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Aquatic macrophytes can be used for wastewater polishing, but not for purification in constructed wetlands

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Abstract

The sequestration of nutrients from surface waters by aquatic macrophytes and soils provides an important service of both natural and constructed wetlands. While emergent species take up nutrients from the soil, submerged and floating macrophytes filter nutrients directly from the surface water, which may be more efficient in constructed wetlands. It remains unclear, however, whether their efficiency is sufficient for wastewater purification, and how plant species and nutrient loading affects nutrient distribution over plants, water, and soil. We therefore determined nutrient removal efficiencies of different vegetation (*Azolla filiculoides*, *Ceratophyllum demersum* or *Myriophyllum spicatum*) and soil types (clay, peaty clay and peat) at three nutrient input rates, in a full factorial, outdoor mesocosm experiment. At low loading (0.43 mg P m^{-2} d^{-1}),

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plant uptake was the main pathway (100 %) for phosphorus (P) removal, while soils showed a net P release. *A. filiculoides* and *M. spicatum* showed the highest biomass production and could be harvested regularly for nutrient recycling, whereas *C. demersum* was outcompeted by spontaneously developing macrophytes and algae. Higher nutrient loading only stimulated *A. filiculoides* growth. At higher rates (≥ 21.4 mg P m^{-2} d^{-1}) 50-90 % of added P ended up in soils, with peat soils becoming more easily saturated. For nitrogen (N), 45-90 % was either taken up by the soil or lost to the atmosphere at loadings ≥ 62 mg N m^{-2} d^{-1}. This shows that aquatic macrophytes can indeed function as an efficient nutrient filter, but only for low loading rates (polishing), not for high rates (purification). The outcome of this controlled study not only contributes to our understanding of nutrient dynamics in constructed wetlands, but also shows the importance of wetland soil characteristics. Furthermore, the acquired knowledge may benefit the application of macrophyte harvesting to remove and recycle nutrients from both constructed wetlands and nutrient-loaded natural wetlands.

Keywords: Eutrophication, nutrient removal, macrophytes, nutrient budgets, purification, water management

1. Introduction

Excess loading of phosphorus (P) and nitrogen (N) from domestic, agricultural and industrial wastewaters is the main cause of eutrophication of aquatic ecosystems, damaging their ecological quality and functioning (Kantawanichkul et al., 2009; Kronvang et al., 2005). Surface water eutrophication can lead to algal and cyanobacterial blooms, die-off of indigenous vegetation and serious decrease in biodiversity (Conley et al., 2009; Pretty et al., 2003). In recent decades, wetlands have been constructed to mitigate eutrophication of

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watercourses, lakes and seas by reducing the nutrient loads in discharge water of wastewater treatment plants, farmlands, households or industries (Brix & Arias, 2005; Mitsch et al., 2005).

Constructed wetland systems (CWS) use macrophytes (free surface flow systems) or a combination of macrophytes and soil (subsurface flow systems), to remove nutrients from the water (Lin et al., 2003). These systems are either used as stand-alone water purification systems (Jing et al., 2001; Vrhovšek et al., 1996) or as a polishing method of pre-treated wastewater (Greenway, 2005; Kaseva, 2004). The most commonly used macrophyte species are emergent genera such as *Typha, Phragmites, Scirpus, Phalaris* and *Iris* (Vymazal, 2011). Advantages of CWS include utilization of natural processes, low cost and energy requirements, and easy operation and maintenance (Brix, 1999; Konnerup et al., 2009). As a result of low maintenance, however, these systems easily become saturated, especially with P, and therefore only work efficiently for a limited amount of time (Drizo et al., 2002).

Although much research has focused on the optimal design of CWS with respect to the most efficient macrophyte species (Lin et al., 2002; Scholz & Xu, 2002), only few have investigated the possibility of using floating or submerged aquatic macrophytes in treatment systems. While helophytes mainly take up nutrients from the soil, floating and submerged aquatic macrophytes, such as *Azolla* spp. or *Myriophyllum* spp., can also take up nutrients from the water layer (Best & Mantai, 1978; Van Kempen et al., 2012). By regularly harvesting these plants, nutrients may be drained from the system. The aquatic biomass can then be used in various bio-based applications, for instance, as a bio-fertilizer or as fodder for livestock (Hauck, 1978).

There is a suite of mechanisms involved in the processes of nutrient removal and recovery in natural and constructed wetlands, including adsorption, precipitation, plant absorption, volatilization, and microbial

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processes such as iron oxidation, nitrification, DNRA (dissimilatory nitrate reduction to ammonium) and anammox (anaerobic ammonium oxidation) (Kadlec & Wallace, 2008; Van Dongen et al., 2001; Van Loosdrecht & Jetten, 1998; Wu et al., 2014). These mechanisms are generally affected by factors such as nutrient loading, plant species and soil characteristics (Gale et al., 1994; Jampeetong et al., 2012; Tanner, 1996). So far, most studies have focused on the effects of only one or two of these factors on nutrient retention in wetlands, whereas little information is available on interactions among plant species, soil type and nutrient loading. Only by including all interactions, however, can nutrient sequestration efficiency of wetland plants and soils under different loads be assessed.

Here, we studied the effects of plant species, nutrient loading and soil type on nutrient uptake rates of aquatic macrophytes and nutrient retention rates of soils. Using a full-factorial outdoor mesocosm experiment, we studied the nutrient uptake rates of three different aquatic macrophytes, *Azolla filiculoides*, *Ceratophyllum demersum* and *Myriophyllum spicatum*, growing on peat, peaty clay or clay soils. Three different, environmentally relevant, nutrient loadings of P (0.43, 21.4 and 85.7 mg P m^{-2} d^{-1}) and N (1.3, 62 and 249 mg N m^{-2} d^{-1}) were applied to the mesocosms, representing pre-treated (low nutrient loading), and eutrophic and hypertrophic wastewater input (medium and high nutrient loading). By studying the resulting distribution of P and N among the different soil, macrophyte and water compartments, we aimed to determine whether nutrient removal by floating or submerged aquatic macrophytes may be an efficient approach for polishing or purifying wastewater.

