Distribution and functional traits of charophytes and vascular plants
-Biodiversity in aquatic systems and along flooding gradients

Lars Båstrup-Spohr
Cover photo: A mix of charophytes, flooded terrestrial vascular plants and the amphibious species *Littorella uniflora* all of which are treated in the present thesis. Photo: Ole Pedersen
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List of papers

The thesis includes the following papers:


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Abstract

A large variety of plant species of very different evolutionary origin are found within and along the margins of aquatic ecosystems. These species have very different adaptations depending on the particular environmental condition under which they grow. This thesis examines the role of these adaptations or functional traits for the distribution on large scales and along specific environmental gradients. Characean algae (charophytes) are an ancient group of aquatic plants found in most aquatic ecosystems. I confirmed that they have declined markedly during the 20th century, most likely as a consequence of widespread eutrophication, and that declining species were characterized by traits such as being obligate perennial and having preference for alkaline lakes. Partly as a consequence of this, there was an exceptionally high proportion of rare charophyte species in Denmark and Scandinavia. These rare species are specialists in particular environments, while the abundant species have traits such as broad salinity tolerance, tall shoots, vegetative reproduction and variable life form. Vascular plants, in contrast to charophytes, occupy the entire gradient from submerged to drained conditions. Along this gradient their functional traits vary markedly due to the environment conditions filtering away species lacking suitable adaptations. I found that the filtering of traits was highly dependent on specific traits and position along the gradient and the traits experiencing the strongest environmental filtering also proved to be the most important in predicting the species composition under local conditions. The results stress the importance of environmental filtering as mechanism determining community assembly in plant communities. The zone from submerged to drained conditions along the margins of softwater, oligotrophic lakes is often dominated by isoetids. They have the remarkable adaption of using sediment CO₂ taken up through permeable roots. This trait makes isoetids vulnerable to sediment anoxia caused by degradation of organic sediments following eutrophication. I investigated if sediment desiccation leads to high mineralization without stressing the plants. The sediment mineralization increased following desiccation and plants continued to grow well under drained, fluctuating and submerged conditions stressing the potential use of desiccation as a sediment restoration tool in eutrophied systems. In conclusion, species functional traits have a large influence on distribution of species both on large scales and along specific environmental gradients.
**Resumé**


De første to kapitler fokuserer på kransnålalger, der til trods for deres ofte betydelige effekt i de økosystemer, hvor de vokser, er dårligt undersøgte og ofte er overset i forhold til de mere grundigt beskrevne karplanter. Kapitel I beskriver udviklingen af artssrigdom og hyppighed af kransnålalger i danske ferske vande siden 1940erne. Generelt har der været en nedgang i artsantallet i en række søer i løbet af 1900-tallet og tre arter er uddøde i Danmark, men en enkelt ny er indvandret. Denne udvikling skyldes med stor sandsynlighed den kraftige forurening med næringsstoffer gennem perioden, som har medført en udbredt eutrofiering. For de enkelte art er mønsteret, at arter der før var almindelige, er blevet endnu mere almindelige, mens de sjældne arter er gået tilbage. De karaktertræk, der er typiske for de arter der er gået tilbage arter, er at de er flerårige og har forkærledighed for hårdvandede søer. Disse forhold er typisk for arter tilknyttet hårvandede klare og dybe søer, hvilket er en sjælden habitattype i nutidens Danmark. Et lyspunkt er dog, at artssrigdommen har en tendens til at stige i de seneste 20 år som følge af en reduceret næringsbelastning af søerne.

I kapitel II bescribes udbredelsen af kransnålalger i de Nordiske lande og hvordan den enkelte arts nichespecialisering og karaktertræk har betydning for arternes hyppighed. Analysen viste at der er en meget stor andel af sjældne arter af kransnålalger (50-87 %) også i forhold til karplanterne (30-35 %). De sjældne og fåtalige arter er karakteriseret af specialiserede nicher og en lavere skudhøjde, mindre salttolerance, dårligere vegetativ formering samt en specialiseret livsform.

Den enkelte arts karaktertræk kan have stor betydning for dens udbredelse under forskellige miljøforhold. Denne mekanisme er beskrevet som miljøfiltering hvor miljøforholdene virker som en række filtre, der fjerner arter uden de rette træk. I kapitel III undersøgtes, hvilke træk, hos karplanter, der var mest filteret af miljøforholdene langs en vand gradient. Analysen viste, at filtreringen af de enkelte karaktertræk generelt ændrede sig med miljøforholdene, mens nogle træk som rodporositet og det specifikke bladareal var udsat for filtering langs hele gradienten. De mest filtrerede træk var også afgørende for at kunne forudsige artssammensætningen under specifikke miljøforhold. Dette resultat viser empirisk at miljøfiltrering er af afgørende betydning for artssammensætningen i plantesamfund.

Vandgradienten fra permanent neddykkede til kun tidsvis vandækkede områder langs oligotrofe blødvandede søer, hvor vandstanden svinger, udgøres vegetation ofte af grundskudplanter, især af planten strandbo *Littorella uniflora*. Grundskudplanterne er pga. deres gaspermeable rødder særligt sensitive over for dårlige iltforhold i sedimentet, hvilket ofte er

I appendikset indgår et dansk og et svensk kapitel, begge udgivne i populærvideneskabelige tidskrifter. Det første kapitel omhandler alle danske arter af kransnålalger og deres udbredelse. Der gives en vurdering af arternes risiko for at uddø nationalt. Denne vurdering, en rødlistevurdering viser, at 85 % af de danske arter har en forøget risiko for at uddø, og at tre arter allerede med stor sandsynlighed er uddøde i Danmark.

Summary

Natural freshwater ecosystems contain a large variety of plants with very different structure and evolutionary origin. These species include representatives from a large number of vascular plant families and one family of characean algae, or charophytes. Plants play a pivotal role for ecosystems by supporting a large biodiversity of associated organisms and by their structure and metabolism affecting the chemical and physical environment. The diversity of the plants themselves changes drastically along environmental gradients as a consequence of species specialization to more or less specific habitat conditions. The particular requirements of a given species, the species niche, are the result of morphological and physiological adaptations. These adaptations are termed functional traits and have decisive influence on species success under particular environmental conditions. This Ph.D.-thesis is about plant distribution and the influence of functional traits.

The first two chapters focus on charophytes, which despite their extensive distribution and considerable ecosystem effects often have been overlooked in ecological studies. Chapter I analyses the changes in species richness and abundance of charophytes in Danish freshwaters during the 70-years long period from about 1940 to 2010. Overall, the richness has declined in reinvestigated lakes and three species have gone nationally extinct during the period while one species has immigrated. This development is most likely due to the widespread eutrophication occurring throughout the 20th century as a result of greater anthropogenic input of nutrients to aquatic ecosystems. The pattern of single species is such that common species have become even more common and rare species have become even rarer. While species increasing in abundance tend to have wide tolerances to alkalinity and nitrogen content, the declining species has functional traits such as a perennial life form and a preference for alkaline lakes, typical of deep-growing species in large alkaline lakes, a rare contemporary habitat. A positive trend in species richness of lakes in recent years, however, gives hope for the future.

Chapter II analyses the distribution of charophyte species in Scandinavia and how niche specialization and functional traits influence species abundance. The analysis revealed that a high proportion of species are rare and Red-listed (50 to 87 %) compared to aquatic vascular plants (30 to 35%). Rare charophyte species are characterized by a high degree of niche specialization and low shoot height, low salt tolerance, poor vegetative reproduction and a specialized life form.

Functional traits can have a profound impact on the distribution of particular species along environmental gradients. The mechanism has been described as environmental filtering where the environmental conditions act as a set of filters removing species without well suited traits. In chapter III, it was investigated to what extent functional traits of vascular plants experienced filtering along a hydrological gradient from below water in ponds to very dry conditions on shallow soils covering the limestone pavements on Øland’s alvar. The analysis showed that the filtering of single traits changed depending on the environmental conditions, while traits such as root porosity and specific leaf area were significantly filtered throughout the investigated gradient. The traits experiencing the strongest environmental filtering also proved to be the most important in predicting the species composition under particular conditions. The results stress the importance of environmental filtering as a mechanism driving community assembly in plant communities.

The hydrological gradient from permanently submerged to areas only occasionally flooded along the margins of oligotrophic, softwater lakes are often dominated by plants of the isoetid life form, particularly Littorella uniflora. Isoetids are particularly sensitive to organic sediment deposition.
following eutrophication because of gas-permeable roots and poor anchorage in organic unconsolidated sediments. As drained sediments have higher oxygen availability and, thus, a higher mineralization rate it was investigated in chapter IV if desiccation could improve sediment conditions without stressing L. uniflora. The species developed the highest biomass under drained conditions. The biomass also increased under fluctuating water levels, where the plants are inclined to change between aerial and submerged leaves, showing a high tolerance across the entire hydrological gradient. The sediment mineralization rate measured as efflux of inorganic carbon was markedly higher under drained conditions which also led to significantly more consolidated sediments both conditions potentially improving isoetid plant growth. Therefore, lakes should fluctuate freely and periods of sediment desiccation can be used as a restoration tool at locations where too high organic content has made lake sediments unsuitable for plant growth.

The appendix includes a Danish and a Swedish chapter dealing with popularization of science. The paper in Svensk Botanisk Tidskrift does, however, include original data. The Danish chapter considers all Danish species of charophytes and their distribution along with an assessment of the risk of species going nationally extinct, a Red List evaluation. The assessment showed that 85 % of the Danish charophyte species have an increased risk of going extinct nationally and three species have most likely already been lost from Denmark. In the second appendix chapter in Swedish, the distribution of plants, their functional traits and the species richness were investigated along a gradient in water cover and soil depth on Öland’s alvar. Along the gradient, there was a large species turnover and species found within meters from each other had markedly different functional traits adapted to the particular micro-environment where they were found. Species richness was highest in deep soils with good water supply as consequence of more a more productive environment and a larger regional species pool.

In conclusion, species functional traits have a large influence on the distribution of species both on large scales and along specific environmental gradients. The knowledge of charophyte distribution and traits, the prediction of species abundance through their traits and the effect of water levels on plant performance and sediment conditions all have potential consequences for ecosystem management. For aquatic vascular plants and particularly for the charophytes only a minority of traits have been identified and even less is known about their role along the multitude of environmental gradients. For vascular plants, improved understanding of how species traits are filtered by environmental conditions can potentially ensure more accurate future predictions of community assembly.
Introduction

*Biodiversity of aquatic plants*

Natural freshwater systems contain a large variety of plants of different growth form and phylogenetic origin. The macroscopic plant communities of freshwaters in the Northern temperate regions include representatives from many families of vascular plants and one family of characean algae (Chambers et al. 2008). The presence of aquatic plants in lakes and ponds support a large biodiversity of associated organisms and plays an important role for the ecological state of water bodies by stabilizing bottom sediments and providing shelter for zooplankton (Jeppesen et al. 1998). The diversity of the aquatic plants themselves responds strongly to natural gradients in habitat conditions but also to anthropogenic alterations of environmental conditions (Sand-Jensen et al. 2000, Vestergaard and Sand-Jensen 2000). During the last century, aquatic plant richness has declined in many water bodies of the intensively used landscapes of Europe because of habitat deterioration and destruction (Lachavanne et al. 1992, Sand-Jensen et al. 2000, James et al. 2005). The response of individual species to environmental conditions varies considerably resulting in distinct patterns, with some species being widespread and common and others rare. These patterns of commonness and rarity have been shown to be related to species niche specialization with generalist species being more widespread and abundant than specialists (Thompson et al. 1999, Gaston and Spicer 2001).

The mechanism by which environmental conditions affect the presence and abundance of particular species can be characterized as the environment acting as a set of filters, only letting through well adapted species with suitable functional traits (Keddy 1992, McGill et al. 2006, Shipley et al. 2006). A functional trait is a well-defined measurable property of organisms, usually measured at the species level that strongly influences organismal performance and, thus, can be used comparatively across species (McGill et al. 2006). Functional traits are thus important for species niche properties such as specialization and tolerance to various environmental conditions (Hutchinson 1957, Violle and Jiang 2009).

In the following chapters I will give an introduction to plants along hydrological gradients with emphasis on the highly understudied group of charophytes and also present some of the environmental factors regulating presence and abundance of plant species. Firstly, I will introduce the concepts of the species niche and environmental filtering of species traits which is the conceptual framework ensuring the understanding of species response to environmental gradients.

*Species niche*

A species’ niche was historically described as an n-dimensional space where each dimension represents one range in abiotic conditions under which a species can survive and reproduce (Hutchinson 1957). The conditions under which a species can survive without the presence of other species is termed the fundamental niche while the conditions under which the species is actually found in nature is referred to as the realized niche (Hutchinson 1957). Often of interest is
the breadth (or width) of a species niche and its location compared to niches of other species (Thompson et al. 1999, Gaston and Spicer 2001). The location of niche compared to the average environmental conditions in the area in focus can be viewed as a measure of niche specialization where species having niches far from the mean conditions are more specialized than species with niches close to the average conditions (Doledec et al. 2000). Species with specialized niches are often found to be rarer than species with more general niches; a pattern existing if species that have general niches and are able to use a wide variety of resources will achieve high local densities and be able to survive in more places over larger areas (Gaston and Spicer 2001). Specialist species have been found to decrease in abundance compared to generalists as a result of historically increasing anthropogenic impact in both terrestrial and aquatic ecosystems (McKinney and Lockwood 1999, Clavel et al. 2011).

Environmental filtering through species traits
Because of niche differences between plant species, the environmental conditions can be viewed as a set of filters sorting which species can occur in a given community (Keddy 1992). The niche is an expression of a species tolerance along different environmental axes and these tolerances are regulated by the morphological and physiological characteristics, or functional traits, of the species. The environmental filtering can thus be viewed as operating on species functional traits rather than species themselves (McGill et al. 2006, Shipley et al. 2006). Along environmental gradients, the average and variability of the traits expressed in communities change and these changes are associated with shifting strength of environmental filtering (Cornwell and Ackerly 2009, Bernard-Verdier et al. 2012). A smaller range in trait values than expected from by random – trait clustering – may be taken as an indication of trait filtering, because species having trait values outside a given allowed trait range succumb (Keddy 1992, Cornwell and Ackerly 2009). The great advantage of using functional traits and not species entities is that traits respond similarly to environmental gradients across large geographic regions thus increasing our knowledge on more general patterns (Diaz et al. 2004, Wright et al. 2004).

The recent theoretical developments regarding traits and environmental filtering have not yet been fully implemented in our understanding of vascular plant distribution along hydrological gradients. For aquatic vascular plants in general and charophytes in particular, the knowledge of the influence of traits on species tolerance and distribution is very limited (Blindow 1992, Sand-Jensen et al. 2000).

Charophytes and aquatic vascular plants
The vascular plants and charophytes we meet today originate from single celled green algae living 700-800 million years ago (Butterfield et al. 1988). These unicellular algae evolved into multicellular organisms eventually yielding the group of charophytes already before the Silurian period more than 450 million years ago (Martin-Closas 2003). The charophytes are the closest living relatives to the vascular plants dominating especially in terrestrial ecosystems (Karol et al. 2001).
Vascular plants are, however, also important representatives of most aquatic systems but this group is thought to have evolved from land plants re-entering the aquatic environment the last 120 million years (Sculthorpe 1967). It is noteworthy, that charophytes dominated shallow freshwater and coastal marine waters during the entire preceding period from 450-120 million years ago.

The charophytes of today are 5-200 cm tall macro-algae living attached to soft sediments by rhizoids in both freshwater and marine environments (Krause 1997). Although their appearance is similar to several aquatic vascular plants their morphology is very different. Charophytes consist of single extraordinarily long internodal cells connected by shorter nodal cells in an axial section from which branches radiate above the sediment and rhizoids below the sediment surface. Contrary to charophytes the vascular plants with which they share habitat have highly specialized multicellular tissues enabling fast internal transport of nutrients, photosynthates and gases (Pedersen and Sand-Jensen 1993, Raven et al. 2003).

**Functional traits of plants along gradients**

Despite the morphological differences, charophytes and aquatic vascular plants are found in the same habitats and share many functional adaptations or traits (Fig. 1). Among the most severe restrictions to plants living submerged in water is the $10^4$-fold lower gas diffusion coefficient in water than in air potentially limiting the supply of CO$_2$ for photosynthesis. In water it is particularly advantageous to have a large surface to volume ratio to increase uptake of dissolved ions and gases and aquatic vascular plants often have thinner and more serrated leaves than their terrestrial counterparts while charophytes have notoriously thin photosynthetic tissues because of the single-celled basic structure. Several species of both charophytes and aquatic vascular plants compensate for the low CO$_2$-concentration by actively using dissolved HCO$_3^-$ found in much higher concentrations in alkaline waters than dissolved CO$_2$ (Madsen and Sand-Jensen 1991, Maberly and Madsen 2002).

![Charophytes growing together with the vascular plant Littorella uniflora in a shallow lake on the island of Öland, Sweden. Photo: Ole Pedersen.](image)
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In water, light attenuation is often high and it can be greatly increased by particles or dissolved colored compounds (Kirk 1994). A trait that can partly compensate for poor light condition is a tall upright structure allowing leaves to grow close to the surface and tall species are thus favored under turbid conditions (Sand-Jensen et al. 2008).

While charophyte species are only capable of growing under water, vascular plants are found all along the gradient from submerged to dry conditions. The traits of vascular plant species distributed at different parts of the gradient tend to differ predictably because of the strong abiotic selection (Violle et al. 2011, Bernard-Verdier et al. 2012). Traits such as specific leaf area (SLA) change along hydrological gradients as plant species living on moist soils tend to have thin leaves with a large surface area while species living under drier conditions have small and thick leaves to limit water loss (Bernard-Verdier et al. 2012).

Environmental conditions
One of the main environmental drivers in aquatic ecosystems is eutrophication triggered by high nutrient concentrations from large anthropogenic inputs of waste water and farmland fertilizers (Moss 1998, Wetzel 2001). In eutrophied waters, the high nutrient concentration increases growth of both microscopic and filamentous algae impoverishing the light conditions and leading to increased organic matter in sediments as the algae die and sink to the bottom (Kalff 2002). Decreased light availability reduces the bottom area with sufficient light available for plant growth while organically enriched sediments negatively affects root development and root anchorage in the sediment (Schutten et al. 2005, Raun et al. 2010). Eutrophication has thus caused decreased species richness of both charophytes and aquatic vascular plants and in the most severe instances led to complete loss of submerged vegetation (ie. Lachavanne et al. 1992, Sand-Jensen et al. 2000, Sand-Jensen et al. 2008). As a result, a large proportion of aquatic vascular plants (30-35%) and particularly charophyte species (50-87%) have entered the national Red Lists in Scandinavia due to their high risk of extinction (Gärdenfors 2010, Kålås et al. 2010, Koistinen 2010, Wind and Pihl 2010).

The decline of abundance due to eutrophication is not the same for all species because some are more tolerant to the poorer light and changed sediment conditions than others (Sand-Jensen et al. 2008). An anthropogenic disturbance such as eutrophication can lead to homogenization of the plant communities with most communities dominated by a few tolerant species (McKinney and Lockwood 1999). While the eutrophication effects on vascular aquatic plants are well documented, the long term consequences of eutrophication for the highly threatened charophytes have not been studied in detail. It is therefore of great importance to describe the effects on this group of organisms and investigate which species traits are involved in regulating both the overall species abundance and the response to specific stressors. These questions are the focus of papers I and II in my thesis.

Another important environmental factor along the margins of freshwater
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systems is the location and fluctuation of the water table driven by seasonal climatic variations in natural systems or by impoundment in impacted water bodies (van der Valk 2005, Mjelde et al. 2013). The change of hydrological conditions from permanently submerged to dry conditions plays a major role in individual species distribution and the species richness along hydrological gradients (Silvertown et al. 1999). Changes are caused by each species being, to some degree, specialized to certain hydrological conditions, i.e. having a specific hydrological niche. This specialization is a function of the traits possessed by a given species. A better understanding of the species distribution patterns along hydrological gradients can thus be gained by investigating which functional traits are important in determining the community assembly at particular hydrological conditions. This is the aspect is addressed in paper III.

In the zone of the water level fluctuation aquatic plants and sediments are exposed to both submerged and drained conditions. These alterations require plant species with specific traits enabling survival under both conditions such as leaf dimorphism and drought resistant seeds (Robe and Griffiths 1998, Brock et al. 2003). Fluctuating water levels also have profound implications for the sediment chemistry and mineralization (Baldwin and Mitchell 2000). Plants with an amphibious life form and being able to change their leaf morphology as a consequence of the changing water cover are often benefitted by fluctuating conditions (Farmer and Spence 1986, Casanova and Brock 2000). In sediments, lowering of the water level greatly increases the oxygen availability potentially increasing the mineralization rate of sediment organic matter (Degroot and van Wijck 1993, James et al. 2001). Desiccation can also increase sediment consolidation (James et al. 2001). Fluctuating water levels can thus to some degree counteract the chemical and physical stresses associated with increased organic sedimentation following eutrophication. Desiccation and subsequent re-submergence have however, also been shown to result in large nutrient fluxes from sediments and substantial acidification due to oxygenation of nutrient-rich organic substances and metal-sulphides (James et al. 2001, Van Wichelen et al. 2007). Despite the potential downsides, water level fluctuation has been suggested as a tool to restore sediment conditions and improve plant growth (Coops and Hosper 2002). In paper IV we investigated how water level fluctuations affected plant growth and sediment conditions in isoetid populations particularly vulnerable to organic enrichment (Sand-Jensen et al. 2005, Møller and Sand-Jensen 2011).
**Aims**
The overall aim of this thesis was to examine the species distribution and the importance of species functional traits for charophytes and vascular plants in water and along hydrological gradients. Particular focus was on analysing the role of traits and niche characteristics in shaping the present distribution of charophytes in Scandinavia and how the abundance has changed in Denmark during the last century. Emphasis was also given to determining the importance of in-lake ecological conditions and landscape variables for the changes in species richness following a century long, anthropogenically imposed, large scale alteration of the Danish landscape.

Along hydrological gradients, the objective was to investigate to what extent specific traits are subjected to environmental filtering and if traits experiencing filtering were important in predicting community assembly. The aim was also to describe, in detail, the response of a single plant species (e.g. *Littorella uniflora*) and the sediment to differing hydrological conditions. As desiccation can lead to improved sediments conditions for plants, focus was on the potential role of water level fluctuations as a restoration tool.

The data background for the analyses was a combination of data available in databases or in published literature and field investigations combined with experiments performed in the laboratory.

By consolidating the use of species functional traits into aquatic plant ecology I anticipate to gain a better understanding of the processes regulating species distribution and abundance. I also hope that the obtained knowledge can be of use to authorities by improving management of aquatic ecosystems.

![Fig. 2. Analysing the vegetation and collecting samples for trait analysis near a desiccating pond on Öland, Sweden. Photo: Hans Henrik Bruun.](image-url)
**Paper synopsis**

*Seventy years of changes in the abundance of Danish charophytes (paper I)*

Charophytes grow attached to soft bottoms in ponds, streams, lakes and estuaries. Like the aquatic vascular plants, charophytes have been reported to decline during the 20th century, but the causes and species specific changes are poorly understood (Blindow 1992, Simons and Nat 1996, Auderset Joye et al. 2002). We used Danish studies on freshwater charophyte distribution conducted around 1940 (Olsen 1944) and repeated measurements performed by the national monitoring programme and ourselves during recent years to evaluate the historical development of species richness and dominance patterns. We also tested to what extent historical changes of species abundance in 29 water bodies were related to landscape features, water quality and species traits.

We found that three species of freshwater charophytes (*Chara filiformis, Tolypella intricata and Nitella gracilis*) have apparently disappeared from Denmark while one species (*Chara connivens*) has immigrated. The national species richness of freshwater charophytes has thus declined from 21 to 19 species.

