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Reasons for the dramatic loss of *Lobelia dortmanna*, a keystone plant species of softwater lakes in the Northern Hemisphere

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Abstract

1. *Lobelia dortmanna* is an iconic keystone species of northern softwater lakes in Europe as well as North America. It has suffered a dramatic decline in distribution in recent decades and the root causes are not well-known, although elements such as eutrophication, acidification and brownification have been suggested as underlying reasons for the decline.

2. In order to quantify the causes of change in the occurrence of *L. dortmanna* in Danish lakes, we firstly mapped the historical distribution of the species and secondly collected the available recent information on the presence of *L. dortmanna* along with data on environmental variables and plant community composition from the lakes. We identified 168 original Lobelia lakes of which *L. dortmanna* had, by now, gone extinct in 48%. A subset of 80 lakes formed the backbone of this study owing to the availability of data related to environmental conditions and aquatic plant communities.

3. Using piecewise structural equation models, we identified that only the mean height of the lake plant communities had a direct negative influence on the occurrence of *L. dortmanna*. The expected adverse effects of eutrophication and alkalinity were all indirect, and funnelled through the trophic affinity of the plant community, which in turn influenced the mean plant height. Direct effects of eutrophication via shading from phytoplankton, acidification or brownification were not observed.

4. We show that eutrophication, particularly in more alkaline water, is likely to have caused the dramatic decline in occurrence of *L. dortmanna*, but our models indicated that the effect of eutrophication acted via interspecific competition from other larger rooted aquatic plants.

5. Conservation efforts aimed at protecting small aquatic plant species should be prioritised in more alkaline lakes where such species are most at risk. Furthermore, managers should be aware of the threat of interspecific competition posed by larger aquatic plants on their smaller counterparts.

KEYWORDS
bicarbonate, competition, eutrophication, isoetids, rare species

1 | INTRODUCTION

Lobelia lakes are named after the characteristic isoetid, *Lobelia dortmanna*, that inhabits oligotrophic lakes of low alkalinity and low total hardness (Murphy, 2002). Lobelia lakes also host other slow-growing isoetids such as species of *Isoetes* and *Littorella uniflora* (Farmer & Spence, 1986; Sand-Jensen & Søndergaard, 1979). These lakes are still common in Scandinavia and parts of North America, and many of these still host healthy populations of *L. dortmanna*. However, as a consequence of the low stature of the plant and its inherently slow growth (Moeller, 1978), *L. dortmanna* is particularly vulnerable to abiotic stress and competition from fast-growing species (Arts, 2002; Roelofs, 2002). Also in the specific case of Danish Lobelia lakes, these lakes were much more common only 100 years ago (Schou et al., 2017) but allegedly as a result of changes in land use and water chemistry, are now rare habitats. This is not only the case in the Danish landscape, but also in the Netherlands and Germany where *L. dortmanna* is classified as endangered (Metzing et al., 2018). The following will address eutrophication, alkalinisation, brownification and loss of habitat, and how these may have contributed to the extinction of local populations of *L. dortmanna*.

The disadvantage of being a small plant with its leaves in a rosette is that *L. dortmanna* is unable to stretch towards the sunlight as a response to shading from tall plants, phytoplankton or coloured dissolved organic matter (cDOM; Sand-Jensen et al., 2018). The maximum colonisation depth is determined by the amount of light reaching the lake bottom and a recent study showed that it may take up to 50% of the surface irradiance to maintain healthy populations of *L. dortmanna* (Borowiak et al., 2017). Consequently, accelerated growth of tall plants and phytoplankton, both common system responses to eutrophication of lakes (Sand-Jensen & Borum, 1991), pose a threat to *L. dortmanna* as the tiny plants are unable to elongate and thereby reach the light in a deteriorating light climate. Furthermore, epiphytes (filamentous or unicellular algae) also respond to eutrophication while the inherent slow growth prevents *L. dortmanna* from responding to increased coverage of epiphytes by increasing the leaf turnover, which is otherwise a common response to epiphyte growth by other submerged plants (Sand-Jensen & Søndergaard, 1981). Interestingly, most isoetids including *L. dortmanna* are evergreen, so part of the reduced growth during summer can be compensated for during the winter when epiphyte cover tends to decline (Sand-Jensen & Borum, 1984). Nevertheless, the lack of capacity to elongate as a response to declining light makes *L. dortmanna* particularly vulnerable to eutrophication when epiphytes, taller plants with high nutrient affinity and phytoplankton all respond positively to excess nutrients (Roelofs, 1983).

The growth of aquatic primary producers is, however, not only regulated by the amount of available nutrients. Commonly, inorganic carbon is in short supply in the aquatic environment, limiting growth and biomass development (Kragh & Sand-Jensen, 2018). In addition to CO_2, bicarbonate (HCO_3^-) is a main source of inorganic carbon in many aquatic systems (Maberly & Gontero, 2017; Maberly & Madsen, 2002), and in submerged aquatic plants the ability to use bicarbonate as an inorganic carbon source is present in about half of the species tested so far (Iversen et al., 2019). Species with the bicarbonate-use trait are typically large species of the elodeid growth form, and bicarbonate-use has not been observed in *L. dortmanna* or any other isoetid species; these obtain inorganic carbon from the sediment via root uptake of CO_2 (Madsen, 1987; Wium-Andersen, 1971). Therefore, in environments rich in bicarbonate (measured as alkalinity), *L. dortmanna* might be exposed to heavy competition from the taller elodeid species fuelled by an extra carbon source. However, inorganic carbon and nutrients can co-limit primary production (Kragh & Sand-Jensen, 2018), including plant growth, potentially reducing the competitive advantage of the generally fast growing bicarbonate users under oligotrophic conditions, allowing tall elodeids and *L. dortmanna* to co-exist even in moderately alkaline lakes. Thus, particularly in alkaline lakes increasing nutrient concentrations can promote a dense plant community of taller plants with higher nutrient affinity.

