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SPECIAL FEATURE: VEGETATION RESTORATION

Liming induces changes in the macrophyte vegetation of Norwegian softwater lakes by mitigating carbon limitation: results from a field experiment

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Keywords
Alkalinity generation; Carbon dioxide; Eco-engineer; Iron reduction; Isoetids; Norway; Restoration

Abbreviations
DIC = dissolved inorganic carbon.

Nomenclature
Heukels 2005 (vascular plants).

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Abstract

Question: Does liming mitigate carbon limitation to the original submerged vegetation of Norwegian softwater lakes?

Location: Dybingsvatn (Dalane region, Southern Norway).

Methods: Eight plots (1 m^2) were permanently marked in the deeper part (1.5 m) of the littoral zone of Dybingsvatn in 1994. Half of the plots were limed four times with dolocal (1 kg m^{-2}) in the period 1994–1998. Pore water, surface water chemistry and the occurrence of new plant species were monitored until 2008. In 2008, the composition and cover of the vegetation were estimated and the dominant isoetid plant species were harvested for determination of additional plant characteristics.

Results: Liming lowered the redox potential of the sediment and led to higher concentrations of dissolved inorganic carbon, Ca^{2+}, Mg^{2+}, Fe^{2+} and Mn^{2+} in the sediment pore water. Submerged macrophytes that could in some way profit from the higher carbon dioxide concentrations developing in the pore water (e.g. *Juncus bulbosus*, *Sparganium angustifolium* and *Callitriche hamulata*) expanded or colonized the limed plots at different time-points. Four years of liming did not affect the cover and composition of the original isoetid vegetation, but led to development of taller and heavier plants with higher molar carbon to nitrogen ratios in the shoots, indicating a higher availability and uptake of carbon dioxide from the sediment.

Conclusions: Liming leads to a higher availability of carbon dioxide in the sediment, partly owing to dissolution of carbonates, and partly by inducing anaerobic decomposition of organic matter and the consequent reduction of iron (IIII) hydroxides in the iron-rich sediment (in-lake alkalinity generation). All observed changes in the vegetation indicate that liming leads to mitigation of carbon dioxide limitation in Norwegian softwater lakes. Liming may be beneficial for fish populations by reducing concentrations of labile aluminium, but it does not preserve the characteristic chemical sediment properties and original vegetation of softwater lakes. We wish to point out that liming may, in the worst case, lead to an alternative stable lake state with anaerobic sediments as relatively highly productive isoetid plant species have a risk of uprooting.

Introduction

Acidification of softwater lakes is one of the most serious environmental problems in Scandinavia in the last decades of the previous century. Acidification is acknowledged as the main cause for the decline of Atlantic salmon (*Salmo salar* L.) populations in Norwegian rivers since the 1960s and 1970s (Hesthagen & Hansen 1991; Sandøy & Langåker 2001). Liming of surface waters is Norway's main strategy to counteract acidification at least until the sulphur emissions have dropped below the critical value (Hindar et al. 1998; Larssen et al. 2010). Since

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the end of the 1980s hundred thousands of tons of fine-grained limestone ($CaCO_3$) are spread yearly in lakes and streams to increase pH values and reduce concentrations of toxic metals such as highly cationic (labile) aluminium. Positive effects of liming on the re-establishment of Atlantic salmon in Norwegian rivers have been documented since that time (Sandøy & Romundstad 1995; Hesthagen & Larsen 2003).

The lakes in SW Norway are fed by rainwater and shallow local groundwater, which interacts with acidic carbonate-poor bedrocks (such as gneiss and granite) that are covered with very thin soil layers. Therefore, the lakes are originally oligotrophic with very low concentrations of dissolved inorganic carbon (DIC). Consequently, HCO_3^- concentrations are generally very low and do not contribute to the carbon (C) demand of macrophytes capable of HCO_3^- uptake (Madsen & Sand-Jensen 1994, Madsen et al. 1996). Consequently, macrophytes have to rely on the uptake of CO_2 to fulfil their C requirements. In fact, the CO_2 levels in softwater lakes are more or less in equilibrium with the atmosphere (18–20 μmol l^{-1}) or only slightly oversaturated (Dillon & Molot 1997; Riera et al. 1999; Jonsson et al. 2003). As diffusion rates of gases in water are much lower compared with air, this results in a very strong C limitation of submerged macrophyte production (Roelofs 1983; Murphy 2002). The original submerged vegetation of softwater lakes in SW Norway consists of macrophytes with various physiological and morphological adaptations to a low C availability, comprising species from a group of isoetids, such as *Isoëtes* spp., *Lobelia dortmanna*, *Littorella uniflora* and *Subularia aquatica* (Roelofs et al. 1994). These plants can take up CO_2 from the sediment pore water with their roots, where concentrations are 10 –100 times higher than in the surface water (Smolders et al. 2002).