2. Materials and methods

2.1. Experimental set-up

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Twenty-seven mesocosms (185 cm Ø, 90 cm depth) were sunk into the ground outside the greenhouse facility at Radboud University (Nijmegen, The Netherlands). All mesocosms were filled with 20 cm (135 L) of clay (originating from Lalleweer, 53°16' N, 6°59' E; n=9), peaty clay (originating from De Deelen, 53°01' N, 5°55' E; n=9) or peat (originating from Ilperveld, 52°27' N, 4°56' E; n=9), after which they received a layer of 50 cm of Nijmegen tap water. Soil characteristics are displayed in Table 1, expressed per unit volume to enable comparison among soil types with respect to nutrient exchange and plant nutrient availability. In all mesocosms, crossed transparent carbon fiber plates were used to create four fully isolated quarters. We did not include non-vegetated treatments because: 1) our focus was on complete ecosystems in constructed and natural wetlands, i.e. including soil and vegetation; 2) bare soils always show spontaneous vegetation development if light and nutrient conditions suffice (see section 2.2); 3) continuous plant removal would lead to significant soil disturbance; and 4) dark conditions would affect soil biogeochemistry. Mesocosms were randomly assigned to "low", "medium" or "high" nutrient loading treatment (n=3 for all). To create these, treatment solutions were added three times a week to enable loading rates of 0.43, 21.4 and 85.7 mg P m^{-2} d^{-1} (added as $NaH_2PO_4 \cdot H_2O$ and atmospheric deposition of 0.1 kg P ha^{-1} y^{-1}) (Furnas, 2003) and 1.3, 62 and 249 mg N m^{-2} d^{-1} (added as NH_4NO_3 and atmospheric N deposition of 20 kg N ha^{-1} y^{-1} in this part of the Netherlands; TNO)(De Leeuw et al., 2001). In the results and discussion sections, treatments will be called 0.43 (low), 21.4 (medium) and 85.7 (high) mg P m^{-2} d^{-1}, according to their respective P loading.

2.2. Plant measurements

In July 2013, environmentally relevant densities (based on personal field observations) of *Ceratophyllum demersum* (5.03 ± 0.24 g DW m^{-2}; rigid hornwort, submerged macrophyte), *Chara hispida* (8.66 ± 0.69 g DW m^{-2}; bristly stonewort, submerged macroalga) and *Myriophyllum spicatum* (5.31 ± 0.60 g DW m^{-2}; Eurasian water-

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milfoil, submerged macrophyte) were planted randomly in each of three quarters of every mesocosm to establish. In April 2014, patches of *Azolla filiculoides* (28.39 ± 0.88 g DW m^{-2}; water fern, floating macrophyte) were added to the water layer of the remaining quarter. Apart from these four introduced species, other species colonized the quarters, including *Zanichellia* spp. and floating algae. During the experimental period, 20 % of the total plant biomass was harvested when vegetation reached 100 % cover to avoid space limitation. During the final harvest, biomass of all present species was harvested separately and dried (48 h at 60 °C), after which they were weighed, ground and homogenized. As *C. hispida* was completely outcompeted by spontaneously developing vegetation, the quarters with this species were excluded from the results.

2.3. Chemical analyses

Surface water samples were collected every week between May and October 2014, whereas pore water samples were collected anaerobically every month using ceramic soil moisture cups (SMS rhizons, Eijkelkamp, Giesbeek, Netherlands). pH of water samples was measured using a combined Ag/AgCl electrode (Orion, Thermo Fisher Scientific, Waltham, MA, U.S.A.) with a TIM840 pH meter (Radiometer Analytical, Lyon, France). Total inorganic carbon (TIC) of water samples was measured using an Infra-red Gas Analyzer (IRGA; ABB Analytical, Frankfurt, Germany). Concentrations of PO_4^{3-}, NO_3^- and NH_4^+ in the surface water and pore water were measured colorimetrically on an Auto-Analyzer III system (Bran & Luebbe, Norderstedt, Germany) by using ammonium molybdate (Henriksen, 1965), hydrazine sulphate (Kamphake et al., 1967) and salicylate (Grasshoff & Johannsen, 1972), respectively. Concentrations of total Al, Fe, Ca, and P were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES; IRIS Intrepid II, Thermo Fisher Scientific, Franklin, MA, U.S.A.).

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Soil samples were collected at the end of the experiment, and subsequently volume weighted and dried for 48 h at 60 °C to determine bulk density. Dry soil samples were heated for 4 h at 550 °C and re-weighed to determine organic matter content. Furthermore, 200 mg of dry soil was digested in a microwave oven (MLS-1200 Mega, Milestone Inc., Sorisole, Italy) with 4 mL 65 % HNO_3 and 1 mL 30 % H_2O_2, after which digestates were analyzed by ICP-OES (see above). Plant available P was determined by extraction according to Olsen et al. (1954), whereas an NaCl-extraction was performed to determine exchangeable N ions (NO_3^- + NH_4^+) as described in Tomassen et al. (2004). Total P concentrations in plants were determined by digestion of 200 mg of dry plant material and analyzed as described above. Furthermore, 3 mg of dry plant material was combusted to determine C and N content using an elemental analyzer (Carlo Erba NA 1500, Thermo Fisher Scientific, Waltham, MA, USA).

2.4. *Budget calculations*

For both N and P, nutrient budgets were calculated to determine the distribution among biomass, soil and other components. Cumulative biomass production and nutrient content of submerged or floating macrophytes (target species and others) were used to calculate plant uptake rates of N and P. Furthermore, nutrient changes in surface water and pore water were calculated from changes of N (NO_3^- and NH_4^+) and P concentrations (end minus start). After subtracting the N and P uptake of plants and water components from the external loading, we assume that the remainder is either stored in the soil or, in case of N, lost through denitrification or anammox.

2.5. Statistical Analyses

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All analyses were performed using the software program R (version 3.2.1; R development Core Team, 2015). The effects were considered significant if $P < 0.05$. In order to meet the assumption that residuals fit a normal distribution and homogeneity of variance, we transformed soil characteristics, N (NO_3^- and NH_4^+) and P concentrations in surface water, biomass production rates, N: P ratios in macrophytes, N and P budgets and N and P sequestration rates (response variables) by log (response variable) or log (response variable+1) in case the lowest value of a variable was below one. Linear mixed models were used to test the main effects and interactions of treatments on soil characteristics, biomass production rates, the ratios between N and P, N (NO_3^- and NH_4^+) and P concentrations in surface water (except for treatments also including time as a main effect in this model), and nutrient budgets with mesocosm number as a random effect, by using R package nlme. Tukey tests were used to find differences between treatments by using R package multcomp. We analyzed the influence of nutrient loadings on P and N sequestration rates using linear and logistic regression models. All graphs were plotted using R package ggplot2.