A more recent threat to Lobelia lakes and their pristine submerged vegetation is the ongoing brownification of fresh water in the Northern Hemisphere (Roulet & Moore, 2006). Brownification is caused by cDOM absorbing strongly in the PAR (photosynthetically active radiation) spectrum (Peacock et al., 2019) with severe implications for submerged aquatic vegetation (Bociąg et al., 2011). Brownification of Lobelia lakes supposedly increased following the cessation of acid rain; during acidification of the catchment, cDOM produced in the terrestrial environment has been retained in the soils and subsequently released as pH again increased in the soil water (Ekström et al., 2011; Graneli, 2012). However, the brownification of Scandinavian lakes has continued beyond the point that is likely to be explained by leakage of accumulated cDOM in the soils and the brownification is now primarily driven by changes in land use (increase in coniferous forest; Kritzberg, 2017). Also, rising temperatures and increased atmospheric N deposition and precipitation stimulate terrestrial primary production and the subsequent production of cDOM (Hongve et al., 2004). Regardless, the tea-coloured Lobelia lakes are losing the pristine vegetation (Bociąg et al., 2011), or the plants are restricted to the uppermost parts of the littoral zone where sufficient light is still available (Madsen-Østerbye et al., 2018).

The most dramatic deteriorating human impact on aquatic ecosystems has been the massive draining of shallow lakes and wetlands throughout both North America (Dahl, 2011) and Europe where up to 80% of the areas have been lost (Verhoeven, 2014). It was not known to what extent Danish Lobelia lakes also had been lost in this process of extensive drainage of wetlands.

In the present study, we aimed at identifying the main causes for the observed dramatic decline in Danish Lobelia lakes. We based our analyses on 80 Lobelia lakes in which *L. dortmanna* was still present (44 lakes) or had gone extinct (36 lakes) over a period of approximately 100 years. We hypothesised that some lakes had been completely lost as a result of drainage, whereas extinction from the remaining lakes was driven by eutrophication and the subsequent consequences related to shading from phytoplankton or competition from tall and fast-growing aquatic plants. Using path analysis,

we were able to identify the effects of TP, Chlorophyll-*a* (Chl-*a*), alkalinity, trophic affinity of lake vegetation and the height of the plant community, as well as external factors such as soil types and land use in the catchment on the presence of *L. dortmanna*. Moreover, we used principal coordinates analysis (PCoA) to visualise differences in plant communities between lakes where *L. dortmanna* was still present and those where *L. dortmanna* had gone extinct.

2 | MATERIALS AND METHODS

2.1 | Occurrence of *Lobelia dortmanna*

In order to quantify the causes of change in the occurrence of *L. dortmanna* in Danish lakes, we firstly mapped the historical distribution of the species and secondly collected the available recent information on the presence of *L. dortmanna* along with data on environmental variables and plant community composition from the lakes.

Data on the occurrence of *L. dortmanna* in Danish lakes was obtained from a combination of various sources. A key botanical survey covering all of Denmark forms the backbone of the present dataset (Emsholm, 1992; Gravesen, 1976, 1979, 1982, 1983, 1986; Wind, 1990, 1992, 1994). We also used data from the Botanical Museum of Denmark (mostly excursion reports) and other private or public botanical surveys. The most recent data on the distribution of *L. dortmanna* were gathered from the botanical survey "Atlas Flora Danica" (Hartvig & Vestergaard, 2015) and vegetation data from the Danish Environmental Monitoring program (NOVANA, Svendsen & Norup, 2005) collected in the period 1995 to 2019.

The above approach resulted in identification of 168 lakes in which *L. dortmanna* had been registered at least once from around 1850 up to 2019. Of these 168 lakes, populations of *L. dortmanna* were still present in 88 lakes but had been lost in the remaining 80 lakes. For a subset of 95 lakes, it was possible to obtain additional data on vegetation composition and environmental variables such as water chemistry, soil types and land use. Owing to a few scattered missing values of the collected environmental variables, a core sample of 80 lakes with complete data coverage formed the backbone of the present study (Table S1). Of these, 44 lakes had recent populations of *L. dortmanna* (recent lakes) whereas the plant had gone extinct in 36 (extinct lakes).

2.2 | Vegetation data

Composition of lake vegetation was extracted from the database of the Danish Environmental Monitoring Program (Danish environmental portal) for recent and extinct Lobelia lakes in which a comprehensive vegetation survey had been conducted. If more than one survey was available, only the most recent was used. All of the surveys were conducted during the period from 2004 to 2019. Data included rooted and free-floating aquatic plants, aquatic mosses, macroalgae including charophytes, and terrestrial plants for which a

submerged lifeform is known. If a plant was identified only to genus, all species in that genus were merged to prevent the same species from occurring more than once (see Table S2 for the list of species included in this study). To study the effect of the plant community on the presence of *L. dortmanna*, we calculated the tropic affinity and plant height of the plant community as measures of competitive ability towards small plants such as *L. dortmanna*. Each identified species or genus was assigned a trophic affinity using the intercalibration common metric (ICM) value according to Kolada et al. (2014). The species-specific ICM values are based on plant occurrence and total phosphorus (TP) concentration in European lakes and normalised to a scale from 1 to 10 (1=lowest TP optima and 10=highest TP optima; Kolada et al., 2014). Each species or genus was assigned a maximum height (Schou et al., 2017). We expect plant species with a higher ICM and plant height to have higher growth rates and tolerate higher levels of eutrophication. Plant community traits of ICM and plant height were calculated as a mean value of the species being present in a lake.

2.3 | Environmental data

2.3.1 | Land use and soil types

We extracted data on land use and soil composition from the entire catchment and buffer zones around each lake to quantify the potential direct or indirect effects on the presence of *L. dortmanna*. The topographical catchment was delineated using a national digital elevation model (10m resolution; SDFE, 2020) and the TauDEM software (Tarboton, 2017). The hydrological conditioning of the elevation model is described in detail by Liu et al. (2018). We extracted soil type (Pedersen et al., 2011) and land use based on Corine Land Cover 2012 (Bossard et al., 2000) of both buffer zones and lake catchments. Although we tested several buffer zones around the lakes (10, 25, 50, 100, 200, 400, 800, 1,600 or 3,200m), we used topographical catchments for further analyses as correlation between land use and soil properties to water chemistry at the catchment scale were better or similar to those of the buffer zones. We identified the proportion of natural vegetation and moraine clay to be good predictors of TP and alkalinity, respectively, and used these for the path analysis (see below).

Using only the smallest buffer zone (10 and 25m), we also extracted the average height of objects above terrain to quantify shoreline sheltering. However, there was no apparent relationship between this and the presence–absence of *L. dortmanna* (Mann–Whitney U-test, $W=922$, p-value$=0.21$) and this variable was not use in further analyses.

