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RESEARCH ARTICLE

Improved prediction of vegetation composition in NW European softwater lakes by combining location, water and sediment chemistry

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Abstract Isoetids, as indicators of near-pristine softwater lakes, have a high priority in national and international (European Water Directive Framework) assessments of ecological lake quality. Our main goal was to identify the most important environmental factors that influence the composition of plant communities and specifically determine the presence and abundance of the isoetid *Lobelia dortmanna* in NW European softwater lakes. Geographical position and composition of surface water, porewater, sediment and plant communities were examined in 39 lakes in four regions (The Netherlands, Denmark, West Norway and East Norway) distributed over a 1,200-km long distance. We confirmed that lake location was accompanied by significant changes in environmental variables between NW European lakes. Lake location was the single most important determinant of vegetation composition and it had significant individual contributions independent of the coupling to environmental variables. This influence of location was supported by a significant decline of community similarity with geographical distance between pairs of lakes at regional, inter-regional and international scales. Combining the geographical position with environmental variables for surface water, porewater and sediment significantly improved prediction of vegetation composition. Specifically, the combination of latitude, surface water alkalinity, porewater phosphate and redox potential offered the highest correlation (BIO ENV correlation 0.66) to vegetation composition. This complex analysis can also account for high sediment variability in the littoral zone of individual lakes, by using site-specific physico-chemical sediment factors, and offer better predictions of vegetation composition when lake water chemistry is relatively homogeneous among lakes within regions.

Keywords Softwater lakes · Isoetids · Latitude · Alkalinity · Phosphate · Redox potential · Porewater · Surface water · Sediment mineralisation

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Introduction

Near-pristine oligotrophic softwater lakes in Northern Europe and North America are dominated by a submerged vegetation with isoetids in the shallow littoral zone (Hutchinson 1975). Isoetids have small leaves in a rosette and well-developed roots from a short stem and extensive air channels allowing efficient intra-plant transport of O_2 and CO_2 between roots and leaves (Pedersen et al. 1995). Most CO_2 for plant photosynthesis is derived from the richer CO_2 source in the sediment and not from the lake water (Wium-Andersen 1971; Søndergaard and Sand-Jensen 1979) thanks to high gas permeability of root surfaces compared to leaf surfaces and fast intra-plant gas transport. Isoetids are evergreen, stress-selected plants (Grime 1977) with the lowest growth and mortality rates among aquatic species (Nielsen and Sand-Jensen 1991). While these traits are essential adaptations to clear-water lakes low in inorganic nutrients and carbon, they also make the isoetid vegetation very susceptible to acidification, alkalinisation, eutrophication or brownification (i.e. increasing water color) of the lakes from human impact (Arts 2002; Murphy 2002).

Isoetids have experienced profound restrictions in their distribution, particularly in lowland regions with intense agricultural activity and high population density (Roelofs 1983). Depending on the exact type and intensity of stressor(s), key isoetid species such as *Lobelia dortmanna*, *Littorella uniflora* and *Isoetes* spp. have declined or disappeared entirely and been replaced by nutrient-demanding, fast-growing algae and rooted species (Sand-Jensen et al. 2000; Arts 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 quality and biodiversity and international evaluation of high ecological quality such as the European Water Directive Framework (Moss et al. 2003; Stelzer et al. 2005). Our main goal here was, therefore, to identify the most important environmental factors that influence the vegetation composition and specifically determine the presence and abundance of *L. dortmanna*, which is the species that best exemplifies the essential characteristics of isoetids in near-pristine oligotrophic softwater lakes (Arts et al. 1989).

Species distribution and abundance are influenced by the geographical setting of lakes and locally by water and sediment quality within the lakes (Rørslett 1991; Smolders et al. 2002). However, the combined influence of geographical location, water quality and sediment quality has not been tested as yet. Many studies have related water quality (e.g., light attenuation, alkalinity, pH, nutrient concentrations) to the vegetation of softwater lakes (Toivonen and Huttunen 1995; Pedersen et al. 2006; McElarney et al. 2010), but only few have considered the influence of sediment type and geographical setting (Duarte and Kalff 1987; Rørslett 1991; Crow 1993). The geographical location should be important for the presence of species and the composition of communities because it determines the vegetation history within regions, in addition to climate, geology and composition of major ions. Therefore, we expect the presence and abundance of individual plant species to vary between regions for historical reasons unrelated to water and sediment chemistry. The composite property, species richness, nonetheless, appears to be predictable for Scandinavia as well as for the individual countries from variables such as water alkalinity (conductivity), pH, water clarity and lake area (Rørslett 1991), while species composition and relative abundance are expected to vary among regions.

