Ecological Engineering 122 (2018) 263–270

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Nutrient kinetics in submerged plant beds: A mesocosm study simulating constructed drainage wetlands

Annica Olesen[a], Solvei M. Jensen[a], Anette Baisner Alnoee[a,b], Annette Baattrup-Pedersen[b], Torben L. Lauridsen[b,c], Brian K. Sorrell[a], Tenna Riis[a,*]

[a] Department of Bioscience, Aarhus University, Ole Worms Alle 1, 8000 Aarhus C, Denmark
[b] Department of Bioscience, Aarhus University, Vejlsøvej 25, 8600 Silkeborg, Denmark
[c] Sino-Danish Centre for Education and Research, Beijing, China

ARTICLE INFO

Keywords:
Constructed wetlands
Drainage water
Tile drainage
Aquatic plants
Macrophytes
Nutrient uptake kinetics

ABSTRACT

Constructed wetlands have become a widespread mean to reduce nutrient loading from tile drained agricultural areas. As a supplement to emergent plants usually present in these wetlands, submerged plants may, if present, enhance nutrient retention by occupying the deeper zones of the wetland basins. The nutrient retention efficiency may, however, vary among submerged species and also vary between multi-species communities compared to single-species communities. In this study we performed a mesocosm experiment to quantify the inorganic nitrogen (NH_4-N and NO_3-N) and phosphorus (PO_4-P) uptake kinetic parameters (V_{max} and C_{min}) in constructed wetlands (1) in habitats with and without plants; and (2) in multi-species communities and single-species communities using four submerged plant species relevant for use in these wetlands. We found that uptake rates of PO_4-P and NH_4-N was three and five times higher, respectively, in habitats with plants compared to habitats without plants, whereas the uptake rates of NO_3-N was similar. Multi-species communities were not more efficient in nutrient retention than single-species communities, although a residual analysis indicated that multi-species communities might be better in taking up and depleting NH_4-N but not PO_4-P and NO_3-N. Overall, our study shows that submerged plants in deeper waters of drainage wetlands can be an important nutrient retaining component, and that a high biomass of one efficient plant species (e.g. *R. aquatilis*) is working similarly well as multi-species communities in this context.

1. Introduction

Intensive use of nitrogen (N) and phosphorus (P) fertilizers in agricultural areas mediate high nutrient loadings to streams and lakes either as surface runoff or through tile drainage systems (Kronvang et al., 2005). To improve the ecological status of aquatic ecosystems in Europe, the Water Framework Directive (WFD) was ratified in 2000 with an objective to meet at least good ecological status in all surface waters by 2015 (European Commision, 2000), with countries implementing their own national action plans to reach this goal. However, by 2015 only 53% of all surface waters in the European Union were in good or high ecological status (Voulvoulis et al., 2017). Therefore, innovative management options and optimization of those already existing are needed to reduce nutrient loadings to aquatic ecosystems.

A promising management option is the establishment of constructed wetlands as a mean to reduce nutrient loading from drained agricultural areas to aquatic environments (Tanner et al., 2005). Constructed wetlands can reduce the direct transport of nutrient enriched water from drained agricultural areas to natural streams or lakes, by creating a temporary residence for water before reaching natural aquatic systems downstream (Kovacic et al., 2000). Nitrogen and P can temporally be retained in constructed wetlands through sedimentation and biological uptake in plants and biofilm (Gumbricht, 1993; Brix, 1997; Vymazal, 2007) until the biomass decay, and inorganic nutrients are released (Bernot et al., 2006; Levi et al., 2015). Additionally plants can stimulate denitrification, where nitrate (NO_3-N) is reduced to free nitrogen (N_2) (Poe et al., 2003) due to release of root exudates (Veraart et al., 2011; Zhang et al., 2017).

A majority of studies investigating the role of plants for nutrient uptake and removal in constructed wetlands have concentrated on emergent plant species and knowledge on the role that submerged species can play is therefore sparse. However, nutrient retention and removal is likely to be higher in a wetland if both emergent and submerged plants are present as they may inhabit different parts of the

* Corresponding author.
E-mail address: tenna.riis@bios.au.dk (T. Riis).

https://doi.org/10.1016/j.ecoleng.2018.08.012
Received 31 May 2018; Received in revised form 10 August 2018; Accepted 13 August 2018

wetland. Thus, emergent plants have roots in waterlogged sediment, while most leaves and stems are aerial, and therefore these plants will be limited to the shallower parts of the wetland. Submerged plants, on the other hand, have all plant parts under water and therefore grow in deeper waters where the depth limit is typically determined by light availability. Additionally, submerged species can take up nutrients from both the sediments and water column (Gumbricht, 1993), whereas emergent plants are restricted to sediment uptake by roots. The nutrient uptake kinetics of the plants may affect the retention capacity of the wetland. It is well established that emergent plant species vary in their efficiency in taking up nutrients, while fewer studies have investigated the efficiency of submerged species. Thus, for emergent plants the maximal nutrient uptake rates reported (V_{max}) were in the range 0.07–13.6 μmol PO_4-P g^{-1} dry weight (DW) h^{-1} (Li et al., 2015; Christiansen et al., 2016), and in the range 0.14–0.93 μmol NO_3-N g^{-1} DW h^{-1} (Li et al., 2015). The ability of species to deplete nutrients (C_{min}) may also vary but to our knowledge, this parameter has not yet been reported in the literature for submerged plant species. Together V_{max} and C_{min} may indicate the efficiency of species specific nutrient uptake in constructed wetlands. At the same time, however, the concomitant occurrence of several species within a constructed wetland may also affect the nutrient uptake efficiency due to niche complementarity or selection effects (Loreau and Hector, 2001) although very sparse knowledge exist especially for submerged species. Niche complementarity occurs when differences between plant species leads to for instance a more efficient nutrient acquisition, caused by resource partitioning or facilitation, and resulting in enhanced nutrient uptake in the ecosystem. Niche complementarity can occur because of species specific differences in morphological as well as physiological characteristics that may affect nutrient uptake kinetics (Levi et al., 2015).

In the present study we aim to explore the potential role of submerged species for nutrient uptake in constructed wetlands to evaluate to what extent they can serve as biofilters both as single- and multi-species communities using a mesocosm approach. Specifically we 1) quantify nutrient uptake kinetics of different species of submerged plants using depletion where nutrient concentration in a solution is reduced over time due to plant uptake (Claassen and Barber, 1974), and 2) compare nutrient uptake kinetics in single-species communities with multi-species communities containing two, three or four submerged plant species. We hypothesize that nutrient uptake is considerably higher when plants are present compared to a situation without plants and that multi-species communities show higher nutrient uptake compared to single-species communities.

