

Long-term effects of liming in Norwegian softwater lakes: the rise and fall of bulbous rush (*Juncus bulbosus*) and decline of isoetid vegetation

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SUMMARY

1. Acidification has been recognised as a serious environmental problem in Scandinavia since the 1970s, and liming has been the main strategy to counteract negative effects on biota by improving water quality. We studied the short- and long-term effects of liming on sediment and water quality, as well as macrophyte development, by comparing five limed with five unlimed lakes during the period 1993–2013.
2. In the limed lakes, massive development of bulbous rush (*Juncus bulbosus*: Juncaceae) occurred during the first 6–9 years after the start of liming (1984–1993). *Juncus bulbosus* then started to decline around 1999 and eventually reached its original abundance in 2010. In addition, the cover of the original isoetid vegetation declined by around 60% between 1995 and 2010. In contrast, changes in submerged aquatic vegetation in the unlimed lakes were minor.
3. Liming initially stimulated anaerobic breakdown of sediment organic matter with Fe as the electron acceptor. *Juncus bulbosus* probably benefitted from the higher CO₂ fluxes from the sediment to the water layer in combination with a high NH₄⁺ concentration in the limed sediment. However, the organic matter became less reactive over time, thus eventually providing less CO₂. Together with the significant increase in water pH over time, this would have led to a decline in C availability in sediment and water, thus discouraging excessive growth of *J. bulbosus*. This may explain why *J. bulbosus* thrived only during the first years after liming.
4. The decline of isoetids in limed lakes was probably caused by a combination of uprooting and sediment anoxia. In limed lakes, isoetids developed a shoot : root ratio that was above the threshold known to promote uprooting of these high buoyancy species on organic sediment. In addition, isoetids eventually became covered by algae and dead *J. bulbosus*, affecting light conditions and suffocating the remaining isoetid vegetation on limed reductive sediments.
5. In the future, renewed excessive growth of *J. bulbosus* seems unlikely due to the lack of sufficient carbon sources. It remains, however, unclear whether the original isoetids vegetation can recover. We hypothesise that the isoetid *Littorella uniflora* may eventually recolonise the lake from the shore and improve the oxygen content of limed sediments; this, in turn will facilitate the germination of other plant species, through high radial oxygen loss in combination with asexual reproduction.

Keywords: acidification recovery, ammonium, carbon dioxide, isoetids, *Juncus bulbosus*

Introduction

Soft water lakes frequently occur in boreal and temperate regions and at higher elevations in the subtropics, mostly on siliceous bedrock or on non-calcareous sandy soils (Murphy, 2002). They are mainly fed by rainwater and shallow ground water and are nutrient-poor (oligotrophic) with very low concentrations of dissolved inorganic carbon (Smolders, Lucassen & Roelofs, 2002). The growth of submerged macrophytes of soft water lakes is carbon limited (Madsen, Olesen & Bagger, 2002; Smolders *et al.*, 2002) and the vegetation is dominated by small isoetid species with low intrinsic growth rates and morphological and physiological adaptations to low water carbon concentrations (Robe & Griffiths, 1990; Pedersen, Sand-Jensen & Revsbech, 1995; Madsen *et al.*, 2002).

As carbon dioxide (CO₂) concentrations in the sediment can be 100 times higher than in the water layer, isoetids have special adaptations that enable effective diffusion of CO₂ from the sediment into the plants as well as restrict losses of CO₂ and O₂ to the water layer (Roelofs, Schuurkes & Smits, 1984; Smits *et al.*, 1990). Isoetids generally grow in sediments with a low nitrogen availability and nitrate as the dominant nitrogen source (Schuurkes, Kok & Den Hartog, 1986). Due to their specific high radial oxygen losses (ROL) from the roots (Sand-Jensen, Prahl & Stokholm, 1982), they promote nitrification of ammonium (NH₄⁺) and so stimulate net nitrogen losses in the sediment by promoting coupled nitrification–denitrification (Risgaard-Petersen & Jensen, 1997; Smolders *et al.*, 2002). In addition, ROL by isoetids results in the oxidation of iron in the sediment, which stimulates co-precipitation of phosphate to highly insoluble Fe(III)phosphates and thus decreases P availability (Tessenow & Baynes, 1975; Christensen, 1997). So, isoetids can be seen as eco-engineers in the way that they can keep their environment oligotrophic and thereby prevent competition with other faster growing plant species (Smolders *et al.*, 2002).

Isoetids have experienced profound reductions in their distribution, particularly in lowland regions with intense agricultural activity and high human population density (Roelofs, 1983; Murphy, 2002). Being indicators of near-pristine clear water lakes, the isoetid vegetation, and the rare species it includes, have a high priority in national assessments of lake biodiversity and ecological status, for example, according to the Water Framework Directive (EU, 2000; Stelzer, Schneider & Melzer, 2005).

Since the 1970–1980s, many shallow soft water lakes in Western Europe originally dominated by isoetid plant species have become dominated by bulbous rush (*Juncus*

bulbosus [Juncaceae]) and/or *Sphagnum* spp. This was attributed to increased atmospheric sulphur and nitrogen deposition resulting in water acidification and accumulation of NH₄⁺ and CO₂ in (slightly) calcareous sediments (Roelofs, 1983; Roelofs *et al.*, 1984; Arts *et al.*, 1990). This unwanted development continued until the carbonate pool of the sediment became exhausted (Smolders *et al.*, 2006).

In the 1970–1980s nuisance growth of *J. bulbosus* in Norway was much less severe than in western European lakes. This has been attributed to the naturally very low carbonate content of Norwegian lake sediments. Therefore, acidification did not result in increased CO₂ levels in the sediment and water layer (Roelofs, Brandrud & Smolders, 1994). However, since the late 1980s, nuisance growth of *J. bulbosus* has become a problem in an increasing number of soft water lakes in SW Norway (Brandrud & Roelofs, 1995). In this region, the atmospheric deposition of S was relatively high and exceeded critical loads (Tørseth & Semb, 1995). A common factor linking the lakes with dense *J. bulbosus* growth was the repeated application of calcium-carbonates (limestone) to the surface water during the 5–7 years before the nuisance growth became apparent (Brandrud & Roelofs, 1995). Whole lake liming was applied since the 1980s to increase the pH of the water in order to protect Atlantic salmon (*Salmo salar*) populations in Norwegian rivers and lakes (Sandøy & Langåker, 2001). Roelofs *et al.* (1994) concluded that liming stimulated the mineralisation of organic matter that is abundant in SW Norwegian lakes. As a consequence, the sediment became anaerobic leading to increased rates of iron reduction and NH₄⁺ accumulation in the sediment. Due to the iron-richness of the sediments, the phosphorus concentration in the pore water remained very low and no releases of phosphorus to the water layer were detected, indicating that there was still sufficient oxidised iron in the sediment to bind available P despite the anoxic conditions (Smolders *et al.*, 2006). *Juncus bulbosus* was able to benefit from the increased nitrogen and carbon availability in limed sediment (Lucassen *et al.*, 1999).