The original motive for liming was concern for the fish population for recreational fishing. Nowadays, preservation and recovery of biodiversity, including the submerged vegetation, are also taken into account by the Norwegian Climate and Pollution Agency. In contrast to western European countries, the macrophyte community in softwater lakes of Norway had remained unaffected by acidification (Roelofs 1983; Roelofs et al. 1984; Roelofs et al. 1995). In large parts of western Europe, 60–80% of total acidifying precipitation was deposited as ammonium sulphate in the last decades of the twentieth century. Nitrification of ammonium in the water layer led to acidification of the water layer. Once the water layer is acidified to pH 4.5, nitrification is inhibited and nitrogen (N) accumulates as ammonium. Acidification of (slightly) calcareous sediments also leads to increased carbon dioxide concentrations in both the sediment and water layer. Hence, because of the increased atmospheric deposition of ammonium, C as well as N availability are highly increased in surface water on slightly calcareous sediments. *Juncus bulbosus* will benefit from this situation. The species is generally present in isoetid vegetation but its growth is highly limited in the (extremely) oligotrophic environment. When N and C levels increase, *J. bulbosus* can show spectacular growth and can completely overgrow the isoetid vegetation in a very short time (Roelofs 1983; Roelofs et al. 1984; Arts et al. 1990).

The different response to acidification of the Norwegian lakes could be attributed to the relatively low ammonium content of the acid precipitation, in combination with the more calcareous-poor character of the lake sediments in Norway compared with western European countries. As a result, there is no accumulation of ammonium and the fluxes of carbon dioxide from the sediment to the overlying (slightly) acid water layer, remained relatively low in Norway (Roelofs et al. 1994; Lucassen et al. 1999). Liming, however, appeared to induce strong changes in the aquatic macrophyte structure of Norwegian softwater lakes (Roelofs et al. 1994; Roelofs et al. 1995; Brandrud 2002). Liming appeared to lead to strongly increased CO_2 fluxes from the sediment while the increased alkalinity in the sediments resulted in strongly increased ammonium and phosphate levels owing to increased decomposition of accumulated organic material (Roelofs et al. 1994; Roelofs et al. 1995; Lucassen et al. 1999).

Limestone is only poorly soluble and therefore a large part of the added lime is expected to accumulate in the sediments of lakes, especially after many years of repeated liming. Grøterud & Haaland (2010) showed that the hypolimnion of four dimictic limed Norwegian lakes existed for 90% of ions originating from lime products (e.g. Ca^{2+} and alkalinity) after 10 yr of liming. More alkaline conditions resulted in enhanced microbial decomposition of organic matter and concomitant oxygen depletion of the hypolimnion. This indicates that liming can change the inorganic C availability for plants, especially in case the littoral zone is also limed.

The aim of this study was to investigate whether liming can induce changes in the vegetation composition and characteristics of Norwegian softwater lakes. In this study we wanted to investigate the long-term effects on vegetation via changes in sediment characteristics as a result of liming. Therefore, we limed relatively small plots in a large unlimed lake thereby preventing changes in overall surface water quality. We hypothesize that dissolution of limestone and enhanced decomposition processes following liming will enhance C availability in the sediment and might result in changes in macrophyte community and/or structure in the originally carbon limited lakes. A small part of this research has been described in a paper dealing specifically with the occurrence of *Sparganium angustifolium*

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in Norwegian softwater lakes as studied by a combination of (experimental) field investigations and eco-physiological experiments (Lucassen et al. 2009).

Materials and methods

Location and experimental design

Lake Dybingen is a softwater lake situated in the Dalane region (SW Norway) near Hauge i Dalane (58º28′°N, 6º18′° E). It consists of granite rocks with some shallow sandy shores. In this region, many lakes became acidified in the period 1960–1990 as a result of a relatively high atmospheric deposition of sulphuric acid. Lake Dybingen has never been limed and, as a consequence, its littoral zone is still dominated by a submerged vegetation of isoetids (Roelofs et al. 1994). In Jul 1994, eight plots of 1 m^2 were marked in the littoral zone of a bay located at the most northern part of the lake. The bay receives run-off from the surrounding area via a small inlet and was selected because of its accessibility. The water table hardly fluctuates (maximum fluctuation ±0.25 m) as the lake is continuously fed with water from the surrounding catchment and has an overflow to lakes situated at a lower altitude. As a consequence, the water depth at the experimental site remains relative stable around 1.5 m. The vegetation in the bay was very homogeneous and dominated by the isoetids *Isoetes lacustris* L. (±50% cover), *Littorella uniflora* (L.) Ascherson (±10% cover) and *Lobelia dortmanna* L. (±10% cover). *Juncus bulbosus*, *S. aquatica*, *I. echinospora* Durieu, *Sphagnum palustris* and *Utricularia* spec. were also present but did not reach a significant cover (in total ±5% cover).