2. Methods

2.1. Mesocosm set-up

The study was conducted near Aarhus, Denmark (56°13′42.8″N 10°07′34.0″E) during summer 2016. The mesocosm design consisted of 56 partially buried 90 L plastic tubs being 67 cm wide and 53 cm high. To each mesocosm about 24 L of washed beach sand sediment was added (sediment depth of 5 cm), followed by 50–55 L tap water until an overflow pipe was reached at 20–23 cm water level (Fig. 1). Each mesocosm was aerated using an air pump to secure mixing and oxic conditions, and connected to a water system where a drip tube provided each mesocosm with 1.2 L tap water per hour (corresponding to two days retention time) (Fig. 1). Four submerged plant species (*Potamogeton perfoliatus* L., *Potamogeton obtusifolius* Mert. & Koch, *Ranunculus aquatilis* L. and *Elodea canadensis* Michx.) were planted in the mesocosms in different species treatment combinations of four replicates each: all single-species combinations, four two-species combinations, all three-species combinations and one four-species combination in a full randomized set-up (Table 1). One control treatment without plants was also conducted. We chose these plant species as they are commonly found in open agricultural landscapes, are tolerant to high nutrient conditions, and therefore are relevant for use in constructed drainage wetlands.

The submerged plant species were collected in Jutland (Denmark) in mid-June 2016, either from a stream (*R. aquatilis, E. canadensis*), a lake (*P. perfoliatus*) or a constructed wetland (*P. obtusifolius*). Apical shoots were shortened to 20 cm and placed in aquariums (27 L) containing tap water and oxygen tubes. The shoots were then acclimatized for three days with renewed water after 1.5 day. The planting of shoots in the outdoor mesocosms took place by dividing each mesocosm into three (three species treatments) or four (single-, two- and four species treatments) proportions, with one species planted in each section, so the distribution of shoots was uniform in all mesocosms. Shoots were planted 5 cm into the sediment and was placed either separately (*P. perfoliatus, E. canadensis*) or in bundles of 2–4 shoots (*R. aquatilis, P. obtusifolius*). Each mesocosm contained approximately 50 g of fresh weight (FW) of plant divided among 1–4 species, so that each species contributed equally to the start biomass in each treatment.

2.2. Plant growth in mesocosms, maintenance and measurements

The plants grew in the mesocosms for eight weeks (ultimo June-ultimo August 2016). A mixture of commercial fertilizer (NPK Macro 14-3-23 and Micro with iron, Pioner, Denmark) was supplied to each mesocosm 2–3 times weekly. The supplied nutrients corresponded to 0.5 mg PO_4-P L^{-1}, 0.5 mg NH_4-N L^{-1} and 1.5 mg NO_3-N L^{-1}. The dosage was doubled after five weeks and was hereafter supplied three times a week. Algae growth in the mesocosms were removed every fourth day in the growth period.

Conductivity, pH, temperature and oxygen content (Supplementary material S1) were measured weekly from nine randomly chosen mesocosms to ensure that conditions were stable throughout the experimental period. Likewise, water samples were collected from the nine randomly chosen mesocosms to measure alkalinity, PO_4-P, NH_4-N and NO_3-N. Alkalinity was analyzed on a titrator (TIM850 titration manager, Radiometer analytical, Hach, CO, USA) by titrating with 0.05 M HCl via Gran plot titration. Nutrient concentrations were measured by a flow injection analyzer (Quikchem FIA + 8000 series, Lachat Instruments, CO, USA).

We measured internal N and P content in plant tissues on a vario EL cube (N; Elementar Analysensysteme, Langenselbold, Germany) and by ICP-method on an Optima 2000 spectrometer (P; PerkinElmer, MA, USA) before shoots were planted in the mesocosms. All N measurements were > 20 mg N g^{-1} (> 2% N) and content > 1 mg P g^{-1} (> 0.1% P) indicating that plants were not nutrient limited for growth when they were established (Willby et al., 2001). Generally the plant N and P concentrations were in the high end of the range found in freshwater plants in general (Duarte, 1992).

2.3. Nutrient uptake experiment

In late August a nutrient uptake experiment was conducted to estimate maximum uptake rate (V_{max}) and minimum concentration (C_{min}) of PO_4^{3-}, NH_4^+ and NO_3^- for the different treatments. The week prior to the experiment, the plants were grown without nutrient addition to reduce the saturation of N and P in plant tissues. Uptake rates and C_{min} were determined from the decrease in nutrient concentrations in each mesocosm over time with the use of depletion curves (Claassen and Barber, 1974). To detect the decrease in nutrient concentration, the water level was lowered to 15 cm (about 37 L) prior to the experiment. Three different nutrient solutions (KH_2PO_4, $(NH_4)_2SO_4$ and KNO_3) were supplied to each mesocosm, resulting in a concentration of approximately 1 mg PO_4-P L^{-1}, 1 mg NH_4-N L^{-1} and 3 mg NO_3-N L^{-1}, simulating drainage runoff (Kronvang et al. 2005). A commercial micronutrient solution (Tropica Plant nutrition, Tropica Aquacare, Denmark) of 0.1 ml L^{-1} was supplied to each mesocosm before the addition of

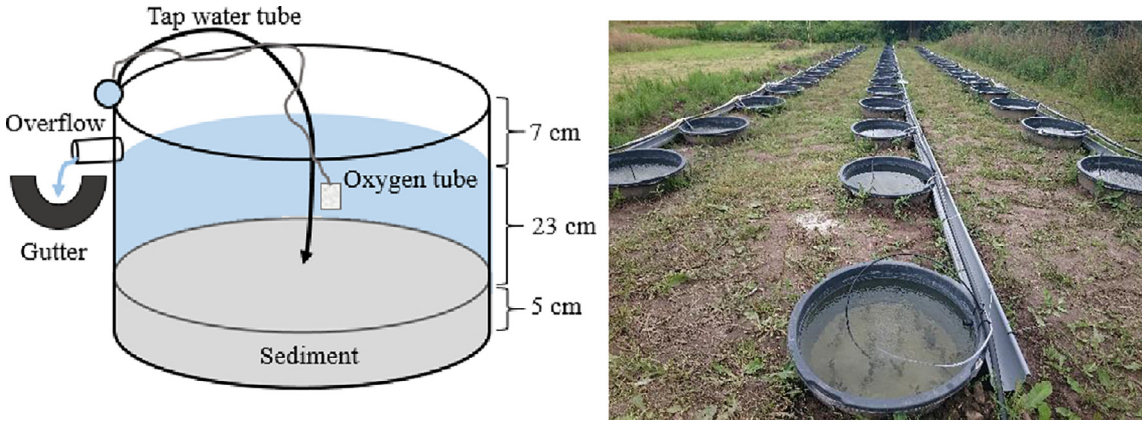

Fig. 1. Mesocosm design. Each mesocosm contained 24 L sediment (5 cm depth) and 50–55 L tap water (20–23 cm water level). An air pump was used for aeration of the mesocosms and fresh water was supplied via a tap water tube connected to a water hose. Excess water went through an overflow tube and into a gutter.