Lake waters are mixed regularly and most physico-chemical properties are easy to characterize for the entire system based on a few samples. Sediment parameters, in contrast, are site specific and more difficult to analyze and apply as an integrative measure. This can explain why surface water variables (Vestergaard and Sand-Jensen 2000a) more than sediment variables (Smolders et al. 2002; Gacia et al. 2009) have been used extensively in multivariate analysis to predict composition of the submerged plant community. However, porewater chemistry and sediment characteristics can have a strong impact on growth and survival of rooted plants in general and isoetid species in particular (Raun et al. 2010). Isoetids have roots in close association with mycorrhiza fungi (Søndergaard and Laegaard 1977) and derive virtually all of their CO_2 and inorganic nutrients via the roots from the sediments (Christiansen et al. 1985). When labile organic matter accumulates in surface sediments, isoetid species face reduced growth, lower survival and greater risk of uprooting (Møller and Sand-Jensen 2010; Pulido et al. 2011), but the performance of non-isoetid species is also restricted in highly organic sediments (Barko and Smart 1983; Raun et al. 2010). Hence, to better understand and predict the impact of environmental variables, we here include porewater chemistry and sediment properties together with lake water chemistry in multivariate analyses to predict the distribution and presence of submerged species in oligotrophic softwater lakes. To evaluate the consequence of geographical setting, we analysed 39 lakes in four regions (The Netherlands, Denmark, West Norway and East Norway) distributed over a 1,200-km long distance.

Methods

Sites and vegetation

During three summers (2007, 2008, and 2009), 48 littoral sites (water depth 0.5 m), were sampled in 39 softwater lakes where *L. dortmanna* is present or has been present not long ago. Sites were deliberately selected away from sports activities and in the least wind exposed site of the lakes to minimize the effect of physical disturbance. However, the effect of wind-exposure among countries cannot be ruled out because lake size differed; Norwegian lakes tended to be larger. Although some of the lakes were water table regulated, none of them experience dramatic drawdown zones. The data set included nine Dutch lakes (nine sites; one site was sampled in each lake), 12 Danish lakes (13 sites; Lake Hampen was sampled at two sites) and 18 Norwegian lakes (26 sites; because two sites were sampled in Lake Dybingen, three sites in Lake Barstad and Haptajorna and four sites in Lake Fagervatn). Vegetation composition was determined in 41 sites, while *L.*

dortmanna abundance was determined in all 48 sites. Vegetation composition as relative cover of species within a 2 m^2 plot was determined following a modified Transley scale (Oliver and Tansley 1904). Both vegetation composition and *L. dortmanna* abundance were transformed to Van der Mareel's scale (Table 1S; Van der Mareel 1979). Geographical position (latitude and longitude), surface water, porewater, sediment, and vegetation data were collected from each site (Table 1, Table 2S).

Surface water, porewater and sediment

Surface water (sw) was collected in glass bottles, kept at 4°C and analysed within a week at Radboud University for alkalinity, Fe, K, Mn, Ca, Cl, Mg, Na, S, and Si as in Pulido et al. (2011). Bicarbonate (HCO_3) was calculated as total inorganic C (DIC) minus free CO_2 and CO_3 derived from pH. DIC was measured by an infrared gas analyzer after converting DIC to CO_2 by addition of acid. Certain variables (NO_3, NH_4, PO_4, and CO_2) and pH, were not included in the subsequent data analysis because they are highly variable in time. Thus, timing of sampling could hide or cause non-reproducible differences among sites. Instead, porewater NO_3, NH_4, PO_4, CO_2 and pH were considered less variable over time and more site specific than surface water analysis.

Porewater (pw) samples were collected without air contact from the rhizosphere (5 cm depth) using ceramic lysimeter cups. Porewater was extracted by connecting the soil moisture samplers to 60-mL syringes, exposed to underpressure and subsequently transferred to air-tight glass bottles. Samples were analysed for pH, DIC, NO_3, NH_4 and PO_4, Fe, K, Mn, Ca, Cl, Mg, Na, S and Si as in Pulido et al. (2011). Redox potential was measured (during field work) by a redox combination electrode. The electrical potentials measured were converted to redox potentials relative to the standard hydrogen potential measured (E_h) by adding the reference and correcting for temperature and porewater pH.

Sediment (sed) cores (10 cm in diameter and 15 cm in depth) were withdrawn from the rhizosphere, placed in ziplock bags, and kept cold (approx. 4°C) until further analysis. Water content, density, organic matter content, plant available NO_3, NH_4 and P, total C, N, P, Fe, Ca, Mn, Mg, Na, and Si, and mineralisation rates (CO_2 and CH_4^+ production) under anaerobic conditions were measured as in Pulido et al. (2011).

