 2006; Morris et al., 2006) did not bring general applicable insights as the results were ambiguous. Moreover, lake managers in NW Europe often lack experience as submerged macrophytes were missing for a long time, while formal decision support schemes are basically absent (Hilt et al., 2006). We argue that there is a need for an integrated analysis to obtain a better understanding of the general consequences of plant removal in relation to trophic state and ecosystem resilience.

In this research we use a comprehensive dynamic ecosystem model - PCLake - to study the effect of mowing on shallow lake ecosystems with alternative stable states. This model describes the main nutrient and food web dynamics of a non-stratifying shallow lake in response to eutrophication and re-oligotrophication (Janse & Van Liere, 1995; Janse, 1997), including many feedback mechanisms and processes that have been associated with plants and alternative stable states in lakes. PCLake is frequently used by scientist and water quality managers, mainly in the Netherlands and Denmark, to analyze the complex dynamics of shallow lake ecosystems and to evaluate the effectiveness of potential restoration measures (e.g. Van Liere & Janse, 1992; Janse et al., 1993; Janse et al., 1998; Nielsen et al., 2014; Trolle et al., 2014). The model has been calibrated with data from more than 40 temperate shallow lakes located in the Netherlands, Belgium and Ireland (Janse et al., 2010). The aim of this calibration exercise was to obtain a best overall fit for the whole set of lakes, rather than achieving an optimal fit for one specific lake at the expense of others. As a result, the model has a fairly wide geographic applicability and is suitable for generalized studies on temperate shallow lakes (Janse et al., 2010). Hence, PCLake provides a consistent framework that can be used to study how alternative stable states come about, and how they affect ecosystem functioning and ecosystem management. For example, Janse et al. (2008) used the model to study how general lake features, such as depth, fetch and sediment type determine the resilience of shallow lakes to external nutrient loading. Likewise, PCLake has been used to evaluate the importance of rising temperatures (Mooij et al., 2007; Mooij et al., 2009), littoral-pelagic coupling (Sollie et al., 2008), allochthonous particulate organic matter (Lischke et al., 2014), tube-dwelling invertebrates (Hölker et al., 2015) and herbivory by birds (Van Altena et al., 2016).

We designed our study to cover several important aspects of mowing that are relevant to ecosystem managers. Firstly, we evaluate how the impact of mowing depends on the trophic status of the lake (i.e. external nutrient loading), mowing intensity and timing of mowing during the growing season. We express the effect of mowing both in terms of remaining plant cover, and in terms of days without nuisance caused either by macrophytes or cyanobacteria. This exercise also allows us to evaluate under which conditions mechanical cutting of macrophytes results in a critical regime shift to the alternative turbid state. Secondly, we use the model to obtain quantitative estimations of the amount of P that can be removed from the system via harvesting of macrophytes. Removal of P may help to remediate eutrophication effects in the lake, and potentially can be recovered for sustainable reuse. Finally, we explore the long term impacts of mowing to analyze whether mowing is a measure that also can be applied to help prevent undesired eutrophication effects in shallow lakes.

Methods

Model description

General features

PCLake consists of a number of coupled ordinary differential equations and auxiliary equations which describe the most important biotic and abiotic components of both the water column and the sediment top-layer of a non-stratifying shallow lake (Janse, 1997). By putting equal emphasis on the biotic and abiotic components, the model is unique in its kind (Janssen et al., 2015). Primary producers are represented by submerged macrophtyes and three groups of phytoplankton (diatoms, green algae and cyanobacteria). The food web is completed by detrivorous macrozoobenthos, zooplankton, zooplanktivorous fish, benthivorous fish and piscivorous fish. The abiotic components in the sediment and in the water column are detritus, inorganic material, dissolved phosphorus, ammonium and nitrate. All organic components are modelled in dry-weight (DW), nitrogen (N) and phosphorus (P), and hence the nutrient-to-dry-weight ratios of the organic components are variable. Internal fluxes of nutrients between the sediment layer and the pelagic zone, including internal loading, are accounted for and modelled dynamically. Processes such as diffusion, adsorption, burial, sedimentation and resuspension are included (see Bryhn & Hakanson, 2007 for details). The main inputs to the model are: dimensions (depth and fetch), water inflow, nutrient loading, particulate loading, temperature, irradiation and sediment characteristics. PCLake has been calibrated following a beyasian approach to parameter estimation and uncertinty analysis (Aldenberg et al., 1995; Janse et al., 2010). The calibration focussed on higher level variables that are of interest to water quality mangers, including chlorophyll-*a*, Secchi depth, vegetation cover and nutrient concentrations in the water column (Janse et al., 2010). In a recent multi-model ensemble study using an independent dataset, PCLake came out as the most accurate model out of a set of three tested aquatic ecosystem models (Trolle et al., 2014). Although PCLake has mainly been applied to temperate lakes in NW Europe, successful case studies in Mediterranean Greece (Mellios et al., 2015) and Subtropical China (Kong et al., 2016) suggest that the model may also be of value outside the temperate zone. A full description of the model is presented by Janse (2005). A schematic overview of PCLake is presented in Supplementary Fig. S 1.

Alternative stable states

The PCLake model shows a nonlinear response to changing nutrient loadings, similar to examples studied in the field (Janse, 1997). Lakes with a low external nutrient loading are in the clear-water macrophyte-dominated state with low chlorophyll-*a* concentrations. Lakes that receive a high external nutrient input reside in a turbid phytoplankton dominated state. In between, a fairly abrupt shift between the contrasting states takes place. The *critical nutrient loading* for a shift from a clear to a turbid state during eutrophication (CNL_{eu}) is at a much higher value than the critical nutrient loading where the reverse switch takes place, back to clear conditions during re-oligotrophication (CNL_{oligo}). Hence, at intermediate loading levels both the clear-water state and the turbid water state can exist as alternative stable states and the prevalent state depends on the foregoing conditions - a phenomenon known as hysteresis. Between the critical nutrient loading values, strong perturbations, such as discrete mowing events, may instigate a regime shift from one state to the other (Janse et al., 2008). Classical alternative stable states theory predicts that a lake is more vulnerable to disturbances closer to a tipping point, while the time it takes to recover from a perturbation increases (Van Nes & Scheffer, 2007). Previous analyses with PCLake indicated that alternative stable states are most likely to occur in lakes that are shallow (<4 m depth) and have a relatively small fetch (<3000 m) (Janse et al., 2008).

Macrophytes

The submerged macrophytes in PCLake represent Waterweeds in general (*Elodea* spp.). Waterweed species are non-native yet widespread in NW Europe and they are often among the first macrophytes to return after restoration measures have been taken (Heimans & Thijsse, 1895; Perrow et al., 1997; Pot & ter Heerdt, 2014; Immers et al., 2015). They are documented to cause nuisance by their mass development and are subject to mowing management (Hilt et al., 2006; Zehnsdorf et al., 2015). In PCLake, the growth of the submerged macrophytes (Fig. 1) is dependent on nutrient availability, temperature and under-water light availability. Plants take up phosphate, ammonium, and nitrate from both water column and soil pore water to achieve optimal P:biomass and N:biomass ratios (Droop, 1974; Madsen & Cedergreen, 2002; Angelstein & Schubert, 2008; Baldy et al., 2015; Christiansen et al., 2016). Ammonium is preferred, but when the ammonium concentration is low, the plants switch to nitrate uptake. The available light for primary production forms a gradient with depth (Lambert–Beer's law) and is controlled by the light intensity at the water surface, which is set by a seasonal sine curve (based on long-term averages for Dutch solar irradiance), and by the light attenuation by the plants themselves (self-shading), phytoplankton, detritus and inorganic matter in the water column as well as background extinction. It is assumed that the growing season starts when a critical spring water temperature (9°C) is reached. This happens in mid-April, given the long-term averaged seasonal water temperature in Dutch lakes. The

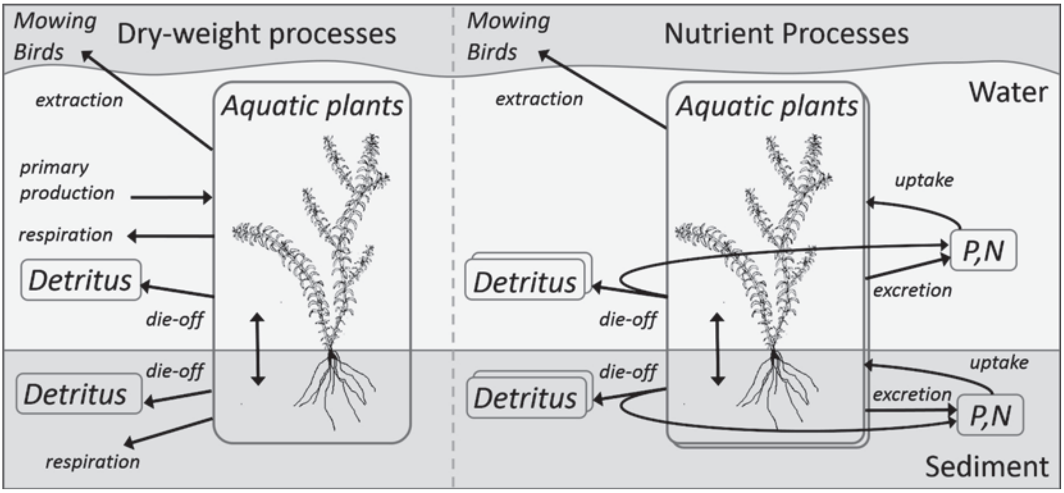


Figure 1. Basic processes of the aquatic plants in PCLake. The modelled processes are nutrient uptake, production, respiration and nutrient excretion, mortality, grazing by birds and mowing. The nutrient processes are modelled both in phosphorus and nitrogen. Herbivory by birds was not considered in this study. The figure is adapted from Janse (2005).

growing season ends half September onwards, when part of the above-ground biomass is allocated to the below ground biomass, and the mortality of the plants is raised for two weeks such that 30% of the original biomass survives, i.e. the over-wintering parts.

The submerged macrophytes are involved in several positive feedbacks with water clarity that have been linked to the emergence of alternative stable states in shallow lakes (Sondergaard & Moss, 1998; Scheffer, 1999; Horppila & Nurminen, 2003; Janse et al., 2008). For example, they are able to suppress phytoplankton growth by being strong competitors for nutrients while having a relatively low light extinction coefficient. Moreover, they provide shelter for phytoplankton grazing zooplankton, and reduce the resuspension caused by wind and benthivorous fish. Furthermore, vegetation promotes growing conditions for piscivorous fish which exert top-down pressure on zooplanktivorous fish. Finally, aquatic plants have the potential to lower the total amount of available nitrogen in the system by promoting denitrification.

A mowing function is available in PCLake, which requires defining a date when the mowing event takes place, the duration of the mowing event and a mowing intensity (i.e. fraction of the biomass that is removed). The mowing intensity is independent of the duration of the mowing event: a natural logarithm is used to calculate the amount of biomass that is removed per day: $h = -ln(1.0-f)/p*V$, where h is the harvested biomass (g m^{-2} day^{-1}), f is the intensity (-), p is the duration (days) and V is the total aquatic plant biomass in the lake (g m^{-2}). We applied a ‘clean’ mowing strategy throughout this study, whereby all biomass is removed from the lake. We did briefly consider potentially harmful side effects of mowing, including enhanced resuspension and incomplete removal of plant material from the water column, but present these findings as an appendix as they did not affect the conclusions of our main analyses (see Supplementary S 2).

Implementation

We used default parameter settings describing a lake that is representative for many shallow lakes in the temperate zone, with a mean depth of 2 m, a 1000 m fetch, a water inflow of 20 mm d^{-1} (100 day residence time), a lightly clayish soil (30% dry matter, of which 10% organic matter, and 10% lutum), no infiltration or seepage and no surrounding wetland area (c.f. Janse et al., 2010). The N:P ratio of the external nutrient loading was set at 13, i.e. the estimated average N:P ratio for agricultural runoff in the Netherlands (Wolf et al., 2003). In this set-up, the calculated CNL_{eu} and CNL_{oligo} values are 1.6 and 0.9 mg P m^{-2} d^{-1} respectively. To run simulations we used a C++ compiled version of the PCLake model called from GRIND for MATLAB (Mooij et al., 2014).

Model simulations

Nutrient loading, mowing intensity and timing

In this study on the impact of mowing on the lake we varied three independent variables of the model that can be controlled by lake managers: (1) external nutrient loading, (2) mowing intensity and (3) timing of the mowing. We first focused on the interplay between the first two. We simulated different combinations of external P loading, ranging from 0.7 to 1.7 in steps of 0.05 (mg m^{-2} d^{-1}), and mowing intensity, ranging from 0 to 0.9 in steps of 0.1 (-). We did not consider P loadings above 1.7 mg m^{-2} d^{-1} as the modelled lake then resides in the turbid water state without macrophytes. Each simulation was started from a clear water state and we ran the model for 20 years before starting the mowing procedure to ensure the lake to be in (seasonal) equilibrium. Note that internal nutrient loading in PCLake is not an independent variable, and by running the model 20 years we achieve that the internal loading in the system associates with the corresponding levels of external P loading. The initialization period was followed by three succeeding years where a mowing event took place. We considered three years to include the effect of mowing on the biomass in the next year (Kimbel & Carpenter, 1981). Each of the mowing years comprised one discrete mowing event, taking place on July 1st. This is in compliance with the guidelines provided by Rijkswaterstaat, responsible for the management of the main waterways and water systems in the Netherlands, who discourage mowing during the avian breeding season (Rijkswaterstaat, 2012). The duration of the mowing event (p) was kept at the default value of 10 days in all of these and subsequent cases. Next, we repeated the foregoing simulations, but this time focusing on different combinations of mowing intensity and timing. Again the mowing intensity ranged from 0 to 0.9 in steps of 0.1, while the mowing dates ranged from June 1st to September 1st in steps of 7 days. We performed this analysis for three different nutrient loading settings (0.8, 1.1 and 1.4 g P m^{-2} d^{-1}, respectively).

To evaluate the effects of the mowing actions we analyzed the summer average (June 10th to September 15th) vegetation cover and total and cyanobacterial chlorophyll-*a* concentration in the final year of the simulations. In the model, the vegetation cover increases linearly with the dry weight (DW) of submerged plants until 200 g DW m^{-2} is reached and the cover is 100%. Also, we calculated the days with nuisance during the peak of the holiday season (beginning of July until the end of August) caused by either submerged water plants or cyanobacteria. We presumed that water plants cause nuisance when they cover more than 40% of the area (Gettys et al., 2014). For the cyanobacteria, we followed the Dutch cyanobacteria protocol and took 12.5 mg m^{-3} cyano-chlorophyll as a limit above which nuisance occurs (Nationaal Water Overleg, 2012). Short-time human exposure to concentrations higher than this value can cause skin rashes or gastrointestinal sickness, and this risk should be communicated to bathing guests.

Additionally, we zoomed in on one intermediate nutrient loading (1.3 mg P m^{-2} d^{-1}) and present the within-season dynamics of the vegetation cover and chlorophyll-*a* in response to several different mowing intensities, to also obtain a more detailed view on the dynamics of the lake.

Nutrient removal by harvesting

We kept track of the amount of P stored in aquatic plant biomass which was harvested from the system in the final (third) year of mowing, to evaluate the potential to impoverish the lake. The amount of P removed from the system via harvesting provides an indication of the P that can potentially be recovered for reuse. In addition, we calculated the relative removal of P, that is, the ratio of P in the harvested biomass to the total amount of P added to the system via external loading. The relative removal thus allows to assess the extent to which harvesting may contribute to the closing of the P cycle.

Prolonged mowing and the resilience to nutrient loading

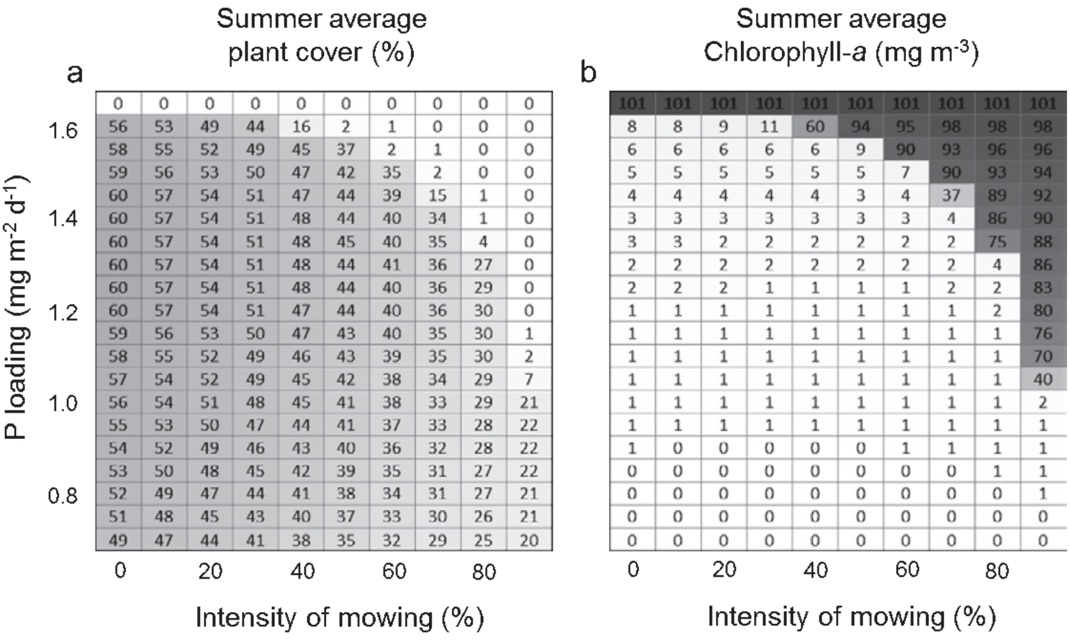
We used PCLake to analyze whether harvesting of macrophytes has the potential to forestall eutrophication effects in the long run. More precisely, we analyzed how repeated annual harvesting changes the CNL_{eu} of the lake, that is, the amount of external nutrient loading the lake can withstand without switching to a phytoplankton-dominated turbid state. Following Janse et al. (2008), we calculated CNL_{eu} values for different combinations of mowing intensity and timing, for which we took the same ranges as presented in the foregoing analysis. For each combination the model was evaluated for P loading rates ranging from 0.1 to 4 mg P m^{-2} day^{-1} in steps of 0.1. Each simulation started with a clear and oligotrophic lake. The summer average Secchi depth (m) after 20 years was used to evaluate the state of the lake, to determine which P loading is the CNL_{eu}. Previous analyses have shown that the ratio of Secchi depth to lake depth is a suitable response variable to determine the CNL_{eu} (c.f. Witteveen+Bos, 2010; Lischke et al., 2014): above a ratio of 0.5 the lake is defined as clear, while below this ratio the lake is defined as turbid. Mowing took place in each of the 20 years and comprised one discrete mowing event lasting the standard 10 days.

Results

Nutrient loading and mowing intensity

The model shows that the summer average plant cover can be reduced by mowing (Fig. 2a). When external nutrient loading is low, and no alternative equilibrium exists, plant cover shows an almost linear decrease with increasing mowing intensity. At high nutrient loadings however, mowing can trigger a regime shift to an alternative state with high phytoplankton concentrations (Fig. 2b). The mowing intensity that leads to a regime shift shows a nonlinear relationship with nutrient loading; the critical mowing intensity decreases sharply when the external loading approaches the critical nutrient loading (1.61 mg m^{-2} d^{-1}). In the vicinity of the critical nutrient loading, a mowing intensity of >30% is sufficient to trigger a collapse.

Zooming in on the seasonal dynamics clearly reveals the time window where plant cover is reduced due to mowing lasting for at least several weeks (Fig. 3a). It also shows that, apart from the average plant cover, the maximum plant cover reached during the growing season is also lowered with increasing mowing intensity. A detailed look reveals the importance of considering three succeeding years: the 90% mowing treatment triggers a regime shift, which only becomes apparent in the second and third year, when the plant community collapses and phytoplankton blooms start to occur (Fig. 3b).



0	0	0	0	0	0	0	0	0	0
56	53	49	44	16	2	1	0	0	0
58	55	52	49	45	37	2	1	0	0
59	56	53	50	47	42	35	2	0	0
60	57	54	51	47	44	39	15	1	0
60	57	54	51	48	44	40	34	1	0
60	57	54	51	48	45	40	35	4	0
60	57	54	51	48	44	41	36	27	0
60	57	54	51	48	44	40	36	29	0
60	57	54	51	47	44	40	36	30	0
59	56	53	50	47	43	40	35	30	1
58	55	52	49	46	43	39	35	30	2
57	54	52	49	45	42	38	34	29	7
56	54	51	48	45	41	38	33	29	21
55	53	50	47	44	41	37	33	28	22
54	52	49	46	43	40	36	32	28	22
53	50	48	45	42	39	35	31	27	22
52	49	47	44	41	38	34	31	27	21
51	48	45	43	40	37	33	30	26	21
49	47	44	41	38	35	32	29	25	20

101	101	101	101	101	101	101	101	101	101
8	8	9	11	60	94	95	98	98	98
6	6	6	6	6	9	90	93	96	96
5	5	5	5	5	5	7	90	93	94
4	4	4	4	4	3	4	37	89	92
3	3	3	3	3	3	3	4	86	90
3	3	2	2	2	2	2	2	75	88
2	2	2	2	2	2	2	2	4	86
2	2	2	1	1	1	1	2	2	83
1	1	1	1	1	1	1	1	2	80
1	1	1	1	1	1	1	1	1	76
1	1	1	1	1	1	1	1	1	70
1	1	1	1	1	1	1	1	1	40
1	1	1	1	1	1	1	1	1	2
1	1	1	1	1	1	1	1	1	1
1	0	0	0	0	0	1	1	1	1
0	0	0	0	0	0	0	0	1	1
0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

Figure 2. Combined effects of mowing intensity and nutrient loading on summer average plant cover (**a**) and chlorophyll-a (**b**) in the final year of the simulations. Mowing starts on July 1st.

An important question is how the response of the ecosystem to mowing translates to nuisance experienced by lake users. Our approach illustrates that there is a sharp boundary between nuisance caused by macrophytes and nuisance caused by cyanobacteria when the nutrient loading is high (Fig. 4a-c). On the other hand, when the nutrient loading is fairly low (<1 mg m^{-2} day^{-1}), mowing can create conditions where hardly any nuisance is experienced during the peak of the summer holiday season (Fig. 4c), given that a substantial fraction of the submerged macrophytes is removed (>50%).

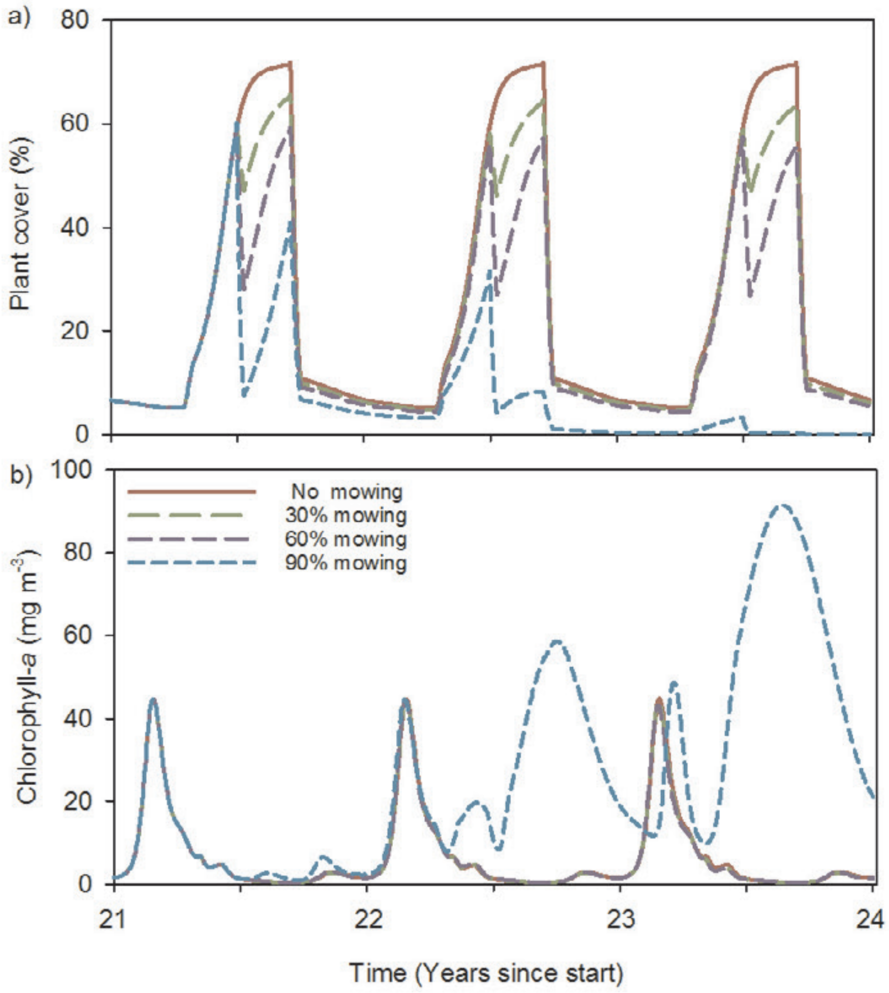
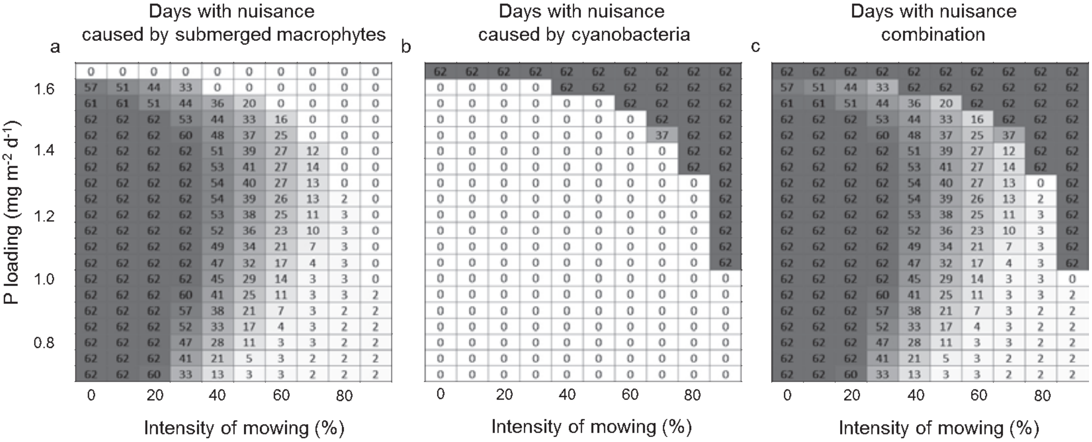


Figure 3. Effects of mowing on July 1st on summer average plant cover (**a**) and chlorophyll-a (**b**) in three succeeding years for a lake receiving 1.3 mg P m^{-2} day^{-1}.



a

Days with nuisance caused by submerged macrophytes

0	0	0	0	0	0	0	0	0	0
57	51	44	33	0	0	0	0	0	0
61	61	51	44	36	20	0	0	0	0
62	62	62	53	44	33	16	0	0	0
62	62	62	60	48	37	25	0	0	0
62	62	62	62	51	39	27	12	0	0
62	62	62	62	53	41	27	14	0	0
62	62	62	62	54	40	27	13	0	0
62	62	62	62	54	39	26	13	2	0
62	62	62	62	53	38	25	11	3	0
62	62	62	62	52	36	23	10	3	0
62	62	62	62	49	34	21	7	3	0
62	62	62	62	47	32	17	4	3	0
62	62	62	62	45	29	14	3	3	0
62	62	62	60	41	25	11	3	3	2
62	62	62	57	38	21	7	3	2	2
62	62	62	52	33	17	4	3	2	2
62	62	62	47	28	11	3	3	2	2
62	62	62	41	21	5	3	2	2	2
62	62	60	33	13	3	3	2	2	2

b

Days with nuisance caused by cyanobacteria

62	62	62	62	62	62	62	62	62	62
0	0	0	0	62	62	62	62	62	62
0	0	0	0	0	0	62	62	62	62
0	0	0	0	0	0	0	62	62	62
0	0	0	0	0	0	0	37	62	62
0	0	0	0	0	0	0	0	62	62
0	0	0	0	0	0	0	0	62	62
0	0	0	0	0	0	0	0	0	62
0	0	0	0	0	0	0	0	0	62
0	0	0	0	0	0	0	0	0	62
0	0	0	0	0	0	0	0	0	62
0	0	0	0	0	0	0	0	0	62
0	0	0	0	0	0	0	0	0	62
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

c

Days with nuisance combination

62	62	62	62	62	62	62	62	62	62
57	51	44	33	62	62	62	62	62	62
61	61	51	44	36	20	62	62	62	62
62	62	62	53	44	33	16	62	62	62
62	62	62	60	48	37	25	37	62	62
62	62	62	62	51	39	27	12	62	62
62	62	62	62	53	41	27	14	62	62
62	62	62	62	54	40	27	13	0	62
62	62	62	62	54	39	26	13	2	62
62	62	62	62	53	38	25	11	3	62
62	62	62	62	52	36	23	10	3	62
62	62	62	62	49	34	21	7	3	62
62	62	62	62	47	32	17	4	3	62
62	62	62	62	45	29	14	3	3	0
62	62	62	60	41	25	11	3	3	2
62	62	62	57	38	21	7	3	2	2
62	62	62	52	33	17	4	3	2	2
62	62	62	47	28	11	3	3	2	2
62	62	62	41	21	5	3	2	2	2
62	62	60	33	13	3	3	2	2	2

Figure 4. Combined effects of mowing intensity and nutrient loading on days with nuisance caused by aquatic plants (**a**), cyanobacteria (**b**) or both aquatic plants and cyanobacteria (c) during July and August (peak of the holiday season in the temperate region) in the final year of the simulations. Mowing starts on July 1st.

Timing of mowing

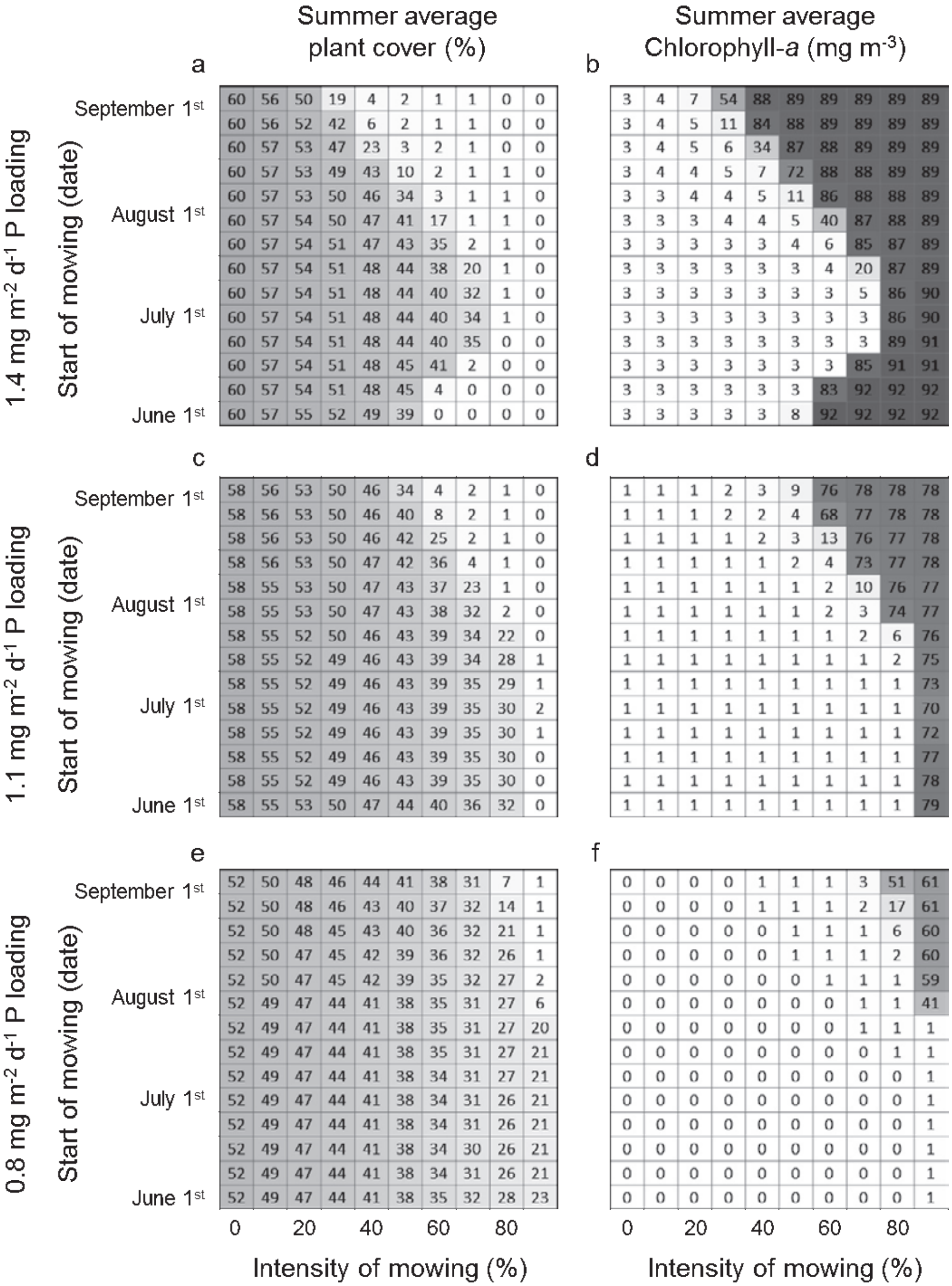
The impact of harvesting varies during the growing season (Fig. 5), particularly when the external nutrient loading is high (Fig. 5a-b) and the lake is susceptible to a regime shift (Fig. 2a-b). When the nutrient loading is high, the modelled lake is most vulnerable in late summer, when harvesting a fraction of 40% is sufficient to instigate a regime shift to the phytoplankton dominated state. To a somewhat lesser extent, also mowing in early summer eases a shift to the turbid state. The resilience to perturbations of the modelled lake is highest during mid-summer, as up to 80% of the vegetation can be removed, resulting in a halving of the summer average plant cover (Fig. 5a-b). The timing of mowing is not particularly important when the external nutrient loading is low (Fig. 5e,f). Large fractions of the plant biomass can be removed almost the entire growing season without risking a regime shift, allowing to reduce the summer average plant cover up to 40%.

Nutrient removal by mowing

The amount of P harvested from the lake during a mowing event increases with mowing intensity and nutrient loading, and is highest close to the point where mowing leads to a regime shift, reaching a maximum of almost 230 mg P m^{-2} (Fig. 6). The relative removal of P increases with mowing intensity and can be as high as 58%. However, the relative removal decreases with increasing nutrient loading. The associated dry-weight of the harvested plant biomass is presented in Supplementary S 3.

Prolonged mowing and resilience

Our model exercises show that in the long run repeated mowing is able to enhance the



a

Start of mowing (date)	0		20		40		60		80	
September 1st	60	56	50	19	4	2	1	1	0	0
	60	56	52	42	6	2	1	1	0	0
	60	57	53	47	23	3	2	1	0	0
	60	57	53	49	43	10	2	1	1	0
	60	57	53	50	46	34	3	1	1	0
August 1st	60	57	54	50	47	41	17	1	1	0
	60	57	54	51	47	43	35	2	1	0
	60	57	54	51	48	44	38	20	1	0
	60	57	54	51	48	44	40	32	1	0
July 1st	60	57	54	51	48	44	40	34	1	0
	60	57	54	51	48	44	40	35	0	0
	60	57	54	51	48	45	41	2	0	0
	60	57	54	51	48	45	4	0	0	0
June 1st	60	57	55	52	49	39	0	0	0	0

b

Start of mowing (date)	0		20		40		60		80	
September 1st	3	4	7	54	88	89	89	89	89	89
	3	4	5	11	84	88	89	89	89	89
	3	4	5	6	34	87	88	89	89	89
	3	4	4	5	7	72	88	88	89	89
	3	3	4	4	5	11	86	88	88	89
August 1st	3	3	3	4	4	5	40	87	88	89
	3	3	3	3	3	4	6	85	87	89
	3	3	3	3	3	3	4	20	87	89
	3	3	3	3	3	3	3	5	86	90
July 1st	3	3	3	3	3	3	3	3	86	90
	3	3	3	3	3	3	3	3	89	91
	3	3	3	3	3	3	3	85	91	91
	3	3	3	3	3	3	83	92	92	92
June 1st	3	3	3	3	3	8	92	92	92	92

c

Start of mowing (date)	0		20		40		60		80	
September 1st	58	56	53	50	46	34	4	2	1	0
	58	56	53	50	46	40	8	2	1	0
	58	56	53	50	46	42	25	2	1	0
	58	56	53	50	47	42	36	4	1	0
	58	55	53	50	47	43	37	23	1	0
August 1st	58	55	53	50	47	43	38	32	2	0
	58	55	52	50	46	43	39	34	22	0
	58	55	52	49	46	43	39	34	28	1
	58	55	52	49	46	43	39	35	29	1
July 1st	58	55	52	49	46	43	39	35	30	2
	58	55	52	49	46	43	39	35	30	1
	58	55	52	49	46	43	39	35	30	0
	58	55	52	49	46	43	39	35	30	0
June 1st	58	55	53	50	47	44	40	36	32	0

d

Start of mowing (date)	0		20		40		60		80	
September 1st	1	1	1	2	3	9	76	78	78	78
	1	1	1	2	2	4	68	77	78	78
	1	1	1	1	2	3	13	76	77	78
	1	1	1	1	1	2	4	73	77	78
	1	1	1	1	1	1	2	10	76	77
August 1st	1	1	1	1	1	1	2	3	74	77
	1	1	1	1	1	1	1	2	6	76
	1	1	1	1	1	1	1	1	2	75
	1	1	1	1	1	1	1	1	1	73
July 1st	1	1	1	1	1	1	1	1	1	70
	1	1	1	1	1	1	1	1	1	72
	1	1	1	1	1	1	1	1	1	77
	1	1	1	1	1	1	1	1	1	78
June 1st	1	1	1	1	1	1	1	1	1	79

e

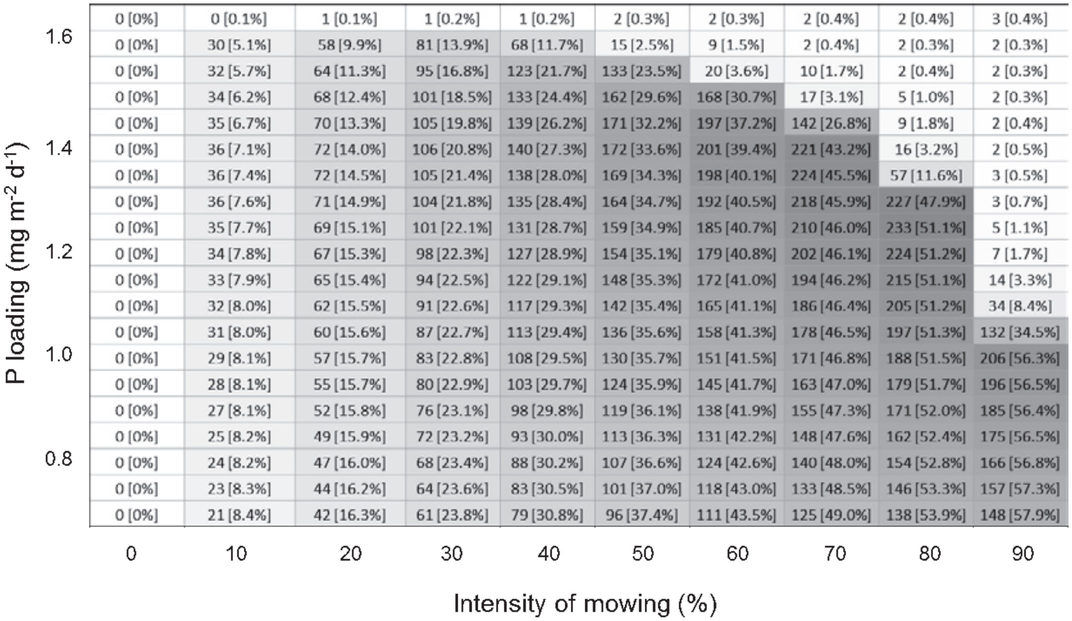
Start of mowing (date)	0		20		40		60		80	
September 1st	52	50	48	46	44	41	38	31	7	1
	52	50	48	46	43	40	37	32	14	1
	52	50	48	45	43	40	36	32	21	1
	52	50	47	45	42	39	36	32	26	1
	52	50	47	45	42	39	35	32	27	2
August 1st	52	49	47	44	41	38	35	31	27	6
	52	49	47	44	41	38	35	31	27	20
	52	49	47	44	41	38	35	31	27	21
	52	49	47	44	41	38	34	31	27	21
July 1st	52	49	47	44	41	38	34	31	26	21
	52	49	47	44	41	38	34	31	26	21
	52	49	47	44	41	38	34	30	26	21
	52	49	47	44	41	38	34	31	26	21
June 1st	52	49	47	44	41	38	35	32	28	23

f

Start of mowing (date)	0		20		40		60		80	
September 1st	0	0	0	0	1	1	1	3	51	61
	0	0	0	0	1	1	1	2	17	61
	0	0	0	0	0	1	1	1	6	60
	0	0	0	0	0	1	1	1	2	60
	0	0	0	0	0	0	1	1	1	59
August 1st	0	0	0	0	0	0	0	1	1	41
	0	0	0	0	0	0	0	1	1	1
	0	0	0	0	0	0	0	0	1	1
	0	0	0	0	0	0	0	0	0	1
July 1st	0	0	0	0	0	0	0	0	0	1
	0	0	0	0	0	0	0	0	0	1
	0	0	0	0	0	0	0	0	0	1
	0	0	0	0	0	0	0	0	0	1
June 1st	0	0	0	0	0	0	0	0	0	1

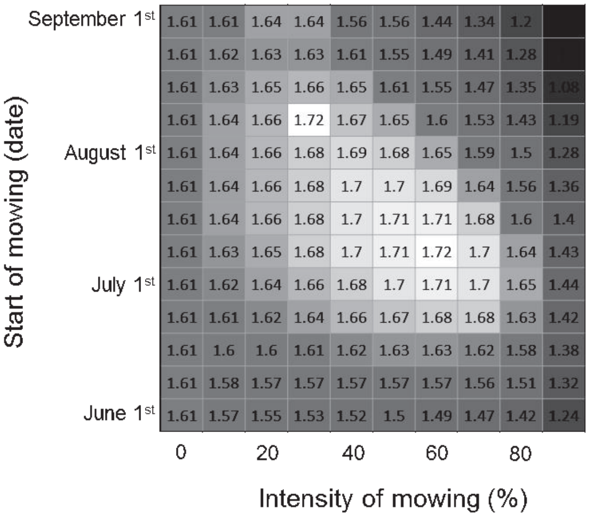
Figure 5. Combined effects of mowing intensity and mowing date on summer average plant cover and chlorophyll-a in the final year of the simulations, for three different nutrient loadings: 1.4, 1.1 and 0.8 mg m^{-2} day^{-1}, respectively.

resilience of the clear water state to nutrient loading for a wide range of mowing intensities and mowing dates, as it leads to an increase (max. 7%) of the critical nutrient loading (CNL_{eu} > 1.61 mg P m^{-2} d^{-1}; Fig. 7). Mowing during July and August in combination with an intermediate mowing intensity is most beneficial for enhancing the CNL_{eu}. Mowing in early-summer or in late-summer can lead to a reduced resilience to nutrient loading (CNL_{eu} < 1.61 mg P m^{-2} d^{-1}).



P loading (mg m-2 d-1)	0	10	20	30	40	50	60	70	80	90
	0 [0%]	0 [0.1%]	1 [0.1%]	1 [0.2%]	1 [0.2%]	2 [0.3%]	2 [0.3%]	2 [0.4%]	2 [0.4%]	3 [0.4%]
1.6	0 [0%]	30 [5.1%]	58 [9.9%]	81 [13.9%]	68 [11.7%]	15 [2.5%]	9 [1.5%]	2 [0.4%]	2 [0.3%]	2 [0.3%]
	0 [0%]	32 [5.7%]	64 [11.3%]	95 [16.8%]	123 [21.7%]	133 [23.5%]	20 [3.6%]	10 [1.7%]	2 [0.4%]	2 [0.3%]
	0 [0%]	34 [6.2%]	68 [12.4%]	101 [18.5%]	133 [24.4%]	162 [29.6%]	168 [30.7%]	17 [3.1%]	5 [1.0%]	2 [0.3%]
	0 [0%]	35 [6.7%]	70 [13.3%]	105 [19.8%]	139 [26.2%]	171 [32.2%]	197 [37.2%]	142 [26.8%]	9 [1.8%]	2 [0.4%]
1.4	0 [0%]	36 [7.1%]	72 [14.0%]	106 [20.8%]	140 [27.3%]	172 [33.6%]	201 [39.4%]	221 [43.2%]	16 [3.2%]	2 [0.5%]
	0 [0%]	36 [7.4%]	72 [14.5%]	105 [21.4%]	138 [28.0%]	169 [34.3%]	198 [40.1%]	224 [45.5%]	57 [11.6%]	3 [0.5%]
	0 [0%]	36 [7.6%]	71 [14.9%]	104 [21.8%]	135 [28.4%]	164 [34.7%]	192 [40.5%]	218 [45.9%]	227 [47.9%]	3 [0.7%]
	0 [0%]	35 [7.7%]	69 [15.1%]	101 [22.1%]	131 [28.7%]	159 [34.9%]	185 [40.7%]	210 [46.0%]	233 [51.1%]	5 [1.1%]
1.2	0 [0%]	34 [7.8%]	67 [15.3%]	98 [22.3%]	127 [28.9%]	154 [35.1%]	179 [40.8%]	202 [46.1%]	224 [51.2%]	7 [1.7%]
	0 [0%]	33 [7.9%]	65 [15.4%]	94 [22.5%]	122 [29.1%]	148 [35.3%]	172 [41.0%]	194 [46.2%]	215 [51.1%]	14 [3.3%]
	0 [0%]	32 [8.0%]	62 [15.5%]	91 [22.6%]	117 [29.3%]	142 [35.4%]	165 [41.1%]	186 [46.4%]	205 [51.2%]	34 [8.4%]
	0 [0%]	31 [8.0%]	60 [15.6%]	87 [22.7%]	113 [29.4%]	136 [35.6%]	158 [41.3%]	178 [46.5%]	197 [51.3%]	132 [34.5%]
1.0	0 [0%]	29 [8.1%]	57 [15.7%]	83 [22.8%]	108 [29.5%]	130 [35.7%]	151 [41.5%]	171 [46.8%]	188 [51.5%]	206 [56.3%]
	0 [0%]	28 [8.1%]	55 [15.7%]	80 [22.9%]	103 [29.7%]	124 [35.9%]	145 [41.7%]	163 [47.0%]	179 [51.7%]	196 [56.5%]
	0 [0%]	27 [8.1%]	52 [15.8%]	76 [23.1%]	98 [29.8%]	119 [36.1%]	138 [41.9%]	155 [47.3%]	171 [52.0%]	185 [56.4%]
	0 [0%]	25 [8.2%]	49 [15.9%]	72 [23.2%]	93 [30.0%]	113 [36.3%]	131 [42.2%]	148 [47.6%]	162 [52.4%]	175 [56.5%]
0.8	0 [0%]	24 [8.2%]	47 [16.0%]	68 [23.4%]	88 [30.2%]	107 [36.6%]	124 [42.6%]	140 [48.0%]	154 [52.8%]	166 [56.8%]
	0 [0%]	23 [8.3%]	44 [16.2%]	64 [23.6%]	83 [30.5%]	101 [37.0%]	118 [43.0%]	133 [48.5%]	146 [53.3%]	157 [57.3%]
	0 [0%]	21 [8.4%]	42 [16.3%]	61 [23.8%]	79 [30.8%]	96 [37.4%]	111 [43.5%]	125 [49.0%]	138 [53.9%]	148 [57.9%]

Figure 6. The amount of P (mg m^{-2} $year^{-1}$) extracted from the system via harvesting of plant biomass during the last year of mowing, for different combinations of external nutrient loading and mowing intensity. The shade indicates the quantity. The relative removal, that is, the ratio of P in the harvested biomass to the total amount of P added to the system via external loading, is presented between squared brackets (%).