Biotic and abiotic measurements

Four of the plots were limed with dolocal (1 kg m^{-2}) in Jul 1994, Aug 1995, Sep 1997 and Jun 1998 (composition of the dolocal: MgO–$CaCO_3$–$MgCO_3$, 1:17:2). The four limed plots were linked and covered within a transect of 5m × 2 m that was completely limed in order to prevent border effects within the plots. The control plots were situated at approximately 1 m distance of both sides of this transect. The amount of lime added corresponded with the yearly lime applications in Norway, in other lakes. Lime was carefully added just above the sediment surface by a scuba-diver. The other four plots remained untreated (control). In the summers of 1995, 1997, 1999, 2004, 2006, 2007 and 2008, sediment pore water of all plots was collected in the centre of the plot. In addition, one surface water sample was collected at a depth of 0–0.5 m in a 500 ml glass bottle representing surface water quality of all plots. Sediment pore water was collected anaerobically using ceramic cups (Eijkelkamp Agrisearch, Giesbeek, The Netherlands) that were installed in the upper 10 cm of the sediment. Sediment pore water was sucked out of the sediment by connecting the cups to vacuum syringes (60 ml). In 2004, the upper sediment layer (0–10 cm) was sampled from the centre of each plot with a PVC tube and redox potential was measured in each plot at a depth of 5–10 cm. In summer 2008 the vegetation composition (in% cover) of all plots was estimated by the same person as at the start of the experiment. Plants were harvested non-destructively to enable further future monitoring of the plots. From the dominant plant species present, 10 representative individuals (if present) were carefully collected by a scuba diver. The length (cm) and dry weight (g) of the roots and shoots were determined. The sediment and plant samples were dried for 24 h in an oven at 100 and 75 °C respectively.

Chemical analyses

The pH of the (pore) water samples was measured with a Cyberscan pH 300 series pH meter (Eutech, Singapore) with a double Ag/AgCl reference pH electrode (Orion 9156BNWP; Thermo Scientific, Beverly, USA). The DIC analyses of the (pore) water samples were carried out using an Infrared Carbon Analyser (Model IRGA ABB; Advance Optima, Zürich, Switzerland). Carbon dioxide levels were calculated from the DIC concentrations and pH according to Stumm & Morgan (1996). The redox potential (Eh in mV) was measured at 5–10 cm depth in the sediment with 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. NH_4^+ and PO_4^{3-} in the surface and sediment pore water samples were analysed with Auto Analyser III systems (Bran & Luebbe, Norderstedt, Germany) using a salicylate method for ammonium (Grasshoff & Johannsen 1977) and ammonium molybdate for phosphate (Henriksen 1965).

Total C and N concentrations in the dried sediment and plant material were determined with a CN elemental analyser (type NA1500; Carlo Erba/ThermoFisher Scientific, Milan, Italy). For the sediment samples, additional analyses were performed. Two-hundred milligrams of dried and ground material was digested for 17 min with 4 ml concentrated nitric acid and 1 ml 30% hydrogen peroxide in a Milestone type mls 1200 Mega microwave (Sorisole, Italy). The total concentrations of elements (Ca, Mg, Mn, Fe, P, S, K, Al, Si and Zn) in the sediment, surface and sediment pore water samples were analysed using an ICP-OES (type Spectroflame, Spectro Analytical Instruments, Kleve, Germany). Quality assurance measures included blanks, replicate analyses and matrix spikes. Recoveries from matrix spikes ranged from 95% to 107%. Repeated analyses did not reveal differences greater than 5%.

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Statistical analyses

Effects of liming, isoetid plant species type and their interaction were assessed by a two-way analysis of variance [generalized linear model (GLM) procedure] using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The effect of liming on pore water chemistry was tested by a one-way analysis of variance (GLM repeated measures). Effects of liming on sediment chemistry, the final cover of plant species and the characteristics of the dominant isoetid plant species was tested with a Mann–Whitney test.

Results

Abiotic parameters

The sediment of the limed plots developed a significant lower redox potential, and significantly higher concentrations of elements originating from lime products such as total Ca, total Mg and total C. There were no significant differences in the organic matter content of the sediment or concentrations of other elements (Table 1).