Statistical analysis

To determine which combination of a maximum of four environmental variables could best account for the variability of vegetation composition ($n = 41$) and *L. dortmanna* abundance ($n = 48$) species, data were first analysed by non-metric multidimensional scaling (NMDS, Clarke 1993) by the program package PRIMER (Clarke and Warwick 1994). Community similarities were calculated using the Bray–Curtis similarity coefficient (Bray and Curtis 1957) directly on the data. Nonparametric Spearman rank correlations among distance matrices on four log-transformed environmental variables (single and in combination) and similarity matrices on species associations were tested with the BIO ENV procedure (Clarke and Ainsworth 1993). The BIO ENV procedure uses the Spearman correlation when correlating the two similarity matrices, i.e., the similarity matrix of species composition and the similarity matrices of environmental variables. High Spearman correlation means that lakes with the same species composition also have the same environmental conditions. PRIMER does not accept missing values; therefore we filled the lack of data by medians when necessary. To cope with geographical patterns, the groups were classified according to their distances measured as the physical distance between lakes based on GPS data. The significance of differences of groupings were tested using one-way ANOSIM analysis ($P < 0.001$). Graphs were drawn with Prism 5.01. Environmental variables were individually correlated by Spearman's rank correlation.

Results

Multiple and single determinants of vegetation composition

The combination of latitude, surface water alkalinity, porewater phosphate and redox potential offered the highest correlation (0.66) to vegetation composition when all sites and environmental data were included in the multivariate analysis (Table 2). Latitude describes the geographical position of the lake, and thus the overall climate, geology and historical development of the vegetation, landscape and land use. Besides the direct effect, latitude can be expected to have indirect effects through significant correlations to several chemical variables in surface waters and sediments (Table 3). Higher latitude in the transect from The Netherland over Denmark to Norway was accompanied by falling concentrations of alkalinity and Ca surface waters; and declining concentration of PO_4, Ca and Mg in porewater due to less carbonate and clay minerals in the soils surrounding the lakes and less agricultural application of phosphorus (Table 3).

Alkalinity of surface waters is mainly determined by HCO_3, and because it mainly derives from dissolution of carbonate and clay minerals at relatively high soil pH, many macro-ions in surface and porewater were positively correlated to alkalinity (e.g. Ca, Mg, Na), while Fe was negatively related because iron minerals mainly undergo dissolution at low soil pH (Table 3). Phosphate in the sediment porewater

Table 1 Environmental variables

	All sites $n = 48$			East Norway $n = 8$			West Norway $n = 18$			Denmark $n = 13$			The Netherlands $n = 9$		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
Latitude	56.6	59.8	51.4	59.0	59.8	58.4	58.4	58.5	58.4	56.1	56.5	55.6	51.6	52.4	51.4
Longitude	11.9	11.9	5.2	9.2	11.9	8.4	6.3	6.5	6.0	9.4	9.7	8.8	5.5	7.0	5.2
Surface water															
Alkalinity (μM)	171.3	1,977.5	1.0	88.6	111.0	53.5	92.9	219.2	1.0	768.6	1,977.5	88.9	205.7	1,199.0	46.1
Fe (μM)	2.9	29.5	bd	2.9	4.9	1.4	2.6	29.5	0.3	1.4	7.7	bd	5.9	17.5	1.1
Mn (μM)	0.3	1.1	bd	0.2	0.3	0.1	0.2	0.4	bd	0.3	1.1	0.1	0.5	0.7	0.2
Ca (μM)	131.8	1,019.5	11.7	26.5	41.5	14.9	25.6	79.3	17.2	384.7	1,019.5	23.5	71.5	497.3	11.7
Cl (μM)	275.9	774.4	41.2	80.4	99.7	69.2	219.1	445.0	41.2	511.7	774.4	247.4	213.5	597.2	98.8
Na (μM)	256.3	716.7	27.4	80.1	100.5	65.3	207.6	377.6	27.4	469.9	716.7	212.5	193.2	468.2	88.0
Si (μM)	31.1	226.6	1.1	32.2	48.6	9.3	19.2	28.9	1.6	62.4	226.6	2.1	7.5	23.5	1.1
Porewater															
PO_4 (μM)	1.0	6.9	bd	0.7	1.9	0.1	0.4	3.6	bd	2.4	6.9	0.4	na	na	na
Fe (μM)	60.1	784.8	0.1	27.8	104.8	0.1	109.4	784.8	0.2	27.0	232.3	0.7	37.8	71.5	12.5
Mn (μM)	9.1	173.2	0.1	1.9	10.3	0.3	1.7	8.1	0.1	28.0	173.2	0.4	3.1	10.3	0.5
Ca (μM)	252.5	1,263.0	30.9	106.2	236.3	30.9	154.2	588.0	49.2	559.1	1,263.0	35.7	136.5	592.6	36.0
Mg (μM)	73.7	287.2	12.8	35.0	97.3	12.8	64.2	134.9	27.4	121.3	287.2	20.3	58.5	183.2	27.0
S (μM)	78.8	512.8	9.9	77.6	296.5	19.0	54.1	213.9	12.2	144.5	512.8	22.9	34.2	87.1	9.9
Redox (mV)	141.1	293.7	−72.0	na	na	na	59.5	134.6	9.8	62.1	293.7	−72.0	107.7	160.9	87.2
Sediment															
NH_4 (μM FS)	258.7	4,115.5	0.1	144.0	377.9	26.0	268.2	1,005.5	11.3	368.2	4115.5	8.3	183.4	434.0	0.1
Mg (μM FS)	37.7	152.5	0.8	42.3	113.2	4.6	69.6	152.5	0.9	5.2	17.1	0.8	16.7	85.0	2.1
Fe:P (molar ratio)	8.2	37.4	0.4	6.0	11.8	2.7	9.8	37.4	1.9	4.1	17.4	0.4	13.0	20.5	3.5
CO_2 (μmol CO_2 $L^{-1}h^{-1}$)	5.7	14.5	1.1	na	na	na	6.8	14.5	1.1	4.9	14.0	1.3	5.1	9.7	2.5
CH_4 (μmol CH_4 $L^{-1}h^{-1}$)	1.0	9.8	bd	na	na	na	0.5	5.3	bd	2.0	9.8	bd	1.1	5	bd
Ca (μM FS)	45.1	286.5	0.5	27.7	45.7	7.1	87.4	286.5	2.1	11.7	48.3	0.5	24.3	142.0	3.2