Start of mowing (date)	0		20		40		60		80	
September 1st	1.61	1.61	1.64	1.64	1.56	1.56	1.44	1.34	1.2	
	1.61	1.62	1.63	1.63	1.61	1.55	1.49	1.41	1.28	
	1.61	1.63	1.65	1.66	1.65	1.61	1.55	1.47	1.35	1.08
	1.61	1.64	1.66	1.72	1.67	1.65	1.6	1.53	1.43	1.19
August 1st	1.61	1.64	1.66	1.68	1.69	1.68	1.65	1.59	1.5	1.28
	1.61	1.64	1.66	1.68	1.7	1.7	1.69	1.64	1.56	1.36
	1.61	1.64	1.66	1.68	1.7	1.71	1.71	1.68	1.6	1.4
	1.61	1.63	1.65	1.68	1.7	1.71	1.72	1.7	1.64	1.43
July 1st	1.61	1.62	1.64	1.66	1.68	1.7	1.71	1.7	1.65	1.44
	1.61	1.61	1.62	1.64	1.66	1.67	1.68	1.68	1.63	1.42
	1.61	1.6	1.6	1.61	1.62	1.63	1.63	1.62	1.58	1.38
	1.61	1.58	1.57	1.57	1.57	1.57	1.57	1.56	1.51	1.32
June 1st	1.61	1.57	1.55	1.53	1.52	1.5	1.49	1.47	1.42	1.24

Figure 7. Effect of prolonged (long term) mowing on the CNL_{eu} (mg P m^{-2} d^{-1}), i.e. the amount of nutrient input the lake can withstand without shifting to the turbid water state, for different combinations of mowing intensity and timing (start of the mowing procedure). The colors indicate whether mowing leads to an increase (white) or decrease (dark grey) of the critical nutrient loading (default 1.61 mg m^{-2} day^{-1}).

Discussion

Temporal relief of nuisance

Our modelling study shows that mowing can result in a temporal reduction of plant cover for a range of nutrient loadings and mowing intensities. These reductions of plant cover can reduce nuisance for up to several weeks, especially when the mowing intensity is fairly high and the external nutrient loading is low or moderate. Our model thus indicates that mowing can facilitate multi-usage of shallow lake ecosystems. At lower intensities mowing also reduces the summer average plant cover, but this may be not sufficient to actually reduce nuisance as the remaining cover still exceeded the threshold level, which we fixed at 40%. Our model analyses indicate that it becomes more difficult to design a convenient mowing strategy when the external nutrient loading is high; the attraction of the alternative equilibrium is so strong that a rather small reduction in plant volume may be sufficient to trigger a shift to phytoplankton dominance. Interestingly, our results elucidate that a reduction of external nutrient loading alone is not an effective measure to drive back nuisance caused by aquatic plants (Fig. 4), which emphasizes the need for mowing. Because the risk of inducing a regime shift by mowing increases with external nutrient loading, the successfulness of mowing to reduce macrophyte nuisance goes hand in hand with the reduction of external nutrient loading. At what percentage of cover lake users perceive plants as a nuisance will vary between lakes and types of users. We took 40% because this number is frequently used in the grey literature, mostly in relation to growth of largemouth bass - a popular target species for sport fisheries (e.g. Gettys et al., 2014). However, we can hypothesize that when lake users already perceive nuisance at a lower plant cover (<40%) it will become increasingly difficult, or even impossible, to manage the vegetation successfully by harvesting while maintaining clear water. Vice versa, if local lake users would be more tolerant to the aquatic vegetation and perceive nuisance at higher percent cover (>40%), it will be more easy to reduce nuisance and maintain a clear-water ecosystem, especially when the external nutrient loading is not close to the critical nutrient loading level (Fig. 4). Hence, before designing a management scheme it is important to identify which stakeholders need to be served and at what percentage of plant cover they actually perceive plants as a nuisance.

The importance of timing

Our model analyses indicate that the highest reductions of plant biomass can be achieved by mowing in mid-summer, while mowing in late summer appears to be least recommendable. The latter is not just because the peak of the holiday season (and thus recreational usage) is in mid-summer, but also because the risk of inducing a regime shift increases when mowing is conducted later in the growing season. In our model, mowing late in the growing season provides the aquatic plants with little opportunity to regain biomass before the growing season ends. This is in line with Engel (1990), who observed slow regrowth after mowing in July compared to mowing in June, and ascribed this to declining day length and water temperature. Consequently, in the following spring the macrophytes may start the competition with phytoplankton on their back foot, which eases a shift to phytoplankton dominance (Scheffer, 2004: p.280). Mowing too early in the growing season also bears a certain risk of triggering a regime shift, as our study showed, particularly when the external nutrient loading is high. We hypothesize that this is because the *inter*-specific competition with phytoplankton in early June is still rather strong, and setting back the submerged macrophytes favors phytoplankton growth. At the peak of the growing season, on the other hand, the *intra*-specific competition among macrophytes becomes more controlling, and mowing reliefs this intraspecific competition. Hence, the net growth rate of the macrophytes directly after mowing relates positively to mowing intensity (e.g. Fig. 2: the net growth rate after 30% and 60% mowing is 0.017 and 0.022 day^{-1}, respectively). This compensatory growth is not sufficient however to compensate for the entire loss of biomass, as plant cover does not recover to pre-harvesting levels (Fig. 2).

The effect of timing on the impact of mowing may be different in field situations, particularly when the macrophyte community comprises growth forms that - unlike e.g. *Elodea canadensis* - produce overwintering organs (Scheffer, 2004: p.279). Hence, in case of propagule forming macrophyte species such as several *Potamogeton* and *Myriophyllum* species, these propagules may have already been formed when harvesting takes place late in the growing season, wherefore the impact on the next growing season is much smaller. Harvesting earlier in the season would then be an effective way to reduce the potential for macrophyte plant growth in the succeeding year, as that would prevent the formation of propagules (Wade, 1990). Interestingly, a reduction of plant volume in the succeeding year is generally considered as a positive result of harvesting (e.g. Dall'Armellina et al., 1996), while our modelling study hints that this strategy is not without risks when lakes have alternative stable states and the external nutrient loading is high.

Restrictions to harvesting

In our model study we harvested fractions of the macrophytes to levels that may be unfeasible in real field situations. For example, there are practical reasons which frustrate harvesting large quantities of aquatic plants, as it is a labor-intensive and expensive activity. A simple calculation learns that for our modelled (circular) lake with a diameter of 1000 m, when receiving 1.2 mg P m^{-2} day^{-1}, a harvesting intensity of 80% implies removing more than 650 tons of fresh biomass in just a short time span, assuming a fresh-weight:dry-weight ratio of 10 (e.g. Boiché et al., 2011; Dorenbosch & Bakker 2011; Supplementary S 3). Secondly, local laws and regulations, such as the Dutch flora- and fauna law, may impose restrictions on harvesting intensity and timing. Plants provide habitat and food for many species and it has been reported that significant amounts of fish and macroinvertebrates are removed along with the plants during harvesting (Engel, 1990), which may include protected species. Furthermore, removing large quantities of plants may conflict with the protection of waterbirds that feed on the plants or the fauna living in macrophyte beds. A third reason is that in a field situation it will always be difficult to estimate the amount of aquatic plants that should be present to safeguard a clear water state, forcing lake managers to take a conservative approach when designing their plans. Hence, even though a submerged plant cover as low as 20% may coincide with good water quality (e.g. Portielje & Van der Molen, 1998; Yanran et al., 2012), Hilt et al. (2006) advise to take 50% vegetation cover as a rule of thumb, and suggest that remaining stands after harvesting should still cover 50% of the lake. Also the Dutch authorities advise to remove maximally 50% of the plant cover, and even suggests to mow only 10% in case of native plant species (Rijkswaterstaat, 2012). In our study we used *Elodea* sps. as model macrophytes, which are invasive in Europe. Our results show that part of the macrophytes should be retained under mowing management to prevent phytoplankton blooms under more eutrophic conditions. Implicitly this suggests that non-native macrophytes may be able to fulfil some of the ecosystem functions of native submerged macrophytes, in this case maintaining water clarity (Carpenter & Lodge, 1986). This is in line with recent findings that non-native macrophytes may fulfil ecosystem functions similarly to their native counterparts and that their effectiveness depends rather on species traits than their origin (Grutters et al., 2015; Grutters et al., 2016). Hence in management, complete removal of non-native macrophytes may be counterproductive for the ecosystem, if there are no native macrophytes to fill the empty place (Hussner et al., 2016).

Spatial heterogeneity

From our analyses it appears that harvesting 10% of the standing crop has only a marginal effect on reducing nuisance. This situation may change however when it is possible and desirable to spatially divide functions over the lake area. By harvesting in such a way that only certain patches are cleared, it may become possible to reduce nuisance locally e.g. in a zone designated for swimming or a channel for navigation. The model we used (PCLake) is not spatially explicit and is therefore not suited to evaluate the effect of a local disturbance by harvesting, as it is intended to provide a general indication of the harvesting pressure the lake can withstand. There is only little known about the effect of spatial heterogeneity on alternative stable states in shallow lakes. Theoretical studies suggest that the potential of local disturbances to instigate an ecosystem-wide regime shift increases with interconnectedness (dispersion) within the system (Van Nes & Scheffer, 2005), and decreases with spatial heterogeneity (Van de Leemput et al., 2015). These studies thus suggest that alternative stable states are unlikely to persist side by side in lakes which are very homogenous. This means that local mowing becomes risky as over-harvesting has catastrophic consequences for the entire lake, albeit the regime shift may be gradual (Bel et al., 2012; Van de Leemput et al., 2015). When lakes do exhibit spatial heterogeneity e.g. in terms of depth, fetch or sediment composition, the response to a local perturbation becomes much more difficult to predict (Van de Leemput et al., 2015), but this heterogeneity can potentially lead to coexistence of contrasting states. The latter would create opportunities for localized harvesting practices. A follow up step is to couple the ecological modules of PCLake to 2D-hydrodynamic models to analyze harvesting in a spatial hydrodynamic context. This development is still in its infancy however (e.g. Van Gerven et al., 2015).

Collateral effects

Generally, not all cut plant biomass is removed from the lake due to inefficiency of the harvesting equipment (Hussner et al., 2016). The fragments that are not collected start to decompose in the water column, thereby releasing nutrients and contributing to the depletion of oxygen which in turn can stimulate internal nutrient loading from the sediment (Hilt et al., 2006). Additionally, cutting machinery may cause resuspension of sediments, which may reduce transparency and stimulate nutrient recycling. These side-effects of mowing are expected to be detrimental to ecosystem functioning (Rijkswaterstaat, 2012), but it is difficult to quantify their true importance in the field. For simplicity reasons, we did not consider the effect of collateral disturbance in our main analyses. Yet, we did briefly look into their relative importance (presented in Supplementary S 2), which revealed that, for the modelled circumstances and assumptions, the effect of collateral damage is marginal. This finding is in line with Carpenter & Gasith (1978) who reported short lived or insignificant effects on the littoral environment after clearing a 0.2 ha patch. Only when a regime shift has already been initiated, our model shows that the collateral effects of mowing stimulate the upheaval (Supplementary S 2). However, we did not consider all potential side effects of mowing invasive aquatic macrophytes. For example, a factor we did not consider in this study is that many nuisance species (including *Elodea* spp.) spread by vegetative fragmentation (Hilt et al., 2006; Redekop et al., 2016). Mowing can stimulate dispersal of non-native nuisance species when fragments are produced that easily ride with the flow and settle at new places (Abernethy et al., 1996; Zehnsdorf et al., 2015). Especially when surrounding lakes or waterways are still free of these exotics, the vegetative dispersal capacity of the nuisance species that is being managed should be taken into consideration (Zehnsdorf et al., 2015). Recently, Hussner et al. (2016) reviewed how management aimed at the reduction or eradication of invasive aquatic plants can impact other (native) species present in the ecosystem. Interestingly, these effects can be both positive and negative. For example, Dawson et al. (1991) reported a case where 30 macroinvertebrate individuals were removed per gram dry weight of cut aquatic plants, while Bickel & Closs (2009) showed that total invertebrate biomass and abundance was significantly higher in the areas where mowing took place compared to the untreated macrophyte beds. Moreover, while Engel (1990) reported that up to 450 fish were removed per 100 kilogram fresh weight of cut aquatic plants, the potential for improving growth and size structure of fishes by reducing macrophyte density has long been recognized (e.g. Wiley et al., 1984; Olson et al., 1998). Furthermore, vegetation is a major food source for many waterfowl species aquatic and it is known that herbivorous birds such as coots (*Fulica*) can have a large impact on vegetation density (Van Altena et al., 2016). Interestingly, this trophic interaction may give rise to an interaction effect between mowing and herbivory. Hence, if a large quantity of vegetation is removed by means of mechanical mowing, this may either cause waterfowl to leave, but it may also cause birds to put extra pressure on the remaining vegetation, potentially triggering a critical regime shift to the turbid state (Van Altena et al., 2016).

Removal and recovery of nutrients

Because there are nutrients stored in plant tissue, as well as in material attached to the plant surface such as periphyton and calcite incrustations, the removal of submerged macrophytes may help to remediate the detrimental effects of eutrophication, both in the lake where the plants are removed from, and in downstream aquatic ecosystems (Carpenter & Adams, 1977b). Our modelling scenarios indicate that the highest amount of P is extracted from the system when both the external P loading and the mowing intensity are high. The relative removal however, which tells more about the capacity to actually prevent further enrichment of the system via harvesting, increases with decreasing nutrient loading, maximally reaching 58% in our analyses. While it should be noted that periphyton and calcite incrustations are not explicitly modelled by PCLake, we find these numbers to be grossly in line with estimations presented in the literature. For example, for a eutrophic lake with 30% plant cover, Carpenter & Adams (1977b) estimated that a relative removal of 37% of the P loading could be established if all plants would be harvested. Conyers & Cooke (1983) reported that a relative removal of 44% could be reached in a mesotrophic lake with 43% plant cover. Moreover, Wile (1978) presented a case where harvesting operations resulted in the removal of 560 kg P, and estimated the relative removal to be 47%. It is important to realize however that these numbers refer to the relative removal of P from the system as a whole, and not solely from the water column (Burton et al., 1978). Although many rooted macrophytes species are well capable of assimilating nutrients directly from the water column through their shoots (Madsen & Cedergreen, 2002; Angelstein & Schubert, 2008; Christiansen et al., 2016), at least part of their nutrients may be obtained from the sediment, especially in systems where large amounts of P are available in the sediment. As a consequence, removal of plant biomass does not axiomatically offset the external loading of P into the water column, and hence the incoming nutrients may perpetuate eutrophication. We postulate that the effect of harvesting rooted macrophytes on ecosystem functioning is highest when the macrophytes take up most of their nutrient directly from the water column. Furthermore, while harvesting alone may not be able to completely offset the incoming nutrient fluxes (Burton et al., 1978), we argue that the usefulness may be enhanced by the joint application of complementary management measures, such the application of phosphorus adsorbing natural soil and modified clay.

Harvested plant tissue can potentially serve as a source of nutrients, instead of only being waste material. The use of aquatic plant biomass to fertilize agriculture fields is an old practice (Roger & Watanabe, 1984), which is still carried out in many parts of mainly the developing world. Recently harvesting aquatic plant biomass has been put forward as a way to close the P cycle (Quilliam et al., 2015). Although excessive growth of macrophytes indicates a local surplus of nutrients, P is a scarce element in many places, leading to phosphate starvation in crops, and global phosphate sources are declining rapidly (Cordell et al., 2009; Childers et al., 2011). The recovery of valuable P thus has the potential to increase the viability of harvesting as a management measure, which is otherwise a costly procedure (Hilt et al., 2006). Currently there is no agreement on how to maximize P uptake and removal by macrophytes (Quilliam et al., 2015). Our model results suggest that it is beneficial for lake managers to reduce the external nutrient loading as much as possible, as that will reduce the possibility of harvesting triggering an unwanted regime shift to a state without macrophytes, and increase the relative removal of P.

Prolonged harvesting

Model analysis of the long-term effects of harvesting suggests that harvesting can potentially be used to prevent nutrient over-enrichment by increasing the resilience of the system to external loading, that is, by increasing the CNL_{eu} (Fig. 7). It is important to note however that in this analysis harvesting was executed every year, and that we started off with a clear and oligotrophic lake - in the domain where no alternative state is apparent. Because of the latter, almost all macrophytes can be removed at the start of the analysis without risking a shift to the alternative state, as there simply is none. In turn, the removal of macrophytes prevents the accumulation of nutrients in the system, postponing the formation of an alternative equilibrium and hence increasing the CNL_{eu}. This implies that the history of the lake is an important factor to consider when designing a mowing strategy. If nutrients have been able to accumulate in the lake prior to the mowing activities, as in our first analyses, the resilience of the lake to perturbations such as mowing may have already decreased and fairly small fractions of macrophyte removal may be enough to instigate a regime shift (see Figure S 4 for an illustrative example). Thus, based on the long term mowing scenarios we argue two points. The first is that phytoremediation can be a worthwhile measure to prevent a lake from becoming eutrophic when it is still oligotrophic and its capacity to withstand perturbations is still high. The second is that it is much more difficult to use phytoremediation to impoverish a lake when it is already eutrophic, even though the absolute removal of nutrients is high, because the capacity to withstand perturbation is much reduced. As many vegetated lakes in NW Europe have only recently recovered from the turbid state, and their sediments are likely to be saturated with nutrients, mowing schemes should be designed with great care.

From model to practice

An important question is how the results of this theoretical exercise should be interpreted by managers and can be useful in contemporary ecosystem management. Our point of departure is that every water system is unique (n=1), but that there are general mechanisms that are key to the ecological functioning of every lake. PCLake has been developed to include the most important biotic and abiotic processes and lake characteristics (Janse et al., 2008). Moreover, to strive for generality, the model has been calibrated with data from >40 lakes with the aim to get the best overall fit (Janse et al., 2010). Hence, PCLake provides a coherent framework to investigate the effect of mowing within an ecosystem context with alternative stable states, allowing us to focus on important aspects of mowing, such as the intensity and timing, while keeping other factors constant. An important purpose of such analysis is to provide scientists and managers with working hypothesis about the way ecosystems function, and to contribute to the development of theory. The insights that are obtained by simulations cannot easily be derived from any other type of study, as the analyses would be too costly or unethical to do in natural systems. As such, these insights complement the insights obtained by alternative approaches, such as lab experiments and field observations (Peck, 2004; Scheffer, 2004: p.313). PCLake is one of the very few integrated ecosystem models available for this kind of simulations (Janssen et al., 2015)

On one hand, we argue that the insights obtained by our simulations are widely applicable, as the model is built up of many general prevailing processes and principles. For example, although Elodea is used as a model species, we expect that, at least in qualitative terms, the response of other yet similar submerged angiosperms, such as *Lagarosiphon major* or *Egeria densa*, will be comparable. Moreover, lake characteristics have been chosen such that the model describes a hypothetical lake that is representative for many small and shallow lakes in the temperate zone. Interestingly, PCLake has even shown to be useful outside the temperate zone (e.g. Mellios et al., 2015; Kong et al., 2016).

On the other hand we acknowledge that the results of PCLake are highly dependent on the lake characteristics modelled. For example, Janse et al. (2008) showed that the resilience of shallow lakes decreases with increasing depth and fetch, implying that in larger and deeper lakes mowing can more readily result in a regime shift to the phytoplankton-dominated turbid state. Also the choice for a specific threshold level where lake users perceive plants as nuisance has implications for our results. When for a given case study, these controlling factors deviate too much from the settings used in this study, the calculations presented here should be redone for the new setting. Please note that such limitations apply to any type of experiment focusing on few independent variables.

Of course, it is conceivable that the ecology of certain lakes may differ fundamentally from the system currently portrayed by PCLake. For example, Blindow et al. (2014) distinguish between a charophyte-dominated clear water state and an angiosperm-dominated clear water state, and report on notable differences in the strengths of the exerted positive feedback loops with water clarity. Effects may be even more profound when a certain process has a strong effect on the functioning of a specific lake, but is not covered by the model. For example, grass carp (*Ctenopharyngodon idella*) has a strong trophic interaction with aquatic plants (Hussner et al., 2016), however this fish species is currently not included in PCLake. In all cases, a customized PCLake study, whereby the model is adapted, calibrated and validated for a specific case, will provide the most accurate predictions which can be readily employed in ecosystem management (e.g. Witteveen+Bos, 2010; Nielsen et al., 2014; Trolle et al., 2014; Kong et al., 2016). The present study provides a clear example of how to set up a model analysis with PCLake to evaluate the effect of mowing on shallow lake ecosystem functioning.

Conclusions

Our integrated modelling analysis of a typical shallow lake in the temperate zone indicates that harvesting submerged macrophytes can be effective in temporarily reducing nuisance in lakes which are oligo- or mesotrophic, particularly when mowing is executed in mid-summer. Designing a successful mowing strategy becomes less easy with increasing nutrient loading. More eutrophic lakes are less resilient to perturbations, and when the external nutrient loading approaches the critical level, relatively small reductions in plant cover are sufficient to trigger an unwanted shift to the alternative phytoplankton dominated state. By extracting nutrients from the lake, negative effects of eutrophication may be partially remediated. Our modelling indicates that the largest amounts of P can be recovered close to the tipping point, although the highest removal of P relative to the input of P is realized when the external P loading is low. Particularly when a lake is still oligotrophic, phytoremediation can be an effective measure to counteract slowly increasing nutrient inputs, while it appears more difficult to use harvesting to impoverish a lake which is already eutrophic, as more eutrophic lakes are also more sensitive to perturbations. These insights provide a basis for more tailored studies on the effects of harvesting in specific lakes systems.

Acknowledgements

We thank Jan H. Janse, Sebastiaan Schep and Martin Droog for their helpful comments and suggestions during the development of this paper.

Supplementary

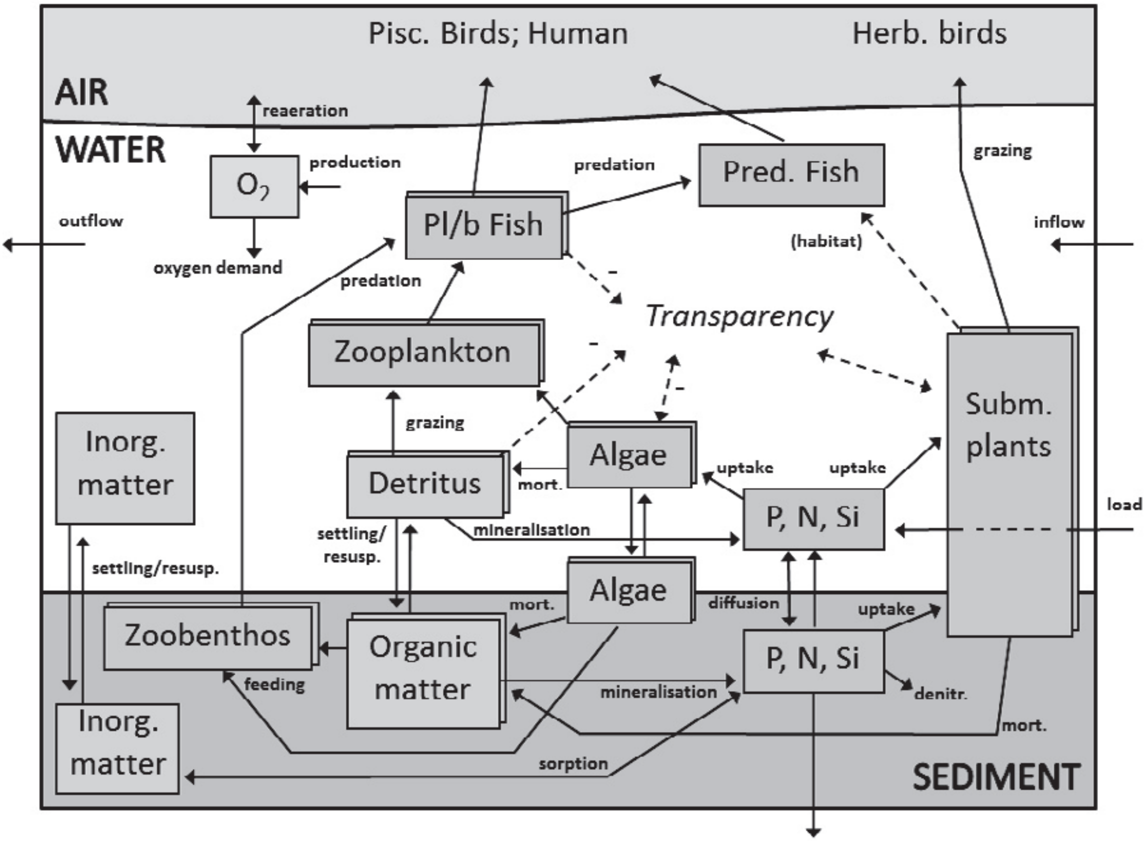


Figure S 1. Schematic view of the structure of PCLake. Blocks denote the state variables of the model. Shaded blocks denote compartments modelled in dry weight, phosphorus and nitrogen (and silica in case of diatoms). Arrows denote mass fluxes. Respiration fluxes are not shown. Dotted arrows denote 'empirical' relations. The biota in PCLake are modeled as functional groups. The submerged macrophytes are assumed to be homogeneously distributed over the complete water column and are rooted in the sediment. Other groups in the water column are phytoplankton (three groups: 'diatoms', 'green algae' and 'cyanobacteria'), zooplankton, planktivorous fish, benthivorous fish and piscivorous fish. The biotic groups in the upper layer of the sediment include the zoobenthos and the settled fractions of the three types of phytoplankton. The abiotic components in the water column and in the sediment are detritus, inorganic material, dissolved phosphorus, ammonium, and nitrate. A full description of the model is presented by Janse (2005). The figure is modified after Janse (1997).

Supplementary S 2: The importance of collateral disturbance

Although the aim of water managers is to remove cut biomass from the water, part of the plant material is often left in the water due to inefficiency of the cutting machinery. This plant material in the water leads to increased light attenuation and stimulates nutrient recycling, disfavoring the growth of the remaining submerged water plants. Another factor that is potentially detrimental to the remaining vegetation is temporarily enhanced resuspension caused by the mowing procedure. This can for example result from thrust engines on mowing boats that stir up the sediment, or because roots are pulled out from the sediment during the cutting.

In the default version of PCLake only 'clean' mowing is considered, whereby all the mown biomass is removed from the system, without additional resuspension. Therefore, we modified the PCLake model equations in such a way that a defined fraction of the clippings remains in the system as detritus. We estimated the fraction to be 20%. Analogous to the detritus resulting from natural mortality, the largest share of this plant material (90%) sinks to the bottom to become part of the detritus pool in the sediment (Janse, 2005). Furthermore, we developed a function that causes the resuspension of the sediment to increase linearly with mowing intensity, maximally reaching an additional 5 g m^{-2} d^{-1} of resuspended material, which is about 2.5 times the amount of sediment that is on average resuspended due to benthivorous fish in a turbid lake (Janse, 2005 p.291). The resuspension is only enhanced during the mowing period. We analyzed the effects of these collateral disturbances on the within-season dynamics of the vegetation cover and chlorophyll-*a* for two different mowing intensities (60 and 90% respectively), an intermediate nutrient loading (1.3 mg P m^{-2} d^{-1}) and a single mowing date (July 1st). We compared the results with the default simulations without collateral disturbance caused by mowing.

For the used parameter settings, this analysis reveal no clear sign of collateral damage (Fig. S 2a-d). According to our model, enhanced resuspension and remaining of plant material in the water column has a negligible effect when 60% of the submerged plants is cut (Fig. S 2a,b), and this is still the case for a mowing intensity of 80% (results not shown). Only when the mowing activity instigates a regime shift, which is the case for a mowing intensity of 90%, the modelled collateral disturbances speed up the regime shift (Fig. S 2c,d). Particularly the enhanced resuspension propels the lake faster to the alternative state. A more elaborated (sensitivity) analysis is needed to elucidate the importance of collateral disturbance. Important to note here is that we did not consider the dispersal aspect of plant fragments generated by the mowing action: vegetative plant fragments can easily spread with the water flow, potentially contributing to new invasions of waterweed in uninhabited waters (Zehnsdorf et al., 2015). This factor should be taken into consideration by water managers dealing with exotic species.

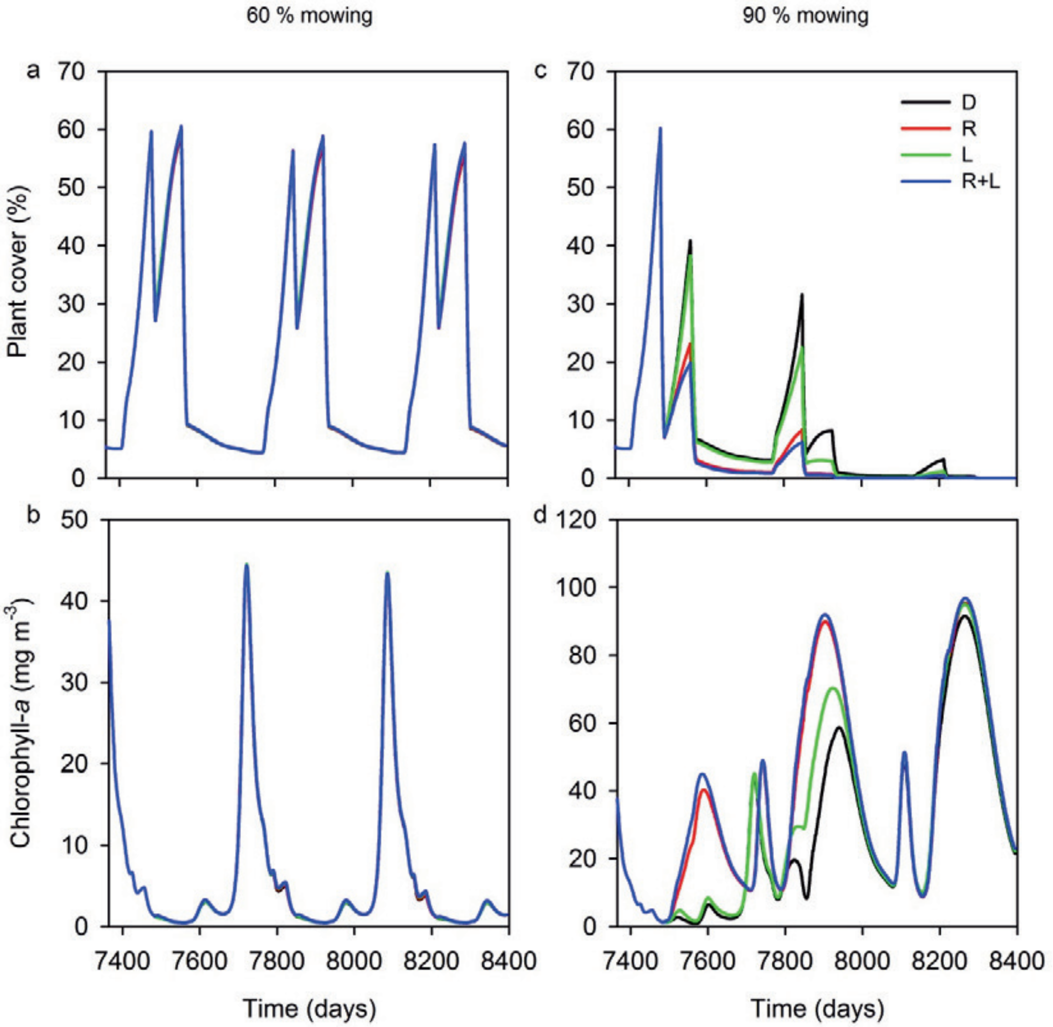
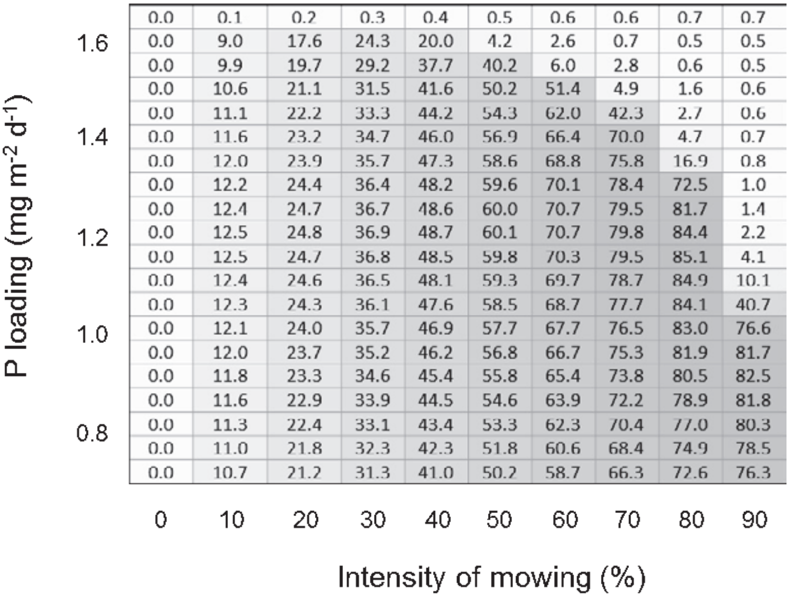


Figure S 2. Effects of collateral disturbance caused by the mowing procedure on July 1st the dynamics of plants and phytoplankton in three succeeding years for a lake receiving 1.3 mg P m^{-2} day^{-1}, for 60 percent mowing and 90 % mowing. D is default (black line), R is enhanced resuspension (red line), L is leaving 20 % of the mowed plant biomass in the water column (green line) and R+D is a combination of the latter two (blue line).



0	10	20	30	40	50	60	70	80	90
0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.6	0.7	0.7
0.0	9.0	17.6	24.3	20.0	4.2	2.6	0.7	0.5	0.5
0.0	9.9	19.7	29.2	37.7	40.2	6.0	2.8	0.6	0.5
0.0	10.6	21.1	31.5	41.6	50.2	51.4	4.9	1.6	0.6
0.0	11.1	22.2	33.3	44.2	54.3	62.0	42.3	2.7	0.6
0.0	11.6	23.2	34.7	46.0	56.9	66.4	70.0	4.7	0.7
0.0	12.0	23.9	35.7	47.3	58.6	68.8	75.8	16.9	0.8
0.0	12.2	24.4	36.4	48.2	59.6	70.1	78.4	72.5	1.0
0.0	12.4	24.7	36.7	48.6	60.0	70.7	79.5	81.7	1.4
0.0	12.5	24.8	36.9	48.7	60.1	70.7	79.8	84.4	2.2
0.0	12.5	24.7	36.8	48.5	59.8	70.3	79.5	85.1	4.1
0.0	12.4	24.6	36.5	48.1	59.3	69.7	78.7	84.9	10.1
0.0	12.3	24.3	36.1	47.6	58.5	68.7	77.7	84.1	40.7
0.0	12.1	24.0	35.7	46.9	57.7	67.7	76.5	83.0	76.6
0.0	12.0	23.7	35.2	46.2	56.8	66.7	75.3	81.9	81.7
0.0	11.8	23.3	34.6	45.4	55.8	65.4	73.8	80.5	82.5
0.0	11.6	22.9	33.9	44.5	54.6	63.9	72.2	78.9	81.8
0.0	11.3	22.4	33.1	43.4	53.3	62.3	70.4	77.0	80.3
0.0	11.0	21.8	32.3	42.3	51.8	60.6	68.4	74.9	78.5
0.0	10.7	21.2	31.3	41.0	50.2	58.7	66.3	72.6	76.3

Figure S 3. Amount of vegetation dry weight biomass that is harvested from the system (g m^{-2} $year^{-1}$). The shade indicates the quantity.

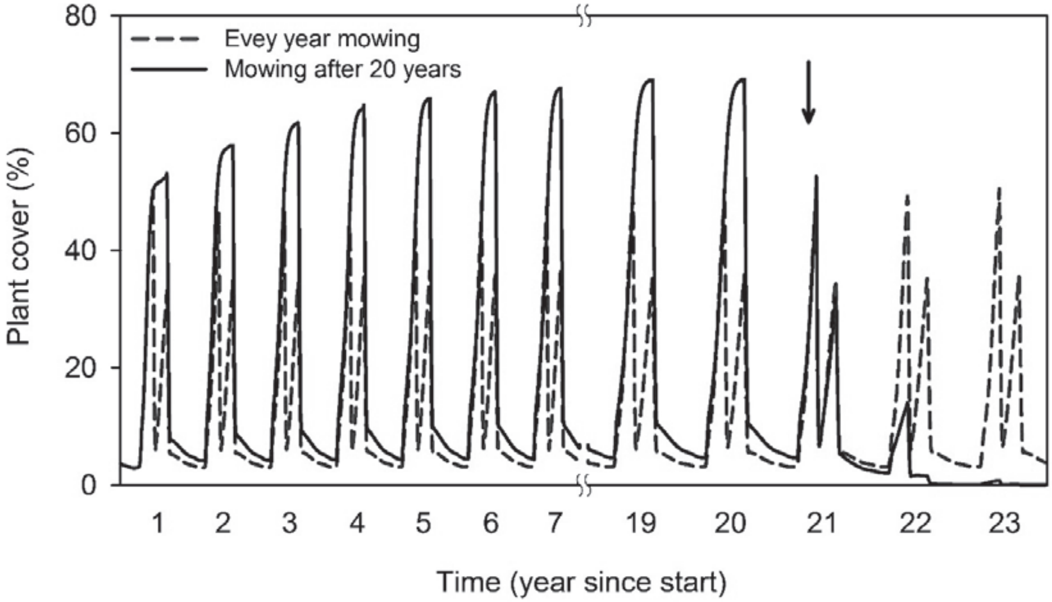


Figure S 4. We modelled a lake receiving 1.2 mg P m^{-2} day^{-1} and applied a mowing intensity of 80%. The only difference between the two scenario's is that in one scenario mowing starts right from the beginning, while in the other scenario mowing starts after twenty years. When mowing is applied directly from the start, the systems moves to an equilibrium situation in which large reductions in plant cover (80%) can be achieved (dashed line). When in the first twenty years no mowing is applied, the system goes to a different equilibrium: after twenty years, when mowing is applied for the first time, the same mowing intensity (80%) instigates a regime shift to the turbid state (solid line). Hence, in the first twenty years nutrients have been able to accumulate in the lake, which lowered the resilience of the lake to perturbations such as mowing.

Chapter 7

Managing successional stage heterogeneity to maximize landscape-wide biodiversity of aquatic vegetation

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E.S. Bakker & S.A.J. Declerck

* Authors contributed equally to this study.

Abstract

The presence of a high diversity of different successional stages in a landscape may help to conserve and promote landscape-wide biodiversity. A strategy to achieve this is using Cyclic Rejuvenation through Management (CRM), an approach employed in a variety of different ecosystems. For this approach to be useful, (a) successional stages are required to be different in community composition and (b) these differences need to be caused by true replacement of species between stages. While potentially valid, these assumptions are not commonly tested prior to application of CMR. In this study, we test these assumptions to explore the usefulness of managing successional stage heterogeneity to maximize landscape-wide aquatic plant diversity.

We carried out vegetation surveys in 21 landscapes in the Netherlands and surveyed 24 individual ditch reaches in each landscape. Using a clustering approach combined with knowledge from literature about vegetation succession in these systems, we assigned our sampled communities to different successional stages. First, using state-of-the-art biodiversity partitioning methods, we quantified the relative importance of species replacement among successional stages in shaping the aquatic plant communities in Dutch polder systems. Next, through scenario analyses based on simulations we studied the effects of reducing successional stage heterogeneity by management on landscape-wide biodiversity. Results showed that differences in aquatic plant community composition among successional stages were an important factor contributing to landscape-wide aquatic plant diversity. Shifts between early successional stages were characterized by higher replacement of species compared to late successional stages. In a scenario of gradual decrease of heterogeneity through the systematic loss of the earliest successional stages we found that 20% of the species richness in a polder was lost, pointing towards the importance of maintaining early successional stages in a polder landscape. This makes a compelling case for application of CRM within agricultural drainage ditch landscapes to maximize the regional aquatic plant diversity, offering potential for biodiversity in these often heavily modified anthropogenic landscapes.

We argue that CRM can maintain and promote biodiversity without compromising the hydrological function of the systems. While applied to drainage ditch systems, this data-driven approach is broadly applicable and may yield first indications of the potential efficiency of applying a CRM scheme to optimize biodiversity.

Introduction

Land use intensification and global change have led to decreasing biotic diversity (Foley et al., 2005). Much of this biodiversity loss is caused by increasing homogenisation of communities (i.e. biotic homogenisation), and not by loss of local diversity (Dornelas et al., 2014). This would imply that the diversity on a landscape scale decreases due to the disappearance of differences between local communities (Smart et al., 2006). This threatens the multifaceted functioning of ecosystems at both local and landscape scale (van der Plas et al., 2016).

Disturbance events create biotic diversity through resetting unidirectional natural succession (Sousa 1984) in natural systems. Examples of such an event include fire (Vandvik et al., 2005), scouring by peak river discharges (Tockner et al., 2000) or landslides (Walker et al., 1996). In absence of these natural dynamics, for example through human management of the ecosystem, there is a risk of loss of successional stage heterogeneity within the landscape (Baptist et al., 2004). Different stages of ecological succession may exhibit different species richness levels. A classic example on forest succession (Odum, 1969, Whittaker, 1970) shows higher species numbers in intermediate stages of succession. Furthermore, different successional stages may harbour very different sets of species. Consequently, the landscape's species diversity is not a function of the local diversity alone, but also of the complementarity between stages present in the landscape. Hence, to maximize the diversity of a landscape, both the local diversity of successional stages, as well as the difference between communities of the different stages needs to be considered. A line of though well represented in the classical partitioning of the landscape diversity (γ) into a local component (α) and a turnover component among local communities (β) (Whittaker, 1960; Jost et al., 2010).

To obtain ecologically diverse sites within a landscape that has lost its natural dynamics due to human influence, management efforts need to be directed towards maintaining a landscape with a variety of successional stages present. This has been widely applied in a variety of ecosystems (Baptist et al., 2004; Vandvik et al., 2005) and is known by different names (e.g. cyclic rejuvenation, rotational management, periodic ecosystem reset). Here we use the term cyclic rejuvenation through management (CRM),which is the practice of periodically resetting part of the habitat in a landscape to a (mostly) bare state to create an ever changing dynamic spatial mosaic of habitat patches in different stages of succession (Hinsch & Poethke, 2007). CRM in floodplain management, aimed at systematic removal of part of the floodplain forests, increased biodiversity in channels where natural reset of succession was absent (Baptist et al., 2004). Likewise, controlled fire management can help preserve grassland diversity through CRM (Richards et al., 1999). While good results have been obtained, the inherent success of this management approach rests strongly on the assumption that successional stages are complementary to one another. The more unique stages are with respect to their community composition, the larger the gain for the regional species pool and thus overall landscape diversity. Conversely, when successional stages are highly similar, CRM will have little effect on landscape-wide species diversity. Management is often a given in many anthropogenic ecosystems, aimed at maintaining the ecosystem services we require (e.g. water storage or food production; Power, 2010). CRM offers an approach for this management to take place, while also realising an increase in biodiversity.

Manmade water systems such as agricultural drainage ditches are under continuous management to maintain the hydrological drainage of agricultural land (Hill et al., 2016). However, their value for conservation of biodiversity is increasingly recognized as well (Armitage et al., 2003; Herzon & Helenius, 2008; Clarke, 2015). Reshaping ditch banks, removing vegetation and dredging organic sediment helps to maintain hydrological functioning of the ditches, but may also support ecological function and diversity (Twisk et al., 2003). Dredging removes nutrient rich sediments (Whatley et al., 2014) that would otherwise result in a eutrophic ecosystem with high duckweed coverage and low biodiversity (Liere et al., 2007; Gerven et al., 2015). Management in these systems is constantly resetting the succession of these waterways (Van Strien et al., 1991; Clarke, 2015; Hill et al., 2016), making an often unorganized form of CRM the norm in these systems.