Table 1
The 14 different treatments and the species within each treatment (n = 4).

Treatment	Species in treatment
Single species	
Pp	*Potamogeton perfoliatus*
Po	*Potamogeton obtusifolius*
Ra	*Ranunculus aquatilis*
Ec	*Elodea canadensis*
Two species	
Pp + Ra	*P. perfoliatus* + *R. aquatilis*
Pp + Ec	*P. perfoliatus* + *E. canadensis*
Ra + Ec	*R. aquatilis* + *E. canadensis*
Po + Ec	*P. obtusifolius* + *E. Canadensis*
Three species	
Pp + Ra + Ec	*P. perfoliatus* + *R. aquatilis* + *E. canadensis*
Pp + Po + Ec	*P. perfoliatus* + *P. obtusifolius* + *E. canadensis*
Pp + Po + Ra	*P. perfoliatus* + *P. obtusifolius* + *R. aquatilis*
Ra + Po + Ec	*R. aquatilis* + *P. obtusifolius* + *E. canadensis*
Four species	
Pp + Po + Ra + Ec	*P. perfoliatus* + *P. obtusifolius* + *R. aquatilis* + *E. canadensis*
Without plants	None

macronutrients.

The experiment was initiated when macronutrients were supplied to each mesocosm. Prior to each sampling, the water was stirred to obtain a homogenic water column. Samples were taken at increasingly longer time intervals (20, 30 or 60 min) for eight hours after which no samples were taken until the following morning, where 3 samples with 30 min interval were taken such that the experiment lasted 22 h in total. For each sample the first 10 ml sample was discarded through a filter (using 0.20 µm, Supor 200, Pall Corporation, NY, USA) and the remaining 10 ml was filtered into an acid washed centrifuge tube (Techno Plastic Products (TPP), Trasadingen, Switzerland). A total of 19 water samples from each mesocosm were frozen until nutrient analysis. PO_4-P, NH_4-N and NO_3-N was analyzed on a flow injection analyzer (Quikchem FIA + 8000 series, Lachat Instruments, CO, USA). The water temperature was measured every 10 min by six temperature loggers (Tinytag Aquatic TG-2100, Gemini Data loggers, Chichester, England) placed in six randomly chosen mesocosms. Light intensity was measured during the experimental period using a LI-1400 Datalogger + LI-192 Quantum sensor, LI-COR, NE, USA). The average light intensity and water temperature in the first day of sampling was 512 (± 250) µmol m^{-2} s^{-1} and 21.4 (± 1.1) °C, respectively.

2.4. Harvest of above- and belowground biomass

Above- and belowground biomass in each mesocosm was harvested after termination of the nutrient uptake experiment. The aboveground biomass was either cut off at the sediment surface or whole shoots were pulled up and separated from where the aboveground biomass visually differed from the belowground biomass. Both above- and belowground biomass was separated by species. All harvested biomass was dried for five days at 60 °C and weighed (dry weight; DW). Results from harvested aboveground biomass in each treatment can be found in Supplementary material (S2).

3. Data analysis and statistics

3.1. Nutrient uptake kinetics

From the nutrient uptake experiment V_{max} and C_{min} were determined for each of the three nutrients (PO_4-P, NH_4-N and NO_3-N)

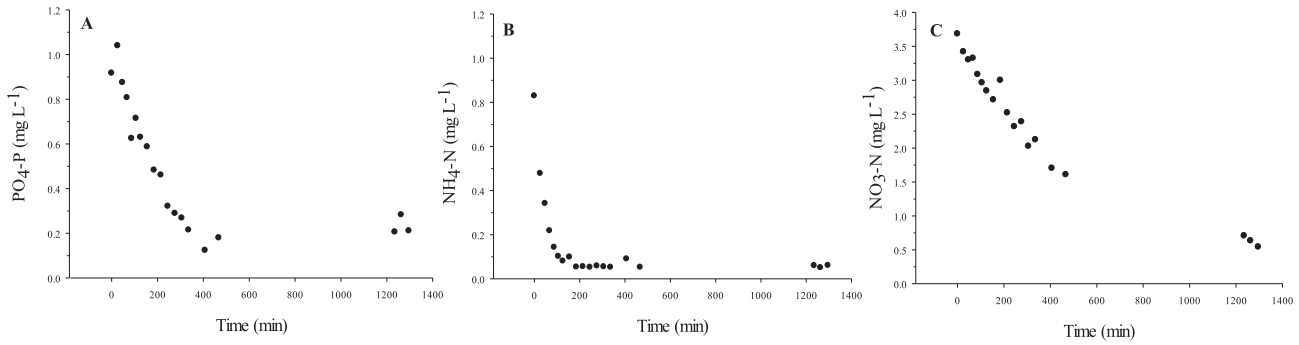

Fig. 2. Depletion curves from the nutrient uptake experiment. Depletion of concentrations of (A) PO_4-P, (B) NH_4-N and (C) NO_3-N (mg L^{-1}) in a mesocosm over time (min).

based on nutrient depletion curves (Claassen and Barber, 1974) (Fig. 2). For each nutrient V_{max} was calculated for each community by fitting depletion curves with linear regression analysis, where the measurements resulting in the steepest initial slope were chosen for each replicate, following Konnerup and Brix (2010). The uptake rates were corrected for volume as 20 ml was removed from each mesocosm at each sampling. Furthermore, the uptake rates were temperature corrected to 20 °C by using the Arrhenius equation with a Q_{10} of 2.0 as a general Q_{10} for biological processes:

$$V_{max} = V_{max(a)} * e^{(20\,^{\circ}C - \text{average temp.}) * 0.0693} \quad (1)$$

where V_{max} is the calculated V_{max} at 20 °C, $V_{max(a)}$ is the calculated rate at the average ambient temperature (average temp.) and 0.0693 ($^{\circ}C^{-1}$) is a temperature coefficient corresponding to a Q_{10} of 2.0 (Sand-Jensen et al., 2007; Alnoee et al., 2015). The temperature corrected rate was then related to sediment surface area (μmol nutrient $m^{-2} h^{-1}$).