The most relevant surface water, porewater and sediment variables are given as means, maxima, and minima for all sites with *Lobelia dortmanna* abundance data, and for the four geographical regions considered in the analysis, East Norway, West Norway, Denmark, The Netherlands

bd Below detection limit, *na* not available, *FS* fresh sediment

Fe < 0.0054 μM, Mn < 0.004 μM, PO_4 < 0.1 μM, CH_4 < 0.1 μmol CH_4 $L^{-1}h^{-1}$

is a measure of the nutrient status of the lake, while sediment redox potential measures the availability of oxygen and other electrons acceptors. Low redox potential under reducing conditions may constrain root development.

When only one parameter was considered at a time in the BIO ENV analysis, latitude gave the highest correlation (0.47) with vegetation composition, followed by porewater PO_4 (0.40), water alkalinity (0.40), and porewater redox potential (0.37, Table 4).

Water, porewater and sediment as determinants of plant composition

One main objective was to test if introduction of porewater and sediment parameters alone or in combination with surface water parameters could offer the same or better predictions of plant composition in multivariate analyses. When only surface water was considered, the combination of alkalinity, Ca, Si and Mn provided the highest correlation (0.54, Table 2b). When only porewater was considered, the combination of PO_4, Ca, S, and redox potential provided the best and slightly higher correlation to vegetation cover (0.55, Table 2c) than water parameters. Finally, when only general sediment variables were considered, the correlation coefficient decreased (0.35, Table 2d). The production rate of CO_2 and CH_4 in the sediment under anoxia is a measure of organic matter lability. Organic matter lability with the Mg content and phosphorus mobility (expressed as the quotient of total iron to total phosphorus) was the best sediment predictor of vegetation composition. Overall, the variables (and inter-correlated variables in parentheses) showing the highest correlations to vegetation composition were alkalinity (Ca, Mg), phosphorus porewater concentrations and mobility in

Table 2 The best combination of four environmental variables in BIO ENV analysis is correlated to vegetation composition or *Lobelia dortmanna* abundance

Type of analysis; sites and env. variables	*n*	Correlation coefficient Env. variables
Vegetation composition		
a) All sites, all env. variables	41	**0.662**
		Latitude, alkalinity-sw, PO_4-pw, redox-pw
b) All sites, only surface water	41	**0.541**
		Alkalinity-sw, Ca-sw, Si-sw, Mn-sw
c) All sites, only porewater	41	**0.554**
		PO_4-pw, Ca-pw, S-pw, redox-pw
d) All sites, only sediment	41	**0.348**
		CO_2-sed, CH_4-sed, Mg-sed, Fe:P-sed
e) East Norwegian sites, all env. variables	8	**0.603**
		Longitude, Cl-sw, Ca-sed, Mn-pw
f) West Norwegian sites, all env. variables	18	**0.608**
		Fe-sw, Fe-pw
g) Danish sites, all env. variables	13	**0.540**
		Alkalinity-sw, PO_4-pw, redox-pw
Lobelia dortmanna abundance		
h) All sites, all env. variables	48	**0.559**
		Latitude, PO_4-pw
i) East Norwegian sites, all env. variables	8	**0.518**
		Na-sw, NH_4-sed, Mg-pw, Mn-pw
j) West Norwegian sites, all env. variables	18	**0.319**
		Fe-sw, Fe-pw
k) Danish sites, all env. variables	13	**0.651**
		PO_4-pw

Correlation coefficients are shown in bold

n Number of sites used in the analysis, *env. variables* environmental variables, *sw* surface water, *pw* porewater, *sed* sediment, *CO_2-sed and CH_4-sed* production of CO_2 and CH_4, respectively, by sediment mineralisation, *NO_3-sed and NH_4-sed* plant available NO_3 and NH_4 respectively

sediments (Fe:P quotient), and sediment reduction status and decomposition rate (redox potential, CO_2 and CH_4 production). Consideration of all environmental variables in water, porewater and sediment offered the highest correlation, while surface water or porewater variables alone had almost the same correlation (Table 2a–d).

