

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 ($\sim 5.6 \text{ mg N.m}^{-2}.\text{d}^{-1}$ and $\sim 1.32 \text{ mg P.m}^{-2}.\text{d}^{-1}$) 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.

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1. 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, 1991b; 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 and 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).

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1.1. Constructed wetlands

Traditionally, emergent and free floating macrophyte species have mainly been used for nutrient removal in constructed wetlands and can remove around $250\text{--}630\text{ g N.m}^{-2}\cdot\text{y}^{-1}$ and $45\text{--}70\text{ g P.m}^{-2}\cdot\text{y}^{-1}$ 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 $<10\text{--}100\text{ g N.m}^{-2}\cdot\text{y}^{-1}$ and $<2\text{--}10\text{ g P.m}^{-2}\cdot\text{y}^{-1}$ 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 and Puijalón, 2009; Van Gerwen et al., 2015). Second, submerged macrophytes have higher tissue nutrient concentrations than emergent species (Demars and 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 $1\text{ kg dry mass m}^{-2}$ (e.g. Schwarz and Howard-Williams 1993; Di Nino et al., 2005; Morris et al., 2006; Van Zuidam and 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.

1.2. 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 and 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; HowardWilliams et al., 1996; Zhang et al., 2014). The change in species composition and abundance may also alter the nutrient removal efficiency.

1.3. 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.

2. Materials and methods

2.1. Study system

We used two shallow ponds of $30 \times 15\text{ m}$ and a water depth of approximately 75 cm as experimental ecosystems, located near Bommel, 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.

2.2. Nutrients and other environmental parameters

Water samples were collected weekly near the inflow and outflow 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).

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 metre. 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 and Johannsen, 1972), orthophosphate (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 $3.38\text{--}7.91$ and $1.16\text{--}1.48\text{ mg.m}^{-2}\cdot\text{d}^{-1}$, respectively for N and P during the experimental period.

2.3. 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,

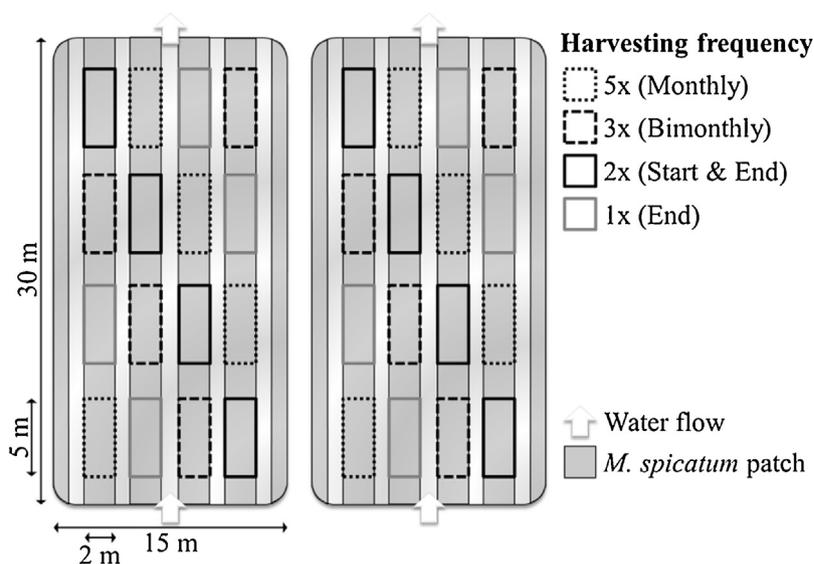


Fig. 1. Schematic of the experimental setup in the two ponds of the constructed wetland.

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 $\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$ and TIC as $\text{CO}_2\text{-C} + \text{HCO}_3\text{-C}$. 'n' indicates the number of data points for pond 1 and pond 2 separate over which the mean and SD are calculated. Italic values for PO_4 indicate the presence of too many identical values near the detection limit; therefore no statistics could be performed. See Appendix Table S1 for additional variables.

Sample type	Variable	Pond 1		Pond 2		n	Df	Test-stat.	p-value	Test
		mean	SD	mean	SD					
Surface water input ($\mu\text{mol.L}^{-1}$)	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 ($\mu\text{mol.L}^{-1}$)	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 ($\mu\text{mol.L}^{-1}$)	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.

with 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 5×2 m plots within the vegetated sections of the ponds. The plots were separated from each other by 2 m of uncut vegetation on the longitudinal sides and by a 1 m wide uncut 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.

2.4. 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 1 ml 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).

2.5. Vegetation survey

The vegetation cover and species composition was determined in all experimental plots 0–3 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).

2.6. 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.test* 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 differed 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. $\frac{\alpha=0.05}{n=4}$). 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.

3. Results

3.1. 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 g of N, 1 g of P, and 3 g of K per square metre was removed from the ecosystem, when harvesting 2x per season.

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 ($F=4.60$, $p=0.044$; Appendix Fig. S1). 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

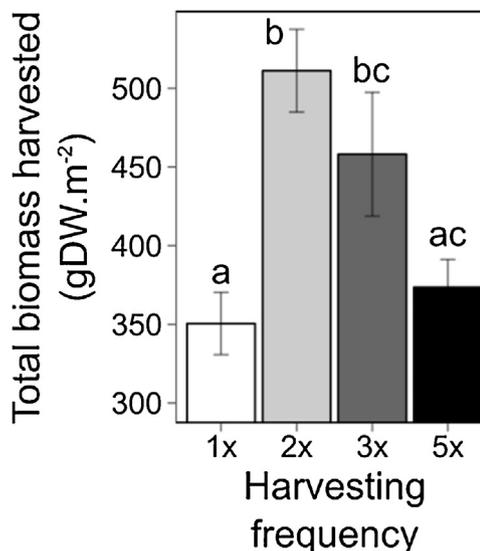


Fig. 2. Cumulative amount of biomass (mean \pm SE of dry mass) harvested per m² per harvesting frequency. Different letters indicate statistically significant differences in the amounts of harvested biomass among harvesting frequencies at $p < 0.05$ (see Table 2).

experiment in pond 2, but not in pond 1 (Appendix Fig. S2). This gradient was similar for all treatments, owing to our experimental design.

