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RESEARCH PAPER

Massive uprooting of *Littorella uniflora* (L.) Asch. during a storm event and its relation to sediment and plant characteristics

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ABSTRACT

During spring storms massive uprooting of *Littorella uniflora* occurred in a shallow Dutch softwater lake. The aim of this study was to test whether changes in plant morphology and sediment characteristics could explain the observed phenomenon. Uprooting was expected to occur in plants having a high shoot biomass and low root to shoot ratio (R:S), growing on sediments with a high organic matter content. Normally, uprooting of the relative buoyant *L. uniflora* is prevented by an extensive root system, expressed as a high R:S. This was studied by sampling floating and still rooted *L. uniflora* plants, as well as sediment and sediment pore water, along a gradient of increasing sediment organic matter content. Increasing organic matter content was related to increasing *L. uniflora* shoot biomass and consequently decreasing R:S. Furthermore, the results indicated that uprooting indeed occurred in plants growing on very organic sediments and was related to a low R:S. The increased shoot biomass on more organic sediments could be related to increased sediment pore water total inorganic carbon (TIC; mainly CO_2) availability. Additionally, increased phosphorus availability could also have played a role. The disappearance of *L. uniflora* might lead to higher nutrient availability in the sediments. It is suggested that this could eventually promote the expansion of faster-growing macrophytes.

INTRODUCTION

Softwater lakes frequently occur in boreal and temperate regions, and at higher elevations in the subtropics, mostly on poorly buffered siliceous bedrock or on non-calcareous sandy soils (Murphy 2002). They are mainly fed by rainwater and shallow local groundwater flows, are generally nutrient-poor and contain very low amounts of dissolved inorganic carbon (DIC) in the water column. As the diffusion rate of gases in water is much lower compared to air, this results in carbon limitation in the water layer for most submerged macrophytes (Smolders *et al.* 2002).

The submerged macrophyte communities in oligotrophic and carbon-limited softwater lakes are often dominated by isoetid plant species, such as *Littorella uniflora* (L.) Asch., *Lobelia dortmanna* L., *Eriocaulon aquaticum* (Hill) Druce and *Isoëtes* spp. L. (Murphy 2002). These isoetids possess morphological and physiological adaptations to low carbon availability, such as low intrinsic growth rates, the ability to utilise sediment CO_2 (Wium-Andersen 1971; Roelofs *et al.* 1984; Boston & Adams 1987; Madsen *et al.* 2002) and the recycling of photorespired CO_2, captured in their extensive lacunal system (Richardson *et al.* 1984; Raven *et al.* 1988). The high O_2 permeability of the roots results in a high radial oxygen loss (ROL) towards the sediment (Sand-Jensen & Prahl 1982; Sand-Jensen *et al.* 1982) that often leads to oxidation of the entire rhizosphere (Wium-Andersen & Andersen 1972; Andersen *et al.* 2006). The resulting stimulation of mineralisation provides extra CO_2 for plant growth, and greatly reduces the availability of other nutrients (nitrogen and phosphorus) by maintaining a high sediment redox potential (Smolders *et al.* 2002). For instance, constant oxidation of Fe(II) and Mn(III) and co-precipitation with phosphates, forms highly insoluble Fe(III) or Mn(IV) phosphate complexes (Boström *et al.* 1988; Christensen & Andersen 1996). When the rhizosphere is completely oxidised, Fe(III) phosphates accumulate below the root zone and phosphate becomes unavailable for most rooted macrophytes (Tessenow & Baynes 1975). Phosphate is, however, still available for many isoetid species, which commonly live in symbiosis with vesicular-arbuscular mycorrhizas (VAM) (Wigand *et al.* 1998; Baar *et al.* 2011). Due to the high ROL, nitrogen availability is also reduced. On one hand nitrification of ammonium to nitrate is promoted, giving isoetids with nitrate-dominated nitrogen use an advantage over most other macrophytes that are unable to produce enough nitrate reductase for efficient nitrate uptake (Melzer 1980; Schuurkes *et al.* 1986; Risgaard-Petersen & Jensen 1997). On the other hand, the diurnal coupling of nitrification and denitrification results in nitrogen loss from the system (Olsen & Andersen 1994; Risgaard-Petersen & Jensen 1997).

During the past century, isoetid vegetation has declined considerably in many European countries and North America, and many species are now endangered Red List species. This decline is related to carbon enrichment and eutrophication providing faster-growing rooted macrophytes with nutrients (Grahn 1977; Roelofs 1983; *Lucassen et al. 1999*; Lucassen *et al.* 2009; Pedersen *et al.* 2006). Moreover, high organic matter production rates under relatively eutrophic conditions may increase the risk of isoetids being buried or uprooted (Smolders *et al.* 2002). Pulido *et al.* (2011) showed in a laboratory experiment that the chance of two isoetid species (*L. uniflora* and *Echinodorus repens*) being uprooted increases with increasing organic matter content. Uprooting of plants may happen when pulling forces overcome the strength of anchorage (Schutten *et al.* 2005). Anchorage forces can be related to root characteristics (biomass, total root length and 3-D architecture) and sediment cohesive properties. The manual force required to uproot *L. uniflora* over a range of sediment types ranged from 0.4 to 2.1 Newton (N; Pulido *et al.* 2011). For comparison, it is known that hydraulic pulling forces of 0.6-m high waves may range between 0.47 and 1.69 N for taller elodeids such as *Myriophyllum* and *Potamogeton* (Schutten *et al.* 2005). This force may be too small to uproot tiny isoetids. On the other hand, in contrast to elodeids, the highly porous air-filled isoetid shoots have a very low specific weight, giving the plant high buoyancy. Furthermore, in contrast to elodeids, isoetids frequently grow in very shallow water. On more productive sediments isoetids tend to increase their shoot biomass while their root biomass remains the same or even becomes smaller (Roelofs *et al.* 1994; Smolders *et al.* 2002). Increased shoot biomasses and decreased root to shoot ratios (R:S) in more nutrient-rich organic sediments probably increase the chance of being uprooted during a wind storm (Smolders *et al.* 2002).