Distance between lakes and vegetation similarity

Bray–Curtis similarity of vegetation composition between all pairs of lakes was significantly negatively correlated to distance between lakes at both regional, inter-regional and inter-national scales (Fig. 1). Similarity between lakes varied greatly from very low to the highest values at regional scales, while the variation of similarity gradually declined at inter-regional and inter-national scales because the highest similarities vanished. At the regional scale, the similarity in vegetation composition varied more between pairs of Danish lakes than lakes in East Norway in accordance with greater environmental variability (e.g. alkalinity, PO_4) in the Danish lakes (Table 1). The four geographical regions differed significantly (ANOSIM; $P < 0.001$).

Table 3 Spearman's correlations coefficient among latitude, surface water alkalinity, porewater PO_4 and redox potential and the most relevant surface water, porewater and sediment variables

	Latitude	Alkalinity-sw	PO_4-pw	Redox-pw
Latitude		**−0.41****	**−0.38***	−0.14 ns
Longitude	−0.18 ns	**0.56*****	**0.56*****	**−0.33***
Surface water				
Alkalinity	**−0.41****		**0.43****	−0.20 ns
Fe	**0.38***	**−0.42****	−0.14 ns	0.24 ns
Mn	−0.01 ns	−0.2 ns	0.13 ns	−0.06 ns
Ca	**−0.48*****	**0.76*****	**0.45****	−0.28 ns
Cl	**−0.61*****	**0.61*****	0.18 ns	−0.03 ns
Na	**−0.61*****	**0.61*****	0.19 ns	−0.04 ns
Si	0.28 ns	0.13 ns	0.05 ns	−0.15 ns
Porewater				
PO_4	**−0.38***	**0.43****		**−0.37***
Fe	0.00 ns	−0.01 ns	0.01 ns	0.02 ns
Mn	**−0.52*****	**0.47***	**0.36***	0.01 ns
Ca	**−0.49*****	**0.39***	**0.34***	−0.28 ns
Mg	**−0.42****	**0.45***	0.23 ns	−0.14 ns
S	−0.14 ns	0.28 ns	0.29 ns	−0.07 ns
Redox	0.14 ns	−0.2 ns	**−0.37***	
Sediment				
NH_4	**0.38***	−0.07 ns	−0.15 ns	0.03 ns
Mg	**0.43****	**−0.41****	−0.23 ns	−0.04 ns
Fe:P	0.14 ns	**−0.42****	**−0.55*****	0.14 ns
CO_2	0.18 ns	−0.28 ns	−0.20 ns	0.05 ns
CH_4	−0.09 ns	0.24 ns	0.16 ns	−0.08 ns
Ca	**0.39***	**−0.37***	−0.14 ns	−0.20 ns

Bold values are statistically significant

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

ns Not significant

Environmental determinants of vegetation composition at regional scales

The variation in vegetation composition should be sensitive to the environmental gradients realized within regions. Thus, when only East Norway was considered, the

Table 4 BIO ENV correlation coefficients for the selected environmental variables

Environmental variable	BIO ENV correlation coefficient
Vegetation composition ($n = 41$)	
Latitude	0.467
Alkalinity	0.401
PO_4-pw	0.402
Redox-pw	0.370
Lobelia dortmanna abundance ($n = 48$)	
Latitude	0.516
Alkalinity	0.128
PO_4-pw	0.207
Redox-pw	0.194

BIO ENV correlation coefficients are shown for selected environmental variables in relation to vegetation composition and *Lobelia dortmanna* abundance similarity matrixes

combination of longitude, Cl in surface waters, Ca in sediments and Mn in porewater showed the highest correlations to vegetation composition (Table 2e). In West Norway, Fe in surface water and porewater showed the highest correlations (Table 2f). In Denmark, the combination of surface water alkalinity and porewater PO_4 and redox potential offered the highest correlation to vegetation composition (Table 2g) in accordance with the pattern for the entire international dataset (Table 2a), and the fact that environmental variability was also strongest among Danish lakes (Table 1). Only two sites in The Netherlands contained the necessary information, they were too few to perform the necessary analysis.

Environmental variables in relation to presence and abundance of *L. dortmanna*

With all sites included, the combination of latitude and porewater PO_4 gave the highest correlation to abundance of the key isoetid species, *L. dortmanna* (Table 2h). At the regional scale in Norway, unexpected combinations of environmental variables showed the highest correlations (i.e., Na in surface water and Mg in porewater and sediment NH_4 in East Norway; Fe in surface water and porewater in West Norway; Table 2i, j). For Danish sites, porewater PO_4 showed the highest correlation to *L. dortmanna* abundance (Table 2k).