When the concentration of PO_4-P, NH_4-N or NO_3-N in the media no longer changed over time (where the depletion curve leveled off), a minimum concentration (C_{min}) was reached. In cases where C_{min} was not reached an actual concentration (C_{act}) was calculated. It was tested using a Students *t*-test whether the last three evening samples were 1) statistically equal to or 2) statistically different from the three morning samples, meaning it was a C_{min} or a C_{act}, respectively. C_{act} was calculated as the average of the last three evening samples, because C_{min} treatments already attained their C_{min} during this period. Because the treatment without plants never reached C_{min}, a comparable concentration was calculated as a minimum concentration at 22 h (C_{22h}) by finding the average nutrient concentration in the last three water samples. This was done for all three nutrients. The methods to determine V_{max} and C_{min} were chosen in order to compare rates and concentrations between treatments in the study. However, using these methods to determine the parameters can result in situations where V_{max} is not achieved or a C_{min} is not reached during the experiment.

3.2. Effect of plant presence on kinetic parameters

We estimated the effect of presence and absence of plants on V_{max} and C_{min} by calculating the proportional V_{max} and C_{min} in treatments with plants constituted of the average V_{max} and C_{min} in the treatment without plants:

$$\text{Effect} = \frac{\text{Value of replicate in treatment with plants}}{\text{Average value in treatment without plants}} \quad (2)$$

Effect values were plotted in box-plots for every treatment with a standard line (y = 1) representing the habitat without plants. If nutrient uptake was > 1 for V_{max} and < 1 for C_{min} there was an effect of plants since high V_{max} indicates high nutrient uptake capacity and low C_{min} indicates more effective depletion.

3.3. Multi-species versus single-species community nutrient uptake kinetics

The effect of increased species richness on both V_{max} and C_{min} was tested with linear regression analysis. Bärlocher and Corkum (2003) was used to calculate the expected V_{max} and C_{min} in multi-species communities based on V_{max} and C_{min} in single-species communities. The expected V_{max} and C_{min} for every replicate in a multi-species community was calculated as the sum of V_{max} or C_{min} of the component single-species communities, weighted by the species proportional contribution to the multi-species community (i.e. 1/2, 1/3 or 1/4). Residuals (obs − exp) were afterwards tested against the null hypothesis, that the average residual equaled zero (*t*-test). If average residual of V_{max} was > 0 and C_{min} was < 0 there was an effect of species richness.

3.4. Statistics

Statistic tests were conducted in the software program JMP version 13.0 (*t*-tests, linear regressions and one-way ANOVA) or Sigmaplot version 12.5 (Michaelis-Menten trend). Differences between V_{max} and C_{min} in the treatments were tested with one-way ANOVA for each nutrient. Before applying one-way ANOVAs, data were tested for homogeneity of variance with Levene's test and log transformed if necessary. We tested if there was a correlation between aboveground biomass and V_{max} for the three nutrients by plotting a saturation curve based on a Michaelis-Menten fit. The biomass does not increase infinitely with the uptake rate, why it makes best ecological sense to test the correlation with a saturation curve instead of a linear trend. Furthermore, we tested if above- and belowground biomasses were significantly different in the treatments with a one-way ANOVA. The shown results are untransformed data. A *post-hoc* test, Tukey HSD, was applied to significant results from ANOVAs. All statistic tests were tested at 0.05 significance level.

4. Results

4.1. Effect of plant presence

Mesocosms with plants had three and five times higher P and N uptake rates, respectively, compared to treatment without plants. The average maximum uptake rate (V_{max}) in treatments with plants ranged from 344 to 1042 μmol PO_4-P $m^{-2} h^{-1}$, 2952–7091 μmol NH_4-N $m^{-2} h^{-1}$ and 1199–2794 μmol NO_3-N $m^{-2} h^{-1}$ and these were generally higher than V_{max} in treatment without plants except for NO_3-N (Table 2). In treatments with plants average V_{max} exceeded treatments without plants in 8 out of 13 and 7 out of 13 treatments for PO_4-P and NH_4-N, respectively. For NO_3-N, only 3 out of 13 treatments with plants had significantly higher V_{max} than treatments without plants (Fig. 3).

The same pattern was evident for minimum concentrations (C_{min}, C_{act} or C_{22h}), where minimum nutrient concentrations in treatments with plants ranged from 5.1 to 19.0 μmol PO_4-P L^{-1}, 4.1–17.6 μmol NH_4-N L^{-1} and 40.4–117.8 μmol NO_3-N L^{-1}, which was considerably lower than the minimum concentration reached in the treatment without plants (Table 2). At the same time, C_{min} in treatments with plants were lower than the average C_{min} of treatments without plants, in particular for NH_4-N, where all plant treatments had significantly lower C_{min} compared to treatments without plants. C_{min} for NO_3-N was also lower in treatments with plants in 9 out of 13 treatments, whereas C_{min} for PO_4-P, was lower in 5 out of 13 treatments with plants compared to treatments without plants (Fig. 3, Table 2). Furthermore, few species specific differences in nutrient uptake was observed. Thus, *R. aquatilis* had significantly higher V_{max} for NH_4-N and lower C_{min} for PO_4-P compared to the other species, while there was no significant difference in kinetic parameters for NO_3-N among the four species included in the experiment (Table 2).

Data for V_{max} was plotted as a function of aboveground biomass to examine to what extent differences in V_{max} between treatments could be explained by differences in biomass. Significant positive correlations were found between the aboveground biomass (g DW) and PO_4-P V_{max} (μmol $m^{-2} h^{-1}$) ($p < 0.0001$; Fig. 4A), and NH_4-N V_{max} ($p < 0.0001$; Fig. 4B), but no significant correlation was found between the aboveground biomass (g DW) and NO_3-N V_{max} ($p = 0.51$, Fig. 4C).

4.2. Effect of species richness

There was no significant relationship between aboveground biomass and species richness (Supplementary material S3). There were also no significant positive correlations between number of species and V_{max} for either PO_4-P ($p = 0.66$), NH_4-N ($p = 0.12$), nor NO_3-N ($p = 0.12$). Similarly, there were no significant correlations between number of species and C_{min} for PO_4-P ($p = 0.13$), NH_4-N ($p = 0.48$), nor NO_3-N

Table 2
Maximum habitat specific nutrient uptake rates (V_{max}; μmol $m^{-2} h^{-1}$) and minimum nutrient concentrations (C_{min}, C_{act}, C_{22h}; μmol L^{-1}) for the three nutrients PO_4-P, NH_4-N and NO_3-N in the different treatments (mean ± SD, $n = 4$) including results from one-way ANOVA (F-ratio, p value). See Table 1 for abbreviation explanations. Letters show differences between all treatments.