The sediment pore water in the limed plots showed significantly higher concentrations of CO_2, HCO_3^-, Ca, Mg, Fe^{2+} and Mn^{2+} throughout the experimental period compared with the reference plot (Table 2) The concentrations of HCO_3^- and Fe^{2+} increased strongly after the last liming event in 1998. Since 2006, the concentrations have gradually declined. The overall NH_4^+ concentration in the pore water between the limed and reference plots did not significantly differ but the concentration was significantly higher in the limed plots in 2004 and 2006 (up to 125 μM) compared with the reference plots (5 μM). For details on the chemistry of the water layer and pore water in time see Lucassen et al. (2009).

Table 1. Sediment composition of the limed ($n = 4$) and unlimed ($n = 4$) plots sampled in 2004. Mean values (± standard error) are given. Concentrations are given in μmol g^{-1} dry weight with the exception of the redox potential (mV) and organic matter content (%). Plots were limed four times with 1 kg dolocal m^{-2} in the period 1994–1998.

	Limed	Unlimed	P
Redox potential	175.4 (26.5)	333.2 (20.7)	***
Total calcium (Ca)	300.0 (47.9)	194.8 (20.1)	*
Total magnesium (Mg)	126.0 (31.9)	69.5 (8.2)	*
Total carbon (C)	3077.5 (437.1)	1907.6 (412.6)	*
Total iron (Fe)	269.8 (19.1)	269.4 (25.0)	n.s.
Total silicon (Si)	23.6 (1.4)	19.3 (2.3)	n.s.
Total zinc (Zn)	2.2 (0.9)	1.2 (0.1)	n.s.
Total potassium (K)	10.6 (0.7)	12.4 (1.3)	n.s.
Total phosphorus (P)	108.9 (7.5)	125.6 (13.3)	n.s.
Total manganese (Mn)	4.3 (0.5)	3.9 (0.8)	n.s.
Total aluminium (Al)	223 (20.8)	211.6 (31.2)	n.s.
Total sulphur (S)	12.9 (1.9)	12.3 (3.5)	n.s.
Total nitrogen (N)	133.8 (22.9)	126.2 (43.4)	n.s.
Organic matter	6.2 (1.1)	6.1 (1.9)	n.s.

***$P < 0.001$; *$0.01 < P < 0.05$.
n.s. = not significant (Mann–Whitney test).

Table 2. Mean chemical composition of the surface water in lake Dybingen and the pore water of the limed and control plots during (1994–2008). Mean values ± SE of the seven time-points are given (μmol l^{-1}).

	Surface water	Pore water control	Pore water, limed	P
Ca	18.6 (1.6)	89.7 (11.6)	342.0 (74.0)	***
CO_2	31.7 (8.9)	274.5 (37.7)	1063.1 (141.3)	***
HCO_3^-	0.9 (0.4)	135.7 (22.4)	762.0 (192.9)	**
Fe	1.2 (0.1)	7.1 (3.0)	143.5 (53.7)	**
Mg	22.6 (1.6)	57.3 (3.3)	157.2 (26.5)	**
Mn	0.3 (0.04)	1.4 (0.5)	23.2 (5.9)	*
Si	25.0 (1.7)	128.7 (14.3)	162.0 (12.1)	*
pH	4.97 (0.16)	6.0 (0.2)	6.1 (0.1)	n.s.
PO_4^{3-}	0.07 (0.02)	0.9 (0.4)	0.4 (0.2)	n.s.
NH_4^+	4.1 (2.1)	5.3 (1.9)	25.4 (17.4)	n.s.
NO_3^-	7.8 (1.2)	11.3 (3.3)	13.4 (5.3)	n.s.
Na^+	149.2 (15.1)	233.5 (33.6)	214.4 (29.0)	n.s.
K^+	7.4 (0.9)	50.0 (15.4)	22.7 (5.4)	n.s.
Cl^-	175.2 (30.4)	250.7 (45.3)	202.3 (34.7)	n.s.
Zn	1.2 (0.7)	2.6 (1.2)	2.2 (0.4)	n.s.
P	0.2 (0.04)	3.1 (1.3)	2.4 (0.7)	n.s.
S	28.3 (4.0)	61.1 (11.1)	69.3 (18.5)	n.s.
Al	11.9 (7.4)	9.0 (3.9)	5.7 (1.8)	n.s.

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.
n.s. = not significant (GLM, repeated measures analysis). For details in time see Lucassen et al. (2009).

Table 3. Statistical analysis of liming, the type of isoetid plant species and their interaction effects on plant morphology characteristics and carbon (C):nitrogen (N) and N:phosphorus (P) ratio of the shoots (generalized linear model, two-way analysis of variance).