In order to reveal the long-term effects of lake liming, we here analyse >20 years of data on water and sediment pore water quality, as well as macrophyte vegetation, in five limed and five unlimed lakes in SW Norway as selected and described by Roelofs *et al.* (1994). We expected that (i) in the limed lakes the isoetid vegetation would decline due to the increased dominance of *J. bulbosus* and (ii) that the massive *J. bulbosus* biomass in limed lakes would eventually collapse due to changes in carbon availability related to changes in sediment quality.

Methods

We studied the same 10 softwater lakes in southern Norway that were studied by Roelofs *et al.* (1994) and Brandrud & Roelofs (1995). Five of the ten lakes have repeatedly been limed with calcium carbonate, starting in 1984–1990 until recently. Liming occurred in summer and was carried out by helicopter and/or by boat (Fig. 1a). Detailed information on the timing and amounts of lime applied is given in Table 1. In 1993, the

limed lakes were all characterised by isoetid vegetation mainly consisting of *Lobelia dortmanna*, *L. uniflora* and *Isoetes lacustris* (Fig. 1c,d) with a local presence of dense *J. bulbosus* stands consisting of large columns with annual shoots as well as surface mats as described by Roelofs *et al.* (1994) and Moe *et al.* (2013) (Fig. 1e,f). In 1993, the unlimed lakes were characterised by the dominance of the same type of isoetids as present in the limed lakes accompanied by small rosettes of *J. bulbosus* (Roelofs *et al.*, 1994).

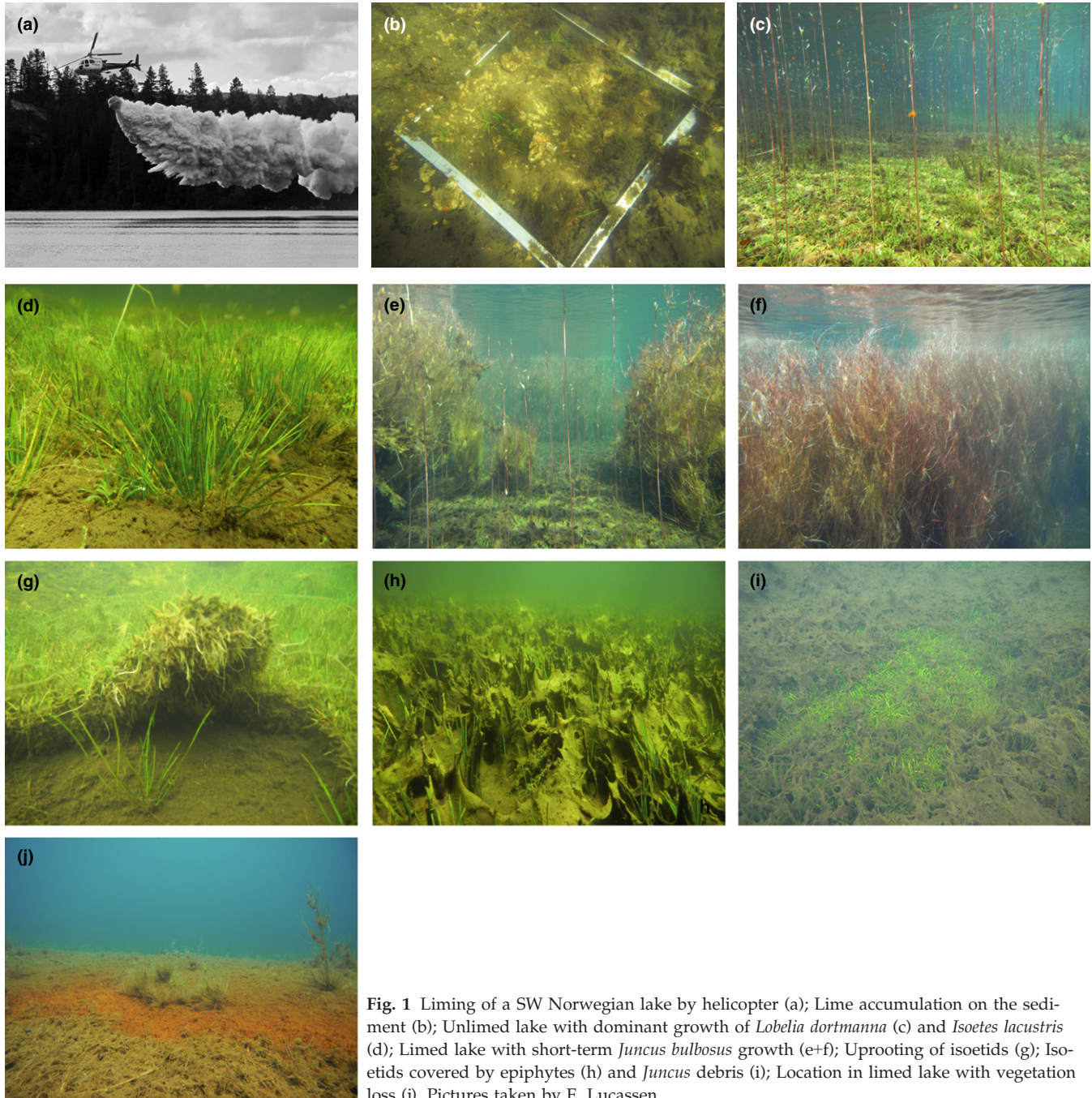


Fig. 1 Liming of a SW Norwegian lake by helicopter (a); Lime accumulation on the sediment (b); Unlimed lake with dominant growth of *Lobelia dortmanna* (c) and *Isoetes lacustris* (d); Limed lake with short-term *Juncus bulbosus* growth (e+f); Uprooting of isoetids (g); Isoetids covered by epiphytes (h) and *Juncus* debris (i); Location in limed lake with vegetation loss (j). Pictures taken by E. Lucassen.

Table 1 Lake information including amounts of lime (tons) applied to the limed lakes from 1990 until now (data from KPS Norway) and the alkalinity ($\mu\text{eq L}^{-1}$) of the surface water in 1995/2013.