When only one parameter was considered at a time in the BIO ENV analysis, latitude returned the highest correlation (0.52) with *L. dortmanna* abundance, followed by porewater PO_4 (0.21), water alkalinity (0.13), and porewater redox potential (0.20, Table 4).

To establish the positive or negative influence of significant predictor variables that appeared in the BIO ENV analysis, we examined their individual relationship to the

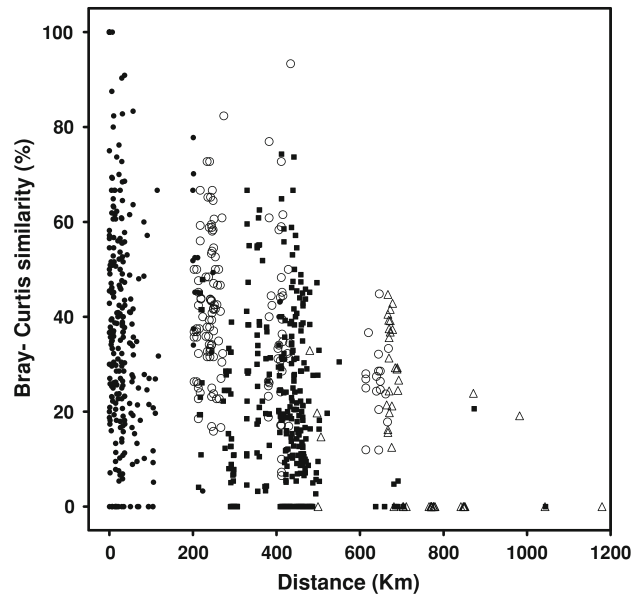


Fig. 1 Bray–Curtis similarity versus distance among sites. *Closed circles*—within a region ($r = -0.41$***), *open circles*—within Norway ($r = -0.25$ **), *closed square*—Norway–Denmark or Denmark–The Netherlands ($r = -0.17$ **), *open triangle*—Norway–The Netherlands ($r = -0.67$ ***). *r* Spearman's correlation. **$P < 0.01$; ***$P < 0.001$

presence and abundance of *L. dortmanna* (Fig. 2). Significant negative correlations were observed for surface water alkalinity and porewater PO_4, and significant positive correlations for higher latitude and sediment redox potential.

Discussion

Latitude, environmental variables and vegetation composition

Latitude was the single variable that correlated most strongly to species composition of submerged communities and abundance of the key isoetid, *L. dortmanna.* Although the importance of lake location for species richness is widely recognized (Duarte and Kalff 1987; Crow 1993; Gacia et al. 1994), only two studies (Rørslett 1991; Gacia et al. 1994) have verified the importance of lake location for the presence and abundance of isoetids in softwater lakes. Gacia et al. (1994) studied the influence of altitude in The Pyrenees, while Rørslett (1991) evaluated the influence of both altitude and latitude in Scandinavia. In both studies, as well as in the present study (Table 2), relative abundance of isoetids increased with higher altitude or latitude within temperate regions accompanied by falling temperature, shorter growth season, lower alkalinity and lower nutrient status of lakes. Still, we found latitude to be the strongest single determinant of vegetation composition and abundance of *L. dortmanna*, though combining latitude with alkalinity of surface water and PO_4 and redox potential of porewater produced the strongest overall prediction.

Even within regions, lake location was the strongest determinant of vegetation composition (i.e. longitude within East Norway; Table 2e). The presence and abundance of *L. dortmanna* was significantly higher in the two Norwegian regions than in Denmark and The Netherlands compared with other isoetid species typical of oligotrophic softwater lakes (Table 1S). The studied lake sites in The Netherlands did not differ significantly in alkalinity from the Norwegian sites (Mann–Whitney-U test) and location appeared to have a significant independent contribution to distribution and abundance of isoetids (Tables 1, 2). Also, the presence and abundance of *L. uniflora* was significantly higher in The Netherlands, Denmark and West Norway than in East Norway compared with *L. dortmanna*, probably reflecting the more Atlantic distribution of *L. uniflora* in contrast to the more boreal distribution of *L. dortmanna* (Table 1S, Hylander 1955). In The Netherlands, *L. dortmanna* is close to its southern continuous distribution limit (Arts and den Hartog 1990) and populations here are more susceptible to extinction than Norwegian populations. If global warming progresses, this difference will be accentuated as *L. dortmanna* may fall outside its distribution range in The Netherlands while West and East Norway may fall in the centre of the future distribution. The number of *Lobelia* lakes in The Netherlands is already so low that the likelihood of recovery after disappearance of yet another *L. dortmanna* population is very low, whereas the recruitment potential is high in Norway where *L. dortmanna* is widespread. The higher human impact in Dutch lakes should further restrain recovery here.

