Influence of quantity and lability of sediment organic matter on the biomass of two isoetids, *Littorella uniflora* and *Echinodorus repens*

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SUMMARY

1. Despite real improvement in the water quality of many previously eutrophic lakes, the recovery of submerged vegetation has been poor. This lack of recovery is possibly caused by the accumulation of organic matter on the top layer of the sediment, which is produced under eutrophic conditions. Hence, our objective was to study the combined effects of quantity and lability of sediment organic matter on the biomass of *Echinodorus repens* and *Littorella uniflora* and on the force required to uproot plants of *L. uniflora*.

2. Lake sediments, rich in organic matter, were collected from four lakes, two with healthy populations of isoetids and two from which isoetids had disappeared. The four lake sediments were mixed with sand to prepare a range of experimental sediments that differed in quantity and lability of sediment organic matter. Two isoetid species, *E. repens* and *L. uniflora*, were grown in these sediments for 8 weeks. Sediment quality parameters, including elemental composition, nutrient availability and mineralisation rates, were determined on the raw sources of sediment from the lakes. Porewater and surface water were analysed for the chemical composition in all mixtures. At the end of the experiment, plants were harvested and their biomass, tissue nutrient concentration and (for *L. uniflora*) uprooting force were measured.

3. For both species, all plants survived and showed no signs of stress on all types of sediment. The biomass of *E. repens* increased as the fraction of organic matter was increased (from 6 to 39% of organic content, depending upon sediment type). However, in some of the sediment types, a higher fraction of organic matter led to a decline in biomass. The biomass of *L. uniflora* was less responsive to organic content and was decreased significantly only when the least labile sediment source was used to create the gradient of organic matter. The increase in shoot biomass for both species was closely related to higher CO_2 concentrations in the porewater of the sediment. The force required to uproot *L. uniflora* plants over a range of sediment organic matter fitted a Gaussian model; it reached a maximum at around 15% organic matter and declined significantly above that.

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4. Increasing organic matter content of the sediment increased the biomass of isoetid plants, as the positive effects of higher CO_2 production outweighed the negative effects of low oxygen concentration in more (labile) organic sediments. However, sediment organic matter can adversely affect isoetid survival by promoting the uprooting of plants.

Introduction

In the northern hemisphere, the vegetation of softwater lakes is often dominated by isoetid species such as *Lobelia dortmanna* L., *Littorella uniflora* (L.) Aschers and *Isoëtes* spp.; hence, these lakes are often referred to as isoetid lakes (Rørslett & Brettum, 1989). Isoetid lakes are oligotrophic ecosystems with very clear water and are characterised by low concentrations and availability of dissolved inorganic carbon (HCO_3^- + CO_2 <1 mM) in the water column (Arts, 2002). This low C availability in the water column limits the growth of elodeids, which use bicarbonate, leading to the dominance of isoetids that are specialised in using sediment-derived CO_2 for photosynthesis (Wium-Andersen & Andersen, 1972a; Murphy, 2002; Madsen & Sand-Jensen, 1991; Spierenburg *et al.*, 2009; Winkel & Borum, 2009).

Isoetids have several morphological and physiological adaptations enabling efficient uptake and use of sediment-derived CO_2. They form rosettes and have a large gas permeable biomass belowground (Raven *et al.*, 1988). Air-filled lacunae occupy a large proportion of the plant volume and ensure rapid diffusion of CO_2 and O_2 between above- and belowground tissues (Sand-Jensen, Prahl & Stokholm, 1982; Smits *et al.*, 1990). Thus, oxygen produced by photosynthesis diffuses from shoots to roots and enters the rhizosphere via radial oxygen loss (ROL) (Sand-Jensen *et al.*, 1982; Pedersen, Sand-Jensen & Revsbech, 1995). ROL from isoetid roots promotes mineralisation of the sediment and immobilisation of P, because P is bound to oxidised Fe in iron-rich sediments (Wium-Andersen & Andersen, 1972b; Smolders, Lucassen & Roelofs, 2002). In addition, ROL promotes nitrification of ammonium in the oxidised rhizosphere, which benefits species that take up N mainly in the form of nitrate, such as isoetids (Sand-Jensen *et al.*, 1982; Roelofs, Schuurkes & Smits, 1984), but it also leads to net losses of N in isoetid vegetation via enhanced denitrification (Risgaard-Petersen & Jensen, 1997).

Therefore, isoetids are key ''chemical engineers'' that help maintaining oligotrophic water and sediment conditions in addition to high water transparency.

In the 20th century, eutrophication, along with acidification and alkalinisation, has led to the disappearance of isoetids in many softwater lakes (Roelofs, 1983; Roelofs *et al.*, 1984; Arts *et al.*, 1989; Arts, 2002). Eutrophication promoted mass development of fast-growing submerged macrophytes, increased epiphyton and plankton blooms, decreased light penetration and eventually caused the loss of isoetids (Roelofs, 1983; Roelofs, Brandrud & Smolders, 1994; Arts, 2002). Degradation of isoetid vegetation in lakes led to a decrease in sediment mineralisation rates as oxidation was no longer enhanced by ROL from isoetid roots (Smolders *et al.*, 2002). This decrease in mineralisation, coupled with increased sedimentation of detritus from macrophytes and phytoplanktons, led to the accumulation of labile organic matter in the top layer of the sediment (Brouwer & Roelofs, 2001). This might explain the final disappearance of isoetids and/or the lack of their recovery (Sand-Jensen, Borum & Binzer, 2005), despite a real improvement in water quality has been achieved (Jeppesen *et al.*, 2005; Sand-Jensen *et al.*, 2008). Therefore, we investigated the effect of sediment organic matter on isoetids.

