Phosphate uptake kinetics for four species of submerged freshwater macrophytes measured by a 33P phosphate radioisotope technique

Christiansen, Nina H.; Andersen, Frede O.; Jensen, Henning S.

Published in:
Aquatic Botany

DOI:
10.1016/j.aquabot.2015.10.002

Publication date:
2016

Document version:
Final published version

Aquatic Botany 128 (2016) 58–67

Contents lists available at ScienceDirect

Aquatic Botany

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

Phosphate uptake kinetics for four species of submerged freshwater macrophytes measured by a ^{33}P phosphate radioisotope technique

Nina H. Christiansen*, Frede Ø. Andersen, Henning S. Jensen

Department of Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

ARTICLE INFO

Article history:
Received 16 March 2015
Received in revised form 8 October 2015
Accepted 15 October 2015
Available online 20 October 2015

Keywords:
Macrophytes
Nutrients
Competition
Oligotrophic
Lake
Softwater
Root-shoot ratio
Littorella uniflora
Potamogeton perfoliatus
Myriophyllum alterniflorum
Elodea canadensis

ABSTRACT

Phosphate (P*i*) uptake kinetics were determined in shoot and root tissues for four freshwater macrophyte species, *Littorella uniflora*, *Potamogeton perfoliatus*, *Myriophyllum alterniflorum* and *Elodea canadensis*, using a radioactive ^{33}P phosphate technique. Collection of plant material in the oligotrophic softwater lake, Lake Hampen, Denmark, where P*i* limits macrophyte growth, enabled us to characterize and compare the P*i* uptake kinetics and competitive characteristics of the four species in a low level P*i* environment. The maximum P*i* uptake rates (V_{max}), the half saturation constants (K_m) together with the affinity at low P*i* concentrations (V_{max}/K_m) were determined by fitting data to the Michaelis-Menten kinetics.

L. uniflora showed the highest V_{max}/K_m in the root tissue and the lowest K_m. *M. alterniflorum* showed the highest and *E. canadensis* and *P. perfoliatus* the lowest V_{max}/K_m in leaf tissue. *M. alterniflorum* had the highest V_{max} and, as the only species, a higher V_{max} in leaves than in roots. Surface area explained about half of V_{max} in *M. alterniflorum* leaves. Roots were the dominant organ for P*i* assimilation for all species at the Lake Hampen P*i* concentrations. K_m showed positive correlation to%P content in root tissue.

The results indicate that at low lake water P*i* concentrations *L. uniflora* is able to survive on the P*i* pools in the sediment porewater. *M. alterniflorum* showed high affinity for P*i* at both low and high P*i* concentrations by both roots and shoots, and suggests that *M. alterniflorum* is a strong competitor at both low and high P*i* concentrations.

1. Introduction

Softwater lakes are characterized by low inorganic carbon concentrations, low to neutral pH, and low nutrient concentrations (Roelofs et al., 1984; Murphy 2002; Pedersen et al., 2006). The aquatic submersed vegetation is typically dominated by isoetids such as *Littorella uniflora* (L.) Asch., *Lobelia dortmanna* L., and *Isoetes lacustris* L. (Roelofs et al., 1984). These slow-growing, stress-tolerant, and short-leafed evergreen rosette species form dense stands with a high root to shoot ratio and are able to exploit the carbon dioxide pool in the sediment (Brouwer et al., 2002; Murphy 2002; Pedersen et al., 2006). The plant communities in northern European softwater lakes have changed during the last century and continue to change in a direction where, filamentous algae and fast-growing, short living submerged aquatic macrophytes (elodeids) are spreading in the lakes and are believed to compete with the isoetids for nutrients and light (Roelofs 1983; Sand-Jensen et al., 2000; Brouwer et al., 2002; Szoszkiewicz et al., 2014). In Denmark, eutrophication has led to a major decline in the *L. uniflora* population; prior to 1990 it was found in 472 lakes, but now it only occurs in 218 lakes (Pedersen et al., 2006). It is important to obtain an understanding of why these community shifts happen to ensure maintenance of the high species diversity in these habitats. Factors such as eutrophication and acidification are believed to be some of the main reasons why more than 90% of these habitats have disappeared in the 20th century (Arts et al., 1990; Brouwer et al., 2002; Murphy 2002; Szoszkiewicz et al., 2014). The increasing phosphorus (P) concentration in lakes during eutrophication may also affect the macrophytes both directly and indirectly because growth of both planktonic and benthic algae is enhanced. The deteriorating light climate may influence the distribution of the macrophyte species since they have different light demands (e.g. Middelboe and Markager 1997). Other parameters, like the inorganic carbon concentration, have also been found to be important for the distribution and growth of freshwater macrophytes (e.g. Vestergaard and Sand-Jensen, 2000).

Aquatic plants take up P in the inorganic dissolved fraction phosphate (P*i*) and since eutrophication in softwater lakes is driven by P

* Corresponding author. Present address: Department of Agroecology, Aarhus University, Blichers Allé 20, Postboks 50, DK–8830 Tjele, Denmark.
E-mail addresses: nina.hoej.christiansen@agro.au.dk (N.H. Christiansen), foa@biology.sdu.dk (F.Ø. Andersen), hsj@biology.sdu.dka (H.S. Jensen).

http://dx.doi.org/10.1016/j.aquabot.2015.10.002

(e.g. Christiansen et al., 1985; Arts 2002), a better understanding of the adaptation of different macrophyte species to low P*i* availability and an identification of species benefitting from increasing P*i* concentrations is essential. Macrophytes are able to take up nutrients through both roots and shoots (Carignan and Kalff, 1980; Brix and Lyngby 1985). In softwater lakes, where the P*i* concentration in the surface water is low, P*i* in the porewater is a more easily accessible P source for rooted macrophytes (Carignan and Kalff, 1980; Barko and Smart 1981; Roelofs et al., 1984). Therefore, macrophytes with a well developed root system and a high root to shoot ratio presumably have a competitive advantage in taking up P*i* in softwater lakes. The relationship between eutrophication and the community shift to more fast-growing, short-lived species suggests that fast-growing species require higher nutrient supplies than slow-growing, long-living species, the latter being expected to be better adapted to growth at low P*i* concentrations (Pedersen and Borum 1996, 1997). Several studies have also shown that different indices and metrics for submerse macrophyte correlate well with the total phosphorus concentrations in lake water (e.g. Søndergaard et al., 2010; Kolada et al., 2014; Szoszkiewicz et al., 2014).

The aim of the study was to test the hypothesis that slow-growing, long-living isoetid species are better adapted to grow at low P*i* availability compared to fast-growing, short-living elodeid species by having a higher affinity for P*i* at low P*i* concentrations and to test the hypothesis that roots have higher affinity for P*i* than shoots at low P*i* concentrations. We also wanted to test the hypothesis that the macrophytes P*i* uptake kinetics will change during the growing season. We investigated these expectations by comparing the short-term uptake kinetics at low P*i* concentrations for roots and shoots of four macrophyte species, a slow-growing, long-living isoetid, *L. uniflora*, and three faster-growing elodeids, *Potamogeton perfoliatus* L., *Myriophyllum alterniflorum* DC. and *Elodea canadensis* L. C. Rich., in relation to dry weight, but also in relation to surface area since a larger surface area may result in a higher uptake rate. Phosphate is taken up via transport proteins in the plant cell membrane (Schachtman et al., 1998; Nussaume et al., 2011) and therefore we used the Michaelis–Menten model to describe the characteristics of the P*i* uptake kinetics. The maximum P*i* uptake rates (V_{max}) and the half saturation constants (K_m) was calculated from the Michaelis–Menten kinetics. Determination of the P*i* kinetics is essential because P*i* often is limiting for macrophyte growth. To determine the short-term uptake kinetics at low P*i* concentrations we used a ^{33}P radioisotope technique (Nielsen et al., 2006), allowing determination of P*i* affinity at the lowest P*i* concentrations, equal to V_{max}/K_m (Healey 1980; Pedersen and Borum 1997).

2. Methods

2.1. Study site

Lake Hampen (surface area 76 ha; maximum depth 13.2 m; mean depth 4 m; mean summer total P concentration 0.05 mg L^{-1};mean summer total nitrogen 0.16 mg L^{-1}; annual mean alkalinity 0.15 mM) is situated in central Jutland, Denmark (N 56°1.083′, E 9°23.259′). The catchment area (993 ha) is mainly covered by nature areas dominated by forest (62%); however, there is a small coverage of agricultural land (30%) along the eastern shoreline of the lake, but there are no urban areas (Frandsen et al., 2012; Kidmose et al., 2011; Ommen et al., 2012). The soil in the catchment area and the sediment in the littoral zone of the lake mainly consist of sand, gravel, silt and peat (Kidmose et al., 2011). Lake Hampen is a nutrient poor groundwater and rainwater fed softwater lake, which hosts different macrophyte populations including both isoetids and elodeids (Vestergaard and Sand-Jensen, 2000). Since the lake accumulates nutrients over time, it is expected to be in the initial stage of eutrophication (Ommen et al., 2012).