As far as we know, no documented observations have been published on (strong) uprooting events for isoetids. During regular spring storms (24-h mean wind speed 7.5–9.9 $m \cdot s^{-1}$; KNMI 2009) in March 2008, *L. uniflora* plants were massively uprooted in the northwest bay of the Dutch softwater Lake Beuven (Fig. 1). It has previously been shown that *L. uniflora* can increase it shoot biomass on more organic and fertile sediments (Sand-Jensen & Søødergaard 1979; Robe & Griffiths 1992; Pulido *et al.* 2011). In addition, Pulido *et al.* (2011) showed that the manual force to uproot *L. uniflora* was relatively low on organic-rich sediments. The event in Lake Beuven provided a good opportunity to study sediment and morphological plant characteristics and interactions related to uprooting of isoetids under real field conditions. We expect that especially isoetids with a relatively high shoot biomass and low R:S growing on relatively organic and nutrient-rich sediments will be vulnerable to uprooting. To study this constructively, we sampled the gradient in increasing organic matter content as present from open water (with *L. uniflora*) to *Phragmites* vegetation (without *L. uniflora*) in this specific wind-exposed bay of Lake Beuven.

Fig. 1. Floating mats of uprooted *L. uniflora*, 3 months after the plants were uprooted in the littoral zone during spring storms in Lake Beuven (the Netherlands) (picture: E. Lucassen).

MATERIAL AND METHODS

Lake Beuven (51°24′00″ N, 5°38′44″ E, 23 m.a.s.l.) is the largest softwater lake in the Netherlands (about 80 ha) and one of the few Dutch lakes still holding large populations of endangered isoetid species, including *L. uniflora* and *L. dortmanna.* The lake is slightly acidic (pH 5.9) and oligotrophic (0.4 μM PO_4) with relatively low inorganic carbon concentrations (115 μM). Due to high summer precipitation rates, the summer water level has been relatively high during the past 5 years, which has led to increased organic matter accumulation in the sediments due to a lack of oxidation processes during desiccation of the lakes shores (Brouwer *et al.* 2008).

After a spring storm at the end of March 2008, massive uprooting of *L. uniflora* took place in one of the bays of Lake Beuven. Patches of the floating *L. uniflora* plants were randomly collected near the shoreline along a transect of about 25 m. In addition, still rooted *L. uniflora* plants were collected along a more or less parallel transect from open water (with *L. uniflora*) to the less air-exposed (NW) side of the bay (with *Phragmites* vegetation). This transect ultimately represented a gradient from minerogenic to organic sediments present in this bay and described by Buskens (1989). Five points, located at a distance of 7.5 m from each other and at a water depth of 0.8–1.0 m, were sampled. At each location, four sub-locations (at a radius of 0.5 m) were sampled carefully without damaging the roots, using a rack. In total, between 30 and 63 (mean 53) plants were collected at every sampling point.

After careful cleaning, the maximum root and shoot length (cm) and number of leaves of every individual plant were determined. After separating the roots and shoots, the dry mass (DM in g, 70 °C for 24 h) was determined. For elemental analysis of the shoots and roots, samples were first ground in stainless steel jars in a ball mill (Retsch, MM301; Haan, Germany). For carbon and nitrogen analyses, five shoots or roots originating from the same sample point were randomly pooled, after which approximately 3 mg of the ground material was weighed in pressed tin cups. The measurement was conducted using an elemental analyser (Carlo Erba, NA1500; Thermo Fisher Scientific, Waltham, MA, USA). For determination of shoot and root phosphorus content, the material from 5 to 10 dried individuals was pooled and ground to obtain approximately 100 mg material, which was digested for 17 min in 4 ml 65% HNO_3 and 1 ml 30% H_2O_2 in an Ethos D Microwave (Milestone, Sorisole, Lobardy, Italy; Kingston & Haswell 1997). The element concentrations within the digests were measured using an ICP-OES (see below).