Several previous studies have demonstrated the main role of water alkalinity and trophic state as predictors of species distribution in lakes (Srivastava et al. 1995; Vestergaard and Sand-Jensen 2000a; Pedersen et al. 2006). Species richness increases significantly with alkalinity because more tall-growing species capable of using HCO_3 for photosynthesis exist in the species-rich group of tall elodeid growth form (Vestergaard and Sand-Jensen 2000b). In contrast, isoetids tend to decline with rising alkalinity, not because alkalinity per se is harmful to isoetids (Seddon 1965) but because tall elodeids thrive better at higher HCO_3 concentrations and tend to overgrow isoetids. Submerged plant species richness in general and isoetid species richness in particular are negatively related to phosphate concentrations in surface waters (Vestergaard and Sand-Jensen 2000a; Smolders et al. 2002; Pedersen et al. 2006). Alkalisation and eutrophication that facilitate the faster grow of competitors (e.g., phytoplankton, filamentous algae, tall elodeids and helophytes) can cause the disappearance of *L. dortmanna* and other isoetids (Arts 2002). The isoetid, *L. dortmanna*, is particularly successful in nutrient-poor softwater lakes since it is highly efficient in using sediment-derived CO_2 and attaining sufficient PO_4 because of slow growth, low nutrient requirements in the tissue and cooperation with mycorrhiza fungi in the sediments for nutrient retrieval (Wigand et al. 1998). Alkalinisation of lake waters and enrichment of sediments with phosphorus and organic carbon can specifically reduce growth and survival of *L. dortmanna* by stimulating mineralisation rates, nutrient supply to competitors and reducing redox potentials and oxygen availability in sediments. This is particularly critical for *L. dortmanna* because of the extraordinarily high dependence of sediment properties and negligible oxygen uptake across leaf surfaces that increases the risk of tissue anoxia if sediments become deprived of oxygen during night respiration (Møller and Sand-Jensen 2010).

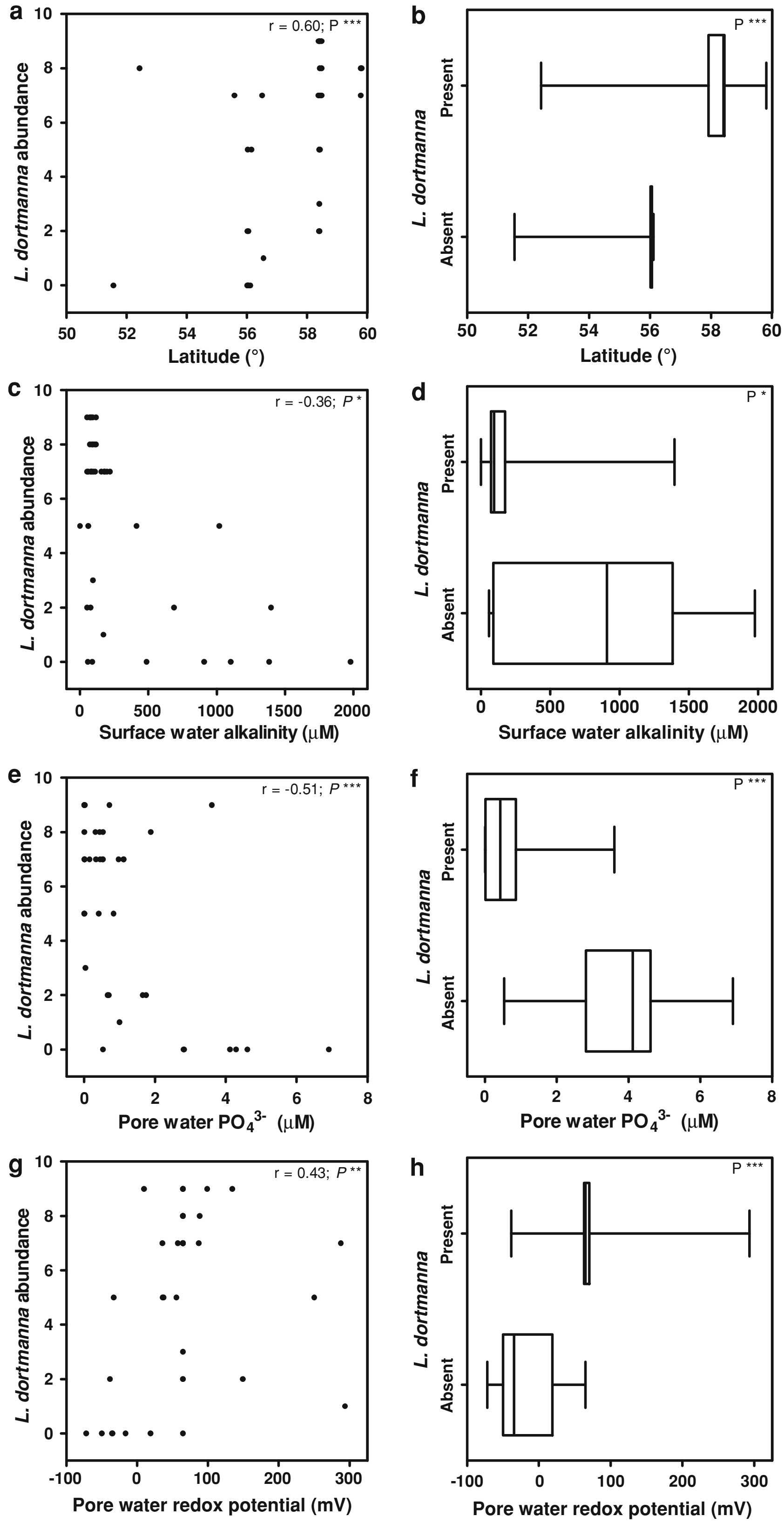
Lake distance and vegetation composition