For ditch ecosystems, the application of CRM has been suggested to positively influence diversity at a landscape scale (Watson & Ormerod, 2004; Clarke, 2015; Hill et al., 2016). Landscape level species diversity (γ) in these ecosystems is largely caused by differences in community composition between individual sites (Goldenberg Vilar et al., 2014; Whatley et al., 2014), indicated by a large β-diversity component. This high contribution of β-diversity to the γ-diversity points in the same direction, stressing the importance of landscape heterogeneity. Despite these strong claims on the usefulness of CRM (Clark, 2015), no formal evaluation has been carried out, nor has it been widely adopted in ditch management practices. A first step in the evaluation and adoption of such a management practice is to illustrate the importance of successional stage heterogeneity for landscape-wide diversity. Differences in community composition may be caused by two inherently different underlying scenarios, namely one of species richness difference and one of species replacement (Baselga, 2010; Legendre, 2014). Richness difference refers to a pattern in which a species rich stage exists in the landscape that holds all species, and all other stages in the landscape are a subset of the community of this richer stage. The only difference between the two stages is that one stage is richer than the other stage. In contrast, a pattern of species replacement refers to a stage having a unique set of species that does not occur in another stage. The stages differ in their species composition, showing a replacement of species from one stage to the other. For CRM to truly be useful for increasing landscape biodiversity, species replacement between different successional stages is required (i.e. complementarity) and not difference in richness alone.

In this study, we created a dataset of 504 vegetation surveys in ditch reaches spread over 21 different regions (or landscapes) in the Netherlands to study the importance of maintaining successional stage diversity on the regional species diversity (γ) of aquatic vegetation. We characterised the dissimilarity among successional stages and its underlying patterns to assess the complementarity of communities of different successional stages for landscape biodiversity. We continued by examining the potential effect of landscape-wide heterogeneity in the presence of different successional stages on the landscape-wide diversity. This was done by calculating the biodiversity of simulated landscapes with different combinations of successional stages. Based on these results we evaluated the hypothesized merit of CRM on promoting regional diversity in ditch systems (Clarke, 2015) through proliferation of a diverse mosaic of habitats.

Methods

Study region and site selection

Our study took place in the peat meadow polder landscapes in the west of the Netherlands. These landscapes were historically created by drainage of peatlands to reclaim land for agriculture. This has led to the distinctive polder landscapes in which long, narrow fields are intersected by a network of drainage ditches, with the whole system being surrounded by dykes. Water level fluctuations within these landscapes are strictly controlled. The ditches in these systems are generally eutrophic to hypertrophic due to the long-time agricultural use of the adjacent fields. The fields are used primarily for cattle farming, though there is still variation in land use intensity due to governmental subsidies (agri-environmental schemes) that compensate farmers for less intensive land use and aim to stimulate nature values (Catry et al., 2017). Additionally, some fields are owned by nature management organisations and are managed extensively with the aim of creating and sustaining moist natural grassland vegetation and its associated biota. In all cases the ditches are regularly managed to sustain their hydrological function. This is done through removal of plant biomass (e.g. mowing) and dredging of organic sediments from the ditch bottom. Within our study we selected 21 different polder landscapes of roughly 200 hectares along a gradient of land management intensity. Within each of these 21 landscapes 24 ditch reaches of 100 meters long were selected according to a stratified random design (Figure S 1.1a). Vegetation surveys were carried out along the 100 meter transects using a Tansley scale (Tansley 1946), encompassing all species growing in the water. These Tansley dominance classes of each plant species found were converted to cover percentages using Table S 1.1. Furthermore, the thickness of the organic sediment layer was measured in the centre of the ditch at five locations (every 20 m) within each ditch reach (Figure S 1.1b).

Progression of natural succession

The succession of vegetation within these ditches is strongly influenced by dredging (Caspers & Heckman, 1981) and eutrophication (Portielje & Roijackers, 1995). Vegetation and channel characteristics move from an open and deep system towards a shallow and choked system filled with emergent and amphibious vegetation (Watson, 2004) and eventually a carr, if left unmanaged (Barendregt et al., 1992; Lamers et al., 2002). The latest stages are unlikely to be present in an agricultural setting, as they hamper the hydrological function of the system. Relationships between succession and nutrient loading (Portielje & Roijackers, 1995) and, more specifically, between successional stages and nutrient loading (Van Zuidam & Peeters 2013) have been found and generally follow the following patterns (Figure 1). First, succession of aquatic vegetation generally starts

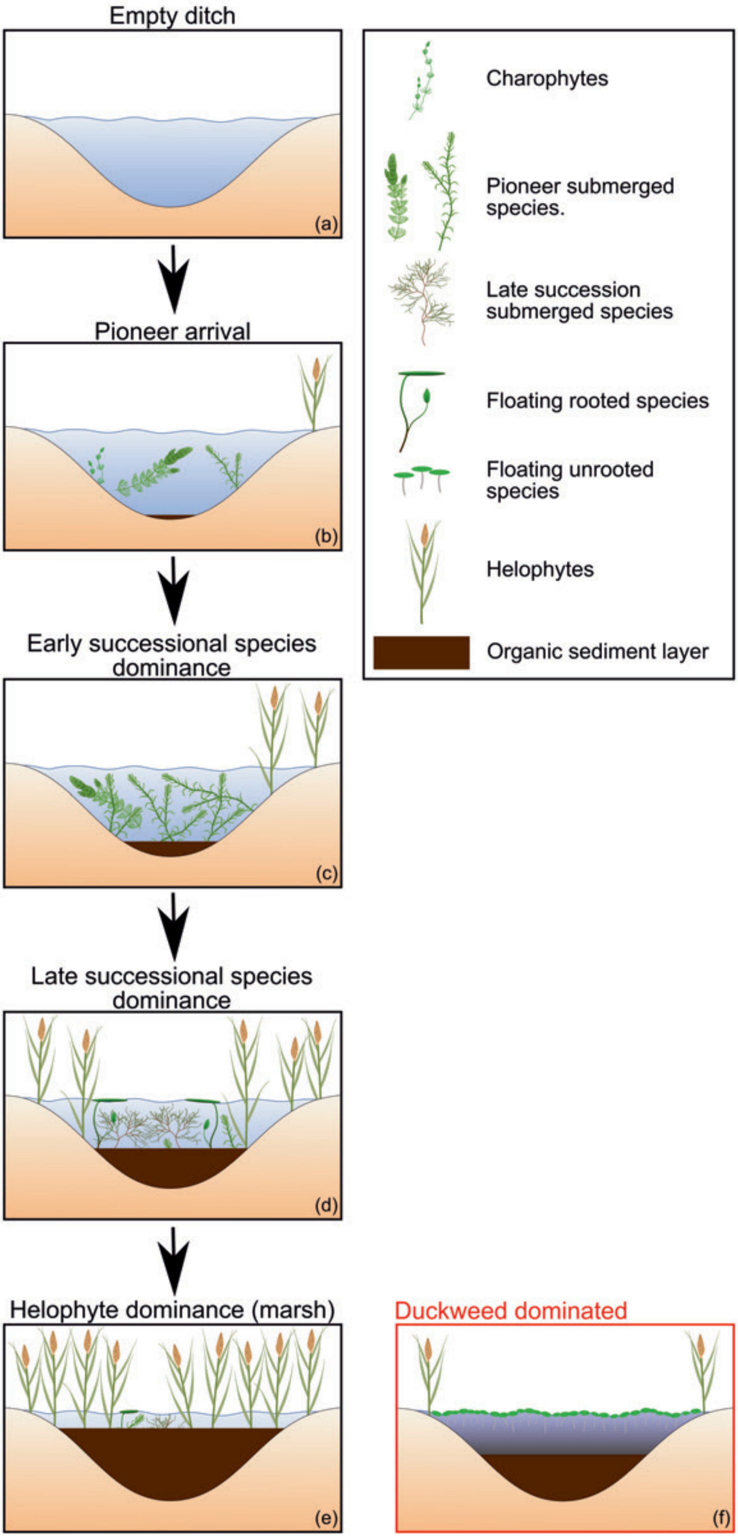


Figure 1. Schematic representation of the theoretical progression of succession in a drainage ditch system based on progression of functional vegetation classes and build-up of organic sediment through time (a-e). (f) Duckweed dominated ditch impairs succession.

off with low abundance of early successional submerged species, such as charophytes and vascular plants such as *Ceratophyllum demersum* and *Elodea nuttallii*. At this stage, the sediment only holds a thin organic sediment layer. The fastest growing species eventually become dominant and organic sediment builds up in the ditch. As succession progresses, organic sediment accumulates further and additional submerged species such as *Potamogeton* sp. and rooted floating species such as *Nuphar lutea* are found. Eventually, as in other shallow aquatic systems (Carpenter, 1981), the organic sediment layer will build up to the point that helophytes are able to occupy the centre of the ditch. If left unmanaged, a swamp will develop. Hypertrophic ditches can have high duckweed coverage, creating anoxic conditions and greatly reducing light irradiance to the ditch bottom, thereby hampering the development of submerged and rooted floating-leaved plants (Gerven et al., 2015). This threatens the existence of diverse aquatic life as a whole due to oxygen depletion (Scheffer et al., 2003). This alternative stable state with high duckweed coverage (Scheffer et al., 2003) will break the natural progression of vegetation succession.

Defining successional stages

Based on the natural progression of succession, we defined functional types of vegetation known to be indicative for different successional stages (Barendregt, Stam & Wassen 1992; Lamers, Smolders & Roelofs 2002; Watson & Ormerod 2004): charophytes, submerged pioneer vegetation, submerged late successional vegetation, rooted floating vegetation, unrooted floating vegetation (e.g. duckweeds) and helophyte vegetation (Figure 1). Through cluster analysis we grouped sites together according to successional stage properties (i.e. vegetation functional types and increasing sediment thickness). To select the most suitable cluster technique and the optimal number of clusters we compared k-means-, fuzzy-, self-organizing tree algorithm-, and model based clustering approaches with the clValid package in R (Brock et al., 2011) using silhouette width and connectivity as the selection criteria. This yielded a fuzzy clustering approach with 9 clusters as the best fitting solution with a connectivity of 192.8 and silhouette width of 0.241. We calculated the average coverage of the defined functional vegetation types and the sediment thickness per cluster and organized them along a gradient of succession as theoretically defined previously (Figure 1).

Successional stages and diversity

The regional, or landscape-wide, biodiversity (γ) can be mathematically partitioned into different components (Jost, 2007; Jost et al., 2010), i.e. the local average α-diversity of ditch reaches and the β-diversity component describing community dissimilarity among different ditch reaches. If different successional stages have a different plant community, variation in successional stage will affect the γ-diversity through the β-component. Part of the β-diversity of a community may be attributed to within successional stage (β_{within}), while another part may be contributed to plant community differences between successional stages ($\beta_{between}$). To determine β_{within} we first calculated the β-diversity for all sites within the same successional stage in a polder landscape. The mean of these values gives β_{within} at the polder level. To determine $\beta_{between}$ we first took the mean cover of each species of all sites within the same successional stage in a polder. We then calculated the $\beta_{between}$ from the resulting averaged species cover matrix using the standard method of Jost (2007).

Because not all successional stages had equal number of sites within a landscape, we used a random resampling procedure to correct for this bias as follows. Prior to calculating β_{within} and $\beta_{between}$, an equal number of sites for all successional stages were randomly sampled from the total pool of sites within a landscape. This procedure was replicated 1000 times, each time selecting a set of sites from the data in equal number for each successional stage. The outcome of all resampling runs were averaged and used for further analyses.

The $\beta_{between}$ component can be further partitioned into two additive components, a component of true species replacement (repl) and a richness difference component (rich), using a method first proposed by Baselga (2010) and Podani & Schmera (2011) and its extensions (Legendre, 2014; Ensing & Pither, 2015). We partitioned the $\beta_{between}$ component based on a Jaccard index and calculated a multi-site β-diversity index and its partitions for presence/absence data (Ensing & Pither, 2015). As no such indices have been developed for our abundance data, we instead used the total variance of a dissimilarity matrix of the community data based on a Ruzicka index (Jaccard based index for abundance data) as per Legendre (2014).

Differences in community composition among different successional stages was tested using a distance-based Redundancy Analyses using Jaccard and Ruzicka indices (dbRDA, Legendre & Anderson, 1999). The same procedure was applied to test for differences in species replacement (repl) and richness differences (rich) in the community among different successional stages. Significant differences among successional stages were assessed using random 1500 permutations. To deal with the unbalanced nature of the data, we used the random resampling procedure mentioned before.

Scenario analyses to evaluate the effect of removal of successional stage heterogeneity on γ-diversity

To test the effect of removing successional stages from the landscape on γ-diversity we defined two scenarios with respect to different management strategies. Scenario 1 assumes that the system is actively managed by removing late successional stages from the landscape. In this scenario, successional stage heterogeneity in the landscape decreases to the point where a homogeneous landscape with only the first stage of succession remains. This scenario will be referred to as the intensive management scenario. We progressively remove successional stages, starting from stage 7 (i.e. the highest in our dataset) and working back (e.g. removing stage 6 and 7, stage 5 to 7 etc.) up to the point that a landscape consists solely of communities of stage 1. Scenario 2 assumes that the system is not managed and will progress towards the last stage of succession. This scenario will be referred to as the extensive management scenario. In this scenario, we progressively remove successional stages, starting from stage 1 and working forward (e.g. removing stage 1 and 2, stage 1 to 3, etc.) up to the point that a landscape consists solely of communities of stage 7. The point of reference for both scenarios is the most heterogeneous situation in which all 7 stages of succession are present.

For the scenario analyses we generated 21 artificial landscapes by assigning 12 ditch reaches from our entire dataset to the landscape. We randomly generated frequencies of successional stage occurrence in the landscape from all possible combinations of successional stages present in the scenario definition at hand (e.g. Scenario 1: stages present: 1 to 3). This approach was taken to remove any bias by choosing a given frequency beforehand, though this conservative approach did increase variation of the landscapes diversity parameters (α, β, γ). The entire analysis was repeated 42000 times to obtain 2000 sets of 21 landscapes, the same number of landscapes as present in the original data. Changes in the diversity parameters were analyzed using linear mixed effect models (lme) with the landscape identity as a random variable for both scenarios because frequencies of successional stages were fixed within a generated landscape. This was done for each of the 2000 sets of landscapes and the model parameters and significance test values were averaged.
All analyses were performed in R version 3.3.2 using *ggplot2* and *vegan* (Oksanen et al., 2015) packages and custom code supplied with (Legendre, 2014).

Results

Of the nine defined successional clusters resulting from our fuzzy clustering approach, we were able to organize seven clusters along a gradient of ditch succession based on the expected development (Figure 1) of functional vegetation types and increasing thickness of the organic sediment layer (Figure 2, Figure S 1.2). Organic sediment was initially relatively thin and increases towards the end of the successional gradient in our clusters (Figure 2). Furthermore, we found a progression from low abundant, early successional submerged plant species towards late successional submerged and rooted floating-leaved species. Eventually, most submerged species declined in the final successional stage 7. Stage 1 was characterized primarily by low abundance of submerged vegetation and a relatively thin organic sediment layer, compared to the other stages. Stage 2 and 3 exhibited a thicker sediment layer while showing increased abundance of early pioneer species. These two stages are different in their cover of floating unrooted vegetation while otherwise being largely similar (Figure S 1.2). Stage 4 showed a further increase of submerged pioneers and the occasional low abundant late successional species that increased further in abundance in stage 5. In stage 6, late successional submerged species became dominant while early pioneers decreased in abundance. In stage 7, a thick organic sediment layer was present and an overall decline in true aquatic vegetation. One successional cluster (9) was not included due to high duckweed cover leading to a

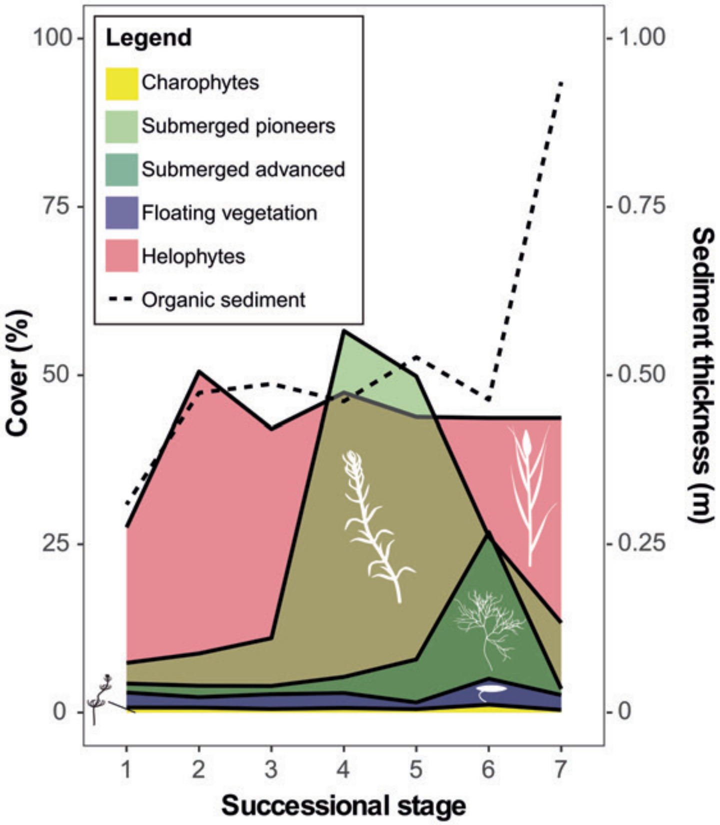


Figure 2. Progression of plant cover (%) and organic sediment thickness (m) through the different identified successional stages (1-7). Results shown per functional vegetation type and sediment data are averaged values over all ditch reaches in the given successional stage cluster. For specific cover per species see Figure S 1.2.

stagnation of the vegetation succession. Successional cluster 8 was characterized by relatively a thick organic sediment layer and overall low vegetation cover. This unusual combination of thick organic sediment with a low production of vegetation places it outside of the natural succession gradient (Figure 1), and hence this cluster was also removed. A cause for this unexpected system state (cluster 8) could be a high removal of vegetation (artificial or natural) with a lack of dredging activities, resulting in the thick organic sediment layer found.

The partition of γ-diversity into its components of alpha and beta (Figure 3a) revealed that only a small part of the γ-diversity could be attributed to the mean α-diversity of ditch reaches (SR=11.9, H'=4.8). The remaining γ-diversity was attributed to the β-diversity component (>60%), illustrating a large difference in community composition among ditch reaches within a landscape. When examining the relative importance of successional stage diversity within this β-diversity component, we found that a large part of the β-diversity could be attributed to differences among successional stages ($\beta_{between}$: SR=80.4%, H'=77.3%, Figure 3b). This β-diversity, present among successional stages ($\beta_{between}$), could be attributed to replacement of species (51.7% (SR) and 40.4% (H'); Figure 3c).

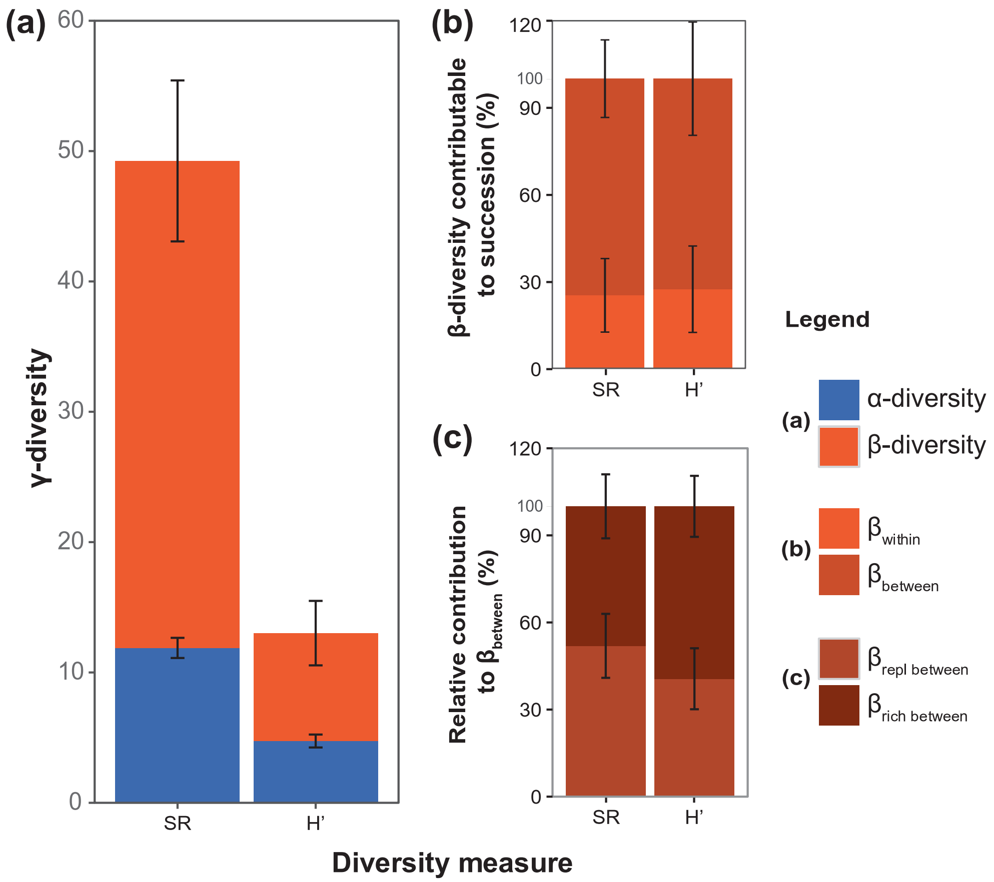


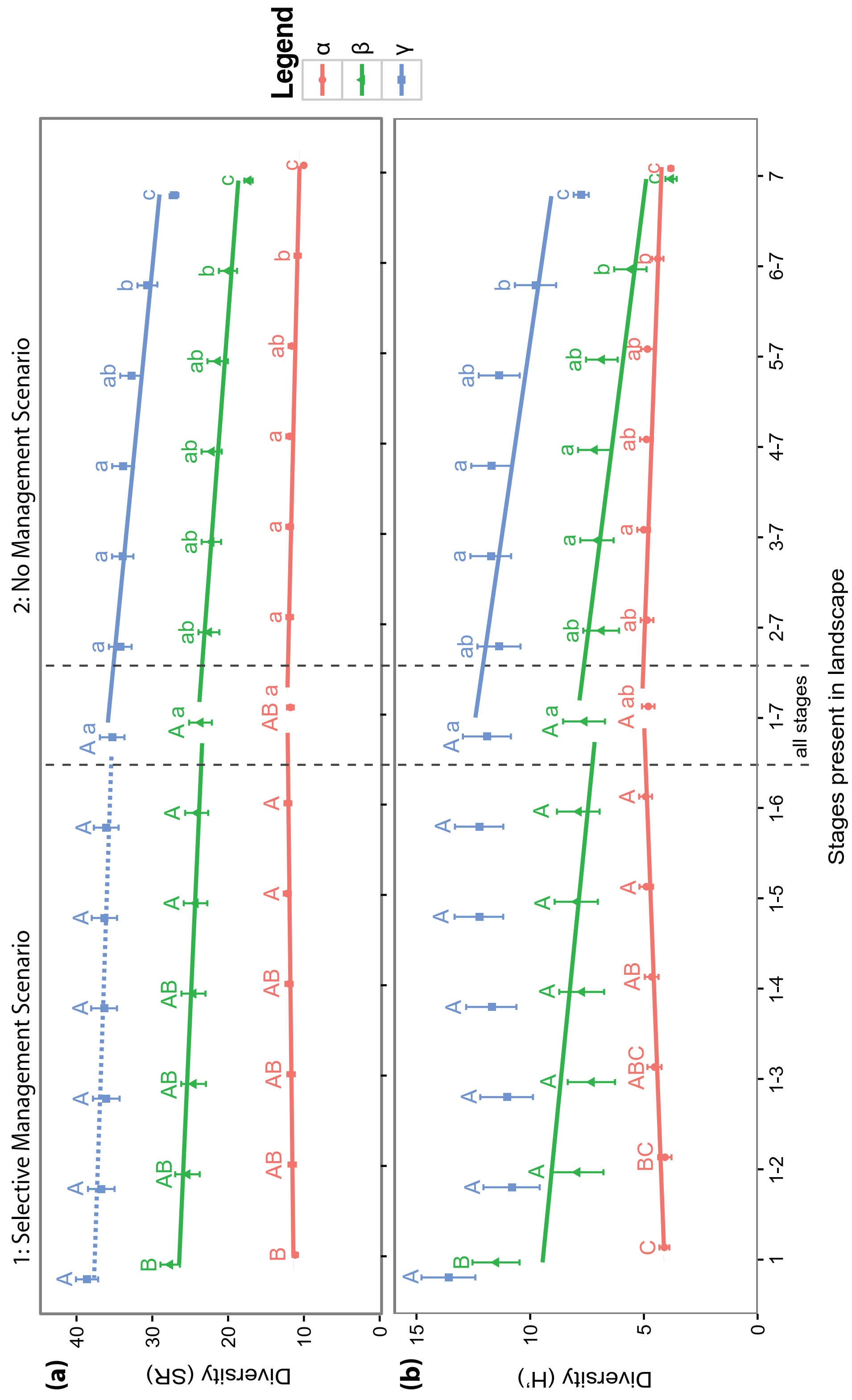
Figure 3. (**a**) Total landscape diversity (γ) of field data on ditch vegetation split into components of: 1) mean local diversity of ditch reaches (α-diversity), 2) difference in community composition between reaches within regions (β-diversity). (**b**) The β-diversity is further divided into a component attributable to within successional stage differences between communities (βwithin) and between successional stage differences (βbetween). (**c**) Relative contribution of species replacement (β repl) and richness difference patterns (β rich) of the βbetween component as resulting from a Jaccard-based partitioning method (Legendre, 2014). Both species richness (SR) and the exponent of the Shannon-Wiener index (H') diversity measures are shown for both graphs. Error bars show 2x standard errors around the mean.

Successional stages differed significantly in their aquatic plant community composition, as shown by an RDA analysis of explaining community dissimilarity by successional stages (Jaccard: R^2_{adj}=5.2%***, Ruzicka: R^2_{adj}=25.6%***). Species replacement was significantly different among successional stages (Jaccard: R^2_{adj}=4.4%***, Ruzicka: R^2_{adj}=8.6%***). Likewise, species richness difference was also significantly different among successional stages (Jaccard: R^2_{adj}=6.2%***, Ruzicka: R^2_{adj}=62.3%***). When comparing successional stages pairwise (Figure S 1.3, Figure S 1.4), we found varying underlying patterns of these two components of β-diversity. For the species presence/absence data (Jaccard) we found that β-diversity consisted of higher replacement in the earlier successional stage combinations compared to later successional stage combinations. For the abundance based analyses (Ruzicka), these patterns were less clear, though very high replacement as well as high richness differences occurred between some pairs of successional stages.

Through scenario analyses, we assessed potential changes in the landscape γ-diversity through decreasing landscape-wide successional stage heterogeneity. A landscape with all seven successional stages had a mean γ-diversity of 35 species. In scenario 1, where late successional stages were progressively removed from the landscape, we did not find a significant change in the γ-diversity (LME: intercept=35.21, slope=0.40, p=0.0502, marginal R^2_{adj} =5.2%, conditional R^2_{adj} =6.8%). Paradoxically, we could even observe an increase in species richness of the landscape when only the first successional stage remains (Figure 4a, table S 1.2). Removal of early successional stages (scenario 2) did show a clear negative effect on the γ-diversity (LME: intercept=36.36, slope=-1.17, $p<0.001$, marginal R^2_{adj} =35.9%, Conditional R^2_{adj}=36.5%). This was caused by the stark decline in β-diversity (Figure 4b, Table S 1.2), leading to a total loss of 7 species; 20% of the species pool of the landscape.

To further elucidate what caused the significant decrease found in scenario 2, we partitioned the between successional stage β-diversity ($\beta_{between}$) into its replacement and richness difference components. Figure S.1.5 (Table S 1.3) showed that the decline in β-diversity of the sites in the landscape ($\beta_{between}$) was driven by a decline in the loss of complementary stages from the landscape, a decline in replacement (β_{repl}; LME: intercept=0.39, slope=-0.045, $p<0.001$, marginal R2=32.3%, Conditional R2=33.7%). β_{rich} was not significantly affected by the loss of successional stage heterogeneity (p=0.401). In contrast to species richness, similar analysis on species abundance which gives greater weight to highly abundant or dominant species, showed no significant effect on the total $\beta_{between}$ (p=0.124). Results between species richness and Shannon diversity were similar (Table S 1.3).

Figure 4 (on righthand page). Simulation results of scenario analyses showing the effects of decreasing landscape-wide successional stage heterogeneity on the landscape aquatic plant diversity (γ) and its partitions (α, β) for both species richness (**a**) and Shannon diversity (**b**). Numbers on the x-axis represent the successional stages included, e.g. 1-4 indicates stages 1 to 4 were included. On the left hand side, a scenario of intensified management is shown, where late successional stages are progressively removed from the landscape (Scenario 1). On the right hand side, a scenario of extensified management is shown, where early successional stages are progressively removed from the landscape (Scenario 2). Error bars show 2x standard errors around the landscape-wide mean diversity values, based on 21 simulated landscapes with 12 ditch reaches per landscape. Different letters indicate statistically significant differences between successional group means. Solid lines show significant trends ($p<0.05$) along the gradient of management, based on a linear mixed effect model where diversity effects are nested within polder identity. Dashed lines show marginal trends ($p<0.10$).


(a)
Diversity (SR)
(b)
Diversity (H')
1: Selective Management Scenario
2: No Management Scenario
Legend
α
β
γ
Stages present in landscape
all stages
1
1-2
1-3
1-4
1-5
1-6
1-7
2-7
3-7
4-7
5-7
6-7
7
0
10
20
30
40
0
5
10
15
A
B
C
AB
BC
ABC
a
b
c
ab

Discussion

Ecological succession is a prime force creating heterogeneity at a landscape level (Amoros, 2001), which may be managed for optimal landscape-wide species diversity. Increasing landscape-wide heterogeneity of successional stages may be accomplished by temporally resetting successional stages through management activities, such as CRM (Sousa, 1984; Vandvik et al., 2005). As CRM is a costly exercise, it is worthwhile to first explore the potential of this method to maximize landscape diversity. We showed that, rather than a trial an error approach, a comparative study of the species composition present in the different successional stages within a system under consideration for this management type can already give estimates of its potential effectiveness. We were able to cluster the vegetation of different sites into successional stages matching prevailing theory on vegetation succession based on sediment organic matter and plant functional groups. Because we found differences in species composition among the different stages, CRM may be an effective tool to increase aquatic plant biodiversity in ditch ecosystems. When applied with an optimal spatial scheme, CRM can lead to a maximization of the landscape-wide diversity of aquatic plants in this heavily modified anthropogenic landscape.

Complementarity of successional stages

A comparative study of the species composition present in the different successional stages within a system under consideration for CRM is vital, because it provides information on the relative contribution of the species present in different successional stages to the landscape wide species pool. We found that β-diversity accounted for most of the landscape ɣ-diversity (for both SR and H'), that β-diversity was primarily composed of differences among successional stages, and that β-diversity was at least in part caused by species replacement (Figure 3). This is a first indication that heterogeneity in successional stages could be of value in promoting and conserving aquatic plant biodiversity at a landscape scale.

Assessing the effect of heterogeneity of successional stages on landscape diversity

Picket and colleagues (Pickett et al., 2008) stated that no vegetation community (or any community, for that matter) is static and acknowledged that this property is vital for management success. Management may intercede in community dynamics in four basic ways (Rosenberg & Freedman, 1984) through (1) designed disturbance, (2) controlled competition, (3) controlled colonization and (4) controlled extinction (e.g. exotic species management; Hussner et al., 2017). CRM is an artificial method of designed disturbance aimed to replace the lack of natural disturbances. Additionally, species competition and extinction, or more broadly 'species interactions' (Pickett et al., 2008), are influenced as well through removal of vegetation by for example CRM. Finally, colonization is facilitated by maintaining a diverse range of viable source populations for seeding newly reset habitat.

Heterogeneity of successional stages at a landscape scale has been shown to contribute to the landscape's diversity in different ecosystems (this study; Richards et al., 1999; Ward et al., 2002). However, management aimed at maintaining a particular successional stage is difficult, if not impossible, as natural succession will progress the community towards later stages of succession over time. Hence, the most viable, long term, management method lies in periodically resetting succession completely. By doing this in phases across the landscape, an optimal rotation may be found in which all stages are present, i.e. the optimal CRM strategy. In practice, management may, however, be more intensive or less intensive than this optimal CRM, leading to loss of heterogeneity of successional stages. This landscape homogenisation may support lower levels of biodiversity (Ward et al., 2002) and may lead to biotic homogenisation between localities within the landscape. By carrying out a scenario analysis using different combinations of existing communities, we gained understanding of the implications and potential loss of diversity from ditch systems through homogenisation of the landscape.

Our scenario analysis showed that more intensive management (Scenario 1), that moves all sites towards earlier successional stages, had relatively little effect on the regional diversity. This was contradictory to our expectation, but likely caused by a high β-diversity within successional stage 1. Pioneer stages are often characterized by a lack of species dominance, allowing for colonisation by a diverse set of species from the surrounding landscape that are likely to be outcompeted as succession progresses (e.g. Hassall et al., 2012). Species composition in early stages of succession are known to be strongly affected by the distance to (Brederveld et al., 2011), and diversity of, source populations (Pickett et al., 2008) and the presence of a diverse and viable seed bank (Sarneel et al., 2014; Van Leeuwen et al., 2014). When all connected reaches in a landscape are reset simultaneously, there is no nearby source population remaining to recolonize the site and landscape diversity would likely plummet (e.g. Prach et al., 2014). This is especially true in hard resets where most of the existing seed bank is also removed together with the sediment. Through CRM, the colonization potential of reset sites is facilitated by creation of a diverse set of source populations. This also suggests that our scenario results may well be an underestimation of the landscape-wide diversity loss due to intensive management (Scenario 1), as our scenario analysis does not account explicitly for the loss of source populations from the landscape.

The other scenario (Scenario 2) is one of extensive management effort, where natural succession moves all sites towards later successional stages. This extensive management lead to clear declines in the landscape-wide diversity (γ) in our scenario analysis, caused by a drop in the β-diversity. Further analysis showed that this reduction in landscape wide aquatic plant diversity was mainly caused by the loss of rare species from the ecosystem. Conservation effort using a spatiotemporal heterogeneous management method, such as CRM, will likely avoid this detrimental effect of homogenisation of the landscape.

Implications for ditch management aiming to increase plant biodiversity

Complete succession from empty ditch to a carr has been suggested to take between 10 and 27 years in our ditch systems (Bakker et al., 1994), and maximum aquatic plant diversity may take 3-4 years to develop after management (Milsom et al., 2004). Combining these timeframes indicates that CRM should revisit each site every 10-odd years and that source populations for plant propagules should be available for at least 3-4 years to realize the highest plant diversity on a landscape scale. However, we suggest that although these timeframes can be true for some ecosystems, factors such as productivity of the ecosystem can severely impact the speed of succession (Carpenter, 1981; Portielje & Roijackers, 1995). Hence, in high productive systems CRM should likely revisit a site sooner than in a less productive system. If the site becomes hypertrophic and the entire water surface is covered with floating plants such as duckweeds, growth of submerged plants is hampered and submerged and emergent vegetation succession may be impaired. In this case, management should restore this site directly by tackling the duckweed problem to realize an increase in plant diversity. We therefore propose that the timing to apply CRM should be assessed by surveying the vegetation and sediment organic matter instead of using a fixed timeframe.

So far, we have focussed on how management may increase landscape wide aquatic plant diversity, however, ditches have an important hydrologic transport function. This function needs to be guaranteed at all times, hence it may often not be possible to let vegetation succession reach the highest successional stages. Fortunately, hydrological functioning and aquatic plant biodiversity do not appear to be mutually exclusive in the sampled ditch systems. As our scenario analysis indicates, landscape wide aquatic plant biodiversity was not severely reduced when the latest successional stages were excluded. CRM could thus already reset the succession well before the latest successional stage is reached and the ditch is filled up with organic sediment and helophytes, which would impair the water transport function.

Conclusions

Agricultural drainage ditches are a non-natural aquatic landscape that, nonetheless, can contribute to the biodiversity of the landscape (Armitage et al., 2003; Verdonschot et al., 2011; Clarke, 2015). Through a data-driven approach we demonstrate the usefulness of successional stage heterogeneity of the landscape and discussed how CRM could maintain this heterogeneity. Our approach allowed a stringent check of the inherent requirements (difference and replacement of species between successional stages) for useful application of CRM, before extensive management activities are carried out that may be hit-or-miss. Our scenario analysis showed that decreasing successional heterogeneity present in the landscape may reduce regional plant species richness by 20% compared to a reference state in which all successional stages are present in the landscape. As management of ditch systems is needed to preserve their hydrological functioning, the adoption of a spatially explicit management scheme that employs the principles of CRM is both viable and cost-effective. Furthermore, spatiotemporal heterogeneous management, such as CRM, will help to ensure that viable source populations of the different aquatic plants are present locally, from which species can recolonize the managed sites. This combination of drainage and ecological function seems achievable and fits well within the context of reconciliation ecology (Rosenzweig, 2003), allowing for coexistence of relatively high levels of biodiversity in a seemingly unhospitable landscape of anthropogenic activity.

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Supplementary

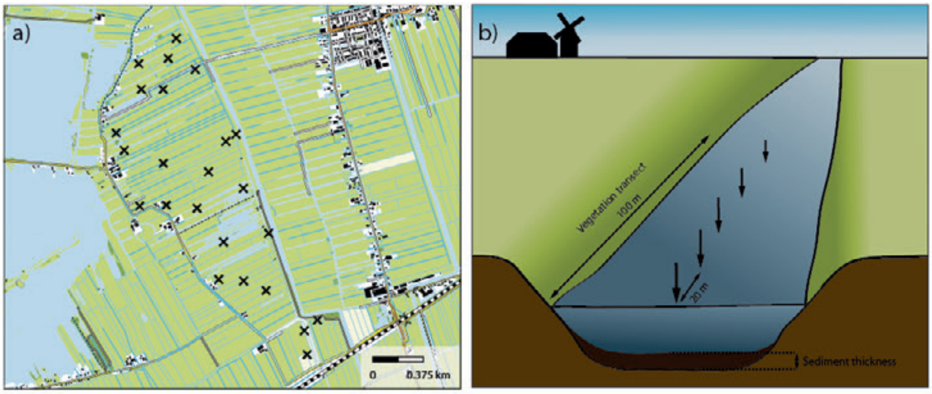
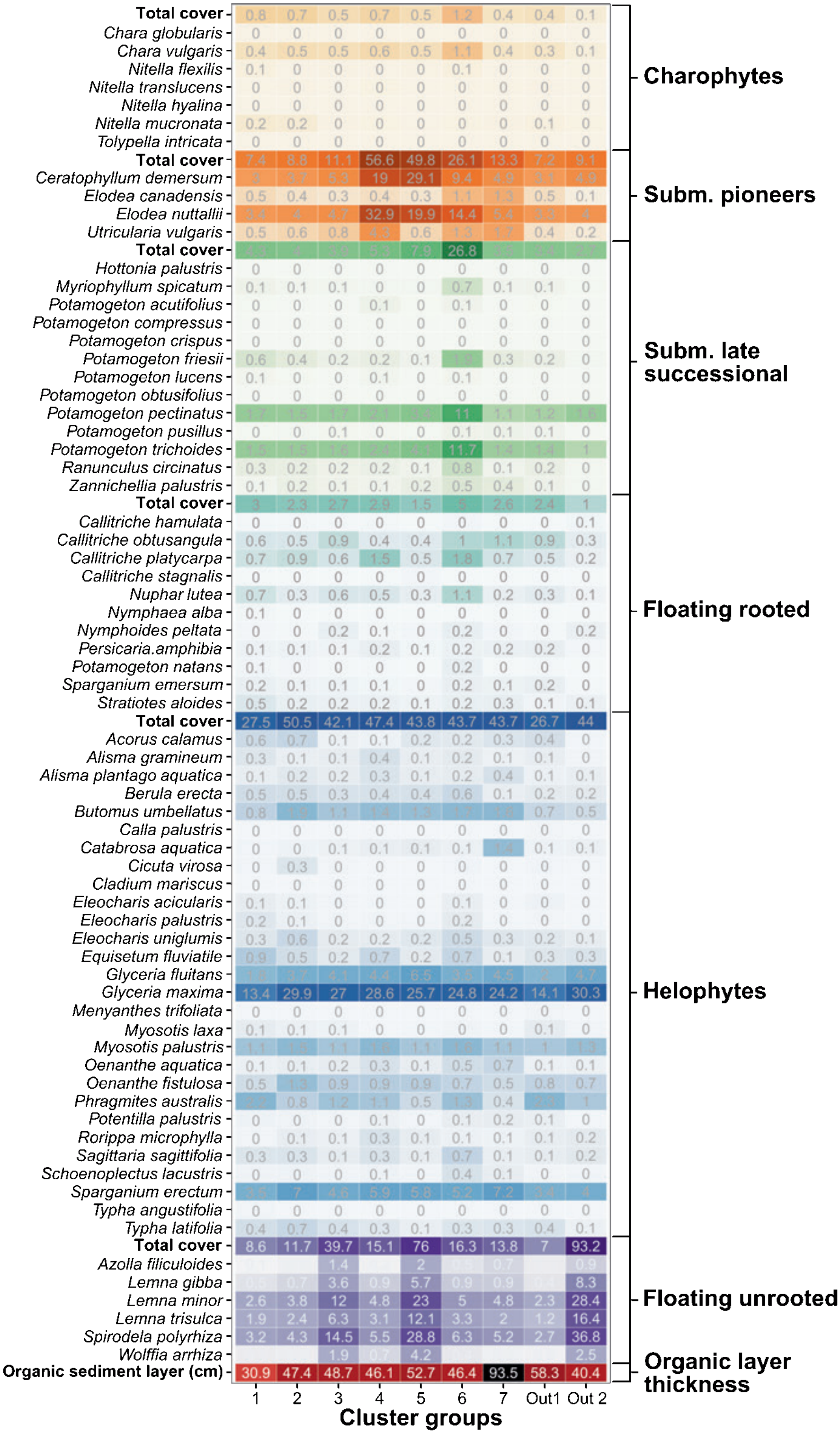


Figure S 1.1. (**a**) Example of one of the 21 polder landscapes with crosses indicating the sampled ditch reaches. (**b**) Illustration of the ditch reach with the vegetation transect (100m) and the five points at which organic sediment thickness was measured.