Species abundance based on occurrence in many water bodies followed a linear rank – log abundance relationship both in the historical and the recent studies (Fig. 3). The dominance structure was stronger today than historically as common species have become relatively more abundant and uncommon species relatively rarer (Fig. 2).

Among species traits, perennial life form and preference for alkaline waters typical of deep-growing species in large alkaline lakes, a rare contemporary habitat, were significantly related to the historic species decline. Species increasing in abundance had wide tolerances to alkalinity and water nitrogen content. Twenty-nine lakes and ponds studied repeatedly showed a significant decline of mean species richness from 3.4 to 2.4 during the 70 years. A small increase of species richness has taken place during the recent 15-20 years in several lakes experiencing reduced nutrient loading. However, many species survive today in relict populations and may find it difficult to recolonize lakes in which water quality has improved. The historical decline of species richness was significantly related to higher nutrient concentrations, higher phytoplankton biomass and lower transparency of eutrophied water bodies. In contrast, the amount of wetlands and openness of the landscape close to a water body did not predict the historical development perhaps because local processes or long distance dispersal determine species richness.

![Abundance vs. species rank](image)

*Fig. 3. Abundance vs. species rank (x-axis) of the fifteen most abundant Danish freshwater charophytes in 1940 (open circles) and in 2010 (closed boxes). Abundance was calculated as log (x + 1), where x is the % species frequency of occurrence. The linear regression line for the fifteen most abundant species was significantly steeper in 2010 than 1940 (p < 0.01).*
Considering together the loss of former freshwater habitats and the deterioration of surviving habitats, we conclude that charophyte occurrence has declined by about 56% in Denmark during the last 70 years. Species reductions determined from reinvestigated water bodies (29%) and the national species list (10%) are much lower and are less suitable measures of the developmental status of charophytes.

**Niche specialization and functional traits regulate abundance of Scandinavian charophytes (paper II)**

Given the historic loss of charophyte abundance, eutrophication of their habitats and their competitive inferiority to tall rooted plants under nutrient rich conditions (Van den Berg et al. 1998), it should be anticipated that charophytes are among the most threatened phototrophs. Because species with specialized niches are by definition restricted to a few special, but very suitable habitats they are suggested to remain rare overall because the majority of habitats would be unsuited for them (Brown 1984, Clavel et al. 2011). We thus hypothesize that within the charophyte group, species with specialized niches and functional traits such as low salinity tolerance and low shoot height should be restricted because of few growth habitats and low competitive capability.

This prediction was tested by examining the distribution of charophytes in Scandinavia and the relationship between species abundance and species traits. Abundance values were assigned to each species based on their status on the national Red Lists. The specialization of species niches were evaluated by the Outlying Mean Index (OMI, Doledec et al. 2000) using a large Danish dataset on charophyte distribution and environmental variables while species functional traits were derived from the literature.

The results supported our predictions showing that 50-87% of the species were on the national Red Lists in the four Scandinavian countries. These proportions are much greater than of aquatic (30-35%) and terrestrial plants (18-28%). Species abundance of charophytes in Scandinavia decreased significantly with niche specialization in separate analyses of marine and freshwater sites (Fig. 4). Four functional traits: shoot height, salinity tolerance, bulbil production and ability to express both perennial and annual life form were significantly positively related to Scandinavian abundance.

![Graph showing relationship between niche specialization and Scandinavian abundance](image_url)

Fig. 4. The relationship between niche specialization and Scandinavian abundance. The niche specialization was calculated as the Outlying Mean Index (OMI) using specific environmental conditions for Danish growth sites (a large OMI indicate a high specialization). The niche specialization was calculated based on two datasets, using marine (>0.5 g NaCl L⁻¹) growth sites and freshwater sites. The lines shown are model II regressions. The relationships between abundance and OMI are significant (Spearman, p<0.05).
We conclude that charophytes are generally rare species restricted by habitat loss and competition with fast-growing phytoplankton, macroalgae and tall vascular plants at eutrophic sites. The few charophyte species maintaining a high abundance in Scandinavia are generalists tolerant of a wide range of conditions, while specialists are often short, have a restricted life form and are rare in the disturbed contemporary landscape.

From pond to alvar: predicting plant community composition along a steep hydrological gradient (paper III)
Conceptually, community assembly may be viewed as a sequence of filters, sieving off species according to their functional traits and thus restricting the range of trait values within a community (Keddy 1992, Cornwell and Ackerly 2009). This conceptual framework may aid in achieving one of the core aims to ecology: the prediction of species’ occurrence and abundance from knowledge of the environmental conditions (McGill et al. 2006, Shipley et al. 2006). One suggested path towards this goal is the maximum entropy (maxent) model developed to predict species’ abundances in local communities (Shipley et al. 2006, Shipley 2010). The strength of filtering of particular traits along environmental gradients is, however, still not clarified and it is also not known if filtered traits are better predictors of community assembly.

We therefore investigated plant community composition and six functional traits along a pronounced hydrological gradient and studied the strength of environmental filtering on traits and the importance of the different traits to community assembly.

The results showed that the functional traits expressed by plant communities change along the hydrological gradient and that the range of trait values observed was restricted at particular parts of the gradient indicating environmental filtering (Fig. 5). Further analysis showed that root porosity is significantly filtered by the environment along the gradient, as is specific leaf area and resistance to water loss on drying. For individual traits, the strength of filtering wax and wane along the gradient, strongly suggesting that the mechanisms through which species are filtered into communities act through different traits as environmental conditions change.

The maxent model predicted 66% of the variation in species’ relative abundances using six traits. The traits subject to filtering also were most important in predicting species abundance.

In summary we showed that few plant traits are subject to environmental filtering across the entire gradient in water coverage and soil depth and that most traits experience strong filtering in only part of the gradient. Evidence for congruence between trait dispersion indices and the maxent model was established, underpinning the importance to plant community assembly of environmental filtering of species through their traits.

Water level fluctuations affect plant growth and sediment dynamics of isoetid populations in oligotrophic lakes (paper IV)
Amphibious species growing in the zone of water level fluctuations often have traits allowing them to cope with strong abiotic changes accompanying alterations from drained to submerged conditions. Along the margins of oligotrophic softwater lakes the isoetid species, Littorella uniflora, survives this stress by changing leaf morphology upon emergence (Hostrup and Wiegleb 1991, Robe
Fig. 5. Community-weighted mean trait values (CWM, black dots) and ranges of trait values (bars) in local communities along a hydrological gradient (expressed as PC1) of increasingly drier conditions. The curves represent least square regression models and $r^2$-values indicate strength of model fit. Linear regression were used for leaf dry matter content (LDMC) while second order polynomial regression lines were fitted to specific leaf area (SLA), water loss on drying (WLOD), plant height, leaf area (log), and root porosity.

...and Griffiths 1998) and it may be particularly successful because of its ability to take up $CO_2$ across root surfaces under both submerged and drained conditions (Fig. 6, Nielsen et al. 1991).

Accumulation of labile organic matter in the sediments from phytoplankton blooms accompanying eutrophication represents a threat to isoetids because of insufficient root oxygenation, reduced anchorage and enhanced growth of taller competitors. We hypothesized that *L. uniflora* tolerates different water levels but grows better and maintains dominance when sediments are periodically drained because air exposure assists oxygenation, mineralization and consolidation of sediments. If so, drainage offers a possible restoration tool. We tested this hypothesis by measuring plant performance and sediment metabolism under drained, fluctuating and submerged conditions in the laboratory and along elevation gradients in the field in...
organically enriched sandy sediments from an oligotrophic Danish lake.

Laboratory and field experiments confirmed that *L. uniflora* maintained high leaf chlorophyll and photosynthesis and grew in biomass under all conditions. Increase of biomass and shoot weight was highest under drained conditions and most so for roots as a suitable response to higher sediment oxygenation and leaf transpiration. The increase of biomass was lowest under fluctuating conditions as a logical consequence of higher costs of producing aerial leaves to replace unsuitable aquatic leaves upon changes from submerged to drained conditions.

Efflux of inorganic carbon from mineralization in un-vegetated standard sediments increased 5.5-fold from submerged, over fluctuating to drained treatments.

In submerged vegetated sediments, where part of sediment CO$_2$ is incorporated into a growing plant biomass, the sum of inorganic carbon efflux and plant growth had more than doubled compared to bare sediments probably reflecting stimulated sediment oxygenation and mineralization because of root oxygen release. Under drained, well-oxygenated conditions the increase of carbon in the biomass was accompanied by an equivalent decline of the inorganic carbon efflux relative to bare sediments. Still, the sum of inorganic carbon efflux and additional carbon trapped in the biomass was 2-fold higher under drained than submerged conditions.

The proportion of reduced Fe$^{2+}$ to total Fe as a measure of the oxygen status of sediments declined from submerged, over fluctuating to permanently drained sediment. Also the proportion of reduced Fe$^{2+}$ was lower in plant covered than bare sediments reflecting root release of O$_2$. Drainage and air contact also increased sediment density and consolidation and, thereby, improved root anchorage compared to submerged conditions.

Periodic drainage and air exposure of isoetid covered sediments is a natural phenomenon in oligotrophic seepage lakes that should be reinstalled as a possible restoration tool if the water has been regulated to a constant level. Water level fluctuations of a moderate frequency can benefit isoetid populations because they grow and survive better than competitors and potential harmful organic matter can be mineralized without sediment anoxia.

**Appendices**

In the appendix are a Danish and a Swedish article. The first addresses all the Danish species of charophytes and their distribution along with an assessment of the risk of
species going nationally extinct, a Red List evaluation. Danish charophytes are found in most types of freshwater through brackish to fully marine environments. The knowledge of the distribution of species found in marine and larger freshwater habitats are fairly good, but the distribution of species with a preference for habitats such as ponds and fens is not well known. The assessment of species extinction risk showed that 85% of the Danish charophyte species has an increased risk of going extinct nationally and three species have most likely already been lost from Denmark.

In the second appendix chapter the distribution of plants, their functional traits and the species richness were investigated along a gradient in water cover and soil depth in an abandoned limestone quarry on Öland. Along the gradient, there was a large species turnover and species found within meters from each other had markedly different functional traits adapted to the local micro-environment where they were found. Some of the most extraordinary plants found was the colonial cyanobacteria *Nostoc commune* surviving on bare limestone pavements as dry crusts only to be revitalized by rain events and winter flooding (Fig. 7) and several charophyte species totally dominating even desiccating pond and re-sprouting from spores upon re-submergence. The species richness was highest at high soil depth and good water supply as a consequence of more a more productive environment and a richer regional species pool.

Fig. 7. Typical vegetation on the limestone pavements in the investigated quarry on Öland. The colonial cyanobacteria *Nostoc commune* and the vascular plant *Sedum album* surviving the tough conditions between submergence in winter and complete dessication in summer. Photo: Ole Pedersen.
Conclusions and perspectives

Several concrete results, new questions and possible applications have emerged from this Ph.D.-thesis. Concerning charophytes, I found that they were indeed rare and many species are declining in abundance in the contemporary Scandinavian landscape. The results of the influence of traits and niche specialization on abundance and distribution could hopefully inspire further research to fill the gap in our knowledge of charophyte ecology. For most species, data on their basic biology stem from anecdotal studies most of them performed more than half a century ago and for quite a few species new molecular methods have questioned previously accepted taxonomical entities. It is thus urgent to gain a better understanding of the basic ecology, taxonomy and distribution of species in small water bodies such as ponds, fens and bogs from which there is less data available. This knowledge is of pivotal importance to ensure that the proper management can be implemented to prevent that rare and declining species are not lost entirely.

Because knowledge of vascular plants is much more advanced, it is now possible to make predictions about the abundance of these species along environmental gradients. I have provided a better understanding of how species traits are filtered by environmental conditions and provided a link potentially ensuring more accurate future predictions of community assembly. Fully implemented, this knowledge can lead to well-considered decisions in ecological restorations, because effects of a given intervention can be accurately predicted beforehand.

One such restoration could be the use of sediment desiccation of lake sediments that proved to have the prospective to improve conditions for plant growth where these have been deteriorated by organic enrichment. Eutrophication leading to organic enrichment has been widespread along with stabilization of water levels to ensure human control over the environment, however letting go of control would lead to a richer and more diverse nature.
Acknowledgments

I am indebted to numerous people supporting me through the last three and a half years. Firstly I want to thank Tone for bearing with me all these years and for helping me through the periods where everything looked impossible with strong help from Alfred’s smiles. To my supervisor Kaj, I am deeply grateful for your invaluable help, guidance and prompt feedback. You always take the time needed to get your students in the right direction and that is a great personal trait. I have really enjoyed our numerous fruitful discussions on plants, birds and nature in general, it is a pleasure to know a man that knows (almost) everything. To Claus, with whom I have shared path with since our first days of introductory chemistry even before the first semester in biology, thanks for the many years. I know we will keep hanging out and go fishing (we still lack the big one), but hopefully we can keep on collaborating scientifically as well. Big thanks to Hans Henrik, for introducing me to the fascinating world of terrestrial botany and for always having an open door and mind for a young aquatic ecologist. Thanks to Jens, for your always open ears and for calling me a bastard (skrummel) in the morning, to Ole P, for help, in particular with the pictures of this thesis, to Lars Iversen, for valuable statistical assistance, to Birgit, Ayoe and Anne, for skilful laboratory assistance, and to Mikkel, for help in the field even though the data did not get published (yet). Last but not least to my fellow young scientists at FBL: Anders, Ane, Jesper, Matteo, Theis, Laci, Nina, Frandsen and all the students thanks for making life as a PhD-student a joy.
References


References


Chapter I
Seventy years of changes in the abundance of Danish charophytes

Photo: Martin Kielland (livetunderoverfladen.dk)
Seventy years of changes in the abundance of Danish charophytes

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SUMMARY

1. Charophytes grow attached to soft bottoms in ponds, streams, lakes and estuaries and are highly threatened throughout Europe according to the national Red Lists. We used Danish studies on freshwater charophyte distributions conducted around 1940 and repeated measurements during recent years to evaluate the historical development of species richness and dominance patterns. We also tested to what extent historical changes of species abundance in 29 waterbodies were related to landscape features, water quality and species traits.

2. We found that three species of freshwater charophytes (Chara filiformis, Tolypella intricata and Nitella gracilis) have apparently disappeared from Denmark while one species (Chara connivens) has immigrated. National species richness has thus declined from 21 to 19 species.

3. Species abundance based on occurrence in many waterbodies followed a linear rank–log abundance relationship both in the historical and the recent studies. The dominance structure was stronger today than historically as common species have become relatively more abundant and uncommon species relatively rarer.

4. Among species traits, perenniality and preference for alkaline waters typical of deep-growing species in large alkaline lakes, a rare contemporary habitat, were significantly related to the historic species decline. Species increasing in abundance had wide tolerances to alkalinity and water nitrogen content.

5. Twenty-nine lakes and ponds studied repeatedly showed a significant decline of mean species richness from 3.4 to 2.4 during the 70 years. A small increase in species richness has taken place during the recent 15–20 years in several lakes experiencing reduced nutrient loading. However, many species survive today in relict populations and may find it difficult to recolonise lakes in which water quality has improved.

6. The historical decline of species richness was significantly related to higher nutrient concentrations, higher phytoplankton biomass and lower transparency of eutrophied waterbodies. In contrast, the amount of wetlands and openness of the landscape close to a waterbody did not predict the historical development perhaps because local processes or long-distance dispersal determine species richness.

7. Considering together the loss of former freshwater habitats and the deterioration of surviving habitats, we conclude that charophyte occurrence has declined by about 56% in Denmark during the last 70 years. Species reductions determined from the reinvestigated waterbodies (29%) and the national species list (10%) are much lower and are less suitable measures of the developmental status of charophytes.

Keywords: charophytes, eutrophication, freshwater stoneworts, habitat deterioration, historical changes

Introduction

Charophytes are 5–200 cm tall macroalgae which are apparently highly threatened by disappearance from soft-bottom sediments in lakes, ponds, streams and brackish waters in Nordic and most other European countries (Blaženec et al., 2006; Siemenska et al., 2006; Langangen, 2007). The abundance of freshwater macrophytes in their
characteristic environment is significantly related to the specific ecological traits of the species (Sand-Jensen et al., 2000). Among charophytes, the largest species appear to be found in clear lakes while the smaller species can also be found in turbid waters (Blindow, 1992a; Sand-Jensen et al., 2000). Because of the rarity, the threatened status and the markedly different ecological traits of charophyte species, it is particularly important to explore how their distribution and relative abundance have developed during the previous century characterised by intensive human impact on aquatic ecosystems. Historical analyses of their distribution in Switzerland and the Netherlands have shown the decline of particularly the rare species during the last 100–200 years (Simons & Nat, 1996; Auderset Joye, Castella & Lachavanne, 2002). In Sweden, a reduction in charophyte populations has been observed during the 20th century, probably due to worsened light conditions caused by eutrophication (Blindow, 1992a). These changes have not, however, been directly related to habitat changes or to multiple ecological species traits. Here, we attempt to fill this gap in our knowledge by establishing the possible causes behind changes in the abundance of Danish freshwater charophyte species during the last 70 years.

A comprehensive study of the distribution of charophytes in Danish waters is available from the early 1900s with the most extensive data being sampled around 1940 (Olsen, 1944). Recent investigations (1990–2011) with most data available for 2009–10 have allowed us to quantify the development of charophyte vegetation between 1940 and 2010. The historical analysis is complicated in Denmark by the fact that eutrophication peaked during the period 1980–95, but subsequently declined up to 2010 (Jensen et al., 2011). While increasing nutrient richness and water turbidity from 1900 to 1980–95 may have led to a reduction of species richness of charophytes, the recent decline of nutrient concentrations and water turbidity may have increased species richness of charophytes in those habitats experiencing the greatest improvement of water quality. Therefore, we expect a mixed response of species richness and composition in those 29 lakes and ponds that have been examined repeatedly during the past 70 years.

Habitat loss will reduce the possibility of dispersal of charophytes to existing habitats and increase the probability of extinction of the rare species. Widespread deterioration of freshwater habitats has mainly involved cultural eutrophication by organic matter, nitrogen and phosphorus from households, fish farms and agriculture (Moss, 1998). In Denmark, loading of nitrogen and phosphorus to aquatic environments increased during the 20th century with loadings starting to decrease in the 1970s for phosphorus and 1990s for nitrogen (Miljøstyrelsen, 1991; Kronvang, 2001), although nutrient loadings are still markedly above pre-1900 levels (Jensen et al., 2011). Higher concentrations of nitrogen and phosphorus in fresh waters have generally stimulated the growth and biomass development of phytoplankton and fast-growing filamentous algae. The resultant increased shading upon submerged flowering plants and charophytes led either to a decline of their depth penetration or to total disappearance, depending on the severity of eutrophication and the type of organism (Sand-Jensen & Borum, 1991; Middelboe & Markager, 1997). Thus, in waters experiencing intermediate levels of nutrient enrichment, tall, robust flowering plants capable of developing their canopy just below the water surface may have survived, while competitively inferior bottom-dwelling mosses and charophytes may have disappeared (Middelboe & Markager, 1997; Van Den Berg et al., 1998a; Sand-Jensen et al., 2008). Several charophytes have a high reproductive potential and, in turn, a high dispersal capacity via birds and for that reason can become established in new or recently disturbed lakes with a restricted coverage of rooted plants (Bonis & Grillas, 2002). We therefore anticipate that the charophyte vegetation has changed during the last 70 years towards a stronger dominance of species tolerant of nutrient-rich and more perturbed conditions, while species specialised to oligotrophic, stable conditions should have declined in abundance because this habitat type has become much rarer.

Aside from habitat-specific eutrophication, the occurrence of aquatic plants has also been shown to be positively affected by the species richness in well-connected aquatic habitats because of better chances of colonisation (Dahlgren & Ehrlen, 2005; Akasaka & Takamura, 2011). Therefore, we expect that, in the reinvestigated Danish lakes, loss of charophyte species has been less pronounced in lakes surrounded by many other lakes and ponds.

Drainage of lowland areas to increase the area of arable land and eutrophication are virtually universal and have played a pivotal role in reducing the ecological status of freshwater systems across the whole of Europe (Moss, 1998). We therefore predict that the same tolerant species have become dominant throughout Europe and that the species undergoing relative increases or reductions in abundance in Denmark during the last 70 years should have behaved similarly in other parts of Europe experiencing the same environmental impact.
Materials and methods

Data collection and taxonomy

The historical distribution of charophyte species was examined in 114 waterbodies with charophyte vegetation during the period 1937–42 (Olsen, 1944). The contemporary study between 1990 and 2011 included 208 localities with charophyte vegetation examined by the environmental agencies, university researchers (Nygaard & Sand-Jensen, 1981; Vestergaard & Sand-Jensen, 2000; Riis & Sand-Jensen, 2001; Sand-Jensen et al., 2008; Anonymous, 2012) gifted botanists (K. Buchwald, M. Holmen, B. Moeslund and J. C. Schou, pers. comm. 2010–12) and recently by ourselves (Dahl-Nielsen, 2011). In addition, the examination yielded the national species richness in fresh water in the two periods, including rare species only found outside the waterbodies under focus. Data from the old and contemporary studies are referred to as 1940 and 2010, respectively because these were the years with most information available.

Charophytes were searched for in lakes, ponds, pits, streams and ditches. To avoid confounding effects of salinity, we focused on data from freshwater localities with salinity lower than 0.5% NaCl. Contemporary vegetation analyses followed national guidelines (Kristensen et al., 1990) and are regarded as more thorough in large lakes than old studies because of the recent use of Scuba diving and sampling along several transects. In the other waterbodies, thoroughness was probably the same, but Sigurd Olsen (1944), being an international expert on charophytes, was perhaps more skilled at identifying charophyte species than recent investigators. Altogether there were high numbers of sites and charophyte species records in both the old investigation (114 sites, 205 records) and the recent investigation (170 sites, 295 records) allowing comparative measurements of the presence and abundance of species.

Charophyte species were identified according to Olsen (1944) and Blindow et al. (2007). When vegetation analyses were available from several visits, a cumulative species list was established. Nomenclature followed German recommendations (Arbeitsgruppe Characeen Deutschlands, 2010) in both the old and the recent study. Chara fragilis was historically the most common Danish species; it is now named Chara globularis. Historically, Chara virgata was viewed as a variety of C. fragilis and its specific distribution is thus not known (Olsen, 1944). Accordingly, we regard C. virgata as being present in 1937–42, although we are unable to determine its relative abundance. To be consistent, we regard findings of C. virgata as C. globularis in the further analysis.

Changing abundance and species traits

We determined the frequency of occurrence of every species in the two studies as the number of records of a species divided by the number of sites investigated. The frequency of occurrence is a measure of how abundant a given species is. To facilitate comparison among all species, some of which are very common while others are very rare or absent in one of the studies, we transformed the data logarithmically. We used log (x + 1) to ensure that species that are absent from one of the two studies are included in the analysis.

We constructed rank-abundance diagrams of species in historic and recent studies from Denmark by plotting log (x + 1) versus falling rank of species and comparing the negative linear slope (Tokeshi, 1993). To compare the dominance pattern of species in old and recent studies, we also calculated Pielou’s evenness index (PEX):
A series of specific traits that could affect the species abundance could be obtained from the literature on Scandinavian charophytes (Langangen, 2007) and supplemented by a Polish investigation in the few cases where Scandinavian data were missing (Gabka, 2009). The traits included were shoot height, life form, and production of oospores and bulbils. The maximum shoot height (cm) was converted to a numerical scale (0: 0–20 cm, 1: 21–40 cm and 3: >41 cm) to account for the variation within and among species. Life form data were included as (i) obligate annual, (ii) obligate perennial and (iii) both annual and perennial. The intensity of oospore production was provided by Langangen (2007) for species with low (0) and high (2) production while species lacking detailed information were considered intermediary (1). Some species produce bulbils as a means of vegetative reproduction and these species were given the value 1, while the rest were assigned the value 0. The oospore and bulbil production were added together forming an index of reproductive potential (0–3).