2.2. Sampling

Sampling of the four macrophyte species (*L. uniflora*, *P. perfoliatus*, *M. alterniflorum* and *E. canadensis)* was conducted in the beginning of June 2011 for all four species, in end of May, June and August 2012 for *E. canadensis*, and in the end of June 2012 for *P. perfoliatus* in the southern middle bay of Lake Hampen. Only one of the four species (*E. canadensis*) was tested three times during the growing season because of limiting capacity in laboratories and in time.

Elodeid material was collected by scuba diving and was gently dug up by hand to make sure that the tissue would be intact. *L. uniflora* was brought back as turfs. Plant material was taken to the laboratory in lake water and kept outside in open containers in water from Lake Hampen and in a room with a constant temperature (15 °C). Lake water was transported back in containers and kept at 4 °C prior to use. All four species were sampled and analyzed in June. Additionally, *E. canadensis* was collected in May and August to allow determination of seasonal changes in this species.

The *L. uniflora* vegetation was found along the shore line in sandy sediments. The three elodeid species grew in deeper water mixed in between each other in softer more organic sediment. To analyze sediment characteristics ten centimeter deep turfs ($n = 3$) were sampled within the *L. uniflora* vegetation, and undisturbed sediment cores ($n = 5$) from the elodeid habitat were taken to the laboratory and kept at 4 °C. Sediment samples were analyzed the next day.

2.3. Uptake kinetics

To be able to determine affinity in roots and leaves at low P*i* concentrations we used a ^{33}P radioisotope technique (Nielsen et al., 2006) to determine the short-term uptake rates. Time series incubations were conducted as a part of the ^{33}P uptake kinetics incubation experiment in order to determine an adequate incubation time with a nearly constant ^{33}P*i* uptake rate. The time series incubations were conducted in June 2011 where root and leaf fragments of similar size and age were cut off and carefully rinsed prior to incubation. For an incubation was used either root or leaf fragments from a single species: (a) *L. uniflora*: four to six non-branching whole roots of 3-4 cm and one to two whole leaves, (b) *M. alterniflorum*: single non-branching roots of 7-12 cm including root tip and stem of 4-6 cm cut off near the stem tip with three leaf-rosettes, and (c) P. perfoliatus: single whole leaves of 3–5 cm. Each replicate originated from different individual plants. The tissue was incubated in 100 or 200 ml media (91.7$CaCl_2$, 69.0 $MgSO_4$, 58.4 $NaHCO_3$, 15.4 $KHCO_3$, 1.29 NH_4NO_3 mg L^{-1}, Smart and Barko 1985) with two P*i* concentrations (0.032 μmol L^{-1} and 3.23 μmol L^{-1}) added in the form Na_2HPO_4. Three incubation times (30, 60 and 120 min) were used for *P. perfoliatus* leaf tissue (three replicates), 10 incubation times up to 60 min (one replicate) for *L. uniflora* root tissue, and 10 incubation times up to 30 min (one replicate) for *L. uniflora* leaf tissue and *M. alterniflorum* tissue. All incubations were added 1 μCi carrier free ^{33}P*i* (half-life 25.4 days). If epiphytes (algae growing on tissues) were present, they were carefully removed by scraping. All time series for *P. perfoliatus* leaf tissue and *L. uniflora* root tissue showed a linear relationship between phosphate uptake per dry mass as a function of incubation time at an incubation time of up to 50 min, indicating that the P*i* uptake by the tissue was not limited within this time frame, and an incubation time of 30 min was finally chosen for all species. *L. uniflora* leaf tissue and *M. alterniflorum* tissue time series showed a linear relationship in the 30 min incubation period. Time series were not conducted on *E. canadensis*

tissue and root tissue of *P. perfoliatus* on the assumption that these would not be limited in P*i* within the 30 min time frame based on our previous experience with the other species.

Incubations for the P*i* uptake kinetics were conducted in June and July 2011 and in May, June and August 2012. The experimental design for the P*i* uptake kinetics corresponded to that of the time series experiment, except that the incubation time was constant (30 min) and the P*i* concentrations varied. Three replicate root and leaf fragments of similar size and age were cut off and carefully rinsed prior to incubation. For an incubation was used either root or leaf fragments from a single species: (a) *L. uniflora*: four to six non-branching whole roots of 3–4 cm and one to two whole leaves, (b) *M. alterniflorum*: single non-branching roots of 7–12 cm including root tip and stem of 4–6 cm cut off near the stem tip with three leaf-rosettes, and (c) *P. perfoliatus*: single whole leaves of 3–5 cm. Each replicate originated from different individual plants. The tissue was incubated in 100 or 200 ml media (Smart and Barko 1985) and the P*i* concentrations in the media were 0.016, 0.032, 0.065, 0.161, 0.323, 0.807, 1.61, 3.23, 6.46 and 12.9 μmol L^{-1} (and for *P. perfoliatus* also 25.8 μmol L^{-1}) added in the form Na_2HPO_4. During all incubations the P*i* concentrations in the media remained relatively constant and did not decline with more than 10%. 1 μCi carrier free ^{33}P*i* was added to all incubations. If epiphytes were present, they were carefully removed by scraping.

The experiment was conducted in a constant temperature room at 15 °C. The incubations were conducted in polycarbonate bottles (150 and 250 ml bottles) which have a very low adsorption of P*i* onto the walls. The bottles were mounted on a vertical rotating disc to optimize mixing in the bottles and thereby minimize the boundary layer. A light–dark incubation (n = 3, 90 min, 0.032 and 3.23 μmol P L^{-1}, 1 μCi ^{33} P*i*) was conducted in light at 379 μmol photons m^{-2} s^{-1} and in darkness (aluminum foil covered bottles) to test if light had an effect on the P*i* uptake. No significant differences in P*i* uptake were observed between light and dark treatments during the 90 min experimental period. Following this, we decided to run the P*i* uptake kinetics incubations at the room background light (PAR = 2 μmol quanta m^{-2} s^{-1}).

After incubation the plant tissue was rinsed following a three step washing procedure: (1) in 2 mg P L^{-1} Na_2HPO_4 to remove adsorbed ^{33}P*i*, (2) in 0.02 mol L^{-1} HCl, and (3) in distilled H_2O for approximately 30 s in each wash. After rinsing and drying (on paper tissues), the plant material was freeze dried and dry weight was determined. Freeze dried tissue was bleached and destructed in NaClO after which scintillation fluor (HionicFluor) was added prior to counting. Radioactivity was determined by counting the tissue in a PerkinElmer Liquid Scintillation Analyzer Tri-Carb 2910 TR. Counts were corrected for background using blank samples and quenching was corrected using external standard.

Radioactivity in the media was determined before and after each incubation. Samples were counted in the liquid scintillation analyzer (100 μL media and 5 ml HionicFluor). P*i* analyses of the media were also conducted and measured spectrophotometrically as dissolved inorganic phosphorus with the molybdenum-blue method (Koroleff 1983).

From the relationship between the uptake and the available ^{31}P*i* and ^{33}P*i*, the assimilated ^{31}P*i* by the macrophyte was determined accordingly:

$$^{31}\mathrm{P}ia = \frac{^{33}\mathrm{P}it \times {}^{31}\mathrm{P}im}{^{33}\mathrm{P}im} \quad (1)$$

where, ^{31}P*i*a represents assimilated ^{31}P*i*, ^{33}P*i*t represents the activity [DPM] in the tissue, and ^{33}P*i*a represents the activity [DPM] of ^{33}P*i* added in the media and where 31P*i*m represents the added amount [μmol] of ^{31}P*i* in the media.

2.4. Plant and sediment analysis

Plant material was analyzed for root to shoot relationship (ratio) (n = 5) and for total phosphorus (P), total nitrogen (N) and total carbon content (C). In the nutrient analyses the five subsamples of plant material were pooled to one sample due to lack of material (n = 1; Table 1). Dry mass from roots and shoots were used to determine the root to shoot ratio (rhizomes were not included). However, no material was available for *E. canadensis* June and therefore this ratio (0.12) was estimated as an average between May and August ratios and used in further calculations. Phosphorus in root and shoot material was measured spectrophotometrically as dissolved inorganic phosphorus with the molybdenum-blue method (Koroleff 1983) after extraction in boiling 1 M HCl (120 °C, 0.5 h) of combusted (520 °C, 2 h) plant material. Nitrogen and C content in dried root and shoot material was analyzed using a CarloErba CHN EA1108-Elemental Analyzer (Table 1). WinRhizo was used to determine the plant surface area to dry weight ratio (n = 10; Table 1), and this ratio was used to convert uptake rates per dry weight to per surface area.