Treatment	PO_4-P		NH_4-N		NO_3-N	
	V_{max} (μmol $m^{-2} h^{-1}$)	C_{min} (μmol L^{-1})	V_{max} (μmol $m^{-2} h^{-1}$)	C_{min} (μmol L^{-1})	V_{max} (μmol $m^{-2} h^{-1}$)	C_{22h} (μmol L^{-1})
Single species						
Pp	458 ± 143^{abc}	*11.8 ± 0.5^{bc}	2975 ± 871^{bc}	17.0 ± 1.2^{b}	1213 ± 763^{ab}	101.4 ± 29.7^{ab}
Ra	1042 ± 88^{a}	5.1 ± 0.9^{d}	7091 ± 648^{a}	15.1 ± 0.9^{bc}	1268 ± 739^{ab}	90.7 ± 29.5^{b}
Ec	497 ± 159^{abc}	12.4 ± 2.1^{abc}	4134 ± 422^{abc}	15.1 ± 0.9^{bc}	1308 ± 229^{ab}	64.2 ± 12.4^{b}
Po	594 ± 175^{ab}	*14.7 ± 2.3^{abc}	2952 ± 551^{bc}	12.1 ± 1.5^{bcd}	1684 ± 330^{ab}	50.1 ± 28.8^{b}
Two species						
Pp + Ra	721 ± 171^{ab}	6.0 ± 2.3^{d}	6287 ± 1225^{ab}	4.1 ± 0.2^{e}	2562 ± 874^{a}	40.4 ± 24.9^{b}
Pp + Ec	664 ± 148^{ab}	11.6 ± 2.5^{bc}	4825 ± 297^{abc}	8.6 ± 3.2^{de}	1551 ± 647^{ab}	62.0 ± 36.3^{b}
Ra + Ec	716 ± 291^{ab}	13.1 ± 3.5^{abc}	6185 ± 1228^{ab}	17.6 ± 0.1^{b}	1199 ± 848^{ab}	117.8 ± 38.7^{ab}
Po + Ec	344 ± 69^{bc}	14.1 ± 2.0^{abc}	3118 ± 123^{abc}	11.9 ± 0.5^{bcd}	1766 ± 631^{ab}	78.8 ± 38.4^{b}
Three species						
Pp + Po + Ec	621 ± 83^{ab}	15.0 ± 1.9^{abc}	5392 ± 3033^{ab}	12.8 ± 1.2^{bcd}	2283 ± 315^{a}	72.6 ± 36.7^{b}
Pp + Po + Ra	825 ± 378^{ab}	13.9 ± 6.2^{abc}	5115 ± 2308^{abc}	15.2 ± 0.4^{bc}	2794 ± 1135^{a}	69.6 ± 45.9^{b}
Pp + Ra + Ec	811 ± 83^{ab}	12.8 ± 3.2^{abc}	6085 ± 1129^{ab}	15.9 ± 0.9^{bc}	2041 ± 414^{ab}	58.8 ± 28.4^{b}
Ra + Po + Ec	434 ± 124^{abc}	19.0 ± 3.5^{ab}	5227 ± 2113^{ab}	15.4 ± 0.2^{bc}	1318 ± 993^{ab}	107.7 ± 22.5^{ab}
Four species						
Pp + Po + Ra + Ec	484 ± 121^{abc}	9.8 ± 1.8^{cd}	5191 ± 969^{ab}	10.8 ± 0.7^{cd}	1423 ± 516^{ab}	56.6 ± 21.1^{b}
Without plants	226 ± 91^{c}	*23.2 ± 1.9^{a}	1179 ± 566^{c}	*30.2 ± 10.7^{a}	146 ± 253^{b}	194.5 ± 43.2^{a}
F-ratio	4.81	9.11	4.28	34.42	2.89	4.38
p value	< 0.0001	< 0.0001	0.0002	< 0.0001	0.0046	0.0001

* Indicates C_{act} instead of C_{min}.

($p = 0.62$). However, we found that the residuals for V_{max} (obs V_{max} − exp V_{max}) were significantly higher than zero for NH_4-N (t-ratio = 3.07, $p = 0.0021$) and NO_3-N (t-ratio = 3.39, $p = 0.0009$) but not for PO_4-P (t-ratio = 0.43, $p = 0.66$). The residuals for C_{min} (obs C_{min} − exp C_{min}) were significantly lower than zero for NH_4-N (t-ratio = 3.26, $p = 0.0012$), but not for NO_3-N (t-ratio = 0.47, $p = 0.32$) or PO_4-P (t-ratio = 2.57, $p = 0.99$).

5. Discussion

In accordance with our first hypothesis, we found that submerged macrophytes contribute significantly to nutrient uptake in our experiment which is in accordance with previous findings (Engelhardt and Ritchie, 2002; Gustafsson and Boström, 2011; Riis et al., 2012; Levi et al., 2015). Based on the nutrient uptake kinetics of the plants in this study we conclude that submerged aquatic plants can mediate a fivefold enhancement of the NH_4^+ uptake and a threefold enhancement of the PO_4^{3-} uptake in habitats with plants compared to habitats without plants. The finding that NO_3-N uptake was less affected by the presence of macrophytes indicate that the investigated species had a preference for NH_4^+ compared to NO_3^- as also found in previous studies (e.g. Miller and Cramer, 2005; Li et al. 2015).

The stimulating effect of plants on nutrient uptake is likely to reflect assimilation by the plants themselves as well as assimilation by the epiphytic biofilm associated with the surface of the plants (Riis et al., 2012; Levi et al., 2015). Thus we found that V_{max} for PO_4-P and NH_4-N increased with increasing aboveground biomass of the plants and also that differences between V_{max} of the various treatments disappeared when expressed on the basis of surface area. This may also explain why the nutrient uptake capacity for PO_4-P was higher in *R. aquatilis* that has highly dissected leaves and, consequently, a high specific leaf area compared to the other species. In accordance with our findings, Engelhardt and Ritchie (2002) found that the reduction in PO_4-P from inlet to outlet water was related to the biomass of submerged plants including the epiphytic biofilm, also indicating that nutrient uptake is highly controlled by the total autotrophic biomass.

To our knowledge area-based nutrient kinetics values for V_{max} and C_{min} have not been reported earlier for submerged aquatic plants, which makes it difficult to compare our results with any previous measurements. Other studies using different methods have reported area-based nutrient uptake rates in the range 0.03–0.27 g N m^{-2} day^{-1} and 0.003–0.027 g P m^{-2} day^{-1}, respectively (Pelton et al., 1998; Brix, 1994, 1997; Eriksson and Weisner, 1997; Levi et al., 2015), which was 10–20 times lower than the V_{max} values in our study (2.27 g N m^{-2} day^{-1} ($NH_4^+ + NO_3^-$) and 0.47 g P m^{-2} day^{-1}). Comparison of nutrient uptake rates between studies can therefore be difficult, which stresses the need for measurements across a range of species under similar conditions.