Site-specific changes

An ANOSIM analysis (Clarke, 1993) was applied to evaluate whether the composition of charophyte species in the Danish waters changed between the historic and the contemporary investigations. This analysis yields a Global-R indicating the separation between groups, and a significance level can be determined for an unbalanced number of samples. The analysis is therefore well suited for our type of data. The analysis was performed on species presence/absence data in the investigated waterbodies using the program PRIMER 6 (Clarke & Warwick, 2001).

A number of the sites visited by Olsen (1944), primarily lakes, were also included in the contemporary study (called reinvestigated lakes) allowing an evaluation of the development in species richness and its possible causes during the last approximately 70 years.

Changes in species richness between the two different periods were tested with a paired t-test. Also available for these reinvestigated lakes were several environmental parameters (total phosphorus, total nitrogen, phytoplankton chlorophyll a, Secchi transparency and alkalinity) collected by the counties and other Danish environmental agencies during the last 30–40 years (Anonymous, 2012). Correlation analysis was used to analyse whether the changes in richness of charophytes were related to the environmental parameters. In order to determine whether changes in richness of charophytes were related to past or present environmental lake conditions, we created simple multiple linear regression models. The environmental variables were split into pre- and post-1996 observations, normalised and added into a PCA. Using relative changes in richness as a response variable and the primary axis score from the two PCAs as the explaining variables, the model was reduced by an F-test using a pre-specified significance level of 5%.

To investigate whether landscape features influenced the development of the charophyte species richness in the reinvestigated lakes, we used a GIS-based methodology (ArcGIS 10, www.ESRI.com) to derive landscape variables from a contemporary national landcover map (AIS landcover map, www.ais.dmu.dk). We selected a group of features known to affect the viability and distribution of biodiversity at the landscape level. Firstly, we determined possible sources of species emigration within three lag distance to each study lake; these were expressed as (i) the % coverage of wetlands near a reinvestigated lake, (ii) the number of lakes >1 ha and (iii) the number of ponds and lakes <1 ha. The three lag distances were chosen to express local landscape-level effects from the three variables mentioned above. These distances were chosen in order to cover neighbouring effects (1 km buffer zone from each study site), long-distance effects (buffer zones from 5 to 10 km from each site) and intermediate distance effects (buffer zones from 1 to 5 km from each site). We also determined the distance to a potentially large dispersal source (distance to a lake >10 ha), distance to nearest inner coastal zone and lake surface area, and we determined whether a locality was interconnected with other lakes since this might also affect the contemporary charophyte richness. Plant species depending on wind dispersal (Nathan et al., 2002) and charophyte dispersal depending on water birds travelling across open areas might be negatively affected by the amount of forest and thereby the structure of the landscape. Hence, to express the openness of the surrounding landscape, the amount of forest was calculated within the three lag distances from each study lake.

To describe the relationship between landscape variables and trends in charophyte species richness, we conducted two selective model reduction steps. Firstly, we fitted four individual logistic regression models of the four variables described in three lag distances from each site (amount of forest, amount of wetlands, number of ponds and number of lakes >1 ha). We used a coding
(1/0) as response variable, where 1 represents a positive or stable trend in species diversity, and 0 represents a decline in diversity. In order to obtain convergence in the models, only main effects of the three lag distances of each variable were used (Table 1). We performed model reduction via a $\chi^2$-test and removed the least significant variable until only significant variables remained. All resulting $P$-values were evaluated at a 5% significance level. Secondly, we created a final logistic regression model containing significant variables from the first selection step together with distance to inner coast, distance to a lake larger than 10 ha, lake surface area and whether the locality was interconnected with other lakes (Table 1). Since the viability and current richness of charophytes are affected by nutrient conditions within each locality (Blindow, 1992a; Van Den Berg et al., 1998b), we adjusted for this by adding the primary axis score from a PCA of the basic environmental variables within the lakes into the model as a confounding variable. The final model underwent the same reduction procedure as the four initial models. All statistical analyses were conducted in R v. 2.14.2 (www.r-project.com).

As a subset of the reinvestigated lakes was analysed several times during the 70-year period, it was possible to investigate changes in species richness in the lakes during the period of progressive eutrophication from 1940 up to 1975–95 followed by the most recent period of oligotrophication up to 2010.

Results

Loss and gain of species

Three charophyte species have apparently disappeared from Danish fresh waters and one species has appeared.

Table 2 The abundance (frequency of occurrence, %) of freshwater charophyte species in the historic (1940) and recent investigations (2010). Also shown are categorical traits for shoot height, life form and reproductive potential. Recordings with asterisk (*) are species not present in the investigated lakes, but found in Denmark within the focal period. N/A indicates no information found in the literature.

<table>
<thead>
<tr>
<th>Species</th>
<th>Shoot height</th>
<th>Life form</th>
<th>Reproductive potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chara aspera</td>
<td>11.4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Chara convivens</td>
<td>0.0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Chara contraria</td>
<td>12.3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Chara denudata</td>
<td>2.6</td>
<td>1</td>
<td>N/A 1</td>
</tr>
<tr>
<td>Chara filiformis</td>
<td>0.9</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Chara globularis</td>
<td>59.6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Chara hispida</td>
<td>16.7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Chara intermedia</td>
<td>11.4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Chara polyacantha</td>
<td>1.8</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Chara rudis</td>
<td>7.0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Chara tomentosa</td>
<td>7.9</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Chara vulgaris</td>
<td>26.3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Nitella capillaris</td>
<td>0.0*</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nitella flexilis</td>
<td>3.5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Nitella gracilis</td>
<td>0.9</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Nitella mucronata</td>
<td>0.9</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Nitella opaca</td>
<td>5.3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Nitella trunctens</td>
<td>2.6</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Nitelopsis obtusa</td>
<td>6.1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Tolypella glomerata</td>
<td>2.6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tolypella intricata</td>
<td>0.0*</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tolypella nidifica</td>
<td>0.0*</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

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Species abundance and composition patterns

As anticipated, the relative abundances of species in the old and recent study were significantly positively correlated (Fig. 1). Negative slopes of species abundance versus falling rank for all species were nonetheless significantly steeper in the recent than the old studies (Fig. 2), reflecting a contemporary community more characterised by dominance. This interpretation is supported by Pielou’s evenness index being higher in the 1940 study (0.79) than in the 2010 study (0.75).

The ANOSIM analysis allowed investigation of changes in species composition at the sites in the two periods. Using the 114 historic and 168 contemporary sites with known charophyte species composition, the ANOSIM yielded a Global-R of 0.047 (P = 0.01), showing a significant change in species composition between the early and the recent period. The relatively low Global-R value indicates some degree of overlap between the periods.

It is likely that species preferences and species traits influence changes in species abundance over the last 70 years. We tested the correlation between the environmental optimum and tolerance values (median and coefficient of variation) of the environmental variables measured at the sites where each species were present with the differences in abundance between the old and the recent study (Table 3). The species optimum and tolerance of alkalinity was significantly correlated with changes of abundance, implying that species preferring high alkalinity have declined in abundance from 1940 to 2010 and that species increasing in abundance have a broad alkalinity tolerance. Also, the increasing species have a wider tolerance of lake nitrogen content, suggesting an ability to thrive under different trophic conditions. The trophic status values from the independent

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Table 3 The environmental optimum and tolerance values of nine Danish charophyte species (median value/coefficient of variation). The values are calculated from the environmental variables at all the growth sites of the species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Alkalinity* (meq L⁻¹)</th>
<th>TP (µg L⁻¹)</th>
<th>TN† (µg L⁻¹)</th>
<th>Chlorophyll a (µg L⁻¹)</th>
<th>Secchi depth (m)</th>
<th>Number of sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chara aspera</td>
<td>1.99/0.43</td>
<td>50.7/0.80</td>
<td>1220/0.56</td>
<td>12.1/1.17</td>
<td>1.32/0.73</td>
<td>27</td>
</tr>
<tr>
<td>Chara contraria</td>
<td>2.18/0.33</td>
<td>52.7/0.79</td>
<td>1044/0.52</td>
<td>13.1/0.74</td>
<td>2.13/0.56</td>
<td>17</td>
</tr>
<tr>
<td>Chara globularis</td>
<td>2.15/0.45</td>
<td>62.1/1.26</td>
<td>1227/0.55</td>
<td>14.1/1.03</td>
<td>1.65/0.67</td>
<td>90</td>
</tr>
<tr>
<td>Chara hispida</td>
<td>2.60/0.33</td>
<td>21.1/0.81</td>
<td>951/0.37</td>
<td>8.3/0.81</td>
<td>0.98/0.82</td>
<td>10</td>
</tr>
<tr>
<td>Chara tomentosa</td>
<td>2.43/0.24</td>
<td>44.0/0.47</td>
<td>1038/0.33</td>
<td>9.9/0.66</td>
<td>1.38/0.74</td>
<td>7</td>
</tr>
<tr>
<td>Chara vulgaris</td>
<td>2.45/0.36</td>
<td>50.8/0.69</td>
<td>1189/0.63</td>
<td>14.6/0.90</td>
<td>1.84/0.66</td>
<td>55</td>
</tr>
<tr>
<td>Nitella flexilis</td>
<td>0.93/0.88</td>
<td>43.0/1.45</td>
<td>1014/0.75</td>
<td>12.6/1.11</td>
<td>1.64/0.72</td>
<td>46</td>
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<tr>
<td>Nitella translucens</td>
<td>0.42/1.19</td>
<td>29.2/0.75</td>
<td>897/0.78</td>
<td>8.8/0.94</td>
<td>1.64/0.70</td>
<td>6</td>
</tr>
<tr>
<td>Nitellopsis obtusa</td>
<td>2.19/0.26</td>
<td>54.5/0.51</td>
<td>1143/0.44</td>
<td>15.0/0.63</td>
<td>1.27/0.72</td>
<td>13</td>
</tr>
</tbody>
</table>

*Both optimum and tolerance values are significantly correlated with the change of species abundance (Spearman P < 0.05).
†The tolerance values are significantly correlated with the change of species abundance (Spearman P < 0.05).
Dutch investigation (Simons & Nat, 1996) did not correlate with the changes in abundance of the Danish charophyte species (data not shown).

Changes in species abundance could be tested against the independently collected categorical traits (Table 2). The analyses showed that obligate perennial species declined significantly more than either obligate annuals or species capable of exhibiting both life forms (Kruskal-Wallis $P = 0.004$). Shoot size and reproductive potential did not appear to influence the changes in abundance (Spearman rank, $P > 0.05$). Accordingly, it appears that perennial species and species with preference for highly alkaline waters are the most vulnerable and subject to a significant decline, while species tolerant of variable alkalinity and nitrogen content have increased in abundance.

**Species number and composition in revisited Danish localities**

Mean species richness of charophytes in the 29 freshwater ponds and lakes that were investigated in both 1940 and 2010 was 3.4 in the early investigation and significantly lower at 2.4 (paired t-test, $P = 0.03$) in the recent investigation. Nine of these waterbodies lost their charophyte vegetation entirely and eight other waterbodies had reduced species richness in the present investigation resulting in a decrease in species richness in 58% of the sites (Fig. 3). In 21% of the sites, no change in richness was observed while an increase in richness was found in the remaining 21% sites.

There was a significant linear correlation between several of the investigated environmental variables (total phosphorus (TP), total nitrogen (TN), chlorophyll $a$, Secchi transparency and alkalinity) and the relative decline of species richness in the reinvestigated lakes during the recent decades (1980–95 to 1996–2010) where environmental measurements were available (Table 4). All relationships suggested that higher nutrient concentrations increased the loss of original species. The environmental variables derived from the strongest eutrophication period (1980–95) had a stronger effect ($F = 10.06$, $P < 0.01$) on the relative changes in richness of charophytes than the contemporary environmental variables from 1996–2010 ($F = 0.03$, $P = 0.87$).

The four initial models of landscape features indicated that forest within 5–10 km, number of lakes $> 1$ ha within 5–10 km and number of ponds within 0–1 km from each site had an effect on the trends in charophyte richness (Table 5). However, the final model showed that none of the spatial variables had any significant effects on changes in diversity ($L = 2.77$, $P = 0.09$). This result suggests that the changes in charophyte diversity in Denmark are not controlled by any of the spatial variables of the landscape surrounding the investigated sites.

**Recent oligotrophication**

Recent oligotrophication of Danish lakes resulting from the decrease in nutrient loading might have caused an increase in species richness of charophytes. Thirteen of the reinvestigated lakes had botanical inventories made in 1940, 1980–95 and after 2000 making it possible to search for an effect of the recent oligotrophication (Fig. 4). Paired tests showed a significant decline in species richness from 1940 to 1980–95 (Wilcoxon, $P = 0.03$) while no differences were found between the other periods for this

### Table 4

<table>
<thead>
<tr>
<th>Environmental variable</th>
<th>Correlation coefficient</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phosphorus 1980–95</td>
<td>0.55 (0.008)</td>
<td></td>
</tr>
<tr>
<td>Total phosphorus 1996–2010</td>
<td>0.47 (0.028)</td>
<td></td>
</tr>
<tr>
<td>Total nitrogen 1980–95</td>
<td>0.44 (0.041)</td>
<td></td>
</tr>
<tr>
<td>Total nitrogen 1996–2010</td>
<td>0.44 (0.04)</td>
<td></td>
</tr>
<tr>
<td>Chlorophyll $a$ 1980–95</td>
<td>0.25 (0.27)</td>
<td></td>
</tr>
<tr>
<td>Chlorophyll $a$ 1996–2010</td>
<td>0.33 (0.18)</td>
<td></td>
</tr>
<tr>
<td>Secchi depth 1980–95</td>
<td>$–0.26$ (0.27)</td>
<td></td>
</tr>
<tr>
<td>Secchi depth 1996–2010</td>
<td>$–0.47$ (0.028)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3 Species richness of charophytes in 29 reinvestigated ponds and lakes in the past (1940) and recently (2010). The dotted line is the 1 : 1 line.

subsample. In five of the 13 lakes, however, an increase in species richness was observed between 1980–95 and after 2000, while only one lake lost species (Fig. 4).

Discussion

Historic changes of charophytes

Charophytes were still widespread and abundant in European countries up to 100–300 years ago in resource-limited and physically stable habitats such as cold, low-light bottom waters of deep oligotrophic lakes and phosphorus-limited calcareous lakes (Hasslow, 1931; Iversen, 1929; Olsen, 1944; Nygaard & Sand-Jensen, 1981; Klein, 1993). Thanks to rapid dispersal and colonisation by oospores, some charophyte species were and still are transiently abundant in newly established ponds and pits before rooted plants gradually take over and outcompete them (Olsen, 1944; Crawford, 1977; Wade, 1990). Resource-limited, physically stable habitats or newly established ephemeral habitats have become much rarer in the more densely populated and intensively cultivated European landscapes of today and charophytes have found themselves in more marginal and threatened positions than before (Agger & Brandt, 1988; Biggs et al., 2005; Munsterhjelm, Henricson & Sandberg-Kilpi, 2008; Sand-Jensen et al., 2008). In Denmark, 85% of the species are proposed to belong to Red List categories (Bastrup-Spohr et al., 2013) and the percentage of species on the Red List is 50–87% across European countries (Schmidt et al., 1996; Blazencic et al., 2006; Siemenska et al., 2006). These percentages are higher than for other aquatic plants species groups that we know of (Gärdnors, 2010; Kalas et al., 2010). The extremely threatened status of charophytes can be explained by loss of their habitats in transparent oligotrophic lakes at the greatest water depths of submerged macrophyte growth and inability to compensate for turbid waters by developing tall shoots and to compete with flowering plants in dense communities in nutrient-rich habitats (Blindow, 1992b; Van Den Berg et al., 1999).

We document here an overall historic decline in species number of freshwater charophytes in Denmark from 21 to 19 species during the last 70 years. Species richness has also declined in Switzerland (26 to 22), (Auderset Joye et al., 2002). The modest overall decline of national species richness sounds much less alarming than the high

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**Table 5** Effect of landscape features on the trends in charophytes species richness during the last 70 years. The initial landscape, significant variables in the initial models and the final landscape model are shown. The included variables in the model were calculated in three lag zones surrounding the lake (0–1 km, 1–5 km and 5–10 km). The variables are forest (% cover), wetlands (% cover), lakes >1 ha (count), ponds <1 ha (count), distance to a large (>10 ha) lake (km), distance to inner coastline (km), connection to other waterbodies (yes/no) and the score of a primary PCA axis on environmental conditions in the lakes.

<table>
<thead>
<tr>
<th>Model</th>
<th>Initial model variables</th>
<th>Significant variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest</td>
<td>Richness trend ~ forest 0–1 km + forest 1–5 km + forest 5–10 km</td>
<td>Forest 5–10k ($L = 4.00$, $P &lt; 0.05$)</td>
</tr>
<tr>
<td>Wetland</td>
<td>Richness trend ~ wetland 0–1 km + wetland 1–5 km + wetland 5–10 km</td>
<td>None ($L = 1.54$, $P = 0.21$)</td>
</tr>
<tr>
<td>Lakes &gt; 1 ha</td>
<td>Richness trend ~ lakes 0–1 km + lakes 1–5 km + lakes 5–10 km</td>
<td>Lakes 5–10k ($L = 8.19$, $P &lt; 0.01$)</td>
</tr>
<tr>
<td>Ponds</td>
<td>Richness trend ~ ponds 0–1 km + ponds 1–5 km + ponds 5–10 km</td>
<td>Ponds 0–1 km ($L = 12.32$, $P &lt; 0.001$)</td>
</tr>
<tr>
<td>Final</td>
<td>Richness trend ~ PCA1 + area_lake + lakes 5–10 km + ponds 0–1 km + forest 5–10 km + distance to coast + distance to lake &gt;10 ha + lake_connection</td>
<td>None ($L = 2.77$, $P = 0.09$)</td>
</tr>
</tbody>
</table>

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**Fig. 4** Species richness of charophytes in thirteen lakes measured three times between 1940 and 2010. Five lakes have experienced a recent increase of species richness (upper panel), whereas no recent changes occurred in the other lakes aside from one lake experiencing a minor richness reduction (lower panel). Overall, there was a significant decline of species richness from 1940 to 1980–95 (Wilcoxon test $P = 0.02$), but no significant change between either 1940 or 1980–95 and the contemporary investigation ($P = 0.24$ and $P = 0.14$).
proportions of species on the national Red Lists because most species are still present within the countries though in diminishing populations and at fewer localities. In that respect, changes in species number in the same localities offer a more precise quantitative descriptor of the development. In 29 of such Danish localities, the mean species number of charophytes per locality declined from 3.4 to 2.4 (29%) over the past 70 years due to habitat deterioration. Still, this comparison does not include former charophyte localities, mostly small ponds and pits, which have been lost entirely by being drained or filled in. Fifty-four such localities contained, on average, 1.43 species in Olsen’s (1944) historical study. If we assume that lost ponds and pits amounted to twice the number of retained localities (Sand-Jensen et al., 2000; Fog, 2011), represented by the aforementioned 29 localities with a mean decline of species richness from 3.4 to 2.4, then the average historical decline of charophyte occurrences in Denmark as a combined result of habitat deterioration and habitat loss would amount to 56%. In comparison, Danish species richness declined by only 10% and is a much less sensitive measure of the overall status of charophytes because one growth site for a species contributes with the same score as numerous growth sites.

Our comparisons among localities document a significant correlation between the decline of species richness and the impoverished water quality due to eutrophication (i.e. higher nutrient concentrations, phytoplankton biomass and water turbidity) in accordance with numerous former investigations (Jupp & Spence, 1977; Lachavanne, Perfetta & Juge, 1992; Sand-Jensen et al., 2000; James et al., 2005). In 13 lakes investigated three times during the 70 years, we also observed an increase in species richness in five of the lakes after nutrient loading has been reduced during the last 15 years. Although this overall increase of charophyte richness was not statistically significant, it has been quite distinct in lakes experiencing a great improvement of water quality (Van Den Berg et al., 1999; Jeppesen et al., 2005; Sand-Jensen et al., 2008). These observations stress that the charophyte vegetation can recover, although it is questionable whether full recovery to the pre-1940 level of richness is possible given that some species have gone nationally extinct, that several species only survive in relict populations in a few localities, and that it is doubtful whether lakes fully return to their former nutrient status.

Processes behind charophyte losses

It is not the eutrophication of waterbodies alone, but a combination of deterioration of waterbodies (i.e. by eutrophication and physical disturbance) and total loss of suitable waterbodies that has led to decline of the charophyte vegetation in European countries. The loss or decline of 38 populations of Chara tomentosa and the stable occurrence or positive development of only seven populations in the low-saline brackish waters of SE Finland between the 1890s and 2003 illustrate the harmful human impact (Munsterhjelm et al., 2008). In 27 of the Finnish localities, the charophyte decline could be linked to either eutrophication (19 cases of stimulated growth of filamentous algae and nine cases of higher input of nutrient-rich drainage water), higher physical impact (10–11 cases of shore activity, dredging, sediment spread and boat traffic) or a combination of the two impacts (i.e. 18 cases of higher water turbidity from phytoplankton and sediment particles). The general loss of waterbodies with charophyte vegetation can be illustrated by the vast decline of ponds in Great Britain from 1.2 million in 1880 to only 0.3 million in 1990 (Biggs et al., 2005), and the same 70–80% decline of ponds and shallow lakes has taken place in Denmark from 1900 to 1990 (Kristensen, Reenberg & Pena, 2009; Fog, 2011).

We initially proposed, but were unable to confirm, the existence of significant relationships between the richness of charophytes in focal waterbodies and features of the landscape surrounding the lakes. These findings suggest that species richness of the individual waterbody is primarily dependent on its water quality and that dispersal from waterbodies located close by is not a critical factor for the occurrence because either long-distance dispersal or local processes are sufficient to sustain species richness.

Like terrestrial mosses, lycopods and ferns, charophytes have ended up in more marginal and resource-poor habitats than the more recently evolved flowering plants (Bates, 1998). Charophytes and mosses can be expected to be restricted by the lack of roots, solid stems and vascular tissue for efficient nutrient uptake and translocation while lycopods and ferns can be expected to be restricted by the lack of secondary growth and well-protected seeds compared with flowering plants. Although these functional shortcomings have excluded charophytes, mosses, lycopods and ferns from resource-rich, unperturbed habitats, their long-term evolutionary histories have adapted them to the marginal, resource-poor habitats through their exceedingly low growth rates, high persistence and capacity for long-distance dispersal of spores between widely scattered habitats (Hedderson, 1992; Longton, 1992). Charophytes and aquatic mosses have systematically lower growth and photosynthetic rates than aquatic flowering plants (Kautsky, 1988; Nielsen & Sand-Jensen,
Abundance patterns and coupling of abundance to species traits

The study confirmed our initial prediction that common charophyte species have become relatively more abundant and rare species relatively less abundant in Denmark during the historic loss and deterioration of suitable charophyte habitats. The same development has taken place in the Netherlands and Switzerland (Simons & Nat, 1996; Auderset Joye et al., 2002). Our analysis showed that rank-abundance diagrams became significantly steeper and indices of evenness smaller in all three countries (data not shown). A parallel development has been observed at the local scale among submerged macrophytes upon eutrophication of Lake Fure (Sand-Jensen et al., 2008). Terrestrial grassland communities likewise respond to increasing soil richness by developing stronger dominance structure among species in gradually increasing competition for light in denser communities (Stevens & Carson, 1999).