2.3.2 | Chemical parameters and lake area

Data on water chemistry were obtained mainly from the Danish Environmental Monitoring Program (NOVANA; Danish

environmental portal). Mean summer values (May to September) based on 1–5 water samples were extracted from the years 2004 to 2019 covering all vegetation surveys. Only surface water observations between 0 and 3 m were used. Furthermore, supplementary water samples were collected in August 2019 for two lakes not included in the NOVANA program and analysed according to Danish Standards (DS, 2021). Only variables that were relatively constant over the year, or were to be analysed during the summer, were included in this study. This included alkalinity, pH, conductivity, TP, total nitrogen (TN), Chl-*a* (a proxy for phytoplankton biomass) and water colour (cDOM). Data on lake area were extracted as digital polygons from the Danish Areal Information System (Danish environmental portal).

2.4 | Data analyses

2.4.1 | Pairwise comparison of recent versus extinct lobelia lakes

Possible differences in environmental conditions and plant community characteristics between recent and extinct Lobelia lakes were tested by Mann–Whitney U-tests, owing to highly skewed distributions.

2.4.2 | Piecewise structural equation model

A multivariate analysis in the form of a piecewise structural equation model (SEM) was conducted to identify direct or indirect links between land use and soil type in the catchment, water chemistry, plant community traits and presence of *L. dortmanna*. The model was fitted using the R package *piecewiseSEM* (Lefcheck, 2016). In order to apply piecewise structural equation models, the hypothesised causal relationships must be proposed to indicate how the explanatory variables are linked to the response variables (Shipley, 2000). Thus, existing knowledge on Lobelia lakes was used to phrase an initial hypothesis on different pathways and interactions between the explanatory variables used in the model. Furthermore, a prescreening of water chemical analyses revealed that that TP and TN were highly collinear, and thus we used TP as a measure of eutrophication. Specifically, we hypothesised that eutrophication (TP concentration) would be most strongly associated with the loss of *L. dortmanna*, and that this could be either a direct effect (e.g. through the promotion of epiphytes) or an indirect result of an impoverished light climate (Chl-*a* concentration) or through the promotion of a more competitive plant community (ICM and plant height). Water colour (cDOM) also might directly affect light climate and thus strongly influence the loss of *L. dortmanna*. Alkalinity, pH and conductivity also were highly collinear ($r > 0.6$), and we choose to use only alkalinity in the analyses. Alkalinity, a measure of water bicarbonate concentration, proposedly acts synergistically with eutrophication as this supplementary inorganic carbon source promotes growth of

both epiphytes, phytoplankton and taller bicarbonate-using elodeid plants. We hypothesised that nutrient-poor conditions were caused primarily by land use such as natural vegetation. The alkalinity of the lake water stems from mineral weathering in the catchment and thus should be promoted by a soil type such as moraine clay. These hypotheses were used as a framework when creating and optimising the best model to fit the data. The model consists of several linear models each accounting for a predictor variable. The direct impact of explanatory variables on the presence of *L. dortmanna* was described using a binomial generalised linear model with a logit link function. As we predicted that alkalinity and TP were going to be significantly linked (Kolada et al., 2014), we specified a "correlated error" between the covariates as we were interested in including both of them as predictors. We applied square root (moraine clay, natural vegetation), $\log_{10}$ (TP, Chl-*a*) and $\log_{10}(x + 1)$ (alkalinity) transformations owing to skewed distributions of some of the explanatory variables. All individual models were validated by residual and quantile–quantile plots.

Build into the *piecewiseSEM* package is a "test of directed separation", which identifies missing statistically significant pathways and their impact on the quality of the overall model. This was used when optimising the model while monitoring the Akaike information criterion (AIC) when adding or changing a pathway to obtain the highest robustness of the model. If a pathway generated a lower AIC score, thereby increasing the strength of the model, it was included in the model. Relevant non-significant pathways ($p < 0.05$) were included in the final model. Fisher's C statistics were used to evaluate the model's goodness-of-fit. A non-significant *p*-value ($p > 0.05$) indicated that the model fitted the data well. Furthermore, pseudo R^2 values for each linear and generalised linear model were calculated.

2.4.3 | Principal coordinates analysis

In order to visualise the relationship between plant community and the underlying environmental gradients in recent and extinct Lobelia lakes, an unconstrained ordination analysis was conducted. The analysis uses the dissimilarity between plant communities in lakes to construct a graphical representation of the data in a low-dimensional space while explaining most of the variance in the original dataset. In this study, a PCoA (also known as classical multidimensional scaling) was conducted. This was chosen instead of ordination techniques such as principal component analysis and nonmetric multidimensional scaling, as it has proven to better preserve the distance among objects (Borcard et al., 2018). Another advantage of PCoA is that the input data have no assumed relationship and that any distance matrix can be applied. The ordination plot of a PCoA can be interpreted like other ordinations where similar objects are being positioned close to each other and dissimilar objects are positioned far apart (Borcard et al., 2018).

A matrix of dissimilarities between plant communities was calculated using a Sørensen distance on incidence data. A posteriori projection of environmental variables used in the fitted SEM was added

as vectors to the ordination plot. The length of the vectors defines the strength of the influence of variables on the plant communities. The PCoA was conducted using the R package *vegan* (Oksanen et al., 2019). To test for differences between the two lake groups (recent and extinct *L. dortmanna* populations) a permutational multivariate analysis of variance was applied using the calculated Sørensen distances.

3 | RESULTS

The 168 lakes where *L. dortmanna* had been observed previously are not distributed evenly across the country, but instead cluster in regions with aeolian sand deposits or sandy deposits from the Weichsel glacial period (Figure 1).

3.1 | Total loss of habitat

Of the original Lobelia lakes, 17 lakes that used to hold *L. dortmanna* do not exist any more because of conversion into arable land, plantations of coniferous forest or urban land. This complete loss of habitat accounts for 10% of all lakes in our dataset, and the case

presented in Figure 2 describes what could have happened to these lakes. During the 1800s, Lake Tarm Kær was situated in a heathland setting and the land was likely used for grazing. In neighbouring areas, draining activities had already begun but Tarm Kær itself was not directly affected until the mid-1900s when the land had been almost completely drained. In the 1960s the calamities continued as the area was further drained and the map from the late 1900s shows the now channelised river, Skjern Å, cut through the former lobelia lake. Beginning in 1999, restoration measures of the river valley have partly brought the river back to its original meandering path, and new wetlands and lakes have appeared. However, *L. dortmanna* has not been found in any of the restored wetlands.