The upper ten centimeters of the turf and sediment cores were cleaned for plant material and were homogenized prior to analysis. Homogenized wet sediment samples were dried to determine dry weight (105 °C, 24 h). Dried sediment was used to determine loss on ignition (520 °C, 5 h). The sediment P concentration was measured spectrophotometrically on extracts of the ignited sediment (boiled in 1 mol L^{-1} HCl at 120 °C, 1 h) as dissolved inorganic phosphorus with the molybdenum-blue method (Koroleff 1983) after extraction in boiling 1 M HCl (120 °C, 0.5 h) of combusted (520 °C, 2 h) plant material.. H_2O P*i* was extracted from 1 g wet sediment by shaking twice in 25 ml DI water for 1 h and finally measured as dissolved inorganic phosphorus after filtration. Porewater was retrieved by centrifugation and filtration and analyzed for dissolved inorganic phosphorus, and lake water was filtered and analyzed for dissolved inorganic phosphorus (Table 2). *L. uniflora* grew in conditions characterized by a slightly lower ambient P*i* concentration in the porewater and a lower pool of H_2O P*i* than the elodeid species (Table 2).

2.5. Calculations and statistics

Uptake rates were calculated with the assumption that the uptake of P*i* was unidirectional, with no loss of P*i* from the tissue back into the media (equation 1). This assumption can be made under P limiting conditions and at short-term incubations because net P uptake is much greater than P loss. V_{max} and K_m were determined by plotting the P*i* uptake rate to the P*i* concentration and fitting the data to the nonlinear Michaelis–Menten model. The Michaelis–Menten kinetics was fitted in GraphPad Prism 5 and the specified standard errors (SE), 95% confidence intervals, goodness of fit (calculated from the sum of squares) and comparisons of fits between leaf and root tissue models for each species were calculated by this program as well. The fitted values were considered different when there was no overlap between the 95% confidence bands, since the 95% confidence bands corresponds to 95% chance of enclosing the true curve. Six outliers from a total of 246 numbers in the dataset were removed when fitting the data to the model (following in μmol L^{-1}, μmol g DW^{-1} h^{-1}: 0.016,0.04 and 0.032,1.45 for *M. alterniflorum* root; 12.9,0.55 for *P. perfoliatus* root; 6.46,0.16, 12.9,0.86 and 12.9,2.53 for *E. canadensis* May root Jun root and Aug root, respectively). Several K_m values in Table 3 were changed to a ">value" when V_{max} was not reached at the highest applied P*i* concentration or when the model estimated an unlikely high K_m value. K_m was then estimated by choosing the substrate concentration at the measured ½ V_{max}. When using the

Table 1
Tissue content of phosphorus (P), total nitrogen (N) and total carbon (C) in percentage of dry weight for *L. uniflora, P. perfoliatus, M. alterniflorum* and *E. canadensis* in June and additionally for *E. canadensis* in May and August 2012 ($n = 1$, one pooled sample from 5 subsamples). Additionally, the surface area to dry weight ratio is presented (mean ± SE, $n = 10$). Letters indicate significant differences in surface area to dry weight ratios (One-way ANOVA and Tukey's multiple comparison test, $p < 0.05$), where leaves and roots were tested separately.

Species	Tissue	Month	*P*	*N*	*C*	Surface area to dry weight ratio
			%	%	%	$cm^2\ mg\ DW^{-1}$
L. uniflora	Leaf	June	0.28	2.39	36.38	$^{a}0.72 \pm 0.05$
L. uniflora	Root	June	0.18	3.13	40.89	$^{x}0.88 \pm 0.07$
P. perfoliatus	Leaf	June	0.26	2.68	33.20	$^{c}3.07 \pm 0.25$
P. perfoliatus	Root	June	0.31	3.82	39.94	$^{y}2.18 \pm 0.45$
M. alterniflorum	Leaf	June	0.43	4.68	44.46	$^{b}1.82 \pm 0.13$
M. alterniflorum	Root	June	0.15	1.37	38.68	$^{y}1.28 \pm 0.21$
E. canadensis	Leaf	May	0.34	3.56	36.33	–
E. canadensis	Root	May	0.27	-	-	–
E. canadensis	Leaf	June	0.27	4.01	40.96	$^{b}1.55 \pm 0.07$
E. canadensis	Root	June	0.25	2.57	36.17	$^{x}0.99 \pm 0.04$
E. canadensis	Leaf	August	0.30	3.31	40.36	–
E. canadensis	Root	August	0.22	2.30	37.97	–

Table 2
Surface water and sediment characteristics (mean ± SD, $n = 3$ for isoetids, $n = 5$ for elodeids). Percent dry weight (DW), percent loss on ignition (LOI), total phosphorus (P), dissolved inorganic P*i* in surface and pore water and easily (water) extractable P*i* (H_2O P*i*).

	May		June		August	
	Isoetid	Elodeid	Isoetid	Elodeid	Isoetid	Elodeid
Surface water						
Pi ($\mu mol\ L^{-1}$)	0.07		0.06		0.12	
Sediment						
DW (%)	71.7 ± 5.20	17.3 ± 10.00	80.2 ± 3.30	39.5 ± 8.61	74.6 ± 0.47	8.45 ± 2.80
LOI (%)	0.57 ± 0.12	19.8 ± 12.51	0.48 ± 0.13	6.51 ± 2.48	0.83 ± 0.58	32.1 ± 8.36
P ($\mu mol\ cm^3$)	1.76 ± 0.19	4.4 ± 0.99	1.65 ± 0.17	3.14 ± 1.43	3.93 ± 1.58	2.68 ± 1.47
Pi ($\mu mol\ L^{-1}$)	1.3 ± 0.31	1.5 ± 00.63	1.33 ± 0.34	1.75 ± 0.56	4.78 ± 0.73	1.76 ± 0.85
H_2O P*i* ($\mu mol\ dm^{-3}$)	14.3 ± 5.11	19.5 ± 13.08	18.2 ± 2.53	38.0 ± 17.5	11.3 ± 1.56	12.9 ± 6.36

Table 3
Maximum P*i* uptake rates (V_{max}) and half saturation constants (K_m), P*i* affinity at low P*i* concentrations (V_{max}/K_m) calculated from the Michaelis–Menten model for leaf and root tissue for *L. uniflora, P. perfoliatus, M. alterniflorum* and *E. canadensis* in June and additionally for *E. canadensis* in May and August 2012 (mean ± SE, $n = 3$). Parameters are calculated per dry weight (g DW) and per surface area (cm^2). 95% confidence intervals are given in parentheses and goodness of fit is given by the *R* square value (R^2), ($P < 0.0001$).

		Uptake rates based on dry weight			Uptake rates based on surface area		
		V_{max}	K_m	V_{max}/K_m	V_{max}	V_{max}/K_m	R^2
		$\mu mol\ g\ DW^{-1}\ h^{-1}$	$\mu mol\ L^{-1}$		$\mu mol\ cm^2\ h^{-1}\ 10^{-3}$		
Leaf tissue							
L. uniflora	June	0.38 ± 0.08 (0.22–0.54)	2.46 ± 1.41 (0.0–5.35)	0.16	0.53	0.22	0.63
P. perfoliatus	June	0.59 ± 0.16 (0.26–0.94)	>12.9[3*]	0.02	0.19	0.01	0.90
M. alterniflorum	June	13.6 ± 3.75(5.91–21.33)	>3.23[4*] (0.58–25.03)	2.96	7.48	1.63	0.88
E. canadensis	May	>0.98[1*]	>6.46[5*]	0.15	>0.63[8*]	0.10	0.95
E. canadensis	June	0.20 ± 0.17(-0.16–0.56)	>6.46[6*]	0.04	0.13	0.02	0.62
E. canadensis	August	0.83 ± 0.13 (0.56–1.10)	0.87 ± 0.51 (0.0–1.91)	0.95	0.53	0.61	0.58
Root tissue							
L. uniflora	June	1.5 ± 0.15 (1.20–1.79)	0.85 ± 0.31 (0.22–1.48)	1.76	1.69	2.00	0.79
P. perfoliatus	June	>2.51[2*]	>12.9[7*]	0.25	>1.16[9*]	0.12	0.97
M. alterniflorum	June	2.95 ± 0.49(1.94–k3.97)	4.08 ± 1.65 (0.69–7.46)	0.72	2.37	0.54	0.81
E. canadensis	May	1.11 ± 0.24 (0.62–1.60)	8.82 ± 3.61(1.41–16.22)	0.13	1.12	0.13	0.88
E. canadensis	June	0.32 ± 0.07 (0.17–0.47)	4.51 ± 2.34 (0.0–9.35)	0.07	0.33	0.07	0.77
E. canadensis	August	1.46 ± 0.18 (1.09–1.84)	3.05 ± 0.95 (1.10–5.0)	0.48	1.46	0.48	0.87

[1,2,3,....9]The Michaelis-Menten estimated parameters: [1]5.92, [2]9.17, [3]31.4, [4]12.8,[5]65.0, [6]23.3, [7]67.9, [8]3.82, [9]4.2

[*] When the model estimated unlikely high K_m values or when V_{max} was not reached, parameters were changed to a ">" value, estimated by choosing the substrate concentration at the measured ½V_{max}. V_{max}/K_m is calculated from these estimated values.