At every plant sampling site (water depth 0.8–1.0 m), four samples of undisturbed sediment (0–10 cm) were taken in a PVC tube (diameter 8 cm). Additionally, four sediment samples were taken from a site where *L. uniflora* had disappeared due to uprooting during the spring storm. Fresh sediments were used for analysis of NaCl-extractable NH_4^+ and NO_3^-. For this purpose, 17.5 g FM was shaken with 50 ml 0.01 M NaCl for 1 h at 100 rpm. Plant available sediment phosphorus was extracted according to the bicarbonate method (Olsen *et al.* 1954). The remaining sediments were dried within a day (105 °C for 24 h), after which the organic matter content was determined by loss on ignition (550 °C, 4 h). For elemental analysis, 200 mg dried (105 °C, 24 h) and ground sediment was digested in a similar way as the plant material.

At every sampling point, the sediment redox potential (Eh in mV) was determined in situ in the upper 5–10 cm of undisturbed sediment using a multimeter (p901; Consort, Turnhout, Belgium), a platinum electrode and an Ag/AgCl reference electrode (Metrohm, Herisau, Switzerland). The measured values were corrected for the reference electrode used. Moreover, sediment pore water was collected anaerobically using ceramic cups (Eijkelkamp, Agrisearch, Giesbeek, the Netherlands) that were installed in the upper 10 cm of the undisturbed sediment. The cups were connected to 30-ml glass bottles, in which a vacuum had been created prior to sampling, to extract the pore water. After sampling, pore water pH was determined in the laboratory using a titration workstation (TitraLab 840; Radiometer analytical SAS, Villeurbanne, France) with a double Ag/AgCl reference pH electrode (Orion 9156BNWP; Thermo Scientific, Nijkerk, the Netherlands). Pore water dissolved inorganic carbon (DIC) was immediately measured with an infrared carbon analyser (Advance Optima; ABB, Cary, NC, USA). Based on pH and DIC concentrations, the CO_2 and HCO_3^- content of the pore water was calculated according to Stumm & Morgan (1996).

For total element composition (P, Ca, K, S, Al, Fe, Mg, Mn and Zn) of the sediment pore water, the Olsen-P extract and sediment and plant material digests, the samples were analysed using an ICP-OES (model IRIS Intrepid II XDL; Thermo Fisher Scientific). The sediment pore water NH_4^+, NO_3^- and PO_4^{3-} content were analysed with an auto analyser system (model III; Bran & Luebbe, Nordstedt, Germany) using a salicylate method for NH_4^+, a hydrazine sulphate method for NO_3^- (NO_2^-), and an ammonium molybdate method for PO_4^{3-}.

Statistics

All statistical analyses were performed in the SPSS v. 16.0 for Windows (SPSS inc., Chicago, IL, USA). Normality was tested using a Kolmogorov–Smirnov test, homogeneity of variance was tested using Levene's test. A one-way ANOVA was used to test for differences in plant root and shoot DM and R:S per sediment. To test overall differences between sediments, a MANOVA was used. Differences in individual sediment variables were tested with one-way ANOVA, followed by a *post-hoc* test (Tukey HSD or Games–Howell). The relationships between shoot DM, R:S and maximum root and shoot length, as well as number of leaves, were determined using a Kruskal–Wallis test followed by several Mann–Whitney tests. To prevent Type I error inflation, a Bonferroni correction was used, implying that the tests had 99.7% confidence ($P < 0.003$). The relationship between shoot DM, R:S of rooted plants and sediment characteristics was tested with Pearson correlations. Many sediment characteristics showed high correlations with shoot DM and R:S. To obtain more information on the structuring of sediment variables on plant variables, a multiple regression with forward selection was carried out.

RESULTS

Sediments

Properties of the sediment (chemical and physical) and sediment pore water are given in Table 1. Sediments 1–5 are arranged from minerogenic to organic sediment, all having *L. uniflora* growing on them. With increasing organic matter content, the total concentrations of most elements in the sediment tended to increase; in addition, the redox potential decreased with increasing organic matter (Table 1; ANOVA). TIC concentrations in the sediment pore water increased with increasing organic matter content, while HCO_3^- did not change significantly. Regarding other pore water measurements TP, Fe and Al increased with increasing organic matter content. The other measured pore water variables mostly differed significantly between sediments, but were not related to sediment organic matter content in a simple manner. In general, many sediment characteristics were highly correlated with each other, with correlation coefficients (r) between 0.7 and 0.9. Only the concentration of pH, TN, K and Mg in the pore water had lower correlations with the other parameters ($r < 0.2$–0.7). Sediment number 6 was different from the others in that it no longer contained *L. uniflora*. This sediment had a similar organic matter content (59%) as the most organic sediment with *L. uniflora* vegetation present (sediment 5). The bare sediment, however, contained higher concentrations for Olsen-P, Ca, Mn, Zn and total NaCl-extractable TN compared to the vegetated sediments. This was also true for TN, NH_4^+, Ca, S, HCO_3^- and Mg concentrations in the sediment pore water.

Plants

Shoot DM and R:S were significantly different per sediment type (ANOVA: $P < 0.0005$). Shoot biomass increased with increasing organic matter content of the sediment. Although the R:S of *L. uniflora* growing on sediments 1–3 did not differ, plants growing on sediments 4 and 5, as well as the floating plants showed statistical significantly lower R:S. These differences were mainly the result of increased shoot biomass, and not due to a lower root biomass. Only the root DM of floating *L. uniflora* was significantly lower compared to the plants from sediments 2, 4 and 5 (Fig. 2, Table 2).