When comparing the single-species treatments in V_{max} and C_{min} for three nutrient solutes we found that *Ranunculus aquatilis* had significantly higher NH_4-N uptake rate and lower C_{min} for PO_4-P compared to the other species. No other species-specific differences was present. This finding is important in the context of planning establishment of macrophytes in wetlands. *R. aquatilis* is a fast-growing species with a high biomass accumulation and along with the efficient NH_4-N and PO_3-P uptake it would therefore be suitable for planting in constructed wetlands for enhancing nutrient uptake.

Although we did not find any significant differences in nutrient uptake in terms of V_{max} and C_{min} between single- and multi-species communities for any of the nutrients measured, we did find that V_{max} for NH_4-N and NO_3-N was higher and C_{min} for NH_4-N was lower than expected in multi-species communities based on the performance of single-species communities. Therefore our second hypothesis was only partly supported. Other studies have also found indications of enhanced nutrient uptake in multi-species communities in wetlands. Engelhardt and Ritchie (2002) found that the amount of P loss from inflow to outflow in a mesocosm experiment was reduced with increasing species richness of submerged plants, and Wang et al. (2013) also found enhanced N removal in wetlands with increasing species richness. In studies from grasslands it has been shown that presence of more

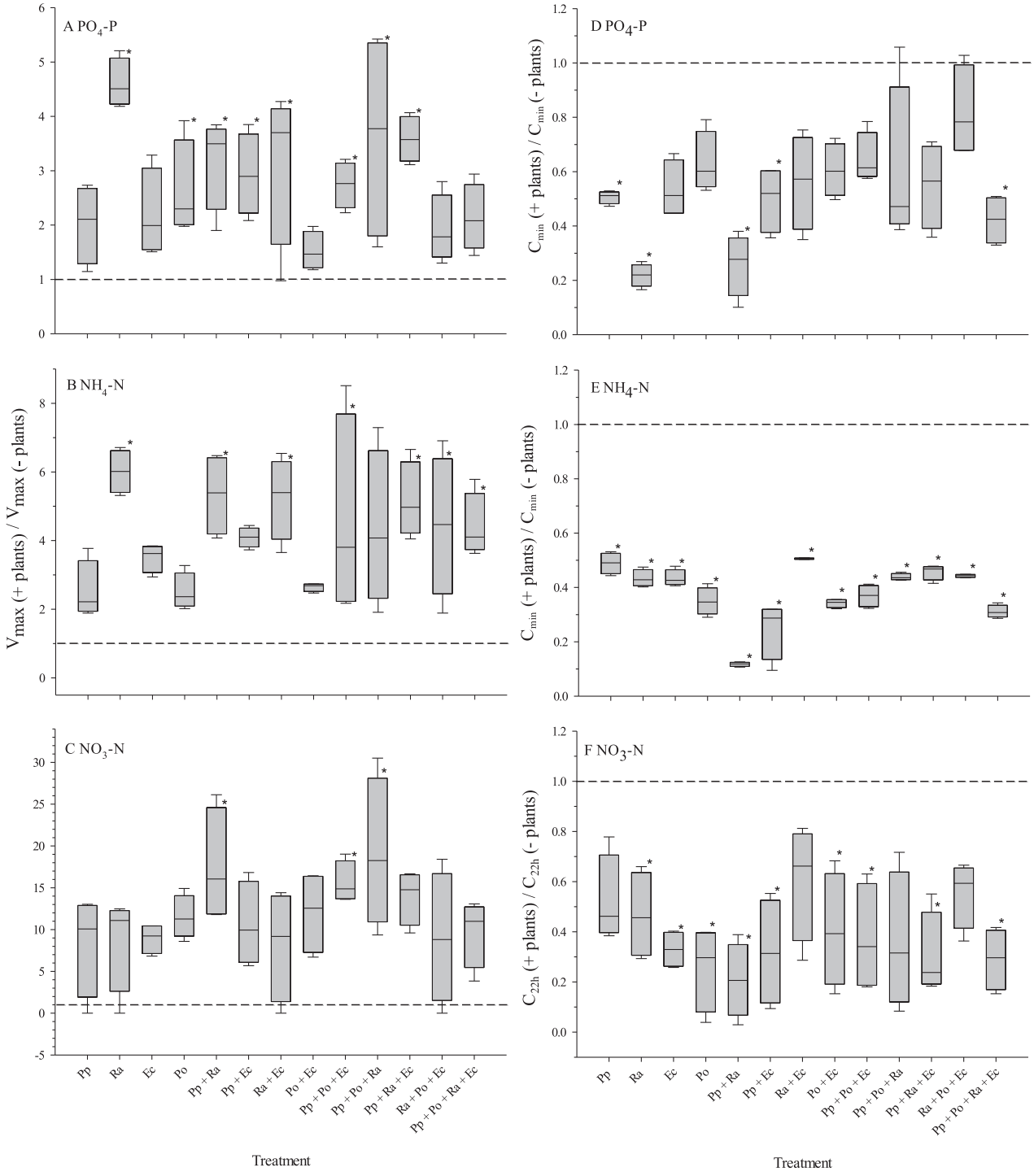

Fig. 3. Maximal nutrient uptake rates (V_{max}; μmol m^{-2} h^{-1}) (A, B, C) and minimum nutrient concentrations (C_{min}, C_{act} and $C_{22}h$; μmol L^{-1}) (D, E, F) for treatments with plants compared to the average V_{max} in treatments without plants for PO_4-P (A, D), NH_4-N (B, E), and NO_3-N (C, F). The dotted line (y = 1) represents the treatment without plants. Asterisks indicate treatments that were significantly different from the treatments without plants (one-way ANOVA; Tukey HSD, Table 2). Boxes states 1st quartile, median, 3rd quartile, minimum and maximum (n = 4). See Table 1 for abbreviation explanations.

functional groups with a large variation in resource acquisition strategies (i.e. C_4 plants and N_2 fixing legumes) among the groups can explain a higher nutrient uptake in species rich communities (Roscher et al. 2008). In the case of aquatic submerged macrophytes the variation in nutrient acquisition is low as all species are able to assimilate nutrient by aboveground biomass as well as by roots and thus increasing species richness will therefore not substantially increase nutrient uptake. However, as the uptake rates of the multi-species communities reflects the physiological uptake rates of both macrophytes and epiphytes, it is possible that a combination of emerged, floating leaved and submerged plants in a wetland will increase the uptake rate of the system because the plants show differences in 1) primary sites of nutrient uptake (leaves or roots), 2) nutrient kinetics (some with high V_{max}, some with low C_{min}), 3) preferences for nitrogen, 4) substrate for epiphytic biofilm