3.2 | Water chemistry, land use and soil types

We tested key water chemical parameters such as alkalinity and TP along with lake phytoplankton (measured as Chl-*a*) and found that they were all significantly different in the two types of lakes (Figure 3). Lakes with recent populations of *L. dortmanna* had lower alkalinity, lower TP and therefore lower Chl-*a* compared to lakes with extinct populations. Likewise, pH and electrical conductivity also were significantly lower in lakes with a recent population
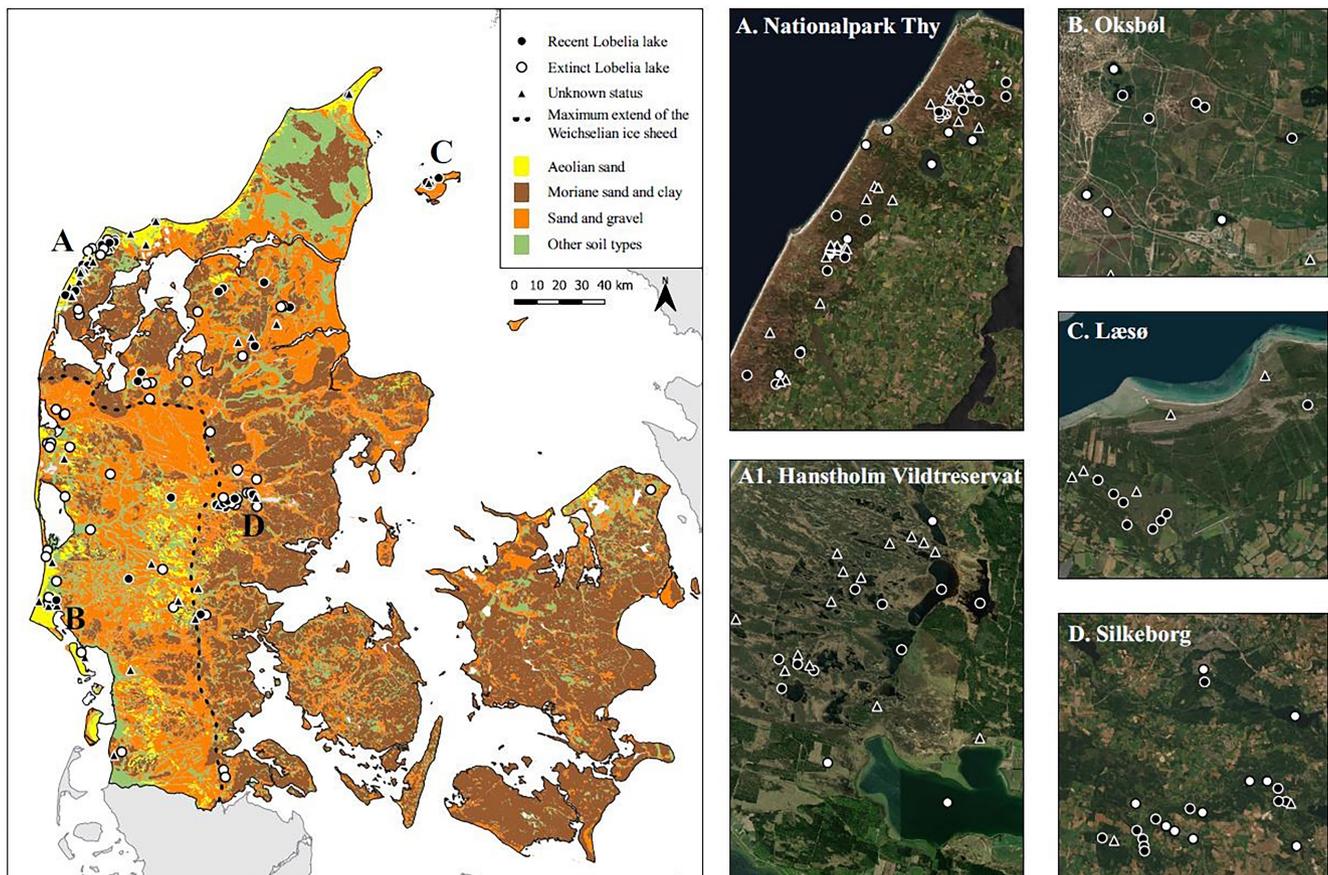