Shoot DM increased with increasing sediment organic matter content and was correlated most strongly to pore water TIC and CO_2, followed by the organic matter content and sediment Fe content (Table 3, Fig. 3). No significant correlation was found between shoot DM and pore water nitrogen (TN, NH_4^+) or HCO_3^-. Multiple regression with forward selection between rooted *L. uniflora* shoot DM and all measured sediment parameters selected only pore water TIC as a predictor variable (Model performance: $r = 0.598$; ANOVA: $P < 0.0005$; Constant < 0.0005). After incorporation of TIC in the regres-

Table 1. Sediment characteristics at the sampling sites as arithmetic mean based on four measurements ±SE. The column denoted with 'ANOVA' shows *P*-value after one-way ANOVA. The letters in superscript show homogeneous groups between sediments (*post-hoc*, Tukey HSD or Games-Howell, $P < 0.05$). All sediments, except sediment 6, were densely vegetated with *L. uniflora*.

		sample location					
parameter	ANOVA	1	2	3	4	5	6
sediment							
redox (mV)	<0.0005	13.75 ± 20.73^{a}	63.75 ± 12.32^{a}	−46.25 ± 3.47^{b}	−71.25 ± 2.10^{b}	−45.25 ± 8.53^{b}	−96.25 ± 12.09^{b}
organic (% DM)	<0.0005	1.94 ± 0.09^{a}	2.59 ± 0.11^{a}	10.76 ± 1.83^{b}	33.12 ± 2.59^{c}	62.45 ± 1.19^{d}	59.13 ± 0.34^{d}
olsen-P (mM)	<0.0005	0.1 ± <0.01^{a}	0.14 ± 0.02^{a}	1.11 ± 0.01^{b}	0.95 ± 0.06^{b}	1.57 ± 0.09^{c}	3.07 ± 0.02^{d}
TP (mM)	<0.0005	1.56 ± 0.04^{a}	1.66 ± 0.04^{a}	7.24 ± 0.87^{b}	9.01 ± 0.37^{bc}	9.83 ± 0.29^{c}	8.52 ± 0.18^{bc}
TN* (mM)	<0.0005	0.43 ± 0.05^{a}	0.37 ± 0.02^{a}	1.37 ± 0.14^{b}	1.58 ± 0.05^{b}	1.61 ± 0.12^{b}	3.34 ± 0.10^{c}
K (mM)	<0.0005	3.01 ± 0.57^{a}	4.20 ± 0.63^{ab}	6.10 ± 0.62^{bc}	5.22 ± 0.23^{b}	7.95 ± 0.28^{c}	5.40 ± 0.24^{b}
S (mM)	<0.0005	3.85 ± 0.16^{a}	4.54 ± 0.12^{a}	21.91 ± 2.54^{b}	26.65 ± 1.13^{bc}	32.94 ± 1.03^{d}	29.99 ± 0.20^{d}
Ca (mM)	<0.0005	11.22 ± 2.40^{a}	11.55 ± 1.08^{a}	20.35 ± 2.67^{b}	19.3 ± 0.40^{b}	17.72 ± 0.75^{ab}	29.50 ± 0.29^{c}
Al (mM)	<0.0005	13.21 ± 1.12^{a}	14.9 ± 0.55^{a}	26.88 ± 2.54^{b}	34.46 ± 2.06 ^{cd}	41.22 ± 1.25^{d}	32.21 ± 0.42^{bc}
Fe (mM)	<0.0005	6.67 ± 0.41^{a}	6.43 ± 0.23^{a}	15.51 ± 1.79^{b}	30.81 ± 1.82^{c}	35.65 ± 1.02^{c}	18.92 ± 0.24^{b}
Mg (mM)	<0.0005	2.03 ± 0.19^{a}	2.24 ± 0.07^{a}	5.73 ± 0.59^{b}	6.42 ± 0.31^{b}	6.92 ± 0.20^{b}	7.01 ± 0.06^{b}
Mn (mM)	<0.0005	0.23 ± 0.01	0.32 ± 0.01^{a}	0.94 ± 0.11^{b}	0.60 ± 0.04^{c}	0.58 ± 0.02^{c}	1.46 ± 0.01^{d}
Zn (mM)	<0.0005	0.29 ± 0.05^{a}	0.35 ± 0.02^{ab}	0.76 ± 0.08^{c}	0.49 ± 0.01^{b}	0.49 ± 0.01^{b}	1.14 ± 0.02^{d}
sediment pore water							
pH	<0.0005	5.62 ± 0.12^{ab}	5.42 ± 0.14^{a}	5.85 ± 0.04^{bc}	5.32 ± 0.07^{a}	5.54 ± 0.08^{ab}	6.12 ± 0.04^{c}
TIC (μM)	<0.0005	381.64 ± 32.50^{a}	402.24 ± 23.24^{a}	415.57 ± 26.70^{a}	571.45 ± 58.66^{ab}	624.72 ± 61.43^{bc}	800.16 ± 43.92^{c}
CO_2 (μM)	0.001	320.68 ± 26.02^{a}	355.61 ± 5.53^{ab}	321.10 ± 20.31^{a}	526.47 ± 59.97^{bc}	546.95 ± 66.14^{c}	514.75 ± 17.91^{bc}
HCO_3^- (μM)	<0.0005	60.96 ± 15.70^{a}	46.62 ± 17.93^{a}	94.47 ± 9.54^{a}	44.99 ± 3.13^{a}	77.76 ± 7.20^{a}	285.42 ± 31.94^{b}
TP (μM)	<0.0005	4.70 ± 0.53^{a}	3.38 ± 0.52^{ab}	2.83 ± 0.38^{bc}	1.73 ± 0.27^{c}	2.04 ± 0.27^{c}	4.34 ± 0.40^{b}
PO_4 (μM)	0.004	2.97 ± 0.73^{ab}	2.24 ± 0.28^{ab}	1.36 ± 0.23^{a}	1.64 ± 0.55^{a}	1.15 ± 0.30^{a}	3.83 ± 0.39^{b}
TN (μM)	<0.0005	64.31 ± 5.99^{a}	42.17 ± 5.84^{abc}	21.80 ± 2.77^{c}	32.16 ± 7.62^{bc}	50.77 ± 6.69^{ab}	131.60 ± 3.77^{d}
NH_4^+ (μM)	<0.0005	50.45 ± 6.33^{a}	19.42 ± 3.51^{b}	19.07 ± 2.60^{b}	31.19 ± 4.44^{ab}	42.06 ± 6.11^{a}	124.83 ± 2.10^{c}
NO_3^- (μM)	0.003	13.86 ± 1.69^{ab}	22.75 ± 2.73^{b}	2.72 ± 0.37^{a}	8.77 ± 1.66^{a}	8.71 ± 3.00^{a}	6.75 ± 5.42^{a}
K (μM)	n.s.	160.21 ± 6.24	125.76 ± 4.08	139.54 ± 7.39	122.32 ± 2.94	132.54 ± 7.36	138.78 ± 7.28
S (μM)	<0.0005	78.63 ± 0.89^{ab}	82.23 ± 2.40^{b}	64.18 ± 6.60^{a}	77.46 ± 1.36^{ab}	68.75 ± 2.87^{ab}	27.02 ± 2.23^{c}
Ca (μM)	<0.0005	110.29 ± 15.19^{a}	85.49 ± 21.48^{a}	74.90 ± 4.10^{a}	56.55 ± 1.63^{a}	78.33 ± 4.74^{a}	189.5 ± 22.38^{b}
Al (μM)	<0.0005	2.58 ± 0.26^{ac}	4.35 ± 0.^{36b}	3.79 ± 0.38^{bc}	4.54 ± 0.40^{b}	5.05 ± 0.33^{b}	1.53 ± 0.15^{a}
Fe (μM)	<0.0005	3.24 ± 0.34^{a}	3.07 ± 0.44^{a}	6.76 ± 0.93^{a}	8.72 ± 0.80^{bc}	17.46 ± 1.69^{d}	12.63 ± 0.97^{c}
Mg (μM)	<0.0005	40.72 ± 2.22^{a}	29.82 ± 1.76^{a}	37.22 ± 3.26^{a}	28.23 ± 0.14^{a}	39.36 ± 3.71^{a}	65.03 ± 8.42^{b}
Mn (μM)	0.007	1.81 ± 0.34^{a}	1.46 ± 0.11^{a}	2.21 ± 0.71	0.85 ± 0.05^{a}	2.60 ± 0.47^{ab}	7.01 ± 2.36^{b}