Michaelis–Menten model for calculating the total P*i* uptake in a whole plant, the estimated parameters were used. As the model parameters are estimated from mainly low P*i* concentrations, estimated P*i* uptake corresponds well with the measured uptake rates at these low concentrations and is therefore proper for use in such calculations. V_{max} /K_m values were calculated on the basis of the model values.

Carignan (1982) presented an empirical model for estimating the relative importance of roots in the phosphorus uptake by aquatic macrophytes and the relative phosphorus availability,

$$P = \frac{9.8}{1 + 2.66(s/w)^{-0.83}} \quad (2)$$

where, *P* represents the percentage contribution by the root to the whole plant phosphorus uptake, and s and w are the dissolved reac-

tive phosphorus concentrations in the sediment porewater and the overlying water, respectively. We used this model with the measured P*i* concentrations in Lake Hampen to compare Carignan's *P* with our own calculations of percentage P*i* uptake by root tissue using our own model with the estimated Michaelis–Menten parameters, porewater and lake water P*i* concentrations, and root and shoot dry weight. The described experiment met the criteria applied by Carignan (1982) in his model; thus, (1) the measurements were conducted on plants recently collected from their natural sites, (2) P*i* concentrations in the medium in contact with the tissue were specified, (3) uptake of ^{33}P per weight of the plant was transformed into ^{31}P uptake per total plant weight, and (4) epiphytic communities were removed, if present.

Statistics were performed in GraphPad Prism 5. Means were compared (if equal between two or more treatments) using One-way ANOVA with a significant level of 5% followed by a Tukey's multiple comparisons test.

Pearson's two tailed correlations ($\alpha = 0.05$) were made with root K_m and V_{max}/K_m values from the uptake kinetics experiment in relation to the root P and N concentrations measured. No correlations emerged for leaf tissue and these are not presented here. The isoetid *L. uniflora* with the very low K_m differed greatly from the other species and was not included in the correlations, except for the correlation between K_m and%P content.

3. Results

In general, all species showed a positive non-linear relationship between the P*i* uptake rate and the P*i* concentration, except *P. perfoliatus* for root tissue and *M. alterniflorum* for leaf tissue that did not reach V_{max} and showed almost linear relationships with the applied P*i* concentrations (Figs. 1 and 2). The maximum P*i* uptake rates (V_{max}) and the half saturation constants (K_m) calculated from the Michaelis–Menten kinetics together with the affinity at low P*i* concentrations (V_{max}/K_m) for leaf and root tissue are summarized for the four different species in Table 3.

Root to shoot ratio for *L. uniflora, P. perfoliatus, M. alterniflorum* and *E. canadensis* in June were (0.91 ± 0.09, 0.04 ± 0.01, 0.11 ± 0.01, and 0.11 ± 0.02, respectively; mean ± SE, $n = 5$) and for *E. canadensis* in May and August (0.12 and 0.14 ± 0.01, respectively; mean ± SE, $n = 5$). In June *L. uniflora* had the highest ratio compared to the elodeids (about 20 and 10 times higher compared to *P. perfoliatus* and the other elodeids, respectively; $p < 0.0001$).

For leaf tissue in June, V_{max} varied 68-fold among the three elodeids with *M. alterniflorum* reaching the highest rate (13.6 ± 3.75 μmol g DW^{-1} h^{-1}; Table 3), and *E. canadensis* the lowest (0.20 ± 0.17 μmol g DW^{-1} h^{-1}; Table 3). *M. alterniflorum* also had the highest V_{max} in root tissue together with *P. perfoliatus* (2.95 ± 0.49 and >2.51 μmol g DW^{-1} h^{-1}, respectively; Table 3) and *E. canadensis* the lowest (0.32 ± 0.07 μmol g DW^{-1} h^{-1}; Table 3). *M. alterniflorum* showed, as the only species, a higher V_{max} in the leaf tissue than in the root tissue (Fig. 1). For *L. uniflora*, representing the isoetids, V_{max} was 16 times lower in leaf tissue and 2 times lower in root tissue (0.38 ± 0.08 and 1.5 ± 0.15 μmol g DW^{-1} h^{-1}, respectively; Table 3) in relation to *M. alterniflorum*. For K_m *P. perfoliatus* showed highest values in both leaf and root tissue among the elodeids (>12.9 and >10.1 μmol L^{-1}, respectively; Table 3) and *M. alterniflorum* the lowest (>4.6 and 4.08 ± 1.65 μmol L^{-1}, respectively; Table 3). *L. uniflora* exhibited lowest K_m values in both leaf and root tissue (2.46 ± 1.41 and 0.85 ± 0.31 μmol L^{-1}, respectively; Table 3) compared to the elodeids. The comparison of fits between models for leaf and root tissue within each species resulted in significantly different curves for each dataset ($p < 0.001$ for *L. uniflora, P. perfoliatus, M. alterniflorum* and *E. canadensis*; Table 3; Fig. 1).

The V_{max}/K_m ratio for leaf tissue in June was highest for *M. alterniflorum* (2.96; Table 3) among the elodeids, and up to 150 times lower for *P. perfoliatus* and *E. canadensis* (0.02 to 0.04, respectively; Table 3). *M. alterniflorum* also had the highest V_{max}/K_m ratio for root tissue where *E. canadensis* had the lowest (0.72 and 0.07, respectively; Table 3). *L. uniflora* demonstrated the highest V_{max}/K_m in the root tissue compared to leaf tissue (1.76 and 0.16, respectively; Table 3) and thereby the highest V_{max}/K_m in root tissue among all four species.

When using the surface area to dry weight ratio (Table 1) to calculate uptake rates per surface area (Table 3), the difference in V_{max} between the four species was still evident, although the difference between, for instance, shoots of *M. alterniflorum* (7.48 μmol cm^2 h^{-1} 10^{-3}) and *E. candensis* (0.13 μmol cm^2 h^{-1} 10^{-3}) decreased from a factor 68 to a factor 58, while the difference between *M. alterniflorum* and *P. perfoliatus* increased from a factor 23 to a factor 39 (0.19 μmol cm^2 h^{-1} 10^{-3}). Another interesting result was that the difference between shoots of *M. alterniflorum* and *L. uniflora* (0.53 μmol cm^2 h^{-1} 10^{-3}) decreased from a factor 38 to a factor 15. For root tissue the V_{max} for *P. perfoliatus* became relatively smaller when calculated per surface area (>1.16 μmol cm^2 h^{-1} 10^{-3}). Otherwise, the differences among species remained nearly unchanged. *E. canadensis* exhibited a seasonal change from May to August (Fig. 2; Table 3), V_{max} being lowest in June compared to May and August in both root and leaf tissue, whereas May and August had similar V_{max} values (Table 3). V_{max} did not show any pronounced difference between leaf and root tissue at the 3 dates. K_m decreased over the season in both root and leaf tissue (8.82–3.05 and 6.46–0.87 μmol L^{-1}, respectively; Table 3). *E. canadensis* showed highest V_{max}/K_m in August in both leaf and root tissue, V_{max}/K_m of root tissue being a factor 2 higher than that of leaf tissue. May and June had similar V_{max}/K_m in leaf and root tissue. The comparison of fits between models for leaf and root tissue for each time of year resulted in significantly different curves for each dataset ($p < 0.001$ for *E. canadensis* May and June; $p < 0.05$ for *E. canadensis* August; Table 3; Fig. 2).

The Michaelis–Menten model based uptake rates calculated from Lake Hampen P*i* concentrations were very similar to the actual measured rates (Table 4), rendering the Michaelis–Menten estimates applicable for these low P*i* concentrations. When calculating the total P*i* uptake in the whole plant in Lake Hampen using the Michaelis–Menten parameters, the measured P*i* concentrations in lake and porewater, and the total dry weight of root and shoot tissue in the macrophytes, we were able to determine the root contribution tototal P*i* uptake (Table 4). In June *L. uniflora* showed the highest and *M. alterniflorum* the lowest percentage uptake by roots (99 vs. 62%; Table 4). When using Carignan's 1982 model (Eq. (2)) for calculating the fraction of total P*i* uptake that the roots were responsible for, all four species showed similar values (83–86%; Table 4).