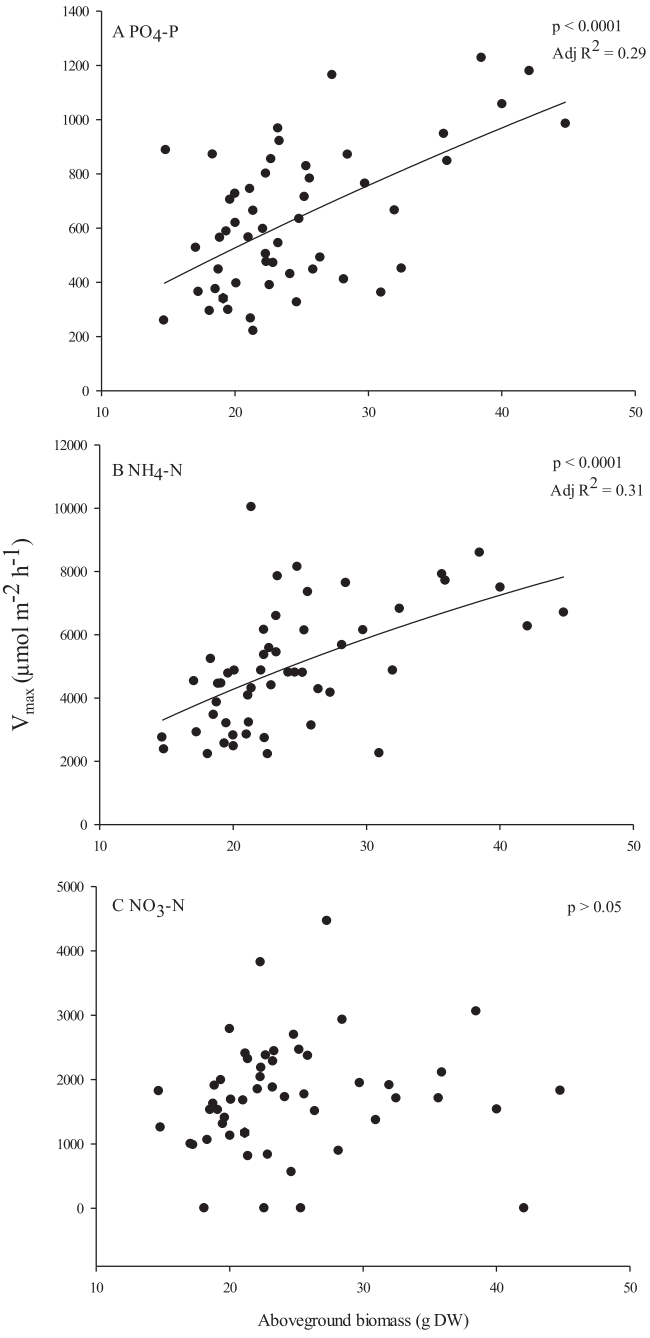

Fig. 4. Regression based on a Michaelis-Menten fit (R^2) between maximum uptake rate (V_{max}, µmol m^{-2} h^{-1}) and the aboveground biomass (g DW) of PO_4-P (A), NH_4-N (B), and NO_3-N (C). There was a significant positive relation for PO_4-P and NH_4-N ($p < 0.05$) but not for NO_3-N ($p > 0.05$). Note the different scales on the y-axis.

and 5) biomass productivity. Thus by including emerged and floating leaved plant groups that are assimilating nutrient primarily from sediment it could enhance nutrient uptake more substantially in a wetland plant community as was also found by Kahmen et al. (2006).

6. Conclusion and perspective

We found that submerged plants enhance N and P uptake three to fivefold, respectively, compared to habitats without plants, and presence of submerged plants can therefore enhance total wetland nutrient uptake if they colonize the deeper parts of the wetland unsuited for emergent plant growth. Among the four species included in the experiment we found that *R. aquatilis* performed most efficiently in NH_4-N and PO_4-P uptake, leading to a potential target species if submerged plants are actively introduced in constructed wetlands. Our data suggest that multi-species communities enhance nutrient uptake compared to single-species communities but the effect was weak, which might reflect that the study species belonged to the same nutrient uptake functional group. Overall we conclude that ensuring establishment of submerged plants in parts of the wetland where emergent plant are unable to colonize will increase the overall nutrient uptake in the wetland.

Acknowledgement

We thank Danish Ministry of Environment and Food for financial support through a Green Development and Demonstration Project (GUDP).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2018.08.012.

References

Alnoee, A.B., Riis, T., Andersen, M.R., Baattrup-Pedersen, A., Sand-Jensen, K., 2015. Whole-stream metabolism in nutrient-poor calcareous streams on Öland. Sweden. Aquat. Sci. 77, 207–219. https://doi.org/10.1007/s00027-014-0380-5.

Bärlocher, F., Corkum, M., 2003. Nutrient enrichment overwhelms diversity effects in leaf decomposition by stream fungi. Oikos 101, 247–252. https://doi.org/10.1034/j.1600-0706.2003.12372.x.

Bernot, M.J., Tank, J.L., Royer, T.V., David, M.B., 2006. Nutrient uptake in streams draining agricultural catchments of the midwestern United States. Freshwater Biol. 51, 499–509. https://doi.org/10.1111/j.1365-2427.2006.01508.x.

Brix, H., 1994. Functions of macrophytes in constructed wetlands. Water Sci. Technol. 29.

Brix, H., 1997. Do macrophytes play a role in constructed treatment wetlands? Water Sci. Technol. 35, 11–17. https://doi.org/10.1016/S0273-1223(97)00047-4.

Christiansen, N.H., Andersen, F.Ø., Jensen, H.S., 2016. Phosphate uptake kinetics for four species of submerged freshwater macrophytes measured by a 33P phosphate radioisotope technique. Aquat. Bot. 128, 58–67. https://doi.org/10.1016/J.AQUABOT.2015.10.002.

Claassen, N., Barber, S.A., 1974. A method for characterizing the relation between nutrient concentration and flux into roots of intact plants. Plant Physiol. 54, 564–568. https://doi.org/10.1104/PP.54.4.564.

Duarte, C.M., 1992. Nutrient concentration of aquatic plants: patterns across species. Limnol. Oceanogr. 37, 882–889. https://doi.org/10.4319/lo.1992.37.4.0882.

Engelhardt, K.A.M., Ritchie, M.E., 2002. The effect of aquatic plant species richness on wetland ecosystem processes. Ecology 83, 2911. https://doi.org/10.2307/3072026.