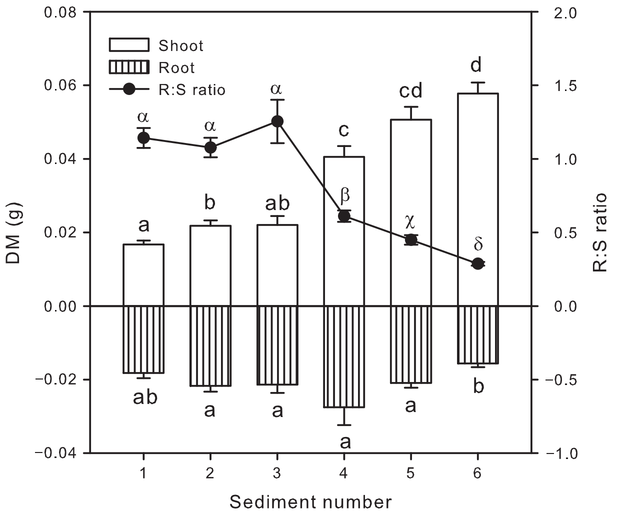

Fig. 2. Mean (±SE) shoot (white bars) and root (shaded bars) biomass (in g DM, left y-axis), and the root:shoot (mass) ratio (filled circles, right y-axis) of *L. uniflora* growing on different sediments (1–5) or in uprooted plants (6). The letters denote statistically homogeneous groups within a series.