K_m and V_{max}/K_m in the elodeid species showed positive and negative correlations with % P ($r^2 = 0.68$ and $r^2 = 0.77$, respectively) and % N ($r^2 = 0.73$ and $r^2 = 0.65$, respectively) content in the root tissue (Fig. 3), whereas no correlations emerged for leaf tissue. Even though the correlations showed a strong trend they only showed significance for K_m correlated to % P content ($p = 0.0446$), maybe due to the low number of data points in the correlations.

4. Discussion

We hypothesized that P*i* concentrations in porewater and lake water could be an important factor in structuring the macrophyte community at the earliest stage of eutrophication because of differences in P*i* uptake kinetics by different macrophytes. We described uptake kinetics by the three parameters V_{max}, K_m, and V_{max}/K_m. If a macrophyte has a high V_{max} it means that it has a competitive

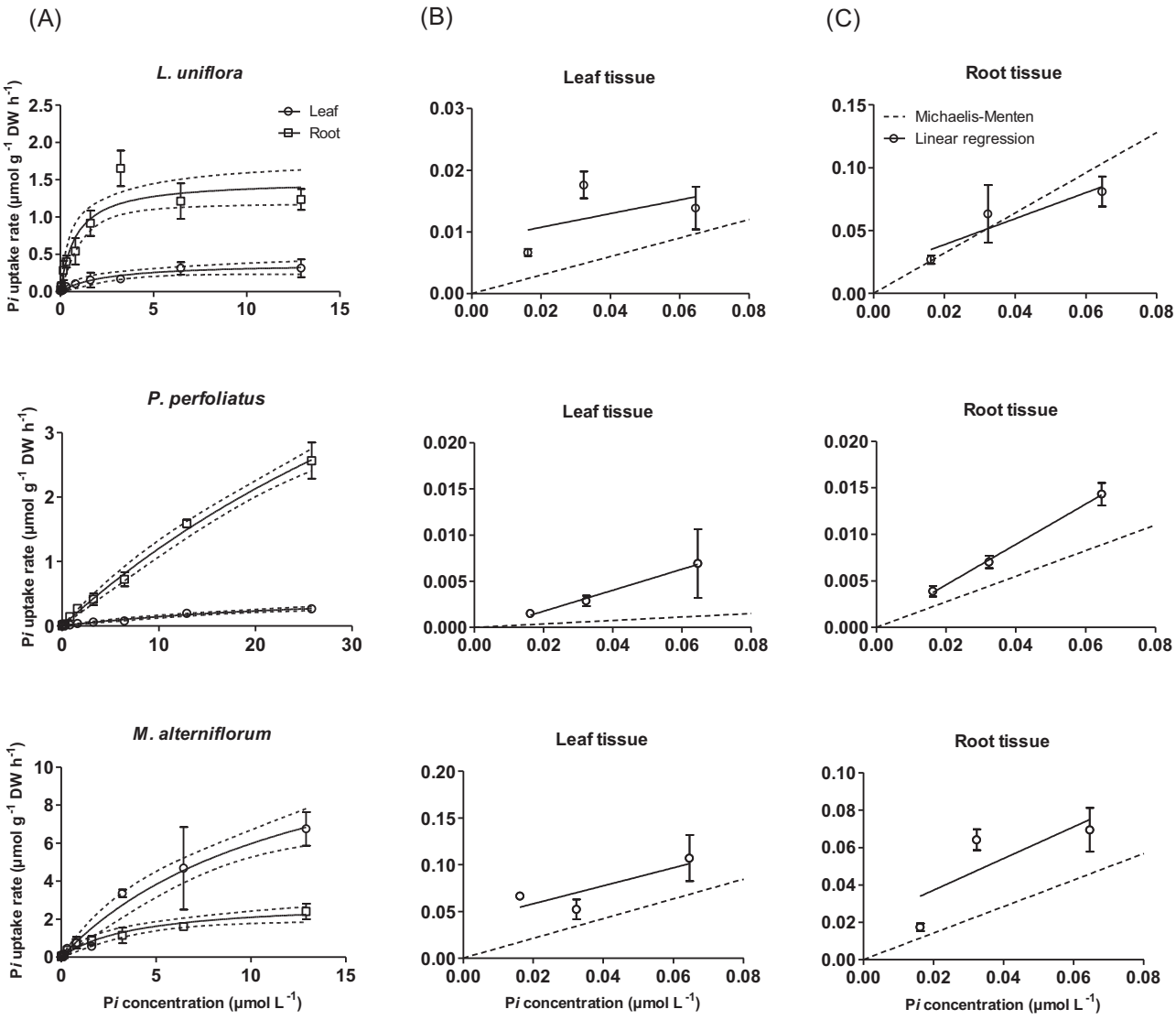

Fig. 1. A) Phosphate (Pi) uptake rates (µmol g^{-1} DW $^{h-1}$) as a function of Pi concentration (µmol L^{-1}; mean ± SE, $n = 3$) illustrated by Michaelis–Menten saturation kinetics for root and leaf tissue of *L. uniflora*, *P. perfoliatus* and *M. alterniflorum* in June, species represented by row 1–3, respectively. Solid lines are best fit to the Michaelis–Menten model and dotted lines are 95% confidence intervals. (B) P*i* uptake rates at low P*i* concentrations (0.016, 0.032 and 0.065 µmol L^{-1}; mean ± SE, $n = 3$). Dotted lines indicate best fit to the Michaelis–Menten model and solid linesindicate linear regression models (leaf and root respectively: $p = 0.29$, $R^2 = 0.16$ and $p = 0.05$, $R^2 = 0.45$ for *L. uniflora*; $p = 0.08$, $R^2 = 0.37$ and $p = 0.06$, $R^2 = 0.48$ for *M. alterniflorum*; $p = 0.29$, $R^2 = 0.35$ and $p < 0.0001$, $R^2 = 0.93$ for *P. perfoliatus*). Notice that axes scales are not the same.

advantage over other species when P*i* concentrations are high, i.e. in eutrophic water bodies. K_m represents a substrate concentration where P*i* is potentially limiting the growth rate because maximum P*i* uptake rates cannot be reached. A low K_m means that a species has a competitive advantage when P*i* availability is low, but at substrate concentrations well below K_m, where uptake rates responds linear to substrate concentrations, the affinity for the substrate is best described by V_{max}/K_m (Healey 1980; Pedersen and Borum 1997) or the slope of a linear fit (Nielsen et al., 2006).

Despite some uncertainty on estimates of V_{max} and K_m (26% and 45%, respectively, as average for both roots and shoots of the four species) the study revealed that the four different macrophyte species had different strategies for P*i* uptake relative to P*i* availability. At low P*i* concentrations *L. uniflora* showed highest affinity (V_{max}/K_m) for P*i* in the root tissue and the lowest K_m in both roots and shoots indicating that *L. uniflora* is well adapted to live in habitats with low porewater P*i* concentrations and competitive to the three other species at low P*i* concentrations. Also at low lake water P*i* concentrations, leaf tissue from *L. uniflora* had a high affinity, about ten times higher than *E. canadensis* and *P. perfoliatus*. These findings corresponds well with our hypothesis, that isoetids are better adapted to grow at low P*i* availability compared to elodeids by having a higher affinity for P*i* at low P*i* concentrations, also in roots. However, *M. alterniflorum* had the highest affinity for shoots, more than six times higher than that of *L. uniflora*, indicating that both *L. uniflora* and *M. alterniflorum* are well adapted to low lake water P*i* concentrations, but *M. alterniflorum* can better take advantage of low P*i* concentrations in lake water. For root tissue, *M. alterniflorum* had the second highest affinity of the four species, which suggests that it is the most severe competitor to *L. uniflora* at intensifying eutrophication. High affinity for P*i* in lake water by the shoot tissue gives the species a competitive advantage when a lake is in the early stage of eutrophication. *P. perfoliatus* and *E. canadensis* both showed high K_m values and low affinity for P*i* at low P*i* concentrations, suggesting that they are less competitive for P*i* at low concentrations than the two other species. In our experiment *P. perfoliatus* never reached V_{max} for root uptake, even when incubated at a P*i* concentration twice as high than for the