3. Results

3.1. Surface water and pore water quality

Over time, surface water P and N (NH_4^++NO_3^-) concentrations increased (Figs. 1 and 2; $X^2=4.26$; $P < 0.05$ and $X^2=35.61$; $P < 0.000$ for P and N respectively), especially towards the end of the growing season. When macrophytes were growing on peat or peaty clay soils, P concentrations in the surface water increased with increasing external P loading ($X^2=115.87$; $P < 0.000$ and $X^2=88.94$; $P < 0.000$ for peat and peaty clay soils respectively).

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Porewater nutrient concentrations depended on soil type. Peat soils had the highest P concentrations in the pore water, whereas the lowest were found in clay soils (X^2=12.07; P < 0.01; data not shown) even though their total P and Olsen P concentrations were much higher than for the other two soils (Table 1). In addition, mesocosms filled with peat soils had higher N concentrations in the pore water than those with peaty clay and clay (X^2=7.13; P < 0.05; data not shown). Surface water and porewater together never contained more than 12 % of total P and N added to the system at P loadings ≥ 21.4 mg P m^{-2} d^{-1} (Figs. 4 and 5).

3.2. Macrophyte productivity and nutrient ratio

Due to their high biomass production rates, *A. filiculoides* and *M. spicatum* could be harvested weekly and biweekly, respectively. *A. filiculoides* had the highest biomass production rates of all three macrophyte species (X^2= 55.45, P<0.000), whereas *C. demersum* grew best on peaty clay soils (X^2=10.67, P < 0.01), but almost disappeared when growing on clay and peat soils due to competition with algae and other non-target species (Fig. 3). Biomass production rates of *A. filiculoides* were significantly higher at high nutrient loading than at low nutrient loading (X^2=11.39, P < 0.01), whereas no effect of nutrient loading was found for the other macrophytes. In quarters with *C. demersum* there was a higher production rate of non-target species than in quarters with *A. filiculoides* and *M. spicatum* (X^2=6.28, P < 0.05). *A. filiculoides* showed high N: P ratios (> 24 mol mol^{-1}) when grown at ≤ 21.4 mg P m^{-2} y^{-1} (P < 0.000), whereas all other species generally showed N: P ratios ranging from 8 to 17 mol mol^{-1}, without an effect of soil type (Table 2).

3.3. Plant nutrient uptake

A. filiculoides and *M. spicatum* accumulated much more P than *C. demersum* (X^2=23.66, P < 0.000; Fig. 4). At a P loading of 0.43 mg m^{-2} d^{-1} around 100 % of added P and N were accumulated by the targeted macrophytes (Figs.

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4 and 5). For the quarters with *A. filiculoides* and *M. spicatum,* around 20-40 % and 10-20 % of the P added was taken up by target species at P loadings of 21.4 and 85.7 mg m^{-2} d^{-1}, respectively, regardless of soil types. *C. demersum*, on the other hand, never took up more than 20 % of the P added at these loadings. Still, at a loading of 85.7 mg P m^{-2} d^{-1}, removal rates by macrophytes were significantly higher than at 0.43 mg P m^{-2} d^{-1} (X^2=7.22, P < 0.05; Fig. 4). The average P sequestration rates by *A. filiculoides* and *M. spicatum* were 3 to 9 mg m^{-2} d^{-1} at P loadings ≤ 21.4 mg m^{-2} d^{-1}. At a high P loading of 85.7 mg m^{-2} d^{-1}, the average P removal rates by *A. filiculoides* and *M. spicatum* were 16 to 20 and 6 to 14 mg m^{-2} d^{-1}, respectively. In addition, *C. demersum* had higher P and N uptake rates in mesocosms with peaty clay compared to mesocosms with clay (X^2=10.50, P < 0.01; X^2=10.43, P < 0.01). In quarters with *C. demersum*, more P was taken up by other, spontaneously developing species than in quarters with *A. filiculoides* and *M. spicatum* (X^2=6.89, P < 0.05). In addition, these non-target plants in *C. demersum* quarters had lower P uptake rates on peaty clay than on peat and clay soils (X^2=6.92, P < 0.05). *A. filiculoides* and *M. spicatum* absorbed much more N than *C. demersum* and the final biomass of *A. filiculoides* had the highest N content (including N_2 fixed) among all macrophyte species (X^2=10.28, P < 0.01; Fig. 5). At high N loadings, less than 21 % of added N was removed by the targeted macrophytes.

3.4. Mobilization and adsorption of nutrients by the soil

At a P loading of 0.43 mg m^{-2} d^{-1}, soils were sources of P, whereas soils became P sinks at P loading ≥ 21.4 mg m^{-2} d^{-1} (Fig. 4). On average, 50 to 80 % and 70 to 90 % of P added accumulated in soils at medium and high nutrient loadings, respectively (Fig. 4). In quarters with *C. demersum*, more P accumulated in the soil than in quarters with *A. filiculoides* (X^2=11.25, P < 0.01). As P loading increased, more P accumulated in the soils (X^2=566.40, P < 0.000). At medium and high N loads, 45 to 90 % and 80 to 90 %, respectively, was either taken up by the soil or lost to the atmosphere through denitrification/anammox.

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4. Discussion

In our mesocosm experiment, we show that at low nutrient input (≤ 0.43 mg P m^{-2} d^{-1}), 100 % of external loading could be removed through macrophyte uptake, whereas with loadings ≥ 21.4 mg P m^{-2} d^{-1}, 50 to 90 % of added P ended up in soils. Differences exist, however, between binding abilities of soils, with clay soils being able to immobilise P better than peaty clay or peat soils. Apart from P, macrophytes were able to remove no more than 65 % and 21 % of added N at loadings of 62 mg m^{-2} d^{-1} and 249 mg m^{-2} d^{-1}, respectively, while the remaining N was either stored in the soil or lost to the atmosphere through denitrification and/or anammox.

4.1. Growth and nutrient uptake of macrophyte species in constructed wetlands

With average biomass production rates of 3.4 and 1.0 g DW m^{-2} d^{-1}, respectively, *A. filiculoides* and *M. spicatum* showed the highest growth rates and therefore the best potential for being used to remove nutrients in constructed wetlands. Due to their high growth rates, these species could be harvested biweekly or even weekly. *C. demersum,* on the other hand, appeared to be less suitable, since this species was readily outcompeted by other species, such as floating algae and *Zanichellia spp*. P was removed most efficiently by *A. filiculoides,* followed by *M. spicatum and C. demersum*. Although a high P load (85.7 mg m^{-2} d^{-1}) resulted in increased uptake rates of 6 to 14 and even 16 to 20 mg P m^{-2} d^{-1} for *M. spicatum* and *A. filiculoides*, respectively, these rates were not sufficient to efficiently filter all added P from the system.