	1	2	3	4	5	6	7	Out1	Out 2	
Total cover	0.8	0.7	0.5	0.7	0.5	1.2	0.4	0.4	0.1	**Charophytes**
Chara globularis	0	0	0	0	0	0	0	0	0	
Chara vulgaris	0.4	0.5	0.5	0.6	0.5	1.1	0.4	0.3	0.1	
Nitella flexilis	0.1	0	0	0	0	0.1	0	0	0	
Nitella translucens	0	0	0	0	0	0	0	0	0	
Nitella hyalina	0	0	0	0	0	0	0	0	0	
Nitella mucronata	0.2	0.2	0	0	0	0	0	0.1	0	
Tolypella intricata	0	0	0	0	0	0	0	0	0	
Total cover	7.4	8.8	11.1	56.6	49.8	26.1	13.3	7.2	9.1	**Subm. pioneers**
Ceratophyllum demersum	3	3.7	5.3	19	29.1	9.4	4.9	3.1	4.9	
Elodea canadensis	0.5	0.4	0.3	0.4	0.3	1.1	1.3	0.5	0.1	
Elodea nuttallii	3.4	4	4.7	32.9	19.9	14.4	5.4	3.3	4	
Utricularia vulgaris	0.5	0.6	0.8	4.3	0.6	1.3	1.7	0.4	0.2	
Total cover	4.3	4	3.9	5.3	7.9	26.8	3.5	3.4	2.7	**Subm. late successional**
Hottonia palustris	0	0	0	0	0	0	0	0	0	
Myriophyllum spicatum	0.1	0.1	0.1	0	0	0.7	0.1	0.1	0	
Potamogeton acutifolius	0	0	0	0.1	0	0.1	0	0	0	
Potamogeton compressus	0	0	0	0	0	0	0	0	0	
Potamogeton crispus	0	0	0	0	0	0	0	0	0	
Potamogeton friesii	0.6	0.4	0.2	0.2	0.1	1.9	0.3	0.2	0	
Potamogeton lucens	0.1	0	0	0.1	0	0.1	0	0	0	
Potamogeton obtusifolius	0	0	0	0	0	0	0	0	0	
Potamogeton pectinatus	1.7	1.5	1.7	2.1	3.4	11	1.1	1.2	1.6	
Potamogeton pusillus	0	0	0.1	0	0	0.1	0.1	0.1	0	
Potamogeton trichoides	1.5	1.5	1.6	2.4	4.1	11.7	1.4	1.4	1	
Ranunculus circinatus	0.3	0.2	0.2	0.2	0.1	0.8	0.1	0.2	0	
Zannichellia palustris	0.1	0.2	0.1	0.1	0.2	0.5	0.4	0.1	0	
Total cover	3	2.3	2.7	2.9	1.5	5	2.6	2.4	1	**Floating rooted**
Callitriche hamulata	0	0	0	0	0	0	0	0	0.1	
Callitriche obtusangula	0.6	0.5	0.9	0.4	0.4	1	1.1	0.9	0.3	
Callitriche platycarpa	0.7	0.9	0.6	1.5	0.5	1.8	0.7	0.5	0.2	
Callitriche stagnalis	0	0	0	0	0	0	0	0	0	
Nuphar lutea	0.7	0.3	0.6	0.5	0.3	1.1	0.2	0.3	0.1	
Nymphaea alba	0.1	0	0	0	0	0	0	0	0	
Nymphoides peltata	0	0	0.2	0.1	0	0.2	0	0	0.2	
Persicaria.amphibia	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0	
Potamogeton natans	0.1	0	0	0	0	0.2	0	0	0	
Sparganium emersum	0.2	0.1	0.1	0.1	0	0.2	0.1	0.2	0	
Stratiotes aloides	0.5	0.2	0.2	0.2	0.1	0.2	0.3	0.1	0.1	
Total cover	27.5	50.5	42.1	47.4	43.8	43.7	43.7	26.7	44	**Helophytes**
Acorus calamus	0.6	0.7	0.1	0.1	0.2	0.2	0.3	0.4	0	
Alisma gramineum	0.3	0.1	0.1	0.4	0.1	0.2	0.1	0.1	0	
Alisma plantago aquatica	0.1	0.2	0.2	0.3	0.1	0.2	0.4	0.1	0.1	
Berula erecta	0.5	0.5	0.3	0.4	0.4	0.6	0.1	0.2	0.2	
Butomus umbellatus	0.8	1.9	1.1	1.4	1.3	1.7	1.6	0.7	0.5	
Calla palustris	0	0	0	0	0	0	0	0	0	
Catabrosa aquatica	0	0	0.1	0.1	0.1	0.1	1.4	0.1	0.1	
Cicuta virosa	0	0.3	0	0	0	0	0	0	0	
Cladium mariscus	0	0	0	0	0	0	0	0	0	
Eleocharis acicularis	0.1	0.1	0	0	0	0.1	0	0	0	
Eleocharis palustris	0.2	0.1	0	0	0	0.2	0	0	0	
Eleocharis uniglumis	0.3	0.6	0.2	0.2	0.2	0.5	0.3	0.2	0.1	
Equisetum fluviatile	0.9	0.5	0.2	0.7	0.2	0.7	0.1	0.3	0.3	
Glyceria fluitans	1.8	3.7	4.1	4.4	6.5	3.5	4.5	2	4.7	
Glyceria maxima	13.4	29.9	27	28.6	25.7	24.8	24.2	14.1	30.3	
Menyanthes trifoliata	0	0	0	0	0	0	0	0	0	
Myosotis laxa	0.1	0.1	0.1	0	0	0	0	0.1	0	
Myosotis palustris	1.1	1.5	1.1	1.6	1.1	1.6	1.1	1	1.3	
Oenanthe aquatica	0.1	0.1	0.2	0.3	0.1	0.5	0.7	0.1	0.1	
Oenanthe fistulosa	0.5	1.3	0.9	0.9	0.9	0.7	0.5	0.8	0.7	
Phragmites australis	2.2	0.8	1.2	1.1	0.5	1.3	0.4	2.3	1	
Potentilla palustris	0	0	0	0.1	0	0.1	0.2	0.1	0	
Rorippa microphylla	0	0.1	0.1	0.3	0.1	0.1	0.1	0.1	0.2	
Sagittaria sagittifolia	0.3	0.3	0.1	0.3	0.1	0.7	0.1	0.1	0.2	
Schoenoplectus lacustris	0	0	0	0.1	0	0.4	0.1	0	0	
Sparganium erectum	3.5	7	4.6	5.9	5.8	5.2	7.2	3.4	4	
Typha angustifolia	0	0	0	0	0	0	0	0	0	
Typha latifolia	0.4	0.7	0.4	0.3	0.1	0.3	0.3	0.4	0.1	
Total cover	8.6	11.7	39.7	15.1	76	16.3	13.8	7	93.2	**Floating unrooted**
Azolla filiculoides	[illegible]	[illegible]	1.4	[illegible]	2	0.5	0.7	[illegible]	0.9	
Lemna gibba	0.5	0.7	3.6	0.9	5.7	0.9	0.9	[illegible]	8.3	
Lemna minor	2.6	3.8	12	4.8	23	5	4.8	2.3	28.4	
Lemna trisulca	1.9	2.4	6.3	3.1	12.1	3.3	2	1.2	16.4	
Spirodela polyrhiza	3.2	4.3	14.5	5.5	28.8	6.3	5.2	2.7	36.8	
Wolffia arrhiza	[illegible]	[illegible]	1.9	0.7	4.2	[illegible]	[illegible]	[illegible]	2.5	
Organic sediment layer (cm)	30.9	47.4	48.7	46.1	52.7	46.4	93.5	58.3	40.4	**Organic layer thickness**

Figure S 1.2. A heatmap of cluster groups (columns) and species (rows) grouped by functional types (colours). The colour intensity and the number in each cell gives the coverage of the plants averaged over all sites within the given cluster. Total cover rows give the cumulative coverage of the given vegetation type which were used as input for the cluster analysis yielding the clusters of successional stages (cluster 1-7) and the two outgroups (8, 9). Clusters 1-7 have been arranged along the successional gradient (early-late).

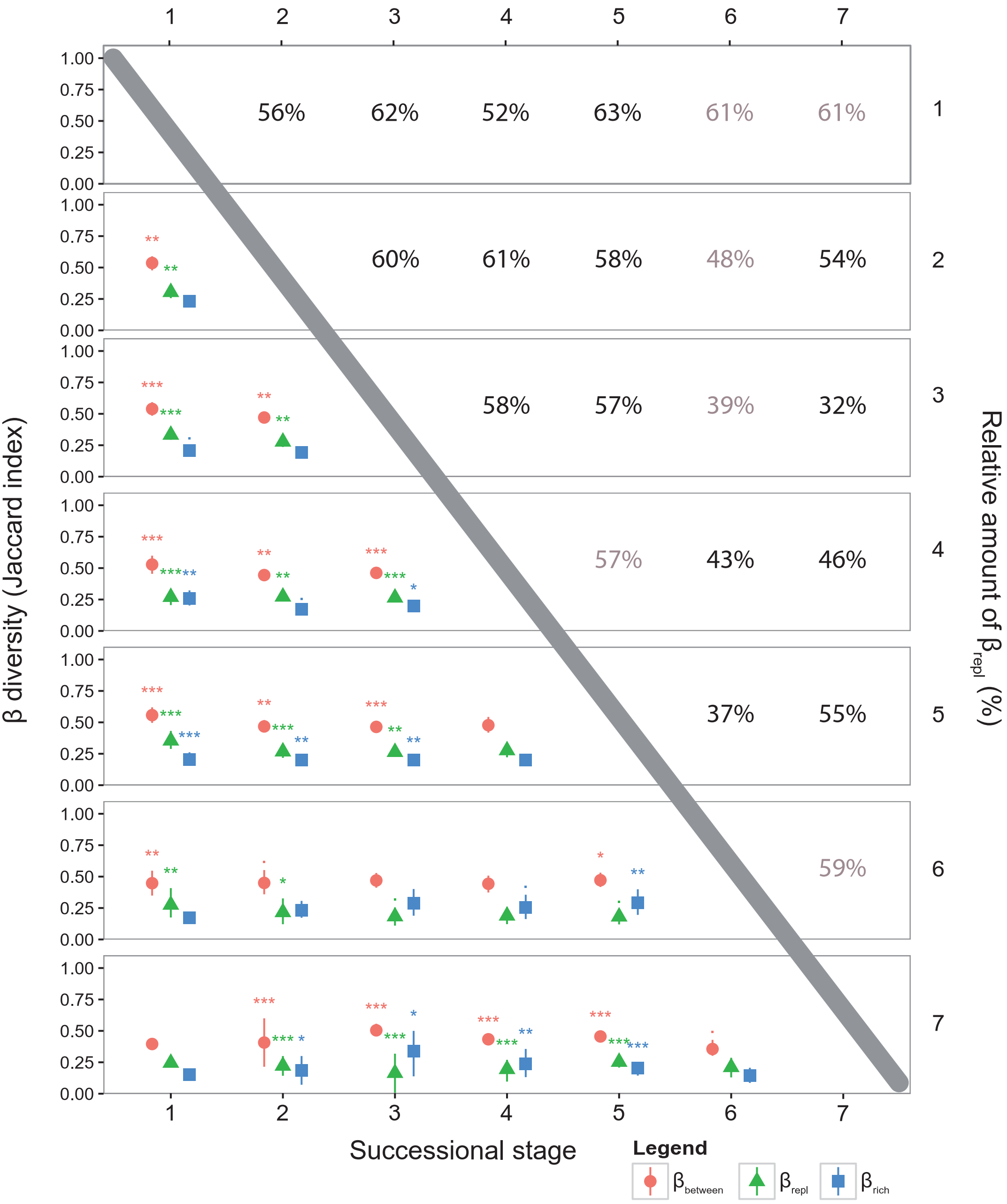


Figure S 1.3. β-diversity (expressed as a multi-site Jaccard dissimilarity index) between communities of different successional stages (βD) and its partitions of species replacement (βrepl) and richness difference patterns (βrich) for presence/absence data. Combinations of each successional stage (1-7) against all other successional stages are shown. Significance against random difference in community composition is indicated using symbols (***:p<0.001, **:p<0.01, *:p<0.05) and error bars give 2 standard errors around the polder mean. The values given in the upper triangle refer to the mean percentage of β-diversity which may be attributed to species replacement. Values given in black indicate that the RDA model was statistically significant.

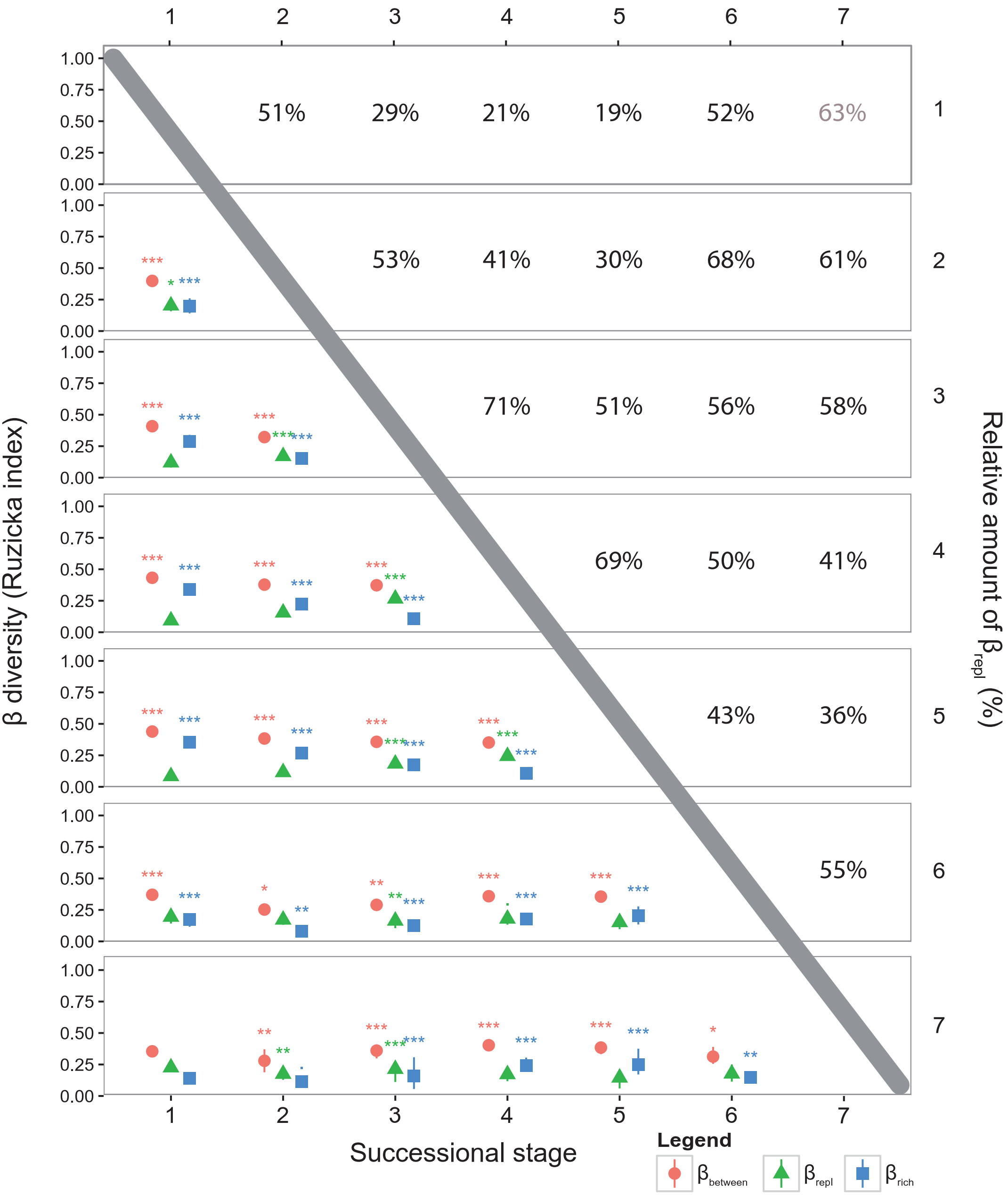


Figure S 1.4. β-diversity (expressed as the total variance of a Ruzicka index distance matrix) between communities of different successional stages (βD) and its partitions of species replacement (βrepl) and richness difference patterns (βrich) for presence/absence data. Combinations of each successional stage (1-7) against all other successional stages are shown. Significance against random difference in community composition is indicated using symbols (***:p<0.001, **:p<0.01, *:p<0.05) and error bars give 2 standard errors around the polder mean. The values given in the upper triangle refer to the mean percentage of β-diversity which may be attributed to species replacement. Values given in black indicate that the RDA model was statistically significant.

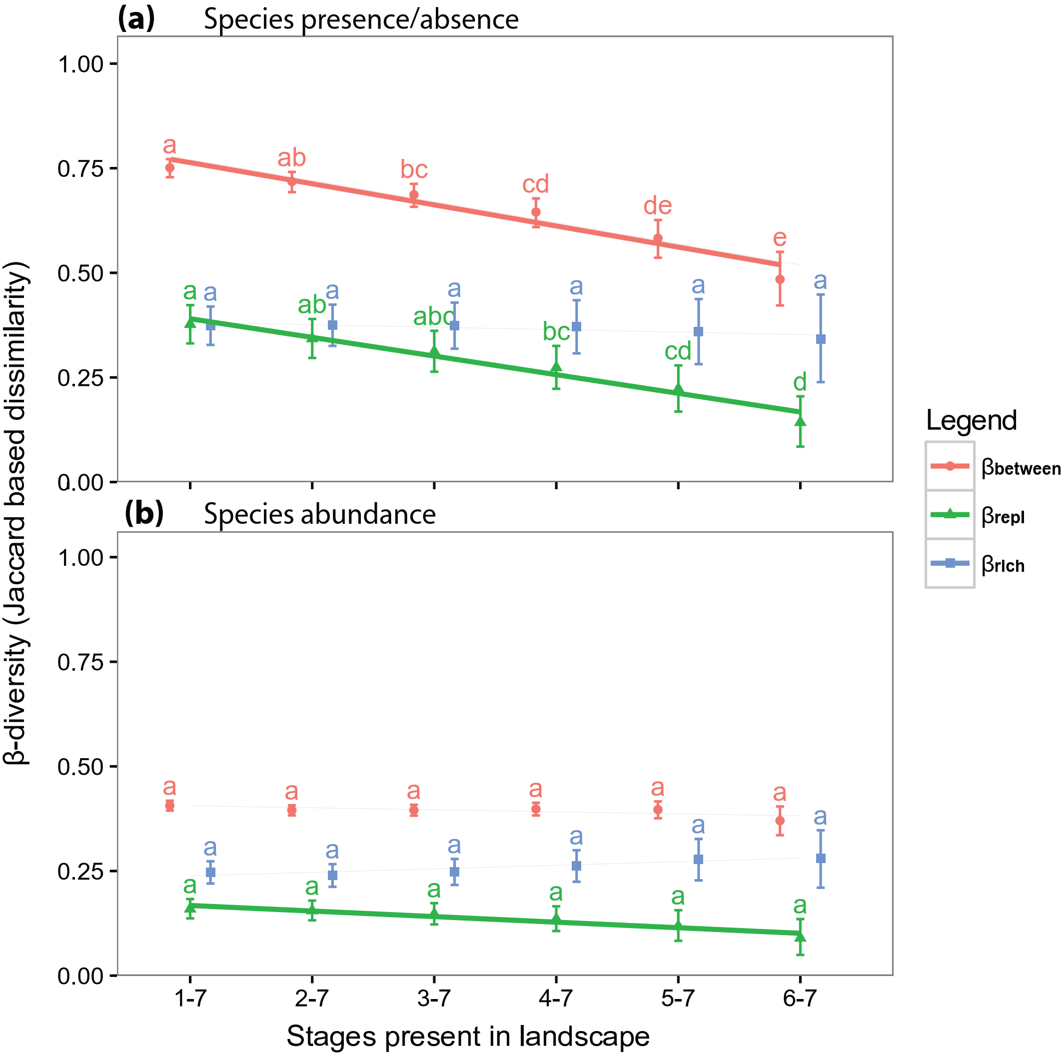


Figure S 1.5. Effects of decreasing landscape-wide successional stage heterogeneity on the between successional β-diversity (βD) and its partitions (βrepl, βrich) based on a scenario of extensified management (scenario 2). (**a**) Changes in diversity based on species incidence data using a multi-site Jaccard dissimilarity index. (**b**) Changes in diversity based on species abundance data using the total variance of a Ruzicka (abundance based Jaccard) dissimilarity matrix. Error bars show the 97.5% confidence limits around the landscape-wide mean diversity values based on 21 simulated landscapes with 12 sites per landscape. Letters indicating pairwise significance between successional group means. Lines show significant trends ($p<0.05$) along the gradient of management based on a linear mixed effect model where diversity effects are nested within polder identity and dashed lines show marginally significant trends ($p<0.10$).

Table S 1.1. Conversion of Tansley abundance classes to numeric classes and percentage cover.

Tansley score	Tansley numeric score	Cover (%)
d	11	60
cd	10	40
ld	9	22
a	8	15
la	7	9
f	6	8
lf	5	3
o	4	2
lo	3	1
r	2	0.5
s	1	0.1

Table S.1.3. Summary table of the different LME regression models and their parameters showing the trends present in the scenario analyses outlined in Figure S 1.5.

Partition	q	parameter	Value	±	se	DF	t-value	p-value
$\beta_{between\ D}$	*SR*	intercept	0.77	±	0.04	102	26.69	<0.001
		slope	-0.05	±	0.00	102	-11.52	<0.001
		marginal R^2	51.18					
		conditional R^2	51.90					
	H'	intercept	0.41	±	0.02	102	21.52	<0.001
		slope	0.00	±	0.00	102	-2.24	0.124
		marginal R^2	4.67					
		conditional R^2	6.38					
$\beta_{between\ repl}$	*SR*	intercept	0.39	±	0.06	102	12.71	<0.001
		slope	-0.04	±	0.01	102	-7.77	<0.001
		marginal R^2	32.30					
		conditional R^2	33.76					
	H'	intercept	0.17	±	0.03	102	7.92	<0.001
		slope	-0.01	±	0.00	102	-3.91	0.012
		marginal R^2	11.33					
		conditional R^2	13.25					
$\beta_{between\ rich}$	*SR*	intercept	0.38	±	0.07	102	5.84	<0.001
		slope	-0.01	±	0.01	102	-0.79	0.401
		marginal R^2	0.01					
		conditional R^2	0.03					
	H'	intercept	0.24	±	0.04	102	3.99	0.005
		slope	0.01	±	0.00	102	1.84	0.197
		marginal R^2	3.57					
		conditional R^2	5.64					

Table S.1.2. Summary table of the different LME regression models and their parameters showing the trends present in the scenario analyses outlined in Figure 4.

Partition	Scenario	q	parameter	Value	±	se	DF	t-value	p-value
α	*1: Intensified management*	*SR*	intercept	12.31	±	0.18	125	67.52	<0.001
			slope	-0.13	±	0.04	125	-3.24	0.019
			marginal R^2	6.99					
			conditional R^2	8.35					
		H'	intercept	5.17	±	0.12	125	44.83	<0.001
			slope	-0.15	±	0.03	125	-5.89	<0.001
			marginal R^2	19.06					
			conditional R^2	20.29					
	2: Extensified management	*SR*	intercept	12.28	±	0.40	125	35.91	<0.001
			slope	-0.28	±	0.04	125	-7.12	<0.001
			marginal R^2	25.63					
			conditional R^2	26.18					
		H'	intercept	5.10	±	0.26	125	23.99	<0.001
			slope	-0.15	±	0.03	125	-5.82	<0.001
			marginal R^2	18.84					
			conditional R^2	19.32					
β	*1: Intensified management*	*SR*	intercept	22.91	±	0.62	125	37.26	<0.001
			slope	0.53	±	0.14	125	3.88	0.005
			marginal R^2	9.56					
			conditional R^2	10.95					
		H'	intercept	6.68	±	0.45	125	15.04	<0.001
			slope	0.40	±	0.10	125	4.02	0.003
			marginal R^2	10.18					
			conditional R^2	10.69					
	2: Extensified management	*SR*	intercept	24.08	±	1.17	125	26.02	<0.001
			slope	-0.89	±	0.11	125	-7.81	<0.001
			marginal R^2	29.14					
			conditional R^2	30.06					
		H'	intercept	7.91	±	0.70	125	16.46	<0.001
			slope	-0.51	±	0.07	125	-7.46	<0.001
			marginal R^2	27.39					
			conditional R^2	27.91					
γ	*1: Intensified management*	*SR*	intercept	35.22	±	0.67	125	53.13	<0.001
			slope	0.40	±	0.15	125	2.71	0.050
			marginal R^2	5.18					
			conditional R^2	6.76					
		H'	intercept	11.78	±	0.47	125	24.98	<0.001
			slope	0.04	±	0.10	125	0.34	0.494
			marginal R^2	0.69					
			conditional R^2	1.79					
	2: Extensified management	*SR*	intercept	36.36	±	1.31	125	34.06	<0.001
			slope	-1.17	±	0.13	125	-9.12	<0.001
			marginal R^2	0.36					
			conditional R^2	0.37					
		H'	intercept	12.45	±	0.86	125	19.34	<0.001
			slope	-0.58	±	0.08	125	-6.93	<0.001
			marginal R^2	24.64					
			conditional R^2	25.17					

Chapter 8

Evidence for a non-linear response of submerged macrophyte tissue nutrient concentration to environmental nutrient availability

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Abstract

In shallow freshwater ecosystems, aquatic plants are an important component of the food web. Plant quality as food source for herbivores and detrivores is partly determined by concentrations of essential elements including nitrogen (N) and phosphorous (P). Changes in these concentrations through changing environmental nutrient availability may affect food quality for consumers, leading to altered rates of consumption and decomposition.

We provide a framework, illustrating mechanisms underlying variation in plant nutrient concentrations within and among species, including differences in homeostasis, nutrient utilization during nutrient limitation, and luxurious uptake. These mechanisms combined potentially result in non-linear associations between environmental nutrient availability and plant tissue nutrient concentrations. In this study, we investigated whether five widespread submerged macrophyte species show this conceptual non-linear association between environmental nutrient availability (N and P) and tissue nutrient concentration in an extensive field survey across 193 sites in the Netherlands.

We found high intra-specific variation in plant N and P concentrations. The plant's tissue P concentration, increased significantly with increasing environmental nutrient availability. However, at high nutrient availability no further increase was observed in two species, providing compelling evidence that the relationship between plant and environmental nutrient concentrations can be non-linear. This result is especially relevant for ecosystems undergoing eutrophication or oligotrophication, since plant nutrient concentration, and thereby food quality for their consumers, may increase during eutrophication, whereas it may show a delayed response to lowering the nutrient status of a eutrophic ecosystem (i.e. oligotrophication).

Introduction

Submerged aquatic plants are at the base of the food web and very important for the functioning of shallow freshwater ecosystems (Carpenter & Lodge, 1986; Phillips et al., 2016). Understanding how tissue nutrient concentrations of submerged aquatic plants are affected by environmental nutrient availability is very important for understanding dynamics in the aquatic food web. Autotrophs in general are considered to have a high degree of flexibility in their tissue elemental concentrations and ratios (Duarte, 1992; Van de Waal & Boersma, 2012; Hessen et al., 2013). The subsequent variation in plant tissue nutrient concentrations may cause a stoichiometric mismatch with its consumers (Miler & Straile, 2010; Hessen et al., 2013), with implications for the performance and functioning of the higher trophic levels (e.g. Rowland et al., 2015). However, most studies to date have focussed on the planktonic food web (Hessen et al., 2013), in which aquatic vascular plants (i.e. macrophytes) are not included. Recently, the importance of herbivory on aquatic vascular plants has been unequivocally confirmed (Bakker et al., 2016; Wood et al., 2017), indicating that aquatic plants are a vital component of aquatic food webs. In turn, the plant's nutrient concentration and stoichiometry can determine the intensity of herbivore impact, with stronger consumption of plants with high tissue nutrient concentrations (Dorenbosch & Bakker, 2011; Bakker et al., 2013a).

Differences in plant tissue nutrient concentrations may stem from: (1) species specific differences in degree of elemental homeostasis, (2) the environmental nutrient availability, and (3) species specific nutrient requirements.
First, different species may show different degrees of elemental homeostasis over an environmental nutrient gradient from being fully homeostatic, i.e. regulators, (Fig. 1 panel A: solid red line) to being fully flexible, i.e. conformers, reflecting environmental nutrient availability (Fig. 1 panel A: dashed blue line) (Meunier et al., 2014). The tissue nutrient concentration of the flexible species will increase with increasing nutrient availability while the nutrient concentration of the homeostatic species will not, leading to differences in elemental composition among these species (Fig. 1: solid red line versus dashed blue line).

Second, the degree of homeostasis within one species may not be uniform over the whole nutrient gradient, but may depend on the environmental nutrient availability (Meunier et al., 2014). We depicted three nutrient ranges in which a single plant species may exhibit varying degrees of elemental homeostasis: (a) the environmental nutrient availability is below the plant's basal nutrient requirements (Fig. 1 panel A: "Negative GR"), (b) the

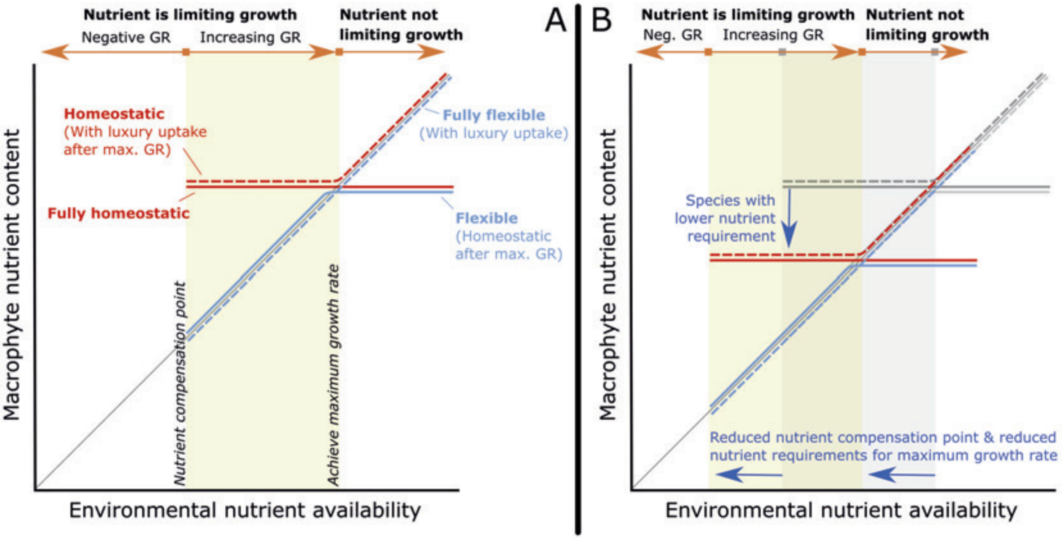

Figure 1. Conceptual framework showing possible responses of aquatic plant nutrient content to a gradient of environmental availability of that nutrient. **Panel A**: The lines only depict the extreme responses (i.e. fully flexible or fully homeostatic over the depicted ranges), but intermediate responses are also possible. GR stands for 'growth rate'. **Panel B**: Identical to panel A, but showing possible contrasting responses of plant species with different nutrient requirements (grey versus coloured, darker lines).

environmental nutrient availability is above the plant’s basal nutrient requirements, but below the concentration needed for maximum growth (Fig. 1 panel A: “Increasing GR”), (c) the environmental nutrient availability is higher than required for maximum growth (Fig. 1 panel A: “Nutrient not limiting growth”). In the first nutrient range (a), the plant requires more nutrients than are available and growth is negative, leading to the eventual death of plant tissue when conditions persist for extended periods of time (Fig. 1 panel A: “Negative GR”). However, plants are able to grow in range b and c and may show varying degrees of elemental homeostasis over both ranges. During the second range (b), the nutrient is abundant enough to satisfy the plant’s basal requirements (i.e. is above its nutrient compensation point) (e.g. Farquhar et al., 1980), but is low enough to limit the growth of the species. In this range, the growth rate will increase under increasing environmental availability of that nutrient, until another factor becomes limiting (Wersal & Madsen, 2011b; Van de Waal & Boersma, 2012). For species with flexible tissue nutrient concentrations, increasing availability of this growth limiting nutrient can increase the tissue nutrient concentration (Duarte, 1992) and thereby increase its growth rate (Ågren & Weih, 2012; Fig. 1: panel A: blue lines). Alternatively, a homeostatic species (i.e. not flexible) will directly utilize the increasing nutrient availability towards new biomass, while maintaining a relatively constant tissue nutrient concentration (Fig. 1: panel A: red lines). During the highest nutrient range (c), nutrients are available in excess and some species may show additional uptake and storage of this nutrient (i.e. luxury uptake; Van de Waal & Boersma, 2012) leading to flexible elemental composition, while others may not (Madsen & Cedergreen, 2002; Pietro et al., 2006; Li et al., 2015; Sistla et al., 2015), resulting in elemental homeostasis in this nutrient range (Fig. 1: panel A: dashed lines and solid lines, respectively).

Third, plants may have different tissue nutrient concentrations due to differences in species specific nutrient requirements. In this case, inherent differences among species in their nutrient requirements (e.g. Gerloff, 1975; Garbey et al., 2004; Gonzales et al., 2010) may shift the entire nutrient uptake and associated growth rate patterns of a plant mentioned in the previous two paragraphs (Sterner & Elser, 2002; Gonzales et al., 2010). For example, one plant has a high nutrient requirement (Fig. 1 panel B: grey lines) while another plant has a lower nutrient requirement (Fig. 1 panel B: coloured lines). The plant with the lower requirements can start growing and reaches its maximum growth under lower environmental nutrient availability than the plant with the higher requirements. This can lead to differences in nutrient concentration between species under similar growing conditions, even if they have similar intrinsic flexibility in tissue nutrient concentrations, because the two species then show a different response over part of the environmental nutrient range (e.g. Fig. 1: panel B).

The concepts depicted in Fig. 1 have so far not been tested for submerged aquatic plants under field conditions. Moreover, field surveys measuring submerged macrophyte and environmental carbon (C), nitrogen (N), and phosphorous (P) concentrations are rare.

The surveys that exist generally found only weak correlations between environmental and aquatic plant nutrient concentration and stoichiometry (e.g. Demars & Edwards, 2007; Meyer et al., 2013; Xing et al., 2013). However, this is in contrast with controlled experiments that have suggested stronger relationships between nutrient addition and plant tissue nutrient concentrations (e.g. Cronin & Lodge, 2003; Xie et al., 2005; Bakker et al., 2013a; Bakker & Nolet, 2014; Christiansen et al., 2016). This discrepancy between experiments and field surveys may be caused by the relatively low numbers of sample sites (~ 1 – 20) for each plant species in the field surveys, potentially resulting in excluding part of the environmental nutrient range in which the species occurs. Additionally, a non-linear response in submerged plant tissue nutrient concentration along an environmental nutrient gradient, as shown in Figure 1, may be expected (Robach et al., 1995; Meunier et al., 2014; Sistla et al., 2015), thus obscuring the overall correlations between the two variables.

In this study, we investigated whether five widespread submerged macrophyte species show this conceptual non-linear association between environmental nutrient availability (N and P) and tissue nutrient concentration in an extensive field survey with 20 – 135 sampled sites per species.

Methods

Field sites and sampling methods

We focussed on *Ceratophyllum demersum* and *Elodea nuttallii* as our main target species, because they are two of the most common and widespread submerged macrophyte species of temperate shallow water ecosystems both occurring over a wide range of aquatic habitats in the Netherlands (De Lyon & Roelofs, 1986). Additional samples of the common species *Myriophyllum spicatum*, *Potamogeton lucens*, and *Potamogeton pectinatus* were added to allow for more general conclusions on the variability in elemental composition of common and widespread submerged macrophyte species. We collected plant, water and sediment samples from 193 different sites in the Netherlands. Most of the samples of *C. demersum* (108 out of 135) and *E. nuttallii* (83 out of 111) originated from drainage channels sampled from the end of May to mid-August of 2014. Additional samples of these species (*E. nuttallii*: 27; *C. demersum*: 28) were collected from a variety of shallow freshwater ecosystems in June and July of 2008 and 2009, while also collecting samples of *M. spicatum*, *P. lucens*, and *P. pectinatus* (22, 21, 20 sites sampled, respectively).

To quantify environmental nutrient availability, we measured inorganic and total N and P in the water and total and extractable N and P in the sediment, as most submerged plants can take up nutrient from both media, depending on the relative nutrient availability (Barko et al., 1991).

Plant analyses

After drying (60 °C), plant dry mass (DW) was ground to a powder using a vegetation grinder with a 0.5 mm mesh (IKA® MF 10 basic, IKA Werke GmbH and Co. KG / Germany). To measure the C and N concentration of the plants, 0.5 mg of dry, ground plant material was inserted into tin capsules and analysed using a CN analyzer (FlashEA 1112 Series, Thermo Scientific, Waltham, MA, USA). Total phosphorous of the plants was measured by ashing (30 min. at 550 °C) 0.5 mg of dry, ground plant material, which was subsequently digested in an autoclave at 121 °C for 30 minutes using a 2.5% persulphate solution. These samples were measured colourimetrically on an Auto-Analyser system (QuAAtro SFA, Seal Analytical, Norderstedt, Germany).

Surface water analyses

At each site, a sample of the water column was taken for nutrient analysis. Water was filtered in the field over pre-washed GF/F filters (Whatman, 0.7 µm pore size), and the filtrate was analysed for dissolved inorganic nutrients (PO_4^{3-}, NO_2^-, NO_3^-, and NH_4^+) using a QuAAtro39 Auto-Analyser (SEAL Analytical Ltd.). The total nitrogen and phosphorous concentration was determined by analysing the suspended solids captured on the GF/F filter after drying at 60 °C. Suspended solid nitrogen concentration was analysed using the CN elemental analyser. Suspended solid phosphorous was determined using the potassium persulphate ($K_2S_2O_8$; 2.5 % w/v) digestion method at 121 °C after a digestion step where samples are placed in a blast furnace at 550 °C. Digested P (as PO_4^{3-}) was measured on the Auto-Analyser. The total N and P concentrations were calculated by adding inorganic water nutrients to the suspended solids nutrients.

Sediment analyses

The top 10 cm of the sediment was collected using a tube sampler (diameter = 13.5 cm). Sediment samples were dried at 60 °C for at least 96 h and analysed for total and available nitrogen and phosphorous. Sediments were put through a grinder (IKA® MF 10 basic, IKA Werke GmbH and Co. KG / Germany) with a 1mm sieve, resulting in a fine powder. Total N was determined through analysis of a 1 mg powdered subsample using the CN elemental analyser. Total P was analysed using the same P-digestion method described above for particulate P determination, using a 5 mg subsample.

A KCl-extraction was performed on the dried sediment to estimate the amount of plant available N. 12.5 ml 1 M KCl was added to 2.5 g of dried sediment and subsequently shaken for 2.5 h at 250 rpm. 2 subsamples (2 ml each) were centrifuged for 10 min at 10,000 rpm and the supernatant was stored at -20 °C for colourimetrical nitrogen analysis on the autoanalyser system. Plant available orthophosphate in the sediment was estimated using an adapted P-Olsen protocol as follows. 50 ml 0.5 M $NaHCO_3$ (at pH 8.50) was added to 2.5 g of dried sediment and subsequently shaken for 30 min and immediately after that, the solution was poured over a filter (Whatman Grade 42, GE Healthcare Europe GmbH, Eindhoven, the Netherlands). Sulphuric acid (1.04 mL, 2.5 M) was added to 10 ml of the filtrate in an Erlemeyer flask. The flask was agitated until no more gas development was visible and the filtrate was filtered again (Whatman Grade 42) and stored at -20 °C until analyses for phosphate concentration on the autoanalyser system. When not enough dried material was available for both extractions, the P-extraction was prioritized and a corresponding reduction in reagent volume was applied in order to keep the sediment:reagent-ratio equal between all samples if needed. See Supplementary Table S 1 for nutrient concentrations of the plants, water and sediment.

Statistical analyses

To assess whether and how the plant tissue nutrient concentrations were associated with environmental nutrient availability, we performed regression analyses. Because we expected that this relationship would not be the same over the entire environmental nutrient gradient (see Fig. 1), we compared the fit of two different models: (1) a linear regression model, and (2) a segmented linear regression model. The best model was selected by comparing the Akaike Information Criterions (AIC) of the models that explained a significant part of the variation in the data ($p < 0.05$). The statistically significant model with the lowest AIC was selected. To identify the best measure for the environmental nutrient availability we first performed PCA analyses on the water nutrient parameters (i.e. inorganic and total N and P) and sediment nutrient parameters (i.e. extractable and total N and P). The first PCA-axis (PC1) represented the environmental nutrient gradient best and captured 64 and 65 % of the variation of environmental N and P, respectively (Supplementary Fig. S 1). In our analyses, we used these PC-axes as proxies for the environmental N and P availability, as the measured nutrient parameters were positively correlated (P: Spearman's rho = 0.35-0.84, $p < 0.001$; N: Spearman's rho = 0.38-0.86, $p < 0.001$). The environmental nutrient data was scaled and centered for the PCA after removal of 3 outliers (i.e. all > 1.5 * interquartile range) from the P-data that strongly affected model outcome.

Pairwise t-tests were used to analyse whether the shoot nutrient concentrations differed among species when they were collected from the same site.

All statistical analyses were performed in R version 3.3.2 using additional *Hmisc*, *car, ggplot2, segmented,* and *vegan* packages.

Results

Variation in plant versus environmental nutrient concentrations

We found large variation in shoot C, N, and P concentration (Fig. 2) and N and P concentrations almost exclusively determined shoot C:N and C:P stoichiometry, respectively (Supplementary Fig. S 2). All species showed a positive association between environmental P and plant P, at least over part of the environmental nutrient range (Fig. 3). For both *C. demersum* and *E. nuttallii* the segmented linear model fitted the data best. These models showed an increase in plant P content with increasing P availability in the lower range of the studied environmental nutrient gradient. Above a certain threshold value (i.e. the segmented model's breakpoint), plant P did not increase with environmental P (Fig. 3). The breakpoint on the PC1-axis between the two lines of the segmented model was similar for both species: 0.89±0.43 (SE) and 0.50±0.63 (SE) for *C. demersum* and *E. nuttallii* respectively (Supplementary Table S 2). These breakpoints correspond to water P concentrations of approximately 11 μmol.L^{-1} inorganic PO4 and approximately 20 μmol.L^{-1} total P, and correspond to sediment P concentrations of approximately 2.8 μmol.g$_{dw}^{-1}$ Olsen-P and approximately 30 μmol.g$_{dw}^{-1}$ total P (i.e. mean concentrations from sites with a PC1 value of between 0-1). The other three species were all collected from sites in the lower part of the environmental nutrient gradient (Fig. 3). The P concentration in *P. pectinatus* shoots significantly increased linearly over the sampled environmental P gradient. The segmented model did fit the data best for *M. spicatum* and *P. lucens* (Supplementary Table S 2). This was caused by only one data point: the site with the highest environmental P concentration (Fig. 3). Without this point the linear model fit the data best for *M. spicatum* (slope = 0.22, p = 0.005, intercept = 0.49), while neither the linear nor the segmented found a significant association between plant and environmental P for *P. lucens* (p > 0.05).

Contrarily to P, no associations between environmental N and plant N were found for any species except *M. spicatum* (Fig. 3), even though the overall variation in both plant and environmental N was large (Fig. 2; Table 1). As for P, the range in environmental N was much larger for *C. demersum* and *E. nuttallii* than for the other three species.

Inter-specific differences in elemental composition

More statistically significant differences among species were found in shoot P concentrations than in shoot N concentration, when growing in the same site (Table 1). *E. nuttallii*'s shoot P concentration was similar to the P concentration of *C. demersum*, but significantly higher than the P concentration of all other species. *C. demersum*'s shoot P concentration was significantly higher than the P concentration of *P. pectinatus*, but not significantly different from the P

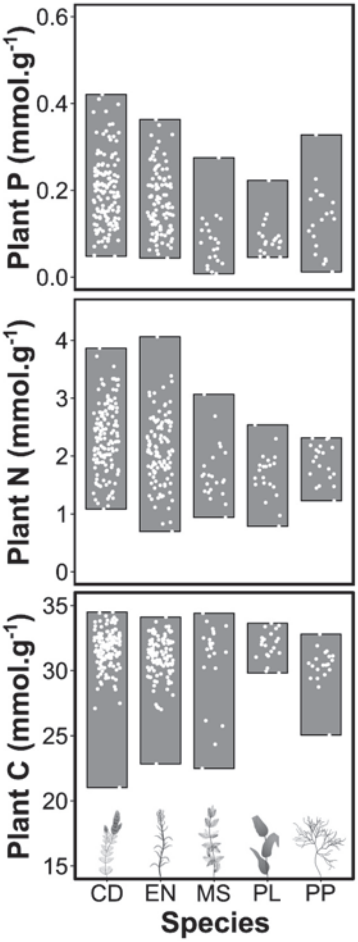

Figure 2. Range of shoot carbon phosphorous (P: **top panel**), nitrogen (N: **middle panel**), and (C: **bottom panel**) concentration (mmol.gdw-1) of the sampled macrophyte species. White dots show the individual data points, bars show the range. CD: *C. demersum*, EN: *E. nuttallii*, MS: *M. spicatum*, PL: *P. lucens*, and PP: *P. pectinatus*.

]

concentration of all other species. *E. nuttallii*'s shoot N concentration was significantly higher than the N concentration in *P. pectinatus* shoots and similar to the shoot N concentrations of all other species. No other significant differences in shoot P or N concentration were found among species growing in the same site.

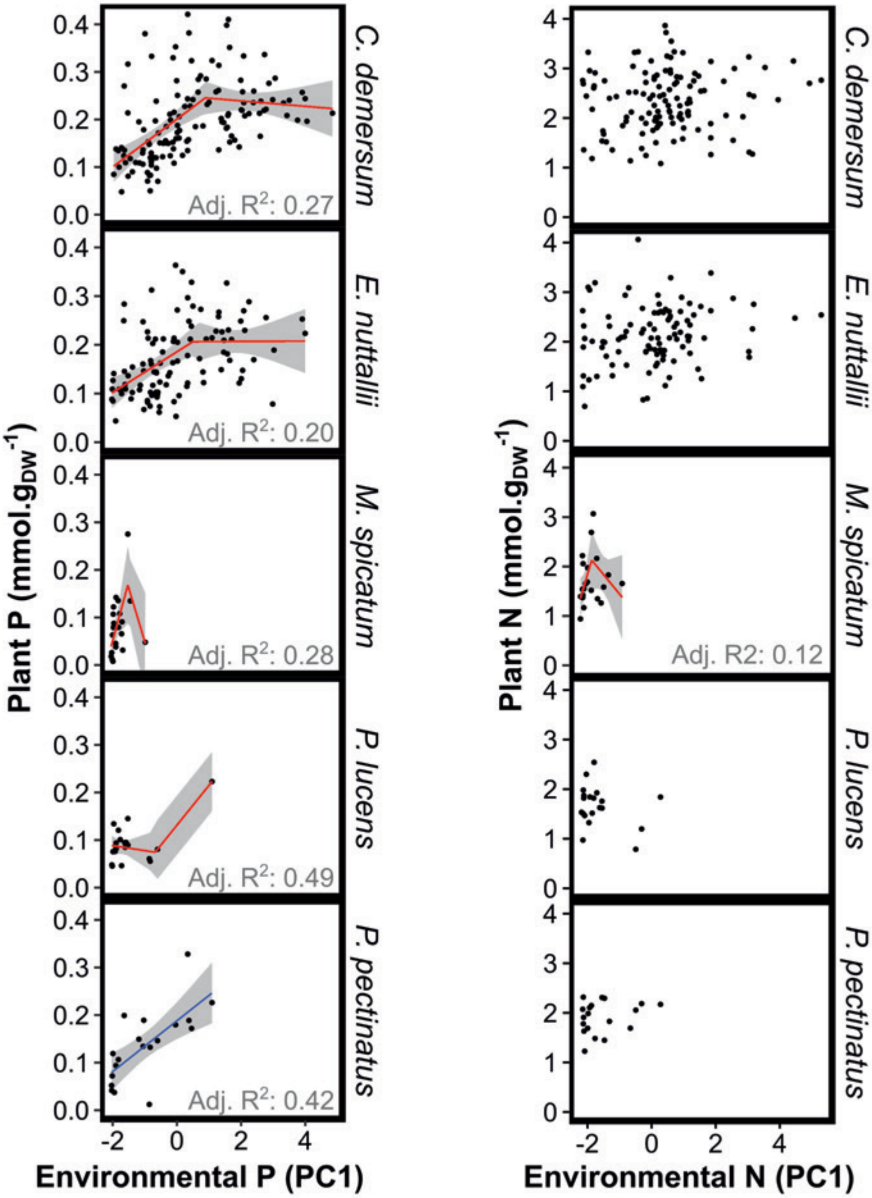

Figure 3. Associations between the environmental phosphorous (P) or nitrogen (N) availability (i.e. axis 1 extracted from the PCA) and shoot P or N concentrations of the five submerged plant species. The dots show the unique data points, the lines show the model fit, and the grey area shows the 95% confidence limits of the model. Only the best fitting model is plotted (blue line for the linear model and red line for the segmented linear model). When environmental nutrient availability could not explain a significant part of the variation in plant tissue nutrient concentration (i.e. $p > 0.05$), no line is drawn. For model details see Supplementary Table S 2.