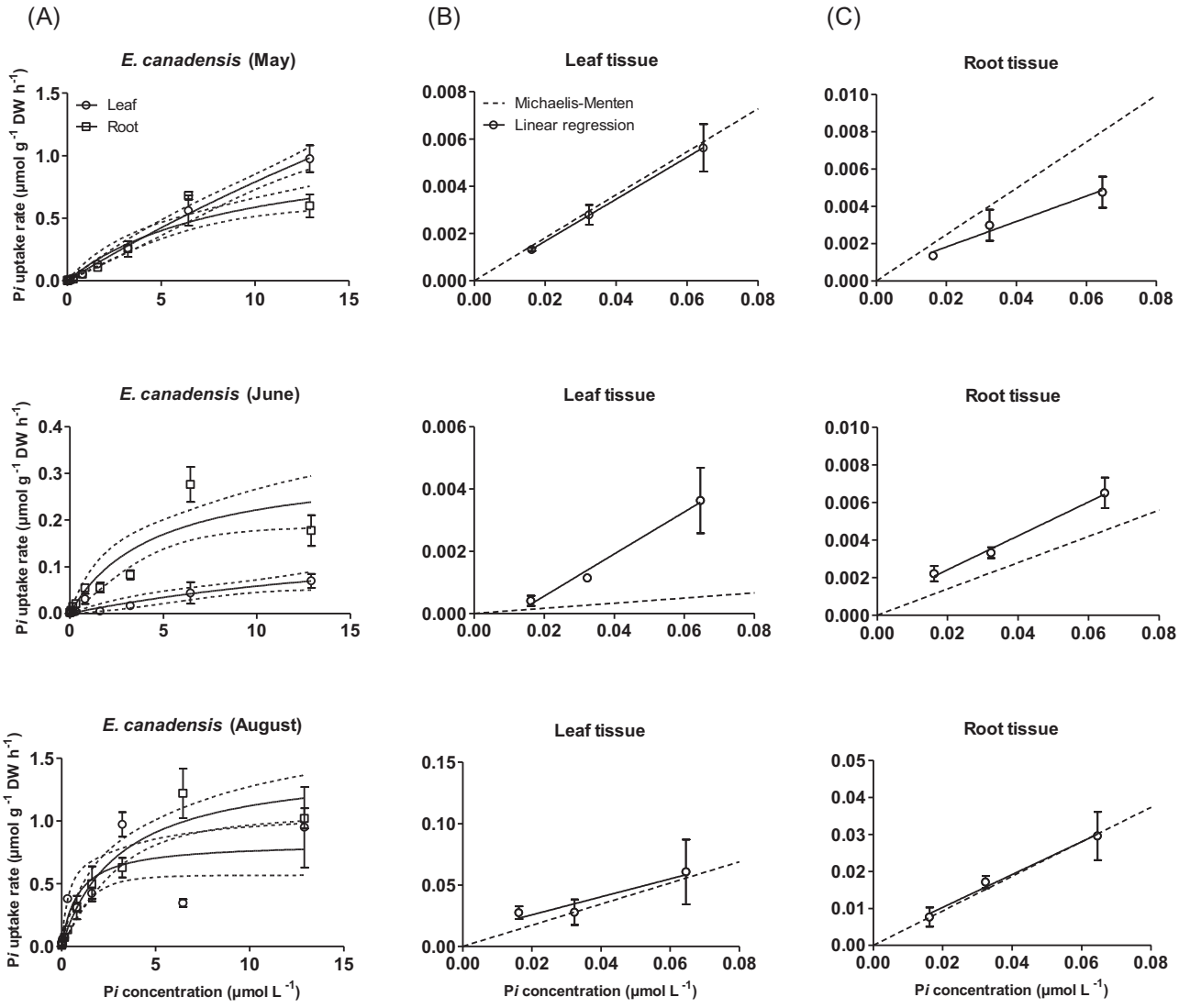

Fig. 2. (A) Phosphate (Pi) uptake rates (µmol $^{g-1}$ DW $^{h-1}$) as a function of P*i* concentration (µmol L^{-1}; mean ± SE, $n = 3$) illustrated by Michaelis–Menten saturation kinetics for root and leaf tissue of *E. canadensis* in May, June and August, dates represented by row 1, 2 and 3 respectively. Solid lines are best fit to the Michaelis–Menten model and dotted lines are 95% confidence intervals. (B) Pi uptake rates at low P*i* concentrations (0.016, 0.032 and 0.065 µmol L^{-1}; mean ± SE, $n = 3$). Dotted lines indicate best fit to the Michaelis–Menten model and solid linesindicate linear regression models (leaf and root respectively: $p = 0.001$, $R^2 = 0.80$ and $p = 0.01$, $R^2 = 0.66$ for *E. canadensis* May; $p = 0.01$, $R^2 = 0.70$ and $p = 0.001$, $R^2 = 0.84$ for *E. canadensis* Jun; $p = 0.15$, $R^2 = 0.27$ and $p = 0.01$, $R^2 = 0.69$ for *E. canadensis* Aug). Notice that axes scales are not the same.

other three species, and it may therefore be able to benefit from very high P*i* concentrations in the sediment. Comparison of our results with another study conducted with a similar ^{33}P*i* addition technique for measuring short-term P*i* uptake reveals somewhat similar uptake kinetics for macrophytes living in marine and freshwater environments low in P*i*. Thus, Nielsen et al. (2006) found that *Thalassia testudinum* was adapted to live at extremely low P*i* concentrations (as low as ~0.010 µmol L^{-1}) from recorded V_{max} (0.58–1.28 µmol g DW^{-1} h^{-1}) and K_m (2.18–7.89 µmol L^{-1}) values. The results by Nielsen et al. (2006) and the present study imply that macrophyte species in general are able to utilize periods of high P*i* availability, except *L. uniflora* that reached V_{max} in the roots at P*i* concentrations close to that found in the sediment porewater.

Macrophytes have several methods for optimizing P*i* uptake e.g. via number and/or species of transport proteins in the membrane (Nussaume et al., 2011), and via morphology of roots and shoots, for example specific surface area and root to shoot ratio. *M. alterniflorum* demonstrated the highest V_{max} of P*i*, and in contrast to the other three species it had a higher V_{max} in leaf tissue than in root tissue. Leaf surface area explained about half of the relative difference between *M. alterniflorum* and the other species, indicating that *M. alterniflorum* has developed a large surface area as an adaptation to potentially optimize P*i* uptake. However, *P. perfoliatus* exhibited the highest surface area to dry weight ratio in both shoots and roots which clearly optimized the P*i* uptake for this species. *L. uniflora* had a root to shoot ratio close to ten times higher than that of *M. alterniflorum* and *E. canadensis* and almost twenty times higher than the ratio of *P. perfoliatus*. *L. uniflora*'s large root system is an advantage for utilizing the much richer nutrient and carbon sources in the sediment (Roelofs 1983; Brouwer et al., 2002). In contrast, the other three species, with their long shoots and large shoot biomass, are well adapted to live in more eutrophic waters with poor light penetration and nutrient- and carbon-rich surface water (Murphy 2002).

Isoetids have several adaptations to life in softwater oligotrophic lakes (see e.g. Smolders et al., 2002; Spierenburg et al., 2010). The findings in the present experiment of low K_m and high affinity for P*i* for *L. uniflora* at low P*i* concentrations add to the known adaptations to low P*i* concentrations in the porewater.

Table 4
Phosphate uptake at ambient P*i* concentrations in Lake Hampen in June 2012 for leaf and root tissue of *L. uniflora*, *P. perfoliatus* and *M. alterniflorum* and in May, June and August for *E. canadensis*. (A) Measured uptake rates (μmol g DW^{-1} h^{-1}) at P*i* concentrations similar to the ambient P*i* concentrations (μmol L^{-1}; mean ± SE, $n = 3$). (B) Michaelis-Menten model estimated uptake rates at ambient P*i* concentrations. (C) Percentage contribution by the root to the whole plant's P*i* uptake. The calculation is based on the parameters V_{max} and K_m estimated from the Michaelis-Menten model, on the ambient P*i* concentrations and on the average root and shoot dry weight (g DW). (D) Estimated parameters from Carignan's (1982) model using ambient P*i* concentrations.

		(A) Measured uptake rate	(B) Michaelis–Menten model estimated uptake rates	(C) Calculated for Lake Hampen	(D) Carignan's model
	Lake water DIP	At 0.065 μmol L^{-1}		P*i* uptake by roots	P*i* uptake by roots
Leaf tissue	μmol L^{-1}	μmol g DW^{-1} h^{-1}	μmol g DW^{-1} h^{-1}	%	%
L. uniflora	0.06	0.014 ± 0.006	0.009		
P. perfoliatus	0.06	0.007 ± 0.006	0.001		
M. alterniflorum	0.06	0.062 ± 0.023	0.061		
*E. canadensis*1	0.07	0.006 ± 0.002	0.006		
*E. canadensis*2	0.06	0.004 ± 0.002	0.001		
*E. canadensis*3	0.12	0.069 ± 0.030*	0.100		
	Porewater DIP	At 1.614 μmol L^{-1}			
Root tissue	μmol L^{-1}	μmol g DW^{-1} h^{-1}	μmol g DW^{-1} h^{-1}		
L. uniflora	1.33	0.918 ± 0.300	0.912	99	83
P. perfoliatus	1.75	0.269 ± 0.021	0.231	89	86
M. alterniflorum	1.75	0.883 ± 0.431	0.888	62	86
*E. canadensis*1	1.54	0.107 ± 0.019	0.165	73	83
*E. canadensis*2	1.75	0.056 ± 0.019	0.090	96	86
*E. canadensis*3	1.76	0.499 ± 0.241	0.535	42	78

[1,2,3] *E. canadensis* in [1] May,[2] June and[3] August.
* Specified at 0.161 μmol L^{-1}, which is more similar to the Lake Hampen August concentration.