	Liming	Plant species	Liming × plant species
Shoot length (cm)	< 0.001	< 0.001	0.014
Root length (cm)	< 0.001	0.178	0.243
Root:shoot	0.888	< 0.001	0.686
Shoot biomass (g)	< 0.001	< 0.001	0.001
Root biomass (g)	< 0.001	< 0.001	0.001
Root:shoot	0.310	0.644	0.762
Shoot C:N	< 0.001	< 0.001	0.002
Shoot N:P	0.018	0.008	0.239

Biotic parameters

The results show that liming led to a significant increase in cover of *J. bulbosus* in the plots from 2% to 10%. In addition, liming enabled settlement of new plant species (e.g. *S. angustifolium* and *C. hamulata*) (Fig. 1). The increase in cover of *J. bulbosus* occurred within 1 yr after the first lime application in 1994. In contrast, the first specimens of

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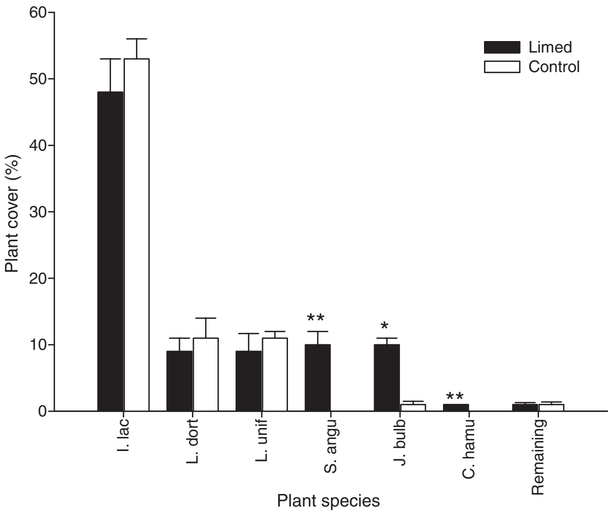

Fig. 1. Final cover (%) of the plant species present on the limed (n = 4) and control (n = 4) plots in summer 2008. Plots were limed four times with dolocal (1 kg m^{-2}) in the period 1994–1998. Mean ± SE (in vertical bars) are given (n = 4). **P < 0.001; *P < 0.01 (Mann–Whitney test). *I. lac* = *Isoetes lacustris*, *L. dort* = *Lobelia dortmanna*; *L. unif* = *Littorella uniflora; S. angu* = *Sparganium angustifolium*; *J. bulb* = *Juncus bulbosus; C. hamu* = *Callitriche hamulata;* Remaining = *Subularia aquatica*, *Isoetes echinospora*, *Sphagnum palustris* and *Utricularia* sp. (no statistical differences for all individual plant species).

S. angustifolium and *C. hamulata* were observed in the limed plots in 2001 – 3 yr after the final lime application in 1998.

Remarkably, four lime applications did not affect the long-term cover of the isoetid plant species. Nevertheless, liming significantly changed the morphology and nutrient composition of the isoetids. Results from the two-way ANOVA indicated that biomasses of shoots and roots of isoetids were significantly affected by liming, the type of isoetid plant species and their interaction (Table 3). Shoot length and shoot biomass of *Lobelia dortmanna*, *Littorella uniflora* and *I. lacustris* were significantly higher in the limed plots than in the reference plots. Also, the length and biomass of the roots was generally higher for the isoetids from the limed plots. The effect on root biomass was only significant for *I. lacustris*. Overall, there was no effect of liming, the type of plant species and their interaction on root–shoot length ratio and root:shoot biomass ratio. All isoetid plant species tended to develop a lower root:shoot ratio on the limed plots but this effect was never significant. Analyses of the shoot material indicated that the C:N and N:phosphorus (P) ratios were significantly affected by liming and the type of isoetid plant species. The C:N ratio of the shoot was also significantly affected by their interactive effect. In the limed plots the C:N ratios of the shoot were significantly higher for *Lobelia dortmanna* and *Littorella uniflora* whereas the N:P ratio of the shoot was significantly lower for *Littorella uniflora* (Fig. 2–3).

Discussion

The results show that 4 yr of lime application induced long-term changes in the vegetation composition and structure by increasing the amount of DIC in the sediment pore water. Apparently, liming enabled the growth of plant species that normally were unable to grow in these highly C-limited lakes. Four years of liming did not affect the long-term cover and composition of the original isoetid vegetation but liming increased the root and shoot development growth of all the isoetid plant species. The increase in C:N ratio of the shoots indicated that this was caused by increased uptake of the carbon dioxide that had become available in the limed sediment [Correction added after online publication 15 Feb 2012: in the preceding sentence the word molar was removed to clarify the C:N ratios].