The impact on isoetids of slowly degrading refractive organic matter in the sediment probably differs from that of highly labile material, and thus, it is essential to distinguish between the quantity and lability of sediment organic matter (Verhoeven, 1986). Mineralisation of refractive organic matter (for example terrestrial leaf litter and peat) may serve as a slow but steady source of CO_2 and inorganic nutrients to the porewater, that isoetids are able to exploit (Smolders *et al.*, 2006). As decomposition is slow, oxygen loss from the roots is sufficient to keep the rhizosphere oxidised (Wium-Andersen & Andersen, 1972b) and, thus, protect against reduced phytotoxins. However, as the lability of sediment organic matter increases (for example through the sedimentation of

phytoplankton or detritus from fast-growing macrophytes), oxygen consumption may eventually exceed ROL from the plant roots, leading to microbial fermentation and production of phytotoxins in the near rhizosphere (Armstrong & Armstrong, 2001; Pedersen, Binzer & Borum, 2004). Thus, even low amounts of highly labile sediment organic matter may have adverse effects on isoetids (Moyle, 1945; Sand-Jensen *et al.*, 2005; Raun, Borum & Sand-Jensen, 2010), whereas the plants may actually benefit from high concentrations of refractive sediment organic matter (Sand-Jensen & Søndergaard, 1979; Roelofs *et al.*, 1984; Lenssen *et al.*, 1999). In addition to adverse chemical effects, high sediment organic matter also affects the physical properties of the sediment by increasing its water content and, thus, decreasing its cohesion, potentially increasing the risk of uprooting.

Our aim was to examine how the quantity and lability of sediment organic matter affects biomass and uprooting of two isoetid species, *Echinodorus repens* Lam. Kern and Reichg and *L. uniflora*. We expected that enrichment of sandy, oligotrophic sediments with organic matter would stimulate sediment mineralisation and consequently also nutrient availability and oxygen demand of the sediment. Therefore, we hypothesised that organic matter would initially increase isoetid biomass until the negative effects of oxygen stress outweighed the positive effects of the higher nutrient availability. At organic fractions above that, isoetid biomass would then decrease and plants might even die. Such a decrease in plant biomass was expected at relatively low organic matter concentrations when labile organic sediment was used and at high concentrations when refractive organic matter was used. Additionally, organic matter would decrease the cohesive forces of the sediment because of the higher water content, and thereby the force necessary to uproot *L. uniflora* plants.

Methods

Sediments from four lakes, differing in lability of the organic content, were mixed with sand from a mesotrophic lake, here named as ''Control'', to prepare gradients of organic matter. Each gradient consisted of seven mixtures created by mixing ''Control'' sediment with 0, 5, 12.5, 25, 50, 75 and 100% volume of fresh lake sediment. Lake sediments, rich in organic matter, were collected from two lakes in Southern Norway, Fagervatnet and Hunsdalvatn, and from two lakes in The Netherlands, Sarsven and Broekse Wielen I. In the Dutch lakes, all isoetids have disappeared over the last few decades as a consequence of eutrophication (Brouwer & Roelofs, 2001). In the Norwegian lakes, Hundsdalvatn is dominated by isoetids, whereas Fagervatnet is a lake where the isoetid vegetation has declined strongly as a consequence of yearly liming (Roelofs *et al.*, 1994). Here, lake sediments are named taking into account their CO_2 production (a measure of lability) as ''Low'' (≤ 15 μmol CO_2 L^{-1} h^{-1}) collected in Fagervatn, ''Medium 1'' and ''Medium 2'' (>15 <20 μmol CO_2 L^{-1} h^{-1}) collected in Hundsdalvatn and Sarsven, respectively, and ''High'' (>20 μmol CO_2 L^{-1} h^{-1}) collected in Broekse Wielen I (Table 1; Fig. 1). Each gradient was replicated three times resulting in a total of 84 cylindrical aquaria (13 cm in diameter and 30 cm high) that were filled with sediment (1 L).

The artificial water column mimicked Dutch softwater lakes and had 1500 μM of Na^+, 500 μM of SO_4^{2-}, 500 μM of HCO_3^-, 500 μM of Ca^{2+} and 1000 μM of Cl^-. Plants were collected from Lake Banen (The Netherlands, 51°16′N, 05°48′E), and three individuals of *E. repens* (8.2 ± 3.5 mg DM m^{-2}; mean ± SD) and three individuals of *L. uniflora* (18.3 ± 4.5 mg DM m^{-2}; mean ± SD) were planted in each aquarium. The aquaria were placed in a water jacket and maintained at 19 ± 1 °C by means of a recirculation chiller (Neslab Merlin M-75; Thermo Scientific, Newington, NH, U.S.A.). Irradiance was 200 μmol m^{-2} s^{-1} and was provided by six Philips-type HP 400 W lamps (Hortilux-Schréder, Monster, The Netherlands) at a photoperiod of 14 h.

Before the experiment, a representative subsample of plants and sediments was characterised to define the initial conditions. Plants were characterised by determining dry shoot and root mass. Then, the plants were planted and completely submerged in artificial lake water.

Sediment analyses

The ''Control'' and treatment lake sediments (''Low'', ''Medium 1'', ''Medium 2'' and ''High'') used to create the four gradients of organic matter were characterised as follows. Organic content was measured as loss on ignition (5 h at 550 °C). Density was

Table 1 Sediment, porewater and plant characterisation of the sand (''Control'') and four lake sediments (''Low'', ''Medium 1'', ''Medium 2'' and ''High'') used to create the mixtures