It is remarkable that Chara vulgaris and C. globularis are among the two most abundant species in Denmark, the Netherlands, the Czech Republic, Switzerland and the Balkans, and that Nitella flexilis is the third most abundant species in the three first-mentioned countries (Simons & Nat, 1996; Auderset Joye et al., 2002; Blazencic et al., 2006; Caisova & Gabka, 2009). These three species are located as numbers 15, 14 and 12 according to increasing trophic rank among 16 species in the Netherlands and are there regarded as being among the most eutrophic species (Simons & Nat, 1996).

The three species lost from Danish waters were already rare in the 1940s, with only 1–6 recorded sites (Olsen, 1944). While both Nitella gracilis and Chara filiformis can be perennial species from more stable habitats, Tolypella intricata is a spring annual characterised by rapid appearance in ephemeral habitats. Tolypella intricata was thus thought to be extinct in Sweden, but spring sampling in former localities revealed the presence of the species in a few localities (Gårdenfors, 2010). This recent finding suggests that the species could still be present in Denmark, although the heavily drained Danish landscape with very few ephemeral waterbodies makes this expectation doubtful.

The relationship between the historical changes of abundance and species traits in Denmark revealed a decline of perennial species and species with preference for highly alkaline waters. These species primarily grow, often to a large depth, in transparent large alkaline lakes, a rare habitat in the contemporary Danish landscape (Bjerring et al., 2011). Species with broader niches capable of growing in lakes of varying alkalinity and nitrogen content and, thus, under more perturbed conditions have better been able to persist.

The observed pattern of success of generalist species and the decline of specialists is consistent with investigations on the response of other types of organisms to habitat homogenisation (Rooney et al., 2004; Devictor et al., 2008). Specialists are defined as having a very high fitness under specific conditions while generalists have a lower fitness, but can thrive under a wider range of conditions (Clavel, Julliard & Devictor, 2011). When conditions change, specialists lose their fitness advantage and disappear while the more versatile generalists survive or even thrive. We suggest that the loss of waterbodies and increases in eutrophication across Europe have caused the observed changes in charophyte community composition and dominance patterns all over the continent.

In conclusion, habitat loss and habitat deterioration by eutrophication have reduced the total occurrence of charophytes by about 56% in Denmark while mean species richness in reinvestigated waterbodies declined by 29% and national species richness by only 10%. Charophytes are critically threatened with 82% of the species on the Danish Red List. The dominance structure has changed such that common species have become more abundant relative to rare species. Species with wide tolerances to both alkalinity and nitrogen content have increased supporting the hypothesis of generalists being better at surviving in disturbed landscapes. Nutrient reduction in several waterbodies during the last 15 years has led to increased species richness, highlighting that historical development is in part reversible, although several species may be unable to recover because they survive only in small relict populations.

Acknowledgments

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References


(Manuscript accepted 2 April 2013)
Chapter II
Niche specialization and functional traits regulate abundance of Scandinavian charophytes

Photo: Martin Mcnaughton (livetunderoverfladen.dk)
Niche specialization and functional traits regulate abundance of Scandinavian charophytes
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Summary
1. Charophytes are slow-growing macroalgae that live attached to soft sediments in lakes, ponds, streams and brackish waters. Given the historic loss and eutrophication of their habitats and their competitive inferiority to tall rooted plants, we predict that charophytes are among the most threatened phototrophs. We also predict that charophyte species with generalist niches and functional traits such as high salinity tolerance and large height should be best able to survive due to more growth habitats and stronger competitive capability.

2. We tested these predictions by examining the distribution of charophytes in Scandinavia and the relationship between species abundance and species traits. Abundance values were assigned to each species based on their status on the national Red List. Niche specialization was evaluated by the Outlying Mean Index (OMI) using a large Danish dataset on charophyte distribution and environmental variables. Species functional traits were derived from the literature.

3. The results supported our predictions showing that 50-87% of the species were on the national Red Lists in the four Scandinavian countries. These proportions are much greater than of aquatic (30-35%) and terrestrial plants (18-28%). Species abundance of charophytes in Scandinavia decreased significantly with niche specialization in separate analyses of marine and freshwater sites. Four functional traits: shoot height, salinity tolerance, bulbil production and ability to express both perennial and annual life form were significantly positively related to Scandinavian abundance.

4. We conclude that charophytes are generally rare species restricted by habitat loss and competition with fast-growing phytoplankton, macroalgae and tall vascular plants at eutrophic sites. The few charophyte species maintaining a high abundance in Scandinavia are generalists tolerant of a wide range of conditions, while specialists are often short, have a restricted life form and are rare in the disturbed contemporary landscape.

Keywords: Charophyte, niche specialization, functional traits, Red List, species abundance

Introduction
Charophytes belong to an ancient group of macroscopic, slow-growing green algae that live attached by hair-like rhizoids to soft sediments in lakes, ponds, streams and brackish waters (Krause, 1997). Some species are widely distributed from tropical to temperate and subarctic waters across the World, others are distributed in temperate or tropical regions and yet others are confined to narrower regions such as Scandinavia (Langangen, 2007). Most charophytes grow in oligo- and mesotrophic waters, while they are often outcompeted by phytoplankton, blanketing filamentous algae and tall rooted angiosperms in eutrophic waters (Blindow, 1992, Sand-Jensen, Pedersen, Thorsgaard et al., 2008). We predict that charophytes should include particularly high proportions of threatened species because widespread eutrophication has increased shading from all three mentioned groups of phototrophic competitors (Sand-Jensen et al., 2008).
However, some charophytes survive thanks to their ability to rapidly colonize and establish temporary dominance in newly constructed or perturbed waters because of high production of oospores that are efficiently dispersed by water birds (Crawford, 1977, Bonis & Grillas, 2002, Brochet et al., 2010). Here we examine both the status of charophytes on national Red Lists in Scandinavia and test how variability in species niche specificity and species traits such as shoot height, reproductive investment and life form influence their abundance in Scandinavia.

Charophytes have experienced a dramatic decline in abundance during the last 150 years in all countries with dense human populations and intensive agriculture (Simons & Nat, 1996, Blindow, 2000, Auderset Joye, Castella & Lachavanne, 2002, Baastrup-Spohr et al., 2013b). Widespread cultural eutrophication has been the most important environmental deterioration of charophyte habitats leading to restriction of depth distribution or total eradication of charophyte species in numerous water bodies (Blindow, 1992, 2000, Baastrup-Spohr et al., 2013b). Also mechanical disturbance by dredging, construction work, motor boat traffic and reed cutting can disturb sediment stability, increase turbulence and turbidity and, thereby, eradicate charophytes (Torn et al., 2010). A third environmental deterioration, mainly operating during the 1950-1980s, involved acidification of low-alkaline waters because of high sulfur deposition from the atmosphere leading to replacement of charophytes by acid-tolerant mosses and filamentous green algae (Riis & Sand-Jensen, 1998). A final important threat has been disappearance of numerous small water bodies and establishment of only a few new ones because of intensified agricultural exploitation of the landscape (Biggs et al., 2005). As a consequence, many charophytes have become rare, threatened by extinction and placed on the IUCN Red List in European countries, while the remaining common species have not declined to the same extent (Blazencic et al., 2006, Gärdenfors, 2010, Baastrup-Spohr et al., 2013b). Although charophytes have been subject to widespread and severe historical decline (Simons & Nat, 1996, Blindow, 2000, Baastrup-Spohr et al., 2013b) there has been no concerted effort to document their general status and evaluate the possible determinants of species abundance. Here we provide this information by determining the proportions of nationally Red-listed charophyte species. We determine the similarity in species composition and abundance between the Scandinavian countries. High similarity would support the notion that it is possible to identify mechanisms that determine whether species are common or rare.

High habitat specificity should by definition restrict species to a few special, but very suitable habitats and they are suggested to remain rare overall because the majority of habitats would be unsuited for them (Brown, 1984). This concept of generalists and specialists is thus based on the trade-off between the capacity to exploit or tolerate a range of conditions (generalists) and performing better under specific conditions (Futuyma & Moreno, 1988). Furthermore, in disturbed contemporary landscapes a similar pattern in the change of species composition has been observed for several species groups; i.e. a decline of specialist and increase of generalist species (Clavel, Julliard & Devictor, 2011).

Therefore, we predict that the degree of specialization of species with respect to physico-chemical and biological habitat conditions should be negatively related to the abundance of charophytes in Scandinavia.

The response of species to environmental gradients are mediated through functional traits (Keddy, 1992). Conceptually, environmental conditions can be viewed as a set of filters only letting through those species with the suitable traits for a given filter (Keddy, 1992). As species often face multiple abiotic and biotic gradients several traits can be involved in determining community composition (Shipley, 2010).
For flowering aquatic plants, several traits have been shown to be significantly related to abundance and, in particular, plant height was related to abundance in both lakes and streams (Sand-Jensen et al., 2000). We thus hypothesize that taller charophytes should be more abundant than shorter species due to stronger competitive ability for light and space of the taller species.

Tolerance of salinity in charophyte species is related to the physiological ability of the species to regulate cell turgor pressure (Winter, Soulie-Märsche & Kirst, 1996) and experiments with several species have shown different salinity tolerances (Winter et al., 1996, Blindow et al., 2003). The observed tolerances correspond well with the distribution of the species within different salinity zones of the Baltic Sea (Blindow, 2000). Because of the close proximity of brackish and freshwater habitats in the Scandinavian region and the large quantity of brackish water habitats we expect that increasing salinity tolerance should positively influence regional abundance.

Charophyte propagules are dispersed with the large number of migratory waterfowl (Bonis & Grillas, 2002, Brochet et al., 2010), and we predict that production of oospores and vegetative propagules by charophyte species should be positively related to the likelihood of dispersal and thereby to the regional abundance. Oospore formation requires the involvement of both male antheridia and female oogonia and these are present on the same individual shoot in most charophyte species (monoecious), while fewer species (dioecious) have separate male and female shoots (Langangen, 2007). When species become rare, monoecious species can probably better maintain oospore formation, while dioecious species can ensure outcrossing and better maintain genetic variability and life vitality associated with small populations. Therefore, we have no a priori expectation as to whether common or rare species should have a larger proportion of monoecious or dioecious species.

Other biological traits may also influence charophyte growth and survival in their specific habitats and, thereby, their national rarity or commonness. Certain varieties within species may differ by being either annual or perennial, while other species are reported as being permanently annual or perennial. For example, a summer annual form should be selectively advantageous in shallow pond and lake sediments disturbed by winter ice-scouring, while perennial forms should be advantageous in low-light and physically stable deep waters where long time is required but also available for population development. We, therefore, predict that species housing both annual and perennial life forms can occupy more habitats than species housing only one of the two life forms.

Thus we set out to test how niche specialization and functional traits may account for the abundance of Scandinavian charophytes.

**Materials and methods**

**Study area**

Scandinavia in this context consists of Denmark, Sweden, Norway and Finland (Fig. 1). Denmark (DK) is the southernmost and the smallest country (43,095 km²) with the fewest freshwater bodies. Nonetheless, because of calcareous soils and high-alkaline freshwaters suitable for a high variety of *Chara* species, the country had a very rich charophyte vegetation 70 years ago (Olsen, 1944) before widespread eutrophication and drainage of approximately two-thirds of all water bodies led to profound impoverishment (Baastrup-Spohr et al., 2013b). Sweden (SW) is the largest (449,964 km²) and most variable country with respect to geology, terrain and water types and had the richest charophyte vegetation in Scandinavia 70 years ago (Olsen, 1944).
Eutrophication and acidification have reduced the number and quality of freshwater charophyte localities in Sweden, though not to the same extent as in Denmark apart from the SE-region, Scania, where agriculture is most intense (Blindow, 1992, 2000). Norway (NO, 324,220 km²) and Finland (FI, 337,030 km²) are located furthest to the north and lack charophyte species with northern distribution limits in Denmark and Sweden, while they have a few species with a predominantly northern or eastern distribution (in the case of Finland, Langangen, 2007). Both countries have experienced eutrophication and acidification, but like in Sweden high proportions of the land area are forests or nature areas, though the calcareous regions most suitable for charophytes are cultivated as in the entire Scandinavia.

Denmark, Sweden and Finland have coastlines facing the brackish waters of the Baltic Sea which houses several charophyte species (Schubert & Blindow, 2003). These brackish populations have declined dramatically due to eutrophication of the southern Baltic Sea in Denmark, Germany and southern Sweden. The Swedish and Finnish coastlines in the central and northern parts of the Baltic Sea are less eutrophied and still support extensive, though diminishing, charophyte populations (Schubert & Blindow, 2003).
Measuring abundance, diversity and similarity

As a common measure of the relative species abundance of charophytes in all Scandinavian countries, we used the Red List categories (Langangen, 2007, Gärdenfors, 2010, Koistinen, 2010, Sjøtun et al., 2010). In the case of Denmark, we used a Red List proposal (Baastrup-Spohr, Dahl-Nielsen & Sand-Jensen, 2013a) based on a recent (1989-2010) compilation of the presence of charophytes in 226 water bodies.

Red List categories were translated to a numerical scale appropriate for statistical analysis: O = regionally extinct, 1 = critically threatened, 2 = endangered, 3 = vulnerable and 4 = nearly threatened. Species present in other Scandinavian countries but absent from a particular country were also given the value 0. Abundant species not present on the Red List were designated the value 6. We assume that these values approximate a logarithmic series of species abundances and confirmed this assumption by finding a highly significant (p < 0.001) linear relationship between the logarithm to number of localities (x) and the numerical Red List categories for both the largest (Sweden) and smallest country (Denmark) in the investigation (data extracted from Langangen, 2007, Gärdenfors, 2010, Baastrup-Spohr et al., 2013a) (Supplementary information, Fig. S1). Because of the high resemblance of numerical Red List categories vs. abundance relationship of the two countries (Fig. S1) and the identical criteria in the Red List regarding number of localities, we argue that the applied Red List values reliably reflect both national and Scandinavian abundance. Therefore, the abundance of individual species in the whole of Scandinavia was calculated as the sum of numerical Red List values in each of the four countries. Thus, the maximum possible abundance was 24 (= 4 x 6).

Diversity indices were calculated for the four Scandinavian countries by applying species number and species abundance as the numerical values for species categories on and off the Red List as presented above. Richness was expressed as species number (S), evenness as the Pielou index (J’ = H’/ln S) and the combination of richness and evenness as the Shannon-Wiener index (H’ = Σ p_i ln p_i, where p_i is the relative abundance of species according to numerical Red List categories). The similarity of abundances of species among the four Scandinavian countries was calculated by the Bray-Curtis (BC) index.

Niche parameters

There are multiple ways of measuring niche specialization each with their own advantages and drawbacks (Devictor et al., 2010). Here we used the Outlying Mean Index (OMI) analysis (Doledec, Chessel & Gimaret-Carpentier, 2000) providing a measure of niche position along multiple environmental gradients while searching for the most influential parameters. The advantage of OMI is that it makes no assumption concerning the shape of species response curves to the environment, and that it gives equal weight to species-rich and species-poor sites (Doledec et al., 2000).

The analysis results in a description of the mean niche position of each species, representing a measure of the distance between the mean conditions used by the species and the mean conditions of the study area. In our case, the resulting OMI values describe the distance of the niche for each charophyte species from the mean niche of a hypothetical charophyte occupying all investigated sites. The method has been successfully applied to terrestrial plants (Thuiller, Lavorel & Araujo, 2005) and in several freshwater environments (Doledec et al., 2000, Dole-Olivier, Malard, Martin et al., 2009). OMI analysis uses two matrices, a sampling unit vs. species matrix and sampling unit vs. niche variables.

We used environmental variables to delineate the species niche based on data collected by the Danish Environmental Agencies (Anonymous, 2012) from 1980 to
2011 consisting of coupled registrations of species occurrence and environmental parameters (Table 1). In the case of closed freshwater and brackish water bodies with distinct in- and outlets, sites with both vegetation and environmental registrations were considered the sampling unit. In more open brackish and fully marine sites, the sampling units were marine environmental monitoring stations with nearby vegetation registrations. All vegetation registrations were collected within a 5 km distance of a monitoring station and in the same water body without any type of barrier (islands, isthmuses etc.). A few marine sites were sampled twice, with more than five years in between, if they showed signs of major changes in species composition over the period.

All registrations of species and associated environmental parameters were collected in the same year, except for a few instances where environmental data from the year immediately before or after species registrations were used. Environmental variables were calculated as time-weighted averages based on more than two measurements within the year.

We analyzed the niche position based on recordings in all investigated sites and in the freshwater and marine (> 0.5 g NaCl L⁻¹) sites separately (Table 1).

### Species traits

A series of species specific traits that could affect the species abundance was obtained from the literature on Scandinavian charophytes (Langangen, 2007) and supplemented by a Polish investigation in the few cases where Scandinavian data were missing (Gabka, 2009). The traits included were shoot height, life form, monoecious/dioecious and production of oospores and bulbils. The maximum shoot height (cm) was converted to a categorical scale: (0) 0-20 cm, (1) 21-40 cm and (3) >41 cm to account for the variation within and among species. Life form data were included as (0) obligate annual, (1) obligate perennial and (2) both annual and perennial or as (0) restricted life form (obligate annual or perennial) and (1) flexible life form (both annual and perennial). The same two data sources were used to determine whether species are monoecious (0) or dioecious (1). The intensity of oospore production was provided by Langangen (2007) for species with low (0) and high (2) production while species lacking detailed information were considered intermediary (1). Some species produce bulbils as a mean of vegetative reproduction and these species were given the value 1, while the rest were assigned the value 0.

### Table 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Full dataset</th>
<th>Marine sites</th>
<th>Freshwater sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity (g NaCl L⁻¹)</td>
<td>0-26.7*</td>
<td>0.6-26.7</td>
<td>N/A</td>
</tr>
<tr>
<td>Chlorophyll a (µg L⁻¹)</td>
<td>0.9-295.2</td>
<td>0.9-295.2</td>
<td>1.1-127.8</td>
</tr>
<tr>
<td>Total phosphorus (µg L⁻¹)</td>
<td>7.6-863.6</td>
<td>18.8-407.5</td>
<td>7.6-863.6</td>
</tr>
<tr>
<td>Total nitrogen (µg L⁻¹)</td>
<td>244.9-5729.3</td>
<td>244.9-5729.3</td>
<td>286.4-4982.1</td>
</tr>
<tr>
<td>Alkalinity (meq L⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secchi depth (m)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake depth maximum (m)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake area (ha)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*In all freshwater sites salinity was very low and measured infrequently and all freshwater sites were given the value 0 in the full dataset and the variable was not included in the freshwater OMI analysis.

**Lake area was log transformed in the OMI analysis.
Species salinity tolerance was collected from Schubert and Blindow (2003), and was included as the range in salinity (max-min) inhabited by a given species. The brackish water species, *Tolypella normaniana*, was not included in the analysis as tolerance values could not be obtained from the literature. A summary of the values of the investigated trait and the Scandinavian abundance is provided in the supplementary material (Table S2).

**Results**

**Species richness and similarity**

Thirty five species have been found in Scandinavia within the last 50 years. In historical time, a few more species such as *Chara baueri* and *Nitella tenuissima* have been recorded but are now considered regionally extinct. Species richness of charophytes was highest in Sweden (32) and lower in the other countries (20-25, Fig. 1). A large number of species were on the Red List in all countries (11-17, Fig. 1) corresponding to 50-87% of all species. The Shannon Wiener biodiversity index, combining richness and evenness, was highest in Sweden (3.38) and lower in Norway (3.07), Denmark (3.00) and Finland (2.93) mainly due to changes in species richness. The Pilou evenness index was about the same in all four Scandinavian countries (0.958-0.977).

Similarity in relative abundance of charophytes between the countries was a close reflection of their proximity (Fig. 1). Similarity was high between neighboring countries and declined with distance between countries.

**Niche and abundance**

The OMI analysis used registrations of charophytes in 226 Danish sites with a total of 19 species. Omitting one species only registered once (*Chara rudis*) and using the restricted number of variables sampled across all marine and freshwater sites yielded an OMI first axis explaining 67% of the observed variability with high scores for salinity in particular (Table 2). As several environmental variables were only measured in the freshwater sites, separating the dataset into a marine and a freshwater section allowed a more detailed description of the relative niche position of the freshwater species. Both in the freshwater and marine sites the OMI first axis explained a high percentage of the variability (Table 2). In the marine dataset, the niches separated primarily along a main gradient of salinity, negatively coupled to nutrients and chlorophyll *a* (Table 2). In the analysis of the freshwater sites the separation of species niches was linked to two gradients; one in alkalinity and a second in lake area. This indicates that niches of freshwater species separate along gradients (e.g. alkalinity) that are not relevant in the permanently alkaline marine waters.

To test if the abundance of charophytes in Scandinavia was related to niche specialization, we correlated the abundance with the OMI index values of each species. Using the niche index values from the full dataset we found a tendency towards a negative relationship between niche specialization and Scandinavian abundance ($r = -0.41$, $p = 0.09$) (Fig. 1).

<table>
<thead>
<tr>
<th>Axes</th>
<th>Full dataset</th>
<th>Marine sites</th>
<th>Freshwater sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of variability</td>
<td>78</td>
<td>17</td>
<td>86</td>
</tr>
<tr>
<td>Salinity (g NaCl L⁻¹)</td>
<td>0.78</td>
<td>-0.11</td>
<td>0.61</td>
</tr>
<tr>
<td>Chlorophyll <em>a</em> (µg L⁻¹)</td>
<td>-0.10</td>
<td>-0.25</td>
<td>-0.54</td>
</tr>
<tr>
<td>Total phosphorus (µg L⁻¹)</td>
<td>-0.17</td>
<td>-0.13</td>
<td>-0.04</td>
</tr>
<tr>
<td>Total nitrogen (µg L⁻¹)</td>
<td>-0.19</td>
<td>-0.56</td>
<td>-0.06</td>
</tr>
<tr>
<td>Alkalinity (meq L⁻¹)</td>
<td>-0.37</td>
<td>-0.07</td>
<td></td>
</tr>
<tr>
<td>Secchi depth (m)</td>
<td>0.07</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Lake depth maximum (m)</td>
<td>-0.03</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Lake area (ha)</td>
<td>-0.17</td>
<td>0.36</td>
<td></td>
</tr>
</tbody>
</table>
However, separating freshwater and marine sites, allowing inclusion of variables important to niche separation in freshwaters, yielded a significant ($p<0.05$) relationship between Scandinavian abundance and niche specialization (Fig. 2). We thus found robust evidence supporting the hypothesis that abundant charophytes are less specialized than rarer species.

**Traits and abundance**

The abundance of Scandinavian charophytes was significantly related to several of the investigated species traits. Taller species tended to be more abundant than small species which was also the case for species with wide salinity tolerance (Table 3).

Annual species were significantly less abundant than species capable of expressing both an annual and perennial life form while obligatory perennials grouped in between (Kruskall-Wallis, $p=0.03$). However, merging the two groups of restricted life forms (obligate annual and perennial) and comparing them to species with flexible life form showed that species with flexible life form were generally more abundant (Mann-Whitney, $p=0.03$). The intensity of spore production and presence of bulbils did not affect the abundance. However, species bearing bulbils tended to have wider salinity tolerance, lower sexual reproductive investment and to be dioecious (Table 3).

**Discussion**

**Diversity and similarity**

Our results confirmed the prediction that the proportion of species on the Red List is high in individual Scandinavian countries (50-87%) and this prediction can be extended to Germany and countries on the Balkan Peninsula (Schmidt *et al.*, 1996, Blazencic *et al.*, 2006) emphasizing the highly critical status of charophytes here and elsewhere in Europe (Simons & Nat, 1996, Auderset Joye *et al.*, 2002).