Discussion

Variation in submerged plant tissue nutrient concentration

Autotroph flexibility in tissue elemental composition is generally considered to be high, as reported for phytoplankton (Van de Waal & Boersma, 2012; Hessen et al., 2013). While some common aquatic plant species have been described as being relatively homeostatic (Hao et al., 2013), our study clearly illustrated high variability in tissue nutrient concentration under field conditions when sampled across large environmental nutrient gradients and with sufficient sampling intensity. We found a similar range in intra-specific submerged plant nutrient concentrations in the field as reported in reviews for submerged plants in general (e.g. Duarte, 1992; Garbey et al.,, 2004) and a slightly lower range than reported for phytoplankton in reviews (e.g. Duarte 1992; Van de Waal & Boersma, 2012). Our study also provides compelling evidence for the existence of non-linear responses of plant tissue nutrient concentrations along a gradient of nutrient supply under field conditions (Fig. 3). Additionally, we also found inter-specific

Table 1. Pairwise differences in shoot nitrogen (N) and phosphorous (P) concentrations among five submerged aquatic plant species collected from the same site. The values show the mean differences in shoot nutrient concentrations (δ mmol.gdw-1) between the pair of species and the statistical significance of the difference (at $p < 0.05$ *). When the value is positive, the tissue nutrient concentration of the species in top row was higher than the concentration of the species on the left. Bold values highlight statistically significant differences. The last panel (Cases) shows the number of sites in which each pair of species co-occurred. Species are abbreviated as: CD (*C. demersum*), EN (*E. nuttallii*), MS (*M. spicatum*), PL (*P. lucens*), and PP (*P. pectinatus*). The 'Grand mean' N and P concentrations (mmol.gdw-1) of each species show the overall mean tissue nutrient concentration for each species sampled in this study. Different superscript letters indicate statistically significant differences in shoot nutrient concentrations among species (ANOVA: N: F4,304=11.8 p<0.001; P: F4,304=19.9 p<0.001 with Tukey post-hoc tests).

N		**CD**	**EN**	**MS**	**PL**	**PP**
Difference between species *(δ mmol.g_{dw}^{-1})*	**EN**	0.03				
	MS	-0.11	0.12			
	PL	0.49	0.19	0.15		
	PP	0.47	**0.62***	0.03	-0.33	
Grand mean *(mmol.g_{dw}^{-1})*		2.33^{A}	2.11^{B}	1.72^{C}	1.68^{C}	1.89^{BC}
P		**CD**	**EN**	**MS**	**PL**	**PP**
Difference between species *(δ mmol.g_{dw}^{-1})*	**EN**	0.004				
	MS	-0.021	**0.052***			
	PL	0.054	**0.029***	0.024		
	PP	**0.086***	**0.059***	-0.050	0.010	
Grand mean *(mmol.g_{dw}^{-1})*		0.197^{A}	0.170^{B}	0.082^{C}	0.091^{C}	0.130^{BC}
Cases		**CD**	**EN**	**MS**	**PL**	**PP**
Per pair (n)	**EN**	78				
	MS	5	5			
	PL	9	8	5		
	PP	10	10	4	7	
Grand total (n)		135	111	22	21	20

differences in tissue nutrient concentration among some of the aquatic plant species when sampled from the same site.

Associations with environmental nutrient concentrations

The conceptual framework (Fig. 1) illustrated that the relationship between environmental and plant nutrient concentrations may not be uniform over the entire environmental nutrient gradient for all species (also see: Robach et al., 1995; Meunier et al., 2014; Sistla et al., 2015). We found significant associations between environmental nutrient availability and plant tissue nutrients for P, but less so for N (also reported by Fernandez-Alaez et al., 1999). In our study, the shoot P concentrations of *C. demersum* and *E. nuttallii* were similar when growing in the same site and significantly increased with increasing environmental P concentrations up until halfway along the sampled environmental nutrient gradient (Fig. 3). Both species showed a similar breakpoint of the model segments on the environmental nutrient gradient. In the upper half of the nutrient gradient, the P concentration in the shoots of these two species showed no clear association with environmental nutrient availability, possibly because P was no longer limiting here. This closely matches the conceptual predictions of the 'Flexible' strategy in our framework (Fig. 1: solid blue line). The other three species did not clearly show this strategy in the available data. The P concentration in *P. pectinatus* shoots linearly increased with increasing environmental P without levelling off. The linear association between *P. pectinatus* tissue P and environmental P could indicate that *P. pectinatus* is 'Fully flexible' (Fig. 1: dashed blue line). However, looking at the environmental nutrient range at which the samples of this species were collected (i.e. the lower half of the total range), it is also possible that *P. pectinatus* will become more homeostatic at higher nutrient availability, similar to *C. demersum* and *E. nuttallii*. The P concentration in the shoots of *P. lucens* and *M. spicatum* was statistically significantly associated with environmental P concentrations and the non-linear model (i.e. segmented) fitted the data best, but this was solely caused by one sampled site per species. Without these sites, a linear association between plant and environmental P concentrations was found for *M. spicatum*, while no statistically significant association was found for *P. lucens*. The large effect of a single data point in the statistical models for *P. lucens* and *M. spicatum* is probably caused by the lower sample size combined with the narrow range of environmental nutrient availability under which these species have been collected compared to the range of *C. demersum* and *E. nuttallii*. We can therefore not conclude which of the strategies shown in Figure 1 these species likely possess.

Because we collected plants from sites over a wide range of nutrient availability (i.e. for *C. demersum* and *E. nuttallii*), we expected that the associations between the environmental nutrient availability and the elemental composition of these common macrophyte species would be stronger than those commonly found in previous studies (e.g. Fernández-Aláez et al., 1999; Demars & Edwards, 2007; Xing et al., 2013). Additionally, the use of segmented models within the conceptual framework presented can aid in better understanding when nutrients are driving the elemental composition of plants and when not. Our models showed a reasonably good fit on our field data (adjusted R^2-value of 0.20-0.40), but were not much stronger in explaining the variation in aquatic plant nutrient concentration than the ones reported by others (e.g. Xing et al., 2013: Spearman's rho: 0.4-0.5), perhaps because these authors sampled only part of the environmental nutrient range or because their non-segmented correlation models fitted well over part of the nutrient range (as for *P. pectinatus* in our study). The difficulty to predict the plant nutrient concentrations with high accuracy from the environmental availability may stem from the fact that true environmental nutrient availability is difficult to accurately measure and because factors other than environmental nutrient availability can affect plant nutrient concentration and stoichiometry, for example water depth, temperature, light availability, life-history stage or growth form (Cronin & Lodge, 2003; Demars & Edwards, 2008; Ventura et al., 2008; Elser et al., 2010; Xia et al., 2014; Xing et al., 2015; Zhang et al., 2016).

Implications for food-web ecology

We want to stress that understanding how aquatic plants respond to environmental nutrient availability is challenging, but very important for limnology. Whereas phytoplankton and terrestrial plants mainly take up nutrients from respectively the water column and the soil (but see: Farquhar et al., 1980), aquatic plants can use multiple sources for resource acquisition, depending on their growth form (Lacoul & Freedman, 2006). Rooted submerged plants, as we mostly used, are able to take up nutrients from both the water column as from the sediment. As a consequence, the relationship between environmental nutrient availability and plant nutrient concentrations can be more complex compared to other groups of primary producers. We showed that changing environmental nutrient availability may lead to variation in tissue nutrient concentration of submerged plants, but perhaps not over the entire nutrient gradient or for all elements in the same way (P versus N in our study). Due to inherent differences in tissue nutrient concentration among species and species-specific habitat preferences, changes in environmental nutrient availability may also lead to a different species composition of the aquatic vegetation (Lacoul & Freedman, 2006; Hirzel & Le Lay, 2008; Bornette & Puijalon, 2011) and to subsequent differences in total vegetation elemental composition (Demars & Edwards, 2007; Frost & Hicks, 2012).

Varying nutrient concentration and stoichiometry of the aquatic vegetation affects the palatability (e.g. Dorenbosch & Bakker, 2011) and thereby affects the fitness of its consumers (e.g. Miler & Straile, 2010). This, in turn, may have large effects on systems undergoing eutrophication or oligotrophication (Van Altena et al., 2016) through changes at a food web level (Sardans et al., 2012; Bakker et al., 2016; review mainly on plankton by Hessen et al., 2013). Because we found no association between environmental nutrient availability and plant tissue nutrient concentration in the higher part of the sampled environmental nutrient range, some plants may become more nutrient rich during eutrophication, but only up until a certain level of eutrophication. During continued eutrophication plant nutrient content and thus plant palatability may thus potentially remain unaffected by the increased nutrient availability. Alternatively, during oligotrophication, the plants' nutrient concentration may not be affected by the reduced nutrient availability at first, but might decrease after continued oligotrophication of eutrophic ecosystems. This suggests that at first plants can contain relatively high nutrient concentrations in ecosystems subject to oligotrophication, which may result in high palatability and consumption, potentially inhibiting of plant growth and colonization (c.f. Van Altena et al., 2016).

Conclusions

We have shown that submerged aquatic plants can be very flexible in their intra-specific C, N, and P concentration, and that this variation within several species was associated with nutrient availability in the field, at least for P. We provide compelling evidence that this relationship can be non-linear in aquatic plants, as predicted by the conceptual framework. We found that P concentration in several plant species increased with environmental availability in the lower environmental nutrient range, but not at high environmental nutrient availability. Moreover, we showed that field surveys aimed at assessing possible relationships between environmental and plant elemental nutrient concentrations under field conditions should include plant samples over the entire range of environmental nutrient availability present in the species' niche. Together, this will help to accurately estimate true intraspecific flexibility in tissue elemental composition and thereby help to better understand the consumer-resource dynamics of the food web of shallow freshwater ecosystems and its repercussions on the ecosystem level under changing environmental nutrient availability.

Acknowledgements

We would like to thank Dennis Waasdorp and Erik Reichman for their valuable assistance with the analyses of the plant samples. We also wish to thank Marlies Gräwe, Marta Alirangues, and Annegreet Veeken for their tireless assistance with the field work. S. Teurlincx was financially supported by the Division for Earth and Life Sciences (ALW) with financial aid from the Netherlands Organization for Scientific Research (NWO), by the Biodiversity Works Research Program (OBW) project no. 841.11.009.

Supplementary

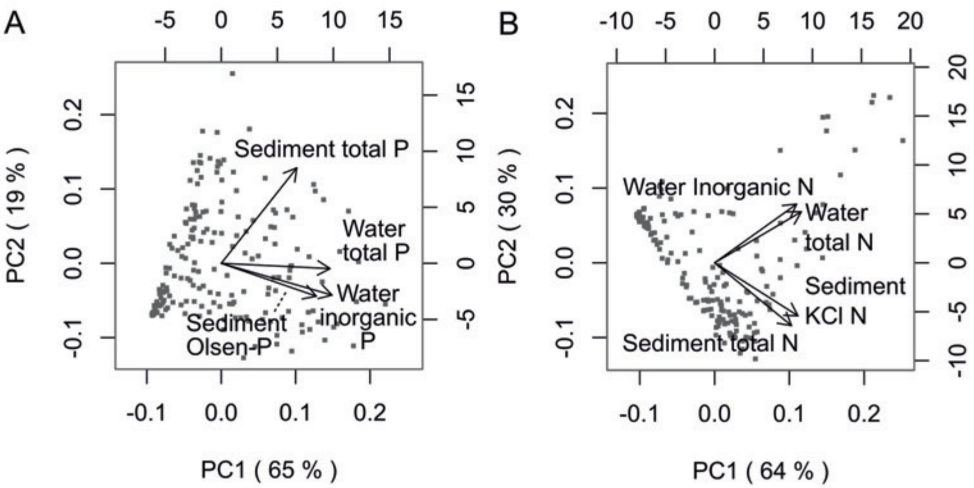

Figure S 1. Principal component analyses of environmental phosphorous (P: **panel A**) and nitrogen (N: **panel B**) parameters (PCA analyses on all data, scaled and centered). Percentages between brackets in the axis title indicate the amount of variation in the data captured by the PC axis. Left and bottom axis labels are for the dots, top and right axis labels for the arrows. PC1 was used as a proxy for environmental nutrient availability in this study.

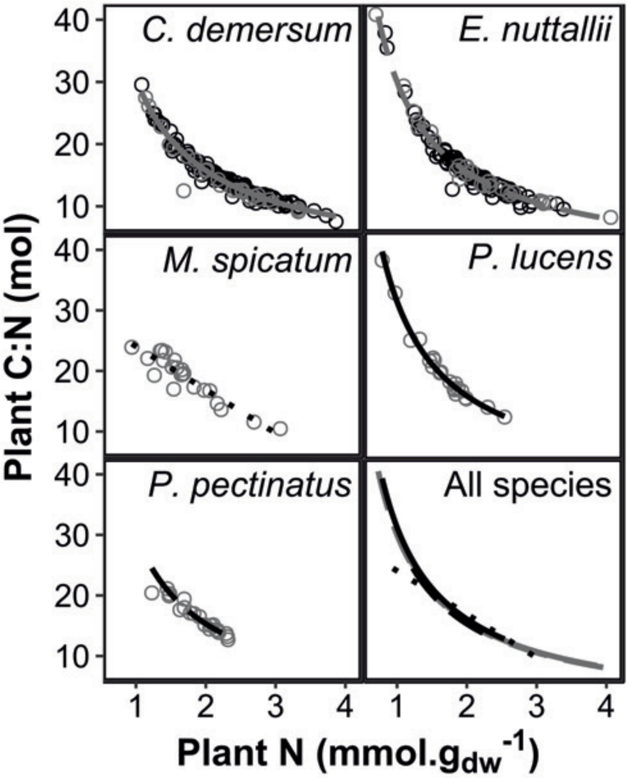

Figure S 2. Plant carbon to nitrogen (C:N, *above*), carbon to phosphorous (C:P, *righthand page top*), and nitrogen to phosphorous (N:P, *righthand page botton*) ratios in relation to plant N or P concentration. The relationships between C:N – N and C:P – P were highly significant for all species ($p < 0.001$; Adjusted R2: 0.86-0.99). Shoot N:P was mainly related to the shoot's P concentration ($p < 0.009$; Adjusted R2: 0.29-0.90) and less so to N.

Figure S 2 (continued).

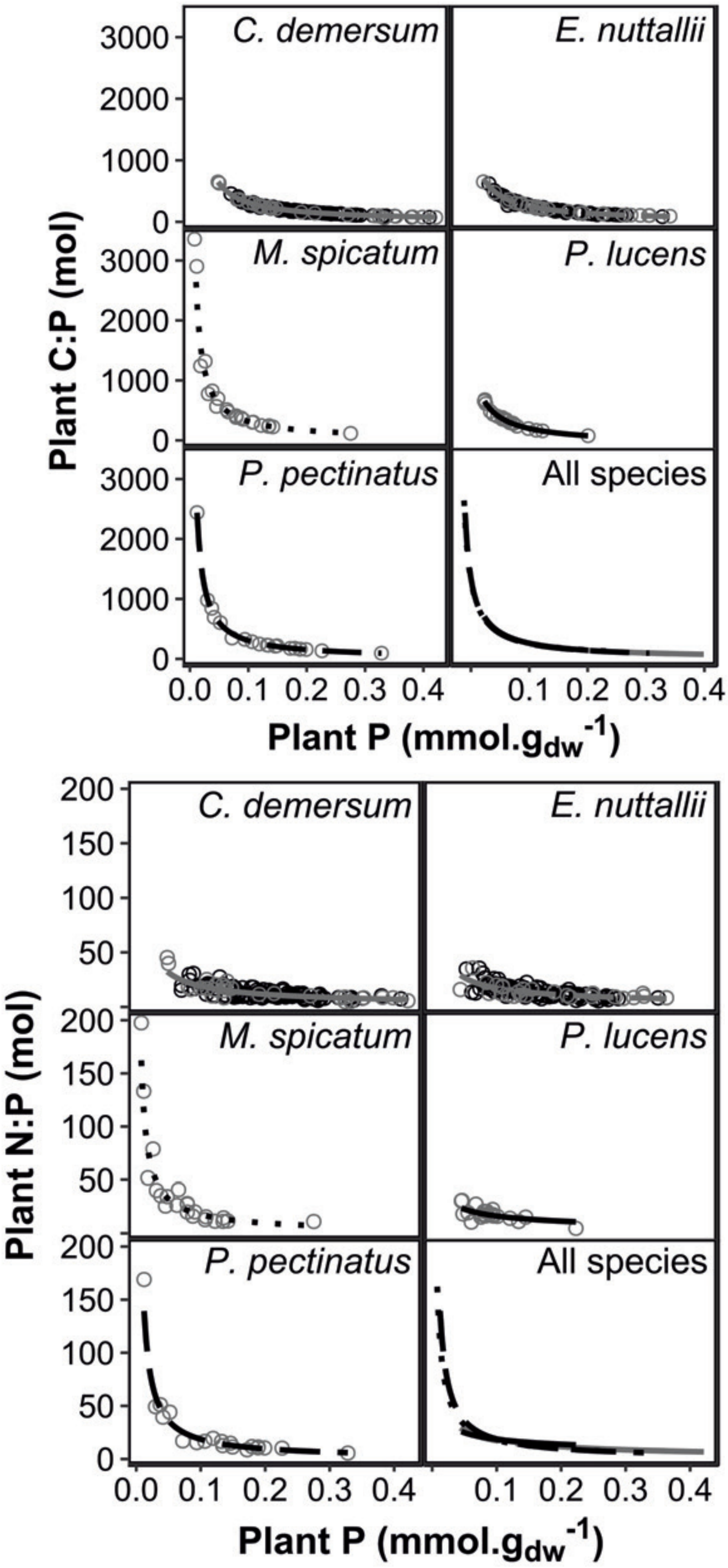

Table S 1. Range (min-max) and **mean ± standard deviation** of the carbon (C) concentration of the shoots of the plants and nitrogen (N), and phosphorous (P) concentration of the plants, water and sediment. For water and sediment, both plant available (free) and total amounts are shown. Number between brackets indicates sample size (n). Exponent indicates the applied power of 10, for example: $9^{-3} = 9*10^{-3} = 0.009$.

		Ceratophyllum demersum	*Elodea nuttallii*	*Myriophyllum spicatum*	*Potamogeton lucens*	*Potamogeton pectinatus*
Plant	C (mmol.g_{dw}^{-1})	21.0-34.5 **31.6±1.67** (135)	22.8-34.1 **31.0±1.65** (111)	22.5-34.4 **30.8±3.20** (22)	29.8-33.6 **31.9±1.12** (21)	25.0-32.8 **30.3±1.55** (20)
	N (mmol.g_{dw}^{-1})	1.08-3.86 **2.33±0.59** (135)	0.70-4.06 **2.11±0.58** (111)	0.94-3.07 **1.72±0.49** (22)	0.79-2.54 **1.68±0.41** (21)	1.23-2.32 **1.89±0.33** (20)
	P (mmol.g_{dw}^{-1})	4.80^{-2}-4.21^{-1} $\mathbf{1.97^{-1}\pm7.99^{-2}}$ (135)	4.40^{-2}-3.63^{-1} $\mathbf{1.70^{-1}\pm7.20^{-2}}$ (111)	7.80^{-3}-2.75^{-1} $\mathbf{8.16^{-2}\pm5.97^{-2}}$ (22)	4.53^{-2}-2.23^{-1} $\mathbf{9.05^{-2}\pm4.04^{-2}}$ (21)	1.20^{-2}-3.28^{-1} $\mathbf{1.30^{-1}\pm7.84^{-2}}$ (20)
Surface water	Free N (mmol.L^{-1})	5.00^{-4}-4.24^{-1} $\mathbf{6.09^{-2}\pm8.89^{-2}}$ (135)	0-3.96^{-1} $\mathbf{5.16^{-2}\pm7.95^{-2}}$ (111)	2.50^{-4}-4.50^{-2} $\mathbf{3.78^{-3}\pm9.37^{-3}}$ (22)	4.40^{-4}-6.72^{-2} $\mathbf{7.98^{-3}\pm1.80^{-2}}$ (21)	2.80^{-4}-6.72^{-2} $\mathbf{9.56^{-3}\pm1.93^{-2}}$ (20)
	Free P (mmol.L^{-1})	0-1.57^{-1} $\mathbf{1.53^{-2}\pm2.02^{-2}}$ (135)	0-1.57^{-1} $\mathbf{1.15^{-2}\pm2.08^{-2}}$ (111)	0-9.11^{-4} $\mathbf{4.93^{-5}\pm1.96^{-4}}$ (22)	0-2.34^{-2} $\mathbf{1.16^{-3}\pm5.10^{-3}}$ (21)	0-2.52^{-2} $\mathbf{5.81^{-3}\pm8.82^{-3}}$ (20)
	Total N (mmol.L^{-1})	6.00^{-3}-5.06^{-1} $\mathbf{9.34^{-2}\pm1.02^{-1}}$ (135)	2.80^{-3}-4.44^{-1} $\mathbf{8.00^{-2}\pm8.77^{-2}}$ (111)	2.00^{-3}-5.37^{-2} $\mathbf{1.35^{-2}\pm1.10^{-2}}$ (22)	4.00^{-4}-6.72^{-2} $\mathbf{1.67^{-2}\pm1.71^{-2}}$ (21)	4.30^{-3}-6.88^{-2} $\mathbf{2.04^{-2}\pm1.81^{-2}}$ (20)
	Total P (mmol.L^{-1})	3.00^{-4}-1.66^{-1} $\mathbf{2.08^{-2}\pm1.99^{-2}}$ (135)	2.00^{-4}-1.66^{-1} $\mathbf{1.87^{-2}\pm2.32^{-2}}$ (111)	2.15^{-4}-1.46^{-3} $\mathbf{6.31^{-4}\pm2.98^{-4}}$ (22)	2.10^{-4}-2.63^{-2} $\mathbf{2.05^{-3}\pm5.58^{-3}}$ (21)	2.10^{-4}-2.71^{-2} $\mathbf{8.04^{-3}\pm1.02^{-2}}$ (20)
Sediment	Free N (mmol.g_{dw}^{-1})	5.00^{-4}-2.43^{-2} $\mathbf{1.15^{-2}\pm5.56^{-3}}$ (132)	3.00^{-4}-2.43^{-2} $\mathbf{1.03^{-2}\pm6.15^{-3}}$ (107)	1.20^{-4}-4.10^{-3} $\mathbf{1.44^{-3}\pm1.14^{-3}}$ (22)	6.00^{-5}-1.28^{-2} $\mathbf{2.56^{-3}\pm3.20^{-3}}$ (20)	2.20^{-4}-1.28^{-2} $\mathbf{2.85^{-3}\pm3.37^{-3}}$ (19)
	Free P (mmol.g_{dw}^{-1})	1.50^{-4}-7.52^{-3} $\mathbf{2.46^{-3}\pm1.79^{-3}}$ (134)	1.00^{-4}-7.52^{-3} $\mathbf{1.82^{-3}\pm1.70^{-3}}$ (108)	3.90^{-5}-1.69^{-3} $\mathbf{3.49^{-4}\pm3.64^{-4}}$ (22)	9.60^{-5}-2.79^{-3} $\mathbf{8.14^{-4}\pm7.97^{-4}}$ (20)	4.60^{-5}-2.79^{-3} $\mathbf{9.31^{-4}\pm9.23^{-4}}$ (19)
	Total N (mmol.g_{dw}^{-1})	0-1.78 $\mathbf{1.20\pm5.30^{-1}}$ (123)	0-1.78 $\mathbf{1.05\pm5.54^{-1}}$ (104)	0-1.14 $\mathbf{2.05^{-1}\pm3.21^{-1}}$ (22)	0-1.81 $\mathbf{3.40^{-1}\pm5.45^{-1}}$ (21)	0-1.81 $\mathbf{4.26^{-1}\pm6.34^{-1}}$ (20)
	Total P (mmol.g_{dw}^{-1})	2.80^{-3}-1.78^{-1} $\mathbf{2.96^{-2}\pm1.91^{-2}}$ (135)	1.10^{-32}-1.78^{-1} $\mathbf{2.89^{-2}\pm2.05^{-}}$ (111)	1.13^{-3}-2.55^{-2} $\mathbf{6.43^{-3}\pm6.38^{-3}}$ (22)	1.10^{-3}-3.35^{-2} $\mathbf{1.03^{-2}\pm1.06^{-2}}$ (21)	1.00^{-3}-3.35^{-2} $\mathbf{1.39^{-2}\pm1.26^{-2}}$ (20)

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Table S 2. Associations between plant and environmental nutrient concentrations as described by the different statistical models used (i.e. linear and segmented linear). For the segmented model, Slope 2 - 1 indicates the difference between the slope of the second segment and the slope of the first segment. The segmented model could not identify any breakpoint in the data of *E. nuttallii*'s N concentration, indicated by 'NA'.

	Nutrient	Model	Parameters	Estimate	SE	t-value	p-value	Adj. R2	AIC
Ceratophyllum demersum	N	Null	Intercept	2.329	0.051				243.1
		Linear	Intercept	2.325	0.058	39.80	<0.001	0.00	225.9
			Slope	0.040	0.037	1.07	0.287		
		Segmented linear	Intercept	1.270	2.629	0.48	0.630	0.00	228.4
			Slope 1	-0.589	1.346	-0.44	0.662		
			Slope 2 - 1	0.655	1.347	0.49	0.628		
			Breakpoint	-1.556	0.871				
	P	Null	Intercept	0.197	0.007				-296.1
		Linear	Intercept	0.186	0.007	28.36	<0.001	0.20	-315.8
			Slope	0.023	0.004	5.78	<0.001		
		Segmented linear	Intercept	0.201	0.009	21.19	<0.001	0.27	-325.5
			Slope 1	0.050	0.010	4.83	<0.001		
			Slope 2 - 1	-0.056	0.015	-3.74	<0.001		
			Breakpoint	0.886	0.430				
Elodea nuttallii	N	Null	Intercept	2.112	0.055				197.5
		Linear	Intercept	2.126	0.059	35.99	<0.001	0.02	181.6
			Slope	0.076	0.041	1.85	0.067		
		Segmented linear	Intercept	NA	NA	NA	NA	NA	NA
			Slope 1	NA	NA	NA	NA		
			Slope 2 - 1	NA	NA	NA	NA		
			Breakpoint	NA	NA				
	P	Null	Intercept	0.170	0.007				-266.2
		Linear	Intercept	0.170	0.006	26.19	<0.001	0.17	-267.2
			Slope	0.022	0.005	4.72	<0.001		
		Segmented linear	Intercept	0.186	0.011	16.67	<0.001	0.20	-269.1
			Slope 1	0.042	0.011	3.83	<0.001		
			Slope 2 - 1	-0.041	0.017	-2.37	0.020		
			Breakpoint	0.500	0.629				
Myriopyllum spicatum	N	Null	Intercept	1.716	0.105				34.3
		Linear	Intercept	2.068	0.633	3.27	0.004	-0.03	36.0
			Slope	0.188	0.332	0.56	0.579		
		Segmented linear	Intercept	6.161	2.403	2.56	0.020	0.12	34.0
			Slope 1	2.188	1.166	1.88	0.077		
			Slope 2 - 1	-2.985	1.324	-2.25	0.037		
			Breakpoint	-1.842	0.133				

Table S 2 (continued).

	Nutrient	Model	Parameters	Estimate	SE	t-value	p-value	Adj. R2	AIC
Myriopyllum spicatum	P	Null	Intercept	0.082	0.013				-58.6
		Linear	Intercept	0.208	0.095	2.18	0.042	0.04	-58.5
			Slope	0.069	0.052	1.33	0.197		
		Segmented linear	Intercept	0.548	0.200	2.74	0.014	0.28	-63.2
			Slope 1	0.248	0.105	2.36	0.030		
			Slope 2 - 1	-0.474	0.161	-2.95	0.009		
			Breakpoint	-1.528	0.119				
Potamogeton lucens	N	Null	Intercept	1.678	0.089				24.71
		Linear	Intercept	1.406	0.244	5.76	<0.001	0.01	24.7
			Slope	-0.154	0.136	-1.13	0.273		
		Segmented linear	Intercept	0.974	0.456	2.14	0.049	0.05	25.5
			Slope 1	-0.382	0.242	-1.58	0.134		
			Slope 2 - 1	1.476	0.988	1.49	0.155		
			Breakpoint	-0.382	0.393				
	P	Null	Intercept	0.091	0.009				-72.2
		Linear	Intercept	0.140	0.018	7.84	<0.001	0.30	-74.0
			Slope	0.032	0.011	3.00	0.008		
		Segmented linear	Intercept	0.067	0.035	1.92	0.073	0.49	-78.9
			Slope 1	-0.010	0.020	-0.52	0.612		
			Slope 2 - 1	0.094	0.032	2.97	0.009		
			Breakpoint	-0.678	0.398				
Potamogeton pectinatus	N	Null	Intercept	1.890	0.073				14.8
		Linear	Intercept	2.078	0.175	11.89	0.000	0.01	14.2
			Slope	0.108	0.103	1.05	0.309		
		Segmented linear	Intercept	-19.293	23.735	-0.81	0.429	-0.01	16.1
			Slope 1	-9.980	11.149	-0.90	0.385		
			Slope 2 - 1	10.147	11.150	0.91	0.377		
			Breakpoint	-2.110	0.028				
	P	Null	Intercept	0.130	0.018				-42.1
		Linear	Intercept	0.188	0.018	10.26	0.000	0.46	-51.4
			Slope	0.053	0.013	4.04	0.001		
		Segmented linear	Intercept	0.487	0.594	0.82	0.425	0.42	-48.6
			Slope 1	0.210	0.302	0.69	0.498		
			Slope 2 - 1	-0.170	0.303	-0.56	0.583		
			Breakpoint	-1.757	0.434				


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Chapter 9

Synthesis

Michiel (M.J.J.M.) Verhofstad

Nuisance submerged aquatic plants

In this PhD-project I aimed to ***(1)*** *identify the main problems caused by nuisance aquatic plant growth and define when these problems may occur.* ***(2)*** *Identify why this problem is occurring at this moment in many parts of the world, including the Netherlands.* ***(3)*** *Identify what ecosystem managers of shallow freshwater ecosystems can do about the problems and finally* ***(4)*** *identify possible economical use the nuisance aquatic plants could provide (****Chapter 1****).*

*After completing the project, it has become clear that nuisance growth of aquatic plants is actually not a new 'upcoming' problem, but has also occurred in the past (e.g. Hasler, 1947; Murphy, 1988a). For example, Dutch newspapers already mention dense stands of plants causing problems in the late 1800's (e.g. Algemeen Handelsblad, 11-July-1883). Here, the invasive Elodea canadensis was blamed. In this thesis, I identified that many native species also caused nuisance. The problem is thus not limited to invasive species (****Chapter 2****). Furthermore, nuisance aquatic plant growth is not unique to the Netherlands, but is also a major problem in other European countries, the USA, Australia, Africa and many other countries all over the world (****Chapter 2****; e.g. Schoonbee, 1991; Mangan & Baars, 2013; Berger et al., 2015; Bickel & Schooler, 2015).*

(1) What is nuisance?

Before I could research the problem, a clear and measurable definition or description of the nuisance problem was required. However, I found out early on in the project that such a definition of at what dimensions (i.e. cover and height) an aquatic plant stand causes problems was not yet available. In **Chapter 2** we therefore defined which characteristics of an aquatic plant stand will determine whether it is considered to be a nuisance or not, and at what threshold levels of these characteristics the plants cause problems. I concluded that plant height, relative to the water surface (i.e. distance of plants to the water surface), and plant cover largely determined whether or not they were deemed a problem. Furthermore, I concluded that the threshold levels of height and cover can be very different for different ecosystem users (i.e. ecosystem services provided by the ecosystem). The general classification assumed that all species will equally be considered a problem if they occur above the set threshold levels.

While this likely is true in general, incorporating species-specific traits may be used to tailor the classification to specific lakes or waterways. Traits, such as tensile strength (i.e. the force needed to break the plant) may cause a stronger species to be considered a problem sooner than a weak species, as fewer stems of the strong species may already impair a motor boat while the same amount of stems of a weaker species will not. I therefore performed a pilot experiment to assess whether the force needed to break the shoots of different species is indeed different among aquatic plants and whether this strength is related to a measurable plant characteristic (BOX 1). Indeed, shoots of different species can differ in strength, but a large variation within species was also identified. I concluded that part of the strength a shoot has is determined by the area of the cross section of the stem, with thicker shoots being stronger (BOX 1: Fig. B1.2). It is therefore likely that not all species will be considered a nuisance at the exact same densities. Species specific information on, for example, their mechanical characteristics could be used to tailor the classification scheme to a specific ecosystem.

BOX 1: Tensile strength of freshwater macrophytes

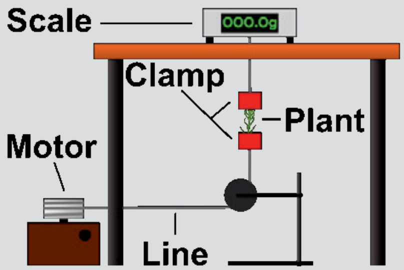


***Figure B1.1**: Schematic representation of the experimental setup.*

To measure the mechanical strength of different macrophyte species, I build a setup capable of measuring the force (in grams) needed to break the plant (for specifications see Fig. B1.1).

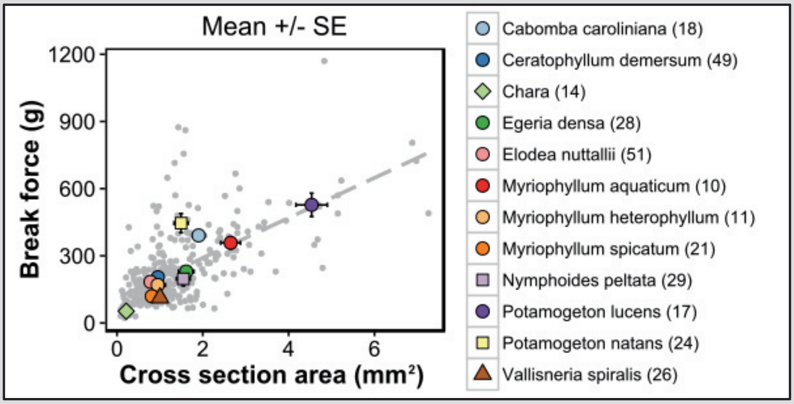


***Figure B1.2**: Force needed to break a shoot related to its cross section area. Colored points show the average per species, while the shape of these points indicates the growth form: submerged (circle), rooted with floating leaves (squares), macro-algae (diamond), rosette (triangle). The number between brackets adjacent to the species name shows the sample size. Grey points show the individual observations. The grey line shows the regression using all the data (Adjusted $R^2 = 0.38$, $p < 0.001$).*

I conclude that different macrophyte species have different breaking strength (ANOVA: $F = 45.3$, $p < 0.001$) and that the force needed to break the plant depends in part on the cross section area of the stem or leaf (Fig. B1.2).

(2) Factors enabling nuisance submerged macrophyte growth

Now that I defined at what dimensions an aquatic plant stand can become a nuisance, I can focus on under what conditions submerged macrophytes will generally grow tall with high areal coverage. In **Chapter 2**, we concluded that many submerged plant species are able to cause nuisance, including both native and non-native species and even charophytes. In **Chapter 3** we concluded that the volume of the submerged macrophytes (i.e. plant height / water depth * plant cover) can be high over a large range of environmental nutrient availability, but the chance of high plant volume was highest when the water transparency was high and the sediment available P was not too low. I argue that these conditions are typical for re-oligotrophicated ecosystems and for ecosystems under mild nutrient loading. I also stressed that the bottom-up processes, enabling submerged plants to grow to nuisance proportions, can be obscured by top-down pressures, such as herbivory or strong water currents.

The window of opportunity for nuisance submerged plant growth is probably relatively short during eutrophication compared to after restoration of clear water. If eutrophication increases nutrient availability in the water to a certain point, phytoplankton can become the dominant primary producer, resulting in the loss of submerged vegetation (Gulati & van Donk, 2002; Jeppesen et al., 2005; Khan & Ansari 2005; Scheffer & van Nes, 2007).

(3.1) Management of nuisance growth

Now that we identified under what conditions nuisance is most likely to occur, I will discuss which management methods can be used to effectively tackle the source of the nuisance problem. Because the problem is likely caused by a too high productivity of the ecosystem (i.e. high light and nutrient availability), changing these parameters will have the best chance of long-term reduction of aquatic plant growth and thus nuisance (**Chapter 4**; Bates and Hentges, 1976; Finlay and Vogt, 2016). Decreasing the light availability (e.g. increase water turbidity, or increase water depth) however is often not a suitable option, as this can adversely impact entire ecosystem functioning and may impair other ecosystem services provided (Nichols, 1991). Therefore, trying to lower the productivity of the ecosystem by decreasing nutrient availability seems most promising (Finlay and Vogt, 2016). Similarly, reduced nutrient loading in eutrophicated ecosystems subject to phytoplankton blooms resulted in vast improvements in ecosystem quality (Jeppesen et al., 2005). Here, decreasing productivity by decreasing nutrient load, reduced phytoplankton biomass and increased water quality and transparency.

However, because I have shown that at least some aquatic plant species are able to grow tall, even under relatively low nutrient availability, it may not always be possible for managers to reduce the productivity of the ecosystem until a level at which the plants will be severely nutrient limited and do not grow tall anymore (**Chapter 2 & 3**). If reducing nutrients is not possible because it is too expensive, too destructive or just not possible due to the external nutrient input into the system, top-down (i.e. effect oriented) measures can help alleviate the problems caused by tall aquatic plants. In this respect, mechanical harvesting can be a suitable management method (**Chapter 5**), as it directly reduces submerged plant height, it removes nutrients from the ecosystem in the form of plant biomass, it is highly controllable, and the harvested biomass can be used for a variety of applications. However, care needs to be taken not to stress the plants too much by too intensive harvesting, as the collapse of the submerged vegetation may result in jumping out of the frying pan into the fire (**Chapter 6**). Only applying plant management in the parts of the ecosystem where the plants actually cause problems may reduce management cost and simultaneously maintain the important ecosystem functions and services the plants perform in the unmanaged parts of the ecosystem.

(3.2) Effects of cutting on nuisance submerged macrophytes

To develop such a sustainable harvesting regime, I first needed to know how much stress the plants can cope with and how the plants respond to cutting and harvesting. During this project I performed literature surveys, controlled laboratory experiments, field experiments, ecosystem modelling experiments and also surveyed actual field harvesting events to assess of the effects of harvesting on submerged aquatic plants.

In the controlled laboratory experiment (**Chapter 4**) we found that major nuisance causing species (i.e. *Elodea nuttallii*, *Myriophyllum spicatum*, *Potamogeton perfoliatus*) are very tolerant to harvesting over a wide range of nutrient concentrations in the environment. This high degree of tolerance was also observed in a large outdoor pond experiment where *M. spicatum* regrew rapidly after harvesting, but high harvesting frequencies (i.e. monthly) reduced *M. spicatum* length and reduced its overall cover (**Chapter 5**). Our analysis with the shallow lake ecosystem model, PCLake, also confirmed that plants are generally tolerant to harvesting and nuisance was reduced only for a short period following a harvesting event (**Chapter 6**). This model simulation also revealed that harvesting a large part of the submerged vegetation in an ecosystem with alternative stable states could be dangerous. Harvesting too many plants, can decrease the vegetation’s competitive ability against phytoplankton and can cause a collapse of the vegetation and potentially toxic phytoplankton blooms. This effect may not be visible after just one year of harvesting, but may take several years, according to our model results.

Focusing on the ecosystem scale, indeed nuisance macrophytes generally seem to be very tolerant to cutting and harvesting, as a literature survey, combined with our own observations, showed that the effects of cutting management on the nuisance species were often only visible within the year of cutting (Murphy, 1988b; BOX 2). However, results varied among and within studies. Some studies showed that cutting may increase the biomass of the nuisance species, while others reported a negative effect (BOX 2). Additionally, the period after which the effects of cutting were visible differed among studies. Some found almost no effects at all, while in others, the effects of cutting on the vegetation remained visible for one more year or more (BOX 2).

But what will affect the response of plants to harvesting? Several factors of the harvesting method will influence how severe and long-lasting the effects of harvesting will be.

BOX 2: Longevity of the effects of cutting on the vegetation in the field

It was not possible within the duration of a single PhD project to assess what effects aquatic plant cutting has on the aquatic vegetation using a representatively large number of ecosystems. I therefore surveyed the literature for studies on the effects of harvesting under natural conditions and extracted data on if, and for how long, the effects were observed (Table B2.1).

***Table B2.1**: Effects of cutting and / or harvesting nuisance submerged plants. 'Target species' refers to the nuisance species on which management is targeted. Symbols indicate: **'0'** = no effect; **'y'** = short-lived effect observed within year of management; **'yy'** = effect observed in subsequent years; **'+'** = increase/positive effect; **'-'** = decrease/negative effect, and **'Fast'** in the 'general regrowth' column indicates the authors reported fast regrowth of the dominant species. If only a 'y' is given in the first column, plant species composition was affected, but not overall diversity (i.e. species are replaced by other species).*

Plant species number / diversity	Target species cover	Target species length	Target species standing biomass	Target species general regrowth	Period described (years)	Reference
y-	0				20+	Baattrup-Pedersen et al., 2002
				y-	2	Bailey & Calhoun, 2008
			y-		1	Dall'Armellina et al., 1996
	y-	y-	y-		1	David et al., 2006
0		0	y-		1	DiNino et al., 2005
y			y-	Fast	5	Engel, 1990
y+			y-		1	Garbey et al., 2003
y		y-		y-	2 to 3	Howard-Williams et al., 1996
0		y-	y-		2	Johnson et al., 1979
			y-		1	Keanel et al., 1998
			y-		1	Keanel & Uehlinger, 1999
			y-		1	Madsen et al., 1988
			y-		2	Monahan & Caffrey, 1996
y-	y+				2	Nielsen et al., 2006
	y-	0	yy-		4	Painter, 1988
y					1	Sabbatini & Murphy, 1996
	y-				2	Van den Berg et al., 2001
		y-	y-		1	Morris et al., 2006
y- | yy-					1	Pedersen et al., 2006
		y- & yy-			4	Unmuth et al., 1998
y- & yy	yy-			yy-	2	Boylen et al., 1996
0 | y	0 & y-		y-		2	Schwartz & Snelder, 1999
			y-	y+	1	Crowell et al., 1994
0 | y+			0 | y+		22	Nichols & Lanthrop, 1994
	y-		y-		1	Bickel and Closs, 2009
0 & y+					5	Palmik et al., 2013
y	y-				1	Pedersen et al., 2011
			y-	Fast	1	Bal and Meire, 2009
			y-	Fast	1	Bal et al., 2006
0 | yy					4	Baattrup-Pedersen and Riis, 2004
		y- & yy-		Fast	3	Olson et al., 1998
			y+	Fast	1	Serafy et al., 1994
yy+	00 & yy-		yy-		2	Eichler et al., 1993
0				0 & y-	1	Fox & Murphy, 1990
00 | yy+	00 | yy+	y-		Fast	5	Verhofstad et al. Oldambtmeer
0	y-	y-		Fast	3	Verhofstad et al. De Munt

I conclude that cutting nuisance vegetation may increase or decrease aquatic plant diversity and that cutting often results in short-term reduction of biomass and plant height of the nuisance species. Long-term effects were scarce and plants often regrew within the growing season. The lack of strong long-term effects may in part be caused by the general short timescale of several of the studies, but there is still overwhelming evidence that fast growing submerged plants are generally highly tolerant to cutting stress.

Factors such as cutting frequency/intensity (i.e. period of cutting), cutting depth, and timing of cutting can affect vegetation response to cutting. First, increasing the frequency of cutting can reduce plant length throughout the season, slow their recovery, and reduce standing biomass (**Chapter 5**, Wile, 1978; Madsen et al., 1988; Nielsen et al., 2006). Second, cutting the plants at the sediment level is more destructive than leaving part of the shoots intact and sensitive species can even die from cutting at the sediment level (Van den Berg et al., 2001; Van Zuidam & Peeters, 2012). Third, plants are able to recover easily if cut early in the growing season, therefore the effects of cutting generally last longer when plants are cut just after peak biomass is reached (Engel, 1990; Garbey et al., 2003; Bal et al., 2006). Overall, there generally is a need for multiple cutting events to keep plant height low throughout the season. Hence Madsen et al. (1988) advised to start harvesting management early in the season to keep the biomass low and decrease the harvesting effort needed in subsequent harvests. However, in ecosystem with alternative stable states, harvesting too many plants early in the season may result in a regime shift towards phytoplankton dominance (**Chapter 6**).

(3.3) Effects of cutting on non-target species

One important subject we have not yet explored is the effect of harvesting on non-target plant species. In some cases, harvesting may decrease the dominance of the nuisance species (e.g. **Chapter 5**) and increase plant biodiversity (BOX 2). But the opposite has also been reported, where cutting strengthens the dominance of the nuisance species, because it is particularly tolerant to stress compared to the other plant species (BOX 2). It remains unclear under what conditions harvesting will increase and when it will decrease plant diversity, but ecosystem productivity may play a role (e.g. **Chapter 4**). Zooming out to the landscape level, management may increase the diversity of the aquatic vegetation on a landscape scale, if different sites are managed at different times; so-called cyclic management (**Chapter 7**, Milsom et al., 2004; Palmik et al., 2013; Zhang et al., 2014, but see Baattrup-Pedersen & Riis, 2004). This temporal-spatial variation in management should aim to create a diverse landscape with a lot of different habitats, thereby increasing the amount of niches and thus the amount of species that can live in this heterogeneous landscape (**Chapter 7**).

Next to affecting non-target plant species, cutting and harvesting aquatic plants can also impact animal species. For example, mechanical management (e.g. cutting) may reduce macro-invertebrate diversity (Habib & Yousus, 2014) and kill fish (Sefary et al., 1994). On the other hand, cutting management can also increase fish stocks in the long-term, by creating more a diverse habitat (Olsen et al., 1998). Last, mowing machinery (e.g. cutter boats) can also affect both flora and fauna indirectly by altering abiotics. For example, mowing machinery may suspend sediment particles and thereby increasing turbidity and reducing oxygen levels, depending on the type of mowing boat, the type of sediment, and on the water depth. Harvesting may also decrease hydraulic resistance in waterways and thereby increase flow (Bal & Meir, 2009), which is why if it so often used in channels and ditches.

BOX 3: Applications of aquatic plant biomass

To find out what harvested submerged plant biomass can be used for, I searched the scientific literature for information. Table B3.1 summarizes the main applications for aquatic plant biomass and lists aquatic plant species that have already been used or tested for this application. It also shows the biomass requirements for the application.