	Area (km ²)	Coordinates	Liming period	Lime (tons)	Alkalinity 1995/2013
Limed lakes					
1. Fagervatnet	0.41	58.24300 6.32608	1987–1997	103	76/89
2. Eiavatnet	4.34	58.24428 6.15849	1990–2001	2110	87/77
3. Juvvatnet	0.04	58.22483 6.30382	1987–2014	176	112/125
4. Åsvolltjørna	0.06	58.22668 6.29749	1987–2011	229	418/108
5. Gaudlandsvatnet	0.17	58.23966 6.14909	1984–2014	334	280/79
Unlimed lakes					
6. Barstadvatnet	1.31	58.24127 6.16077			82/88
7. Dybingsvatnet	0.96	58.28748 6.17560			46/92
8. Evjatjørna	0.15	58.26232 6.16905			87/91
9. Haptatjørna	0.02	58.25276 6.13505			56/80
10. Mjåvatnet	0.07	58.2279 6.6973			41/77

Vegetation

In 1995 and 2010, species composition and percentage cover of submerged aquatic vegetation was estimated at exactly the same locations that were studied by Roelofs *et al.* (1994) in 1993. For each lake, an accessible area (mostly a small bay of about 0.10 ha) was investigated. The coordinates of these lake areas are given in Table 1. In 1995 and 2010, vegetation cover was determined by the same person by snorkeling. The cover of isoetids was estimated in different vegetation sections that were visually present. Based on the cover and surface area of the sections, total cover of plants for each lake area could be calculated. In the years between 1995 and 2010, the vegetation was observed by snorkeling and taking photographs on a regular basis, which allowed major changes to be detected. Total plant cover was estimated by adding up percentage cover of all plants, including those growing underneath, for example, *Juncus* mats floating on the water layer, and could therefore exceed 100%. In each lake, intact specimens of the dominant plant species were collected in quadrats of 1 m² in triplicate. Roots and shoots were separated and lengths were measured in 20 randomly selected individuals from each triplicate quadrat. Roots and shoots were subsequently dried (24 h at 70 °C), and the dry weight was measured.

Water column

Between 1993 and 2013, samples of the water column were collected 14 times during summer (between June and August). The samples were collected from a fixed point in the area where the submerged vegetation was studied in the lake ($n = 1$). Samples were collected half way between the bottom and the surface water at about 0.5 m depth, in 500 mL serum glass bottles. Immediately after sampling pH was measured in the 500 mL bottle and a 30 mL subsample was separated and kept airtight for analysis of total inorganic carbon (TIC). The alkalinity was determined by titrating 40 mL of the sample with 0.01 M HCl down to pH 4.2. The remaining sample was fixed with citric acid (0.6 mg L⁻¹) and kept cool until chemical analyses.

Sediment pore water

In 1995 and in 2010, samples of the sediment pore water were taken in patches of *L. dortmanna*, *L. uniflora* and/or *I. lacustris*. In the limed lakes, locations with large columns and surface mats of *J. bulbosus* were additionally sampled. Three to nine samples were taken in each lake at the same sites where the submerged vegetation was studied, resulting in a total number of 34 and 35 samples in unlimed and limed lakes, respectively. In 1995, three

locations with luxurious growth of *J. bulbosus* were sampled in each limed lake (15 samples in total). In 2010, the dense *J. bulbosus* stands had collapsed leaving dead plant material on the sediment top layer (Fig. 1j). Three to six locations with former luxurious growth were sampled resulting in a total of 21 samples. Sediment pore water was collected using ceramic cups (Eijkelpamp, Agri-search, Giesbeek) 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). The syringes were kept cool; directly after sampling a subsample was taken for analysis of TIC and pH. The remaining sample was fixed with citric acid (0.6 mg L^{-1}) and used for chemical analysis. Data were compared with data from 1993 (Roelofs *et al.*, 1994) taken in the same lakes at the same locations.

Chemical analyses

The pH was determined using a titration workstation (TitraLab 840; Radiometer analytical SAS, Villeurbanne, France) with a double Ag/AgCl pH electrode (Orion 9156BNWP; Thermo Scientific, Waltham). TIC was measured on an infrared carbon analyser (Advance Optima, ABB, Cary). Based on the pH and TIC concentrations, the CO_2 and HCO_3^- content was calculated according to Stumm & Morgan (1996). The total elemental composition (P, Ca, Na, K, S, Al, Fe, Mg, Mn and Zn) of the samples was analysed, using an ICP-OES (models: IRIS Intrepid II XDL, Thermo Fisher Scientific, Waltham). Here NH_4^+ , NO_3^- and PO_4^{3-} content was analysed by an Auto Analyser system (model III; Bran & Luebbe, Nordstedt), using a salicylate method for NH_4^+ , a hydrazine sulphate method for nitrate and an ammonium molybdate method for phosphate. Chloride was analysed by flame photometry according to O'Brien (1962). In addition, data on water quality in 1975 and 1986 were obtained from an RESA-database at the Norwegian Institute of Water Research, an application orientated Norwegian data archive system developed by 'Egil Stoeren Programutvikling'.

Statistical analyses

Values of isoetid dominated and *J. bulbosus* dominated locations were averaged for each lake. Sediment pore water data were subsequently expressed as means \pm standard error of the mean for limed ($n = 5$) and unlimed lakes ($n = 5$). All statistical analyses were performed in the program SPSS 16.0 for Windows (SPSS inc., Chicago). Normality was tested using a Kolmogorov–Smirnov test,

homogeneity of variance was tested using Levene's test. In case data were not normally distributed they were log transformed to approach normality. This was the case for the concentration of HCO_3^- in the pore water, the concentrations of HCO_3^- , CO_2 , NH_4^+ and Al in the water column, as well as the cover of *Potamogeton. natans* and *Sparganium angustifolium* in lakes.

Modern repeated measures analyses (mixed linear analyses) were performed to test (interactive) effects of liming and time on water column quality. For all other data on parameters related to liming of lakes (pore water quality, vegetation cover and *Isoetes* root and shoot dry weight), a two-way ANOVA (with a Tukey's-*b post-hoc* test) was performed to test the effects of liming, time and their interaction.

Results

Vegetation

In 1995, the littoral zones of the investigated areas in the unlimed and limed lakes were almost fully covered with isoetids (Fig. 2a). However, total plant cover was much higher in the limed lakes due to a significantly higher cover of *J. bulbosus* ($\pm 70\%$) (Fig. 2c+f), which mainly consisted of large columns of shoots with more or less continuous, dense, entangled, flowering surface mats spreading on the water surface (Fig. 1e+f). In 2010, plant cover and vegetation composition in unlimed lakes was not significantly different from 1995 (Fig. 2). In contrast, total plant cover in limed lakes significantly decreased between 1995 and 2010 (Fig. 2f). This was due to a collapse of the *J. bulbosus* vegetation (Fig. 2c), starting around 1999 (personal observations), and a significant decline in cover of isoetids by about 60 percentage (Fig. 2a). There was a significant effect of liming (and an interaction with time) on the cover of *I. lacustris*. Also, *L. dortmanna* and *L. uniflora* tended to decline as a consequence of liming and for *L. uniflora* this effect was marginally significant ($P = 0.069$).