	''Control''	''Low''	''Medium 1''	''Medium 2''	''High''	ANOVA
Lake Name	Broekse Wielen III	Fagervatnet	Hunsdalvatn	Sarsven	Broekse Wielen I	
Latitude	51°43′N	58°24′N	58°28′N	51°16′N	51°43′N	
Longitude	5°45′E	6°33′E	8°28′E	5°47′E	5°45′E	
Sediment						
NO_3^- (μM FS)	353 ± 48[a]	72 ± 44[b]	37 ± 30[b]	35 ± 38[b]	65 ± 41[b]	***
NH_4^+ (μM FS)	376 ± 11[c]	294 ± 92[c]	622 ± 202[b]	393 ± 120[c]	1072 ± 50[a]	***
P (μM FS)	791 ± 37[a]	58 ± 8[d]	210 ± 22[c]	335 ± 54[bc]	309 ± 5[b]	***
C:N (M)	11 ± 1[d]	22 ± 0[b]	24 ± 1[a]	20 ± 0[c]	23 ± 2[ab]	***
N:P (M)	6 ± 1[b]	21 ± 2[b]	18 ± 2[b]	39 ± 2[b]	80 ± 61[a]	**
E_h initial (mV)	509 ± 29[a]	222 ± 11[c]	262 ± 13[b]	128 ± 8[e]	170 ± 8[d]	***
E_h final (mV)	611 ± 92[a]	339 ± 87[b]	261 ± 56[b]	247 ± 51[b]	265 ± 65[b]	***
Porewater						
CO_2 (μM)	299 ± 158[b]	229 ± 59[b]	378 ± 158[ab]	507 ± 404[ab]	701 ± 250[a]	*
NO_3^- (μM)	14 ± 37	2 ± 0	1 ± 0	1 ± 0	1 ± 0	ns
NH_4^+ (μM)	169 ± 93[ac]	91 ± 10[c]	274 ± 25[b]	169 ± 5[a]	281 ± 45[ab]	*
PO_4^{3-} (μM)	1 ± 0[c]	0 ± 0[c]	0 ± 0[c]	9 ± 3[b]	32 ± 12[a]	***
Plant						
Echinodorus repens						
C (mol g^{-1})	27 ± 3[b]	31 ± 1[a]	30 ± 2[ab]	32 ± 0[a]	30 ± 1[ab]	*
N (μmol g^{-1})	2187 ± 412[bc]	2525 ± 76[ab]	2848 ± 156[a]	1988 ± 85[bc]	1883 ± 36[c]	**
P (μmol g^{-1})	189 ± 20[b]	239 ± 68[a]	162 ± 17[bc]	245 ± 23[a]	113 ± 15[c]	***
Littorella uniflora						
C (mol g^{-1})	32 ± 1[c]	33 ± 1[b]	34 ± 0[a]	31 ± 0[e]	31 ± 0[e]	***
N (μmol g^{-1})	1773 ± 203[a]	1526 ± 85[bc]	1643 ± 175[ab]	1286 ± 124[cd]	1128 ± 64[d]	***
P (μmol g^{-1})	137 ± 25[b]	87 ± 7[c]	66 ± 15[c]	180 ± 25[a]	81 ± 4[c]	***

Initial fresh sediment (FS) concentrations of plant available NO_3^-, NH_4^+ and P ($n = 3$); C:N and N:P ($n = 4$);
initial and final redox potentials (E_h) as an average of four sediment depths ($n = 3$); initial porewater concentrations of CO_2,
NO_3^-, NH_4^+ and PO_4^{3-} ($n = 3$); final C, N and P content of *E. repens* and *L. uniflora* shoots ($n = 3$). Data are shown as means ± SD.
Means with the same letter do not differ significantly at 95% CI. ANOVA was used to test differences among sediments as indicated
by *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ns: ≥0.05.

measured after drying 45 mL of fresh sediment for 24 h at 105 °C. This enabled us to express all concentrations as per L fresh sediment. Total C and N concentrations of the sediments were determined using a CNS analyser (type NA 1500; Carbo Erla Instruments, Milan, Italy).

Plant available NO_3^- and NH_4^+ in the sediments were determined by NaCl extractions using 50 mL of 0.1 M NaCl per 17.5 g of fresh sediment. Plant available P in the sediments was determined according to Olsen *et al.* (1954). Nitrate, NH_4^+ and PO_4^{3-} were measured by colorimetry (Auto Analyser, model III; Bran & Luebbe, Norderstedt, Germany) using hydrazine sulphate (Kamphake, Hannah & Cohen, 1967), salicylate (Grasshoff & Johannsen, 1972) and ammonium molybdate (Henriksen, 1965), respectively.

To establish the elemental chemical composition of the sediment, a homogenised (Ball mill MM301, Haan, Germany) portion of 200 mg dry sediment was digested in 4 mL HNO_3 (65%) and 1 mL H_2O_2 (30%), using an Ethos D microwave (Milestone, Sorisole Lombardy, Italy). Analyses of Al, Ca, Fe, K, Mg, Mn, Na, P, Si, S and Zn from digestates were carried out using an inductively coupled plasma emission spectrophotometer (IRIS-OES model Intrepid II XDL; Thermo Fisher Scientific, Waltham, MA, U.S.A).