These high proportions of species on the Red List are unprecedented among aquatic submerged plants in Denmark and Norway (30-35%) and terrestrial plants in all four Scandinavian countries (18-28%) (Gärdenfors, 2010, Kålås *et al.*, 2010, Koistinen, 2010, Wind & Pihl, 2010).
We explain the highly critical status of charophytes by the fact that relatively few and small habitats suitable for charophytes are available nowadays in Scandinavia because of extensive habitat loss and deterioration by eutrophication, acidification, and mechanical perturbation (Baastrup-Spohr et al., 2013b). Moreover, charophytes are much more sensitive to eutrophication than aquatic plants because they are competitively inferior and occupy habitats below the canopy of tall plants and at the greatest depth of submerged vegetation being highly sensitive to loss of vegetation (Blindow, 1992, Van den Berg et al., 1998). For example, when Lake Fure became eutrophied and turbid from 1940 to 1985, all 11 charophyte species were lost whereas only 8 of the original 21 rooted plant species vanished (Sand-Jensen et al., 2008).

The number of charophyte species observed within a country is a poor descriptor of their status because many species are only observed in very few localities (Auderset Joye et al., 2002, Blazencic et al., 2006). Although the number of charophyte localities has declined very markedly in Scandinavia during the last 100 years most species previously recorded by Olsen (1944) have persisted (Baastrup-Spohr et al., 2013b). A few species have even been added in some countries in recent years due to either establishment of new species, acceptance of new species following taxonomic reevaluation or because of more intensive field work (Langangen, 2007). However, the great loss of suitable freshwater and brackish sites due to heavier eutrophication, drainage and mechanical deterioration in Denmark account for the loss of species abundance (Baastrup-Spohr et al., 2013b).

### Abundance, specialization and traits

As predicted, charophytes with low niche specialization were more abundant in Scandinavia while specialist species were rarer. This relationship will exist if species that have broad environmental tolerances and are able to use a wide variety of resources will in so doing achieve high local densities and be able to survive in more places over larger areas (Gaston & Spicer, 2001).

Some investigators argue that it is a main analytical problem in studies of abundance versus range size that species are evaluated based on their realized niche, while the fundamental niche is best determined experimentally. Experimental determinations of the fundamental niche breadth with respect to several biological features are few because of tradition and insufficient experimental expertise among investigators. However, even thorough experimental studies, if available, may not provide the necessary answers because they are restricted in time and space, include a limited number of traits and not necessarily the essential ones and never include the greater variety of genotypes that are supposed to be present in widely distributed common species.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Scandinavian abundance</th>
<th>Height</th>
<th>Salinity tolerance</th>
<th>Oospore production</th>
<th>Bulbil production</th>
<th>Life form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>0.46 (&lt;0.01)</td>
<td>0.16 (0.34)</td>
<td>-0.27 (0.11)</td>
<td>0.11 (0.54)</td>
<td>-0.39 (0.02)</td>
<td>0.28 (0.12)</td>
</tr>
<tr>
<td>Salinity tolerance</td>
<td>0.59 (&lt;0.01)</td>
<td>0.35 (0.03)</td>
<td>0.36 (0.03)</td>
<td>0.48 (0.03)</td>
<td>-0.04 (0.82)</td>
<td>0.05 (0.77)</td>
</tr>
<tr>
<td>Oospore production</td>
<td>0.05 (0.74)</td>
<td>-0.27 (0.11)</td>
<td>0.11 (0.54)</td>
<td>0.48 (0.03)</td>
<td>-0.39 (0.02)</td>
<td></td>
</tr>
<tr>
<td>Bulbil production</td>
<td>0.35 (0.03)</td>
<td>0.36 (0.03)</td>
<td>0.48 (0.03)</td>
<td>0.48 (0.03)</td>
<td>-0.39 (0.02)</td>
<td></td>
</tr>
<tr>
<td>Life form</td>
<td>0.40 (0.02)</td>
<td>0.40 (0.02)</td>
<td>0.28 (0.12)</td>
<td>-0.11 (0.54)</td>
<td>0.16 (0.37)</td>
<td></td>
</tr>
<tr>
<td>Monoecious/dioecious</td>
<td>0.12 (0.48)</td>
<td>0.05 (0.77)</td>
<td>0.29 (0.09)</td>
<td>-0.20 (0.24)</td>
<td>0.36 (0.03)</td>
<td>-0.04 (0.82)</td>
</tr>
</tbody>
</table>
Among charophytes, for example, the common species *Chara vulgaris* is highly polymorphic with at least five noticeable varieties in Scandinavia which may ensure greater ecophysiological variability and permitting the species to thrive in many places (Langangen, 2007). It is highly unlikely that all five varieties are ever accounted for in the description of the fundamental niche. Therefore, there is no guarantee that experiments disclose the relevant fundamental niche.

The historical landscape development in, particularly, southern Scandinavia, has been characterized by widespread eutrophication. Such a development can lead to biotic homogenization with a decrease of specialists and an increase of generalists (McKinney & Lockwood, 1999). For charophytes such as *Chara globularis*, *C. vulgaris* and *Nitella flexillis* that have been shown to increase in, or retain high abundance within the last century in Denmark, The Netherlands and Switzerland all have low values of specialization (Simons & Nat, 1996, Auderset Joye et al., 2002, Bastrup-Spohr et al., 2013b). In contrast, highly specialized species such as *Laprothamnium papulosum* has declined dramatically (Schubert & Blindow, 2003).

Recent studies on terrestrial plant species suggest that their success is intimately linked to the functional traits (Mokany & Roxburgh, 2010). Charophytes of the Scandinavian countries support this pattern because species abundance was significantly linked to several of the investigated traits. Shoot height of charophytes as a proxy for competitive capability for light and space in dense stands was positively linked to Scandinavian abundance, similar to findings for vascular *Potamogeton* species (Sand-Jensen et al., 2000). This result indicates that the ability to compete for light plays an important role in shaping the charophyte community. The ability to physiologically tolerate a large range of salinities should also be of great advantage in Scandinavia because of the proximity and close linkage between marine and freshwater habitats and the mere increase of the number of potential growth sites. This was verified by the relationship between abundance and salinity tolerance, implying that species with wide tolerances were generally more abundant than species with smaller ranges. For some species (e.g. *Chara aspera*), distribution maps reveal that luxuriant growth in certain coastal waters is accompanied by the presence of species in inland waters nearby (Olsen, 1944, his Fig. 31). Interestingly, the salinity tolerance seems to co-vary within species with the formation of bulbils suggesting that this mode of reproduction is more essential for dispersal and survival of species in brackish waters than freshwater bodies. Apomictic reproduction has been shown to be more abundant in harsh environments (Richards, 2003). Brackish environments with changing salinity and strong physical disturbance (Idestam-Almquist, 2000) could be regarded as harsher than freshwaters giving rise to more apomictic species.

Abundance of charophyte species in Scandinavia was also positively correlated to the existence of both annual and perennial rather than only one life form within species. This effect could be straightforward because the breath of life forms increases the ability of species to thrive both in perturbed habitats as annual forms and in stable habitats as perennial forms. Charophytes develop dense monospecific stands as winter annuals in ephemeral ponds that dry out during summer thanks to efficient survival of numerous oospores and sprouting during refilling in autumn. Vascular aquatic plants are impeded much more than charophytes by summer desiccation and their contribution increases gradually from temporary to permanent calcareous ponds (Sand-Jensen et al., 2010). In permanent ponds and lakes, summer annual forms may thrive during summer in shallow waters and survive heavy physical disturbance and thick ice cover during the winter as oospores and vegetative propagules. Perennial
forms may grow year-round in the physically more stable deeper waters where more time is needed for population growth because of low light availability. Certain charophytes have an exceptional capability of growing at lower light and temperature than vascular plants in the cold bottom waters of temperate lakes (Nygaard & Sand-Jensen, 1981).

A diagnosis of being common or rare

Our data confirmed that charophyte’s traits and degree of niche specialization are important determinants of whether species are common or rare. In common species the prevalent traits are related to being large and having a flexible life form and a high salinity tolerance. In rare species life traits are associated with having more specialized niches.

Some rare charophyte species have derived from quite common species which they still resemble. Thus, Chara filiformis resembles the common C. contraria and Nitella capillaris resembles the common N. opaca (Langangen, 2007). With reference to the rich terrestrial Cape flora with many endemics, Davies et al. (2011) proposed that if plants speciate via small isolated populations at the edge of larger species ranges, then lineages that are diversifying rapidly will have many threatened species. Already Darwin (1859) noted the pattern of widespread, variable plant species acting as species pumps via peripheral sister species of restricted distribution. If this mechanism also operates in charophytes, then formation of peripheral species in cold regions of Scandinavia could be driven by widespread, common species. If we fragment the ranges of these common species they will lose their ability to operate as species pumps in the future (Knapp, 2011).

In conclusion, most charophytes are rare in Scandinavia because of too few suitable habitats and high niche specificity. In contrast to early believes, charophytes are not solely restricted to stable, resource-poor habitats (ultra-oligotrophy or deep waters of low irradiance). Many charophytes flourish in recently constructed ponds and restored lakes at relatively high nutrient levels before phytoplankton or tall vascular plants eventually take over and outcompete them (Crawford, 1977, Wade, 1990, Baastrup-Spohr et al., 2013b). The best way to set long-term priorities for conservation of charophyte diversity is to offer a flow of new pond habitats, ensure diverse habitats with clean water and avoid physical deterioration for the benefit of both common and rare species.

Acknowledgements

We thank the Villum Kann Rasmussen Centre of Excellence, Centre for Lake Restoration (CLEAR) for financial support, Dr. Dorte Krause-Jensen for providing marine botanical data and Drs. Irmgaard Blindow and Hans Henrik Kehlet Bruun for valuable comments to the manuscript. We are indebted to many Danish field botanists for information on the distribution of charophytes.

References

Niche specialization and traits of charophytes in Scandinavia


Estuarine Coastal and Shelf Science, 87, 27-32.


Supporting information

The supporting information is found in final part of the PhD-thesis.

Fig. S1. Comparison between relationship between Red List status and observed abundance in Sweden and Denmark. Both relationships are highly significant and the two slopes not significantly different.

Table S2. The abundance and functional traits of each charophytes species found in Scandinavian waters.
Chapter III
From pond to alvar: predicting plant community composition along a steep hydrological gradient

Photo: Ayoe Lüchau
Summary

1. Why do plants grow where they grow? Prediction of species’ occurrence and abundance in relation to the environment is a core aim to ecology and so is understanding the link between environmental stressors and adaptive traits. Conceptually, community assembly may be viewed as a sequence of filters, sieving off species according to their functional traits and thus restricting the range of trait values within a community. The filtering concept is the theoretical basis of a maximum entropy (maxent) model developed to predict species’ abundances in communities.

2. We investigated plant community composition and six morpho-physiological plant traits along a pronounced hydrological gradient in an abandoned limestone quarry. We studied the strength of environmental filtering on traits and the importance of the different traits to community assembly, investigating whether the traits experiencing filtering also prove valuable in predicting community composition.

3. We show that root porosity is significantly filtered by the environment along a marked hydrological gradient, as is specific leaf area and resistance to water loss on drying. For individual traits, the strength of filtering wax and wane along the gradient, strongly suggesting that the mechanisms through which species are filtered into communities act through different traits as environmental conditions change along the gradient.

4. The maxent model predicted 66% of the variation in species’ relative abundances using six traits. The traits subject to filtering also were most important in predicting species abundance.

5. Synthesis. Our results show that few plant traits are exposed to environmental filtering across the entire gradient in water coverage and soil depth, and that most traits are subject to strong filtering only in parts of the gradient. Evidence for congruence between trait dispersion indices and the maxent model was established, underpinning the importance to plant community assembly of environmental filtering of species through their traits.

Key-words Alvar, community assembly, environmental filtering, maximum entropy, Öland, plant trait, species richness, trait-environment relationship.
Introduction

An important goal of community ecology is to predict the occurrence and abundance of species inhabiting natural communities (McGill et al., 2006). Attaining this goal requires thorough appreciation of the mechanisms important to community assembly. These mechanisms have been likened to a series of filters determining which species from the regional species pool can complete their life cycle under the prevailing abiotic and biotic conditions (Keddy, 1992, Grime, 2006). The view that filters are operating on functional traits of species - rather than on species themselves, has received great attention recently (McGill et al., 2006, Shipley et al., 2006, Ackerly & Cornwell, 2007, Cornell & Ackerly, 2009), although the idea that the distribution of species must be explained through their functional traits is deeply rooted in ecology (e.g. Warming, 1895).

While the abiotic and biotic environment selects for or against certain plant traits, the traits themselves may determine species interactions and ecosystem function (Grime, 1998, Hooper et al., 2005, McGill et al., 2006, Lavorel, 2013). Along environmental gradients, variability of the traits expressed in communities change and these changes are associated with shifting rules of community assembly (Cornwell & Ackerly, 2009, Bernard-Verdier et al., 2012). A smaller range in traits values than expected from randomness – trait clustering – may be taken as an indication of trait filtering, because species having trait values outside a given allowed trait range succumb (Keddy, 1992, Cornell & Ackerly, 2009). Sorting of species into communities appears to be particularly distinct along environmental gradients between land and freshwaters, which originally sparked the idea of environmental filters (van der Valk, 1981). Here, in particular water coverage (flooding) and sediment conditions (e.g. organic content and anoxia) change over short distances and strongly affect the abundance of plant species and composition of communities (van der Valk, 1981, Silvertown et al., 1999, van Eck et al., 2004). This background stimulated us to study the importance of abiotic factors for the filtering of traits and how this influences the ability to predict species abundance in an area of particularly steep gradients in water coverage and sediment depth along a gradient from permanent ponds to dry shallow soils on the limestone alvar of Öland, SE Sweden.

Water coverage affects plants through the 10^4-fold reduction of gas diffusion rates and the 20-25-fold lower solubility of oxygen at saturation compared to atmospheric air (Crawford, 2009). These basic physical differences impose severe constrains on the supply rates of inorganic carbon for photosynthesis and oxygen for respiration of plants in water relative to air (Madsen & Sand-Jensen, 1991). Plants in submerged or water-logged soils, often have gas filled tissue, to alleviate problems of poor oxygen supply (Justin & Armstrong, 1987, Končalová, 1990). Aerenchyma in roots is essential to transport of oxygen from shoots to the tips of root buried in anoxic sediments (Colmer, 2003). Aerenchyma formation increases substantially both within and between species distributed from dry and well oxygenated soils to water-logged, anoxic soils (Mommer et al., 2006). While submerged plants never experience water shortage, the water available to terrestrial plants tends to decrease with decreasing soil depth (Pérez-Ramos et al., 2012). Along with the decrease in soil moisture, nutrient availability will also decline.

Along a gradient in soil depth and water availability traits such as the wilting point of shoots and the rate of water loss at high temperature, measures of the ability to endure conditions with insufficient external water supply, are expected to respond. The ability to resist water loss on drying (WLOD) should increase from a minimum in deep soils of high water holding capacity to a maximum in shallow soils and it should decline with water coverage as submerged species lack...
Community assembly from pond to alvar

protection against desiccation (Wetzel, 2001) while in occasionally flooded areas both strategies could co-occur leading to increased trait range. Also specific leaf area (SLA) has been shown to decrease along gradients in water availability (Violle et al., 2011, Bernard-Verdier et al., 2012). Among permanently submerged aquatic plants (Nielsen & Sand-Jensen, 1989) and temporarily submerged amphibious or terrestrial plants (Mommer & Visser, 2005), the restricted inorganic carbon supply to photosynthetic tissues has resulted in different strategies, such as high-SLA, often serrate, leaves to maximise the surface area to volume ratio. Other leaf traits such as dry matter content (LDMC) and surface area are also related to soil moisture in terrestrial environments (Hallik et al., 2009, Violle et al., 2011, Bernard-Verdier et al., 2012) and they are likely to change with the frequency of flooding. The different strategies of aquatic plants concerning formation of submerged, floating and aerial leaves may, however, distort this picture by causing trait over-dispersion (Bernard-Verdier et al., 2012).

A methodology for prediction of the relative abundance of species in communities was formulated by Shipley and co-workers (2006) using a maximum entropy model constrained by community aggregated trait values. The model’s concept is that if species filtering processes constrain the composition of a local community in such a way that the individual species possessing suitable functional traits become more abundant than predictable from all regional species available then “community-level properties should emerge” (Laliberté et al., 2012). An important assumption when modelling with community aggregated traits as constraints is that these traits are actually subject to environmental filtering. Trait filtering by the environmental conditions should restrict the range of values of a given trait at a given site and is often evaluated by comparison to what could be expected due to random processes (Cornwell & Ackerly, 2009, Ingram & Shurin, 2009, Bernard-Verdier et al., 2012).

We aim at evaluating whether traits, which are subject to strong environmental filtering, also proves to work in predicting the presence and abundance of species in communities distributed along a hydrological gradient. We do this by testing the following three specific hypotheses: 1) In local communities, using few but biologically relevant functional traits, we can make strong prediction of which plant species from a species pool will be found in which relative abundances, 2) The strength of environmental filtering of traits will change along a steep environmental gradient according to the selective pressure imposed, and 3) Functional traits experiencing environmental filtering are strong predictors in models of community assembly. For the tests, we used the framework of Ingram & Shurin (2009) and Shipley et al. (2006) on a coupled community-trait-environment data set collected in an abandoned limestone quarry surrounded by intact Alvar vegetation on the Baltic island of Öland, Sweden.

Material & Methods

Site

The investigation was conducted in an abandoned limestone quarry on Räpplinge Alvar on the island of Öland, SE Sweden (Sand-Jensen & Jespersen, 2012). The substratum consists of exposed solid limestone pavements, which are either extremely dry and almost devoid of vegetation over large areas, covered by shallow soil or sediment layers (up to a few cm) or covered by more permanent vegetation in slightly deeper soils and sediments in wetter places, in cracks between the limestone plates or in depressions close to and within small ponds. The quarry was abandoned about 30 years ago and has been managed by horse grazing (2 horses on 6 ha in summer) keeping the entire area open and mostly without shrubs and trees.
Table 1. Environmental conditions measured in analyses of trait-environment relationships in an abandoned limestone quarry on Öland, Sweden. Environmental variates 1-5 are calculated from continuous water level registration and assesses the conditions of flooding and drought. Variates 6-14 are based on measurements within each community sample plot.

<table>
<thead>
<tr>
<th>Environmental parameter</th>
<th>Number</th>
<th>Description</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days with water cover</td>
<td>1</td>
<td>Number of days with a positive water value</td>
<td>0-51 days</td>
</tr>
<tr>
<td>Longest water cover</td>
<td>2</td>
<td>Longest period of positive water values</td>
<td>0-51 days</td>
</tr>
<tr>
<td>Days with drought</td>
<td>3</td>
<td>Number of days with a negative water value &lt; -5 cm</td>
<td>0-51 days</td>
</tr>
<tr>
<td>Summed depth</td>
<td>4</td>
<td>Sum of all positive water values</td>
<td>0-1969.1 cm</td>
</tr>
<tr>
<td>Summed water value</td>
<td>5</td>
<td>Sum of all water values</td>
<td>-694.7-1969.1 cm</td>
</tr>
<tr>
<td>Water depth in plot</td>
<td>6</td>
<td>Manually measured water depth in each plot</td>
<td>0-35 cm</td>
</tr>
<tr>
<td>Sediment depth</td>
<td>7</td>
<td>Mean sediment/soil depth</td>
<td>0.7-16 cm</td>
</tr>
<tr>
<td>Standard deviation of sediment depth</td>
<td>8</td>
<td>Standard deviation of soil depth</td>
<td>0.5-7.6 cm</td>
</tr>
<tr>
<td>Vegetation height</td>
<td>9</td>
<td>Mean vegetation height</td>
<td>1.7 -36.6 cm</td>
</tr>
<tr>
<td>Standard deviation of vegetation height</td>
<td>10</td>
<td>Standard deviation of vegetation height</td>
<td>0.6-22.9 cm</td>
</tr>
<tr>
<td>Abundance of moss</td>
<td>11</td>
<td>Presence of moss in subplots</td>
<td>0-100%</td>
</tr>
<tr>
<td>Abundance of pebble</td>
<td>12</td>
<td>Presence of pebble in subplots</td>
<td>0-100%</td>
</tr>
<tr>
<td>Abundance of bare soil/sediment</td>
<td>13</td>
<td>Presence of bare soil/sediment in subplots</td>
<td>0-100%</td>
</tr>
<tr>
<td>Abundance of Nostoc commune</td>
<td>14</td>
<td>Presence of <em>N. commune</em> in subplots</td>
<td>0-100%</td>
</tr>
</tbody>
</table>

The local climate is quite dry (mean annual precipitation 510 mm; 1960-1990), with cold winters (January mean -1.2°C) and relatively mild summers (July mean 16.2°C) (SMHI, 2013). The precipitation is evenly distributed throughout the year (monthly mean range 32-54 mm), but temperature variations lead to large differences in the evapotranspiration and thus water availability (SMHI, 2013). Between April and August in 2010, surface temperature on the exposed limestone pavements exceeded 40°C on 37 days (21%) and reached a maximum of 50°C on two days in July (Sand-Jensen & Jespersen, 2012). Thus, the area is characterized by extreme gradients in water availability between the permanent water-filled ponds and the exposed limestone pavements with mm-thin layers of dust where water is only available during and immediately after wet weather (Sand-Jensen et al., 2010). The drainage water from the limestone soils filling the shallow ponds has a substantial acid neutralizing capacity (2 meq l⁻¹) and a pH of 8.2 at air saturation and pH 7 at 30-fold super-saturation typical of soils (Sand-Jensen & Staehr, 2012, Christensen et al., 2013). The soils in the investigated site had low phosphorus concentrations (0.1-0.14 µg TP g DW⁻¹) and the ponds had very low concentrations of soluble inorganic nitrogen and phosphorus (Christensen et al., 2013). Some representative species are *Sedum album* L. occurring abundantly in the driest places, *Juncus articulatus* L. and *Alopecurus geniculatus* L. growing abundantly in the bank zone of ponds, and *Chara aspera* C.L. Willdenow being common in the ponds.

**Vegetation sampling and water regime**

The vascular vegetation was analysed quantitatively between 23 and 28th of May 2010 in twenty-one 0.25 m² squares (referred to as local communities) placed by stratified random sampling to encompass the different soil depths, water regimes and vegetation physiognomies within a 100 by 200 m area. Every local community was located close to one of four focal ponds in the area. All plants except seedlings were identified to species and recorded by their presence or absence in each of twenty-five 10 × 10 cm subplots within the 0.25 m² square. A total of 525...
subplots were examined and 65 species were recorded.

Ground surface without vascular plants was classified as covered by moss, colonial cyanobacterium *Nostoc commune* Vaucher, pebble or bare soil and the presence of each type was recorded in the subplots. Maximum vegetation height (cm), water depth (cm) and soil depth (cm) were measured in each subplot (soil depth by poking a thin metal peg vertically into the soil (Table 1).

The highly dynamic water regime was presumed to be of utmost importance for plant community assembly due to infrequent precipitation events, shallow soils, impermeability of the limestone, elevation heterogeneity and the hot microclimate of the study site. Therefore, we calculated the daily water depth in each plot by placing two continuously operating barometric pressure sensors along with an atmospheric reference (HOBO U20, Onset, Massachusetts, USA) in two of four ponds in the area in the beginning of the growing season 2010 (April 8). In the two ponds without continuous recordings of water level, four manual recordings were made during the season, and the water level dynamics of these two ponds corresponded very closely to one of the continuously measured ponds. We thus modelled the water level in these two ponds by normalising the continuous measurements of this pond to the manual recordings (for full description, see Supporting Information).

We measured the relative elevation of each local community to the nearest focal pond and calculated water depth as a mean daily value. From these data we calculated five different indices of water cover history for each plot (Table 1). The sampled abiotic parameters were condensed to a single primary axis of environmental variation using a Principal Components Analysis (PCA). The PCA yielded a PC1-axis accounting for 59% of the total variation and with similar loadings for most of the measured parameters (see Table S1). Both water cover parameters and soil/sediment depth decreased with increasing PC1 value and the axis can largely be interpreted as a flooding gradient.