I. lacustris was the dominant isoetid species in the limed lakes in 2010 (Fig. 2a+b). The plants in 2010 were, however, much larger than in 1995 as reflected in a significantly higher shoot and root dry weight (Fig. 3). For both parameters, there was significant effect of liming ($P < 0.001$) and an interactive effect between liming and time (DW shoot $P = 0.002$; DW root $P = 0.013$). *Potamogeton natans* was not observed in the unlimed lakes while in the limed lakes it occurred at a few spots in a low cover (Fig. 2d). *Sparganium angustifolium* was present in unlimed lakes and limed lakes during the period

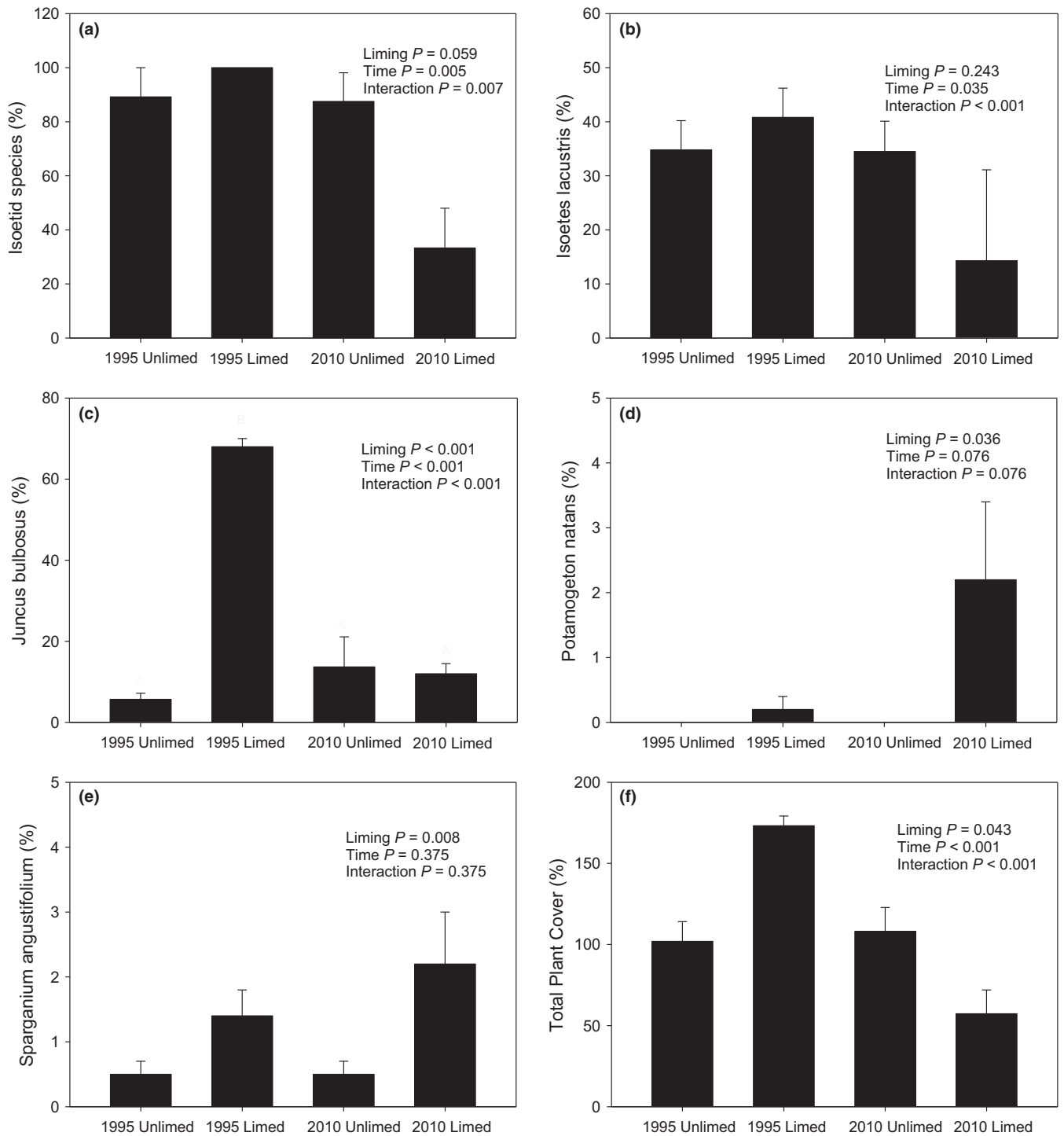


Fig. 2 Plant abundance (cover) in unlimed and limed lakes in 1995 and 2010. Mean values ($n = 5$) \pm standard error of the mean are given. Statistical information on the (interactive) effects of liming and time (1995 versus 2010) on vegetation cover and characteristics (tested by a two-way ANOVA) are given within each panel. Statistical significance was accepted at $P < 0.05$.

1995–2010. Its cover was higher in the limed lakes compared to unlimed lakes but this effect was significant only in 2010 (Fig. 2e). This species mainly appeared as a small rosette plant in 1995 (isoetid growth form) while it was present with floating leaves (vallisnerid growth

form) in 2010. In 2013, patches with *Potamogeton alpinus* were observed in two of the limed lakes (Gjuvatn and Gaudlandsvatn) and some small *Chara globularis* plants were found in one limed lake (Gjuvatn) (data not shown).

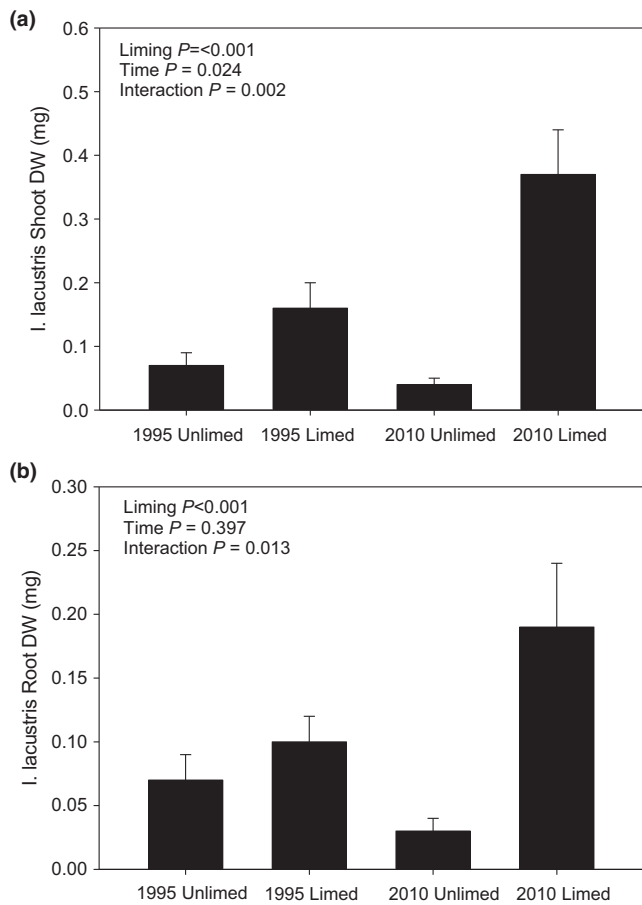


Fig. 3 Root and shoot dry weight of *Isoetes lacustris* in unlimed and limed lakes in 1995 and 2010. Mean values ($n = 5$) \pm standard error of the mean are given. For statistical results (tested by a two-way ANOVA) see panel. Statistical significance was accepted at $P < 0.05$.