Mineralisation rates were used to characterise the lability of the sediments. Mineralisation rates were determined under aerobic and anaerobic conditions over 4 weeks. Glass bottles (100 mL) were filled with 25 g of fresh sediment and 10 mL of deionised water and sealed with gas-tight rubber stoppers. Aerobic
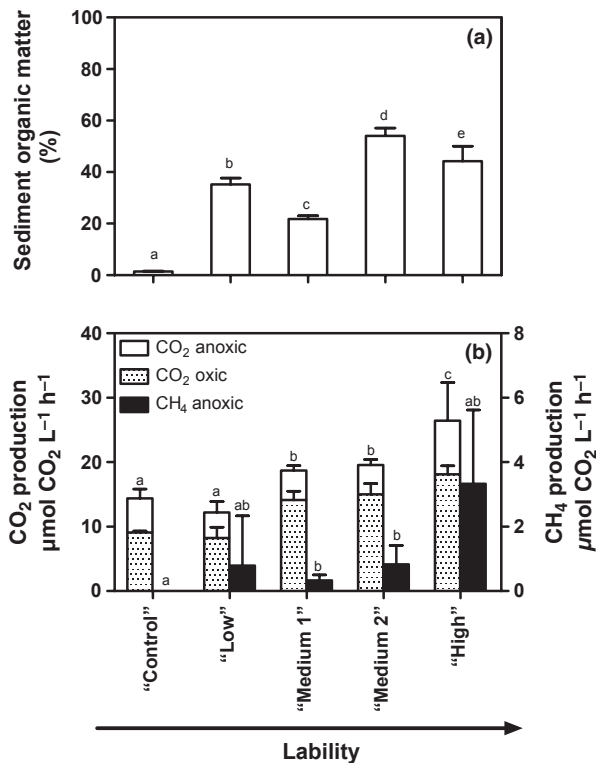
Fig. 1 Sediment organic matter (a) and lability (b) characterisation of the sand (''Control'') and four lake sediments (''Low'', ''Medium 1'', ''Medium 2'' and ''High'') used to create the mixtures. Sediment organic matter (%), and CO_2 and CH_4 production (μmol CO_2 L^{-1} h^{-1} fresh sediment h^{-1}) under oxic and anoxic conditions of the initial sediments are shown as means ± SD, n = 4. Means with the same letter do not differ significantly at 95% CI. ANOVA was used to test differences: $P \leq 0.001$ for sediment organic matter, $P \leq 0.001$ for total CO_2 (sum of oxic and anoxic production) and $P \leq 0.05$ for CH_4 production.

Redox potential was measured using a platinum electrode and a Metrohm Ag reference electrode (Metrohm, Herisau, Switzerland) connected to mV/pH meter (Radiometer Nederland BV, Zoetermeer, The Netherlands). The electrical potentials measured were converted to redox potentials relative to the standard hydrogen potential measured (E_h) by adding the reference (210 mV) and correcting for temperature and porewater pH.

Surface water and porewater analyses

Every 2 weeks, surface and porewater samples were collected. Porewater samples were collected with 5-cm-long sediment moisture samplers (Rhizon SMS-5 cm; Eijkelkamp Agrisearch Equipment, Wageningen, The Netherlands) that were installed vertically at a depth of 2–8 cm. Porewater was extracted anoxically by connecting the samplers to 30-mL vacuum serum bottles. The pH of the water samples was measured using a titration workstation (TitraLab 840; Radiometer analytical SAS, Villeurbanne, France) with a double Ag/AgCl reference pH electrode (Orion 9156BNWP; Thermo Scientific). Nitrate, NH_4^+, PO_4^{3-}, Al, Ca, Fe, Mg, Mn, P, Si, S, Zn, total inorganic C and pH of surface and porewater were analysed as mentioned earlier. Surface and porewater Cl^-, Na^+ and K^+ were determined by flame photometry (Auto Analyser, model III; Bran & Luebbe).

Plant analyses

Before the experiment, shoot and root dry mass of a subsample of plants (n = 3 for each plant species) was measured (24 h at 70 °C). After 54 days, the number of shoots per species was counted. ''Uprooting force'' (the force required to uproot individual *L. uniflora* plants) was measured using a tensiometer (Tensiometer, Aabenraa, Denmark). Plants were harvested and shoots, roots and stolons were separated. Dry mass of each species was determined for each aquarium, and shoot, root and stolon biomass were expressed as the final biomass (g DM m^{-2}). Various key elements (Al, Ca, Fe, K, Mg, Mn, Na, P, Si, S, Zn) in plant tissue were measured by drying and homogenising (Ball mill MM301) 50 mg plant material followed by digestion and chemical analyses, as mentioned earlier. Carbon and N contents of dried ground plant material

conditions were maintained during the 4 weeks, assuming that the consumption of 1 mol of O_2 is followed by the production of 1 mol of CO_2. The linear CO_2 production over the 4 weeks further supports this assumption. To create anaerobic conditions, the headspace of the bottles was flushed with N_2 for 10 min. The bottles were stored in darkness at 20 °C. Headspace gas samples were taken each week using 1-mL syringes and were analysed at once for CH_4 and CO_2 by infrared gas analysis (Advance Optima Infrared Gas Analyser, ABB, Cary, NC, U.S.A.).

Redox potential was measured during the day in the fresh sediment (before planting and just before harvesting) at 1.5, 3, 6 and 9 cm depth. Redox potentials are given as average of the four depths to provide a general picture of the sediment conditions.

were determined using a CNS analyser (type NA 1500; Carlo Erba Instruments, Milan, Italy).

Statistical analyses

Differences in plant, sediment or water parameters were assessed by univariate ANOVA or by a two-way analysis of variance (GLM procedure) depending on the numbers of fixed factors using SPSS 15.0.1 (SPSS Inc., Chicago, IL, U.S.A.). Differences between treatments were assessed with *post hoc* tests following ANOVA. When the requirements of homogeneity of variance were fulfilled (by Levene's test), the *LSD post hoc* test was used. However, when requirements of homogeneity of variance were not fulfilled, a Games–Howell *post hoc* test was applied. Significance was accepted at a probability of ≤0.05. Data were log-transformed where appropriate to improve normality and homogeneity. Graphs and regression lines were drawn with PRISM 5.01 (GraphPad Software, Inc., La Jolla, CA, U.S.A.).

Results

Sediment

Total organic matter (quantity), measured as loss on ignition, ranged from 1.4% in the "Control" sediment to 54.9% in "Medium 2" sediment (Fig. 1a). Lability of the sediment organic matter was determined by total C production (CO_2 and CH_4) of fresh sediment under oxic and anoxic conditions. Methane never contributed substantially to total C production and did not increase from "Low" to "High" lability. In contrast, CO_2 produced under anoxia, increased significantly with increasing lability of sediment organic matter and accounted for 31% of the total CO_2 production (Fig. 1b).