***Table B3.1**: Applications for aquatic plant biomass. The number in front of the species names indicates the number of species mentioned.*

Application	Growth form	Species mentioned	General biomass requirements	References (Incl. cited literature)
Fertilizer / soil conditioner (e.g. Agricultural compost / fish pond fertilizer)	Emergent	[4+] *Aeschynomeme* spp.; *Limnocharis flava*; *Phragmites australis; Sesbania bispinosa*	Preferably fast decomposition, high nutrient concentrations and no toxins.	Edwards, 1980; Köbbing et al., 2013
	Free floating	[4+] *Azolla pinnata*; *Eichhornia crassipes*; *Pistia stratiotes*; *Salvinia* spp.; *Spirodela polyrrhiza*		Edwards, 1980
	Rooted with floating leaves	[1] *Stratiotes aloides*		Dutch newspapers, 1808-1941
	Submerged	[1] *Elodea spp.; Hydrilla verticillata*		Edwards, 1980; Ho et al., 2015
Human food	Emergent	[20+] *Aponogeton* spp. tubers; *Colocasia esculenta*; *Cyperus esculentus* tubers; *Cyrtosperma chamissonis*; *Eleocharis dulcis* tubers; *Ipomoea aquatica*; *Limnocharis flava* shoots; *Ludwigia adscendens* young leaves and shoots; *Ludwigia repens* young leaves and shoots; *Monochoria* spp. leaves; *Nasturtium heterophyllum*; *Neptunia oleracea*; *Oryza sativa* seeds; *Rorippa nasturtium-aquaticum*; *Sagittaria sagittifolia*; *Sium sisarum* roots; *Sphenoclea zeylandica* young plants; *Typha angustifolia* rhizomes; *Zizania aquatica* seeds; *Zizania latifolia* seeds and stems	Preferably good taste, high nutritional value and must be free of pathogens and toxins.	Edwards, 1980
	Free floating	[4] *Eichhornia crassipes* young leaves and flowers (may itch); *Hygroryza aristata* seeds; *Pistia stratiotes*; *Wolffia arrhiza*		Edwards, 1980

	Rooted with floating leaves	[13+]	*Aponogeton* spp. tubers; *Dicraeanthus* spp. stems and leaves; *Euryale ferox* seeds and fruits; *Nelumbo nucifera*; *Nymphaea lotus*; *Nymphaea nouchali*; *Nymphaea stellata*; *Trapa bicornis* nuts; *Trapa bispinosa* nuts; *Trapa incisa* nuts; *Trapa natans* nuts; *Victoria amazonica* seeds; *Victoria cruziana* seeds		Edwards, 1980
	Submerged	[5+]	*Aponogeton* spp. tubers; *Blyxa lancifolia* leaves; *Ottelia alismoides* shoots; *Potamogeton* sp. rhizomes; *Myriophyllum aquaticum* shoot tips		Edwards, 1980
Livestock feed	Emergent	[12+]	*Aponogeton* spp. tubers; *Alternanthera philoxoides*; *Coix aquatica*; *Hygroryza aristata*; *Ipomoea aquatica*; *Leersia hexandra*; *Limnocharis flava*; *Panicum geminatum*; *Paspalidium geminatum*; *Phragmites australis*; *Sagittaria* spp. vegetable parts; *Typha* sp.	In general biomass must be free of pathogens and toxins and should be (partly) dehydrated. Biomass should be rich in carbohydrates, protein, nutrients and fibres. Possibly needs to be mixed with traditional fodder due to relatively low palatability.	Edwards, 1980; Nevel et al., 1997; Dung et al., 2002; Köbbing et al., 2013; Quilliam et al., 2015
	Free floating	[5+]	*Azolla pinnata*; *Eichhornia crassipes*; *Lemna minor*; *Lemna spp.*; *Pistia stratiotes*; *Salvinia* sp.		Edwards, 1980; Dung et al., 2002; Quilliam et al., 2015
	Rooted with floating leaves	[2+]	*Aponogeton* spp. tubers; *Nymphaea stellata*		Edwards, 1980; Quilliam et al., 2015
	Submerged	[4+]	*Aponogeton* spp. tubers; *Ceratophyllum demersum*; *Hydrilla verticillata*; *Potamogeton crispus*		Edwards, 1980; Quilliam et al., 2015; Shaltout et al., 2016
Fish feed	Emergent	[1]	*Ipomoea reptans*	High protein content and protein to energy ratio, potentially has to be mixed with other feeds.	Kalita et al., 2007
	Free floating	[4]	*Echhornia crassipes*; *Lemna minor*; *Salvinia cuculata*; *Spirodela polyrrhiza*		Edwards, 1980; Kalita et al., 2007; Saha & Ray, 2011
	Rooted with floating leaves	[1]	*Trapa natans*		Kalita et al., 2007
	Submerged	[3]	*Ceratophyllum demersum*; *Elodea canadensis*; *Hydrilla verticillata*		Edwards, 1980
Biofuel (e.g. CH4 / bioethanol / in combination with fertilizer)	Emergent	[6+]	*Ceratopteris thalictroides; Cyperas spp.; Phragmites australis; Scirpus grosses; Typha* spp.; *Utricularia reticulata*	Suitability depends in part on particle size, water content, volatile solids content, and nutrient content. Possibly needs to be mixed with other substrates.	Abbasi et al., 1990; Wilkie & Evans, 2010; Köbbing et al., 2013; Quilliam et al., 2015
	Free floating	[4]	*Azolla pinnata*; *Eichhornia creassipes*; *Pistia stratiotes*; *Salvinia molesta*		Edwards, 1980; Abbasi et al., 1990; O'Sullivan et al., 2010; Wilkie & Evans, 2010; Quilliam et al., 2015

BOX 3 (continued)

Application	Growth form		Species mentioned	General biomass requirements	References
Biofuel (continued)	Rooted with floating leaves	[1]	*Nymphaea stellata*		Abbasi et al., 1990; Wilkie & Evans, 2010; Quilliam et al., 2015
	Submerged	[3]	*Cabomba caroliniana*; *Elodea nuttallii*; *Hydrilla verticillata*		Abbasi et al., 1990; Muñoz Escobar et al., 2010; O'Sullivan et al., 2010; Wilkie & Evans, 2010; Quilliam et al., 2015
Building material (e.g. Roofing / fiber / fencing)	Emergent	[1]	*Phragmites australis*	Suitability depends on application, but durability, moisture content, plant size, air content, cellulose content, and fibre tensile strength have been reported.	Nevel et al., 1997; Köbbing et al., 2013
	Free floating	[1]	*Eichhornia crassipes*		Ayudhya, 2016
	Rooted with floating leaves		None specified		
	Submerged		None specified		
Pharmaceutical (e.g. Medicine)	Emergent		None specified	High concentrations of extractable pharmaceuticals should be present, or the parts of the plant should have medicinal properties. Biomass should not contain toxins or bitter taste. *E. nuttallii* contains beta-sitosterol.	
	Free floating		None specified		
	Rooted with floating leaves		None specified		
	Submerged	[3]	*Elodea canadensis*; *Elodea nuttallii*; *Elodea potamogeton*		Muñoz Escobar et al., 2010
Additional: Biochar	Not specified		None specified	Not specified	Quilliam et al., 2015

(4) Useful applications of nuisance aquatic plants

So far, I have mainly discussed the negative aspects of massive growth of aquatic plants, but there may also positive aspects of this massive growth of submerged plants. We showed in **Chapter 5** that the same species that can cause nuisance, can be ideal for constructed wetlands designed for water polishing. These nuisance causing plant species are especially useful because of the characteristics they possess that are also responsible for the nuisance: rapid growth and tolerance to mechanical stress.
Theoretically, harvesting submerged plants may thus reduce nutrient availability in natural ecosystems as well, thereby reducing the productivity of the system and thus reducing nuisance growth of the plants themselves. However, the amount of nutrients removed via harvesting of plant biomass is often low relative to the entire nutrient budget of the ecosystem and will therefore unlikely be a viable method for reducing nutrient availability to the required levels (i.e. oligotrophication) in more natural ecosystems, especially when the ecosystem still receives high nutrient input (**Chapter 6**; Peterson et al., 1974; Carpenter & Adams, 1977; but see **Chapter 5**).

In general, the harvested biomass from any ecosystem where nuisance growth needs to be managed can potentially be used for a wide variety useful application (Bates & Hentges, 1976). All over the world aquatic plants have been, and still are, harvested, sold, and used for a wide variety of applications, for example as agricultural fertilizer, as food for human consumption, as animal feed or as a building material (for more information see: BOX 3). The suitability of the harvested biomass for these different applications depends on biomass characteristics, such as tissue nutrient concentration (Edwards, 1980; Quilliam et al., 2015; BOX 3). I already showed in the introduction of this thesis (**Chapter 1: Fig. 3**), that there is large variation in biomass elemental composition, both among and within aquatic plant species. In **Chapters 4, 5 & 8** I showed that part of the variation can be caused by the environmental nutrient availability and that biomass nutrient concentration varies over the growing season. This information can be used to find the best suited application for the biomass, depending on the location and timing of harvesting.

In the western world, many of these applications of aquatic plant biomass have been forgotten and have become disused. I would like to make a plea that we look towards other parts of the world and to our own history and start reusing this harvested biomass in a sustainable way. This may also help to reduce the net cost of aquatic plant management (Quilliam et al., 2015). Perhaps the most promising application that can be implemented rapidly is the use of the biomass as agricultural fertilizer and soil conditioner. This application can be especially beneficial because it can help to close the local nutrient cycle and reduce our dependency on finite phosphate rock if no additional chemical fertilizers are used on the nearby agricultural lands (Quilliam et al., 2015; Chowdhury et al., 2017). Another advantage of this application is that the biomass can often be used locally, keeping transportation costs to a minimum. The biomass also needs little to no processing before it can be used. Another advantage is that this application can be combined with other sustainable initiatives, such as using the biomass to produce biofuel, as waste product of the biofuel production still holds most of the nutrients that can thus be used as a fertilizer (O'Sullivan et al., 2010; Wilkie & Evans, 2010). Next to biomass characteristics, the demand in the market also largely influences what biomass applications are most viable (Quilliam et al., 2015). We believe that global research on the suitability of biomass for all possible applications can aid in creating new markets.

Conclusions

The amount of plants that are considered problematic differs among different ecosystem services and the perception of the local users of the ecosystem. Quantitatively describing nuisance is essential for both science and ecosystem management as it provides scientists and managers with measurable thresholds levels of nuisance, enabling the definition of management goals and enabling the evaluation of whether the goals are reached.

I also conclude that it will be an important challenge to balance managing the nuisance plant stands and maintaining enough plants to perform their important ecosystem functions, because small plant stands can already be perceived as a nuisance by some ecosystem users and harvesting too many plants may cause nuisance phytoplankton blooms. Therefore, tackling the bottom-up processes enabling nuisance growth of plants is the only viable option for long-term, effective, and predictable management of the nuisance problem, but this may often not be feasible. This low feasibility is expected because nuisance growth can occur in a wide variety of shallow ecosystems, as many ecosystems are not nutrient poor enough to limit the plants to grow tall. Furthermore, many species, both native and invasive, have the potential to grow to nuisance proportions. One possible solution around the problems with bottom-up management could be to spatially separate the ecosystem into parts that are managed top-down where the ecosystem services for humans take place (e.g. recreation) and into parts where plants are left unmanaged to perform their essential functions. Even though this management scheme needs more research, I suggest that harvesting may be a good top-down solution to reduce nuisance locally when the cause of the problem cannot be addressed. If harvesting is applied correctly, it can directly reduce nuisance when it occurs, although the effects are often short-lived. Another advantage of harvesting is that it removes nutrients from the ecosystem. A downside of this method is that it is relatively expensive, but spatially dividing the ecosystem and only managing parts where nuisance actually is a problem may reduce the costs.

Perspectives

Last, I recommend that further research on aquatic plant physiology is vital for successful management of nuisance aquatic plant growth, as this will increase our understanding on how the plants will respond to their environment and to management and thereby increase the predictability and effectiveness of management. Furthermore, it is important to monitor the vegetation for multiple years to gain insight in the ecosystem effects of harvesting. I only found a few studies that monitored the effects of aquatic plant management over many years, while the effects may be only visible over multiple years (e.g. **Chapter 6**; Painter, 1988; as discussed for herbivory by Miler & Straile et al., 2010). Another reason to monitor over multiple years is because factors influencing plant growth, such as weather influences on water temperature, affect the vegetation in general and thus possibly also the effects of harvesting (e.g. Zhang et al., 2016).

A final, but most important message is that we should not focus on the negative sides of nuisance aquatic plants alone, but also always remember the multitude of benefits the plants bring to the ecosystem, such as promoting clear water and providing habitat and food for many species, which in turn also provide many ecosystem services to humans.

Chapter 10

References

References

A

Abbasi S.A., Nipaney P.C. & Schaumberg G.D. (1990) Bioenergy potential of 8 common aquatic weeds. *Biological Wastes,* **34,** 359-366.

Abernethy V.J., Sabbatini M.R. & Murphy K.J. (1996) Response of *Elodea canadensis* Michx and *Myriophyllum spicatum* L to shade, cutting and competition in experimental culture. *Hydrobiologia,* **340,** 219-224.

AERF: Aquatic Ecosystem Restoration Foundation (2005) Best management practices handbook for aquatic plant management in support of fish and wildlife habitat. *Aquatic Ecosystem Restoration Foundation*, Marietta, GA. USA.

Agren G.I. & Weih M. (2012) Plant stoichiometry at different scales: element concentration patterns reflect environment more than genotype. *New Phytologist,* **194,** 944-952.

Aguiar F.C.F. & Ferreira M.T. (2013) Plant invasions in the rivers of the Iberian Peninsula, south-western Europe: A review. *Plant Biosystems,* **147,** 1107-1119.

Aivazidou E., Tsolakis N., Iakovou E. & Vlachos D. (2016) The emerging role of water footprint in supply chain management: A critical literature synthesis and a hierarchical decision-making framework. *Journal of Cleaner Production,* **137,** 1018-1037.

Akhurst D.J., Jones G.B., Clark M. & Reichelt-Brushett A. (2012) Effects of carp, gambusia, and Australian bass on water quality in a subtropical freshwater reservoir. *Lake and Reservoir Management,* **28,** 212-223.

Albright T.P. & Ode D.J. (2011) Monitoring the dynamics of an invasive emergent macrophyte community using operational remote sensing data. *Hydrobiologia,* **661,** 469-474.

Aldenberg T., Janse J.H. & Kramer P.R.G. (1995) Fitting the dynamic model PCLake to a multi-lake survey through Bayesian Statistics. *Ecological Modelling,* **61,** 83–99.

Alwin T.G., Fox M.G. & Cheruvelill K.S. (2010) Estimating Lake-wide Watermilfoil Weevil (*Euhrychiopsis lecontei*) Density: The Roles of Quadrat Size, Sample Size, and Effort. *Journal of Aquatic Plant Management,* **48,** 96-102.

Amoros C. (2001) The Concept of Habitat Diversity Between and Within Ecosystems Applied to River Side-Arm Restoration. *Environmental Management*, **28,** 805–817.

Anderson L.W.J. (2003) A review of aquatic weed biology and management research conducted by the United States Department of Agriculture - Agricultural Research Service. *Pest Management Science,* **59,** 801-813.

Angelstein S. & Schubert H. (2008) *Elodea nuttallii*: Uptake, translocation and release of phosphorus. *Aquatic Biology,* **3,** 209–216.

Angelstein S., Wolfram C., Rahn K., Kiwel U., Frimel S., Merbach I. & Schubert H. (2009) The influence of different sediment nutrient contents on growth and competition of *Elodea nuttallii* and *Myriophyllum spicatum* in nutrient-poor waters. *Fundamental and Applied Limnology,* **175,** 49-57.

Armitage P.D., Szoszkiewicz K., Blackburn J.H. & Nesbitt I. (2003) Ditch communities: A major contributor to floodplain biodiversity. *Aquatic Conservation: Marine and Freshwater Ecosystems,* **13,** 165–185.

Arthur G.D., Stirk W.A., Novak O., Hekera P. & Van Staden J. (2007) Occurrence of nutrients and plant hormones (cytokinins and IAA) in the water fern *Salvinia molesta* during growth and composting. *Environmental and Experimental Botany,* **61,** 137-144.

Ayudhya B.I.N. (2016) Comparison of compressive and splitting tensile strength of autoclaved aerated concrete (AAC) containing water hyacinth and polypropylene fibre subjected to elevated temperatures. *Materials and Structures,* **49,** 1455-1468.

B

Baattrup-Pedersen A., Larsen S.E. & Riis T. (2002) Long-term effects of stream management on plant communities in two Danish lowland streams. *Hydrobiologia,* **481,** 33-45.

Baattrup-Pedersen A. & Riis T. (2004) Impacts of different weed cutting practices on macrophyte species diversity and composition in a Danish stream. *River Research and Applications,* **20,** 103-114.

Bacchus S.T. & Barile P.J. (2005) Discriminating sources and flowpaths of anthropogenic nitrogen discharges to Florida springs, streams and lakes. *Environmental & Engineering Geoscience,* **11,** 347-369.

Bachmann R.W., Horsburgh C.A., Hoyer M.V., Mataraza L.K. & Canfield D.E. (2002) Relations between trophic state indicators and plant biomass in Florida lakes. *Hydrobiologia,* **470,** 219-234.

Bailey J.E. & Calhoun A.J.K. (2008) Comparison of Three Physical Management Techniques for Controlling Variable-leaf Milfoil in Maine Lakes. *Journal of Aquatic Plant Management,* **46,** 163-167.

Bakker E.S., Dobrescu I., Straile D. & Holmgren M. (2013a) Testing the stress gradient hypothesis in herbivore communities: facilitation peaks at intermediate nutrient levels. *Ecology,* **94,** 1776-1784.

Bakker E.S. & Nolet B.A. (2014) Experimental evidence for enhanced top-down control of freshwater macrophytes with nutrient enrichment. *Oecologia,* **176,** 825-836.

Bakker E.S., Pages J.F., Arthur R. & Alcoverro T. (2016) Assessing the role of large herbivores in the structuring and functioning of freshwater and marine angiosperm ecosystems. *Ecography,* **39,** 162-179.

Bakker E.S., Sarneel J.M., Gulati R.D., Liu Z.W. & Van Donk E. (2013b) Restoring macrophyte diversity in shallow temperate lakes: biotic versus abiotic constraints. *Hydrobiologia,* **710,** 23-37.

Bakker E.S., Van Donk E., Declerck S.A.J., Helmsing N.R., Hidding B. & Nolet B.A. (2010) Effect of macrophyte community composition and nutrient enrichment on plant biomass and algal blooms. *Basic and Applied Ecology,* **11,** 432-439.

Bakker E.S., Wood K.A., Pages J.F., Veen G.F., Christianen M.J.A., Santamaria L., Nolet B.A. & Hilt S. (2016) Herbivory on freshwater and marine macrophytes: A review and perspective. *Aquatic Botany,* **135,** 18-36.

Bakker S.A., Berg N.J. van den & Speleers B.R. (1994) Vegetation transitions of floating wetlands in a complex of turbaries between 1937 and 1989 as determined from aerial photographs with GIS. *Vegetatio,* **114,** 161–167.

Bal K., Struyf E., Vereecken H., Viaene P., De Doncker L., De Deckere E., Mostaert F. & Meire P. (2011) How do macrophyte distribution patterns affect hydraulic resistances? *Ecological Engineering,* **37,** 529-533.

Bal K.D. & Meire P. (2009) The Influence of Macrophyte Cutting on the Hydraulic Resistance of Lowland Rivers. *Journal of Aquatic Plant Management,* **47,** 65-68.

Bal K.D., Van Belleghem S., De Deckere E. & Meire P. (2006) The re-growth capacity of sago pondweed following mechanical cutting. *Journal of Aquatic Plant Management,* **44,** 139-141.

Baldy V., Thiebaut G., Fernandez C., Sagova-Mareckova M., Korboulewsky N., Monnier Y., Perez T. & Tremolieres M. (2015) Experimental assessment of the water quality influence on the phosphorus uptake of an invasive aquatic plant: Biological responses throughout its phenological stage. *PLoS One,* **10,** doi: 10.1371/journal.pone.0118844

Baptist M.J., Penning W.E., Duel H., Smits A.J.M., Geerling G.W., van der Lee G.E.M., et al. (2004) Assessment of the Effects of Cyclic Floodplain Rejuvenation on Flood Levels and Biodiversity Along the Rhine River. *River Research and Applications,* **20,** 285–297.

Barendregt A., Stam S.M.E. & Wassen M.J. (1992) Restoration of fen ecosystems in the Vecht River plain : cost-benefit analysis of hydrological alternatives. *Hydrobiologia,* **233,** 247–258.

Barko J.W., Gunnison D. & Carpenter S.R. (1991) Sediment interactions with submersed macrophyte growth and community dynamics. *Aquatic Botany,* **41,** 41-65.

Barko J.W. & Smart R.M. (1986) Sediment-related mechanisms of growth limitation in submersed macrophytes. *Ecology,* **67,** 1328-1340.

Barrat-Segretain M.H. (2004) Growth of *Elodea canadensis* and *Elodea nuttallii* in monocultures and mixture under different light and nutrient conditions. *Archiv Fur Hydrobiologie,* **161,** 133-144.

Barrat-Segretain M.H. & Amoros C. (1996) Recolonization of cleared riverine macrophyte patches: importance of the border effect. *Journal of Vegetation Science,* **7,** 769–776.

Baselga A. (2010) Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography,* **19,** 134–143.

Bates R.P. & Hentges J.F. (1976) Aquatic weeds - eradicate or cultivate. *Economic Botany,* **30,** 39-50.

Bel G., Hagberg A. & Meron E. (2012) Gradual regime shifts in spatially extended ecosystems. *Theoretical Ecology,* **5,** 591–604.

Berger S.T., Netherland M.D. & Macdonald G.E. (2015) Laboratory Documentation of Multiple-Herbicide Tolerance to Fluridone, Norflurazon, and Topramazone in a Hybrid Watermilfoil (*Myriophyllum spicatum* x *M. sibiricum*) Population. *Weed Science,* **63,** 235-241.

Bernes C., Carpenter S.R., Gårdmark A., Larsson P., Persson L., Skov C., Speed J.D. & Van Donk E. (2015) What is the influence of a reduction of planktivorous and benthivorous fish on water quality in temperate eutrophic lakes? A systematic review. *Environmental Evidence,* **4,** 1.

Bernhardt E.S., Band L.E., Walsh C.J. & Berke P.E. (2008) Understanding, managing, and minimizing urban impacts on surface water nitrogen loading. *Annals of the New York Academy of Sciences,* **1134,** 61-96.

Best E.P.H., Woltman H. & Jacobs F.H.H. (1996) Sediment-related growth limitation of *Elodea nuttallii* as indicated by a fertilization experiment. *Freshwater Biology,* **36,** 33-44.

Bickel T.O. & Closs G.P. (2009) Impact of partial removal of the invasive macrophyte *Lagarosiphon major* (hydrocharitaceae) on invertebrates and fish. *River Research and Applications,* **25,** 734-744.

Bickel T.O. & Schooler S.S. (2015) Effect of water quality and season on the population dynamics of *Cabomba caroliniana* in subtropical Queensland, Australia. *Aquatic Botany,* **123,** 64-71.

Bini L.M., Thomaz S.M., Murphy K.J. & Camargo A.F.M. (1999) Aquatic macrophyte distribution in relation to water and sediment conditions in the Itaipu Reservoir, Brazil. *Hydrobiologia,* **415,** 147-154.

Blindow I., Hargeby A. & Hilt S. (2014) Facilitation of clear-water conditions in shallow lakes by macrophytes: differences between charophyte and angiosperm dominance. *Hydrobiologia,* **737,** 99-110.

Bloemendaal F.H.J. L. & Roelofs J.G.M. (1988) *Waterplanten en waterkwaliteit.* Natuurhistorische Bibliotheek no 45, Stichting Uitgeverij van de Koninklijke Natuurhistorische Vereniging te Utrecht en Vakgroep Aquatische Oecologie en Biogeologie van de Katholieke Universiteit te Nijmegen. 189 pp.

Bociag K., Galka A., Lazarewicz T. & Szmeja J. (2009) Mechanical strength of stems in aquatic macrophytes. *Acta Societatis Botanicorum Poloniae,* **78,** 181-187.

Boiché A., Lemoine D.G., Barrat-Segretain M.H. & Thiébaut G. (2011) Resistance to herbivory of two populations of *Elodea canadensis* Michaux and *Elodea nuttallii* Planchon St. John. *Plant Ecolology,* **212,** 1723–1731.

Bole J.B. & Allan J.R. (1978) Uptake of phosphorus from sediment by aquatic plants, *Myriophyllum spicatum* and *Hydrilla verticillata*. *Water Research,* **12,** 353-358.

Bornette G. & Puijalon S. (2009) Macrophytes: Ecology of Aquatic Plants. In: *Encyclopedia of Life Sciences*. John Wiley & Sons Ltd., Chichester, UK.

Bornette G. & Puijalon S. (2011) Response of aquatic plants to abiotic factors: a review. *Aquatic Sciences,* **73,** 1-14.

Boylen C.W., Eichler L.W. & Sutherland J.W. (1996) Physical control of Eurasian watermilfoil in an oligotrophic lake. *Hydrobiologia,* **340,** 213-218.

Brandrud T.E. (2002) Effects of liming on aquatic macrophytes, with emphasis on Scandinavia. *Aquatic Botany,* **73,** 395-404.

Brandrud T.E. & Roelofs J.G.M. (1995) Enhanced growth of the macrophyte *Juncus bulbosus* in S Norwegian limed lakes. A regional survey. *Water Air and Soil Pollution,* **85,** 913-918.

Brate Bryant C. (1973) Control of Aquatic Weeds by Mechanical Harvesting. *PANS Pest Articles & News Summaries,* **19,** 601-606.

Brederveld R.J., Jähnig S.C., Lorenz A.W., Brunzel S. & Soons M.B. (2011) Dispersal as a limiting factor in the colonization of restored mountain streams by plants and macroinvertebrates. *Journal of Applied Ecology*, **48,** 1241–1250.

Brock G., Pihur V., Datta S. & Datta S. (2011) clValid, an R package for cluster validation. *Journal Of Statistical Software,* **25**.

Bruinsma J., Krause W., Nat E. & Van Raam J. (1998) *Determinatietabel voor kranswieren in de Benelux,* Stichting Jeugdbondsuitgeverij, Utrecht, The Netherlands.

Bryhn A.C. & Håkanson L. (2007) A comparison of predictive phosphorus load-concentration models for lakes. *Ecosystems,* **10,** 1084–1099.

Burlakova L.E. & Karatayev A.Y. (2007) The effect of invasive macrophytes and water level fluctuations on unionids in Texas impoundments. *Hydrobiologia,* **586,** 291-302.

Burton T.M., King D.L. & Ervin J.L. (1978) Aquatic plant harvesting as a lake restoration technique. In: *Lake restoration: proceedings of a national conference,* August 22-24, 1978, Minneapolis, Minnesota. pp 177–185

C

Cabrera-Walsh G., Schooler S. & Julien M. (2011) Biology and preliminary host range of *Hydrotimetes natans* Kolbe (Coleoptera: Curculionidae), a natural enemy candidate for biological control of *Cabomba caroliniana* Gray (Cabombaceae) in Australia. *Australian Journal of Entomology,* **50,** 200-206.

Caffrey J.M., Millane M., Evers S., Moran H. & Butler M. (2010) A novel approach to aquatic weed control and habitat restoration using biodegradable jute matting. *Aquatic Invasions,* **5,** 123-129.

Cao J.-J., Wang Y. & Zhu Z.-L. (2012) Growth response of the submerged macrophyte *Myriophyllum spicatum* to sediment nutrient levels and water-level fluctuations. *Aquatic Biology,* **17,** 295-303.

Carignan R. & Kalff J. (1980) Phosphorus sources for aquatic weeds - water or sediments. *Science,* **207,** 987-989.

Carpenter S.R. (1981) Submersed Vegetation : An Internal Factor in Lake Ecosystem Succession. *The American Naturalist,* **118,** 372–383.

Carpenter S.R. & Adams M.S. (1977a) *Environmental Impacts of Mechanical Harvesting of Submersed Vascular Plants,* Center for Biotic Systems, Institute for Environmental Studies, University of Wisconsin.

Carpenter S.R. & Adams M.S. (1977b) The macrophyte tissue nutrient pool of a hardwater eutrophic lake, implications for macrophyte harvesting. *Aquatic Botany,* **3,** 239–255.

Carpenter S.R. & Gasith A. (1978) Mechanical cutting of submersed macrophytes: Immediate effects on littoral water chemistry and metabolism. *Water Research,* **12,** 55–57.

Carpenter S.R. & Lodge D.M. (1986) Effects of submersed macrophytes on ecosystem processes. *Aquatic Botany,* **26,** 341-370.

Carpenter S.R., Ludwig D. & Brock W.A. (1999) Management of Eutrophication for Lakes Subject to Potentially Irreversible Change. *Ecological Applications,* **9,** 751–771.

Carpenter S.R., Stanley E.H. & Vander Zanden M.J. (2011) State of the World's Freshwater Ecosystems: Physical, Chemical, and Biological Changes. In: *Annual Review of Environment and Resources, Vol 36.* (Eds A. Gadgil & D.M. Liverman), pp. 75-99. Annual Review of Environment and Resources. Annual Reviews, Palo Alto.

Carr G.M. & Chambers P.A. (1998) Macrophyte growth and sediment phosphorus and nitrogen in a Canadian prairie river. *Freshwater Biology,* **39,** 525-536.

Carr G.M., Bod S.A.E., Duthie H.C. & Taylor W.D. (2003) Macrophyte biomass and water quality in Ontario rivers. *Journal of the North American Benthological Society,* **22,** 182-193.

Cason C. & Roost B.A. (2011) Species Selectivity of Granular 2,4-D Herbicide When Used to Control Eurasian Watermilfoil (*Myriophyllum spicatum*) in Wisconsin Lakes. *Invasive Plant Science and Management,* **4,** 251-259.

Caspers H. & Heckman C.W. (1981) Ecology of orchard drainage ditches along the freshwater section of the Elbe Estuary. *Archiv für Hydrobiologie,* **43,** 347–486.

Catry I., Marcelino J., Franco A.M.A. & Moreira F. (2017) Landscape determinants of European roller foraging habitat: implications for the definition of agri-environmental measures for species conservation. *Biodiversity and Conservation*, **26,** 553–566.

Chambers P.A., Dewreede R.E., Irlandi E.A. & Vandermeulen H. (1999) Management issues in aquatic macrophyte ecology: a Canadian perspective. *Canadian Journal of Botany-Revue Canadienne De Botanique,* **77,** 471-487.

Charudattan R. (2001) Are we on top of aquatic weeds? Weed problems, control options, and challenges. In: *International Symposium on the Worlds Worst Weeds.* (Ed D.P.P.G.F.L.U.S.A. Univ Florida), pp. 43-68. British Crop Protection Council Symposium Proceedings. Nov 12 2001. British Crop Protection Council, FARNHAM.

Childers D.L., Corman J., Edwards M. & Elser J.J. (2011) Sustainability challenges of phosphorus and food: solutions from closing the human phosphorus cycle. *Bioscience,* **61,** 117–124.

Chmara R., Szmeja J. & Banas K. (2014) Factors controlling the frequency and biomass of submerged vegetation in outwash lakes supplied with surface water or groundwater. *Boreal Environment Research,* **19,** 168-180.

Chowdhury R.B., Moore G.A., Weatherley A.J. & Arora M. (2017) Key sustainability challenges for the global phosphorus resource, their implications for global food security, and options for mitigation. *Journal of Cleaner Production,* **140,** 945-963.

Christiansen N.H., Andersen F.O. & Jensen H.S. (2016) Phosphate uptake kinetics for four species of submerged freshwater macrophytes measured by a P-33 phosphate radioisotope technique. *Aquatic Botany,* **128,** 58-67.

Chunkao K., Nimpee C. & Duangmal K. (2012) The King's initiatives using water hyacinth to remove heavy metals and plant nutrients from wastewater through Bueng Makkasan in Bangkok, Thailand. *Ecological Engineering,* **39,** 40-52.

Clarke S.J. (2015) Conserving freshwater biodiversity : The value , status and management of high quality ditch systems. *Journal for Nature Conservation,* **24,** 93–100.

Clayton J. & Champion P. (2006) Risk assessment method for submerged weeds in New Zealand hydroelectric lakes. *Hydrobiologia,* **570,** 183-188.

Coetzee J.A., Hill M.P., Byrne M.J. & Bownes A. (2011) A review of the biological control programmes on *Eichhornia crassipes* (C.Mart.) Solms (Pontederiaceae), *Salvinia molesta* DSMitch. (Salviniaceae), *Pistia stratiotes* L. (Araceae), *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae) and *Azolla filiculoides* Lam. (Azollaceae) in South Africa. *African Entomology,* **19,** 451-468.

Conyers D.L. & Cooke G.D. (1983) A comparison of the costs of harvesting and herbicides and their effectiveness in nutrient removal and control of macrophyte biomass. *In: Lake Restoration, Protection, and Management*. pp 317–321

Cordell D., Drangert J.O. & White S. (2009) The story of phosphorus: Global food security and food for thought. *Global Environmental Change,* **19,** 292–305.

Cronin G. & Lodge D.M. (2003) Effects of light and nutrient availability on the growth, allocation, carbon/nitrogen balance, phenolic chemistry, and resistance to herbivory of two freshwater macrophytes. *Oecologia,* **137,** 32-41.

Crowell W., Troelstrup N., Queen L. & Perry J. (1994) Effects of harvesting on plant-communities dominated by Eurasian watermilfoil in lake Minnetonka, MN. *Journal of Aquatic Plant Management,* **32,** 56-60.

Cruz C., Silva A.F., Venturini F.P., Garlich N., Pitelli R. & Pitelli R.A. (2015) Food preference and consumption of aquatic macrophytes submerged by snail *Pomacea canaliculata*. *Planta Daninha,* **33,** 433-439.

Cullen P. & Forsberg C. (1988) Experiences with reducing point sources of phosphorus to lakes. *Hydrobiologia,* **170,** 321–336.

Cusell C., Kooijman A., Fernandez F., Van Wirdum G., Geurts J.J.M., Van Loon E.E., Kalbitz K. & Lamers L.P.M. (2014) Filtering fens: Mechanisms explaining phosphorus-limited hotspots of biodiversity in wetlands adjacent to heavily fertilized areas. *Science of the Total Environment,* **481,** 129-141.

D

Dallarmellina A., Gajardo A., Bezic C., Luna E., Britto A. & Dallarmellina V. (1996) Mechanical aquatic weed management in the lower valley of the Rio Negro, Argentina. *Hydrobiologia,* **340,** 225-228.

David N., Greenfield B.K. & Siemering G.S. (2006) Evaluating impacts of Lake Maid (TM) plant control. *Journal of Aquatic Plant Management,* **44,** 60-66.

De Backer S., Teissier S. & Triest L. (2014) Identification of total phosphate, submerged vegetation cover and zooplankton size thresholds for success of biomanipulation in peri-urban eutrophic ponds. *Hydrobiologia,* **737,** 281-296.

De Lyon M. & Roelofs J. (1986) *Waterplanten in relatie tot waterkwaliteit en bodemgesteldheid,* Katholieke Universiteit, Laboratorium voor Aquatische Oecologie.

Declerck S., Vandekerkhove J., Johansson L., Muylaert K., Conde-Porcuna J.M., Van Der Gucht K., Perez-Martinez C., Lauridsen T., Schwenk K., Zwart G., Rommens W., Lopez-Ramos J., Jeppesen E., Vyverman W., Brendonck L. & De Meester L. (2005) Multi-group biodiversity in shallow lakes along gradients of phosphorus and water plant cover. *Ecology,* **86,** 1905-1915.

Demars B.O.L. & Edwards A.C. (2007) Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply. *Freshwater Biology,* **52,** 2073-2086.

Demars B.O.L. & Edwards A.C. (2008) Tissue nutrient concentrations in aquatic macrophytes: comparison across biophysical zones, surface water habitats and plant life forms. *Chemistry and Ecology,* **24,** 413-422.

Di Nino F., Thiebaut G. & Muller S. (2005) Response of *Elodea nuttallii* (Planch.) H. St. John to manual harvesting in the North-East of France. *Hydrobiologia,* **551,** 147-157.

Dionisio Pires L.M., Ibelings B.W., Elger A., Gons H., Lammens E., Nolet B., Noordhuis R., Portielje R. & Vijverberg J. (2004) Re-oligotrophication: effects on carrying capacity and biodiversity state-of-the-art knowledge for shallow lakes in The Netherlands. *Internal RIZA report.*

Dixon M.H., Hill S.A., Jackson M.B., Ratcliffe R.G. & Sweetlove L.J. (2006) Physiological and metabolic adaptations of *Potamogeton pectinatus* L. tubers support rapid elongation of stem tissue in the absence of oxygen. *Plant and Cell Physiology,* **47,** 128-140.

Dodds W.K., Bouska W.W., Eitzmann J.L., Pilger T.J., Pitts K.L., Riley A.J., Schloesser J.T. & Thornbrugh D.J. (2009) Eutrophication of US Freshwaters: Analysis of Potential Economic Damages. *Environmental Science & Technology,* **43,** 12-19.

Dorenbosch M. & Bakker E.S. (2011) Herbivory in omnivorous fishes: effect of plant secondary metabolites and prey stoichiometry. *Freshwater Biology,* **56,** 1783-1797.

Dornelas M., Gotelli N.J., McGill B., Shimadzu H., Moyes F., Sievers C., et al. (2014) Assemblage Time Series Reveal Biodiversity Change but Not Systematic Loss. *Science,* **344,** 296–300.

Droop M.R. (1974) The nutrient status of algal cells in continuous culture. *Journal of the Marine Biological Association of the United Kingdom,* **54,** 825–855.

Duarte C.M. (1992) Nutrient concentration of aquatic plants - patterns across species. *Limnology and Oceanography,* **37,** 882-889.

Duarte C.M. & Kalff J. (1990) Biomass density and the relationship between submerged macrophyte biomass and plant growth form. *Hydrobiologia,* **196,** 17-23.

Dung N.N.X., Manh L.H. & Uden P. (2002) Tropical fibre sources for pigs - digestibility, digesta retention and estimation of fibre digestibility in vitro. *Animal Feed Science and Technology,* **102,** 109-124.

E

Edwards P. (1980) Food potential of aquatic macrophytes. *ICLARM Studies and Reviews,* **5,** 51.

Eichler L.W., Bombard R.T., Sutherland J.W. & Boylen C.W. (1993) Suction harvesting of Eurasian watermilfoil and its effect on native plant-communities. *Journal of Aquatic Plant Management,* **31,** 144-148.

Eighmy T.T., Jahnke L.S. & Fagerberg W.R. (1991) Studies of *Elodea nuttallii* grown under photorespiratory conditions .2. Evidence for bicarbonate active-transport. *Plant Cell and Environment,* **14,** 157-165.

Eiswerth M.E., Donaldson S.G. & Johnson W.S. (2000) Potential environmental impacts and economic damages of Eurasian watermilfoil (*Myriophyllum spicatum*) in western Nevada and northeastern California. *Weed Technology,* **14,** 511-518.

Elser J.J., Fagan W.F., Kerkhoff A.J., Swenson N.G. & Enquist B.J. (2010) Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytologist,* **186,** 593-608.

Engel S. (1990) Ecological impacts of harvesting macrophytes in Halverson lake, Wisconsin. *Journal of Aquatic Plant Management,* **28,** 41-45.

Ensing D.J. & Pither J. (2015) A novel multiple-site extension to pairwise partitioned taxonomic beta diversity. *Ecological Complexity,* **21,** 62–69.

EU legislation (1991a) *Fighting water pollution from agricultural nitrates*. Council Directive 91/676/EEC of 12 December 1991.

EU legislation (1991b) *Urban wastewater treatment*. Council Directive 91/271/EEC of 21 May 1991.

EU legislation (2000) *Good-quality water in Europe (EU Water Directive)*. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000.

European Union (2000) Directive 2000/60/EC of The European Parliament and of the Council of 23 October 2000 – EWFD – Establishing a Framework for Community Action in the Field of Water Policy. *Official Journal of the European Communities*, L327:1–73.

Evans J.M. & Wilkie A.C. (2010) Life cycle assessment of nutrient remediation and bioenergy production potential from the harvest of hydrilla (*Hydrilla verticillata*). *Journal of Environmental Management,* **91,** 2626-2631.

Everitt J.H., Yang C., Escobar D.E., Webster C.F., Lonard R.I. & Davis M.R. (1999) Using remote sensing and spatial information technologies to detect and map two aquatic macrophytes. *Journal of Aquatic Plant Management,* **37,** 71-80.

F

Farquhar G.D., Firth P.M., Wetselaar R. & Weir B. (1980) On the gaseous exchange of ammonia between leaves and the environment - determination of the ammonia compensation point. *Plant Physiology,* **66,** 710-714.

Fernandes T.V., Shrestha R., Sui Y.X., Papini G., Zeeman G., Vet L.E.M., Wijffels R.H. & Lamers P. (2015) Closing Domestic Nutrient Cycles Using Microalgae. *Environmental Science & Technology,* **49,** 12450-12456.

Fernandez-Alaez M., Fernandez-Alaez C. & Becares E. (1999) Nutrient content in macrophytes in Spanish shallow lakes. *Hydrobiologia,* **408,** 317-326.

Filizadeh Y. & Murphy K.J. (2002) Response of sago pondweed to combinations of low doses of diquat, cutting, and shade. *Journal of Aquatic Plant Management,* **40,** 72-76.

Finlay K. & Vogt R.J. (2016) An ecosystem management framework to maintain water quality in a macrophyte-dominated, productive, shallow reservoir. *Hydrobiologia,* **776,** 111-123.

Foley J.A., Defries R., Asner G.P., Barford C., Bonan G., Carpenter S.R., et al. (2005) Glo-bal Consequences of Land Use. *Science,* **309,** 570–574.

Fox A.M. & Murphy K.J. (1990) The efficacy and ecological impacts of herbicide and cutting regimes on the submerged plant-communities of 4 British rivers. 2. A multivariate-analysis of the effects of management regimes on macrophyte communities. *Journal of Applied Ecology,* **27,** 541-548.

Fox J. & Weisberg S. (2011) *An {R} Companion to Applied Regression,* Sage, Thousand Oaks {CA}.

Frost P.C. & Hicks A.L. (2012) Human shoreline development and the nutrient stoichiometry of aquatic plant communities in Canadian Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences,* **69,** 1642-1650.

Fulmer J.E. & Robinson A.T. (2008) Aquatic plant species distributions and associations in Arizona's reservoirs. *Journal of Aquatic Plant Management,* **46,** 100-106.

G

Garbey C., Murphy K.J., Thiebaut G. & Muller S. (2004) Variation in P-content in aquatic plant tissues offers an efficient tool for determining plant growth strategies along a resource gradient. *Freshwater Biology,* **49,** 346-356.

Garbey C., Thiebaut G. & Muller S. (2003) Impact of manual spring harvesting on the regrowth of a spreading aquatic plant: *Ranunculus peltatus* SCHRANK. *Archiv Fur Hydrobiologie,* **156,** 271-286.

Gerloff G.C. (1975) *Nutritional ecology of nuisance aquatic plants,* National Environmental Research Center, Office of Research and Development, US Environmental Protection Agency.

Gerven L.P.a.V., Klein J.J.M.D., Gerla D.J., Kooi B.W., Kuiper J.J. & Mooij W.M. (2015) Competition for Light and Nutrients in Layered Communities of Aquatic Plants. *The American Naturalist,* **186,** 72-83.

Gettys L.A., Haller W.T. & Petty D.G. (eds) (2014) Biology and Control of Aquatic Plants: *A Best Management Practices Handbook, 3rd edn.* Aquatic Ecosystem Restoration Foundation, Marietta, Georgia

Godfrey K.E., Anderson L.W.J., Perry S.D. & Dechoretz N. (1994) Overwintering and establishment potential of *Bagous affinis* (coleoptera, curculionidae) on *Hydrilla verticillata* (hydrocharitaceae) in northern California. *Florida Entomologist,* **77,** 221-230.

Goldenberg Vilar A., Van Dam H., Van Loon E.E., Vonk J.A., Van Der Geest H.G. & Admiraal W. (2014) Eutrophication decreases distance decay of similarity in diatom communities. *Freshwater Biology,* **59,** 1522–1531.

Gonzalez A.L., Kominoski J.S., Danger M., Ishida S., Iwai N. & Rubach A. (2010) Can ecological stoichiometry help explain patterns of biological invasions? *Oikos,* **119,** 779-790.

Goodenberger J.S. & Klaiber H.A. (2016) Evading invasives: How Eurasian watermilfoil affects the development of lake properties. *Ecological Economics,* **127,** 173-184.

Goossen C.M., Langers F. & Lous J.F.A. (1997) *Indicatoren voor recreatieve kwaliteiten in het landelijk gebied.* pp. 132. DLO-Staring Centrum, Wageningen, the Netherlands.

Gopal B. & Goel U. (1993) Competition and allelopathy in aquatic plant-communities. *Botanical Review,* **59,** 155-210.

Grasshoff K. & Johannsen H. (1972) A new sensitive and direct method for the automatic determination of ammonia in sea water. *Journal du Conseil Permanent International pour l'Exploration de la Mer,* **34,** 516–521.

Green J.C. (2005) Modelling flow resistance in vegetated streams: review and development of new theory. *Hydrological Processes,* **19,** 1245-1259.

Gross E.M., Feldbaum C. & Choi C. (2002) High abundance of herbivorous Lepidoptera larvae (*Acentria ephemerella* DENIS & SCHIFFERMULLER) on submersed macrophytes in Lake Constance (Germany). *Archiv Fur Hydrobiologie,* **155,** 1-21.

Grutters B.M.C., Bart B.J., Verberk W.C.E.P. & Bakker E.S. (2015) Native and non-native plants provide similar refuge to invertebrate prey, but less than artificial plants. *PLoS One,* **10,** 1–18. doi: 10.1371/journal.pone.0124455

Grutters B.M.C., Gross E.M. & Bakker E.S. (2016) Insect herbivory on native and exotic aquatic plants: phosphorus and nitrogen drive insect growth and nutrient release. *Hydrobiologia,* **778,** 209–220.

Gu B.H. (2006) Environmental conditions and phosphorus removal in Florida lakes and wetlands inhabited by *Hydrilla verticillata* (Royle): implications for invasive species management. *Biological Invasions,* **8,** 1569-1578.