**Functional plant traits**

Species’ traits were measured on individuals collected throughout their spatial distribution in the study site in May 2010 (with a few additional species collected in 2011 and 2012). Traits were measured on the 32 most dominant species of vascular plant species responsible for more than 80% of the relative abundance in the investigated plots.

We regarded the regional species pool as all the species occurring in the study area and for which the traits were known. The regional abundance could then be calculated as the relative number of subplots occupied by a given species. Because of the small area of the study site (~2 ha), dispersal limitation was considered to have negligible effects on the community assembly.

Because of our emphasis on environmental filtering acting on plant traits (see below), we focussed on functional traits relevant for the environmental gradient and measurable on a continuous scale.

**Table 2.** Covariance of traits within species assessed with Spearman rank correlation. In the upper right-hand triangle, p-values are shown, while r-values are in the lower left-hand triangle. A positive r-value indicates that species with high values for one trait also tends to have high values for the other.

<table>
<thead>
<tr>
<th>Trait</th>
<th>SLA</th>
<th>LDMC</th>
<th>LA</th>
<th>Root porosity</th>
<th>WLOD</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA (cm² g⁻¹)</td>
<td>0.14</td>
<td>0.48</td>
<td>0.39</td>
<td>0.0097**</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>LDMC (mg g⁻¹)</td>
<td>-0.27</td>
<td>0.68</td>
<td>0.50</td>
<td>0.18</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Area cm²</td>
<td>-0.13</td>
<td>0.076</td>
<td>0.016*</td>
<td>0.063</td>
<td>0.047*</td>
<td></td>
</tr>
<tr>
<td>Root porosity (%)</td>
<td>0.16</td>
<td>-0.12</td>
<td>0.42</td>
<td>0.27</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>WLOD (%)</td>
<td>0.45</td>
<td>0.24</td>
<td>-0.33</td>
<td>0.27</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>-0.20</td>
<td>-0.072</td>
<td>0.35</td>
<td>0.22</td>
<td>-0.21</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05; **p<0.01
Leaf area (cm²) was measured on the youngest yet fully developed and expanded leaf from at least 10 individual plants per species (excluding petiole if present) using a flatbed desktop scanner. The same leaves were weighed when fresh and fully hydrated, and subsequently after > 24 hours of drying at 105°C. Leaf dry matter content (LDMC, mg g⁻¹) was assessed as leaf dry weight in relation to fresh weight. Specific leaf area (SLA, cm² g⁻¹) was calculated from leaf area and leaf dry weight. For one species, *Schoenoplectus tabernaemontani* (C.C.Gmel.) Palla, leaf area was estimated from area-length relations made from leaf sections and measured plant height. For a few species, less than 10 individuals were examined (see Table S2).

For the measurement of desiccation resistance and root porosity, plants were collected at the study site by digging up whole sods that were transported to the laboratory, placed in dim light at moderate air temperatures and analysed within one day of collection. Plant resistance to desiccation was measured as initial water loss on drying (WLOD %) at the observed maximum summer temperature. Three entire green shoots were separated from their roots at the soil surface, weighed and placed upright in a rack in an oven at 50°C for 20 minutes and reweighed. Drying was then continued for a minimum of 16 hours at 105°C to ensure loss of all remaining water and final weights were measured. WLOD was calculated as the % of the total water content lost during the initial 20 minutes at 50°C.

Root porosity was assessed using the gravitational method of Visser & Bögemann (2003). To ensure sufficient plant material (min. 100 mg per batch) for accurate determination, we often had to use more than one individual per analysis. The analysis were, except in a few instances performed in triplicates (Table S2). In short, roots were cleaned of all soil particles and debris and cut into ~2 cm long pieces. Fresh weight was recorded. The root fragments were then transferred to a small water-filled 20 ml glass flask closed with a rubber stopper with an attached metal screw hook and then weighed submerged in water using a hanging balance. The flask was then transferred to a desiccator connected to a vacuum pump and subjected to 5 min of 0.8 bar partial vacuum three times in a row to replace the root gas with water. The flasks were then reweighed and the porosity calculated according to the formula:

\[
\text{Porosity} = 100 \times \frac{W_2 - W_1}{FW - W_1},
\]

where \(W_1\) is the weight of the flask with root material before vacuum minus the weight of the flask without roots and \(W_2\) is the weight of the flask with root material after vacuum minus the weight of the flask without roots. \(FW\) is the fresh weight of the used root material.

Plant height was extracted from the LEDA data base (Kleyer et al., 2008). Due to the much skewed distribution of plant height and leaf area these parameters were log transformed before analysis.

**Environmental trait relationship**

Due to environmental filtering, the expression of functional plant traits by local communities are expected to vary along abiotic gradients (Shipley, 2010b). A community-weighted mean value (CWM) can be used as an estimate of the trait of an average individual in a community and is a suitable measure of the trait response along environmental gradients. It is calculated as:

\[
\text{CWM} = \sum_{i=1}^{S} p_i \times t_i,
\]

where \(t_i\) is the trait \(t\) of species \(i\) of \(S\) species in the local community each with a relative abundance \(p_i\). The relationship between the condensed environmental variables (PC1) and CWMs was determined by regression analysis, while the co-variation of traits within species was examined using Spearman rank correlation (only significant results are shown).

Predictability of the CWM values from environmental conditions is essential for the
implementation of the full Shipley model (Shipley et al., 2011) (see below). We quantified to what extent the environmental conditions can be used to accurately predict the CWM values observed in the local communities by generalized additive models (GAM) with CWM of each trait as response variable and the observed environmental conditions as predictor variables. Backward elimination was used for model simplification with the \( p = 0.05 \) level used to sequentially assess models.

To examine to what extent traits were subject to environmental filtering we used the approach of Ingram and Shurin (2009). Here the range of traits within a local community is compared to the range expected according to a null model derived from the regional species pool. In short, we produced the null model for every local community by simulating 999 communities with the same species number, but randomly including species from the regional species pool. The obtained null distribution of a trait range in a community was used to assess if the observed range within a community was smaller than expected for a randomly assembled community, i.e. clustered. For each trait in each community, we calculated the \( p \) value as the proportion of the 999 null communities with a larger trait range value than the observed. If the range was smaller than 97.5\% it was accepted as being significant with \( \alpha = 0.05 \) (Ingram and Shurin 2009).

To alleviate multiple comparisons (6 traits in 21 communities) we also conducted the ‘meta-analysis’ proposed by Ingram and Shurin (2009) by first calculating the Trait Clustering Index (TCI) for each community as:

\[
TCI = - \frac{\text{Ranges}_{\text{obs}} - \text{Range}_{\text{null}}}{\text{SD}(\text{Range}_{\text{null}})},
\]

where \( \text{Ranges}_{\text{obs}} \) is the observed trait range, \( \text{Range}_{\text{null}} \) is the average range of the null model and \( \text{SD}(\text{Range}_{\text{null}}) \) is the standard deviation of the null model. Thus positive values of TCI indicate lower range of a given trait than expected by random sampling. We then tested whether the TCI values of all the analyzed communities deviated from zero using a one sample \( t \)-test to indicate overall tendencies towards trait clustering. Both the GAMs and the trait clustering analysis were performed in the R environment (R development Core Team, 2012) using mgcv library and the library provided by Ingram & Shurin (2009) respectively.

**Trait abundance models**

Shipley et al. (2006) developed a model, using maximum entropy with community aggregated traits as constraints to predict the relative abundance of species within a local community from a regional species pool. We used this maxent (maximum entropy) based model to predict the relative abundances of species in the studied communities from the measured traits. We tested the model with permutation tests (Shipley, 2010c) and used the backward stepwise procedure proposed by Shipley et al. (2011) to create the simplest model that, given the measured traits, still yielded a result significantly better than random. Earlier work has shown that using more informative priors improve the models predictive ability (Sommer et al., 2010, Laliberté et al., 2012). Therefore we ran the model using the regional relative abundance of each species as priors. The resulting model did not have markedly improved prediction capacity, and we thus only present results from the model using the maximally uninformative prior.

The maxent model estimates a series of constants \( (\lambda) \) (Lagrange multipliers) quantifying how much a unit change in a particular trait is associated with a proportional change in the predicted relative abundance in a local community when all other traits are kept constant. Hence, \( \lambda \) assesses the magnitude with which the relative abundance of a species changes as the value of the focal trait changes. Positive \( \lambda \)-values mean that locally abundant species have large values for the focal trait.
Conversely, a negative $\lambda$ means that species with large values for the focal trait are subordinate or absent. Values of $\lambda$ close to zero mean that there is no directional selection of the particular trait in the local community (Shipley, 2010b). To ensure comparability between the $\lambda$–values all traits were standardized to unit variance before analysis. All models were performed in the R environment using the FD library (Laliberté & Shipley, 2009, R development Core Team, 2012).

**Results**

**Environment- trait relationships**

The environmental conditions in local communities were highly variable with some plots being permanently submerged with quite variable average sediment depth (2.3-16.1 cm) while other plots were never flooded and generally had very shallow soil layers (0.7-5.2 cm, Table 1).

Across species, some traits showed indications of covariance. Species with large
leaf area also tended to be tall and have high root porosity, while species with high SLA rapidly lost a large percentage of their water content upon desiccation at 50 °C (WLOD, Table 2).

The community-weighted mean values (CWM) responded to the environmental gradient as indicated by significant regressions (Fig. 1). Leaf dry matter content (LDMC) responded linearly to the gradient while the response of the remaining traits was significantly better described by a quadratic function indicating that most traits respond in a nonlinear fashion to the investigated gradient. Significant GAM models with high predictive ability revealed strong relationships between environmental conditions and CWM trait values (Table 3). All analysed environmental variables contributed significantly to one or more of the models. Variables such as summed water depth and vegetation height contributed to the majority of the models and only the model of leaf area did not include any of the hydrological variables (Table 3).

### Table 3. Generalized additive models (GAM) of the community-weighted mean trait values (CWM) using the 14 environmental variates as predictor terms (as numbered in Table 1).

<table>
<thead>
<tr>
<th>CWM Trait</th>
<th>Environmental variate no.</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA</td>
<td>4, 5, 3, 2, 7, 8, 6, 9, 11</td>
<td>0.87</td>
</tr>
<tr>
<td>LDMC</td>
<td>1, 4, 5, 14</td>
<td>0.81</td>
</tr>
<tr>
<td>LA</td>
<td>8, 9, 10, 12</td>
<td>0.83</td>
</tr>
<tr>
<td>Root porosity</td>
<td>3, 2, 6, 9, 10, 12</td>
<td>0.82</td>
</tr>
<tr>
<td>WLOD</td>
<td>4, 5, 3, 8, 6, 9, 10, 11</td>
<td>0.80</td>
</tr>
<tr>
<td>Height</td>
<td>4, 7, 8, 6, 9, 10, 11, 12, 13</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Displaying the CWM and range of traits along the condensed environmental gradient (PC1) revealed that leaf area and plant height decreased, while LDMC increased, along the gradient, which means that shoot and leaf size decreased towards dryer conditions, while leaves contained more dry mass per fresh weight (Fig 1). Root porosity appeared to show a saturation response being low on dry and shallow soils, while increasing under wetter conditions and saturating at 30% aerenchyma under permanently submerged conditions. SLA and WLOD peaked at intermediate flooding and soil depths (Fig. 1).

### Table 4. Trait filtering and its importance for community assembly along a hydrological gradient. Environmental filtering was evaluated using the trait clustering index (TCI), where values above zero indicate a lower range of trait values within a community than expected from a null model. Significance was tested as deviation from zero by a one sample t-test. Trait importance to community assembly was assessed by the absolute λ-values from a maxent model containing all traits. Consistent change of the degree of filtering and importance along the gradient was assessed by correlation of TCI and λ-values, respectively, with the condensed environmental gradient (PC1). A positive r-value indicates higher trait filtering in the part of gradient with drier conditions and shallower soils, while negative values indicate stronger filtering under conditions with frequent flooding or stagnant water.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Average TCI</th>
<th>TCI : PC1 Spearman r</th>
<th>Absolute λ-values</th>
<th>λ : PC1 Spearman r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific leaf area</td>
<td>0.55 **</td>
<td>0.62 **</td>
<td>33.6</td>
<td>0.07</td>
</tr>
<tr>
<td>Water loss on drying</td>
<td>0.52 *</td>
<td>0.26</td>
<td>41.8</td>
<td>-0.34</td>
</tr>
<tr>
<td>Root porosity</td>
<td>0.81 *</td>
<td>-0.58 **</td>
<td>30.5</td>
<td>-0.44 *</td>
</tr>
<tr>
<td>Leaf dry matter content</td>
<td>0.45</td>
<td>-0.86 **</td>
<td>25.9</td>
<td>0.61 **</td>
</tr>
<tr>
<td>Plant height†</td>
<td>0.27</td>
<td>0.70 **</td>
<td>18.0</td>
<td>-0.12</td>
</tr>
<tr>
<td>Leaf Area†</td>
<td>0.58</td>
<td>0.63 **</td>
<td>27.2</td>
<td>-0.32</td>
</tr>
</tbody>
</table>

† Variable was log transformed before analysis

*p<0.05; **p<0.01
**Trait filtering across the gradient**

In up to seven of the 21 local communities, the observed range of a given trait was significantly smaller than expected from the null models. Within each local community between zero and three traits showed significant clustering (Table S3).

A large standardized trait clustering metric (TCI) causing a significant deviation from zero across communities provides evidence of environmental filtering along the gradient. We found significant evidence for environmental filtering acting through the plant traits root porosity, WLOD, SLA, and a tendency for leaf area to experience filtering as well (Table 4).

SLA and WLOD showed substantial co-variation (Table 2), with species having a high SLA also exhibiting a high WLOD. The two factors are probably significant for the same reason – variation in resistance to water loss. Root porosity - reflecting the ability to supply oxygen to root meristems under wet, anoxic conditions in soils - was not correlated to either SLA or WLOD. Hence, we found evidence for environmental filtering in two independent traits – tolerance to drought versus to flooded soils along the sampled gradient.

**Fig. 2.** Trait clustering index (TCI) in the local communities along the hydrological gradient (expressed as PC1). Values of TCI above zero indicate environmental filtering of species through the measured traits. The TCI values of SLA, WLOD and root porosity are significantly different from zero across the gradient (one-sided t-test, p<0.05)
Displaying the TCI values along the sampled gradient (PC1) allows for an assessment of the degree of environmental filtering of a given trait under different conditions (Fig. 2, Table 4). For all traits, except WLOD, the degree of trait clustering is correlated significantly to the overall gradient (Table 3) showing that although a given trait is not filtered by the environment under all the sampled conditions, it might still be strongly range limited along parts of the gradient.

**Prediction of community structure**

From the observed CWM trait values and the individual trait values of each species in the species pool, we predicted the relative abundance of each species in local communities using the maxent model. The model including all six traits predicted 66% \((p = 0.03)\) of the observed relative abundances of the 32 species included in the species pool (Fig. 3). The backward elimination procedure resulted in a significant model containing only one trait (leaf area, \(p = 0.02\)) and, with a substantially lower predictive ability \((r^2 = 0.10)\) (Fig. 4).

From the maxent model using all the measured traits as constraints, the \(\lambda\) values were obtained for each trait in each local community (see Fig. S5). The \(\lambda\) values varied greatly between the communities with both positive and negative values indicating that selective pressure on traits differs within short spatial distances as a result of the steeply changing environmental conditions. The change in \(\lambda\) values along the gradient was consistent for root porosity and LDMC, as they all correlated significantly with the environmental gradient (PC1-axis, Table 4). \(\lambda_{\text{porosity}}\) changed from positive values to values close to zero, showing that high root porosity is a necessity for growth and survival under waterlogged conditions, while the trait decreased in importance under drier conditions, possibly even becoming disadvantageous in the driest sites. The opposite trend was found for \(\lambda_{\text{LDMC}}\) showing that a large dry mass per fresh mass was undesirable in wet sites, but seemingly unimportant under drier conditions. The \(\lambda\)-values for the remaining traits showed large variations and did not change consistently along the gradient and thus do not appear to play any consistent role along the investigated hydrological gradient.

Overall, using only six traits in the model predicted species’ relative abundance fairly well and root porosity along with LDMC appeared to play the most consistent role for community assembly along the gradient.

**Discussion**

**Relationship between environment and traits**

The environmental conditions at the study site range from very harsh - with plants growing in soils less than one cm deep and often experiencing summer temperatures above 40\(^\circ\)C - through more productive areas with deeper soil, higher availability of soil nutrients and water - to areas with a permanent water cover. Water cover reduces inorganic carbon supply to most plants and generates anoxic sediments. Traits known to be crucial to plant survival and growth under...
these contrasted and adverse conditions, such as root porosity under flooding (Justin & Armstrong, 1987, Colmer & Voesenek, 2009) and leaf dry matter content (LDMC) under drought (Hallik et al., 2009), changed accordingly. Overall, we found that community-weighted mean values (CWM) of measured traits were strongly related to the position along the environmental gradient of the particular community.

Using generalized additive models (GAM), we found a close relationship between CWM trait values and environmental conditions. In particular, hydrological properties associated with water depth and frequency of flooding were important predictors for most CWM trait values. Vegetation height, as a proxy for site productivity, also contributed significantly to models for several traits. This property could probably be better substituted by direct measurements of available soil nutrients (Pérez-Ramos et al., 2012).

The strong correlation of SLA with resistance to desiccation (WLOD) underpins the general importance of this often-measured trait (Wright et al., 2004). Unexpectedly, however, SLA and WLOD showed unimodal responses to the hydrological gradient. In the drier sites, SLA increased with soil depth, which corresponds well with previous findings (Bernard-Verdier 2012). The decrease of SLA with depth and duration of water cover may seem counterintuitive and is at odds with previous findings (Violle et al., 2011). However, there is probably a fundamental difference in the function of SLA between flooded meadows and permanent water with deeper sediments. At the study site, low-SLA species are mainly Typha latifolia L and Potamogeton natans L. For the emergent macrophyte T. latifolia, characteristic of environments with ample nutrients and (excess) water (Wisheu & Keddy, 1992), low SLA may be a trait related to competition attained through accumulation of a recalcitrant litter layer (Christensen et al., 2009).

For the floating-leaved Potamogeton natans, low SLA may express resistance to high mechanical stress from wave action (Puijalon et al., 2011). For permanently submerged species, SLA would indeed be high (Nielsen & Sand-Jensen, 1989).

Environmental filtering and trait clustering

Across the environmental gradient three traits were significantly clustered, indicating that environmental filtering plays a major role for community assembly under the highly variable conditions investigated.

All traits showed some degree of filtering and in five of six analyzed traits, the degree of filtering correlated with the hydrological gradient. Flooding and drought have been known since long to strongly affect trait composition in plant communities (Iversen, 1936, van Eck et al., 2004, Jacobsen et al., 2008). SLA and WLOD seemed to be environmentally filtered across the entire gradient, which corresponds with findings for both woody (Cornwell & Ackerly, 2009) and perennial herbaceous species (Bernard-Verdier et al., 2012).
In contrast to the mentioned studies, we found a relationship between the degree of filtering in SLA and the hydrological gradient, indicating trait clustering on shallower soils with drier conditions. Most likely, very dry sites demand special strategies for leaf water economy, whereas pond borders and submerged sites offer more room for trait variation. Not considering submerged communities with helophytes only, WLOD showed the same pattern as SLA with drier sites having stronger filtering than wetter. Leaf area and plant height, although apparently not environmentally
filtered along the whole gradient, both showed signs of filtering towards the dry end of the gradient, most likely caused by the same factors as described for SLA and WLOD.

Root porosity was subjected to environmental filtering across the hydrological gradient. This is, to our knowledge, the first time that root porosity has been shown to undergo environmental filtering. The trait range was particularly restricted – all species having high root porosity - under submerged conditions, as expected from the function of aerenchyma in root oxygen supply in anoxic sediments (Justin & Armstrong, 1987, Douma et al., 2012). The filtering of root porosity also in some communities at the dry end of the gradient could be caused by a tradeoff between root porosity and nutrient uptake or mechanical strength (Končalová, 1990, Striker et al., 2007).

The maxent model presumes that environmental filtering is important to community structure (Shipley, 2010a, Shipley, 2010b). One should therefore anticipate that traits under strong environmental filtering (i.e. high TCI) would also be the best predictors of community assembly according to the maxent model (large absolute λ values). The three traits that experienced significant environmental filtering (SLA, WLOD and root porosity) across communities also appeared to be the better predictors of community assembly, as these traits had the highest λ-values in the full maxent model. The same three traits, along with leaf area also showing indications of filtering across the gradient, remained in the model simplified by backward elimination suggesting a consistent importance for community assembly. The seeming importance of leaf area was mainly driven by one of the investigated 21 local communities, perhaps putting a question mark to the use of backward elimination for finding the most parsimonious version of the maxent model. Including more community samples could potentially alleviate this effect, as single λ values would have smaller impact on the summed λ used as selection criteria.

Similar to values of the Trait Clustering Index, λ values for local communities taken from the full model varied considerably along the environmental gradient, an observation also made elsewhere (Laliberté et al., 2012, Sonnier et al., 2012). The fact that metrics of both methods from the single local communities correlated with the environmental gradient called for an assessment of TCI and λ values on a local community scale (Fig. 5). For root porosity and LDMC, a significant positive correlation between TCI and the absolute λ values indicated that traits experiencing strong filtering (higher TCI values) are also strong determinants of community assembly (Fig. 5). For the remaining traits and plant height, the relationship between trait filtering and trait importance was either not significant or negative because the λ-values did not respond monotonously along the environmental gradient. Thus, the correspondence between the independent measures of environmental filtering and traits important to community assembly confirms that environmental filtering acts through traits that have great influence on community assembly. Nevertheless, the lack of complete congruence of the two methods may result from the fact that the maxent model does not fully explain the observed relative abundance (r² = 0.66). This in turn could be a result of not including all traits of importance for community assembly (Sonnier et al., 2010). Traits that could have proven valuable for community assembly are seed germination above and under water (van der Valk, 1981) and seed buoyancy (Douma et al., 2012), that previously have been shown to be important for plant distribution along flooding gradients.

In conclusion, we found that both community-weighted mean traits and environmental filtering of functional traits of species responded to a gradient in water coverage and soil depth. Using the maxent
approach, we could make powerful predictions of relative abundance of plant species using only six measured traits. We found evidence for congruence between the degree of filtering experienced by a trait and its importance for predicting community assembly.

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References


Supplementary material
The supplementary material are palced at then end of the thesis.
It consists of a thorough explanation of water indices, PCA-tables, tables of all measured traits, tables of TCI-values for all trait in all communities and a graphs showing the relationships between λ-values and the PCA-axis.
Chapter IV
Water level fluctuations affect plant growth and carbon dynamics of isoetid populations

Photo: biopix.dk
Water level fluctuations affect plant growth and sediment dynamics of isoetid populations in oligotrophic lakes

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1. Several amphibious isoetid species, and Littorella uniflora in particular, exchange O₂ and CO₂ across roots with the sediment and dominate the littoral zone from below to above the water in North temperate oligotrophic, softwater lakes. Accumulation of labile organic matter in the sediments from phytoplankton blooms accompanying eutrophication represents a threat to isoetids because of insufficient root oxygenation, reduced anchorage and enhanced growth of taller competitors. We hypothesized that L. uniflora tolerates different water levels but grows better and maintains dominance when sediments are periodically drained because air exposure assists oxygenation, mineralization and consolidation of sediments. If so, drainage offers a possible restoration tool. We tested this hypothesis by measuring plant performance and sediment metabolism under drained, fluctuating and submerged conditions in the laboratory and along elevation gradients in the field in natural and organically enriched sandy sediments from an oligotrophic Danish lake.