The highest concentration of plant available NO_3^- was found in the "Control" sediment (Table 1), which was also reflected in tissue N content. Nitrate in the "Control" sediment was 5–10 times higher than in other sediments, which all had similar NO_3^- concentrations. The highest concentration of plant available NH_4^+ was found in the "High" sediment, being from two to four times higher than in any other sediment. The "Control" sediment had highest plant available P (Olsen-P) and was 2–14 times higher than in the other sediments. Lowest C:N and N:P molar ratios were

found in the "Control" sediment. The concentration of most other measured elements, except Si, differed significantly among sediments (Supporting Information, Table S1).

Sediment E_h was measured after planting and just prior to harvesting (Table 1). Initially, E_h was significantly different in all sediments, "Control" sediment being the most oxidised. Prior to harvesting, E_h either remained the same ("Medium 1") or had increased during the experiment and, by the end, E_h was effectively the same in all pure sediments (Table 1).

Porewater

Initial CO_2, NH_4^+ and PO_4^{3-} concentrations in the porewater were significantly different among sediments, whereas initial porewater NO_3^- concentration did not differ (Table 1). Porewater CO_2 ranged from 229 μM in "Low" to 701 μM in "High". Shoot biomass of *E. repens* and *L. uniflora* showed a positive linear relationship with initial porewater CO_2 concentration (Fig. 3). Although the relationship between *E. repens* shoot biomass and initial CO_2 concentration had a higher correlation coefficient ($r^2 = 0.44$) than the relationship between *L. uniflora* shoot biomass ($r^2 = 0.30$), both species were analysed together because the slope did not differ between species. No such correlation was found for any other elements measured.

Plants

All plants survived and appeared healthy on all the sediments, regardless of organic content. Nevertheless, plant dry mass of *E. repens* and *L. uniflora* was significantly affected by both quantity and lability of sediment organic matter (Table 2). Quantity of sediment organic matter seemed to have stronger effect on *E. repens* plant mass than on *L. uniflora*. So, quantity of organic matter significantly affected shoot, root, stolon, shoot to root ratio and total biomass of *E. repens* while only stolon mass (and therefore also total plant mass) was affected for *L. uniflora* (Table 2).

Echinodorus repens. When sediment of the lowest total C production (≤15 μmol CO_2 L^{-1} h^{-1}; "Low") was used to generate a gradient of organic matter, quantity of organic matter significantly affected shoots and stolons of *E. repens* (Fig. 2, left-hand column). Root
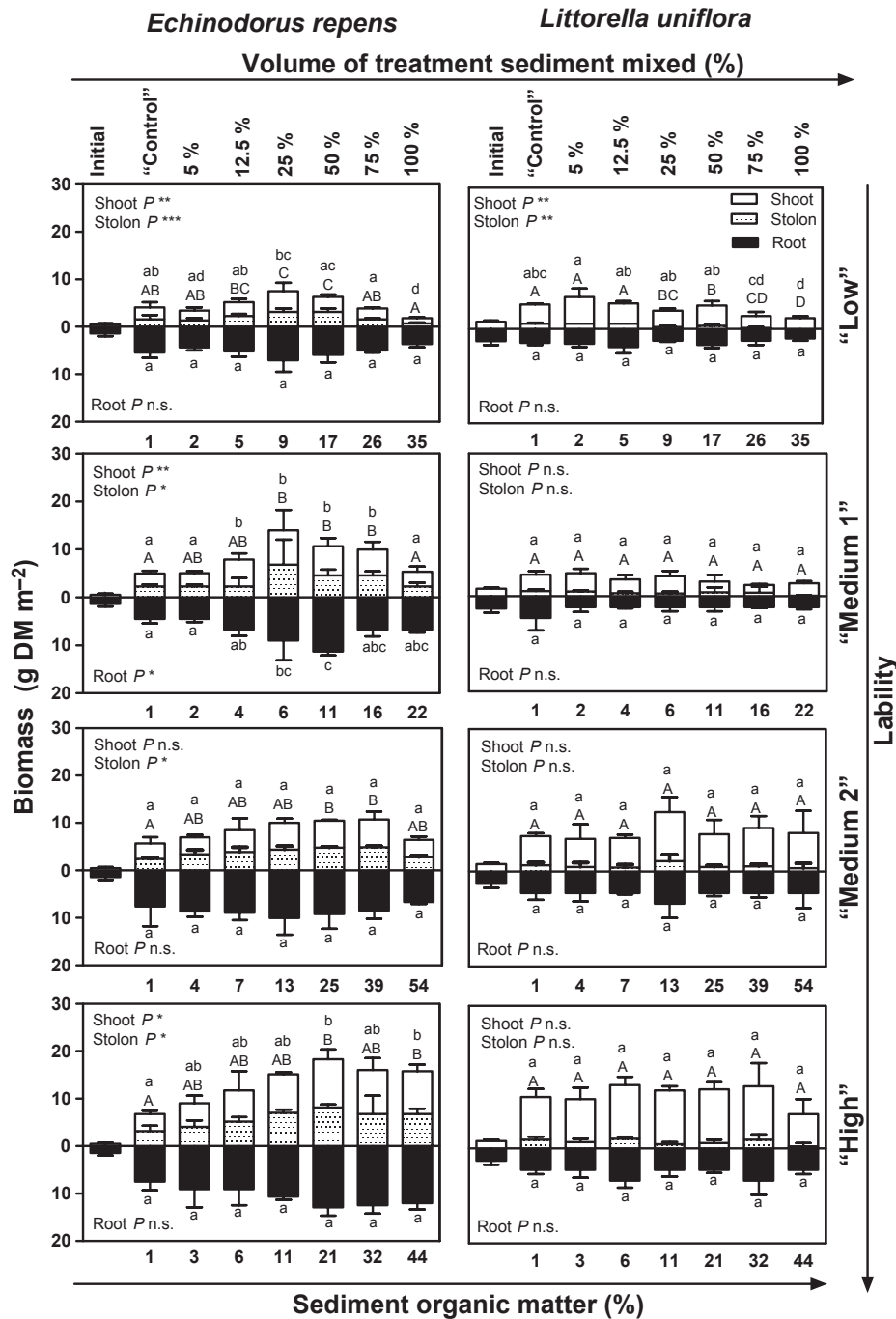
Fig. 2 Biomass of *Echinodorus repens* and *Littorella uniflora* over four gradients of sediment organic matter. *Echinodorus repens* and *L. uniflora* shoot, root and stolon biomass (g DM m^{-2}) at the beginning of the experiment (initial) and at the time of harvesting (54 days after) over four gradients of organic matter created by mixing sand ("Control") with 5, 12.5, 25, 50, 75 and 100% volume of fresh lake sediments ("Low", "Medium 1", "Medium 2" and "High"). The *x*-axes show the % (w/w) of organic matter in the sediment as a result of the seven different mixtures of sand and lake sediment indicated in the upper part of the figure. Gradients are ordered by lability (increasing from top to bottom) of the lake sediment used to created the gradient, as characterised by the production of CO_2 (see text). Data are means ± SD. Means with the same letter do not differ significantly at 95% CI (lower case for shoot and root, upper case for stolon). ANOVA was used to test differences among sediment mixtures: *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ns ≥ 0.05. On average, *E. repens* had an initial plant mass of 0.008 g DM increasing to 0.056 g DM at the time of harvesting. For *L. uniflora*, plant mass increased from 0.018 to 0.043 g DM.
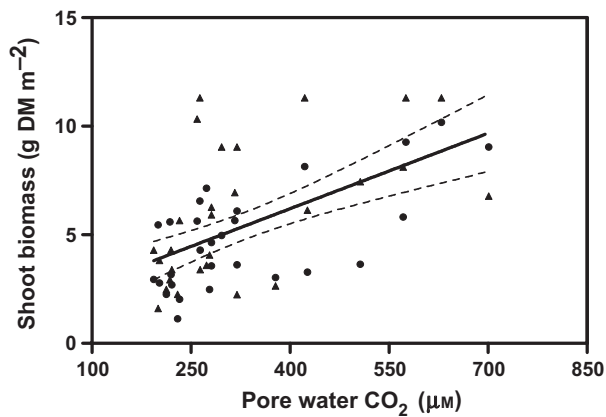
Fig. 3 Shoot biomass versus porewater CO_2. Means of shoot biomass (g DM m^{-2}; $n = 3$) of *Echinodorus repens* (•) and *Littorella uniflora* (▲) versus the means of initial porewater CO_2 (μM; $n = 3$) in sediment mixtures ($n = 28$). Linear regression line (━) and the 95% confidence intervals (- - -); $y = 0.01158 + 1.57$; $r^2 = 0.33$; *P* value < 0.0001.