Gulati R.D. & Van Donk E. (2002) Lakes in the Netherlands, their origin, eutrophication and restoration: state-of-the-art review. *Hydrobiologia,* **478,** 73-106.

Gumbricht T. (1993a) Nutrient removal processes in freshwater submersed macrophyte systems. *Ecological Engineering,* **2,** 30.

Gumbricht T. (1993b) Nutrient removal capacity in submersed macrophyte pond systems in a temperate climate. *Ecological Engineering,* **2,** 49-61.

Gunn I.D.M., Meis S., Maberly S.C. & Spears B.M. (2014) Assessing the responses of aquatic macrophytes to the application of a lanthanum modified bentonite clay, at Loch Flemington, Scotland, UK. *Hydrobiologia,* **737,** 309-320.

H

Habib S. & Yousuf A.R. (2014) Impact of mechanical deweeding on the phytophilous macroinvertebrate community of an eutrophic lake. *Environmental Science and Pollution Research,* **21,** 5653-5659.

Halbedel (Angelstein) S. (2016) Absence of phosphorus at iron coatings on *Elodea nuttallii* roots point toward a redox-mediated phosphorus uptake strategy: implications for a root-microbe relationship. *Inland Waters,* **6,** 343-351.

Hansson L.A., Nicolle A., Bronmark C., Hargeby A., Lindstrom A. & Andersson G. (2010) Waterfowl, macrophytes, and the clear water state of shallow lakes. *Hydrobiologia,* **646,** 101-109.

Hao B.B., Wu H.P., Shi Q., Liu G.H. & Xing W. (2013) Facilitation and competition among foundation species of submerged macrophytes threatened by severe eutrophication and implications for restoration. *Ecological Engineering,* **60,** 76-80.

Hargeby A., Andersson G., Blindow I. & Johansson S. (1994) Trophic web structure in a shallow eutrophic lake during a dominance shift from phytoplankton to submerged macrophytes. *Hydrobiologia,* **279,** 83-90.

Harman W.N., Hingula L.P. & Macnamara C.E. (2005) Does long-term macrophyte management in lakes affect biotic richness and diversity? *Journal of Aquatic Plant Management,* **43,** 57-64.

Hasler A.D. (1947) Eutrophication of lakes by domestic drainage. *Ecology,* **28,** 383-395.

Hassall C., Hollinshead J. & Hull A. (2012) Temporal dynamics of aquatic communities and implications for pond conservation. *Biodiversity and Conservation*, **21,** 829–852.

Heimans E. & Thijsse J.P. (1895) *In sloot en plas*. W. Versluys, Amsterdam

Henriksen A. (1965) An automated method for determining low-level concentrations of phosphate in fresh and saline waters. *Analyst,* **90,** 29–34.

Herzon I. & Helenius J. (2008) Agricultural drainage ditches , their biological importance and functioning. *Biological Conservation*, **141,** 1171–1183.

Hessen D.O., Elser J.J., Sterner R.W. & Urabe J. (2013) Ecological stoichiometry: An elementary approach using basic principles. *Limnology and Oceanography,* **58,** 2219-2236.

Hidding B., Bakker E.S., Hootsmans M.J.M. & Hilt S. (2016) Synergy between shading and herbivory triggers macrophyte loss and regime shifts in aquatic systems. *Oikos*, **125,** 1489-1495.

Hidding B., Brederveld R.J. & Nolet B.A. (2010) How a bottom-dweller beats the canopy: inhibition of an aquatic weed (*Potamogeton pectinatus*) by macroalgae (*Chara* spp.). *Freshwater Biology,* **55,** 1758-1768.

Hill R.P., Chadd N., Morris M.J., Swaine P.J. & Wood J.D. (2016) Aquatic macroinvertebrate biodiversity associated with artificial agricultural drainage ditches. *Hydrobiologia*, **776,** 249-260.

Hilt S. & Gross E.M. (2008) Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? *Basic and Applied Ecology,* **9,** 422-432.

Hilt S., Gross E.M., Hupfer M., Morscheid H., Mahlmann J., Melzer A., Poltz J., Sandrock S., Scharf E.M., Schneider S. & De Weyer K.V. (2006) Restoration of submerged vegetation in shallow eutrophic lakes - A guideline and state of the art in Germany. *Limnologica,* **36,** 155-171.

Hilt S., Kohler J., Adrian R., Monaghan M.T. & Sayer C.D. (2013) Clear, crashing, turbid and back - long-term changes in macrophyte assemblages in a shallow lake. *Freshwater Biology,* **58,** 2027-2036.

Hinsch M. & Poethke H. (2007) Consequences of cyclic vegetation management for arthropod survival : Simulation experiments. *Basic and Applied Ecology,* **8,** 321–331.

Hirzel A.H. & Le Lay G. (2008) Habitat suitability modelling and niche theory. *Journal of Applied Ecology,* **45,** 1372-1381.

Ho A., Reim A., Kim S.Y., Meima-Franke M., Termorshuizen A., De Boer W., Van Der Putten W.H. & Bodelier P.L.E. (2015) Unexpected stimulation of soil methane uptake as emergent property of agricultural soils following bio-based residue application. *Global Change Biology,* **21,** 3864-3879.

Hofstra D.E., Champion P.D. & Dugdale T.M. (2006) Herbicide trials for the control of parrotsfeather. *Journal of Aquatic Plant Management,* **44,** 13-18.

Hogsden K.L., Sager E.P.S. & Hutchinson T.C. (2007) The impacts of the non-native macrophyte *Cabomba caroliniana* on littoral biota of Kasshabog Lake, Ontario. *Journal of Great Lakes Research,* **33,** 497-504.

Hölker F., Vanni M.J., Kuiper J.J., et al. (2015) Tube-dwelling invertebrates: tiny ecosystem engineers have large effects in lake ecosystems. *Ecological Monographs,* **85,** 333–351.

Horppila J. & Nurminen L. (2003) Effects of submerged macrophytes on sediment resuspension and internal phosphorus loading in Lake Hiidenvesi (southern Finland). *Water Research,* **37,** 4468-4474.

Hosper S.H. (1998) Stable States, Buffers and Switches: an Ecosystem Approach to the Restoration and Management of Shallow Lakes in the Netherlands. *Water Science and Technology,* **37,** 151–164.

Hosper H., Pot R. & Portieltje R. (2011) Meren en plassen in Nederland: toestand, trends en hoe verder? *H2O,* **7,** 25-28.

Hothorn T., Bretz F. & Westfall P. (2008) Simultaneous Inference in General Parametric Models. *Biometrical Journal,* **50,** 346-363.

Howard-Williams C., Schwarz A.M. & Reid V. (1996) Patterns of aquatic weed regrowth following mechanical harvesting in New Zealand hydro-lakes. *Hydrobiologia,* **340,** 229-234.

Hussner A. & Jahns P. (2015) European native *Myriophyllum spicatum* showed a higher use capacity than alien invasive *Myriophyllum heterophyllum*. *Hydrobiologia,* **746,** 171-182.

Hussner A., Mettler-Altmann T., Weber A.P.M. & Sand-Jensen K. (2016) Acclimation of photosynthesis to supersaturated CO2 in aquatic plant bicarbonate users. *Freshwater Biology,* **61,** 1720-1732.

Hussner A., Stiers I., Verhofstad M.J.J.M., Bakker E.S., Grutters B.M.C., Haury J., Van Valkenburg J., Brundu G., Newman J., Clayton J.S., Anderson L.W.J. & Hofstra D. (2017) Management and control methods of invasive alien freshwater aquatic plants: A review. *Aquatic Botany,* **136,** 112-137.

I

Ibelings B.W., Portielje R., Lammens E., Noordhuis R., Van Den Berg M.S., Joosse W. & Meijer M.L. (2007) Resilience of alternative stable states during the recovery of shallow lakes from eutrophication: Lake Veluwe as a case study. *Ecosystems,* **10,** 4-16.

Immers A.K., Bakker E.S., Van Donk E., Ter Heerdt G.N.J., Geurts J.J.M. & Declerck S.a.J. (2015) Fighting internal phosphorus loading: An evaluation of the large scale application of gradual Fe-addition to a shallow peat lake. *Ecological Engineering,* **83,** 78-89.

J

Jackson R.B., Carpenter S.R., Dahm C.N., Mcknight D.M., Naiman R.J., Postel S.L. & Running S.W. (2001) Water in a changing world. *Ecological Applications,* **11,** 1027-1045.

Janse J.H. (2005) Model studies on the eutrophication of shallow lakes and ditches. *PhD Thesis.* Wageningen University

Janse J.H. (1997) A model of nutrient dynamics in shallow lakes in relation to multiple stable states. *Hydrobiologia,* **342/343,** 1–8.

Janse J.H., De Senerpont Domis L.N., Scheffer M., Lijklema L., Van Liere L., Klinge M. & Mooij W.M. (2008) Critical phosphorus loading of different types of shallow lakes and the consequences for management estimated with the ecosystem model PCLake. *Limnologica,* **38,** 203–219.

Janse J.H., Van Donk E. & Aldenberg T. (1998) A model study on the stability of the macrophyte-dominated state as affected by biological factors. *Water Research,* **32,** 2696–2706.

Janse J.H., Scheffer M., Lijklema L, Van Liere L., Sloot J.S. & Mooij W.M. (2010) Estimating the critical phosphorus loading of shallow lakes with the ecosystem model PCLake: Sensitivity, calibration and uncertainty. *Ecological Modelling,* **221,** 654–665.

Janse J.H., Van der Does J. & Van der Vlugt J.C. (1993) PCLake: Modelling eutrophication and its control measures in Reeuwijk lakes. *Report.*

Janse J.H. & Van Liere L. (1995) PCLake: a modelling tool for the evaluation of lake restoration scenarios. *Water Science and Technology,* **31,** 371–374.

Janssen A.B.G., Arhonditsis G.B., Beusen A, et al. (2015) Exploring, exploiting and evolving diversity of aquatic ecosystem models: a community perspective. *Aquatic Ecology,* doi: 10.1007/s10452-015-9544-1

Jeppesen E., Jensen J.P., Sondergaard M., Lauridsen T., Pedersen L.J. & Jensen L. (1997) Top-down control in freshwater lakes: The role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia,* **342,** 151-164.

Jeppesen E., Meerhoff M., Jacobsen B.A., Hansen R.S., Sondergaard M., Jensen J.P., Lauridsen T.L., Mazzeo N. & Branco C.W.C. (2007) Restoration of shallow lakes by nutrient control and biomanipulation-the successful strategy varies with lake size and climate. *Hydrobiologia,* **581,** 269-285.

Jeppesen E., Sondergaard M., Jensen J.P., Havens K.E., Anneville O., Carvalho L., Coveney M.F., Deneke R., Dokulil M.T., Foy B., Gerdeaux D., Hampton S.E., Hilt S., Kangur K., Kohler J., Lammens E., Lauridsen T.L., Manca M., Miracle M.R., Moss B., Noges P., Persson G., Phillips G., Portielje R., Schelske C.L., Straile D., Tatrai I., Willen E. & Winder M. (2005) Lake responses to reduced nutrient loading - an analysis of contemporary long-term data from 35 case studies. *Freshwater Biology,* **50,** 1747-1771.

Jeppesen E., Sondergaard M., Lauridsen T.L., Davidson T.A., Liu Z.W., Mazzeo N., Trochine C., Ozkan K., Jensen H.S., Trolle D., Starling F., Lazzaro X., Johansson L.S., Bjerring R., Liboriussen L., Larsen S.E., Landkildehus F., Egemose S. & Meerhoff M. (2012) Biomanipulation as a Restoration Tool to Combat Eutrophication: Recent Advances and Future Challenges. In: *Advances in Ecological Research, Vol 47: Global Change in Multispecies Systems, Pt 2.* (Eds G. Woodward & U. Jacob & E.J. Ogorman), pp. 411-488. Advances in Ecological Research. Elsevier Academic Press Inc, San Diego.

Jeppesen E., Sondergaard M., Sondergaard M. & Christofferson K. (eds) (1998) *The Structuring Role of Submerged Macrophytes in Lakes*. Springer-Verlag, New York.

Johnson R.E. & Bagwell M.R. (1979) Effects of mechanical cutting on submersed vegetation in a Louisiana lake. *Journal of Aquatic Plant Management,* **17,** 54-57.

Jones G.B. & Cooke G.D. (1984) Control of nuisance aquatic plants with burlap screen. *Ohio Journal of Science,* **84,** 248-251.

Jost L. (2007) Partitioning diversit into independent Alpha and Beta components. *Ecological Society of America*, **88,** 2427–2439.

Jost L., Devries P., Walla T., Greeney H., Chao A. & Ricotta C. (2010) Partitioning diversity for conservation analyses. *Diversity and Distributions,* **16,** 65–76.

K

Kaenel B.R., Matthaei C.D. & Uehlinger U. (1998) Disturbance by aquatic plant management in streams: Effects on benthic invertebrates. *Regulated Rivers-Research & Management,* **14,** 341-356.

Kaenel B.R. & Uehlinger U. (1999) Aquatic plant management: ecological effects in two streams of the Swiss Plateau. *Hydrobiologia,* **415,** 257-263.

Kalita P., Mukhopadhyay P.K. & Mukherjee A.K. (2007) Evaluation of the nutritional quality of four unexplored aquatic weeds from northeast India for the formulation of cost-effective fish feeds. *Food Chemistry,* **103,** 204-209.

Kamphake L.J., Hannah S.A. & Cohen J.M. (1967) Automated analysis for nitrate by hydrazine reduction. *Water Research,* **1,** 205–206.

Khan F.A. & Ansari A.A. (2005) Eutrophication: An ecological vision. *Botanical Review,* **71,** 449-482.

Kimbel J.C. & Carpenter S.R. (1981) Effects of mechanical harvesting on *Myriophyllum spicatum* L. regrowth and carbohydrate allocation to roots and shoots. *Aquatic Botany,* **11,** 121–127.

Kissoon L.T.T., Jacob D.L., Hanson M.A., Herwig B.R., Bowe S.E. & Otte M.L. (2013) Macrophytes in shallow lakes: Relationships with water, sediment and watershed characteristics. *Aquatic Botany,* **109,** 39-48.

Köbbing J.F., Thevs N. & Zerbe S. (2013) The utilisation of reed (*Phragmites australis*): a review. *Mires and Peat,* **13**.

Kong X., He Q., Yang B., et al. (2016) Hydrological regulation drives regime shifts: evidence from paleolimnology and ecosystem modeling of a large shallow Chinese lake. *Global Change Biology,* doi: 10.1111/gcb.13416

Körner S. (2002) Loss of submerged macrophytes in shallow lakes in north-eastern Germany. *International Review of Hydrobioly,* **87,** 375–384.

Kuiper J.J., Verhofstad M.J.J.M., Louwers E.L.M., Bakker E.S., Brederveld R.J., Van Gerven L.P.A., Janssen A.B.G., De Klein J.J.M. & Mooij W.M. (2017) Mowing Submerged Macrophytes in Shallow Lakes with Alternative Stable States: Battling the Good Guys? *Environmental Management***, 59,** 619-634.

Kunii H. (1984) Seasonal growth and profile structure development of *Elodea nuttallii* (Planch) St John in pond Ojaga-Ike, Japan. *Aquatic Botany,* **18,** 239-247.

Kwakernaak C., Jansen P., Van Kempen M.M.L., Smolders A.J.P. & Van Rheenen H. (2015) A smart solution for flooding, drought and water pollution. *Water Matters : Knowledge Journal for Water Professionals,* **2,** 44-47.

L

Lacoul P. & Freedman B. (2006) Environmental influences on aquatic plants in freshwater ecosystems. *Environmental Reviews,* **14,** 89-136.

Lamers L., Schep S., Geurts J. & Smolders F (2012) Erfenis fosfaatrijk verleden: helder water met woekerende waterplanten. *H2O,* **13,** 29–31.

Lamers L.P.M., Smolders A.J.P. & Roelofs J.G.M. (2002) The restoration of fens in the Netherlands. *Hydrobiologia,* **478,** 107–130.

Lamers L.P.M., Van Diggelen J.M.H., Op Den Camp H.J.M., Visser E.J.W., Lucassen E., Vile M.A., Jetten M.S.M., Smolders A.J.P. & Roelofs J.G.M. (2012) Microbial transformations of nitrogen, sulfur, and iron dictate vegetation composition in wetlands: a review. *Frontiers in Microbiology,* **3,** 12.

Lamers L.P.M., Vile M.A., Grootjans A.P., Acreman M.C., Van Diggelen R., Evans M.G., Richardson C.J., Rochefort L., Kooijman A.M., Roelofs J.G.M. & Smolders A.J.P. (2015) Ecological restoration of rich fens in Europe and North America: from trial and error to an evidence-based approach. *Biological Reviews,* **90,** 182-203.

Legendre P. (2014) Interpreting the replacement and richness difference components of beta diversity. *Global Ecology and Biogeography,* **23,** 1324–1334.

Legendre P. & Anderson M.J. (1999) Distace-Based Redundancy Analysis: Testing Multispecies Responses in Multifactorial Experiments. *Ecological Monographs,* **69,** 1–24.

Lewis W.M., Wurtsbaugh W.A. & Paerl H.W. (2011) Rationale for Control of Anthropogenic Nitrogen and Phosphorus to Reduce Eutrophication of Inland Waters. *Environmental Science & Technology,* **45,** 10300-10305.

Li E.H., Li W., Wang X.L., Xue H.P. & Xiao F. (2010b) Experiment of emergent macrophytes growing in contaminated sludge: Implication for sediment purification and lake restoration. *Ecological Engineering,* **36,** 427-434.

Li J.H., Yang X.Y., Wang Z.F., Shan Y. & Zheng Z. (2015a) Comparison of four aquatic plant treatment systems for nutrient removal from eutrophied water. *Bioresource Technology,* **179,** 1-7.

Li K.Y., Liu Z.W. & Gu B.H. (2010a) Compensatory growth of a submerged macrophyte (*Vallisneria spiralis*) in response to partial leaf removal: effects of sediment nutrient levels. *Aquatic Ecology,* **44,** 701-707.

Li W., Cao T., Ni L.Y., Zhu G.R., Zhang X.L., Fu H., Song X. & Xie P. (2015b) Size-dependent C, N and P stoichiometry of three submersed macrophytes along water depth gradients. *Environmental Earth Sciences,* **74,** 3733-3738.

Liere L., Janse J.H. & Arts G.H.P. (2007) Setting critical nutrient values for ditches using the eutrophication model PCDitch. *Aquatic Ecology,* **41,** 443–449.

Lischke B, Hilt S, Janse JH, Kuiper J.J., Mehner, T., Mooij W.M. & Gaedke U. (2014) Enhanced Input of Terrestrial Particulate Organic Matter Reduces the Resilience of the Clear-Water State of Shallow Lakes: A Model Study. *Ecosystems,* **17,** 616–626.

Lucassen E., Roelofs J.G.M., Schneider S.C. & Smolders A.J.P. (2016) Long-term effects of liming in Norwegian softwater lakes: the rise and fall of bulbous rush (*Juncus bulbosus*) and decline of isoetid vegetation. *Freshwater Biology,* **61,** 769-782.

M

Maberly S.C. & Madsen T.V. (1998) Affinity for CO2 in relation to the ability of freshwater macrophytes to use HCO3. *Functional Ecology,* **12,** 99-106.

Madsen J.D. (1993) Biomass Techniques for Monitoring and Assessing Control of Aquatic Vegetation. *Lake and Reservoir Management,* **7,** 141-144.

Madsen J.D. (1998) Overview of the Ecological Assessment Technology Area. *Journal of Aquatic Plant Management,* **36,** 25-27.

Madsen J.D., Adams M.S. & Ruffier P. (1988) Harvest as a control for sago pondweed (*Potamogeton pectinatus* L) in Badfish-Creek, Wisconsin - frequency, efficiency and its impact on the stream community oxygen-metabolism. *Journal of Aquatic Plant Management,* **26,** 20-25.

Madsen J.D., Sartain B., Turnage G. & Marko M. (2016) Management of flowering rush in the Detroit Lakes, Minnesota. *Journal of Aquatic Plant Management,* **54,** 61-67.

Madsen T.V. & Cedergreen N. (2002) Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream. *Freshwater Biology,* **47,** 283-291.

Mallin M.A., Mciver M.R., Wambach E.J. & Robuck A.R. (2016) Algal blooms, circulators, waterfowl, and eutrophic Greenfield Lake, North Carolina. *Lake and Reservoir Management,* **32,** 168-181.

Mangan R. & Baars J.R. (2013) Use of life table statistics and degree day values to predict the colonisation success of *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae), a leaf mining fly of *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae), in Ireland and the rest of Europe. *Biological Control,* **64,** 143-151.

Martin G.D. & Coetzee J.A. (2014) Competition between two aquatic macrophytes, *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae) and *Myriophyllum spicatum* Linnaeus (Haloragaceae) as influenced by substrate sediment and nutrients. *Aquatic Botany,* **114,** 1-11.

Mataraza L.K., Terrell J.B., Munson A.B. & Canfield D.E. (1999) Changes in submersed macrophytes in relation to tidal storm surges. *Journal of Aquatic Plant Management,* **37,** 3-12.

Mazzeo N., Rodriguez-Gallego L., Kruk C., Meerhoff M., Gorga J., Lacerot G., Quintans F., Loureiro M., Larrea D. & Garcia-Rodriguez F. (2003) Effects of *Egeria densa* Planch. beds on a shallow lake without piscivorous fish. *Hydrobiologia,* **506,** 591-602.

Mckee D., Hatton K., Eaton J.W., Atkinson D., Atherton A., Harvey I. & Moss B. (2002) Effects of simulated climate warming on macrophytes in freshwater microcosm communities. *Aquatic Botany,* **74,** 71-83.

Meijer M.L., De Boois I., Scheffer M., Portielje R. & Hosper H. (1999) Biomanipulation in shallow lakes in The Netherlands: an evaluation of 18 case studies. *Hydrobiologia,* **408,** 13-30.

Mellios N., Kofinas D., Laspidou C. & Papadimitriou T. (2015) Mathematical Modeling of Trophic State and Nutrient Flows of Lake Karla using the PCLake Model. *Environmental Processes,* **2,** 85–100.

Meuleman A.F., Beltman B. & Scheffer R.A. (2004) Water pollution control by aquatic vegetation of treatment wetlands. *Wetlands ecology and management,* **12,** 459-471.

Meunier C.L., Malzahn A.M. & Boersma M. (2014) A New Approach to Homeostatic Regulation: Towards a Unified View of Physiological and Ecological Concepts. *Plos One,* **9,** 7.

Meyer A., Combroux I. & Tremolieres M. (2013) Dynamics of Nutrient Contents (Phosphorus, Nitrogen) in Water, Sediment and Plants After Restoration of Connectivity in Side-Channels of the River Rhine. *Restoration Ecology,* **21,** 232-241.

Michelan T.S., Silveira M.J., Petsch D.K., Pinha G.D. & Thomaz S.M. (2014) The invasive aquatic macrophyte *Hydrilla verticillata* facilitates the establishment of the invasive mussel *Limnoperna fortunei* in Neotropical reservoirs. *Journal of Limnology,* **73,** 598-602.

Middelboe A.L. & Markager S. (1997) Depth limits and minimum light requirements of freshwater macrophytes. *Freshwater Biology,* **37,** 553-568.

Miler O. & Straile D. (2010) How to cope with a superior enemy? Plant defence strategies in response to annual herbivore outbreaks. *Journal of Ecology,* **98,** 900-907.

Milsom T.P., Sherwood A.J., Rose S.C., Town S.J. & Runham S.R. (2004) Dynamics and management of plant communities in ditches bordering arable fenland in eastern England. *Agriculture Ecosystems & Environment,* **103,** 85-99.

Miranda L.E. & Hodges K.B. (2000) Role of aquatic vegetation coverage on hypoxia and sunfish abundance in bays of a eutrophic reservoir. *Hydrobiologia,* **427,** 51-57.

Mitchell D.S. (1996) Interactions between national and local strategies for the management of aquatic weeds. *Hydrobiologia,* **340,** 153-156.

Moe T.F., Brysting A.K., Andersen T., Schneider S.C., Kaste O. & Hessen D.O. (2013) Nuisance growth of Juncus bulbosus: the roles of genetics and environmental drivers tested in a large-scale survey. *Freshwater Biology,* **58,** 114-127.

Monahan C. & Caffrey J.M. (1996) The effect of weed control practices on macroinvertebrate communities in Irish Canals. *Hydrobiologia,* **340,** 205-211.

Mony C., Puijalon S. & Bornette G. (2011) Resprouting response of aquatic clonal plants to cutting may explain their resistance to spate flooding. *Folia Geobotanica,* **46,** 155-164.

Mooij W.M., Brederveld R.J., de Klein J.J.M., et al. (2014) Serving many at once: How a database approach can create unity in dynamical ecosystem modelling. *Environmental Modelling & Software,* doi: 10.1016/j.envsoft.2014.04.004

Mooij W.M., De Senerpont Domis L.N. & Janse J.H. (2009) Linking species-and ecosystem-level impacts of climate change in lakes with a complex and a minimal model. *Ecological Modelling,* **220,** 3011–3020.

Mooij W.M., Janse J.H., Senerpont Domis L.N., Hülsmann S. & Ibelings B.W. (2007) Predicting the effect of climate change on temperate shallow lakes with the ecosystem model PCLake. *Hydrobiologia,* **584,** 443–454.

Morris K., Bailey P.C.E., Boon P.I. & Hughes L. (2006) Effects of plant harvesting and nutrient enrichment on phytoplankton community structure in a shallow urban lake. *Hydrobiologia,* **571,** 77-91.

Morris K., Boon P.I., Bailey P.C. & Hughes L. (2003) Alternative stable states in the aquatic vegetation of shallow urban lakes. I. Effects of plant harvesting and low-level nutrient enrichment. *Marine and Freshwater Research,* **54,** 185–200.

Moss B. (1990) Engineering and biological approaches to the restoration from eutrophication of shallow lakes in which aquatic plant-communities are important components. *Hydrobiologia,* **200,** 367-377.

Moss B., Stansfield J., Irvine K., Perrow M. & Phillips G. (1996) Progressive restoration of a shallow lake: A 12-year experiment in isolation, sediment removal and biomanipulation. *Journal of Applied Ecology,* **33,** 71-86.

Muñoz Escobar M., Voyevoda M., Führer C. & Zehnsdorf A. (2011) Potential uses of *Elodea nuttallii*-harvested biomass. In: *Energy, Sustainability and Society*. Vol. **4**. Springer.

Murphy K.J. (1988a) Aquatic weed problems and their management - a review .1. The worldwide scale of the aquatic weed problem. *Crop Protection,* **7,** 232-248.

Murphy K.J. (1988b) Aquatic weed problems and their management - a review .2. Physical control measures. *Crop Protection,* **7,** 283-302.

N

Nakamura K., Kayaba Y., Nishihiro J. & Takamura N. (2008) Effects of submerged plants on water quality and biota in large-scale experimental ponds. *Landscape and Ecological Engineering,* **4,** 1-9.

Nationaal Water Overleg (2012) Blauwalgen protocol. URL: http://cyanobacterien.stowa.nl/Upload/Blauwalgenprotocol_2012.pdf (Last accessed 27 SEP 2017).

Nawrocki J.J., Richardson R.J. & Hoyle S.T. (2016) Monoecious hydrilla tuber dynamics following various management regimes on four North Carolina reservoirs. *Journal of Aquatic Plant Management,* **54,** 12-19.

Neel J.K., Peterson S.A. & Smith W.L. (1973) Weed harvest and lake nutrient dynamics. U.S. Environmental Protection Agency, Washington, D.C. 20460.

Nelson L.S., Stewart A.B. & Getsinger K.D. (2002) Fluridone effects on fanwort and water marigold. *Journal of Aquatic Plant Management,* **40,** 58-63.

Nevel B.E., Hanganu J. & Griffin C.R. (1997) Reed harvesting in the Danube Delta, Romania: Is it sustainable? *Wildlife Society Bulletin,* **25,** 117-124.

Newman R.M., Holmberg K.L., Biesboer D.D. & Penner B.G. (1996) Effects of a potential biocontrol agent, *Euhrychiopsis lecontei*, on Eurasian watermilfoil in experimental tanks. *Aquatic Botany,* **53,** 131-150.

Nichols S.A. (1991) The interaction between biology and the management of aquatic macrophytes. *Aquatic Botany,* **41,** 225-252.

Nichols S.A. & Lathrop R.C. (1994) Impact of harvesting on aquatic plant-communities in Lake Wingra, Wisconsin. *Journal of Aquatic Plant Management,* **32,** 33-36.

Nichols S.A. & Shaw B.H. (1986) Ecological life histories of the 3 aquatic nuisance plants, *Myriophyllum spicatum*, *Potamogeton crispus* and *Elodea canadensis*. *Hydrobiologia,* **131,** 3-21.

Nielsen U.N., Riis T. & Brix H. (2006) The effect of weed cutting on *Luronium natans*. *Aquatic Conservation-Marine and Freshwater Ecosystems,* **16,** 409-417.

Nielsen A, Trolle D, Bjerring R, Søndergaard M., Olesen J.E. Janse J.H. Mooij W.M. & Jeppesen E. (2014) Effects of climate and nutrient load on the water quality of shallow lakes assessed through ensemble runs by PCLake. *Ecological Applications,* **24,** 1926–1944.

Nilsson C. & Brittain J.E. (1996) Remedial strategies in regulated rivers: Introductory remarks. *River Research and Applications,* **12,** 347-351.

Noordhuis R., Van Der Molen D.T. & Van Den Berg M.S. (2002) Response of herbivorous water-birds to the return of Chara in Lake Veluwemeer, the Netherlands. *Aquatic Botany,* **72,** 349-367.

O

Odum E.P. (1969) The Strategy of Ecosystem Development. *Science,* **164,** 262–270.

O'hare M.T., Stillman R.A., Mcdonnell J. & Wood L.R. (2007) Effects of mute swan grazing on a keystone macrophyte. *Freshwater Biology,* **52,** 2463-2475.

Oksanen J., Blanchet F.G., Kindt R., Legendre P., Minchin P.R., O'Hara R.B., et al. (2015) vegan: Community Ecology Package. R package version 2.3-1.

Olson M.H., Carpenter S.R., Cunningham P., Gafny S., Herwig B.R., Nibbelink N.P., Pellett T., Storlie C., Trebitz A.S. & Wilson K.A. (1998) Managing macrophytes to improve fish growth: A multi-lake experiment. *Fisheries,* **23,** 6-12.

O'sullivan C., Rounsefell B., Grinham A., Clarke W. & Udy J. (2010) Anaerobic digestion of harvested aquatic weeds: water hyacinth (*Eichhornia crassipes*), cabomba (*Cabomba caroliniana*) and salvinia (*Salvinia molesta*). *Ecological Engineering,* **36,** 1459-1468.

P

Pagano A.M. & Titus J.E. (2004) Submersed macrophyte growth at low pH: contrasting responses of three species to dissolved inorganic carbon enrichment and sediment type. *Aquatic Botany,* **79,** 65-74.

Painter S.D. (1988) Long-term Effects of Mechanical Harvesting on Eurasian Watermilfoil. *Journal of Aquatic Plant Management,* **26,** 25-29.

Palmik K., Maemets H., Haldna M. & Kangur K. (2013) A comparative study of macrophyte species richness in differently managed shore stretches of Lake Peipsi. *Limnologica,* **43,** 245-253.

Peck S.L. (2004) Simulation as experiment: A philosophical reassessment for biological modeling. *Trends in Ecology and Evolution,* **19,** 530–534.

Pedersen M.L., Baattrup-Pedersen A., Rorth F.R., Madsen T.V. & Larsen S.E. (2011) Short-Term Impacts of Weed Cutting on Physical Habitats in Lowland Rivers - the Importance of Initial Environmental Conditions. *Polish Journal of Environmental Studies,* **20,** 1271-1280.

Pedersen T.C.M., Baattrup-Pedersen A. & Madsen T.V. (2006) Effects of stream restoration and management on plant communities in lowland streams. *Freshwater Biology,* **51,** 161-179.

Perna C.N., Cappo M., Pusey B.J., Burrows D.W. & Pearson R.G. (2012) Removal of aquatic weeds greatly enhances fish community richness and diversity: an example from the Burdekin river floodplain, tropical Australia. *River Research and Applications,* **28,** 1093-1104.

Perrow M.R., Jowitt A.J.D., Stansfield J.H. & Phillips G.L. (1999) The practical importance of the interactions between fish, zooplankton and macrophytes in shallow lake restoration. *Hydrobiologia,* **395,** 199-210.

Perrow M.R., Schutten J.H., Howes J.R., Holzer T., Madgwick J.F. & Jowitt A.J.D. (1997) Interactions between coot (*Fulica atra*) and submerged macrophytes: the role of birds in the restoration process. *Hydrobiologia,* **342/343,** 241–255.

Peters J. & Van Emmerik W. (2013) Overdaad schaadt. Relatie waterplanten, vis en visserij complex. *Visionair,* **30,** 17-19.

Peterson S.A., Smith W.L. & Malueg K.W. (1974) Full-scale harvest of aquatic plants - nutrient removal from a eutrophic lake. *Journal Water Pollution Control Federation,* **46,** 697-707.

Phillips G., Willby N. & Moss B. (2016) Submerged macrophyte decline in shallow lakes: What have we learnt in the last forty years? *Aquatic Botany,* **135,** 37-45.

Pickett S.T.A., Cadenasso M.L. & Meiners S.J. (2008) Ever since Clement : from succession to vegetation dynamics and understanding to intervention. *Applied Vegetation Science,* **12,** 9–21.

Pietro K.C., Chimney M.J. & Steinman A.D. (2006) Phosphorus removal by the Ceratophyllum/ periphyton complex in a south Florida (USA) freshwater marsh. *Ecological Engineering,* **27,** 290-300.

Podani J. & Schmera D. (2011) A new conceptual and methodological framework for exploring and explaining pattern in presence - absence data. *Oikos,* **120,** 1625–1638.

Poirrier M.A., Burt-Utley K., Utley J.F. & Spalding E.A. (2010) Submersed Aquatic Vegetation of the Jean Lafitte National Historical Park and Preserve. *Southeastern Naturalist,* **9,** 477-486.

Portielje R. & Roijackers R.M.M. (1995) Primary succession of aquatic macrophytes in experimental ditches in relation to nutrient input. *Aquatic Botany,* **50,** 127–140.

Portielje R. & Van der Molen D.T. (1998) Trend-analysis of eutrophication variables in lakes in The Netherlands. *Water Science and Technology,* **37,** 235–240.

Pot R. (2004) *Veldgids water- en oeverplanten,* KNNV-Uitgeverij/Stowa, Utrecht, The Netherlands.

Pot R. & Ter Heerdt G.N.J. (2014) Succession dynamics of aquatic lake vegetation after restoration measures: increased stability after 6 years of development. *Hydrobiologia,* **737,** 333-345.

Power A.G. (2010) Ecosystem services and agriculture: tradeoffs and synergies. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365,** 2959–2971.

Prach K., Rehounková K., Lencová K., Jírová A., Konvalinková P., Mudrák O., et al. (2014) Vegetation succession in restoration of disturbed sites in Central Europe: the direction of succession and species richness across 19 seres. *Applied Vegetation Science,* **17,** 193–200.

Q

Quilliam R.S., Van Niekerk M.A., Chadwick D.R., Cross P., Hanley N., Jones D.L., Vinten A.J.A., Willby N. & Oliver D.M. (2015) Can macrophyte harvesting from eutrophic water close the loop on nutrient loss from agricultural land? *Journal of Environmental Management,* **152,** 210-217.

R

Rattray M.R., Howard-Williams C. & Brown J.M.A. (1991) Sediment and water as sources of nitrogen and phosphorus for submerged rooted aquatic macrophytes. *Aquatic Botany,* **40,** 225-237.

Raun A.L., Borum J. & Sand-Jensen K. (2010) Influence of sediment organic enrichment and water alkalinity on growth of aquatic isoetid and elodeid plants. *Freshwater Biology,* **55,** 1891-1904.

R Core Team (2014) *R: A Language and Environment for Statistical Computing,* R Foundation for Statistical Computing, Vienna, Austria.

Redekop P., Hofstra D. & Hussner A (2016) *Elodea canadensis* shows a higher dispersal capacity via fragmentation than *Egeria densa* and *Lagarosiphon major*. *Aquatic Botany,* **130,** 45–49.

Reeves J.L., Lorchi P.D., Kershner M.W. & Hilovsky M.A. (2008) Biological Control of Eurasian watermilfoil by *Euhrychiopsis lecontei*: Assessing Efficacy and Timing of Sampling. *Journal of Aquatic Plant Management,* **46,** 144-149.

Richards S.A., Possingham H.P. & Tizard J. (1999) Optimal Fire Management for Maintaining Community Diversity. *Ecological Applications,* **9,** 880–892.

Richardson R.J. (2008) Aquatic plant management and the impact of emerging herbicide resistance issues. *Weed Technology,* **22,** 8-15.

Richter D. & Gross E.M. (2013) Chara can outcompete Myriophyllum under low phosphorus supply. *Aquatic Sciences,* **75,** 457-467.

Robach F., Hajnsek I., Eglin I. & Tremolieres M. (1995) Phosphorus sources for aquatic macrophytes in running waters: Water or sediment? *Acta Botanica Gallica,* **142,** 719-731.

Rijkswaterstaat (2012) Handreiking waterplanten maaibeheer. Aanwijzingen voor het maaien van waterplanten in rijkswateren ten behoeve van recreatie. *Report.*

Roger P.A. & Watanabe I. (1984) Algae and aquatic weeds as source of organic matter and plant nutrients for wetland rice. International Rice Research Insitute. *Report.*

Rosenberg D.B. & Freedman S.M. (1984) Application of a model of ecological succession to conservation and land-use management. *Environmental conservation,* **11,** 323–330.

Rosenzweig M.L. (2003) Reconciliation ecology and the future of species diversity. *Oryx,* **37,** 194–205.

Rowland F.E., Bricker K.J., Mara M.J.V. & Gonzalez M.J. (2015) Light and nutrients regulate energy transfer through benthic and pelagic food chains. *Oikos,* **124,** 1648-1663.

S

Sabbatini M.R. & Murphy K.J. (1996) Response of Callitriche and Potamogeton to cutting, dredging and shade in English drainage channels. *Journal of Aquatic Plant Management,* **34,** 8-12.

Saha S. & Ray A.K. (2011) Evaluation of Nutritive Value of Water Hyacinth (*Eichhornia crassipes*) Leaf Meal in Compound Diets for Rohu, Labeo rohita (Hamilton, 1822) Fingerlings after Fermentation with Two Bacterial Strains Isolated from Fish Gut. *Turkish Journal of Fisheries and Aquatic Sciences,* **11,** 199-207.

Sand-Jensen K. (1998) Influence of submerged macrophytes on sediment composition and near-bed flow in lowland streams. *Freshwater Biology,* **39,** 663-679.

Sand-Jensen K. & Borum J. (1991) Interactions among phytoplankton, periphyton, and macrophytes in temperate fresh-waters and estuaries. *Aquatic Botany,* **41,** 137-175.

Sand-Jensen K., Bruun H.H. & Baastrup-Spohr L. (2016) Decade-long time delays in nutrient and plant species dynamics during eutrophication and re-oligotrophication of Lake Fure 1900–2015. *Journal of Ecology***,** n/a-n/a.

Santos M.J., Khanna S., Hestir E.L., Andrew M.E., Rajapakse S.S., Greenberg J.A., Anderson L.W.J. & Ustin S.L. (2009) Use of Hyperspectral Remote Sensing to Evaluate Efficacy of Aquatic Plant Management. *Invasive Plant Science and Management,* **2,** 216-229.

Sardans J., Rivas-Ubach A. & Penuelas J. (2012) The C:N:P stoichiometry of organisms and ecosystems in a changing world: A review and perspectives. *Perspectives in Plant Ecology Evolution and Systematics,* **14,** 33-47.

Sarneel J.M., Janssen R.H., Rip W.J., Bender I.M.A. & Bakker E.S. (2014) Windows of opportunity for germination of riparian species after restoring water level fluctuations: A field experiment with controlled seed banks. *Journal of Applied Ecology,* **51,** 1006–1014.

Sayer C.D., Burgess A., Kari K., Davidson T.A., Peglar S., Yang H.D. & Rose N. (2010) Long-term dynamics of submerged macrophytes and algae in a small and shallow, eutrophic lake: implications for the stability of macrophyte-dominance. *Freshwater Biology,* **55,** 565-583.

Scheffer M. (2004) *Ecology of shallow lakes*. Springer-Verlag, New York

Scheffer M. (1999) The effect of aquatic vegetation on turbidity ; how important are the filter feeders ? *Hydrobiologia,* **408/409,** 307–316.

Scheffer M., Carpenter S., Foley J.A., Folke C. & Walker B. (2001) Catastrophic shifts in ecosystems. *Nature,* **413,** 591-596.

Scheffer M., Hosper S.H., Meijer M.L., Moss B. & Jeppesen E. (1993) Alternative equilibria in shallow lakes. *Trends in Ecology & Evolotion,* **8,** 275–279.

Scheffer M., Szabo S., Gragnani A., Van Nes E.H., Rinaldi S., Kautsky N., et al. (2003) Floating plant dominance as a stable state. *Proceedings of the National Academy of Sciences of the United States of America,* **100,** 4040–4045.

Scheffer M. & Van Nes E.H. (2007) Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia,* **584,** 455-466.

Schneider S.C., Moe T.F., Hessen D.O. & Kaste O. (2013) Juncus bulbosus nuisance growth in oligotrophic freshwater ecosystems: Different triggers for the same phenomenon in rivers and lakes? *Aquatic Botany,* **104,** 15-24.

Schoonbee H.J. (1991) Biological-control of fennel-leaved pondweed, *Potamogeton pectinatus* (potamogetonaceae), in south-africa. *Agriculture Ecosystems & Environment,* **37,** 231-237.

Schriver P., Bogestrand J., Jeppesen E. & Sondergaard M. (1995) Impact of submerged macrophytes on fish-zooplankton-phytoplankton interactions - large-scale enclosure experiments in a shallow eutrophic lake. *Freshwater Biology,* **33,** 255-270.

Schwarz A.M. & Howard-Williams C. (1993) Aquatic weed-bed structure and photosynthesis in 2 New-Zealand lakes. *Aquatic Botany,* **46,** 263-281.

Schwarz A.M. & Snelder T. (1999) Integrated submerged aquatic vegetation management in an urban New Zealand river. *Hydrobiologia,* **415,** 235-241.

Serafy J.E., Harrell R.M. & Hurley L.M. (1994) Mechanical removal of Hydrilla in the Potomac river, Maryland - local impacts on vegetation and associated fishes. *Journal of Freshwater Ecology,* **9,** 135-143.

Shaltout K.H., Eid E.M. & El-Komi T.M. (2016) Phytomass and nutrient value of *Potamogeton crispus* L. in the water courses of Nile Delta, Egypt. *Rendiconti Lincei-Scienze Fisiche E Naturali,* **27,** 251-259.

Shardendu & Ambasht R.S. (1991) Relationship of nutrients in water with biomass and nutrient accumulation of submerged macrophytes of a tropical wetland. *New Phytologist,* **117,** 493-500.

Shilton A.N., Powell N. & Guieysse B. (2012) Plant based phosphorus recovery from wastewater via algae and macrophytes. *Current Opinion in Biotechnology,* **23,** 884-889.

Sidorkewicj N.S., Cazorla A.C.L., Murphy K.J., Sabbatini M.R., Fernandez O.A. & Domaniewski J.C.J. (1998) Interaction of common carp with aquatic weeds in Argentine drainage channels. *Journal of Aquatic Plant Management,* **36,** 5-10.

Sisneros D., Lichtwardt M. & Greene T. (1998) Low-dose metering of Endothall for aquatic plant control in flowing water. *Journal of Aquatic Plant Management,* **36,** 69-72.

Sistla S.A., Appling A.P., Lewandowska A.M., Taylor B.N. & Wolf A.A. (2015) Stoichiometric flexibility in response to fertilization along gradients of environmental and organismal nutrient richness. *Oikos,* **124,** 949-959.

Slade J.G., Poovey A.G. & Getsinger K.D. (2008) Concentration-exposure time relationships for controlling sago pondweed (*Stuckenia pectinata*) with Endothall. *Weed Technology,* **22,** 146-150.

Smart S.M., Thompson K., Marrs R.H., Duc M.G. Le, Maskell L.C. & Firbank L.G. (2006) Biotic homogenization and changes in species diversity across human-modified ecosystems. *Proceedings of the Royal Society B,* **273,** 2659–2665.

Smith C.S. & Barko J.W. (1990) ECOLOGY OF EURASIAN WATERMILFOIL. *Journal of Aquatic Plant Management,* **28,** 55-64.

Smith V.H. (2003) Eutrophication of freshwater and coastal marine ecosystems - A global problem. *Environmental Science and Pollution Research,* **10,** 126-139.

Smolders A.J.P. & Van Kempen M.M.L. (2015) Azolla: Van plaagsoort tot groenproduct. *Vakblad Natuur Bos Landschap,* **12,** 31-33.

Sollie S., Janse J.H., Mooij W.M., Coops H. & Verhoeven J.T.A. (2008) The contribution of marsh zones to water quality in Dutch shallow lakes: a modeling study. *Environmental Management,* **42,** 1002–16.

Søndergaard M., Jeppesen E., Lauridsen T.L., Skov C., Van Nes E.H., Roijackers R., Lammens E. & Portielje R. (2007) Lake restoration: successes, failures and long-term effects. *Journal of Applied Ecology,* **44,** 1095-1105.

Søndergaard M., Johansson L.S., Lauridsen T.L., Jorgensen T.B., Liboriussen L. & Jeppesen E. (2010) Submerged macrophytes as indicators of the ecological quality of lakes. *Freshwater Biology,* **55,** 893-908.

Søndergaard M. & Moss B. (1997) Impact of Submerged Macrophytes on Phytoplankton in Shallow Freshwater Lakes. In: *The Structuring Role of Submerged Macrophytes in Lakes*. (Eds E. Jeppesen & M. Søndergaard & M. Søndergaard & K. Christoffersen), pp. 115-132. Springer New York, New York, NY.

Søndergaard M., Phillips G., Hellsten S., Kolada A., Ecke F., Maeemets H., Mjelde M., Azzella M.M. & Oggioni A. (2013) Maximum growing depth of submerged macrophytes in European lakes. *Hydrobiologia,* **704,** 165-177.