Limestone is only poorly soluble in pure water and therefore accumulates in the deeper parts of the lakes, as proved by the study of Grøterud & Haaland (2010). In the sediment of the limed plots, dissolution of calcium and magnesium carbonates occurred, as appeared from the significantly higher concentrations of HCO_3^-, CO_2, Ca^{2+} and Mg^{2+} developing in the pore water of the limed plots (Table 2). Remarkably, bicarbonate, calcium, magnesium and iron concentrations became very high in the second monitoring period which started 10 yr after the first lime addition (Lucassen et al. 2009). The dissolution of calcite can be stimulated in relatively acid water due to the reaction of H^+ with the limed sediment interface (Compton et al. 1989). In addition, organisms excreting organic acids locally stimulate dissolution of carbonates (Cai et al. 1995; Martin & Sayles 1996; Müller et al. 2003). The higher alkalinity following carbonate dissolution led to more reducing conditions in the sandy sediment, as indicated by the redox potential that was still lower in the limed plots (175.4 mV) as compared with the unlimed plots (332.2 mV) even 6 yr after the final liming event. This is in agreement with the study of Grøterud & Haaland (2010) who showed that the microbial decomposition of organic matter is stimulated in limed lakes under the more alkaline conditions and that this can, at least temporarily, lead to oxygen depletion of the hypolimnion. The sediments of Norwegian softwater lakes are generally very rich in iron (Lucassen et al. 2009) and high concentrations of Fe^{2+} developed in the limed plots. This indicates that liming not only increases the availability of carbon dioxide by dissolution of carbonates, but also by in-lake alkalinity generation following enhanced anaerobic decomposition of organic matter and the consequent reduction of iron (IIII)hydroxides in the iron-rich sediments.

Liming led to an increase in cover of *J. bulbosus* from 2% to 10% within 1 yr after the first lime application in 1994. The *J. bulbosus* plants on the limed plots were approxi-

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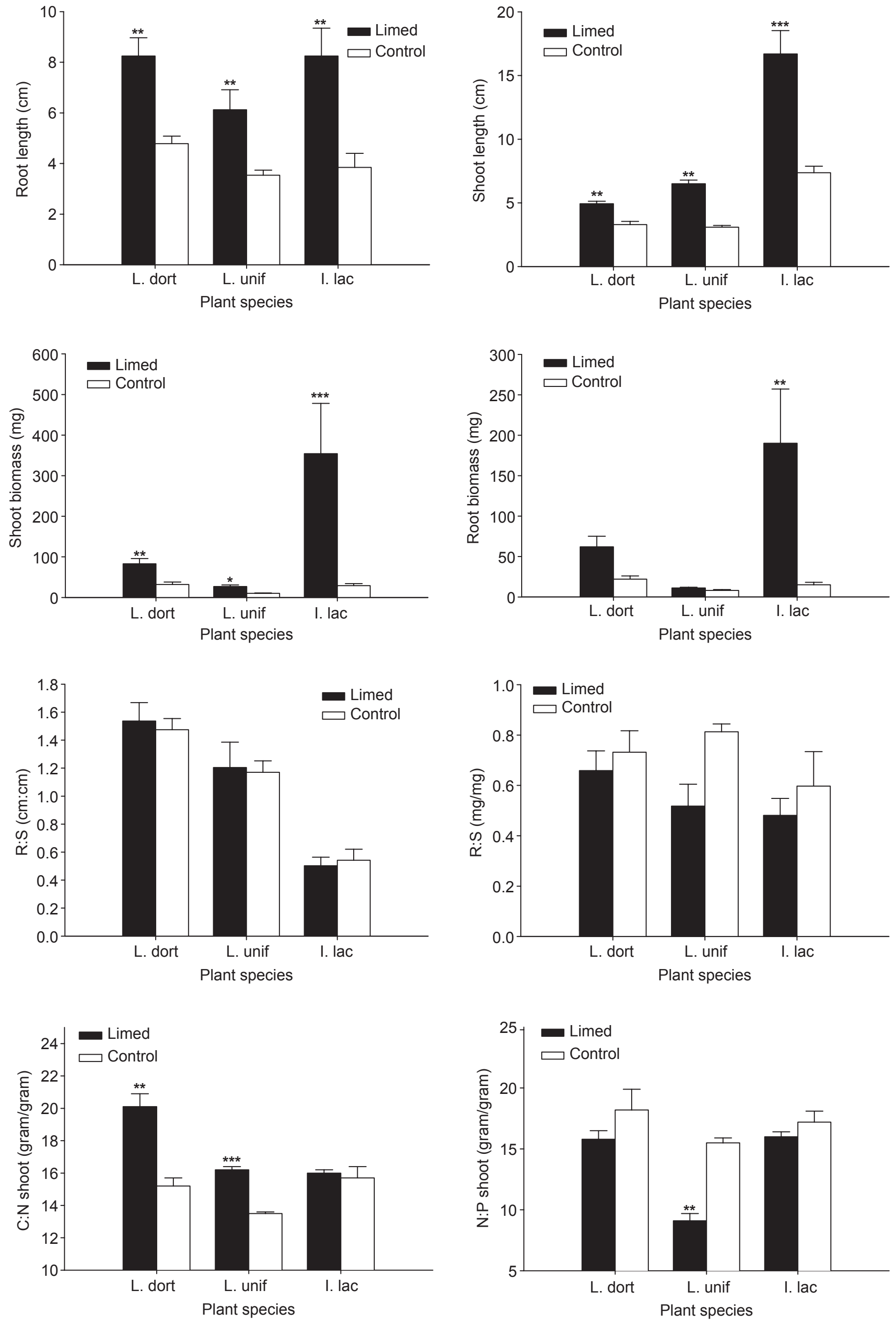