Table 2 Organic matter effects on plant mass

	Quantity	Lability	Interaction	r^2
Echinodorus repens				
Shoot	***	***	*	0.69
Root	***	***	ns	0.59
Stolon	***	***	*	0.73
Shoot to root	***	***	*	0.51
Total biomass	***	***	ns	0.71
Littorella uniflora				
Shoot	ns	***	*	0.72
Root	ns	***	ns	0.40
Stolon	*	**	ns	0.25
Shoot to root	ns	***	ns	0.44
Total biomass	*	***	ns	0.66

Effects of quantity and lability of sediment organic matter, and their interaction, on shoot, root, stolon, shoot to root ratio and total dry mass of *E. repens* and *L. uniflora*. GLM was used to test differences: *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ns: ≥ 0.05. r^2 adjusted.

biomass was not significantly affected but followed the trend for shoots and stolons. Biomass increased up to 9% of organic matter and declined at higher concentrations. When ''Medium 1'' sediment was used to generate a gradient of organic matter, quantity of organic matter significantly affected shoot, stolon and root biomasses. Here, shoot and stolon biomasses increased up to 6% of organic matter, whereas root biomass continued to increase up to 11%. Above these values, biomass declined to values similar to those of

the ''Control'' treatment (i.e. pure sand). When ''Medium 2'' sediment was used, only stolon mass was affected; stolon biomass increased up to 39% of organic matter (Fig. 2). Interestingly, when ''High'' sediment was used to generate a gradient of organic matter, plant mass was affected in a similar pattern as when ''Low'' sediment was used, except that plant mass did not decline after reaching a maximum at 21% organic matter. In summary, increase in organic matter stimulated *E. repens* biomass up to moderate values of organic content, above which *E. repens* biomass declined or remained unaffected (depending on lake source) by higher concentrations of organic matter.

To evaluate the effect of the lability of sediment organic matter on *E. repens*, total biomass from pure (i.e. no added sand) ''Low'', ''Medium 1'' and ''Medium 2'', and ''High'' sediment was compared (indicated by 100% in the upper part of Fig. 2). *Echinodorus repens* biomass in ''Low'' sediment was lowest (6.03 ± 1.31 g DM m^{-2}) and significantly differed from the three other sediments. Total biomass in ''Medium 1'' (11.3 ± 2.26 g DM m^{-2}) did not differ from that of ''Medium 2'' (12.81 ± 1.31 g DM m^{-2}), while biomass in ''High'' (27.14 ± 2.26 g DM m^{-2}) was significantly greater than in the two medium sediments. Therefore, the effect of increasing lability was always positive on the total biomass of *E. repens*. Shoot to root ratio (excluding stolon from shoot) in ''Low'' was 0.34 ± 0.01 and systematically increased from 0.40 ± 0.12 to 0.55 ± 0.10 and 0.76 ± 0.10 in ''Medium 1'', ''Medium 2'' and ''High'', respectively.