FIGURE 1 Map of recent or extinct populations of *Lobelia dortmanna* in Denmark. The five inserts show detailed maps of "hot spots" with particularly high density of Lobelia lakes; these water bodies are all located on aeolian sand or sandy deposits from the Weichsel glacial period.
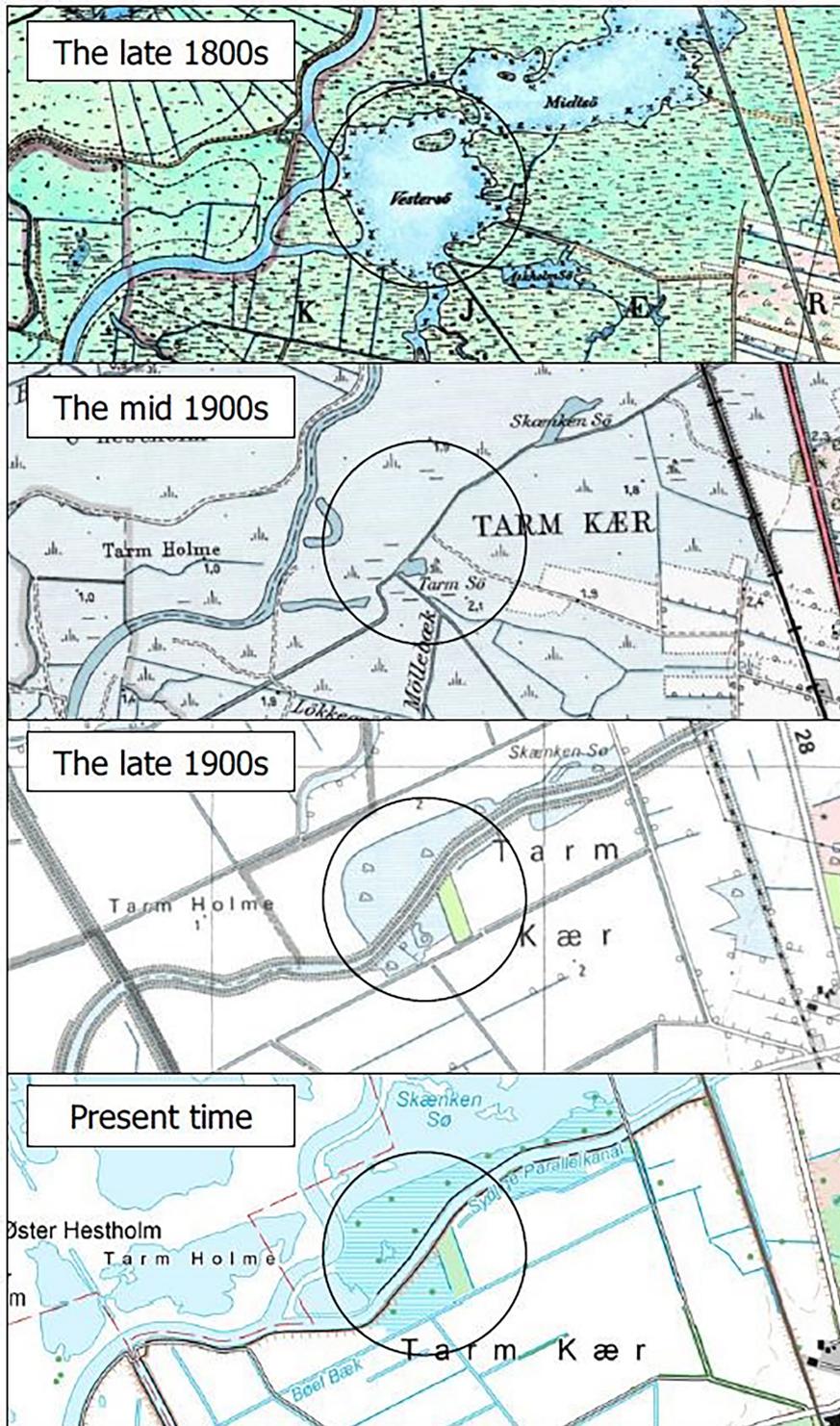
FIGURE 2 Example of Lobelia lakes that are completely lost as a result of changes in land use. *Late 1800s*; Lake Tarm Kær originally consisted by a number of small shallow lakes hosting populations of *Lobelia dortmanna*. *Mid 1900s*; the conversion of wetlands into farmland had begun and only a couple of puddles remain along with two oxbow lakes created by the nearby River Skjern Å. *Late 1900s*; channelisation of the river and drainage of the wetland are complete and the land had been converted into arable land. *Present time*; a large restoration project has partly restored the river valley and again converted the low-lying areas into wetlands.

of *L. dortmanna*, whereas TN and colour did not differ significantly between the two lake types (Figure S1).

Many aspects of land use and soil types are known to both influence the vegetation of softwater lakes, yet we found that the amount of agriculture in the catchment was the only parameter that differed significantly between the two types of lakes; lakes with extinct populations of *L. dortmanna* had more agricultural land in their catchment (Figure S1). By contrast, the amount of natural vegetation showed

no significant differences, and this also was the case for the two soil types of our focus, aeolian sand and moraine clays (Figures 3 and S1).

3.3 | The path leading to loss of *Lobelia dortmanna*

The suggested model of the effects of landscape and in-lake variables on the presence or extinction of *L. dortmanna* was insignificant
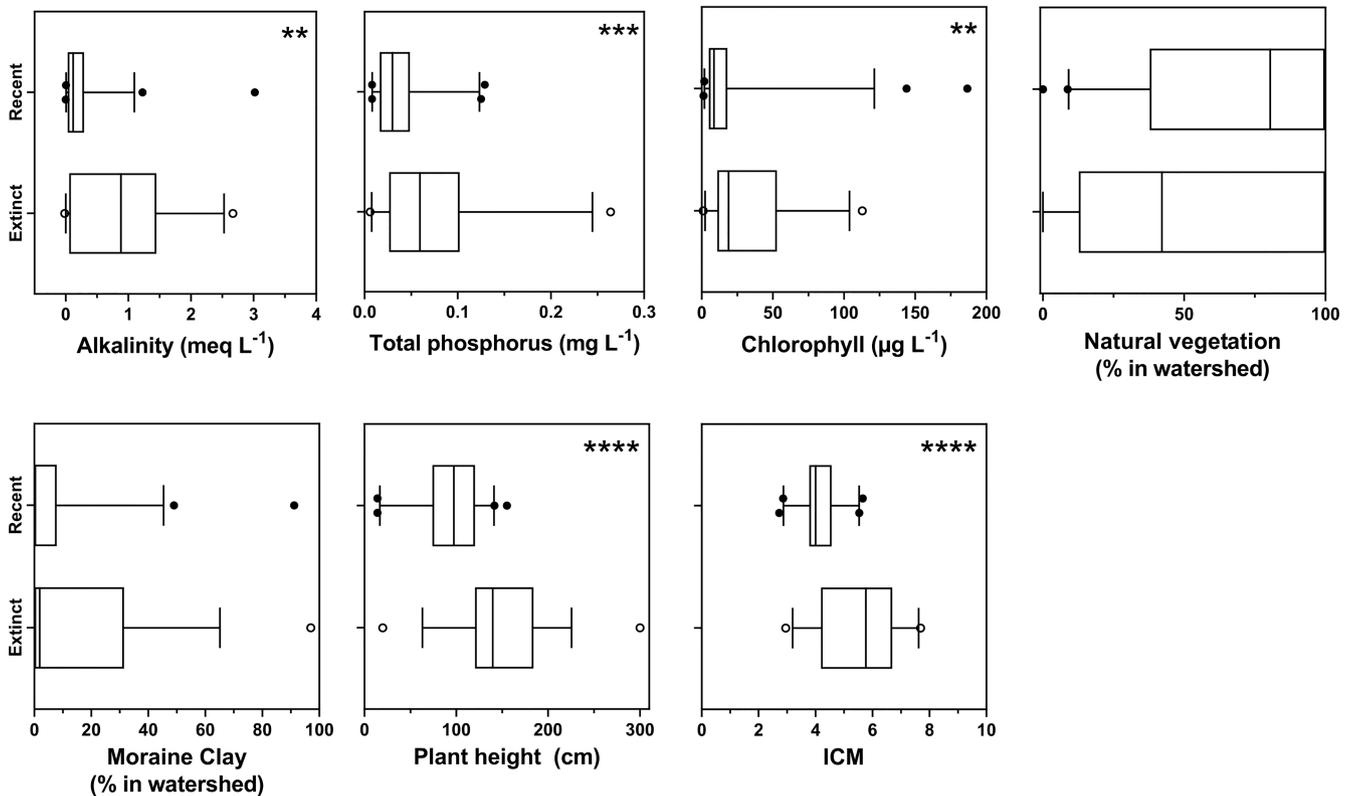
FIGURE 3 Selected environmental variables of water chemistry, land use and plant community metrics of lakes with recent populations of *Lobelia dortmanna* or lakes where the plant has gone extinct. The box-whisker plots show median (vertical line in box), 50% percentiles (extension of box), error bars (90% of observations) and "outliers" (dots). The number of asterisks indicates level of significance (**, $p < 0.01$; ***, $p < 0.001$; and ****, $p < 0.0001$; Mann–Whitney U-test).