For *C. demersum*, nutrient sequestration rates increased linearly with increased nutrient loading, while for *M. spicatum* there was a logistic response to external nutrient loading (Fig. 6). *A. filiculoides* showed linearly increasing P sequestration rates upon increased P loading and a logistic response to external N loading. These different response types between species most likely resulted from differences in main nutrient sources and

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nutrient limitation. For rooted *M. spicatum,* plants mainly rely on soil uptake (Barko & Smart, 1980; Best & Mantai, 1978; Carignan & Kalff, 1980), whereas for non-rooted *A. filiculoides* and *C. demersum* water is the main nutrient source (Denny, 1987; Mjelde & Faafeng, 1997). Our results indicate that at loads ≤ 22 mg P m^{-2} d^{-1}, *M. spicatum* is the most efficient P remover, whereas at loads ≥ 22 mg P m^{-2} d^{-1}, *A. filiculoides* is more efficient (Fig. 6a). In addition, the effective thresholds for P purification (100 % removal) of *C. demersum, A. filiculoides*, and *M. spicatum* are 1.9, 4.8 and 6.8 mg P m^{-2} d^{-1}, respectively (Fig 6a). Threshold values for complete N removal are 8.6 and 31.4 mg N m^{-2} d^{-1} for *C. demersum* and *M. spicatum*, respectively (Fig. 6b). *A. filiculoides*, on the other hand, hardly ever becomes N limited due to its symbiosis with a diazotrophic microbial community (Handley & Raven, 1992). Under low external P loadings, *A. filiculoides* therefore displayed very high N: P ratios indicating P limitation at P loadings ≤ 21.4 mg P m^{-2} d^{-1}. *C. demersum*, on the other hand, having no access to soil or atmospheric N, probably showed N limitation in these systems, as indicated by their low N: P ratios. For all species, N: P ratios decreased with increasing P load.

4.2. Using aquatic macrophytes for polishing of pre-treated wastewater

Due to regular harvesting of *A. filiculoides* and *M. spicatum*, P and N were removed at rates of around 3 to 9 mg P m^{-2} d^{-1} and 31 mg N m^{-2} d^{-1} at loadings of 0.43 mg P m^{-2} d^{-1} and 1.3 mg N m^{-2} d^{-1}. These results are comparable to those found by Van Kempen (2013) who found uptake rates of 3.7 mg P m^{-2} d^{-1} (13.4 kg ha^{-1} $year^{-1}$) and 13.7 mg N m^{-2} d^{-1} (50 kg ha^{-1} $year^{-1}$) in summer, and 4.8 mg P m^{-2} d^{-1} (17.5 kg ha^{-1} $year^{-1}$) and 69.3 mg N m^{-2} d^{-1} (253 kg ha^{-1} $year^{-1}$) in early fall for *A. filiculoides* grown in N-free water with 25 µmol L^{-1} PO_4. For *M. spicatum*, our results are in the same range as those reported by Smith and Adams (1986) and N uptake rates of 0.05-1.26 g N m^{-2} d^{-1} by *Myriophyllum aquaticum* reported by Nuttall (1985). Due to lowering of the O_2 concentration in the water layer, similar to other floating or densely growing submerged macrophytes (Caraco et al., 2006), these

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plants not only take up all P being discharged into the system, but additionally mobilize and take up P from the soil by their roots and the creation of anaerobic conditions (Wetzel, 2001).

Since uptake of nutrients by aquatic macrophytes depends on their biomass production and thus on macrophyte photosynthesis, these systems would only function optimally during the growing season. Under low external loading, soils will take up most of the P during winter, which can subsequently be mobilised and taken up by macrophytes in summer, creating an efficient and sustainable constructed wetland for water polishing in temperate climates.

4.3. Using aquatic macrophytes for wastewater purification

When P loading in the treatment water increases, uptake rates of *A. filiculoides* double or even triple, to rates around 6-24 mg P m^{-2} d^{-1}. The highest value is comparable to results of Reddy and DeBusk (1985), who reported P uptake rates of 43 ± 15 mg P m^{-2} d^{-1} by *A. filiculoides* grown in an N-free, 3 mg L^{-1} PO_4^{3-}-medium. Although plants could not take up all P at medium or high external P loadings, overall surface water quality remained around or below 12 µmol L^{-1} when clay sediments were used for the construction of the wetland. At the end of the growing season, however, plant uptake decreased and P availability in surface waters above peaty clay and peat soils increased strongly to concentrations around 60 and 72 µmol P L^{-1}, respectively, indicating not only inactivity of aquatic macrophytes but probably also P saturation of soils. Due to the 7-8 times higher Fe and Al contents (400 vs. 50-60 mmol L^{-1} FW, 450 vs. 60-70 mmol L^{-1} FW for Fe and Al, respectively) of clay soils, P was most probably immobilized more efficiently by clay (Reddy & DeLaune, 2008), which resulted in lower P concentrations in surface water above clay soils.

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More than 98 % of added N was removed from the surface water during the run of the experiment. As nutrient loading increased, the amount of added N that was removed by plant uptake decreased. Harvested biomass of target plants contained 31 mg N m^{-2} d^{-1} for *M. spicatum*, whereas in the quarters with *C. demersum*, non-target macrophytes or algae absorbed most N. For *A. filiculoides* it was difficult to calculate N removal rates due to unknown N_2 fixation rates leading to an overestimation of N uptake rates by *A. filiculoides*. N that was not taken up by plants, but was still removed from the water layer most likely ended up in the soil or was released to the atmosphere by denitrification and/or anammox (Hao et al., 2002; Van der Star et al., 2007). On average, inorganic N (NH_4^++NO_3^-) concentrations in the surface water were below 8 µmol L^{-1} with external loadings ≤ 62 mg N m^{-2} d^{-1} and around 20 µmol L^{-1} when receiving 249 mg N m^{-2} d^{-1}. At the end of the growing season, N concentrations increased under high nutrient loading, similar to P, suggesting nutrient leaching from senescing plants is more important than soil saturation.