Sousa W.P. (1984) The Role of Disturbance in Natural Communities. *Annual Review of Ecology and Systematics*, **15,** 353–391.

Spears B.M., Mackay E.B., Yasseri S., Gunn I.D., Waters K.E., Andrews C., Cole S., De Ville M., Kelly A. & Meis S. (2015) A meta-analysis of water quality and aquatic macrophyte responses in 18 lakes treated with lanthanum modified bentonite (Phoslock®). *Water Research.*

Spencer D.F. & Ksander G.G. (1999) Influence of dilute acetic acid treatments on survival of monoecious Hydrilla tubers in the Oregon House Canal, California. *Journal of Aquatic Plant Management,* **37,** 67-71.

Spencer D.F., Ksander G.G., Donovan M.J., Liow P.S., Chan W.K., Greenfield B.K., Shonkoff S.B. & Andrews S.P. (2006) Evaluation of waterhyacinth survival and growth in the Sacramento Delta, California, following cutting. *Journal of Aquatic Plant Management,* **44,** 50-60.

Sprecher S.L., Getsinger K.D. & Stewart A.B. (1998) Selective effects of aquatic herbicides on sago pondweed. *Journal of Aquatic Plant Management,* **36,** 64-68.

Stallings K.D., Seth-Carley D. & Richardson R.J. (2015) Management of Aquatic Vegetation in the Southeastern United States. *Journal of Integrated Pest Management,* **6,** 5.

Sterner R.W. & Elser J.J. (2002) *Ecological stoichiometry: the biology of elements from molecules to the biosphere,* Princeton University Press.

Sterner R.W., Elser J.J., Fee E.J., Guildford S.J. & Chrzanowski T.H. (1997) The light:nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. *American Naturalist,* **150,** 663-684.

Strand J.A. & Weisner S.E.B. (2001) Dynamics of submerged macrophyte populations in response to biomanipulation. *Freshwater Biology,* **46,** 1397-1408.

Sukhodolov A.N. & Sukhodolova T.A. (2010) Case Study: Effect of Submerged Aquatic Plants on Turbulence Structure in a Lowland River. *Journal of Hydraulic Engineering*, **136,** 434-446.

Suren A.M. (2009) Using Macrophytes in Urban Stream Rehabilitation: A Cautionary Tale. *Restoration Ecology,* **17,** 873-883.

T

Tamayo M. & Olden J.D. (2014) Forecasting the Vulnerability of Lakes to Aquatic Plant Invasions. *Invasive Plant Science and Management,* **7,** 32-45.

Tang Y., Harpenslager S.F., Van Kempen M.M.L., Verbaarschot E.J.H., Loeffen L.M.J.M., Roelofs J.G.M., Smolders A.J.P. & Lamers L.P.M. (2017) Aquatic macrophytes can be used for wastewater polishing but not for purification in constructed wetlands. *Biogeosciences,* **14,** 755-766.

Tang Y., Van Kempen M.M.L., Van Der Heide T., Manschot J.J.A., Roelofs J.G.M., Lamers L.P.M. & Smolders A.J.P. (2016) A tool for easily predicting short-term phosphorus mobilization from flooded soils. *Ecological Engineering,* **94,** 1-6.

Tansley A.G. (1946) *Introduction to plant ecology*. Allen & Unwin, London.

Tockner K., Malard F. & Ward J. V (2000) An extension of the Flood pulse concept. *Hydrological Processes*, **14,** 2861–2883.

Trebitz A.S., Nichols S.A., Carpenter S.R. & Lathrop R.C. (1993) Patterns of vegetation change in Lake Wingra following a *Myriophyllum spicatum* decline. *Aquatic Botany,* **46,** 325-340.

Trolle D., Elliott J.A., Mooij W.M., Janse J.H., Bolding K., Hamilton D.P. & Jeppesen E. (2014) Advancing projections of phytoplankton responses to climate change through ensemble modelling. *Environmental Modelling & Software,* **61,** 371-379.

Twisk W., Noordervliet M.A.W. & Ter Keurs W.J. (2003) The nature value of the ditch vegetation in peat areas in relation to farm management. *Aquatic Ecology,* **37,** 191–209.

U

Unmuth J.M.L., Sloey D.J. & Lillie R.A. (1998) An evaluation of close-cut mechanical harvesting of Eurasian watermilfoil. *Journal of Aquatic Plant Management,* **36,** 93-100.

V

Van Altena C., Bakker E.S., Kuiper J.J. & Mooij W.M. (2016) The impact of bird herbivory on macrophytes and the resilience of the clear-water state in shallow lakes: a model study. *Hydrobiologia,* **777,** 197-207.

Van de Bund W.J. & Van Donk E. (2002) Short-term and long-term effects of zooplanktivorous fish removal in a shallow lake: a synthesis of 15 years of data from Lake Zwemlust. *Freshwater Biology,* **47,** 2380-2387.

Van de Haterd R.J.W. & Ter Heerdt G.N.J. (2007) Potential for the development of submerged macrophytes in eutrophicated shallow peaty lakes after restoration measures. *Hydrobiologia,* **584,** 277-290.

Van de Leemput I.A., Van Nes E.H. & Scheffer M. (2015) Resilience of Alternative States in Spatially Extended Ecosystems. *PLoS One,* doi: 10.1371/journal.pone.0116859

Van de Waal D.B. & Boersma M. (2012) Ecological stoichiometry in aquatic ecosystems. In: *Oceonography*. (Ed U.-E.J. Committee). Encyclopedia of Life Support Systems. Eolss Publishers, Oxford ,UK.

Van den Berg M., Kolen M. & Coops H. (2001) *Maaien van waterplanten in het Veluwemeer.* RIZA, Lelystad.

Van der Plas F., Manning P., Soliveres S., Allan E., Scherer-lorenzen M., Verheyen K., et al. (2016) Biotic homogenization can decrease landscape-scale forest multifunctionality. *Proceedings of the National Academy of Sciences,* **113,** 3557–3562.

Van Donk E. (1990) Necessity for aquatic plant management after lake restoration by biomanipulation. *Procedings of the 8th Symposium on Aquatic Weeds,* p91–96.

Van Donk E. & Otte A. (1996) Effects of grazing by fish and waterfowl on the biomass and species composition of submerged macrophytes. *Hydrobiologia,* **340,** 285-290.

Van Donk E. & Van de Bund W.J. (2002) Impact of submerged macrophytes including charophytes on phyto- and zooplankton communities: allelopathy versus other mechanisms. *Aquatic Botany,* **72,** 261-274.

Van Gerven L.P.A., Brederveld R.J., De Klein J.J.M., et al. (2015) Advantages of concurrent use of multiple software frameworks in water quality modelling using a database approach. *Fundamental and Applied Limnology,* **186,** 5-20.

Van Gerven L.P.A., De Klein J.J.M., Gerla D.J., Kooi B.W., Kuiper J.J. & Mooij W.M. (2015) Competition for Light and Nutrients in Layered Communities of Aquatic Plants. *The American Naturalist,* **186,** 72–83.

Van Ginkel C.E. (2011) Eutrophication: Present reality and future challenges for South Africa. *Water SA,* **37,** 693–702.

Van Leeuwen C.H.A., Sarneel J.M., Van Paassen J., Rip W.J. & Bakker E.S. (2014) Hydrology, shore morphology and species traits affect seed dispersal, germination and community assembly in shoreline plant communities. *Journal of Ecology,* **102,** 998–1007.

Van Liere L.& Janse J.H. (1992) Restoration and resilience to recovery of the Lake Loosdrecht ecosystem in relation to its phosphorus flow. *Hydrobiologia,* **233,** 95–104.

Van Nes E.H. & Scheffer M. (2007) Slow recovery from perturbations as a generic indicator of a nearby catastrophic shift. *American Naturalist,* **169,** 738–747.

Van Nes E.H. & Scheffer M. (2005) Implications of spatial heterogeneity for catastrophic regime shifts in ecosystems. *Ecology,* **86,** 1797–1807.

Van Nes E.H., Van den Berg M.S., Clayton J.S., Coops H., Scheffer M. & Van Ierland E. (1999) A simple model for evaluating the costs and benefits of aquatic macrophytes. *Hydrobiologia,* **415,** 335–339.

Van Nes E.H., Scheffer M., Van Den Berg M.S. & Coops H. (2002a) Aquatic macrophytes: restore, eradicate or is there a compromise? *Aquatic Botany,* **72,** 387-403.

Van Nes E.H., Scheffer M., Van Den Berg M.S. & Coops H. (2002b) Dominance of charophytes in eutrophic shallow lakes - when should we expect it to be an alternative stable state? *Aquatic Botany,* **72,** 275-296.

Van Strien A.J., Van der Burg T., Rip W.J. & Strucker R.C.W. (1991) Effects of Mechanical Ditch Management on the Vegetation of Ditch Banks in Dutch Peat Areas. *Journal of Applied Ecology,* **28,** 501–513.

Van Zuidam B.G., Cazemier M.M., Van Geest G.J., Roijackers R.M.M. & Peeters E. (2014) Relationship between redox potential and the emergence of three submerged macrophytes. *Aquatic Botany,* **113,** 56-62.

Van Zuidam J.P. & Peeters E. (2012) Cutting affects growth of *Potamogeton lucens* L. and *Potamogeton compressus* L. *Aquatic Botany,* **100,** 51-55.

Van Zuidam J.P. & Peeters E.T. (2013) Occurrence of macrophyte monocultures in drainage ditches relates to phosphorus in both sediment and water. *SpringerPlus,* **2,** 564.

Vandvik V., Heegaard E., Måren I.E. & Aarrestad P.A. (2005) Managing heterogeneity: The importance of grazing and environmental variation on post-fire succession in heathlands. *Journal of Applied Ecology,* **42,** 139–149.

Velthuis M. *et al.* (2017) Impact of temperature and nutrients on carbon: nutrient tissue stoichiometry of submerged aquatic plants: an experiment and meta-analysis. *Frontiers in Plant Science,* **8,** doi: 10.3389/fpls.2017.00655.

Ventura M., Liboriussen L., Lauridsen T., Sondergaard M. & Jeppesen E. (2008) Effects of increased temperature and nutrient enrichment on the stoichiometry of primary producers and consumers in temperate shallow lakes. *Freshwater Biology,* **53,** 1434-1452.

Verdonschot R.C.M., Keizer-Vlek H.E. & Verdonschot P.F.M. (2011) Biodiversity value of agricultural drainage ditches: A comparative analysis of the aquatic invertebrate fauna of ditches and small lakes. *Aquatic Conservation: Marine and Freshwater Ecosystems,* **21,** 715–727.

Verhofstad M.J.J.M. & Bakker E.S. (2017) Classifying nuisance submerged vegetation depending on ecosystem services. *Limnology,* doi: 10.1007/s10201-017-0525-z

Verhofstad M.J.J.M., Alirangues Núñez M.M., Reichman E.P., Van Donk E., Lamers L.P.M. & Bakker E.S. (2017a) Mass development of monospecific submerged macrophyte vegetation after the restoration of shallow lakes: roles of light, sediment nutrient levels, and propagule density. *Aquatic Botany,* **141,** 29-38.

Verhofstad M.J.J.M., Poelen M.D.M. Van Kempen M.M.L. Bakker E.S. & Smolders A.J.P. (2017b) Finding the harvesting frequency to maximize nutrient removal in a constructed wetland dominated by submerged aquatic plants. *Ecological Engineering,* **106,** 423-430.

Vereecken H., Baetens J., Viaene P., Mostaert F. & Meire P. (2006) Ecological management of aquatic plants: effects in lowland streams. *Hydrobiologia,* **570,** 205-210.

Verma V.K., Singh Y.P. & Rai J.P.N. (2007) Biogas production from plant biomass used for phytoremediation of industrial wastes. *Bioresource Technology,* **98,** 1664-1669.

Vymazal J. (2007) Removal of nutrients in various types of constructed wetlands. *Science of the Total Environment,* **380,** 48-65.

Vymazal J. (2011) Plants used in constructed wetlands with horizontal subsurface flow: a review. *Hydrobiologia,* **674,** 133-156.

W

Wade P.M. (1990) Physical control of aquatic weeds. In: Pieterse A.H. & Murphy K.J. (eds) *Aquatic Weeds: The Ecology and Management of Nuisance Aquatic Vegetation.* Exford University Press, Oxford, pp 93–135.

Walker L.R., Zarin D.J., Fetcher N., Myster R.W. & Johnson A.H. (1996) Ecosystem Development and Plant Succession on Landslides in the Caribbean. *Biotropica,* **28,** 566–576.

Wang C., Wang C. & Wang Z. (2010) Effects of submerged macrophytes on sediment suspension and NH4-N release under hydrodynamic conditions. *Journal of Hydrodynamics,* **22,** 810-815.

Ward J. V., Tockner K., Arscott D.B. & Claret C. (2002) Riverine landscape diversity. *Freshwater Biology,* **47,** 517–539.

Washington P.M., Thomas G.L. & Marino D.A. (1992) Successes and failures of acoustics in the measurement of environmental impacts. *Fisheries Research,* **14,** 239-250.

WaterrecreatieAdvies (2005) Report: Onderzoek aantal recreatievaartuigen in Nederland.

Watson A.M. & Ormerod S.J. (2004) The distribution of three uncommon freshwater gastropods in the drainage ditches of British grazing marshes. *Biological Conservation,* **118,** 455–466.

Wells R.D.S., Bannon H.J. & Hicks B.J. (2003) Control of macrophytes by grass carp (*Ctenopharyngodon idella*) in a Waikato drain, New Zealand. *New Zealand Journal of Marine and Freshwater Research,* **37,** 85-93.

Wersal R.M. & Madsen J.D. (2011a) Comparative effects of water level variations on growth characteristics of *Myriophyllum aquaticum. Weed Research,* **51,** 386-393.

Wersal R.M. & Madsen J.D. (2011b) Influences of water column nutrient loading on growth characteristics of the invasive aquatic macrophyte Myriophyllum aquaticum (Vell.) Verdc. *Hydrobiologia,* **665,** 93-105.

Whatley M.H., van Loon E.E., van Dam H., Vonk J.A., Van der Geest H.G. & Admiraal W. (2014) Macrophyte loss drives decadal change in benthic invertebrates in peatland drainage ditches. *Freshwater Biology,* **59,** 114–126.

Whittaker R.H. (1960) Vegetation of the Siskiyou Mountains, Oregon and California. *Ecological Monographs*, **30,** 279–338.

Whittaker R.J. (1970) *Communities and Ecosystems*, 1st edn. Macmillan, New York.

Wile I. (1978) Environmental effects of mechanical harvesting. *J. Aquat. Plant Manage,* **16,** 14-20.

Wiley M.J., Gorden R.W., Waite S.W. & Powless T. (1984) The Relationship between Aquatic Macrophytes and Sport Fish Production in Illinois Ponds: A Simple Model. *North American Journal of Fisheries Management,* **4,** 111-119.

Wilkie A.C. & M. E.J. (2010) Aquatic plants: an opportunity feedstock in the age of bioenergy. *Biofuels,* **1,** 11.

Wilson C.E., Darbyshire S.J. & Jones R. (2007) The biology of invasive alien plants in Canada. 7. *Cabomba caroliniana* A. Gray. *Canadian Journal of Plant Science,* **87,** 615-638.

Wingfield R., Murphy K.J. & Gaywood M. (2006) Assessing and predicting the success of *Najas flexilis* (Willd.) Rostk. & Schmidt, a rare European aquatic macrophyte, in relation to lake environmental conditions. *Hydrobiologia,* **570,** 79-86.

Witteveen+Bos (2010) Ecologische modellering Loenderveense Plas en Terra Nova. *Report.*

Wolf J., Beusen A.H.W., Groenendijk P., Kroon T., Rötter R. & Van Zeijts H. (2003) The integrated modeling system STONE for calculating nutrient emissions from agriculture in the Netherlands. *Environmental Modelling & Software,* **18,** 597–617.

Wood K.A., O'hare M.T., Mcdonald C., Searle K.R., Daunt F. & Stillman R.A. (2016) Herbivore regulation of plant abundance in aquatic ecosystems. *Biological Reviews.*

Wood K.A., Stillman R.A., Clarke R.T., Daunt F. & O'hare M.T. (2012) The impact of waterfowl herbivory on plant standing crop: a meta-analysis. *Hydrobiologia,* **686,** 157-167.

Wu M.Y. & Wu J. (2007) In-vitro investigations on ultrasonic control of water chestnut. *Journal of Aquatic Plant Management,* **45,** 76-83.

X

Xia C.X., Yu D., Wang Z. & Xie D. (2014) Stoichiometry patterns of leaf carbon, nitrogen and phosphorous in aquatic macrophytes in eastern China. *Ecological Engineering,* **70,** 406-413.

Xie D., Yu D., You W.H. & Wang L.G. (2013) Morphological and Physiological Responses to Sediment Nutrients in the Submerged Macrophyte *Myriophyllum spicatum*. *Wetlands,* **33,** 1095-1102.

Xie Y.H., An S.Q. & Wu B.F. (2005) Resource allocation in the submerged plant *Vallisneria natans* related to sediment type, rather than water-column nutrients. *Freshwater Biology,* **50,** 391-402.

Xing W., Wu H., Shi Q., Hao B., Liu H., Wang Z. & Liu G. (2015) Multielement stoichiometry of submerged macrophytes across Yunnan plateau lakes (China). *Scientific Reports,* **5**.

Xing W., Wu H.P., Hao B.B. & Liu G.H. (2013) Stoichiometric characteristics and responses of submerged macrophytes to eutrophication in lakes along the middle and lower reaches of the Yangtze River. *Ecological Engineering,* **54,** 16-21.

Y

Yakandawala K. & Dissanayake D. (2010) *Mayaca fluviatilis* Aubl.: an ornamental aquatic with invasive potential in Sri Lanka. *Hydrobiologia,* **656,** 199-204.

Yanran D., Chenrong J., Wei L., Shenghua H. & Zhenbin W. (2012) Effects of the submerged macrophyte *Ceratophyllum demersum* L. on restoration of a eutrophic waterbody and its optimal coverage. *Ecological Engineering,* **40,** 113–116.

Yeo R.R. & Mchenry W.B. (1977) Hydrilla, a new noxious aquatic weed in California. *California Agriculture,* **31,** 4-5.

Z

Zefferman E. (2014) Increasing canopy shading reduces growth but not establishment of *Elodea nuttallii* and *Myriophyllum spicatum* in stream channels. *Hydrobiologia,* **734,** 159-170.

Zefferman E.P. & Harris D.J. (2016) Predicting drivers of nuisance macrophyte cover in a regulated California stream using boosted regression tree models. *Journal of Aquatic Plant Management,* **54,** 78-86.

Zehnsdorf A., Hussner A., Eismann F., Roenicke H. & Melzer A. (2015) Management options of invasive *Elodea nuttallii* and *Elodea canadensis*. *Limnologica,* **51,** 110-117.

Zhang P., Bakker E.S., Zhang M. & Xu J. (2016) Effects of warming on *Potamogeton crispus* growth and tissue stoichiometry in the growing season. *Aquatic Botany,* **128,** 13-17.

Zhang Q., Xu Y.S., Huang L., Xue W., Sun G.Q., Zhang M.X. & Yu F.H. (2014) Does mechanical disturbance affect the performance and species composition of submerged macrophyte communities? *Scientific Reports,* **4,** 6.

Chapter 11

English summary

To mow or not to mow is the question managers of shallow freshwater ecosystems often have to ask themselves these days. On the one hand, they want submerged vegetation in the ecosystem because these plants provide many important functions and services (**Chapters 1 & 2**). On the other hand, massive growth of these submerged plants is a major problem for people using the ecosystems worldwide (**Chapter 2**). Furthermore, very dense stands of just a few species may also impair the beneficial ecosystem functions submerged plants generally have. Therefore, submerged plants are often managed when growing to nuisance proportions with mechanical cutting and harvesting being one of the most used management methods. However, this type of management of the aquatic vegetation is often not successful or effects are short-lived (**Chapter 9**). Because cutting management is expensive and time consuming, some might wonder whether the plants should be mown at all.

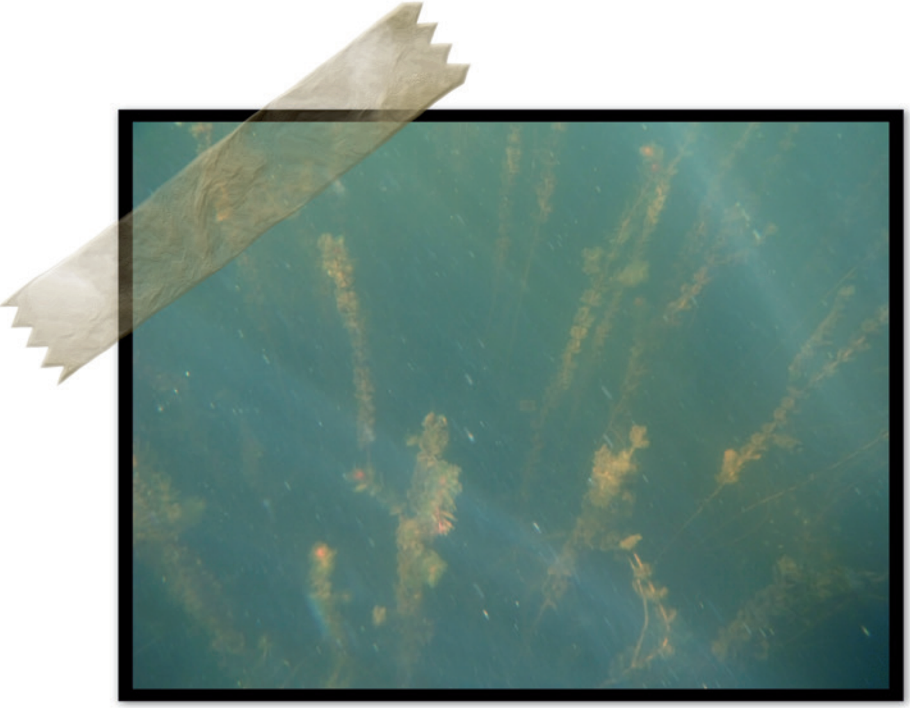
In this thesis, I gathered scientific knowledge on which problems are caused by nuisance submerged aquatic plants, which plant characteristics can be used to separate nuisance from non-nuisance vegetation, which environmental factors affects plant tissue elemental composition, plant growth, and the plant's response to cutting. I discuss how this knowledge can further our understanding of the nuisance problems and how it can aid managers in tackling problems caused by rapid growth of submerged plants, while maintaining the important ecosystem functions these plants provide.

In **Chapter 2** I showed that the height and cover of the plants can best be used to quantitatively differentiate between nuisance and non-nuisance vegetation and that the height and cover threshold nuisance levels differ among the different services provided by the ecosystem to human users. In **Chapter 3** I found that light availability (i.e. clear water) and the presence of viable propagules are essential for submerged plants to grow in the field. The chance of nuisance submerged plant growth is highest when sediment phosphorus was plentiful, but my results indicated that nuisance plant growth is likely to occur over a wide range of environmental nitrogen and phosphorus concentrations (**Chapters 3 & 4**). In **Chapters 4 and 5** I showed that several common nuisance causing species are very tolerant to cutting, thus explaining the short-term effect cutting generally has (**Chapter 9**). However, I proposed that effects of cutting will generally last longer when growing conditions reduce the growth rate of the species, for example when nutrient concentrations in the environment are lower, as this reduces the growth rate of the species (**Chapters 4 & 6**).
Next to environmental factors, cutting intensity also affected the response of the submerged vegetation to cutting management, where a higher intensity decreased the dominance of the nuisance species (**Chapter 5**). Furthermore, I provided proof in **Chapter 7** that cyclic harvesting management in space and time may potentially increase landscape wide aquatic plant diversity. Contrarily, a too high cutting intensity may lead to a loss of the submerged vegetation and possibly result in algae blooms in systems with

alternative stable states, also impairing several ecosystem services (**Chapter 6**). I argue that ecosystem managers should not aim for a complete loss of the submerged vegetation to combat nuisance aquatic plant growth, because the plants perform vital function in shallow ecosystems (**Chapter 1**) that may also be required for several ecosystem services to humans (**Chapter 2**).

Last, I investigated whether the nuisance submerged plant species can be used for beneficial applications. In **Chapter 5** I showed that *Myriophyllum spicatum* (shown on the cover of this thesis) can be successfully used to remove nutrients from a constructed wetland. Furthermore, the biomass that should be collected after cutting management can be used for a wide variety of useful applications, ranging from biofuel to agricultural fertilizer (**Chapter 9**). The suitability of the biomass for each application depends in part on the biomass characteristics, such as tissue elemental composition and water content. The biomass' elemental composition can be highly variable even within a single plant species and, in part, is associated with the availability of the elements in the environment and with the time in the growing season, but may also differ interspecifically (**Chapters 4, 5 & 8**).

I conclude that the best method to tackle nuisance growth of submerged aquatic plants is to tackle the bottom-up processes enabling fast submerged plant growth, in particular the abiotic factors enabling high productivity of the ecosystem (**Chapter 9)**. However, as this may not always be possible, top-down management such as cutting and harvesting, can alleviate the problems caused by these plants if tailored to the ecosystem and location at hand (**Chapters 2, 5, 6 &** 7). Harvesting parts of the plants directly reduces standing biomass and vegetation height, removes nutrients, creates a valuable resource and if applied well, maintains the plants important functions in shallow freshwater ecosystems.



Chapter 12

Nederlandse samenvatting

Dutch summary

Maaien of niet maaien is de vraag die beheerders van ondiepe zoete wateren zich tegenwoordig vaak moeten stellen, zowel in Nederland als elders in de wereld. Aan de ene kant wil men waterplanten in het ecosysteem hebben, omdat deze planten veel belangrijke ecologische functies vervullen en diensten leveren aan de mens (**Hoofdstukken 1 & 2**). Aan de andere kant is de enorme groei van waterplanten een belangrijk probleem geworden voor mensen die gebruikmaken van de ecosystemen (**Hoofdstuk 2**). Bovendien kunnen hoge dichtheden planten, bestaande uit slechts een paar dominante woekersoorten, de gunstige ecosysteemfuncties die o.a. ondergedoken waterplanten in het algemeen vervullen negatief beïnvloeden. Maaien (en verzamelen) is één van de meest gebruikte beheermethoden om de problemen van deze woekerende planten te lijf te gaan. Dit type beheer van de waterplanten is echter vaak niet succesvol in het langdurig terugdringen van de plantengroei (**Hoofdstuk 9**). Mede omdat het maaibeheer duur en tijdrovend is, zou je je kunnen afvragen of de planten wel gemaaid moeten worden.

In dit proefschrift heb ik wetenschappelijke kennis verzameld over welke problemen veroorzaakt worden door woekerende waterplanten, welke eigenschappen van de planten gebruikt kunnen worden om problematische vegetaties van niet-problematische vegetaties te onderscheiden. Bovendien heb ik onderzocht welke milieufactoren de groei van de plant en de reactie van de plant op maaien beïnvloeden. Ik bespreek hoe deze kennis ons begrip van de problemen kan bevorderen en hoe deze kennis beheerders kan helpen de problemen veroorzaakt door de snelle groei van ondergedoken waterplanten aan te pakken, terwijl tegelijkertijd de belangrijke ecosysteemfuncties van planten behouden blijven.

In **Hoofdstuk 2** laat ik zien dat de hoogte en de bedekkingsgraad van de waterplanten het best kunnen worden gebruikt om kwantitatief onderscheid te maken tussen problematische en niet-problematische waterplantenvegetaties. Ook beargumenteer ik dat de drempelwaarden voor dit onderscheid mede bepaald worden door de ecosysteemdiensten die door het ecosysteem geleverd worden (o.a. varen versus zwemmen). Uit **Hoofdstuk 3** blijkt dat de beschikbaarheid van licht (d.w.z. helder water) en de aanwezigheid van levensvatbare propagulen (o.a. zaden) essentieel zijn voor de aanwezigheid van onderwaterplanten in ondiepe meren. Wanneer aan deze voorwaarden voldaan wordt, is de kans op hoge dichtheden aan waterplanten logischerwijs het hoogst op locaties met een ruime hoeveelheid voeding in de bodem (m.n. fosfaat). Mijn resultaten wijzen er echter ook op dat de overlast van planten voor kan komen over een brede gradiënt van voedingstoffen in het milieu, mede omdat relatief lage dichtheden in sommige gevallen al als vervelend worden ervaren. (**Hoofdstukken 2, 3 & 4**). In **Hoofdstukken 4 en 5** heb ik laten zien dat een aantal soorten waterplanten die vaak voor overlast zorgen, erg tolerant zijn tegen knippen (maaibeheer). Dit kan het kortstondige effect dat maaien vaak heeft verklaren (**Hoofdstuk 9**). Ik heb

beargumenteerd dat de effecten van maaien over het algemeen langer duren wanneer de milieuomstandigheden de groeisnelheid van de soort verlagen, bijvoorbeeld wanneer de concentratie voedingsstoffen in het milieu lager zijn, aangezien dit de hergroei van de plant vertraagd (**Hoofdstukken 4 & 6**).
Naast omgevingsfactoren beïnvloedt ook de maai-intensiteit de reactie van de waterplanten op het maaibeheer, waarbij een hogere intensiteit de dominantie van de woekersoort in sommige gevallen kan verminderen (**Hoofdstuk 5**). Anderzijds kan een te hoge maai-intensiteit leiden tot verlies van de ondergedoken waterplanten en eventueel resulteren in (blauw)algenbloei in ecosystemen, waardoor de meeste ecosysteemdiensten ook worden aangetast (**Hoofdstuk 6**). Ik pleit er dus voor dat ecosysteembeheerders niet te rigoureus ondergedoken waterplanten moeten verwijderen ter bestrijding van de overlast, omdat de waterplanten vitale functies vervullen in ondiepe ecosystemen (**Hoofdstuk 1**) die ook nodig zijn voor diverse ecosysteemdiensten voor de mens (**Hoofdstuk 2**). Daarnaast hebben we in **Hoofdstuk 7** beargumenteerd dat cyclisch beheer in ruimte en tijd potentieel de biodiversiteit van de waterplanten op landschapsschaal kan optimaliseren.

Tenslotte heb ik onderzocht of de woekerende waterplanten gebruikt kunnen worden voor maatschappelijk relevante toepassingen. In **Hoofdstuk 5** heb ik aangetoond dat *Myriophyllum spicatum* (weergegeven op de voorkant van dit proefschrift) succesvol kan worden gebruikt om in voedingsstoffen (o.a. N, P, K) uit het milieu terug te winnen. Bovendien kan de biomassa, die na het maaien verzameld dient te worden, gebruikt worden voor een breed scala aan toepassingen variërend van biobrandstof tot landbouwmeststof (**Hoofdstuk 9**). De geschiktheid van de biomassa voor elke toepassing hangt gedeeltelijk af van de eigenschappen van de biomassa, zoals de elementaire samenstelling en het watergehalte. Deze elementaire samenstelling van de biomassa kan zeer variabel zijn in waterplanten, zelfs binnen één plantensoort. De samenstelling van de plant correleert deels met de beschikbaarheid van de elementen in het omliggende milieu en deels met de tijd in het groeiseizoen, maar kan ook intrinsiek verschillen tussen soorten (**Hoofdstukken 4, 5 & 8**).

Ik concludeer dat de beste methode om de overlast door woekerende onderwaterplanten effectief en voor langere termijn tegen te gaan is, om de bottom-up processen (d.w.z. de groeicondities) aan te pakken die de woekerende plantengroei mogelijk maken. Denk vooral aan de abiotische factoren die de hoge productiviteit van het ecosysteem mogelijk maken, zoals het aanbod van meststoffen (**Hoofdstuk 4 & 9**). Aangezien dit lang niet altijd mogelijk is, kan top-down beheer, zoals maaien en verzamelen, de problemen veroorzaakt door woekerende waterplanten op een directe manier verminderen op de locatie waar de problemen zich voordoen. Hierbij is het van belang de methode toe te spitsen op de specifieke situatie van die locatie (**Hoofdstukken 2, 5, 6 & 7**). Over het algemeen is aan te bevelen maar een deel van de waterplanten te maaien. Dit vermindert direct de biomassa en vegetatiehoogte, waardoor de overlast vermindert en zorgt ervoor dat de planten hun belangrijke functies in het ecosystemen blijven vervullen, doordat niet de hele vegetatie wordt verwijderd. Daarnaast is het ook belangrijk om de gemaaide biomassa te verwijderen, omdat dit de voedingsstoffen die aanwezig zijn in de plant uit het milieu verwijdert en daarnaast een potentieel waardevol product (biomassa) creëert.

Chapter 13

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Naast de ‘oude garde’ (niet vervelend bedoeld) hebben de mede PhD’ers een grote rol gespeeld in mijn PhD waardoor ik zo graag op het NIOO was. *Jan*, je bent een held! Bedankt voor de fijne gesprekken onder het genot van een gezamenlijk biertje en tijdens de wandelingen naar de NIOO-vijvers, waar we nog getuigen zijn geweest van necrofilie bij amfibieën. Fijn dat we elkaar via het NIOO hebben mogen ontmoeten, succes met je nieuwe leven in Scandinavië en hopelijk zien we elkaar snel weer. *Luuk*, fijn om een gezellige mede muzikant in jou gevonden te hebben! Ik heb voor altijd een rijtje ‘kwalitatief hoogstaande’ grappen paraat dankzij jou. *Thijs* (bedankt voor de zuidelijke gezelligheid), *Peiyu* (thank you for scaring away the burglar in Italy and keeping me safe in my car with your chinese knot) *Antonella* (thank you for the epic parties), *Sven* (bedankt, ook voor alle hulp in de laatste fase), *Mandy* (we drinken nog eens een Duvel!), *Laura* (we go way back, dreissena!!!), *Annette* (bedankt buurvrouw), *Karen* (bedankt voor de gezelligheid bij het koffieapparaat), *Libin*, *Manqi, Marika* and *Wei*, thank you all for creating such a nice atmosphere at NIOO, at all the parties and at the conferences with lots of ‘ge-ouwehoer’ about everything and nothing. It wouldn’t have been the same without you all. My special thanks goes out to my dear officemates, *Kong*, *Kostas* and lately *Kim*, *Tania* (the parent) and *Joost* (holiday photo quizmaster) thank you all so much for your help, great conversations (both science related and very not-science related, but always open, considerate and honest) and perfect atmosphere created in the ‘Best Office’! *Kim*, thank you for looking after me when I was extremely busy and for letting me stay in my bed after the unforgettable parties in Wageningen. Ook wil ik mijn twee mede Vierlingsbekenaren bedanken waarmee ik al sinds de basisschool optrek. *Bart* en *Ralf*, super tof dat we dit PhD-avontuur voor een groot deel samen hebben kunnen aangaan! Wie had dat in groep 8 gedacht. Ook dank natuurlijk voor de gezelligheid en voor alle antwoorden (ik zat in het team voor de sfeer, niet omdat ik nou zoveel nutteloze feitjes weet) bij de Bèkse pubquiz!

Naast werken aan 'woekerende waterplanten' heb ik ook ongeveer vier jaar in de personeelsvereniging (Party Planners!) gezeten, waar we vele mooie borrels en feesten georganiseerd hebben. Party people: *Jan*, *Maaike*, *Kim*, *Sven*, *Paolo, Vero*, *Marta*, *Laura*, *Kristin*, *Ruth*, *Thomas*, *Rutger* and *Antonella* thank you all for the great time we had planning, preparing and organising all the 'borrels' and parties. What a great group of people! I would also like to thank all the people from the reception/facility, who often helped with parts of the party planning throughout the years. Bedankt *Elly*, *Ninke*, *Gerrie*, *Edith*, *Dick* en overige leden van het ondersteunend personeel, ook voor al het regelwerk en prettige gesprekken bij de balie! *Peter* (van de vogeltjes), bedankt voor je inzichten in het reilen en zeilen van onze huiskoolmees Pluisje.

Naast de mensen waarmee ik samen heb gewerkt op het NIOO waren er nog een aantal mensen die me geholpen hebben om dit werk af te kunnen ronden. Lieve *pap* en *mam*, jullie hebben mij altijd gestimuleerd om te doen wat ik leuk vond. Ik wilde graag biologie studeren, wat volgens sommigen een slechte keus was omdat daar geen werk in zou zijn. Maar jullie hebben altijd gezegd dat ik dat gewoon moest doen, want als daar echt je passie in ligt kom je er wel. Bedankt voor jullie steun! *Suus*, jou wil ik ook bedankt voor je hartelijke berichtjes en interesse, en natuurlijk voor het organiseren van de gezinsactiviteiten, waaronder de jaarlijkse kerstklaasavond, waarbij het hele gezin weer samen komt om ouderwets gezellig slechte gedichten voor te lezen en cadeaus uit te pakken. *Oma*, ook u wil ik graag bedanken voor uw interesse in mijn werk en dat u altijd klaarstaat met een luisterend oor, een open blik en een lekker kopje Engelse thee. Ik waardeer onze open gesprekken zeer!

Elke week is de zondagavond (19:07 precies) weer een hoogtepunt van mijn week. We komen dan samen met een groep geweldige mensen om koffie, overdatum bier en isotonische sportdrank te drinken en om vooral ook veel muziek te maken. *Bram*, *Mathijs*, *Jasper* en *Maik*, ik wil jullie allemaal heel erg bedanken dat jullie na al die jaren nog steeds met net zoveel plezier als ik naar Vierlingsbeek komen om samen nieuwe muziek te maken en ook gewoon gezellig te ouwehoeren onder het genot van een pilsje. Hierdoor heb ik zelfs in de hele drukke tijd op het werk, dit even kunnen loslaten. Hopelijk zitten we als we 90+ zijn nog steeds elke zondagavond in de garage!
Ook wil ik *Onno*, *Marjolein*, *Marco*, *Isa*, *Hilke*, *Clara*, en *Lisanne* (CABOMBA!) bedanken voor de leuke uitjes die we nog steeds doen samen. Allemaal biologen op hun eigen werkveld, waardoor ik vaak weer geprikkeld wordt om de verbreding te zoeken, maar het is vooral gewoon altijd erg gezellig.

Als laatste kom ik bij de belangrijkste persoon die ik tijdens dit proces heb leren kennen. *Annieke*, liefie, dankjewel voor je steun en vertrouwen in mij, niet alleen werk gerelateerd, maar ook privé. Bedankt voor het meegaan op veldwerk, meedenken over nieuwe ideeën, kritisch lezen van de hoofdstukken in dit boek. Bedankt ook voor het geduldig aanhoren van mijn verhalen tijdens moeilijke perioden en het relativeren van de zaken die soms minder liepen, dit zal niet altijd makkelijk geweest zijn. Je hebt van ons huisje een echt thuis gemaakt voor mij. Ook heb ik veel leuke uitstapjes aan jou te danken, die ik zonder zou waarschijnlijk nooit zou maken. Lekker pimpelen in de Bush, op avontuur in China en Canada. Je bent mijn rots in de branding, op naar het volgende avontuur!

Chapter 14

Curriculum vitae

Michiel (M.J.J.M.) Verhofstad

I, Michiel (M.J.J.M.) Verhofstad, was born on the 19th of September 1987 in Boxmeer and grew up in a charming village by the river Meuse called Vierlingsbeek, the Netherlands. Early on, I developed a fascination for both nature and technology and was always making (or breaking) stuff to find out how the world works. After graduating from the Elzendaal College in Boxmeer in 2006, I studied biology at the Radboud University Nijmegen, the Netherlands. In 2011 I received my master's degree '*cum laude*' after completing my theses on (1) dreissenid mussel ecology and physiology and (2) nutrient removal from waste water using duckweeds. After graduating I kept working on this last project as a junior researcher for a few months investigating the suitability of using LED-lights within the project. Hereafter I kept working as a junior researcher at the Radboud University, but on a new project: 'Highport.Eefde'. In this project we aimed to develop a regional development plan with a large and diverse group of local stakeholders focused around the village of Eefde, the Netherlands. From 2012 until 2017 I was first appointed as a junior researcher at the Netherlands Institute of Ecology (NIOO-KNAW), the Netherlands, which was quickly extended to a full PhD-position. During this period, I investigated submerged aquatic plants and the associated problems that occur when they become too abundant. I focused on why and under what conditions these plants may cause problems, if cutting and harvesting parts of these nuisance plants can be a successful management option, and if these nuisance plant species have any useful applications. I am now going to apply and expand this knowledge in my new job at FLORON, in which I will work to protect and improve (mainly Dutch) aquatic flora and ecosystems.

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Scientific publications

Verhofstad M.J.J.M. & Bakker E.S. (2017) Classifying nuisance submerged vegetation depending on ecosystem services. *Limnology,* doi: 10.1007/s10201-017-0525-z

Verhofstad M.J.J.M., Alirangues Núñez M.M., Reichman E.P., Van Donk E., Lamers L.P.M. & Bakker E.S. (2017) Mass development of monospecific submerged macrophyte vegetation after the restoration of shallow lakes: roles of light, sediment nutrient levels, and propagule density. *Aquatic Botany,* **141,** 29-38.

Verhofstad M.J.J.M., Poelen M.D.M. Van Kempen M.M.L. Bakker E.S. & Smolders A.J.P. (2017) Finding the harvesting frequency to maximize nutrient removal in a constructed wetland dominated by submerged aquatic plants. *Ecological Engineering,* **106,** 423-430.

Kuiper J.J., **Verhofstad M.J.J.M.**, Louwers E.L.M., Bakker E.S., Brederveld R.J., Van Gerven L.P.A., Janssen A.B.G., De Klein J.J.M. & Mooij W.M. (2017) Mowing Submerged Macrophytes in Shallow Lakes with Alternative Stable States: Battling the Good Guys? *Environmental Management,* **59,** 619-634.

Hussner A., Stiers I., **Verhofstad M.J.J.M.**, Bakker E.S., Grutters B.M.C., Haury J., Van Valkenburg J., Brundu G., Newman J., Clayton J.S., Anderson L.W.J. & Hofstra D. (2017) Management and control methods of invasive alien freshwater aquatic plants: A review. *Aquatic Botany,* **136,** 112-137.

Verhofstad M.J.J.M., Grutters B.M.C., Van der Velde G. & Leuven R.S.E.W. (2013) Effects of water depth on survival, condition and stable isotope values of three invasive dreissenid species in a deep freshwater lake. *Aquatic Invasions,* **8,** 157-169. doi: 10.3391/ai.2013.8.2.04

Grutters B.M.C., **Verhofstad M.J.J.M.**, Van der Velde G., Rajagopal S. & Leuven R.S.E.W. (2012). A comparative study of byssogenesis on zebra and quagga mussels: the effects of water temperature, salinity and light-dark cycle. *Biofouling,* **28,** 121-129. *(First two authors contributed equally to this study.)*

Awards and certificates

2nd prize for oral presentation at the 2014 International Shallow Lakes Conference (Antalya, Turkey)

Universiteit Utrecht

UMC Utrecht

Graduate School of Life Sciences

CERTIFICATE

The Board of Studies of the Graduate School of Life Sciences
hereby certifies that

M.J.J.M. Verhofstad

born September 19th 1987, Boxmeer, The Netherlands

has successfully completed the

Training Programme of the Graduate School of Life Sciences

Utrecht, December 20th 2017

Prof. J.A.G. van Strijp, PhD

Chair
Executive Board of Studies

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Classifying nuisance submerged vegetation depending on ecosystem services (2014-2017)

Writing of project proposal (4.5 ECTS)

- MacroManagement of nuisance macrophytes (2013-2014)

Post-graduate courses (1.8 ECTS)

- Introduction to R for statistical analysis; PE&RC and SENSE (2014)
- Generalized linear models; PE&RC (2016)
- Mixed models; PE&RC (2016)

Laboratory training and working visits (2.3 ECTS)

- Anaerobic decomposition of plant biomass methods; Opure, Ede, the Netherlands (2013)
- Presented my research on harvesting aquatic plants; Witteveen&Bos (2014)
- Collaborated on harvesting aquatic plants projects; Collaborate with 2 waterboards (2014-2016)
- Collaborated on harvesting and useful applications of aquatic plants; B-Ware / RUN (2015-2017)
- Presented my research on harvesting aquatic plants; Deltares (2016)

Invited review of (unpublished) journal manuscript (5 ECTS)

- Limnologica: wetland plant ecology (2014)
- Fresenius Environmental Bulletin: harvesting submerged plants (2015)
- Hydrobiologica: aquatic plant physiology (2016)
- Biogeochemistry: nutrient recovery using aquatic plants (2016)
- Landscape Online: aquatic plant management (2017)

Competence strengthening / skills courses (2.6 ECTS)

- Efficient writing strategies; Into languages / WUR (2016)
- Scientific writing; Into languages / WUR (2016)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.5 ECTS)

- PE&RC Day (2014, 2015)
- PE&RC Final year weekend (2016)

Discussion groups / local seminars / other scientific meetings (7.1 ECTS)

- Symposia CWE, PEHM, B-ware, RU AqE, dispersal of aquatic organisms by waterbirds, seminar East China Normal University, CoP Beheer en Onderhoud, STOWA (2012-2016)
- NERN-NAEM; oral presentation (2013)
- NIOO Seminar; oral presentation (2013)
- NIOO Seminar (2013-2017)
- NERN-NAEM; poster presentation (2014)
- NERN-NAEM; oral presentation (2015)
- NERN-NAEM; oral presentation (2016)
- CoP Exoten symposium; oral presentation (2016)
- NERN-NAEM; session convener (2017)

International symposia, workshops and conferences (8.1 ECTS)

- International Shallow Lakes conference; oral presentation (*won 2nd prize*); Antalya, Turkey (2014)
- 14th International symposium on aquatic plants; oral presentation; Edinburgh; Scotland (2015)
- 2 Day workshop on invasive aquatic plants in Düsseldorf (2015)
- SIL Conference; oral presentation; Turino, Italy (2016)

Lecturing / supervision of practicals / tutorials (3 ECTS)

- Aquatic ecology (2013-2017)

Supervision MSc students (15 ECTS)

- Effects of cutting on macrophytes
- Anaerobic digestion of submerged plants
- Factors governing explosive macrophyte growth
- Environmental vs macrophyte stoichiometry
- Effects of harvesting on nutrients on aquatic plants

NOTES:

Royal Netherlands
Academy of Arts
and Sciences