indicating a good model fit ($\chi^2 = 16.7$, $p = 0.275$). Lake TP did not directly affect the presence of *L. dortmanna* in the investigated lakes (Figure 4). TP also did not affect the presence of *L. dortmanna* through an effect on Chl-*a*, despite the TP concentration significantly increasing the Chl-*a*, as Chl-*a* itself did not significantly affect the presence of *L. dortmanna*. Instead, the effect of TP was routed through a significant effect on the ICM values and height of the plant communities, which in turn significantly affected the presence of *L. dortmanna* (Figure 4). The model thus suggest that higher TP concentrations led to more eutrophic plant communities (higher ICM values) that were taller and caused a decreased likelihood of *L. dortmanna* presence.

As with TP, lake alkalinity did not directly affect the presence of *L. dortmanna*, but had a strong indirect effect routed through the plant community in the same way as eutrophication. Thus, the joint effect of eutrophication and high alkalinity results in tall plant communities, accounting for 48% of the variability of *L. dortmanna* presence indicating that competition plays a major role in the extinction of *L. dortmanna* in the 80 study lakes. In the model, lake TP was rather weakly ($R^2 = 0.2$) negatively related to the cover of natural vegetation in the catchments, indicating that other land uses such as agriculture or urban areas cause the release of nutrients to the lakes. Using agriculture or urban areas did, however, not improve the prediction of lake TP indicating that point sources might play a major role in these catchments.

Lake alkalinity was positively related to the proportion of moraine clay in the soils of the lake catchment. Again, the relationship was rather weak ($R^2 = 0.19$) indicating that sources of bicarbonate other than weathering of minerals in the surface soil control lake water alkalinity, such as deeper soil layers. Relationships between variables that were not considered causal (alkalinity vs. TP and alkalinity vs. natural vegetation cover) but that could originate from a common unquantified driver were allowed to correlate in the model (see Discussion).

3.4 | Differences in plant communities

Despite an overlap in plant species composition, the PCoA revealed differences in the plant communities between the two lake groups (recent and extinct Lobelia lakes; Figure 5). The difference in species composition between groups was significantly different (PERMANOVA, $F_{1,78} = 6.16$, $p < 0.001$).

Along the first axis, frequent species (i.e. species recorded in >10 lakes) with the highest weighted average scores were *Stuckenia pectinata* and *Hydrocharis morsus-ranae* whereas species with the lowest scores were *Potamogeton polygonifolius* and *Sphagnum* mosses (Table S2). On the second axis *Isoetes lacustris* and *Ranunculus flammula* had the highest scores among the frequent species, while *Potamogeton polygonifolius* and *Sphagnum* mosses had the lowest values.
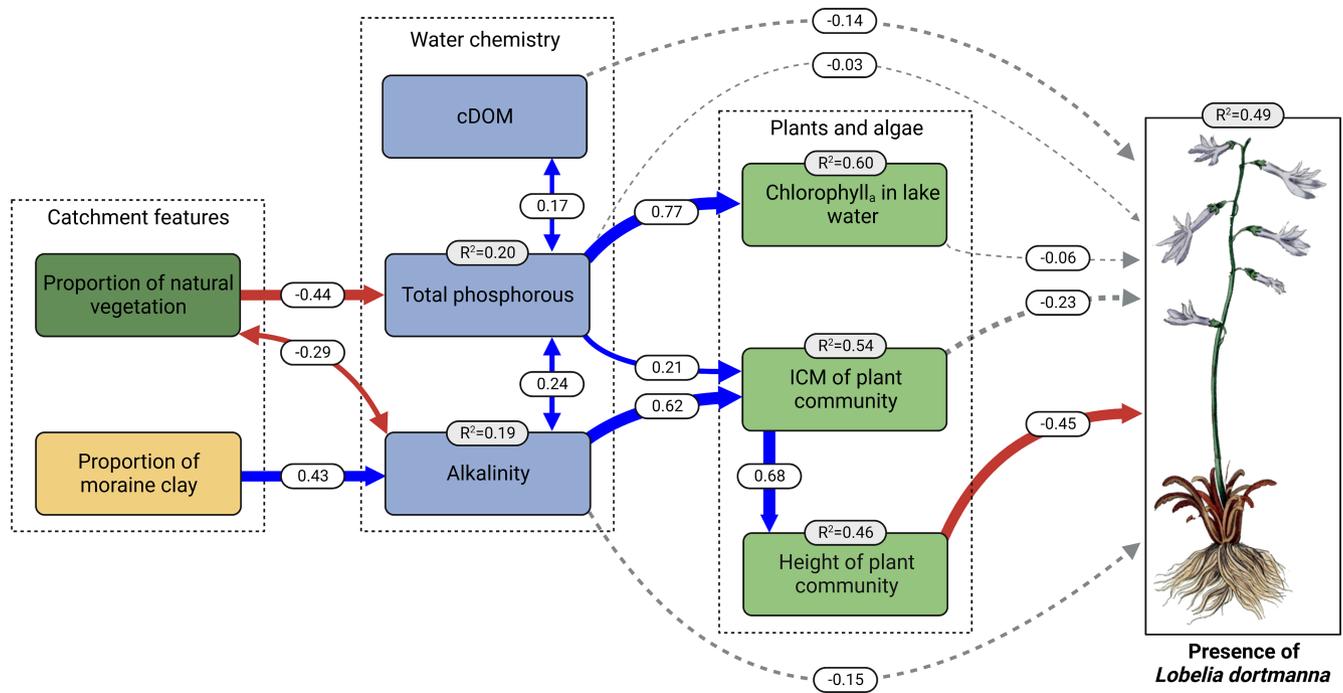
FIGURE 4 Structural equations model of the effects of landscape and in-lake variables on current presence or absence of *Lobelia dortmanna* in lakes that historically contained the species. Red lines indicate negative relationships while blue lines represent positive relationships. Arrow width is proportional to the standardised effect size and significant effects are marked with solid lines while non-significant effects are stippled and in grey colour. Insert boxes at each modelled (endogenous) variable indicate the R^2 of the specific sub-model.