4.4. Implications for management

We showed that in macrophytes-dominated CWS, submerged or floating macrophytes are able to remove most of the added nutrients at low P and N loadings, whereas at higher nutrient loadings, floating or submerged macrophytes can only remove 20-45 % and 10-25 % of the external P loads for 21.4 and 85.7 mg P m^{-2} d^{-1}, respectively. For water management, using fast growing aquatic macrophytes, such as *A. filliculoides* or *M. spicatum* regular mowing allows complete removal of added nutrients at relatively low nutrient loading (≤ 4.8 mg P m^{-2} d^{-1} or ≤ 6.8 mg P m^{-2} d^{-1}, respectively). Although *A. filiculoides* still extracted P and competed with soil adsorption at higher P loads (≥ 21.4 mg P m^{-2} d^{-1}), most external P ended up in the soil, eventually resulting in saturation. While aquatic macrophytes are able to remove this P from the soils by either creating anaerobic conditions or through root uptake, the external load will have to be reduced for this process to occur efficiently.

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Consequently, at these higher P and N loads, the macrophyte stage can only be used as an additional polishing step after a major part of the nutrients have been removed by other ways of water treatment.

5. Conclusions

Here, we show that aquatic macrophytes can be used for polishing, but not as a stand-alone purification treatment for nutrient removal from wastewater. At loads ≤ 22 mg P m^{-2} d^{-1}, *M. spicatum* is the best option, whereas at loads ≥ 22 mg P m^{-2} d^{-1}, *A. filiculoides* removes P more efficiently. Furthermore, we have shown that soil type is a previously underestimated factor influencing the efficiency of nutrient removal and immobilization. Especially at higher P loads, soils form highly important sinks and the saturation potential of the soil is therefore important. Clay soils should be preferred, as these take longer to become saturated than more organic soils.

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Author Contributions

Conceived and designed the experiment: J.G.M.R., A.J.P.S., L.P.M.L. and M.M.L.V.K.; Performed the experiment: E.J.H.V., L.M.J.M.L. and M.M.L.V.K.; Analysed the data: S.F.H., Y.T. and E.J.H.V.; Wrote the paper: S.F.H., Y.T., A.J.P.S., L.P.M.L. and M.M.L.V.K.

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Table 1 Soil characteristics of peat, peaty clay and clay soils used in the experiment (±SE; n=36). pH and Total inorganic carbon (TIC) are derived from porewater analyses, whereas all other analyses were performed using fresh or dry soil (see Sect. 2.3.).

Soil	Bulk density (kg DW.L^{-1} FW)	Organic matter %	pH	TIC (µmol L^{-1})	Salt extractable N (NO_3^- + NH_4^+) (µmol L^{-1} FW)	Olsen-P (umol L^{-1} FW)	Total-P (mmol L^{-1} FW)	Total-Fe (mmol L^{-1} FW)	Total-Al (mmol L^{-1} FW)	Total-Ca (mmol L^{-1} FW)
Peat	0.15 (0.00)^{C}	43.73 (0.80)^{A}	7.20 (0.02)^{A}	8825.84 (120.36)^{A}	551.72 (58.71)^{B}	269.41 (13.16)^{B}	4.98 (0.19)^{B}	47.15 (0.92)^{B}	55.43 (1.80)^{B}	65.05 (1.06)^{B}
Peaty clay	0.23 (0.01)^{B}	34.39 (1.63)^{B}	6.92 (0.03)^{B}	5892.89 (240.56)^{B}	494.11 (70.17)^{B}	153.90 (13.98)^{C}	3.39 (0.19)^{C}	58.72 (4.32)^{B}	67.84 (5.37)^{B}	62.14 (5.02)^{B}
Clay	1.00 (0.01)^{A}	5.07 (0.24)^{C}	7.18 (0.04)^{A}	10189.53 (537.67)^{A}	1063.66 (123.98)^{A}	1104.48 (18.69)^{A}	22.25 (0.41)^{A}	402.74 (5.26)^{A}	438.77 (8.05)^{A}	101.85 (1.31)^{A}

Significant differences among soil types are indicated by different capital letters (A, B and C).

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Table 2 Plant tissue ratios between N and P for different macrophytes subjected to different nutrient loadings (0.43, 21.4 and 85.7 mg P m^{-2} d^{-1}) at the end of the experiment. Average N: P ratios of target species are given with standard error.

Species	Soil type	N : P (mol : mol)		
		0.43	21.4	85.7
A. filiculoides	Clay	34.77 (±1.03)[a]	42.88 (±4.12)[a]	17.87 (±1.28)[b]
	Peaty clay	49.21 (±3.66)[a]	24.10 (±0.64)[b]	11.23 (±0.32)[c]
	Peat	41.94 (±0.23)[a]	24.17 (±1.95)[b]	12.84 (±0.75)[c]
C. demersum	Clay	8.92 (±1.36)	9.16 (±1.12)	NA
	Peaty clay	9.33 (±0.97)	9.04 (±1.59)	8.04 (±0.84)
	Peat	16.95(±4.29)[a]	9.43 (±0.69)[ab]	7.52 (±0.93)[b]
M. spicatum	Clay	10.43 (±1.39)	9.80 (±0.53)	9.22 (±1.92)
	Peaty clay	13.31 (±1.80)[a]	10.24 (±0.56)[ab]	8.40 (±0.74)[b]
	Peat	10.14 (±1.18)	9.66 (±0.38)	8.34 (±0.78)

Significant differences among different nutrient loadings are indicted by different lower case letters (a, b and c); there were no significant differences among soil types. Note that NA means that there were no replicates for this treatment.