3.2. 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 been 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).

4. 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.

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
1 x (end)	103 ± 5 ^{AC}	5.01 ± 0.42 ^{AC}	0.79 ± 0.06 ^A	2.09 ± 0.11 ^A
2 x (start & end)	138 ± 8 ^B	6.17 ± 0.26 ^B	1.02 ± 0.05 ^B	3.04 ± 0.14 ^B
3 x (bimonthly)	119 ± 11 ^{AB}	5.72 ± 0.35 ^{AB}	0.89 ± 0.08 ^{AB}	2.85 ± 0.25 ^B
5 x (monthly)	95 ± 4 ^C	4.59 ± 0.23 ^C	0.74 ± 0.04 ^A	2.29 ± 0.12 ^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 ^A	5.77 ± 0.24 ^A	0.94 ± 0.04 ^A	2.80 ± 0.14 ^A
Pond 2	98 ± 5 ^B	4.81 ± 0.23 ^B	0.75 ± 0.04 ^B	2.26 ± 0.12 ^B

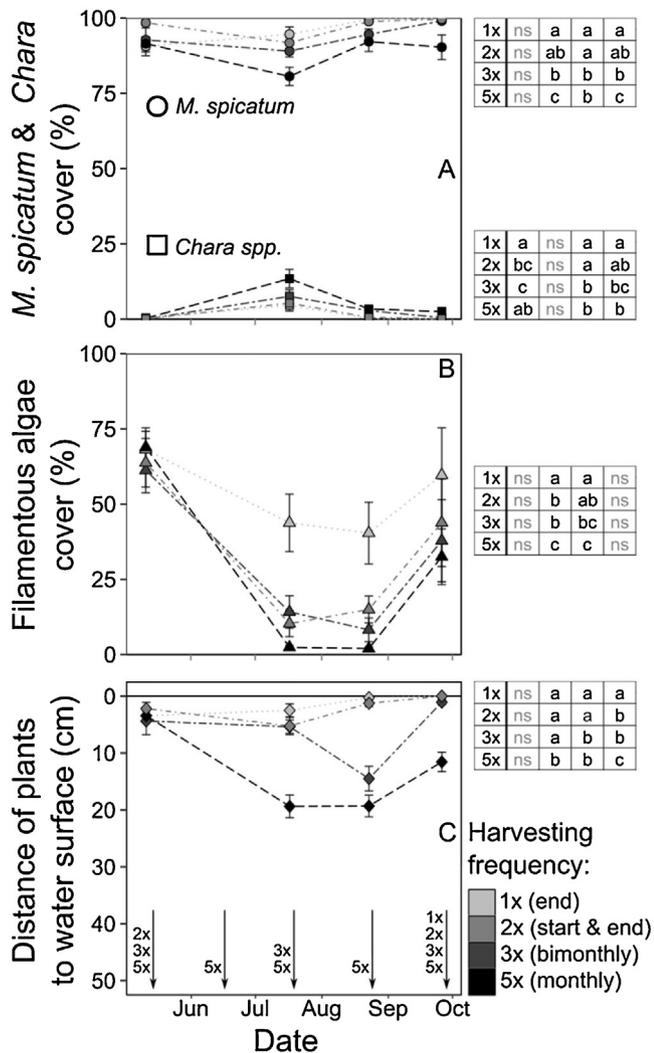


Fig. 3. Mean ± SE cover of *M. spicatum* and charophytes (A), filamentous algae (B) and the average distance between the plant canopy and the water surface (C). Vertical arrows at the bottom of panel C indicate harvesting dates for each of the harvesting frequencies. Different letters in tables next to the graphs indicate statistically significant differences between harvesting frequencies on that one date (Kruskal-Wallis tests ($\alpha=0.0125$ 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.

4.1. Nutrient removal

During this period a total average of 17.0 mg N.m².d⁻¹ and 2.7 mg P.m².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 1 x 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. 2 x or 3 x during the growing season. These harvesting frequencies removed 6 g N, 1 g P, and 3 g K per m² 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 and De Busk 1985; ; 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., 2015; 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. Gumbrecht, 1993a; Abernethy et al., 1996; Barrat-Segretain, 2004; Angelstein et al., 2009), leading to different amounts of nutrients being removed from the ecosystem under identical conditions (Vymazal, 2007; Li et al., 2010, 2011; 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 and 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 and Madsen, 1998; Hussner et al., 2016). CO₂ is the most beneficial form of carbon for the plants to take up (Maberly and Madsen, 1998; Hussner et al., 2016). In both our ponds, output concentrations of CO₂ were almost zero, indicating

total CO₂ removal by primary producers. As the CO₂ 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 and 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 CO₂. 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 metres) and can take up nutrients from the entire water column (Bornette and 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.

4.2. 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 and 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. 20 cm) 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 unown 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; HowardWilliams et al., 1996). However, less tolerant macrophyte species may decrease in abundance under a harvesting regime (e.g. Van Zuidam and Peeters, 2013). 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 5 x (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, 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; HowardWilliams et al., 1996; this study, but see: Johnson and 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.

4.3. 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 requires 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, 1988; 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; Schollemma, 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 and Wilkie, 2010; Quilliam et al., 2015)

5. 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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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecoleng.2017.06.012>

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