Alkalinity was the environmental variable with the highest average loading, indicating the strongest correlation with plant community composition (Figure 5). Alkalinity increased with increasing values of the first axis and deceased values of the second axis, in essence from communities containing *L. dortmanna* towards communities where it had been lost.

4 | DISCUSSION

Modelling the mechanisms behind the widespread extinction of *L. dortmanna* from Danish softwater lakes suggested that only the mean height of the plant communities of the lakes had a direct negative influence on the occurrence of *L. dortmanna*. The expected adverse effects of, for example, eutrophication and alkalinity were all indirect and funnelled through the ICM of the plant community, which was further translated into effects on mean plant height. Direct effects of eutrophication via shading from phytoplankton, acidification or brownification were not observed. Below, we discuss these findings and provide an outlook for the management of Lobelia lakes in the context of the ongoing global change.

Lobelia dortmanna is among the smallest and slowest-growing submerged aquatic plants in the North European flora (Nielsen & Sand-Jensen, 1991) and experiments have shown it to be among the poorest competitors in a test of the competitive ability of 26 wetland plants (Keddy et al., 2000). It is therefore not entirely surprising that competition from the entire plant community lies behind the

widespread extinction of *L. dortmanna*. Furthermore, analyses of the entire aquatic flora of southern Scandinavia reveal that in regions where the majority of lakes are eutrophic, including Denmark, smaller plants preferring oligotrophic conditions have suffered substantial declines and are now rare in the landscape (Pedersen et al., 2006; Sand-Jensen et al., 2018). By contrast, in more oligotrophic regions plant size is not related to species abundance (Sand-Jensen et al., 2018).

While we anticipated an influence of eutrophication (TP) acting directly, or through the promotion of phytoplankton, on the presence of *L. dortmanna*, we did not find support for this in the data. Instead, we found evidence for an indirect effect through promotion of plant community with a higher nutrient affinity (higher ICM-value) and larger average plant height. Increased concentrations of TP from low initial levels in oligotrophic lakes should promote a denser aquatic plant community of fast-growing species (Murphy et al., 2018; Sand-Jensen & Borum, 1991). The lakes in the dataset are predominantly shallow allowing for development of dense aquatic plant populations even at high TP concentration, at least in some instances, because of lower risk of phytoplankton causing light limitation of the taller aquatic plants reaching the surface (Sand-Jensen et al., 2008). Stable clear water states dominated by tall aquatic plants can exist even in very nutrient-rich lakes (Scheffer et al., 1993; Scheffer & Jeppesen, 2007). Furthermore, increased TP could have indirectly affected other ecosystem components known to be detrimental to *L. dortmanna* but not quantified in the present study, such as epiphytic and filamentous algae (Sand-Jensen & Borum, 1984) or
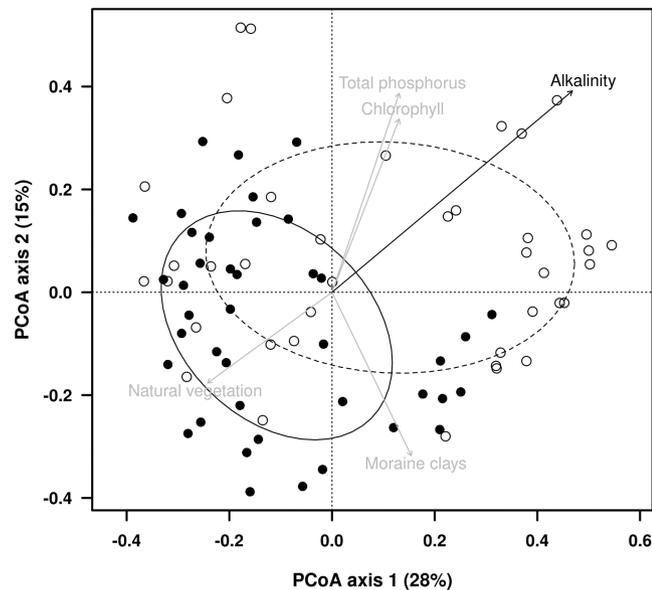
FIGURE 5 Principle coordinates analysis (PCoA) of the plant composition in 80 of the analysed lakes where vegetation surveys were available. All lakes used to harbour *Lobelia dortmanna*, but the plant has gone extinct in 36 of the lakes (open circles, stippled line) while remaining in 44 lakes (black dots, solid line). For each lake group, the superimposed 50% confidence interval show the main trend of the point cloud. Lakes with extinct populations had significant shift in species composition compared to lakes with remaining populations ($p < 0.001$, PERMANOVA). Superimposed vectors show the average weighted loadings of the environmental variables included in the structural equation models (Figure 4). Black vectors are significant whereas grey vectors are not.

sediment organic matter (Møller & Sand-Jensen, 2011). Such effects could account for some of unexplained variation in our model of *L. dortmanna* presence.

As expected, there was a negative influence of alkalinity on the presence of *L. dortmanna*, but again this effect was not direct but rather routed through a taller plant community with a higher nutrient affinity. Experimental evidence shows that elodeids, under favourable growth conditions (with excess inorganic carbon) exert a substantial competitive pressure on isoetids such as *L. dortmanna* (Spierenburg et al., 2010). The typical tall submerged plant species in Danish lakes are elodeids capable of using bicarbonate as a source of inorganic carbon in their photosynthesis, such as native species of *Potamogeton* spp. and the non-native *Elodea* spp. (Maberly & Gontero, 2017; Olesen & Madsen, 2000; Sand-Jensen et al., 2018), unlike *L. dortmanna* that relies on sediment CO_2 (Wium-Andersen, 1971). Thus, as high alkalinity strongly promotes elodeid growth while *L. dortmanna* remains rather unaffected (Pulido et al., 2012; Raun et al., 2010), competitive exclusion in highly alkaline eutrophicated ecosystems seems likely, despite the lack of direct experimental evidence on the effect of alkalinity and nutrients on the competition between elodeids and *L. dortmanna* (or other isoetids).