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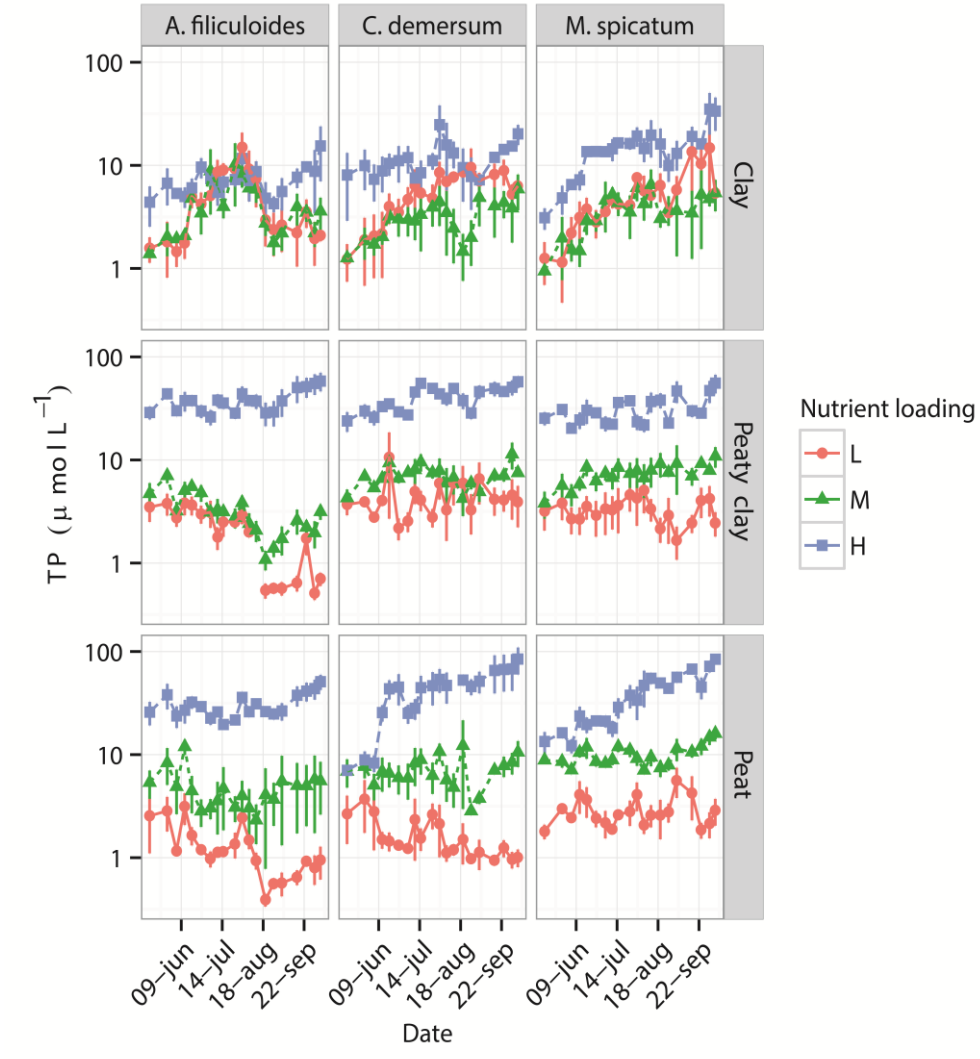

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Figure 1. Surface water TP concentrations subjected to different nutrient loadings (L = 0.43 mg P m^{-2} d^{-1}; M = 21.4 mg P m^{-2} d^{-1}; H = 85.7 mg P m^{-2} d^{-1}) in mesocosms with different plant species (vertical panels) on clay, peaty clay or peat soils (horizontal panels) during the experiment. Average TP concentrations are given with SEM. Note the $\log_{10}$ scale for the y-axis.

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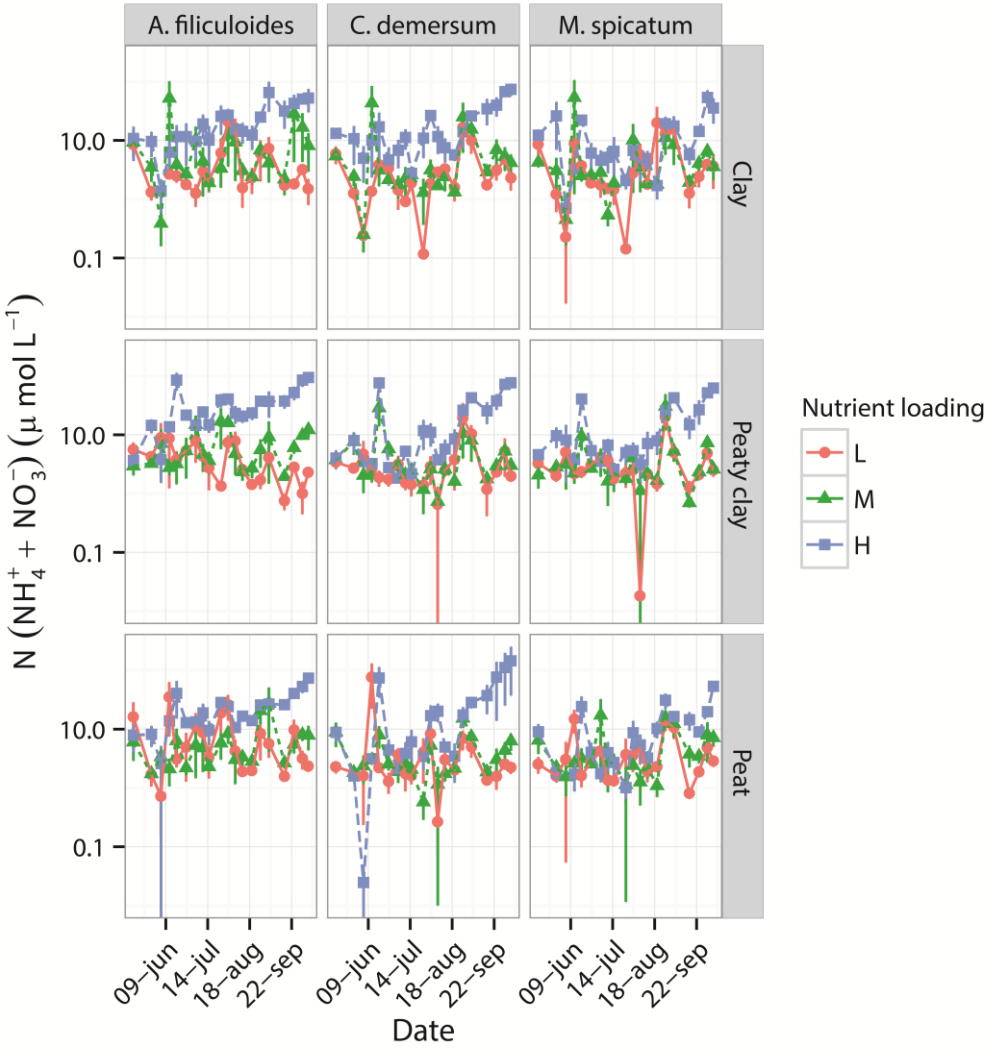

430 Figure 2. Surface water N (NH_4^++NO_3^-) concentrations subjected to different nutrient loadings (L = 0.43 mg P m^{-2} d^{-1}; M = 21.4 mg P m^{-2} d^{-1}; H = 85.7 mg P m^{-2} d^{-1}) in mesocosms with different plant species (vertical panels) on clay, peaty clay or peat soils (horizontal panels) during the experiment. Average N (NH_4^++NO_3^-) concentrations are given with SEM. Note the $\log_{10}$ scale for the y-axis.