Water column

The mean water sulphate concentration in both limed and unlimed lakes decreased by about 50% from 45 μM in 1975 to 22.5 μM in 2013 ($P < 0.001$). In addition, the mean pH of the unlimed lakes increased ($P < 0.001$) from pH 4.85 in 1975 to pH 5.6 in 2013. As expected, the Ca concentration in the limed lakes was higher than in the unlimed lakes (Fig. 4). RESA monitoring data indicated that in the late 1980s – early 1990s the limed lakes (before the liming started) Ca concentrations were similar in all lakes (Fig. 4).

There was a significant effect of time on pH and the concentration of Si, Ca and S in the water column (Table 2). pH and Si concentration increased while the Ca and S concentrations decreased. pH and HCO_3^- concentration of the limed lakes was significantly higher than in the unlimed lakes, while it was the reverse for the concentrations of Mg, Mn, Na^+ and Cl^- (Table 2,

Fig. 4). The concentration of S became significantly lower in the limed lakes than in the unlimed lakes after 2001. No significant differences in the concentrations of PO_4^{3-} , NH_4^+ and NO_3^- in the water column of limed and unlimed lakes were found (Table 2 and Fig. 4).

Sediment pore water

The concentration of Fe^{2+} at locations with *J. bulbosus* in the limed lakes was significantly higher compared to isoetids in (un)limed lakes in 1995 and 2010 (Fig. 5, Table 3). A similar pattern was obvious for NH_4^+ , but there also was an interaction effect with time. In 2010, when *Juncus* had already collapsed, NH_4^+ concentrations were significantly higher compared to 1995. This was accompanied by a significantly lower NO_3^- concentration (Fig. 5, Table 3). The CO_2 concentration at locations with *J. bulbosus* in limed lakes was significantly higher compared to locations with isoetids in unlimed lakes in 1995 and 2010. There was, however, no interaction effect with time. The concentration of HCO_3^- at locations with *J. bulbosus* was significantly higher compared to isoetids in unlimed lakes in 1995 as well as 2010. There was however no interaction effect with time. Locations dominated by *Juncus* had a significantly lower SO_4^{2-} concentration compared to isoetids in unlimed lakes and this effect remained unchanged in 2010. The concentration of Si was higher at both location types (isoetids and *Juncus*) in limed lakes compared to isoetid vegetation in unlimed lakes but only in 2010 (Fig. 5, Table 3). In 1993, the pore water concentrations of Fe^{2+} , HCO_3^- , and CO_2 of the *Juncus* vegetated sediment in limed lakes were much higher (Roelofs *et al.*, 1994) compared to 1995 and 2010.

Discussion

While the unlimed lakes had a stable macrophyte vegetation, we detected significant changes in the limed lakes. A few (3–5) years after liming, luxurious growth of *J. bulbosus* occurred without affecting the cover of the original isoetid vegetation. About 20 years after the onset of liming, the *J. bulbosus* vegetation had collapsed and the original isoetid vegetation had strongly declined.

Short-term increase in *J. bulbosus* in limed lakes

Mass development of *J. bulbosus* is typically related to changes in the C and N budgets of a lake (Roelofs *et al.*, 1984; Schuurkes *et al.*, 1987; Lucassen *et al.*, 1999; Schneider *et al.*, 2013). In 1993, 6–9 years after the first liming events, concentrations of Fe^{2+} , NH_4^+ , HCO_3^- and CO_2 in