Neither acidification nor brownification played a major role in the extinction of *L. dortmanna* from our study lakes. Massive acidification

of the poorly buffered Lobelia lakes has strongly affected populations of *L. dortmanna*, although the present dataset does not indicate that acidification was the causal effect of the extinction from Danish lakes (Figure 4). The atmospheric acid deposition took place primarily during 1960–1980 and resulted in total eradication of isoetids, including *L. dortmanna*, in many of the affected lakes (Farmer, 1990; Roelofs, 1983). The sluggish return to pre-1960 conditions following installations of sulfur filters on coal-fuelled powerplants resulted in hasty management actions such as liming of the acidified lakes, a measure that only worsened conditions for the isoetids. The lime was titrated into free CO_2 stimulating growth of *Sphagnum* mats, *Juncus bulbosus* and *Myriophyllum alterniflorum* (Brandrud, 2002; Roelofs et al., 1994; Spierenburg et al., 2009) overgrowing the few remaining slow-growing isoetids. It also seems that the change in inorganic nitrogen forms from nitrate to ammonium in acidified lakes also favours growth of, for example, *J. bulbosus* further aggravating the stimulating effect of high CO_2 in water (Smolders et al., 2002). More recently, it was hypothesised that once the liming activities are discontinued, there will be a gradual return of the sensitive isoetids as a new equilibrium with the atmosphere will bring down CO_2 in the water to pre-liming levels (Lucassen et al., 2016). In Denmark, however, liming of acidified lakes has never been widely used as a management tool, and since the 1980s the pH has increased substantially, likely reaching pre-acidification levels (Baastrup-Spohr et al., 2017). Therefore, water samples taken in recent times are unlikely to reflect the low pH that some of the lakes with extinct populations of *L. dortmanna* might have experienced back in the 1960s and 1970s and so, our analytical approach would not necessarily capture the possible effect of acidification.

Likewise, brownification also did not contribute to the explanation of presence or absence of *L. dortmanna* in the 80 study lakes (Figure 4). Severe brownification is a more recent threat to isoetids than acidification because leaking of cDOM to surface waters was increased as a result of higher pH in the precipitation (Ekström et al., 2011) as a result of successful scavenging of SO_x in flue gasses. *Lobelia dortmanna* is inherently slow-growing and therefore the die-off is similarly slow. Moreover, being an amphibious plant, populations growing in shallow water may be able to survive even severe brownification for decades in shallow water that occasionally dry out. Such remaining populations of *L. dortmanna* in tea-coloured water have been observed in Lake Tvorup Hul (Madsen-Østerbye et al., 2018) even at depths where the light was insufficient to support growth and survival, a phenomenon referred to as extinction debt (Kuussaari et al., 2009). It is not clear if this extinction debt will ever be redeemed since current management practices are focusing on clearing forest in order to restore the heathlands, which leads to much lower production of cDOM that coniferous forest (McGowan et al., 2018). Thus, brownification may decrease and enable spreading of *L. dortmanna* from the few remaining individuals so that extinction is prevented.

Management of softwater lakes also include in-lake restoration measures such as removal of organically rich sediments in the littoral zone. Labile sediment organic matter has been shown to result

in poor root function in *L. dortmanna* and other isoetids such as *Littorella uniflora* (Møller & Sand-Jensen, 2011; Raun et al., 2010), and sediment removal has been used with success (Van Wichelen et al., 2007). Nevertheless, there are successful restoration attempts from, for example, the Netherlands where the entire focus has been on the water chemistry (Brouwer et al., 2002) and not sediment parameters. Owing to a lack of data, the present investigation did not consider sediment quality in the 80 study lakes and therefore we cannot rule out that the results of the path analysis (Figure 4) would be slightly different if sediment organic matter had been included in the analysis. However, the constant production and loading of labile organic matter to the sediment also is a result of eutrophication (Spierenburg et al., 2013); dead phytoplankton and fast-growing aquatic plants end up in the sediment each autumn where it fuels the O_2 consumption of the sediment (Pulido et al., 2011). Clearly, the tall fast-growing elodeids that we propose outcompete *L. dortmanna* thrive in the sediment and we are therefore convinced that eutrophication is the main driver of extinction, but via the observed indirect actions on the entire plant community.

Ongoing climate change might even have exacerbated the effects of eutrophication as increased temperatures increases processes such as growth rate of tall elodeids (Olesen & Madsen, 2000), sediment respiration and overall eutrophication effects (Jeppesen et al., 2010).

5 | CONCLUSION AND OUTLOOK

Our finding that the adverse effects of eutrophication on the presence of *L. dortmanna* act through indirect effects on the entire plant community is novel. In the present dataset, there was no indication that shading from phytoplankton (the ultimate result of eutrophication) was causing the extinction of *L. dortmanna* in Danish lakes. Nevertheless, lakes with extinct *L. dortmanna* had higher TP and higher alkalinity, but both environmental parameters acted indirectly upon *L. dortmanna* through vegetation ICM and thereafter mean height of the plant community. This points to competition from other rooted aquatic plants as the main driver and not eutrophication per se. Additional effects of acidification or brownification were not supported by the dataset, but we conclude that possible effects from brownification may not yet have kicked in as a consequence of extinction debt.

Lobelia dortmanna, all other isoetids (if evaluated) and other small species, such as *Elatine* spp., are placed on the national red list in Denmark and in most other northwestern European countries (e.g., Metzing et al., 2018) where many lakes are eutrophicated. The observed negative effects of interspecific competition on *L. dortmanna* resulting from high levels of nutrients, particularly in alkaline waters, are likely also part of the cause of the decline of most of these other small species of aquatic plants (Sand-Jensen et al., 2018). In a conservation context, this implies a special focus on the more alkaline lakes where extinction risk seems to be highest. For managers, our results suggest that besides reducing eutrophication, actions that limit large aquatic plants, but are tolerated by the isoetids, such as periodic drawdown in the summer season could help maintain or even strengthen vulnerable populations of *L. dortmanna* and other isoetids (Baastrup-Spohr et al., 2016; Sand-Jensen et al., 2022).

CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

Data and R code used in the manuscript analyses is available at the repository of the University of Copenhagen (ERDA at https://doi.org/10.17894/ucph.b594b3f5-6660-4517-8d0c-bc2ed7ad00f5).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.