The possible nutrient limitation on *E. repens* biomass was evaluated by comparing shoot N and P in ''Control'' plants with those that had grown in pure ''Medium 1'', ''Medium 2'' and ''High'' sediments (Table 1). Nitrogen content in ''Control'' shoots was significantly lower than in ''Medium 1'' but did not differ from the three other sediments. P content in ''Control'' sediment was significantly lower than in ''Low'' and ''Medium 2'', but higher than in ''High''.

Littorella uniflora. The impact of quantity and lability of sediment organic matter was much less pronounced for *L. uniflora* than for *E. repens*. Shoot and stolon biomass was only significantly affected over the gradient based on the ''Low'' sediment (Fig. 2, right-hand column). Shoot biomass was unaffected up to 17% organic matter and declined at higher values;

stolon biomass started declining at 5% organic matter. The quantity of organic matter did not affect *L. uniflora* biomass across the gradients based on ''Medium 1'', ''Medium 2'' and ''High'' sediments.

Influence of the lability of the sediment organic matter was again evaluated by comparing total biomass in pure sediments. Total biomass did not differ significantly among sediments, although the trend was the same as for *E. repens*, i.e. biomass increased with increasing lability (4.5, 4.8, 12.5 and 12.2 g DM m^{-2} in ''Low'', ''Medium 1'', ''Medium 2'' and ''High'', respectively). In contrast to *E. repens*, the shoot to root ratio was always above 1 (''Low'' = 1.08, ''Medium 1'' = 1.3, ''Medium 2'' = 2.01 and ''High'' = 1.88).

The potential contribution of nutrient limitation to the observed pattern was evaluated using the approach described for *E. repens*. Shoot N content in ''Control'' was significantly higher than in ''Low'', ''Medium 2'' and ''High''. Phosphorus content in ''Control'' significantly differed from the four pure sediments so that shoot P in the ''Control'' was lower than in ''Medium 2'' and higher than in ''Low'', ''Medium 1'' and ''High'' (Table 1).

We hypothesised that increasing organic matter could lead to adverse effects on the anchoring of plants. The uprooting force for individual *L. uniflora* plants over the gradient of sediment organic matter fitted a Gaussian model (Fig. 4). The Gaussian model provided a better explanation than a linear model (r^2 = 0.24 for the linear model versus 0.35 for the Gaussian model). The model indicates that uprooting force increased from low to moderate sediment organic content, and after reaching a local maximum at around 15% organic matter, the force then decreased significantly to values below those obtained for ''Control'' (i.e. pure sand) sediments (Fig. 4).

Discussion

We aimed to investigate how the quantity and lability of sediment organic matter affected the biomass of the two isoetids, *E. repens* and *L. uniflora*. In contrast to our expectations, none of the plants died during the experiment. All plants survived and showed no signs of stress, regardless of the quantity or lability of the sediment organic matter to which they were exposed. The plants did not show any visual damage of either roots or shoots caused by anoxia, as previously reported for both *L. dortmanna* and *L. uniflora* (Raun
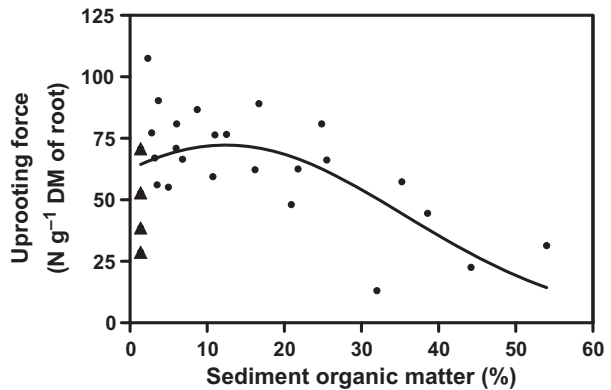
Fig. 4 Uprooting force of *Littorella uniflora* versus sediment organic matter. Means of uprooting force (N g^{-1} DM of root, n = 3) of *L. uniflora* versus means of sediment organic matter (%) in sediment mixtures (▲ for ''Control''; • for the rest of treatments; n = 28). Data were fitted to a Gaussian model (y = 72.3 exp (−05 ((x − 12.46) 23.1^{-1})^2); r^2 = 0.35) providing better explanation than a linear relationship (r^2 = 0.24). Uprooted plants in average had 0.026 g DM shoot and 0.017 g DM root and three to eight leaves ranging from 4 to 12 cm.

et al., 2010). However, although the nature of the organic matter used in Raun *et al.* (2010) was not clearly defined, the estimated CO_2 production in a sediment with only 1.7% organic matter was 22-fold higher than in any of the sediments used in our study. In the present investigation, plants were able to maintain, or even increase, the redox potential of the sediment (Table 1), probably by ROL from their roots. In contrast to previous studies in which plants were adversely affected, or even died, at low sediment organic matter (Barko & Smart, 1983; Sand-Jensen *et al.*, 2005; Raun *et al.*, 2010), all plants in our study grew and increased in number by asexual reproduction, even when grown in sediments with a high organic content (up to 54.9%) or in labile sediments (26 μmol CO_2 L^{-1} h^{-1}; Fig. 1). In oxic environments, decomposition of sediment organic matter causes O_2 consumption followed by CO_2 production (Smolders *et al.*, 2006). The balance between the negative effects of O_2 consumption (Godshalk & Wetzel, 1978a,b,c; Barko & Smart, 1983) and the positive effects of CO_2 production (Sand-Jensen & Søndergaard, 1979; Lenssen *et al.*, 1999) in a carbon-limited system determines net impact on the vegetation. In the present study, the positive effects of CO_2 production clearly prevailed over the negative effects of O_2 consumption, because the plants were able to oxidise the sediment.