Considering the importance of lake location (i.e. latitude and longitude) for vegetation composition, we should anticipate a significant decline of the similarity of vegetation composition with increasing distance between pairs of lakes. Indeed, we observed this decline with distance between lakes within regions, between regions and across the entire 1,200-km latitudinal range (Fig. 1). The similarity between pairs of lakes varied extensively at all spatial levels and it was primarily high similarities that declined with increasing distance between lakes, while low similarities appeared at all spatial scales. Some inter-regional and international spatial scales were sufficiently large to produce substantial changes in the presence and abundance of both common (i.e. *L. uniflora* and *L. dortmanna*) and rare isoetids (i.e. *Elatine hexandra*, Table 1S). Both the high variability and low similarity at all spatial scales are probably a result of our methodological approach because we determined vegetation composition in 2 m^2 large littoral sites. Such sites will usually not include all submerged species within a lake and appreciable dissimilarity can occur among sites if the lake is large and the littoral zone highly heterogeneous. Our approach does have the major advantage, however, that sample size is constant, shallow littoral sites are truly comparable among lakes in terms of water depth and bathymetry, which would have a strong influence if the presence and relative abundance of submerged species had been integrated for an entire lake.

Surface water, porewater, sediment and plant communities

Surface water, porewater or sediment variables alone offered significant correlations to vegetation composition,

Fig. 2 *Lobelia dortmanna* versus environmental variables in sites with vegetation composition data. *Lobelia dortmanna* abundance (*left hand*; shown by *scatter plots*) and presence-absence (*right hand*; shown by *box* and *whiskers* indicating medians, 25 and 75% percentiles and range) versus latitude (**a**, **b**), surface water alkalinity (**c**, **d**), porewater PO_4 (**e**, **f**) and porewater redox potential (**g**, **h**; $n = 4$). Spearman's correlation was used to correlate *L. dortmanna* abundance and Mann–Whitney *U* test was used to test differences for presence-absence: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$



although all variables together improved the interpretation by combining predictor variables from both surface water (e.g. alkalinity), sediment porewater (e.g. PO_4, redox potential) and general sediment properties (e.g. organic matter lability, Table 2). Thus, the more complex analysis can offer better predictions of community structure in lakes, particularly in regions where terrestrial soils and lake water chemistry are relatively homogeneous (Gacia et al. 2009). In the softwater lakes of the Pyrenean mountains, isoetid communities (mainly *Isoetes lacustris* and *I. echinospora*) were associated with higher redox potential and lower PO_4 in the sediments, like in other study (Fig. 2), and in addition with higher NO_3 relative to NH_4 because of stimulation of nitrification by oxygen release from roots.

In our study, some unexpected correlations appeared at regional scales (e.g. lake water Na and Cl and sediment NH_4 in East Norway; Fe in lake water and sediment porewater in West Norway, Table 2), and although these correlations are difficult to explain with our present knowledge, they raise new hypotheses on plant distribution that may be worthwhile testing experimentally in the future. Because species distribution is sometimes highly heterogeneous within lake basins, site-specific physico-chemical sediment factors and historical vegetation development need to be included to account for the variability in vegetation composition (Pearsall 1929; Pedersen et al. 2006; Gacia et al. 1994, 2009).

To conclude, we have confirmed that lake location was accompanied by significant changes in environmental variables between The Netherlands, Denmark and West and East Norway. Lake location was the single most important determination of vegetation composition and it had significant individual contributions independent of the coupling to environmental variables. This influence of location was supported by a significant decline of community similarity with geographical distance between pairs of lakes at regional, inter-regional and international scales. Combining latitude with environmental variables for surface water (e.g. alkalinity), porewater (e.g. PO_4, redox potential) and sediment (e.g. organic matter lability, Fe:P) significantly improved prediction of vegetation composition. This complex analysis can also account for high sediment variability in the littoral zone of individual lakes and offer better predictions of vegetation composition when lake water chemistry is relatively homogeneous among lakes within regions.

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