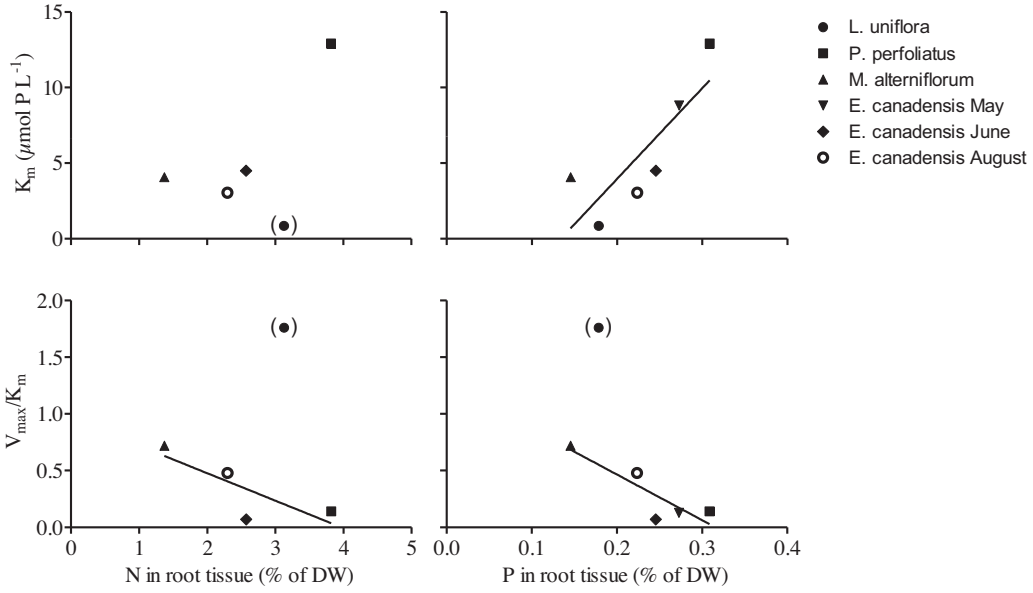

Fig. 3. Half saturation constants (K_m) and phosphate (Pi) affinities (V_{max}/K_m) correlated to root tissue content of (A) nitrogen (N%) and (B) phosphorus (P%) for *P. perfoliatus*, *M. alterniflorum* and *E. canadensis* in June and additionally for *E. canadensis* in May and August 2012. *L. uniflora* were not included in the correlationswhere points are in brackets. Correlation coefficients: K_m and V_{max}/K_m in relation to % N ($R^2 = 0.73$, $p = 0.15$ and $r^2 = 0.65$, $p = 0.20$, respectively) and to % P ($r^2 = 0.68$, $p = 0.045$ and $r^2 = 0.77$, $p = 0.051$, respectively). Trend lines are shown.

It has been discussed which organs are most important for the nutrient uptake by macrophytes, shoots or roots? It is now generally accepted that rooted macrophytes may have an efficient root uptake of P (Barko and Smart 1981; Carignan and Kalff, 1980; Barko et al., 1991). Our experiment showed that both roots and shoots can function as organs for P*i* uptake, but different uptake kinetics appeared for species and organs. For all four species, our experimental results for June reveal roots to be the dominant organ for P*i* uptake (62–99%). For *M. alterniflorum*, however, leaf tissue was responsible for about 40% of the uptake despite the relatively low P*i* concentration in the lake water. When applying Carignan's (1982) model for root and shoot contribution to P uptake at the measured P*i* concentrations in water and sediment in Lake Hampen, roots appeared to be the dominant organ for P*i* uptake (83–86%) for all four species. These numbers are of the same magnitude as those determined from the measured Michaelis–Menten uptake kinetics. However, our results suggest that some species, especially *M. alterniflorum*, may easily shift to leaf tissue uptake to assimilate P*i* at increasing lake water concentrations of P*i*. Carignan and Kalff (1980) found similar results for *Myriophyllum* sp., which may act as an opportunistic species that take up P from the most available source (Nichols and Shaw 1986). Baldy et al. (2015) found that *Elodea nuttallii* (Planch.) St. John shoot tissue was important for P uptake at eutrophic conditions, however the results from the present study on *E. canadensis* indicate that root and shoot tissue are of similar importance at eutrophic conditions for this species.

In contrast, *L. uniflora* was not able to increase the P*i* uptake rate in the leaf tissue to the same degree as *M. alterniflorum* and had a low V_{max}. Thus, *L. uniflora* relies on the roots for assimilation of P*i*. Gerloff (1975) conducted a similar ^{32}P*i* uptake kinetic experiment over a P concentration range of 0.1–100 μmol L^{-1} with four species of macrophytes and two filamentous algae. He found that the filamentous algae would outcompete the macrophytes at low P*i* concentrations (in this case 1 μmol L^{-1}) and that the algae would benefit from an increasing P*i* concentration.

In the present study, together the four species exhibited a positive correlation with K_m in relation to the P content in root tissue and the elodeid species a negative correlation with V_{max}/K_m in relation to the P content in the root tissue. Though the correlations were not all significant, these trends indicated that P*i* uptake may depend on P content in the tissue, with a higher affinity for P*i* when the P content was low. This relationship is in general accepted in the literature (Gerloff 1975) and in accordance to what has been found in other studies. For example, Brix et al. (2010) found for two emergent macrophyte species (*Cladium mariscus* spp. *jamaicense* and *Typha domingensis*) that the affinity for P*i* was up to about 6 times higher for plants with low P tissue content compared to plants with higher P content in tissues. However we measured uptake rates on newly produced leaf material, whereas the P content was measured on the total plant. Older tissue might have other uptake rates and P content than younger tissue. Other factors, such as luxury uptake and growth rate, could also influence tissue P content.

As an average for several species, Gerloff and Krombholz (1966) estimated the critical *P* value to be 0.13%, whereas Christiansen et al. (1985) for *L. uniflora* in Lake Hampen estimated the critical tissue content of P to be 0.28% or more. We found root tissue P content to be 0.18, 0.15, 0.31 and 0.25% for *L. uniflora*, *M. alterniflorum*, *P. perfoliatus* and *E. canadensis*, respectively. This suggests that the growth of all four species was partly P limited. However, when looking at the K_m values found in this experiment and comparing them to the actual P*i* concentrations recorded in the porewater in Lake Hampen (in June 1.33 μmol L^{-1}), *L. uniflora* should be close to V_{max} in the root tissue (K_m = 0.85 μmol L^{-1}), whereas the other three species must have been limited at this P*i* concentration with a K_m at least 4–13 times higher. This implies that P remains a limiting factor for macrophyte growth in Lake Hampen.

Tissue P concentration varies according to plant species, type of tissue, time of year and trophic level of sediment and water (Gerloff and Krombholz 1966; Thiébaut and Muller, 2003). Baldy et al. (2015) found at eutrophic conditions a seasonal change for *E. nuttallii* demonstrating higher shoot P uptake and P content in winter compared to spring and summer, and higher growth rates and nutrient-use efficiency during spring. We cannot explain the seasonal change observed in tissue P content and in V_{max} for *E. canadensis* with a lower P content and a very low V_{max} in late June. This midsummer minimum might indicate that *E. canadensis* was stressed, potentially limiting its physiological performance compared to the other two dates (Table 3; Fig. 2). Similarly, Best (1977) found seasonal changes in organic and mineral components with a V_{max} minimum in midsummer for *E. canadensis*, and Pieczynska and Tarmanowska (1996) demonstrated that high biomasses of both living and decomposing filamentous algae have a negative effect on the growth of *E. canadensis*. In our study, *E. canadensis* was collected in between much taller elodeids and at high densities of filamentous algae (personal observation). Our results indicate that further studies on seasonal changes in uptake kinetics are needed and that the short-term ^{33}P*i* technique is very useful for this type of studies.

In conclusion, our study showed that the slow-growing stress tolerant isoetid *L. uniflora* can exploit scarce P*i* resources by having a higher affinity for P*i* in roots at low P*i* concentrations than faster-growing elodeid species. At increasing P*i* concentrations in the lake water and porewater, elodeids will have an advantage and might outcompete the isoetids. Thus, different P uptake mechanisms may, together with other parameters accompanying eutrophication like light climate and carbon concentration, be important for the development of the macrophyte community.