2. Laboratory and field experiments confirmed that L. uniflora maintained high leaf chlorophyll and photosynthesis and grew in biomass under all conditions. Increase of biomass and shoot weight was highest under drained conditions and most so for roots as a suitable response to higher sediment oxygenation and leaf transpiration. The increase of biomass was lowest under fluctuating conditions as a logical consequence of higher costs of frequently producing aerial leaves to replace unsuitable aquatic leaves upon changes from submerged to drained conditions.

3. Efflux of inorganic carbon from mineralization in un-vegetated standard sediments increased 5.5-fold from submerged, over fluctuating to drained treatments. In submerged vegetated sediments, where part of sediment CO₂ is incorporated into a growing plant biomass, the sum of inorganic carbon efflux and plant growth had more than doubled compared to bare sediments probably reflecting stimulated sediment oxygenation and mineralization because of root oxygen release. Under drained, well-oxygenated conditions the increase of carbon in the biomass was accompanied by an equivalent decline of the inorganic carbon efflux relative to bare sediments. Still, the sum of inorganic carbon efflux and additional carbon trapped in the biomass was 2-fold higher under drained than submerged conditions.

4. The proportion of reduced Fe²⁺ to total Fe as a measure of the oxygen status of sediments declined from submerged, over fluctuating to permanently drained sediment. Also the proportion of reduced Fe²⁺ was lower in plant covered than bare sediments reflecting root release of O₂. Drainage and air contact also increased sediment density and consolidation and, thereby, improved root anchorage compared to submerged conditions.

5. Periodic drainage and air exposure of isoetid covered sediments is a natural phenomenon in oligotrophic seepage lakes that should be reinstalled as a possible restoration tool if the water has been regulated to a constant level. Water level fluctuations of a moderate frequency can benefit isoetid populations because they grow and survive better than competitors and potential harmful organic matter can be mineralized without sediment anoxia.

**Keywords:** Softwater lake, isoetid, restoration, Littorella uniflora, water level fluctuation, sediment desiccation.
Introduction

The shallow littoral zone along rivers and streams and at the margins of lakes and ponds are often characterized by greatly fluctuating water levels from winter to summer, from year to year and over decades (van der Valk, 2005, White et al., 2008) influencing community assembly and growth of plant species (Baastrup-Spohr et al., 2013) and sediment processes (Baldwin & Mitchell, 2000). Fluctuations between submergence and emergence alter the availability of O\textsubscript{2} and CO\textsubscript{2} to photosynthesis and respiration because of 10\textsuperscript{4}-fold lower gas diffusion coefficients in water than in air. These large differences in abiotic conditions have profound effects on plant performance along hydrological gradients from permanently dry to permanently submerged conditions and are, thus, main determinants for the distribution of plant species along the margins of water bodies (van der Valk, 1981, Keddy & Reznicek, 1986, Baastrup-Spohr et al., 2013). In natural lakes, plant species richness have been shown to be positively related to the magnitude of water level fluctuations (Mjelde, Hellsten & Ecke, 2013) while the presence of single species are also highly dependent on the timing and frequency of water level fluctuations determining which species can germinate and thrive (van der Valk, 1981, Riis & Hawes, 2002, Van Geest et al., 2005).

In the zone of fluctuating water levels, amphibious plants often dominate the vegetation because they are capable of photosynthesizing and growing both under water and in air (Pedersen & Sand-Jensen, 1997, Casanova & Brock, 2000). They can accomplish this double-life by forming rigid “aerial leaves” with stomata and a well-developed cuticle to reduce evaporation in air and having flexible, thinner gas-permeable “aquatic leaves” without stomata under water (Nielsen, 1993, Robe & Griffiths, 1998, Mommer & Visser, 2005). Some amphibious species shift between utilizing atmospheric CO\textsubscript{2} by aerial leaves and combing passive CO\textsubscript{2} use with active use of dissolved HCO\textsubscript{3} by aquatic leaves to compensate for lower gas diffusion rates in water (Sand-Jensen, Pedersen & Nielsen, 1992, Sand-Jensen & Frost-Christensen, 1999, Maberly & Madsen, 2002). Most amphibious leaves, however, rely on passive CO\textsubscript{2} use from either air, water or, in some cases, the rhizosphere of alternating importance in response to fluctuating water levels and CO\textsubscript{2} concentrations (Madsen & Sand-Jensen, 1991, Nielsen, Gacia & Sand-Jensen, 1991).

Flooded soils is another strong selective force which requires development of aerenchyma to ensure a sufficient O\textsubscript{2} supply from leaves and stems to apical root meristems buried in anoxic soils (Justin & Armstrong, 1987, Colmer, 2003). Exposure to air for submerged plants, in contrast, represents a strong desiccation risk although some leaves may survive in the damp layer at the sediment surface (Sculthorpe, 1967) or rhizomes and roots may survive for a short while in wet soils without the supply of leaf photosynthates (Engelhardt, 2006). Also, terrestrial species can survive flooding and aquatic species survive drought as resistant seeds and sprout and grow when conditions once again becomes suitable to them (Brock et al., 2003). Thus, the zone of fluctuating water levels is inhabited by a mixture of terrestrial, amphibious and submerged species, although the amphibious species in terms of biomass and cover is the main vegetation component, while many terrestrial species may occur in low numbers scattered throughout the zone (Keddy & Reznicek, 1986, Casanova & Brock, 2000).

The sandy shores and shallow waters with fluctuating water levels in oligotrophic, softwater seepage lakes in the North temperate regions are often inhabited by small Isoetid species of the rosette growth form (e.g., Carex viridula var. viridula, Elatine hexandra, Juncus bulbosus, Littorella uniflora, Lobelia dortmannia and Isoetes spp.; (Sand-Jensen & Søndergaard, 1979,
Smolders, Lucassen & Roelofs, 2002). In this zone of fluctuating water levels, the amphibious isoetid species can benefit from the reduced competition from taller, more nutrient demanding and less disturbance tolerant terrestrial and aquatic plants (Farmer & Spence, 1986). In NW Europe, *Littorella uniflora* is usually the dominant species in this habitat (Arts & Denhartog, 1990, Riis & Sand-Jensen, 1998, Pedersen et al., 2006) and it can form almost monospecific stands from 1 m above to 2-3 m below the mean water level (Sand-Jensen & Søndergaard, 1979). With 2-3-years intervals, minimum summer water level can change by up to 0.5-1.0 m between particularly wet and dry years (Sand-Jensen and Søndergaard 1979). *Littorella uniflora* is apparently well-adapted to fluctuating water levels by forming aerial leaves with stomata and low evaporation and submerged, gas-permeable and thicker leaves without stomata (Hostrup & Wiegleb, 1991, Robe & Griffiths, 1998). Leaves, stems and roots have well-developed air lacunae and the CO₂-rich rhizosphere represents an important CO₂ source to photosynthesis both in air and, even more so, under water (Nielsen et al., 1991).

Because of the highly permeable roots allowing uptake of CO₂, *Littorella uniflora* and other isoetids are highly susceptible to low oxygen concentrations in the sediment often accompanying eutrophication, phytoplankton blooms and associated accumulation of labile organic matter in sediments (Møller & Sand-Jensen, 2011). Accumulation of organic matter not only affects the roots directly but can also lead to poorer root anchorage in looser sediments of higher water content leading to higher risks of uprooting by wave action (Schutten, Dainty & Davy, 2005). Exposure of shallow sediments to air can lead to reduction of the organic content of sediments (Degroot & van Wijck, 1993, James et al., 2001) as a result of faster mineralization perhaps due to a higher O₂ flux from the atmosphere to support microbial respiration. Higher mineralization could in turn lead to a larger efflux of liberated nutrients following re-submergence and potentially fuel eutrophication (James et al., 2001, Steinman et al., 2012). Sediments should also become more compact and consolidated following drainage because of loss of organic matter and stronger gravitation leading to better root anchorage upon re-submergence (James et al., 2001). Oxygenation of reduced sediment compounds, primarily sulfide and Fe²⁺, can, however, lead to acidification following re-submergence (Smolders et al., 2006, Van Wichelen et al., 2007). Despite the potentially downside of increased sediment nutrient release and acidification, it has been proposed to restore sediment conditions by promoting water level fluctuations and periodic drainage of the sediments (Coops & Hosper, 2002). The potential use of water level fluctuations as a restoration tool has, however, not been experimentally investigated in detail for softwater lakes.

We here tested the long-term influence on plant performance and sediment decomposition of permanently aerial, fluctuating aerial-submerged and permanently submerged conditions in controlled laboratory experiments and in field experiments along an elevation gradient with *Littorella uniflora* inhabiting sandy littoral sediments in Lake Ræv, mid-Jutland, Denmark.

The gradient from aerial to submerged conditions offers changing benefits and constraints to *L. uniflora*’s performance and sediment’s decomposition rate. At high elevation under drained conditions and air exposure, we predict that *L. uniflora* should allocate more biomass to roots than leaves to ensure water supply and avoid desiccation. High O₂ supply from the atmosphere to surface sediment should also prevent constraints on root development and assist fast decomposition of labile organic matter provided the soil does not dry up entirely. Fast mineralization in turn should release inorganic nutrients and stimulate plant growth in organically enriched sediments more than
Isoedtids and water level fluctuations

in fluctuating or permanently submerged populations. Under water, in contrast, there is obviously no water shortage and associated constraints on leaf development, while possibly reduced oxygenation of sediments may restrict root development and decomposition of organic matter. We therefore predict that proportions of leaves to roots should be high and decomposition should be lower than under drained conditions. Fluctuating exposure to air and water may result in rates of sediment decomposition between those in treatments permanently in air or under water. Fluctuating conditions may represent an extra cost to replace aquatic leaves by aerial leaves upon exposure to air to avoid desiccation, while aerial leaves can be converted to aquatic leaves upon submergence. We therefore predict that biomass development should be lower under fluctuating conditions than under permanent emergence or submergence.

Material and methods

Laboratory experiment

Experimental treatments – The influence of different water level regimes on growth of Littorella uniflora and sediment chemistry and decomposition was tested in cylindrical cores (diameter 10 cm, height 20 cm) over a 160-days long laboratory experiment. Triplicate sediment cores with no plants or with natural densities of L. uniflora were subjected to either submerged, drained or fluctuating water level (Fig 1A).

Fig. 1. Schematic drawing of laboratory setup. A) The six different treatments: planted and bare sediments exposed to either submerged (1), fluctuating (2) or drained (3) conditions. Fluctuating treatments alternated between submerged and drained conditions. B) DIC flux measurements in submerged (4) and drained (5) treatments. Under submerged conditions the flux was measured as accumulation of DIC within the chamber connected to the sediment core, by retrieving small water-samples with a syringe and analysing them in an IRGA system. Under drained conditions the CO2-flux was measured directly in the air of a larger chamber covering the whole sediment core and connected to a portable IRGA.
Plants and sediment were collected in shallow water (0.5 m) in the oligotrophic Lake Ræv (56.02521 lat., 9.443243 long.) in July 2011. Plants were brought back to the laboratory undisturbed in natural sediment turfs (25 cm diameter and 20 cm deep). Turfs were transferred from the cylinder to buckets and kept submerged in lake water until used for experiments within 8 days. Surface sediment (0-20 cm) was also brought to the laboratory where stones and large pieces of organic matter were removed by sieving through a 0.5 cm mesh. Thereafter, a small amount of standard dry pellets of organic pasture grass was crushed, added to the sediment and carefully homogenized. Pellets contained (as a percentage of DW) 91 ± 1% (n = 4) organic matter, 46.9 ± 0.4% organic carbon (C), 2.3 ±0.15% total nitrogen (TN) and 0.27 ± 0.01% total phosphorus. Added organic matter is less nutrient-rich than plankton particles but will stimulate sediment metabolism (Møller & Sand-Jensen, 2011) and elevated the sediment organic content from 0.38 % to 0.54 % of the dry weight. Sediment was then left waterlogged for one week prior to experiments to allow new sediment chemistry to establish. Each experimental core was then filled with 3 kg of homogenized sediment. In half of the cores twenty *Littorella uniflora* (2550 pants m⁻²) were planted in equally distributed pre-made holes in the sediment (6 cm deep and 0.5 cm wide). Sediment cores were then gently vibrated to allow sediment consolidation around the roots. Cores with no plants were subjected to similar treatment to avoid initial differences in sediment density. The cores were placed in separate aquaria (9 litre) with water levels being either high (10 cm above the sediment surface) in the flooded treatments, low (15 cm below the sediment surface) in the drained treatment or alternating between these two situations in the fluctuating treatment. The set-up was placed in a temperature controlled room at 14°C in an 12 hour light; 12 hour dark cycle with an incident photosynthetically active irradiance (PAR) of 169 µmol photons m⁻² s⁻¹ when submerged and 184 µmol photons m⁻² s⁻¹ when drained. Drained conditions were secured by drilling two 5 mm holes 2 cm above the bottom of these cores before filling it with sediment. Due to capillary forces of sediment particles inside the cores water was drawn in from the aquarium yielding moist but un-flooded conditions at the sediment surface. Drainage holes were covered on the inside with a fine-meshed plastic fabric to allow water to pass but holding sediment particles within the core. When cores were handled during the experiment inserted rubber stoppers prevented leakage.

Because of the large volume of water needed for the experiment we used filtered lake water from nearby Lake Ersrum diluted 20 times with demineralized water to obtain chemical condition similar to those of the softwater, oligotrophic Lake Ræv. The incubation medium contained: 0.2 mM dissolved inorganic carbon (DIC), 6.8 µM total nitrogen and 0.2 µM total phosphorus. Water level was kept constant in the flooded and drained treatment by replacing evaporated water with demineralized water and replenishing the water column at regular intervals to avoid algal growth interfering with measurements of metabolism and nutrient fluxes. The fluctuating treatment alternated between the flooded and drained situation to simulate a natural community positioned near the water line in a lake with variable water level. The treatment was initiated and completed with a drained period of four weeks duration. In between sediments were flooded for two periods of five weeks duration separated by a drainage period of three weeks. In submerged treatments, water was kept at air saturation by bubbling with atmospheric air.

**Plant morphology and performance**

Plant morphology and leaf chlorophyll were measured before and after the laboratory experiment. Initially, twenty individuals were sacrificed and analysed and after the experiment all plants were counted, analysed
morphologically and leaves were used for measurements of chlorophyll and photosynthesis (only measured at the end of the experiment).

Plants of each replicate core were separated into leaves, stem and roots and dried (105 °C) and weighed and a subsequently combusted (550°C) and biomass calculated as organic dry weight. Values are presented as organic dry weight of leaves and roots (roots and stems).

A subsample of dried leaves were homogenized and analysed for the content of organic carbon (C) on a CHN EA1108-elemental analyser (Carlo Erba Instruments, Milan, Italy). This enabled calculation of the net carbon incorporation of the plants during the experiment using the same proportion of carbon to organic dry weight for the roots as for leaves after correction for mineral content.

Maximum photosynthesis of leaves were measured after the experiment according to the method of Møller and Sand-Jensen (2011) with small modifications. In short, leaves were split lengthwise (to secure efficient exchange of CO₂ between leaf and water) and incubated in water in closed glass flasks (volume 50 ml) containing 1000 µM free CO₂ (pH 6.9) to ensure inorganic carbon saturation. The flasks were mounted on a rotating wheel and exposed to 340 µmol photons m⁻² s⁻¹ for 2 hours at 15 °C. Oxygen concentration inside the flasks was measured with a Firebox 3 O₂ optode (PreSens, Regensburg, Germany) after incubation and compared to similarly treated blanks with no leaves. Photosynthetic rate was normalized to leaf dry weight measured after freeze drying.

Chlorophyll a was measured by extraction in ethanol for 24 h and subsequent spectrophotometric analysis (Christoffersen & Jespersen, 1986) of subsamples of the leaves used for photosynthetic measurements.

**Carbon efflux**

Release of organic carbon from the cores was assessed by analysing water samples from each water column taken before and after renewal of water. Dissolved and particulate organic carbon were measured according to Kragh and Søndergaard (2004).

Efflux or uptake of inorganic carbon from cores was measured midway into the light and dark period several times during the experiment. Under drained conditions, CO₂ evolution was measured by inclosing the turf in a large transparent polyethylene cylinder (3.68 litre) preventing contact to the atmosphere (Fig. 1B). The cylinder was connected to a portable IRGA (LI-820, LI-COR biosciences, Lincoln, USA) and a CTS diaphragm pump (Hargraves Technology Corporation, Mooresville, USA) delivering 1 litre of air per minute through gas impermeable tygon-tube (CM scientific, Silsden, UK). The IRGA was connected to a laptop computer for data logging. Concentration change of CO₂ over time and knowledge of volume of chamber and tubing enabled calculation of the CO₂ exchange rate.

In the submerged treatments, DIC exchange was determined by mounting a transparent, water-filled chamber (0.785 litre) on top of the sediment core. The chamber had a flat top equipped with a small hole for extracting minute water samples for DIC analysis (Fig. 1). The hole was closed by a rubber stopper between sampling. The inside of the chamber was equipped with a 1 cm stirrer bar rotated by a large magnet outside the chamber to ensure water mixing. DIC exchange was determined as the concentration change during 20-30 minutes and measured on triplicate 100 µl water samples injected into 3 % HNO₃ in a bubble chamber purged with N₂-gas carrying evolved CO₂ into an Infrared gas analyser (IRGA, ADC-225-MK3, Hoddesdon, UK) (Vermaat & Sand-Jensen, 1987).

All measurements of inorganic C flux were performed in both light and darkness three times during the 160-days experiment enabling calculation of the diurnal mean net CO₂ evolution. The flux from the fluctuating treatment was measured four times to obtain values for both submerged periods. All rates
were expressed relative to sediment surface area.

**Sediment geochemistry** - After the experiment, a 15 cm long sediment core was retrieved with a Perspex tube (5 cm diameter) from each core. Cores were sliced in depth intervals of 0-2 cm, 2-6 cm and 6-10 cm. Sediment water content was measured as weight loss after 48 hours at 105°C. The dried and homogenized sediment was used for determination of organic matter content as loss on ignition at 550°C. Total iron was measured on combusted samples using the phenanthrolin method modified by Möller and Sand-Jensen (2008). To include the potential sink of iron precipitating on plant roots, a subsample of roots from each vegetated core was combusted at 550°C and iron in the residue was dissolved in HCl and measured as described above.

The amount of reduced iron (Fe\(^{2+}\)) adsorbed to sediment particles and dissolved in the pore-water was determined on samples taken with a small perspex tube (1 cm in diameter) split into the same depth intervals as described above. The samples were transferred to 300 ml gas tight glass bottles containing an anoxic 1 M MgCl\(_2\) solution and left for one hour on a shaking table for adsorbed Fe\(^{2+}\) to be fully exchanged with Mg\(^{2+}\). Subsequently, samples were left for particles to settle for one hour and the concentration of dissolved Fe\(^{2+}\) in the supernatant was measured as described above.

**Field experiment**

To evaluate how naturally drained and flooded areas with dense vegetation of *L. uniflora* responded to water level fluctuations and addition of organic matter, a 100-days long field experiment was set up in late summer to autumn (18th July to 25th October 2011) along a steep hydrological gradient in Lake Ræv. The water level in Lake Ræv has in recent years undergone a systematic drawdown of 0.7 m but was relatively constant during the experiment (see below). Six plots (36*60 cm) were distributed perpendicular to the coastline with the deepest plot located 36 cm below the water and the highest plot elevated 71 cm above the water line at the beginning of the experiment. Each plot was divided into six subplots (12*12 cm) half of which were randomly assigned to organic enrichment while the other half served as controls. Organic matter (1000 g DW m\(^{-2}\)) was added as feeding pellets inserted 4 cm into the sediment using a forceps, while in control subplots the forceps was inserted in a similar way to control for the confounding effect of physical disturbance. The subplots were separated by buffer zones ensuring that leakage from organic subplots did not affect the controls. The addition of organic matter was equivalent to 0.69±0.05 % extra organic matter relative to sediment dry weight in the upper 0 -10 cm of the sediment. According to regular water level recordings the driest plot had been emerged for 2 years, the second driest plot for 1.5 years and the third driest plot for one year, whereas the fourth plot had been subjected to fluctuating water for one year prior to the experiment.
The water level was measured by a pressure sensitive water level logger (HOBO U20) placed in the lake and a reference logger placed above the water. The water level gradually rose by 10 cm during the 100-days long experiment flooding the lowest terrestrial plot 80 days into the experiment. The terrestrial plots covered the entire zone in which *L. uniflora* grows terrestrially whereas it extends to a water depth of about 2 m. At regular intervals throughout the experiment, sediment moisture was measured in four replicates within each terrestrial plot with a Theta Probe ML2x (Delta-T Devices Ltd, Cambridge, UK). The probe was inserted into the buffer zones between subplots to prevent plant damage. The sediment moisture of the plots did not change markedly during the experiment until the final sampling date where the lowest terrestrial plot had become submerged (Fig. 2). Sediment moisture content (SM, %) was closely related to elevation (E, m) of each plot relative to the water line (linear regression p<0.001 $r^2=0.85$, SM =-61.6 E + 51.1). To allow inclusion of the two submerged plots and because of low variation in soil moisture during the experiment, the entire flooding gradient was expressed as average elevation relative to the water line; positive values indicated aerial conditions.

At the end of the experiment, a sediment sample (5 cm in diameter, >10 cm long) was retrieved from each subplot in Perspex tubes. Each tube were closed with rubber stoppers to avoid disturbance and brought to the laboratory for analysis. The rest of each subplot was gently excavated collecting all plant material. Plants were placed in sealed plastic bags with moist paper towel, kept cool and brought to the laboratory.

In the laboratory, sediment samples were split and analysed for content of water, organic matter and carbon using the same procedure as for sediments from the laboratory experiment. Knowing the organic matter content of control and enriched plots enabled estimation the sediment carbon loss along the elevation gradient during the experiment period. The loss was calculated as the difference between enriched and control plots subtracted from the amount initially added to sediments. The sediment layer from 2 to 10 cm depth was used in the calculation to avoid the effect of mosses and algae often growing on sediment surfaces and it was assumed that carbon constituted the same proportion of organic matter in initial sediments as in the added organic matter (46.9 %).

All plants from each subplot were counted, split into leaves and below-ground biomass (roots and stem) and the dry weight recorded. The organic dry weight was determine by correcting for the mineral content of submerged plants collected at the site in July showing a low content of both roots and shoots.

Leaves collected at the end of the experiment were analysed for organic C content using the same method as for leaves in the laboratory experiment. As the mineral content of leaves and roots was similar organic C was assumed to have the same proportion of dry weight in above and below ground biomass. Calculating the difference in plant C between control and enriched plots enabled estimation of the proportion of C from sediment mineralization of added organic matter being incorporated in new plant biomass.

Photosynthesis was determined for leaves of the collected plants using the method described for laboratory experiments.

### Statistical analysis

Statistical analysis and graphs were performed in Graph Pad Prism 5 (Graph Pad Software Inc., La Jolla, USA). Differences between single variables in treatments in the laboratory experiment and along the elevation gradient in the field were tested with one-way ANOVA followed by Tukey's test. The effect of the treatments on two variables (e.g. root and shoot biomasses) were analysed by two-way ANOVA on joined data from treatments
with and without plants followed by Bonferroni post-tests. \( F_{\text{max}} \)-tests were used to evaluate variance homogeneity before parametric tests. In a few instances data had to be log transformed to meet the requirements for variance homogeneity. The significance of differences between initial plant biomass of the cores and biomass after the experiment was evaluated by assessing the overlap of 95 % confidence limits, because of the very low variance of initial biomass. Values are presented as ± standard deviation (SD).