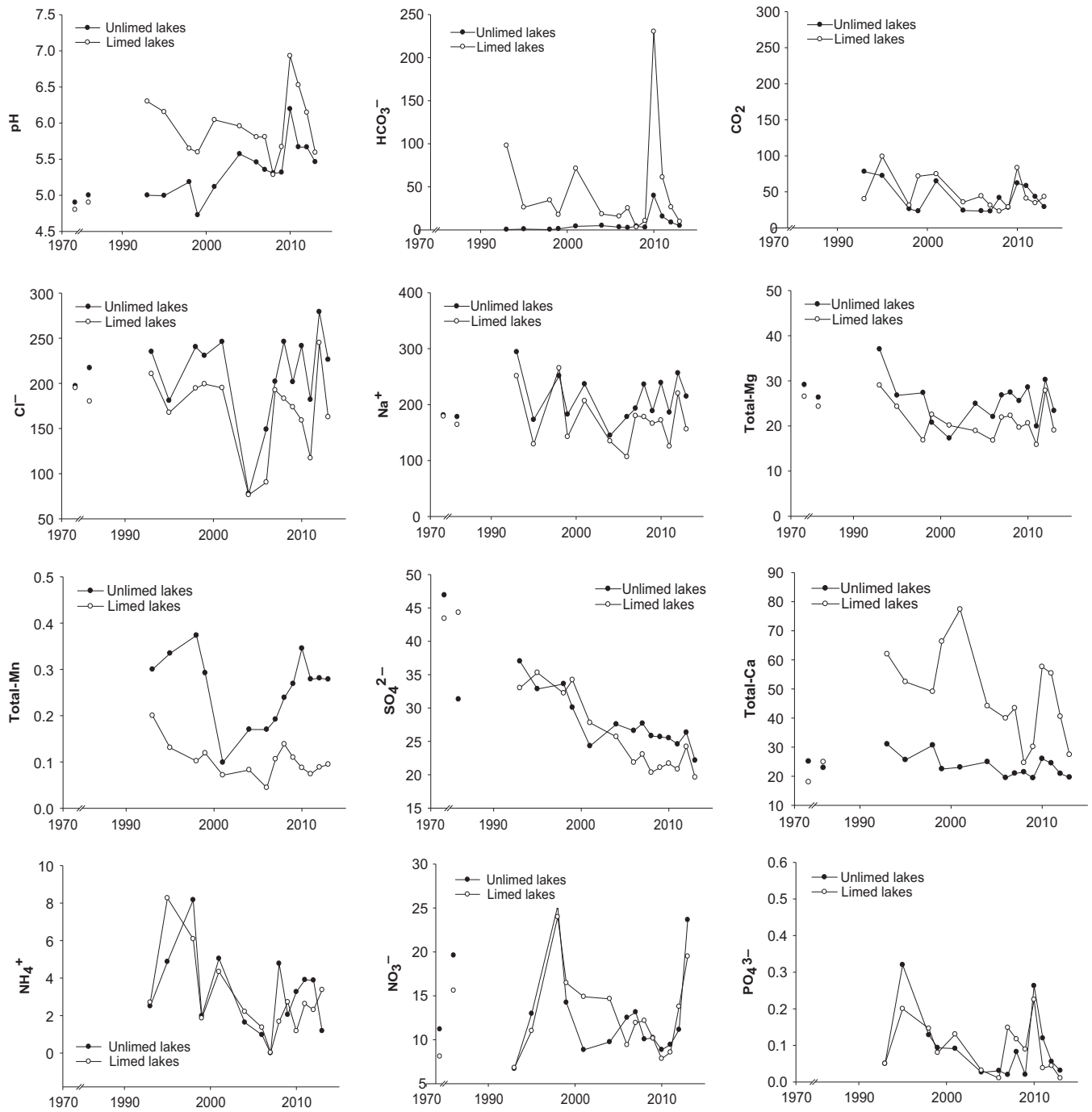


Fig. 4 Mean water column quality of limed and unlimed lakes from 1993 to 2014. The samples were collected in summer (from May to August). Concentrations are given in μM . For statistics see Table 2.

the sediment pore water of limed lakes were significantly higher than in unlimed lakes. This is likely due to an increased availability of carbonates caused by liming, and the concomitant stimulation of mineralisation of sediment organic matter that is abundant in the lakes (up to 20–40% of dry weight) (Roelofs *et al.*, 1994). Since iron(III)(hydr)oxides usually act as the prime electron

acceptors under anaerobic conditions, this commonly results in iron and ammonium mobilisation (Roelofs *et al.*, 1994; Lucassen *et al.*, 2009; Lucassen, Smolders & Roelofs, 2012).

From eco-physiological experiments it is known that *J. bulbosus* can effectively take up CO_2 from the water layer through its thin leaves (Roelofs *et al.*, 1984) while direct

Table 2 Modern repeated measures analyses testing the (interactive) effects of liming and time on surface water quality using mixed linear models in SPSS 10. Statistical significance is indicated in bold and was accepted at $P < 0.05$.

	Liming (<i>P</i> values)	Time (<i>P</i> values)	Interaction (<i>P</i> values)
HCO ₃ ⁻	<0.001	<0.001	0.003
TIC	<0.001	<0.001	0.064
pH	<0.001	<0.001	0.152
Total-Ca	<0.001	0.022	0.118
Total-Mg	<0.001	<0.001	0.279
Total-Mn	<0.001	0.499	0.832
Total-Si	0.120	<0.001	0.337
Na ⁺	0.001	<0.001	0.921
Cl ⁻	0.001	<0.001	0.959
K ⁺	0.047	0.436	0.773
Total-Fe	0.063	0.486	0.995
Total-Zn	0.085	<0.001	0.365
Total-Al	0.112	<0.001	0.724
Total-S	0.120	<0.001	0.337
Total-S*	<0.001	0.043	0.842
CO ₂	0.122	<0.001	0.516
Total-P	0.331	<0.001	0.323
NH ₄ ⁺	0.416	<0.001	0.754
NO ₃ ⁻	0.521	<0.001	0.847
PO ₄ ³⁻	0.427	0.003	0.338

*From 2004 and on.

uptake from the sediment is possible to a limited extent (Roelofs, 1983; Wetzel *et al.*, 1985). A growth experiment with sediments from Norwegian softwater lakes has confirmed that *J. bulbosus* can benefit from increased nitrogen availability in limed sediments in acidic waters, only when carbonate dissolution in the sediment and CO₂ availability in the surface water are relatively high (Lucassen *et al.*, 1999). Although ammonium concentrations increased in the limed sediments, surface water CO₂ concentrations were not significantly higher in the limed lakes. However, Roelofs *et al.* (1995), showed that fluxes of CO₂ from the sediment to the overlying acidified surface water can be 3.5 and 9 times higher in limed lakes as compared to unlimed SW Norwegian lakes. Hence, although CO₂ concentrations in the water layer do not differ, the fluxes of CO₂ can be much higher in limed lakes. Furthermore, it was shown that reacidification of the lakes in autumn could lead to a strong increase in CO₂ availability in limed lakes (36 µM in July 1994 versus 186 µM in October 1994) (Roelofs *et al.*, 1995). Interestingly, the most vital and luxuriant growth of *J. bulbosus* seems to have occurred in the years around 1990, when the climate was milder than normal, with a series of ice-free winters and high precipitation (Brandrud & Roelofs, 1995). Such conditions provided a

relatively long growing season, higher microbial activity, and a more rapid reacidification of the limed lakes.

Long-term development of *J. bulbosus* in limed lakes

After surface mats have developed, the shoots are able to take up CO₂ from the atmosphere, which will make the vegetation much less dependent on dissolved CO₂. So once developed under favourable conditions, the dense *J. bulbosus* vegetation will be able to survive for years even if C availability in the sediment or water layer becomes sub-optimal. In 1999, 12–15 years after the first liming events had taken place, we have observed the first signs of *J. bulbosus* die-back. In parts of the limed lakes, the bottom was covered by dead *J. bulbosus* (Fig. 1j), and mats floating on the water surface were slowly disappearing under the water surface (personal observations). Although the exact cause for the decline of the *J. bulbosus* mats is unknown, an extremely cold winter or a sudden increase in water table (preventing the use of atmospheric CO₂ by the floating mats) could have killed the floating mats at the start of this century. Apparently, floating mats were unable to redevelop after their collapse.

In 1993, the concentrations of reduced Fe, HCO₃⁻, CO₂ and NH₄⁺ in the sediment pore water were much higher than in 1995 and 2010, suggesting higher anaerobic mineralisation rates in the sediment in the first years after liming. These results indicate that the increased buffering of the sediments after liming has strongly increased the decomposition of reactive organic matter (Smolders *et al.*, 2006) leading to a strong decline in the remaining reactive organic matter over time. Growth of small submerged *J. bulbosus* plants mainly depends on the CO₂ flux from the sediment to the overlying water layer (Roelofs *et al.*, 1995) and will be negatively affected by the lower TIC production in the sediment (Lucassen *et al.*, 1999). Furthermore, reacidification of the water layer, resulting in temporary increased water layer CO₂ concentrations in spring or autumn (Roelofs *et al.*, 1995), has become much less frequent as a result of decreased acid deposition (Helliwell *et al.*, 2014). These processes very likely explain why the floating mats were unable to redevelop after their collapse.