Acknowledgements

This study was supported by the Centre for Lake Restoration, a Villum Kann Rasmussen Centre of Excellence and The Danish Council for Independent Research, Natural Sciences grant No. 09-067485. We thank Carina Kronborg Lohmann, Rikke Orluff Holm and Birthe Christensen for their assistance in the laboratory. We also thank Annette Steen Brandsholm and Danish Centre for Food and Agriculture, Aarhus University, for assistance and access to WinRhizo.

References

Arts, G.H.P., van der Velde, G., Roelofs, J.G.M., van Swaay, C.A.M., 1990. Successional changes in the softwater macrophyte vegetation of (sub) Atlantic, sandy, lowland regions during this century. Freshw. Biol. 24, 287–294.

Arts, G.H.P., 2002. Deterioration of atlantic soft water macrophyte communities by acidification, eutrophication and alkalinisation. Aquat. Bot. 73, 373–393.

Baldy, V., Thiebaut, G., Fernandez, C., Sagova-Mareckova, M., Korboulewsky, N., Monnier, Y., Perez, T., Tremolieres, M., 2015. Experimental assessment of the water quality influence on the phosphorus uptake of an invasive aquatic plant: biological responses throughout its phenological stage. PLoS One 10 (3), http://dx.doi.org/10.1371/journal.pone.0118844.

Barko, J.W., Gunnison, D., Carpenter, S.C., 1991. Sediment interactions with submersed macrophyte growth and community dynamics. Aquat. Bot. 41, 41–65.

Barko, J.W., Smart, R.M., 1981. Sediment-based nutrition of submerged macrophytes. Aquat. Bot. 10, 339–352.

Best, E.P.H., 1977. Seasonal changes in mineral and organic components of *Ceratophyllum demersum* and *Elodea canadensis*. Aquat. Bot. 3, 337–348.

Brix, H., Lyngby, J.E., 1985. Uptake and translocation of phosphorus in eelgrass (*Zostera marina*). Mar. Biol. 90, 111–116.

Brix, H., Lorenzen, B., Mendelssohn, I.A., McKee, K.L., Miao, S., 2010. Can differences in phosphorus uptake kinetics explain the distribution of cattail and sawgrass in the Florida Everglades? BMC Plant Biol. 10, 23.

Brouwer, E., Bobbink, R., Roelofs, J.G.M., 2002. Restoration of aquatic vegetation in acidified and eutrophied softwater lakes: an overview. Aquat. Bot. 73, 405–431.

Carignan, R., 1982. An empirical model to estimate the relative importance of roots in phosphorus uptake by aquatic macrophytes. Can. J. Fish. Aquat. Sci. 39, 234–247.

Carignan, R., Kalff, J., 1980. Phosphorus sources for aquatic weeds: water or sediments? Science 207, 987–989.

Christiansen, R., Friis, N.J.S., Søndergaard, M., 1985. Leaf production and nitrogen and phosphorus tissue content of *Littorella uniflora* (L.) Aschers. in relation to nitrogen and phosphorus enrichment of the sediment in oligotrophic Lake Hampen, Denmark. Aquat. Bot. 23, 1–11.

Frandsen, M., Nilsson, B., Engesgaard, P., Pedersen, O., 2012. Groundwater seepage stimulates the growth of aquatic macrophytes. Freshw. Biol. 57, 907–921.

Gerloff, G.C., Krombholz, P.H., 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. Limnol. Oceanogr. 11, 529–537.

Gerloff, G.C., 1975. Nutritional Ecology of Nuisance Aquatic Plants. Technical Publication Office. Environmental Protection Agency, pp. pp78.

Healey, F.P., 1980. Slope of the Monod equation as an indicator of advantage in nutrient competition. Microb. Ecol. 5, 281–286.

Kidmose, J., Engesgaard, P., Nilsson, B., Laier, T., Looms, M.C., 2011. Spatial distribution of seepage to a flow-through lake: Lake Hampen, Western Denmark. Vadose Zone J. 10, 110–124.

Kolada, A., Willby, N., Dudley, B., Nõges, P., Søndergaard, M., Hellsten, S., Mjelde, M., Penning, E., van Geest, G., Bertrin, V., Ecke, F., Mäemets, H., Karus, K., 2014. The applicability of macrophyte compositional metrics for assessing eutrophication in European lakes. Ecol. Indic. 45, 407–415.

Koroleff, F., 1983. Determination of phosphorus. In: Grasshof, K., Erhardt, M., Kremling, K. (Eds.), Method of Seawater Analyses. , 2nd ed. Verlag Chemie, Weinheim, pp. 125–139.

Middelboe, A.L., Markager, S., 1997. Depth limits and minimum light requirements of freshwater macrophytes. Freshw. Biol. 37, 553–568.

Murphy, K.J., 2002. Plant communities and plant diversity in softwater lakes of northern Europe. Aquat. Bot. 73, 287–324.

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

Nielsen, O.I., Koch, M.S., Jensen, H.S., Madden, C.J., 2006. Thalassia testudinum phosphate uptake kinetics at low in situ concentrations using a ^{33}P radioisotope technique. Limnol. Oceanogr. 51, 208–217.

Nussaume, L., Maréchal, E., Thibaud, M.C., Block, M.A., et al., 2011. Plant plasma membrane and phosphate deprivation. In: Murphy, A.S. (Ed.), The Plant Plasma Membrane, Plant Cell Monographs 19. Springer-Verlag, Berlin Heidelberg, pp. pp. 237–251.
Ommen, D.A.O., Kidmose, J., Karan, S., Flindt, M.R., Engesgaard, P., Nilsson, B., Andersen, F.Ø., 2012. Importance of groundwater and macrophytes for the nutrient balance at oligotrophic Lake Hampen, Denmark. Ecohydrology 5, 286–296.
Pedersen, M.F., Borum, J., 1996. Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. Mar. Eco. Prog. Ser. 142, 261–272.
Pedersen, M.F., Borum, J., 1997. Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake. Mar. Eco. Prog. Ser. 161, 155–163.
Pedersen, O., Andersen, T., Ikejima, K., Hossain, M.D.Z., Andersen, F.Ø., 2006. A multidisciplinary approach to understanding the recent and historical occurrence of the freshwater plant, *Littorella uniflora*. Freshw. Biol. 51, 865–877.
Pieczynska, E., Tarmanowska, A., 1996. Effect of decomposing filamentous algae on the growth of *Elodea canadensis* Michx. (a laboratory experiment). Aquat. Bot. 54, 313–319.
Roelofs, J.G.M., 1983. Impact of acidification and eutrophication on macrophyte communities in soft waters in the Netherlands I. Field observations. Aquat. Bot. 17, 139–155.
Roelofs, J.G.M., Schuurkes, J.A.A.R., Smits, A.J.M., 1984. Impact of acidification and eutrophication on macrophyte communities in soft waters. II. Experimental studies. Aquat. Bot. 18, 389–411.
Sand-Jensen, K., Riis, T., Vestergaard, O., Larsen, E.L., 2000. Macrophyte decline in Danish lakes and streams over the last 100 years. J. Ecol. 88, 1030–1040.
Schachtman, D.P., Reid, R.J., Ayling, S.M., 1998. Phosphorus uptake by plants: from soil to cell. Plant Physiol. 116, 447–453.
Smart, R.M., Barko, J.W., 1985. Laboratory culture of submersed freshwater macrophytes on natural sediments. Aquat. Bot. 21, 251–263.
Smolders, A.J.P., Lucassen, E.C.H.E.T., Roelofs, J.G.M., 2002. The isoetid environment: biogeochemistry and threats. Aquat. Bot. 73, 325–350.
Spierenburg, P., Lucassen, E.C.H.E.T., Lotter, A.F., Roelofs, J.G.M., 2010. Competition between isoetids and invading elodeids at different concentrations of aquatic carbon dioxide. Freshw. Biol. 55, 1274–1287.
Szoszkiewicz, K., Ciecierska, H., Kolada, A., Schneider, S.C., Szwabińska, M., Ruszczyńska, J., 2014. Parameters structuring macrophyte communities in rivers and lakes ?results from a case study in North-Central Poland. Knowl. Managt. Aquat. Ecosyst. 415, 08.
Søndergaard, M., Johansson, L.S., Lauridsen, T.L., Jørgensen, T.B., Liboriussen, L., Jeppesen, E., 2010. Submerged macrophytes as indicators of the ecological quality of lakes. Freshw. Biol. 55, 893–908.
Thiébaut, G., Muller, S., 2003. Linking phosphorus pools of water, sediment and macrophytes in running waters. Ann. Limnol. 39, 307–316.
Vestergaard, O., Sand-Jensen, K., 2000. Alkalinity and trophic state regulate aquatic plant distribution in Danish lakes. Aquat. Bot. 67, 85–107.