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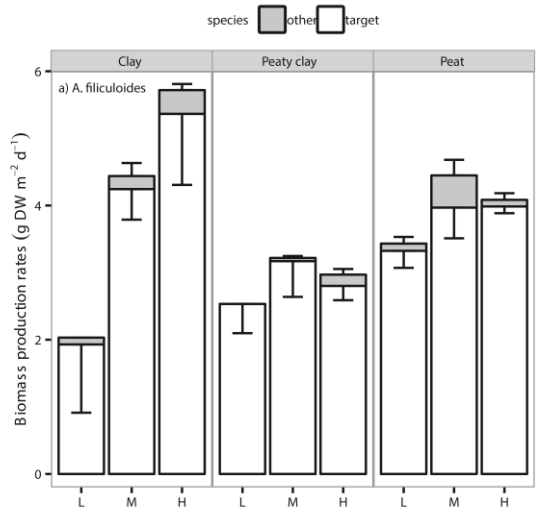

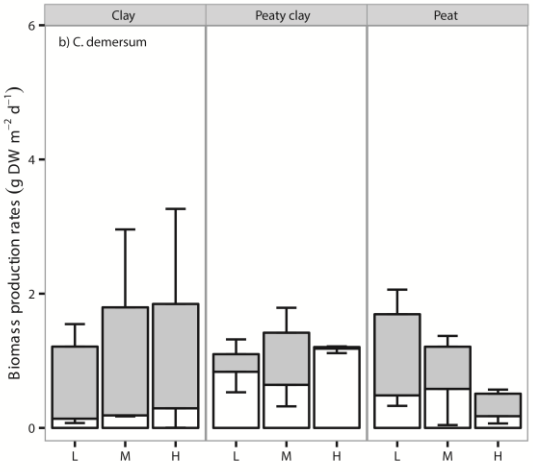

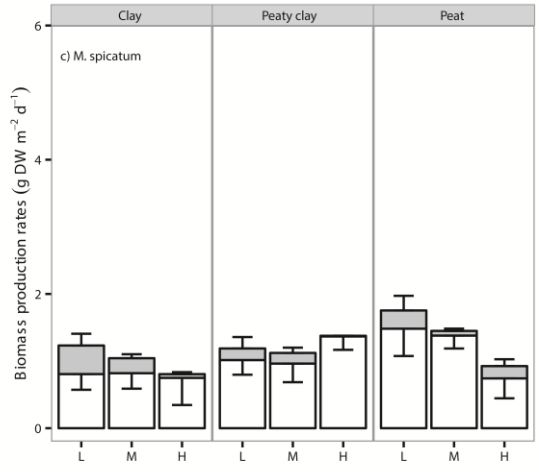

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Figure 3. Biomass production rates (in g DW m^{-2} d^{-1}) of *A. filiculoides* (a), *C. demersum* (b), *M. spicatum* (c) and other, non-target plants
435 (e.g. floating algae, *Zanichellia* spp and other plants) grown on different soil types and subjected to different nutrient loadings (L = 0.43 mg P m^{-2} d^{-1}; M = 21.4 mg P m^{-2} d^{-1}; H = 85.7 mg P m^{-2} d^{-1}). Average biomass production rates of target species (-SEM) and other plants (+SEM) are given.

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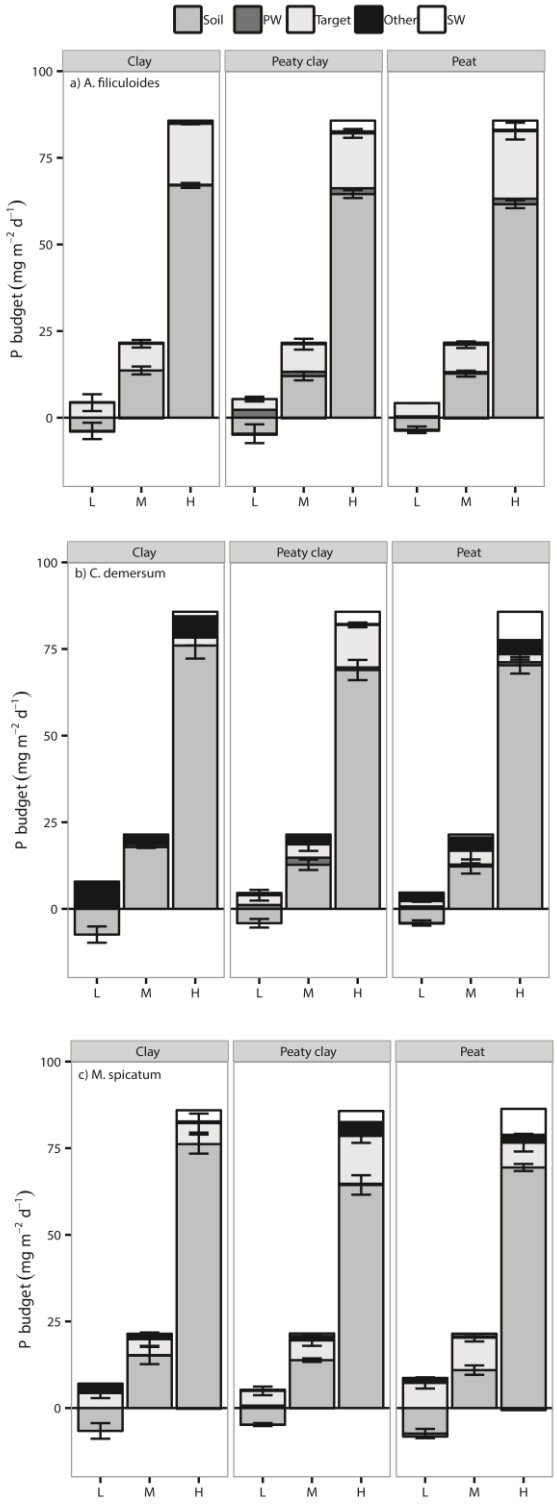

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Figure 4. P budgets of soil, surface water, pore water, target species and other plants subjected to different nutrient loadings (L = 0.43 mg P m^{-2} d^{-1}; M = 21.4 mg P m^{-2} d^{-1}; H = 85.7 mg P m^{-2} d^{-1}) for (a) *A. filiculoides*, (b) *C. demersum*, and (c) *M. spicatum*. Standard errors are given only for soil and target species. PW = pore water, SW = surface water. Positive values represent P accumulation in relative parts; negative values represent P release from respective compartments.