Fig. 2. Effects of liming on plant characteristics of the dominant isoetid plant species growing on the plots. Mean ± SE (in vertical bars) are given (n = 4). ***P < 0.001; **P < 0.01; *P < 0.05 (Mann–Whitney test). [Correction added after online publication 15 Feb 2012: in the bottom two subfigures the C:N shoot and N:P shoot ratios were clarified to be in (gram/gram)]

mately two times higher (±30 cm) compared with the plants on the control plots (results not shown). It was indicated from earlier studies that *J. bulbosus* can, in contrast to isoetids, very efficiently take up carbon dioxide for photosynthesis from the water layer by its thin leaves (Roelofs 1983; Svedäng 1990; Svedäng 1992). Uptake by the roots is possible but only to a very limited extent (Roelofs 1983; Wetzel et al. 1985). In entirely limed Norwegian lakes *J. bulbosus* can develop excessively, often completely filling up the water column (1–2 m). This development is related to 2.5–3.5 times higher carbon dioxide fluxes from the sediment, in combination with a strongly increased

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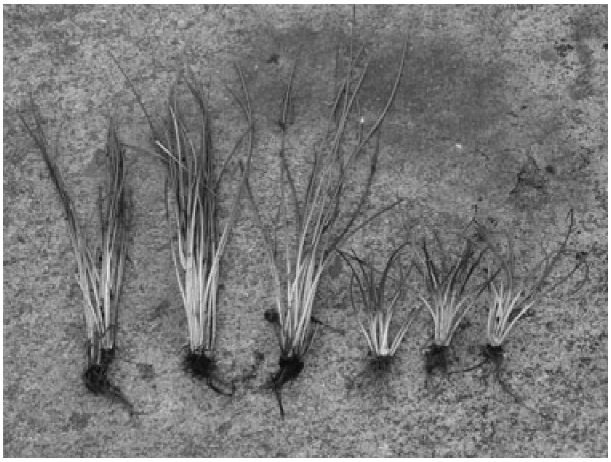

Fig. 3. Appearance of representative *Isoetes lacustris* plants growing on the limed (left) and control plots (right) in summer 2008.

ammonium availability in the sediment (Roelofs et al. 1995). In an ecophysiological experiment it was confirmed that *J. bulbosus* can only grow excessively where both parameters increase strongly (Lucassen et al. 1999). In the period 2004–2006 the concentration of HCO_3^- in the sediment pore water of the limed plots further increased and NH_4^+ concentration also temporarily increased up to 20–130 μM (details see Lucassen et al. 2009). *Juncus bulbosus* could not take advantage of this situation as cover and biomass remained unchanged. This confirms that *J. bulbosus* cannot profit from a higher carbon dioxide availability in the sediment by root uptake: it profits from higher carbon dioxide fluxes from the sediment to the water layer. Apparently, these fluxes remained relatively low because of the small scale to which the sediment was limed. Carbon dioxide fluxes may more easily diffuse horizontally leading to a faster decline of carbon dioxide concentrations in the deeper part of the water layer near the plants growing on the plots.

Three years after the last lime application in 1998, the vallisnerid plant species *S. angustifolium* and the elodeid plant *C. hamulata* were observed for the first time in the limed plots. Ecophysiological experiments have shown that *S. angustifolium* can take up carbon dioxide for photosynthesis by the roots (Lucassen et al. 2009) if concentrations become high enough. Therefore, *S. angustifolium* was able to benefit from the increased carbon dioxide concentrations in the pore water of the limed plots. The plants were able to develop long linear leaves (150–200 cm) that all floated on the water surface. In contrast to the typically small isoetid plant species, *S. angustifolium* invests most of its energy in shoot production, resulting in the production of floating leaves that will enable the additional uptake of atmospheric carbon dioxide. This is an effective strategy to deal with a low CO_2 availability in softwater lakes.