Eriksson, P.G., Weisner, S.E.B., 1997. Nitrogen removal in a wastewater reservoir: the importance of denitrification by epiphytic biofilms on submersed vegetation. J. Environ. Qual. 26, 905. https://doi.org/10.2134/jeq1997.00472425002600030043x.

European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Off. J. Eur. Communities L 327.

Gumbricht, T., 1993. Nutrient removal processes in freshwater submersed macrophyte systems. Ecol. Eng. 2, 1–30. https://doi.org/10.1016/0925-8574(93)90024-A.

Gustafsson, C., Boström, C., 2011. Biodiversity influences ecosystem functioning in aquatic angiosperm communities. Oikos 120, 1037–1046. https://doi.org/10.1111/j.1600-0706.2010.19008.x.

Kahmen, A., Renker, C., Unsicker, S.B., Buchmann, N., 2006. Niche complementarity for nitrogen: an explanation for the biodiversity and ecosystem functioning relationship? Ecology 87, 1244–1255. https://doi.org/10.1890/0012-9658(2006)87[1244:NCFNAE]2.0.CO;2.

Konnerup, D., Brix, H., 2010. Nitrogen nutrition of Canna indica: effects of ammonium versus nitrate on growth, biomass allocation, photosynthesis, nitrate reductase activity and N uptake rates. Aquat. Bot. 92, 142–148. https://doi.org/10.1016/J.AQUABOT.2009.11.004.

Kovacic, D.A., David, M.B., Gentry, L.E., Starks, K.M., Cooke, R.A., 2000. Effectiveness of constructed wetlands in reducing nitrogen and phosphorus export from agricultural tile drainage. J. Environ. Qual. 29, 1262. https://doi.org/10.2134/jeq2000.00472425002900040033x.

Kronvang, B., Jeppesen, E., Conley, D.J., Søndergaard, M., Larsen, S.E., Ovesen, N.B., Carstensen, J., 2005. Nutrient pressures and ecological responses to nutrient loading reductions in Danish streams, lakes and coastal waters. J. Hydrol. 304, 274–288. https://doi.org/10.1016/J.JHYDROL.2004.07.035.

Levi, P.S., Riis, T., Alnøe, A.B., Peipoch, M., Maetzke, K., Bruus, C., Baattrup-Pedersen, A., 2015. Macrophyte complexity controls nutrient uptake in lowland streams. Ecosystems 18, 914–931. https://doi.org/10.1007/s10021-015-9872-y.

Li, J., Yang, X., Wang, Z., Shan, Y., Zheng, Z., 2015. Comparison of four aquatic plant treatment systems for nutrient removal from eutrophied water. Bioresour. Technol. 179, 1–7. https://doi.org/10.1016/J.BIORTECH.2014.11.053.

Loreau, M., Hector, A., 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412, 72–76. https://doi.org/10.1038/35083573.

Miller, A.J., Cramer, M.D., 2005. Root nitrogen acquisition and assimilation. Plant Soil 274 (1–2), 1–36.

Pelton, D.K., Levine, S.N., Braner, M., 1998. Measurements of phosphorus uptake by macrophytes and epiphytes from the LaPlatte River (VT) using ^{32} P in stream microcosms. Freshwater Biol. 39, 285–299. https://doi.org/10.1046/j.1365-2427.1998.00281.x.

Poe, A.C., Piehler, M.F., Thompson, S.P., Paerl, H.W., 2003. Denitrification in a constructed wetland receiving agricultural runoff. Wetlands 23, 817–826. https://doi.org/10.1672/0277-5212(2003) 023[0817:diacwr]2.0.co;2.

Riis, T., Dodds, W.K., Kristensen, P.B., Baisner, A.J., 2012. Nitrogen cycling and dynamics in a macrophyte-rich stream as determined by a 15N-NH4 + release. Freshwater Biol. 57, 1579–1591. https://doi.org/10.1111/j.1365-2427.2012.02819.x.

Roscher, C., Thein, S., Schmid, B., Scherer-Lorenzen, M., 2008. Complementary nitrogen use among potentially dominant species in a biodiversity experiment varies between two years. J. Ecol. 96, 477–488. https://doi.org/10.1111/j.1365-2745.2008.01353.x.

Sand-Jensen, K., Pedersen, N.L., Søndergaard, M., 2007. Bacterial metabolism in small temperate streams under contemporary and future climates. Freshwater Biol. 52, 2340–2353. https://doi.org/10.1111/j.1365-2427.2007.01852.x.

Tanner, C.C., Nguyen, M.L., Sukias, J.P.S., 2005. Nutrient removal by a constructed wetland treating subsurface drainage from grazed dairy pasture. Agric. Ecosyst. Environ. 105, 145–162. https://doi.org/10.1016/J.AGEE.2004.05.008.

Veraart, A.J., de Bruijne, W.J.J., de Klein, J.J.M., Peeters, E.T.H.M., Scheffer, M., 2011. Effects of aquatic vegetation type on denitrification. Biogeochemistry 104, 267–274. https://doi.org/10.1007/s10533-010-9500-z.

Voulvoulis, N., Arpon, K.D., Giakoumis, T., 2017. The EU water framework directive: from great expectations to problems with implementation. Sci. Total Environ. 575, 358–366. https://doi.org/10.1016/J.SCITOTENV.2016.09.228.

Vymazal, J., 2007. Removal of nutrients in various types of constructed wetlands. Sci. Total Environ. 380, 48–65. https://doi.org/10.1016/J.SCITOTENV.2006.09.014.

Wang, H., Chen, Z.-X., Zhang, X.-Y., Zhu, S.-X., Ge, Y., Chang, S.-X., Zhang, C.-B., Huang, C.-C., Chang, J., 2013. Plant species richness increased belowground plant biomass and substrate nitrogen removal in a constructed wetland. CLEAN - Soil, Air, Water 41, 657–664. https://doi.org/10.1002/clen.201200348.

Willby, N.J., Pulford, I.D., Flowers, T.H., 2001. Tissue nutrient signatures predict herbaceous-wetland community responses to nutrient availability. New Phytol. 152, 463–481. https://doi.org/10.1046/j.0028-646X.2001.00274.x.

Zhang, C.-B., Liu, W.-L., Han, W.-J., Guan, M., Wang, J., Liu, S.-Y., Ge, Y., Chang, J., 2017. Responses of dissimilatory nitrate reduction to ammonium and denitrification to plant presence, plant species and species richness in simulated vertical flow constructed wetlands. Wetlands 37, 109–122. https://doi.org/10.1007/s13157-016-0846-4.