Table 2. Maximum leaf and root length (cm) and number of leaves (# leaves) of *L. uniflora* growing in sediments 1–5 or floating *L. uniflora* (F). Values show arithmetic means ± SD. Kruskal–Wallis tests showed a significant effect ($P < 0.005$) on all three variables. Homogeneous groups are denoted per variable with similar letters (Mann–Whitney tests with Bonferroni correction on Type I error inflation; differences were accepted at $P < 0.003$).

		maximum length		# leaves
sampling site	n	root	shoot	shoot
Sediment 1	60	5.82 ± 0.18^{a}	5.36 ± 0.13^{a}	3.58 ± 0.14^{a}
Sediment 2	60	5.16 ± 0.13^{b}	6.83 ± 0.16^{b}	3.7 ± 0.11^{a}
Sediment 3	30	6.18 ± 0.22^{a}	8.19 ± 0.38^{c}	2.7 ± 0.15^{b}
Sediment 4	63	7.67 ± 0.22^{c}	11.21 ± 0.25^{d}	3.57 ± 0.14^{a}
Sediment 5	50	9.16 ± 0.24^{d}	13.81 ± 0.35^{e}	3.76 ± 0.15^{a}
Floating	91	5.47 ± 0.17^{ab}	13.8 ± 0.29^{e}	4.96 ± 0.15^{c}

sion model, other parameters did not significantly improve the model. The same analysis on R:S resulted in pore water CO_2 as the only significant predictor variable included in the regres-

Table 3. Pearson correlations between rooted *L. uniflora* R:S and the measured sediment characteristics for sediments 1–5. For all correlation analyses, n = 263. *significant at $P < 0.05$, **significant at $P < 0.01$. Correlation coefficients (r) < 0.2 are in grey font; correlation coefficients >0.5 are in bold font.

	pore water		sediment	
parameter	r	*P*	r	*P*
TIC (µM)	**−0.528**	**		
CO_2 (µM)	**−0.535**	**		
HCO_3^- (µM)	n.s.			
pH	0.29	**		
Redox (mV)	0.345	**		
organic matter content (% DM)			**−0.505**	**
Fe (µM)	−0.464	**	**−0.504**	**
Al (µM)	−0.402	**	−0.475	**
TP (µM)	0.43	**	−0.437	**
PO_4^{2-} (µM)	0.359	**		
Olsen-P (µM)			−0.404	**
TN (µM)	n.s.		−0.413	**
NH_4^+ (µM)	n.s.		n.s.	**
NO_3^- (µM)	0.185	**		
K (µM)	0.284	**	−0.383	**
S (µM)	0.127	*	−0.446	**
Ca (µM)	0.33	**	−0.3	**
Mg (µM)	n.s.		−0.422	**
Mn (µM)	n.s.		−0.145	*

sion model (Model performance: r = 0.535; ANOVA: $P < 0.0005$; Constant < 0.0005).

Most plants had three to four leaves, and the number was not related to sediment organic matter content, concentration of TIC and CO_2 in sediment pore water or sediment Olsen-P (Table 2). Only plants growing on sediment 3 had significantly less leaves, while the number of leaves of the uprooted *L. uniflora* was significantly higher.

The *L. uniflora* nitrogen content ranged from 1.6% to 5.0% DM in roots and 2.3–3.4% DM in shoots, while phosphorus content ranged from 0.09% to 1.23% DM in roots and 0.13–0.30% DM in shoots. Both tissue nitrogen and phosphorus content differed significantly with sediment type (ANOVA, Tukey HSD and Games-Howell, $P < 0.01$); these increased on more organic and also more nutrient-rich sediments. Only the nitrogen and phosphorus content of plants growing on sediment 3 deviated from this trend and had relatively low tissue nitrogen and phosphorus content (Fig. 4).

DISCUSSION

Substantial amounts of *L. uniflora* plants were uprooted in Lake Beuven during spring 2008, resulting in thinned *L. uniflora* patches or even bare sediment. The results show that the risk of uprooting was mainly related to sediment pore water TIC concentrations, which were strongly determined by the sediment organic matter content. This led to morphological changes in the plants, such as increasing shoot DM and decreasing R:S. Because *L. uniflora* has, compared to other non isoetid macrophytes, a very low specific weight, an increase in *L. uniflora* shoot biomass and R:S might induce uprooting. The relatively low specific weight of isoetids is caused by the presence of an extensive air lacunae system, making up 30–50% of the leaf volume (Robe & Griffiths 1998; Madsen *et al.* 2002). Under nutrient-limited conditions, shoot biomass of isoetids remains low and uprooting of isoetids is prevented because of the anchorage provided by the relatively extensive root system. In the literature, isoetid R:S of 0.5 to even more than 1.0 are often reported (Sand-Jensen & Prahl 1982; Roelofs *et al.* 1994; Bagger & Madsen 2004; Lucassen *et al.* 2012). In more fertile soils, isoetids invest more in shoot biomass to avoid aboveground competition for light from more productive species, which results in a decrease in R:S and in an increased risk of uprooting (Roelofs *et al.* 1994; Smolders *et al.* 2002).