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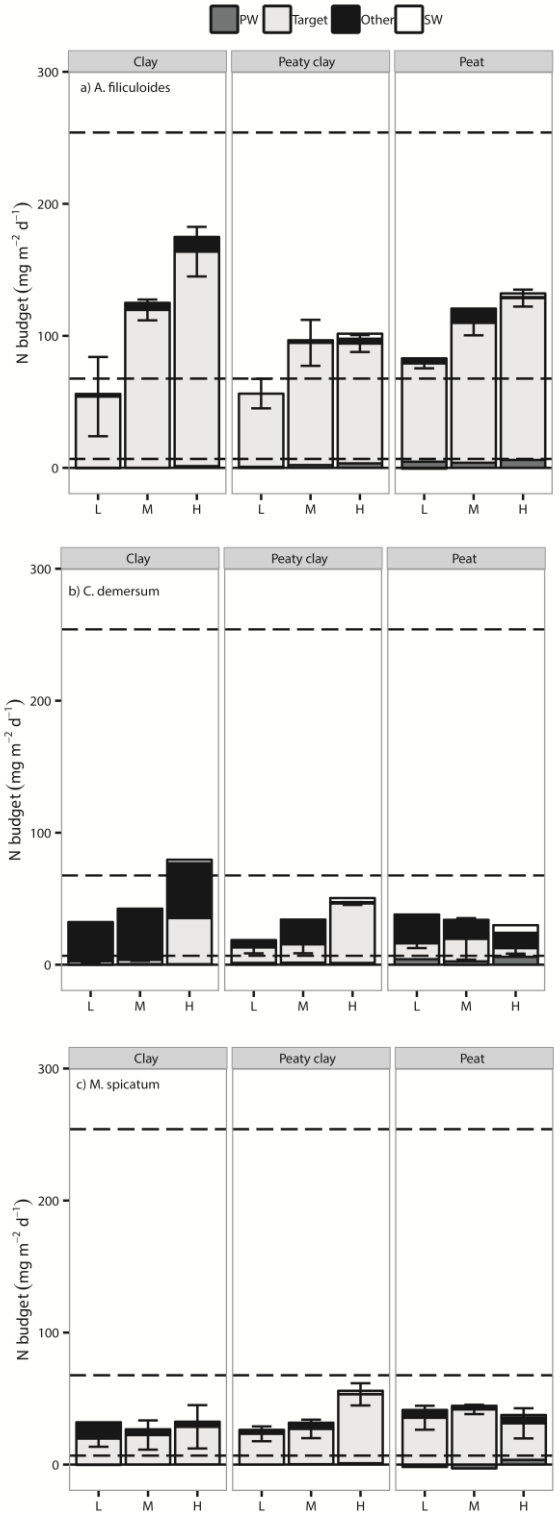

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Figure 5. N distribution in surface water, pore water, target species and other plants subjected to different nutrient loadings (L = 0.43 mg P m^{-2} d^{-1}; M = 21.4 mg P m^{-2} d^{-1}; H = 85.7 mg P m^{-2} d^{-1}) from (a) *A. filiculoides*, (b) *C. demersum* and (c) *M. spicatum* macrophyte systems. Standard errors are given only for target plants. PW = pore water, SW = surface water. Positive values represent N accumulation in relative parts; negative values represent N release from respective compartments. The lowest, medium and highest dashed lines represent external N input at low, medium and high N loadings (including actual atmospheric N deposition), respectively.

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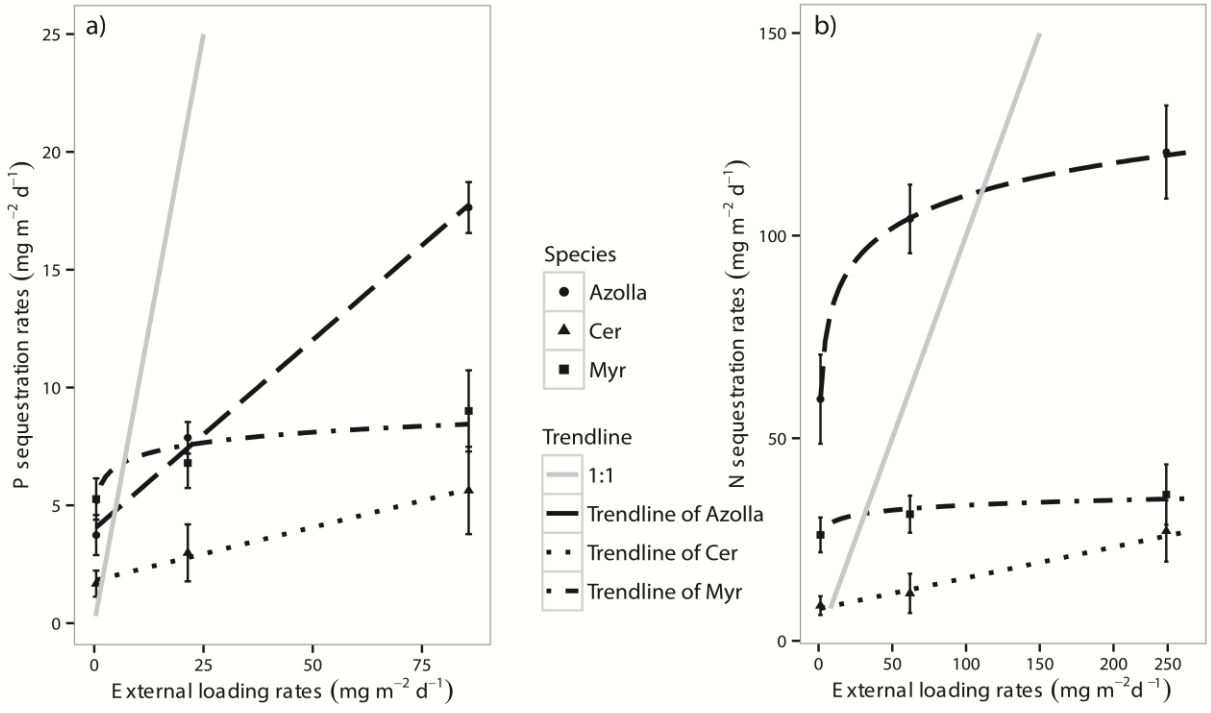

Figure 6. The correlations between external loading and nutrient sequestration rates of P (a) and N (b) by three different aquatic plant species. Standard errors and 1:1 line are given. Note that for *A. filiculoides* N_2 fixation is included in the sequestration rates, overestimating the effects of loading.