Long-term decline of isoetids in limed lakes

In 1995, 8–11 years after the start of liming, the abundance of isoetids was not different between the limed and unlimed lakes (Fig. 2a). Apparently, liming does not affect isoetid cover over this time frame. In 2010, the original isoetid vegetation cover had strongly declined

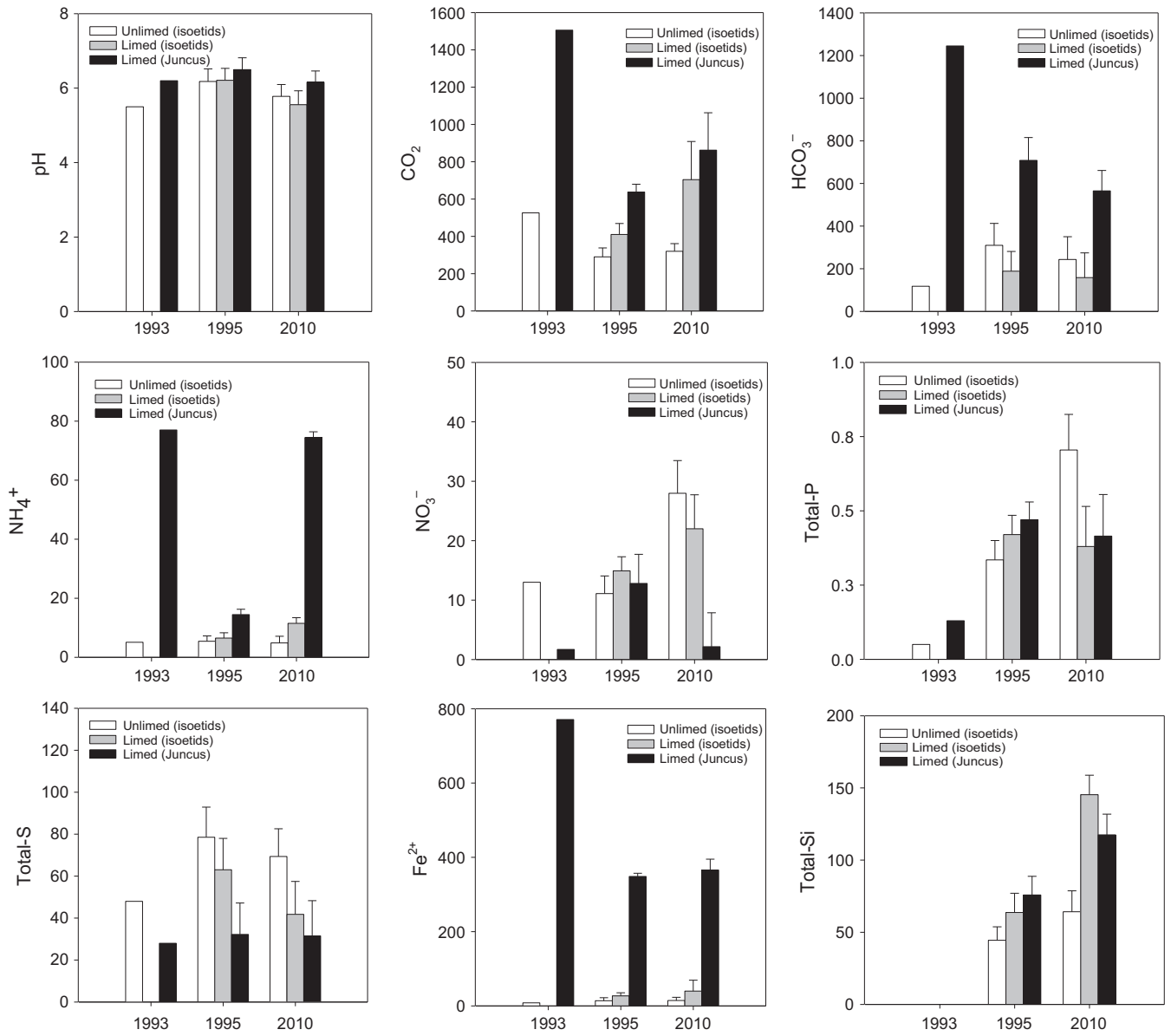


Fig. 5 Sediment pore water quality of limed ($n = 5$) and unlimed lakes ($n = 5$) in SW Norway sampled in summer 1995 and summer 2010. In 2010 all *Juncus* had died back. Also mean values for 1993 (Roelofs *et al.*, 1994) are given (data on Total-Si are missing). Concentrations are given in μM . For statistics see Table 3.

in the limed lakes indicating a long-term negative effect of liming on isoetid vegetation. The decrease in cover can be related to changes in the shoot : root ratio of the isoetids. Roelofs *et al.* (1994) reported a three to five times higher shoot : root ratio of *L. uniflora* and *L. dortmanna* in the limed lakes in 1993.

It is well known that *L. uniflora* plants with an increased biomass and shoot : root ratio are sensitive to uprooting due to wave action, especially on more organic, partially degraded soils (Smolders *et al.*, 2002; Pulido *et al.*, 2011; Spierenburg *et al.*, 2013; Sand-Jensen & Møller, 2014). We have frequently observed signs of

uprooting of isoetids during summer sampling. Partly detached mats of *L. dortmanna* creating patches without plants were found at a depth of 1–2 m in the littoral zone (Fig. 1g) while patches and solitary plants were also observed floating on the water surface and on the shore lines. This sensitivity is related to the high buoyancy of isoetids due to the presence of gas-filled lacunae in their leaves, in combination with a decreased grip on the sediment because of lower cohesive strength in the loose organic sediments (Pulido *et al.*, 2011; Spierenburg *et al.*, 2013). Spierenburg *et al.* (2013) showed that *L. uniflora* plants that were uprooted from organically

Table 3 Two way-ANOVA with Tukey's-b *post-hoc* test for testing the (interactive) effects of location with isoetids and *Juncus* in (un)limed lakes and time (1995 versus 2010) on sediment pore water quality. Statistical significance is indicated in bold and was accepted at $P < 0.05$. Location types with a same letter do not differ significantly.