In Lake Beuven, the R:S of *L. uniflora* was lower when plants were growing on more organic sediments. This was mainly caused by an increase in shoot biomass, as only a slight decrease in root biomass was observed. This indicates that promotion of shoot growth, rather than reduction in root biomass, caused the lowering of R:S when plants grew on more organic sediments. The increase in shoot biomass was due to the production of longer leaves and, to a lesser degree, to a higher number of leaves. Increased shoot biomass of aquatic plants on more organic and fertile sediments has already been reported for *L. uniflora* (Sand-Jensen & Søødergaard 1979; Robe & Griffiths 1992; Pulido *et al.* 2011), and *Isoetës macrospora* (=*I. lacustris*) (Boston & Adams 1987).

For the event in Lake Beuven, uprooting occurred on organic sediments with plants having a mean shoot DM approaching 0.06 g, a R:S of around 0.3 and on average five leaves. Still rooted *L. uniflora* plants always had a R:S $\geq$ 0.5 (Fig. 2). The relation between sediment organic matter content and shoot growth can be viewed as an indirect relationship. Organic matter content as such will not directly influence plant growth, but rather the availability of macro- and micronutrients, which are connected to sediment organic matter content, will result in the observed shoot growth of *L. uniflora*. In this study, pore water TIC and CO_2 concentration showed the highest correlation with shoot DM and R:S. Multiple regression indicate that TIC and CO_2 best explained the variation in shoot DM and R:S of *L. uniflora* respectively, while no other variable added additional explanatory power to the regression model. Next to shoot biomass, sediment structure is of importance for the risk of uprooting. With increasing organic matter content, the sediment water content increases and sediment density strongly decreases. Such highly organic, soft and 'flushy' sediments provide less support for the roots of *L. uniflora*, making them even more vulnerable to uprooting (Schutten *et al.* 2005).

A relationship between pore water CO_2 and shoot DM of isoetids has been reported previously (*e.g.,* Baattrup-Pedersen & Madsen 1999; Madsen *et al.* 2002; Bagger & Madsen 2004) and is linked to the pore water CO_2 utilization by isoetids, which comprises up to 95% of the carbon uptake of *L. uniflora* (Sand-Jensen & Søødergaard 1978). The acquired pore water CO_2 is transported to the shoot by diffusion through the air lacunae system in the roots and shoots (Madsen *et al.* 2002). Due to the relatively high diffusive resistance, the CO_2 flux from roots to shoots is limited. If sufficient CO_2 for positive net photosynthesis at the leaf apex is attained, the diffusion rate of CO_2 will consequently restrict the maximum leaf length (Madsen & Sand-Jensen 1991). The CO_2 diffusion rate towards the leaf is strongly affected by the steepness of the CO_2 gradient from root to shoot. Thus, at higher pore water CO_2 availability,

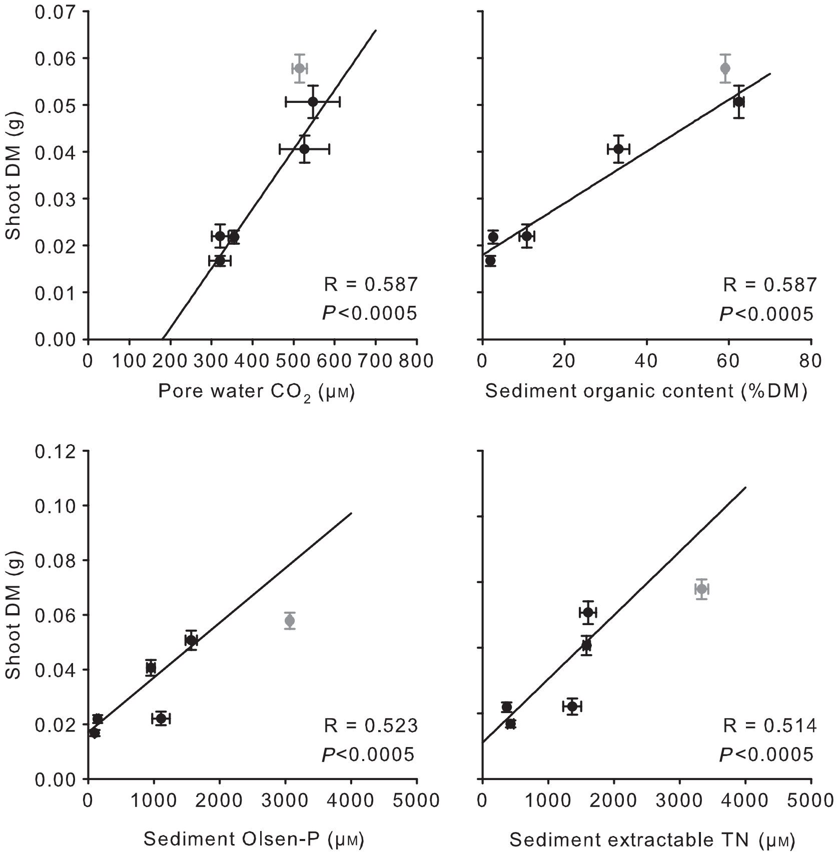

Fig. 3. Regressions of *L. uniflora* shoot DM on sediment and pore water properties (mean ± SE). The regression lines are calculated for the shoot DM and properties of vegetated sediments 1–5. The grey circles show the combined shoot DM of uprooted *Littorella* and properties of sediment 6 without *Littorella* vegetation (and therefore not included in the regression analyses).