The observed increase in biomass could be caused by N, P or inorganic C produced during

mineralisation or a combination of the three (Sand-Jensen & Søndergaard, 1979; Lenssen *et al.*, 1999). In the present experiment, it is unlikely that N or P caused the increase in biomass because (i) plenty of N and P available to plants was present in the ''Control'' sediment (Table 1) and (ii) the plant tissue had a relatively high N and P content. In fact, the ''Control'' sediment had the highest concentration of plant available N and P (Table 1), and therefore, we think it unlikely that the observed increase in biomass would be caused by increased nutrient availability. In addition, neither tissue N nor P indicated that plants grown at low sediment organic matter suffered from nutrient limitation (Table 1). There are no values in the literature for *E. repens* but those for *L. uniflora* do not indicate N or P limitation (Boston, 1986; Christiansen, Friis & Søndergaard, 1985; Andersen, Pedersen & Andersen, 2005; Spierenburg *et al.*, 2010). In fact, nutrient limitation is not easily evaluated by means of tissue nutrient concentration, as nutrient concentrations are diluted in fast-growing tissues (Christiansen *et al.*, 1985). For *E. repens*, however, there was a significant reduction in biomass at high sediment organic matter and we propose that this could possibly be caused by P limitation, as pure ''Low'' sediment showed the lowest plant available P (Olsen-P) of the sediments studied.

In contrast, it is more likely that CO_2 caused the observed increase in biomass with increasing organic matter as the plants were probably C limited. Initial porewater CO_2 ranged from 0.2 to 0.7 mM (Table 1), and hence, the initial concentrations of porewater CO_2 were much lower than the critical concentration to saturate photosynthesis in isoetids, ranging from 2 to 8 mM (Søndergaard & Sand-Jensen, 1979; Roelofs *et al.*, 1984; Pedersen *et al.*, 1995). Moreover, shoot biomass of both *E. repens* and *L. uniflora* was positively correlated with CO_2 availability in porewater (Fig. 3), as shown in previous studies for isoetids from C-limited environments (Pedersen *et al.*, 1995; Smolders *et al.*, 2002; Bagger & Madsen, 2004). Improved C availability tends to increase shoot to root ratio in isoetids (Søndergaard & Sand-Jensen, 1979; Roelofs *et al.*, 1994; Smolders *et al.*, 2002), and in the present study, shoot to root ratios above 1.5 were observed for *L. uniflora* growing in the two most labile sediments. The plants benefit from the high proportion of photosynthetic tissue in relation to the belowground tissue in reduced sediments, as it promotes oxidation

of the sediment (Sand-Jensen *et al.*, 1982). However, tissues of *L. uniflora* are highly porous (promoting buoyancy), and a high relative proportion of root biomass seems necessary to anchor them securely in the sediment (Raven *et al.*, 1988).

Uprooting of plants happens when pulling forces overcome the strength of anchorage (Schutten, Dainty & Davy, 2005). Anchorage may be divided into forces related to root-related parameters (biomass, total root length and 3D architecture) and those related to sediment cohesive forces. In natural lakes and ponds, sediment cohesion has been shown to vary 70-fold (Schutten *et al.*, 2005), probably as a function of water content and, thus, organic matter. However, sediment cohesion forces are a function of packing density of sediment particles, and thus, pure sandy sediments would have lower cohesive forces than sediment with just a small proportion of tiny organic particles (Abdullah & Geldart, 1999). This is also partly reflected in Fig. 4, where the Gaussian model predicts maximum anchoring around 15% organic matter. In the present investigation, the force required to uproot *L. uniflora* ranged from 0.4 to 2.1 N across the range of sediments tested. Hydraulic pulling forces of 0.6 m waves may range between 0.47 to 1.69 N for elodeids, such as species of *Myriophyllum* and *Potamogeton* (Schutten *et al.*, 2005), but the hydraulic pull on a tiny *L. uniflora* would be much lower. Unfortunately, it is not possible to compare the measured uprooting forces in the present study with those from nature, as there are no data available. However, observations of floating mats of isoetids indicate that uprooting forces may, from time to time, exceed the anchorage strength (Lucassen & Roelofs, personal observations). Uprooting of isoetids may happen during storms with extreme wave action and perhaps also during periods of extensive photosynthesis, where buoyancy of the porous tissues reduces the forces necessary to uproot the plants. Although the trend in Fig. 4 is apparent, there is huge variation in uprooting force for the same content of sediment organic matter. The variation is probably real and may reflect the fact that uprooting forces are more related to 3D root architecture, which was not measured in the current study, than to biomass alone.

Our findings may inform future attempts at lake restoration, as the selection of suitable lakes requires analyses of water and sediment characteristics. The present study shows that the quantity of organic

matter is insufficient to predict the performance of isoetid populations. It also shows that, to predict isoetid population performance, it is essential to consider the lability and the physical characteristics of the sediment, as well as organic matter content. Similarly, it is crucial to consider the present isoetid populations. In degraded lakes with a low population density of plants, the negative effects of sediment organic matter presumably prevail over the positive. In bare sediments, organic matter causes highly reduced conditions (Wium-Andersen & Andersen, 1972b; Tessenow & Baynes, 1978; Wigand, Stevenson & Cornwell, 1997) that can reduce germination success and, therefore, hamper the recovery of the isoetid vegetation (Greulich *et al.*, 2000). Under such conditions, when the sediment is not suitable for the re-establishment of isoetids, removal of the organic top layer of sediment can lead to successful restoration of isoetid lakes (Brouwer & Roelofs, 2001).

In conclusion, if plants are able to oxidise the sediment, sediment organic matter can stimulate isoetid biomass, because of increased CO_2 availability. Thus, to understand better the role of sediment organic matter on isoetids, it is essential to consider (i) the lability of the sediment organic matter (because it will provide information on inorganic C production and oxygen consumption) and (ii) the physical properties of the sediment, because uprooting events are more likely on highly organic sediments with low cohesive forces.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Sediment element content of the sand (''Control'') and treatment lake sediments (''Low'', ''Medium 1'', ''Medium 2'' and ''High'') used for the mixtures

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