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

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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

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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