a steeper gradient is present and longer maximum leaf lengths can be obtained without loss in net photosynthetic activity. Because the leaves of *L. uniflora* are relatively permeable for CO_2, high concentrations of CO_2 in the water surrounding the leaves could release some of the restrictions on leaf length imposed by low internal CO_2 diffusion rates. High aquatic CO_2 concentrations can, therefore, also lead to increased leaf length and decreased R:S (Andersen *et al.* 2006). However, the overlying water in Lake Beuven does not show spatial differences in CO_2 concentration (data not shown). Therefore, the observed differences in *L. uniflora* shoot DM and R:S are most likely related to the pore water CO_2 level, which implies that the uptake of sediment CO_2 is still important as a carbon source for photosynthesis of *L. uniflora* growing in surface water with about 115 µmol CO_2 l^{-1}.

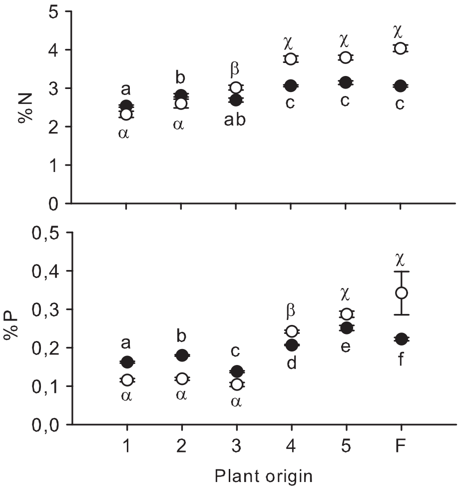

Fig. 4. Nitrogen (upper panel) and phosphorus (lower panel) content (% DM) of *L. uniflora* shoots (closed symbols) and roots (open symbols) sampled from sediments 1–5 and from floating, uprooted plants (F). The letters denote statistically homogeneous groups within the shoot or root samples (ANOVA: $P < 0.001$, Tukey HSD and Games-Howell: $P < 0.01$).

Next to CO_2, sediment phosphorus and, to a lesser extent, sediment extractable-TN content were also correlated to the organic matter content. However, these parameters showed weaker correlations with *L. uniflora* shoot DM and R:S and were, therefore, not selected within the multiple regression due to multicollinearity (Table 3). Nevertheless, increasing nutrient (nitrogen, phosphorus) levels may also have affected the observed increased shoot biomass (Sand-Jensen & Søødergaard 1979; Sand-Jensen *et al.* 2005). A higher nutrient availability can enable increased shoot growth, without the need to extend the root biomass for nutrient acquisition. This argument would only hold if at least shoot production is nutrient-limited. Based on the root and shoot tissue nitrogen content (Fig. 4), however, *L. uniflora* production seemed not to have been nitrogen-limited. The tissue nitrogen content was in the range of non-nitrogen-limited *L. uniflora* (3.3% N; Christiansen *et al.* 1985). Furthermore, in a study reported by Robe & Griffiths (1994), *L. uniflora* was not limited by nitrogen (as NO_3^-) at very low pore water levels of 0.6 µM. In our study, pore water NO_3^- levels always exceeded this level. This implies that in Lake Beuven the higher nitrogen availability with increasing sediment organic matter content does not affect shoot DM and R:S of *L. uniflora*. The production of the sampled *L. uniflora* could, however, have been phosphorus-limited. The tissue phosphorus content was below reported values of 0.28% for phosphorus-limited *L. uniflora* (Christiansen *et al.* 1985). With the exception of sediment number 3, a clear relationship was found between shoot DM and Olsen-P (Fig. 3).

Once isoetids are massively uprooted they are no longer able to oxidise the sediments, leading to a reduction in oxidised Fe (III)-phosphates and Mn(IV)-phosphates, releasing phosphates into the sediment pore water and making them available for rooted macrophytes. Moreover, without isoetids the induced coupling of nitrification and denitrification will be minimal, which on longer time scales can result in a build up of nitrogen concentrations within the sediments (Smolders *et al.* 2002). Bare sediments with high nutrient content can form a suitable substrate for faster-growing macrophytes, such as the elodeids *C. hamulata* and *M. alterniflorum*. Especially when the aquatic CO_2 levels become relatively high, elodeid species can theoretically invade or massively expand in softwater lakes (Spierenburg *et al.* 2009, 2010). Once these species successfully dominate the softwater lakes, re-colonisation with isoetids may become difficult, since at sufficient nutrient availability the slow-growing isoetids will be poor competitors. Moreover, the germination success of isoetids is hampered on reductive, anoxic sediments (Farmer & Spence 1987; Arts & van der Heijden 1990; Bellemakers *et al.* 1996). Finally, in Lake Beuven the high biomass of uprooted *L. uniflora* was blown onto the lakeshore, where it formed thick mats, gradually decomposing over time. Locally, these mats provided a major input of organic matter and nutrients to the littoral zone. This could threaten the submerged plants in the littoral zone by burying and local nutrient enrichment of the sediment. To protect the endangered isoetid vegetation, it is important to prevent the accumulation of nutrient-rich organic matter in sediments by allowing a natural water regime with low water tables in summer. Another option could be to remove the highly organic sediments from the system.

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