Only a few small plants (5–10 cm) of *C. hamulata* developed in the limed plots. Eco-physiological experiments have indicated that this plant species is unable to use bicarbonate (Adamec & Ondok 1992) and can strongly increase in biomass with increased carbon dioxide concentrations in softwater lakes (Spierenburg et al. 2009; Spierenburg et al. 2010). In our lake, however, carbon dioxide concentrations in the water layer did not increase because of liming of the small plots. This suggests that *C. hamulata* may also be able to take up carbon dioxide from the sediment, although to a limited extent, or might have profited, like *J. bulbosus* from, a local increase of carbon dioxide fluxes from the limed sediment.

In general, liming did not affect the cover or composition of the isoetid vegetation but led to significantly taller isoetid plants with significantly higher shoot and root biomasses. A relatively large increase in biomass of the shoots on more fertile sediments has already been reported for *Littorella uniflora* (Sand-Jensen & Søndergaard 1979; Robe & Griffiths 1992; Andersen et al. 2006) and *Isoetes macrospora* (=*lacustris*) (Boston & Adams 1987). The significantly higher C:N ratios of the shoots of isoetids in the limed plots indicate that the increase in growth was enabled by the higher carbon dioxide availability in the sediment pore water and the concomitant increased incorporation of carbon into the plant tissue [Correction added after online publication 15 Feb 2012: in the preceding sentence the word molar was removed to clarify the C:N ratios]. Similar increases in C:N ratio have been found for the elodeids *C. hamulata* and *M. alterniflorum* DC following increased carbon dioxide in softwater mesocosms (Spierenburg et al. 2009). [Correction added after online publication 15 Feb 2012: in the preceding sentence the word molar was removed to clarify the C:N ratios] The effect of liming on development of a lower N:P ratio in plant tissue is very likely related to the increased reduction of iron, including iron phosphates, which leads to an enhanced availability of phosphate (Patrick et al. 1973; Boström et al. 1982).

Changes in vegetation, as described above, indicate that liming leads to a mitigation of carbon dioxide limitation in the original vegetation of Norwegian softwater lakes. Our results reveal that this already occurs in small areas that are limed for a few years. In reality, many lakes in SW Norway have been limed for over 20 yr now. As entire lakes are limed, changes in water quality will also occur. Therefore, we wish to emphasize that the results of our field experiment strongly underestimate the effects of liming entire lakes. For example, in many limed lakes a massive growth of *J. bulbusos* occurred after repeated liming of entire lakes (Roelofs et al. 1994; Roelofs et al. 1995). Later, most of these dense *J. bulbosus* vegetations collapsed leaving the literal zones of the lakes covered with a thick carpet

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of organic debris without plants for at the least the following 10 yr.

Isoetids are typical eco-engineers with high radial oxygen losses (ROL) from the roots, which maintain aerobic and nutrient-poor sediment conditions thereby avoiding competition with other plants (Pedersen et al. 1995; Smolders et al. 2002). The high ROL at the roots is related to the presence of many gas-filled lacunae in their leaves causing high buoyancy. In more fertile sediments with an increased carbon dioxide availability, decreased root:shoot ratios will decrease the plants grip on the soil because of a lower cohesive strength on more loose (organic or degraded) sediments, which may strongly promote uprooting (Smolders et al. 2002; Schutten et al. 2005; Pulido et al. 2011). For example, massive uprooting of the *Littorella uniflora* vegetation has been observed in the Dutch softwater Lake Beuven during a heavy storm. The uprooted plants were growing on the most organic-rich substrates in this lake and were characterized by a low root:shoot ratio (Brouwer et al. 2008). The cover of isoetids is strongly decreased in the limed Norwegian lakes owing to overshadowing by *J. bulbosus* and probably also uprooting, and in some cases no isoetid plants are left. Once isoetids have disappeared they are no longer able to oxidize the sediment. A loss of isoetids results in more reductive sediments which hampers the germination and/or survival of isoetid seedlings (Sand-Jensen et al. 2005). This may complicate recovery of the isoetid vegetation in limed lakes even after liming is stopped. In the worst case, liming may eventually lead to an alternative stable state with anaerobic sediments with a very poor vegetation quality. Although liming may be beneficial for fish populations through reduction of concentrations of labile aluminium in the acid water layer, we warn that it strongly affects sediment properties and thereby the characteristic vegetation of these oligotrophic C-limited lakes.

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