	Location type	Time	Interaction	Unlimed Isoetids	Limed Isoetids	Limed <i>Juncus</i>
pH	0.109	0.012	0.748	a	a	a
CO ₂	0.041	0.030	0.901	a	ab	b
HCO ₃ ⁻	0.043	0.546	0.939	ab	a	b
NO ₃ ⁻	0.050	0.269	0.036	a	a	a
NH ₄ ⁺	<0.001	0.009	0.006	a	a	b
K ⁺	0.002	0.002	0.160	b	a	a
Na ⁺	0.002	<0.001	0.014	b	a	a
Cl ⁻	0.054	0.591	0.059	a	a	a
Total-Al	0.751	<0.001	0.592	a	a	a
Total-Ca	0.469	0.361	0.943	a	a	a
Total-Fe	0.012	0.908	0.998	a	a	b
Total-Mg	0.211	0.046	0.935	a	a	a
Total-Mn	0.433	0.342	0.463	a	a	a
Total-P	0.694	0.369	0.240	a	a	a
Total-S	0.013	0.275	0.406	b	b	a
Total-Si	0.010	0.002	0.234	a	b	b
Total-Zn	0.170	<0.001	0.238	a	a	a

enriched sediments typically had a larger shoot biomass and a shoot : root ratio of 3.2. This is in agreement with our observations on the decline of this species in limed lakes (Roelofs *et al.*, 1994). Therefore, it is likely that uprooting has played a significant role in the long-term decline of most isoetid species in the limed lakes. Morphological changes of the plants such as increased shoot and root weight and a decreased shoot : root ratio are typically caused by increased CO₂ availability in the sediment (Madsen *et al.*, 2002; Smolders *et al.*, 2002; Pulido *et al.*, 2011; Spierenburg *et al.*, 2013). A long-term field experiment showed that these morphological changes also occur in Norwegian softwater lakes as a consequence of liming (Lucassen *et al.*, 2012).

Roelofs *et al.* (1994), did not find changes in shoot biomass or shoot : root ratio for *I. lacustris* in the short-term after liming. From our study it became clear that not only the shoot but also the root biomasses of this species had increased proportionately in limed lakes in the long term. As a result, the shoot:root did not change as dramatically in the long term for this plant species. Therefore, *I. lacustris* might have remained less sensitive to uprooting. Although *I. lacustris* became the dominant isoetid species in the limed lakes in the long term, apart from uprooting, other causes are likely involved in the long-term decline of isoetids in the limed lakes.

Large parts of the sediments of limed lakes became covered by dead *J. bulbosus* biomass after the die-back of the *Juncus* mats at the turn of the century (Fig. 1i+j). This dead litter is recalcitrant due to its low P concentration. It suffocates the remaining isoetid vegetation and does

not provide a favourable rooting substrate for isoetids. Also isoetids were observed to be covered by epiphytes in the limed lakes (Fig. 1h) which could have been an additional reason for isoetid decline (Sand-Jensen & Sondergaard, 1981). In general, reducing conditions prevail in locations where isoetids have disappeared (Smolders *et al.*, 2002), which may strongly hamper the recolonisation of isoetids. Contrary to most other aquatic plant species, isoetids lack suberisation or lignification of the root walls, which makes them highly permeable to gases. In reducing sediments, roots with a high permeability for gases lose oxygen because their environment serves as a high oxygen sink (Smits *et al.*, 1990; Smolders *et al.*, 2002). It is known that isoetids can resist short periods of tissue anoxia but longer periods of anoxia can cause mortality (Sand-Jensen, Borum & Binzer, 2005). This may explain why the isoetids have not regrown.

Implications

Liming has been reported to have a positive effect on the richness of acid-sensitive invertebrates and overall fish abundance (Mant *et al.*, 2013). Based on the number of macrophyte species in the limed lakes in 2010, one could argue that liming has increased the diversity of macrophytes as well. These very oligotrophic SW Norwegian lakes, however, are characterised by a very typical extensive and species-poor isoetid vegetation which is strongly affected as a result of liming. The slow colonisation and expansion of other non-isoetid submerged macrophytes also occurred on experimental

plots that were limed four times in lake Dybingsvatn (Lucassen *et al.*, 2012). Apparently, changes in carbon and nutrient availability due to liming eventually enable growth of non-isoetid submerged macrophytes. The possibility of elodeid plant species to colonise isoetid-dominated softwater lakes by these factors was also shown in competition experiments (Spierenburg *et al.*, 2009, 2010).

It remains unclear if a recovery of the highly typical isoetid vegetation will take place in the future even when liming is permanently stopped. Removing the recalcitrant dead *J. bulbosus* matter now covering a large part of the littoral sediments might be a prerequisite for isoetid recovery. In reducing sediments recovery of the vegetation is difficult as germination of isoetids as well as survival of seedlings is hampered under anoxic sediment conditions (Sand-Jensen *et al.*, 2005). Recolonisation may locally occur from the shore by *L. uniflora*. This isoetid species has the possibility to reproduce asexually by developing stolons and thus to recolonise parts of the limed sediments with less unfavourable sediment conditions. By having a high ROL activity, *L. uniflora* may enable germination of other isoetid plant species that fully depend on reproduction by seeds. However, it remains uncertain whether this type of vegetation development is stable in the long term as isoetids remain sensitive to uprooting on soft degraded organic sediments now present in the limed SW Norwegian lakes.

Interestingly, in 2013 the unlimed and the limed lakes had similar pH and bicarbonate concentrations. Atmospheric S deposition has significantly declined over the last few decades in Southern Norway (over 80% from 1980 to 2010) resulting in pH increases in lakes (from <5 to >5.5) (Skjelkvåle, Wright & Henriksen, 1998; Hesthagen *et al.*, 2011b; Helliwell *et al.*, 2014). Water quality data of our lakes show that the decline of dissolved S concentrations was around 50% while pH increased from 4.8 to 5.6 in the period 1975–2013 (Fig. 4). So the unlimed acidified lakes are clearly recovering from acidification, as a result of decreased sulphur deposition.

The positive effect of liming on fish species such as brown trout (*Salmo trutta*), and Atlantic salmon (*Salmo salar*) are evident (Larsen & Hesthagen, 1995; Hesthagen *et al.*, 2011a). Atlantic salmon is much more sensitive to acidic water than brown trout and liming of surface waters may therefore still be required to preserve the former species. Liming of entire lakes, however, can be prevented by using dosers which apply limestone powder in rivers, depending on the water flow and pH below the liming sites (Hindar *et al.*, 1998; Hesthagen *et al.*, 2011a,b). Regarding the strong negative effects on

the characteristic isoetid vegetation, we suggest that the direct application of lime in lakes should be avoided where possible.

Acknowledgments

We thank J. Eygensteyn, P. van der Ven, R. van der Gaag and E. Piersson of the Department of General Instruments (Radboud University Nijmegen) for technical assistance with the chemical analyses during the last 20 years. We thank the Norwegian authorities (fylkesmannen) for provision of data on lake liming. Further we thank T-E. Brandrud, M. Mjelde, M. Maessen, B. Paffen, M. Oonk and H. Tomassen for their input in an early stage of the project.

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(Manuscript accepted 7 February 2016)