Results

Plant response to water level

Laboratory experiments - Plant biomass increased during the 160-days long experiment in all treatments, but it became much higher under drained than under submerged or fluctuating conditions (Fig. 3, one-way ANOVA, p<0.001). This high biomass under drained conditions was primarily caused by a significantly larger root biomass under drained conditions, while leaf biomass did not differ between treatments (Fig. 3, two-way ANOVA, p<0.001). Because plant density was lowest in the drained treatment, the high biomass was caused by individual plants with significantly larger leaf mass and, in particular, high root mass compared to plants from submerged and fluctuating conditions (Fig. 4, two-ANOVA, p<0.001). Plants biomass in the fluctuating treatment tended to be lower than in the submerged treatment and a t-test of only these two treatments revealed a significant difference (t-test, p=0.033). This finding showed that fluctuating conditions had a negative influence on growth of *L. uniflora* though the biomass still increased during the experiment.

At the end of the experiments, plants in the fluctuating water regime had been in air for 28 days after the last submergence and plants held both aerial and submerged leaf types (sensu Hostrup & Wiegleb, 1991). The two leaf types had leaf chlorophyll contents very similar to those of leaves from permanently submerged and drained
conditions and did not differ significantly from those (Fig. 5, one-way ANOVA p<0.001, Tukey p>0.05). For photosynthesis, the aerial leaves from the fluctuating conditions had significantly higher rates than all other treatments (Fig. 5, one-way ANOVA p<0.001, Tukey p<0.05) and both photosynthetic rate and leaf chlorophyll content suggested a limited direct stress on the plants due to the fluctuations.

Field experiments - The individual plant weight did not differ between emergent plots along the natural gradient in elevation except the second highest plot and soil moisture having a lower plant mass than the plot located just above the water line (one-way ANOVA, p<0.05). Plants in the remaining terrestrial plots were all significantly heavier than in the deepest submerged plots (Fig. 4, Tukey, p< 0.05). Biomass allocation to both leaves and roots differed significantly along the gradient (two-way ANOVA, p<0.05). A strong significant interaction indicated that increasing elevation resulted in opposing trends in biomass allocation, while root biomass increased to shoot biomass tended to decrease (Fig. 4, p<0.05). Leaf photosynthesis under standard conditions did not differ significantly along the elevation gradient (Fig. 5, one-way ANOVA, p=0.57), even though chlorophyll content was significantly higher in the submerged treatment (Fig. 5, one-way ANOVA, p< 0.001, Tukey, p<0.05).

The high individual plant weight in most dry plots did not lead to a higher biomass per surface area because of low plant density (Fig. 3). Instead, the highest biomass was found at intermediate plant density close to the water line, and the two driest plots had a significantly lower biomass compared to all other plot except the deepest plot from which they did not differ (one-way ANOVA, p<0.05, Tukey, p<0.05). The field response generally showed high resemblance to the response in the laboratory experiment, emphasizing the validity of the latter.

Plant response to organic enrichment in the field experiment

Field experiments – Influence of added organic matter on plant performance relative to control plants in natural sediments was
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**Fig. 5.** Leaf chlorophyll (black bars) and net photosynthetic rate (white bars) of leaves in laboratory and field experiments (control plots) along the elevation gradient (mean ± SD). Plants from the fluctuating treatments carried both water leaves (WL) and aerial leaf types (AL). Significant differences in chlorophyll content are indicated by letters while differences between photosynthetic rates are indicated by asterisk. The two leaf types of the fluctuating plants highly resembled those of their counterparts in the two other treatments.

only tested in the field experiment. Addition of labile organic matter increased biomass across treatments (Fig. 6A, two-way ANOVA, p<0.05). Along the elevation gradient the enrichment effect was significantly stronger for leaves than for root development (linear regression, p=0.03). The addition of organic matter did not affect the water content of the sediments, while there was a strong effect of elevation (two-way ANOVA). Thus, the increased plant growth in organically enriched sediments was more likely due to higher sediment concentration of CO₂ and nutrients due to higher decomposition rates.

**Sediment conditions and carbon budget**

*Laboratory experiment* - Different water regimes and plant cover in the laboratory experiment strongly affected carbon metabolism and, thus, the flux of inorganic and organic carbon from the sediment to the surrounding medium during the 160 days. All treatments lost more sediment carbon than they gained by plant uptake of DIC from the

**Fig 6. Field experiment.** Effect of addition of sediment organic matter on plant biomass (mean ± SD) across the investigated elevation gradient. A) Addition of organic matter significantly increased the biomass (black bars) compared to control plots (white bars) across the entire gradient (two-way ANOVA, p<0.05). B) The relative increase in biomass increased markedly under aerial conditions and more for leaves than roots.
Table 1. *Laboratory experiment*. Sediment water content, loss of sediment organic carbon, efflux of DIC and TOC from the sediment, and increase of plant biomass (mean values ± SD) during the 160-days experiment. Differences were tested by one-way ANOVA followed by Tukeys-test and significant differences are indicated by different letters.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plants</th>
<th>Sediment water content (% of WW)</th>
<th>DIC flux (mol m⁻²)</th>
<th>Plant growth (mol m⁻²)</th>
<th>DIC + plant growth (mol m⁻²)</th>
<th>TOC flux (mol m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submerged</td>
<td>+</td>
<td>19.6±0.5</td>
<td>4.4±0.6</td>
<td>3.5±0.06</td>
<td>7.8±0.6ab</td>
<td>4.3±0.7a</td>
</tr>
<tr>
<td>Submerged</td>
<td>-</td>
<td>18.9±0.2</td>
<td>3.1±0.9</td>
<td>3.1±0.9a</td>
<td>3.1±0.9a</td>
<td>3.2±0.2ab</td>
</tr>
<tr>
<td>Fluctuating</td>
<td>+</td>
<td>15.1±2.4</td>
<td>4.8±0.8a</td>
<td>2.4±0.5a</td>
<td>7.2±0.9a</td>
<td>2.3±0.2b</td>
</tr>
<tr>
<td>Fluctuating</td>
<td>-</td>
<td>15.0±2.6</td>
<td>11.4±2.2bc</td>
<td>11.4±2.2bc</td>
<td>2.2±0.5b</td>
<td></td>
</tr>
<tr>
<td>Drained</td>
<td>+</td>
<td>12.7±2.6</td>
<td>7.9±3.3bc</td>
<td>7.8±1.0c</td>
<td>15.7±2.8cd</td>
<td></td>
</tr>
<tr>
<td>Drained</td>
<td>-</td>
<td>13.4±4.6</td>
<td>17.1±2.3d</td>
<td>17.1±2.3d</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*flooded sediments*

water as indicated by a significant reduction of organic matter in all treatments compared to initial sediment conditions (one-way ANOVA, p<0.05, Tukey p<0.05). Bare sediments showed a profound and highly significant increase of the DIC efflux from submerged (3.1±0.9 mol C m⁻²) over fluctuating (11.4±2.2 mol C m⁻²) to permanently drained conditions (17.1±2.3 mol C m⁻², Table 1, one-way ANOVA, p=0.05, Tukey p<0.05). In vegetated sediments part of the CO₂ released by mineralization in the sediment can be incorporated into the growing plant biomass which can account for the reduced DIC efflux observed under fluctuating and permanently drained conditions compared to bare sediments. The sum of DIC efflux and accumulation of plant biomass, however, was at the same level in bare and vegetated sediments under fluctuating (11.4±2.2 and 7.2±0.9 mol C m⁻², respectively) and permanently drained conditions (17.1±2.3 and 15.7±2.8 mol C m⁻², respectively). These combined rates can be regarded as suitable measures of total sediment mineralization to CO₂ and they were much lower under permanently submerged conditions with either bare (3.1±0.9 mol C m⁻²) or plant covered sediments (7.8±0.6 mol C m⁻²). Under submerged and fluctuating conditions dissolved organic carbon (DOC) can be lost from sediments and dead leaves to the water. This loss of organic carbon was particularly high in the submerged treatment with plants, and lower in the submerged treatments without plants. Correcting for the shorter period of submergence in fluctuating treatments, these treatments had a higher daily efflux of organic matter during submergence supporting the hypothesis that a burst of organic material degraded to low molecular compounds under drained conditions is released when submergence follows emergence. When the DOC loss was included in the efflux of DIC and the increase of plant biomass, the total loss of carbon from the sediments still remained lower in submerged vegetated (13.1 mol C m⁻²) and bare sediments (6.3) than in permanently drained sediments (17.7 and 17.0).

The larger efflux of DIC and the larger sum of DIC efflux and increase of plant biomass under drained and fluctuating treatments compared with flooded treatments support the hypothesis that sediment mineralization is higher under the former conditions. The low proportion of reduced Fe²⁺ in sediments of drained and fluctuating treatments is in accordance with the prevalence of oxygenated conditions supporting high and complete mineralization to CO₂ under these conditions (Fig. 7).
Table 2. Field experiment. Water content, loss of organic carbon content and increase in plant biomass (mean values ± SD) in organically enriched sediments relative to control sediments along the elevation gradient during the 100-days experiment. Differences were tested by one-way ANOVA followed by Tukeys-test and significant differences are indicated by different letters.

<table>
<thead>
<tr>
<th>Plot elevation (m)</th>
<th>Sediment water content (% of WW)</th>
<th>Org carbon loss (mol C m⁻²)</th>
<th>Plant biomass increase (mol C m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.39</td>
<td>24.5*±1.8</td>
<td>27.5±4.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.8±1.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>-0.18</td>
<td>30.6*±5.2</td>
<td>29.4±5.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.8±2.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.05</td>
<td>31.2*±1.4</td>
<td>23.0±1.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.3±1.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.27</td>
<td>22.9±1.7</td>
<td>22.7±3.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.0±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.46</td>
<td>9.4±0.4</td>
<td>32.6±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.68</td>
<td>5.4±0.4</td>
<td>36.3±1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4±1.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* flooded at sampling

Fig. 7. Laboratory experiment. The percentage of reduced Fe<sup>2+</sup> of the total Fe pool in the sediment (mean ± SD) subjected to the different water regime and plant cover (white bars) or no plant cover (black bars). Lower percentage of reduced Fe<sup>2+</sup> indicates more oxygen available in sediments. A two ANOVA showed significant effect of both water regime and plant cover (p<0.05).

CO₂ released from organic matter. This amount was highest (6 mol C m⁻²) in the middle of the investigated gradient. Compared to the laboratory experiment the proportion of the degraded organic C being incorporated into plants was substantially lower in the field. This difference could result from enriching the sediment at a single depth rather than exposing plant roots to extra organic matter distributed homogeneously over all depth as in the laboratory experiment.

Discussion

Plant response to water level regimes

Measurements confirmed that Littorella uniflora is well adapted to amphibious life at the margins of oligotrophic, softwater lakes. Plant biomass increased under drained, fluctuating as well as submerged conditions in laboratory experiments and along the elevation gradient from water to air in the field and leaf chlorophyll and photosynthesis remained high under all treatments. Other amphibious species, which are dependent on CO₂ uptake through leaf surfaces for photosynthesis, can survive under water but usually have very low photosynthesis and lose weight when the water contains low CO₂.
concentrations close to air saturation (Sand-Jensen et al., 1992). Mentha aquatica, for example, has a negative relative growth rate (-0.8 d⁻¹) in air-saturated water and it requires 15-fold super-saturation of CO₂, which can be found in groundwater-fed lowland streams, to support appreciable growth rates (1.8 d⁻¹), though growth rates are three times higher in air (4.7 d⁻¹, Sand-Jensen & Lindegaard, 2004, their Table 6.2). Littorella uniflora and other isoetid species grow well in oligotrophic, softwater lakes with low CO₂ concentrations close to air saturation in the water because they exploit the CO₂-rich sediment resources (Raven et al., 1988, Pedersen, Sandjensen & Revsbech, 1995). The small isoetid species can maintain dominance under fluctuating water levels at the margins of oligotrophic, softwater lakes because of low competition from taller species which require higher nutrient levels, are less tolerant to the flooding regime and wave exposure, are unable to utilize the CO₂-rich hydrosoil and have no access to alternative inorganic carbon sources (e.g. HCO₃⁻) under water (Farmer & Spence, 1986).

There was no indication in our experiments that enrichment of sediments with labile organic matter stressed Littorella uniflora. On the contrary, plant growth was greatly stimulated in the field presumably due to higher sediment mineralization supplying extra CO₂ and inorganic nutrients for plant uptake (Sand-Jensen & Søndergaard, 1979, Bagger & Madsen, 2004). Previous laboratory experiments with submerged L. uniflora have documented that enrichment of sediments with organic matter of the same type and amount (0.1 and 0.2 % of sediment DW) stimulates plant growth, while greater organic enrichment (0.4-1.6 %) induces sediment anoxia, reduces plant growth, chlorophyll and photosynthesis and leads to stunted roots having difficulties receiving sufficient O₂ flux to the apical root meristems and supplying the leaves with adequate nutrients (Raun, Borum & Sand-Jensen, 2010, Møller & Sand-Jensen, 2012).

It is likely, that L. uniflora under drained or alternating drained and submerged conditions can better sustain high organic enrichment because exposure to air will prevent leaf anoxia, assist in sediment oxygenation and also lead to lower proportions of reduced Fe²⁺, and other reduced potential phytotoxins (e.g., Mn²⁺, NH₄⁺ and small fatty acids; Armstrong & Armstrong, 2001, Smolders et al., 2002) characteristic of anoxic sediment conditions (Fig. 7).

The highest biomass in laboratory experiment was found under drained conditions and, likewise, the largest plants grew in the dry part of the elevation gradient in the field. This finding is in accordance with previous field investigations of L. uniflora showing higher growth rates above than under water (Robe & Griffiths, 1998). Although individual plants in the field were larger under drained conditions, the biomass was smaller in the driest plots than in the plots located close to the water line having higher plant density.

![Fig. 8. Laboratory experiment. The sediment density of un-vegetated sediments (mean ± SD) of the three different water level regimes. Black squares: submerged, white circles: fluctuating and black circles: drained conditions after the laboratory experiment. The sediments were significantly less condensed in the top layer under submerged conditions compared to the other two conditions. Also the sediment density increased with depth (two-way ANOVA, p<0.05).](image-url)
The lower plant density at the highest elevation, which had been out of the water for more than a year prior to the investigation, can be explained by poor germination because lack of moist sediments and prolonged seed desiccation impair germination (Arts & van der Heijden, 1990) and by lack of vegetative spread by runners under dry conditions (Robe & Griffiths, 1998).

The fluctuating water level in laboratory experiments was accompanied by reduced biomass development relative to permanently emerged or submerged conditions. This finding suggests that fluctuations were costly and reduced biomass development although leaf chlorophyll and photosynthesis were not depressed and did not suggest physiological stress (Møller & Sand-Jensen, 2011). Plants under fluctuating conditions had both aerial and aquatic leaf types conforming to earlier results showing that emerged aquatic leaves can remain active for more than a month (Robe & Griffiths, 1998). While the aquatic leaves can remain active under emerged conditions they are certainly not as suitable as the terrestrial leaves as they lack stomata for CO₂ uptake and have a thin cuticle resulting in a high water loss. The lower biomass under fluctuating conditions is probably due the extra costs linked with higher leaf turnover.

Aquatic submerged leaves of L. uniflora remain active for six-nine months before they are shed (Sand-Jensen & Søndergaard, 1978), while in the present experiments plants are inclined to produce new leaves every five weeks because of the shifting water level.

Leaf and root development of L. uniflora responded in different ways to drainage and flooding. Root mass increased markedly relative to leaf mass under drained conditions in both laboratory and field experiments probably in response to limited water and nutrient availability. This response of increasing biomass allocation to the organ exploiting the most limited resources is widespread among plants (Poorter & Nagel, 2000) and lower root to shoot biomass is also typical of submerged aquatic plants compared with terrestrial plants (Wetzel, 2001). The same root-shoot response as we found for L. uniflora has been observed in earlier Danish work (Nielsen & Sand-Jensen, 1997), while the root-shoot ratio remained constant upon emergence in Scottish studies (Robe & Griffiths, 1998). This difference could be caused by the Scottish plants being regularly exposed to air and therefore, already possessing long roots because of earlier periods of emergence. In the Danish study the submerged plants had not been exposed to air for several years and lacked the special long (15-20 cm), robust roots that can absorb water from deep sediments.

Sediment response to water level regimes

While all sediments exposed to different water level regimes in the laboratory experiment lost organic matter, the amount differed significantly between treatments. The largest direct DIC efflux was observed in the drained treatment without vegetation but if incorporation of carbon into plant biomass was accounted for the combined loss from the drained vegetated sediments was of similar magnitude. This finding corresponds with earlier investigations showing high loss of organic matter under drained conditions (Degrroot & van Wijck, 1993, van Wijck & Degrroot, 1993, James et al., 2001). The lowest loss of organic matter was observed in submerged bare sediments, while the submerged vegetated and both treatments with fluctuating water levels lost intermediate amounts. The low loss of the submerged bare sediment is likely caused by lower mineralization rates due to reduced oxygen depth penetration in organically enriched sediments (Møller & Sand-Jensen, 2011) as also supported by the high proportion of reduced Fe²⁺. It is possible that there was a higher build-up of DIC in the sediment in this treatment because there were no plant roots to incorporate CO₂ in plant biomass or serve as an air channel conduit for transport from the sediment to the water. However, the
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experiment lasted so long (i.e. 160 days) that this sediment accumulation must be low relative to the measured efflux. However, it is possible that part of the anoxic respiration instead of forming CO₂, released methane which we did not quantify. If so, the carbon efflux would have been underestimated.

In the field experiment, the decline of added organic matter was measured directly by weight differences between enriched and control sediments. This decline was also highest under the driest conditions, but lowest in intermediate elevations, while submerged plots were located in between. In the field, we did not measure the DIC efflux. The substantial loss in the submerged plots could be due to release of organic matter from the freshly added dry pellets that are dissolved under these conditions, while this process will not take place to nearly the same extent in naturally formed aquatic detritus.

Restoration of isoetid populations

Seepage lakes is a preferred habitat of isoetid species because they are nutrient-poor and experience widely fluctuating water levels that are tolerated and perhaps even preferred by isoetid species (Sculthorpe, 1967), while competitors among aquatic elodeid species dry out upon emergence and taller amphibious or terrestrial species are unable to photosynthesize or grow under water (Sand-Jensen et al., 1992, Sand-Jensen & Frost-Christensen, 1999, Bailey-Serres & Voesenek, 2008). It is, therefore, a problem when the water level of oligotrophic lakes becomes strictly regulated because isoetid species then lose their competitive advantage of being able to photosynthesize and grow well in the fluctuating transition zone between air and water (Pedersen & Sand-Jensen, 1992, Nielsen & Sand-Jensen, 1997).

There are other advantages for isoetids linked to the influence on sediment properties of fluctuating water levels. This is particularly the situation when high sedimentation of organic matter due to external input or high in-lake phytoplankton production threatens to make the sediments unsuitable to growth and root anchorage of isoetids by preventing oxygenation, increasing formation of potential plant toxins and making the texture less firm and cohesive (Møller & Sand-Jensen, 2008, 2011, 2012). Then, exposure to air can serve, as documented here, to increase the oxygen flux, degradation of organic matter, plant growth and specific density of surface sediments. This did not lead to problems with acidification (Smolders et al., 2006) in our study (data not shown) because FeS levels were low in the sediments. Lower water content and higher specific density, in turn, are known to improve root anchorage (Schutten et al., 2005, Sand-Jensen & Møller, 2013).

In those instances where thick surface layers of muddy organic matter prevent isoetid establishment and growth, a promising, though costly, restoration tool is to combine reduced water level with direct removal of the drained organic surface layer once again exposing the underlying solid mineral sediment to isoetid reestablishment. This procedure has been used with success in several lakes in the Netherlands (Brouwer, Bobbink & Roelofs, 2002) and with the same effect but less well documented in some Danish lakes (Miljøcenter-Ribe, 2008).

As sediment desiccation has also been shown to decrease the level of nitrogen in sediments (James, Barko & Eakin, 2004, Smolders et al., 2006), this tool may prove valuable in lakes where sediments accumulation has not been too excessive.

Acknowledgements

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References


Appendix 1
Kransnålalger rummer mange truede arter

Photo: Martin Mcnaughton (livetunderoverfladen.dk)

Et forslag til rødliste understreger behovet for at forbedre kransnålalgernes livsbetingelser og holde skarpt øje med udviklingen.

Kransnålalgers udbredelse


Kransnålalger rummer mange truede arter

Lars Bastrup-Spohr, Jeppe Dahl-Nielsen & Kaj Sand-Jensen


Et forslag til rødliste understreger behovet for at forbedre kransnålalgernes livsbetingelser og holde skarpt øje med udviklingen.

Kransnålalgnerne dominerer vegetationen i de første år af vandhullets liv for senere at blive udkonkurreret og forsvinde, når rørsumpen etableres, eller højt-voksne undervandsplanter tager over. Kransnålalgnerne hænger dog ved i flere årter i kalkrige, næringsfattige vandhuller, som vi blandt andet møder i Vestskoven og i grus- og kalkgrave. Men kransnålalger er små, langsamt voksende arter som på sigt ofte udkonkurreres af kraftige blomsterplanter.


Billede 1. Bugtet Glanstråd (Nitella flexilis) er en af de få arter af kransnålalger der er gået frem i det seneste århundrede, i både Danmark og andre vesteuropæiske lande. Arten var tidligere mest kendt fra det kalde bundvand i kalkfattige søer, men er nu vidt udbredt også i næringsrige søer og damme. Foto: Jens Christian Schou (biopix.dk)
forureningsalger kvalte dem. Sådanne stjernelokaliteter for kransnålalger er forsvundet overalt både i de danske farvande og ved Tysklands og Polens Østersøkyster. Længere oppe i Østersøen langs de svenske og baltiske kyster, hvor eutrofieringen er mere beskeden, er der fortsat områder med store forekomster af kransnålalger. Samlet set udgør kransnålalgerne imidlertid ét af de mest sjældne og uddybningstruede organisationer i Europa.

Arternes relative hyppighed før og nu
For at kunne opgøre kransnålalgernes status har vi sammenstillet alle informationer om deres forekomst ifølge amternes og miljøcentres optegnelser, videnskabelige studier og dygtige botanikeres observationer. Endvidere har vi (JDN) selv besøgt et stort antal lokaliteter. I vurderingen af arternes nuværende relative hyppighed tager vi udgangspunkt i miljøovervågningens data, da disse er indsamlet efter den samme metodi. I alt indgår 399 artsobservationer fordelt på 226 lokaliteter i vores sammenstilling, mens Sigurd Olsens data rummede 243 artsfun fordelt på 124 lokaliteter. Der voksede med sikkerhed 25 arter af kransnålalger i Danmark for 70 år siden, mens der i dag vokser 23 arter.

Arternes hyppighed før og nu er signifikant korrelerede (Fig. 1). Arternes hyppighed før og nu er samme for 70 år siden og i dag. Både i den gamle og den nye sammenstilling optræder mange arter med en lav hyppighed, og de må derfor betegnes som sjældne og truede og dermed som kandidater til rødlist. I Olsens kvantitative data optræder 8 arter således med en hyppighed på mellem 0,9 og 4% på lokaliteterne og yderligere 2 arter er registreret som sjældne, mens 12 arter i det nye dataset optræder med en hyppighed på mellem 0,8 og 4,2%. Otte af de fåtallige arter er fælles for de to sammenstillinger. For arterne med primær forekomst i brakvand er de historiske data ikke så fyldestgørende, men opptegnelser fra 1980’erne og frem viser, at flere arter er sjældne og i tilbagegang i danske farvande og også i resten af Østersøen.

Forsvundne og nye arter

**Forsvundne arter**

Fem af Sigurd Olsens sjældne arter er ikke genfundet ved de seneste undersøgelser, og fire af arterne er sandsynligvis forsvundet fra Danmark. 

- *Tolypella intricata* er sjælden i Norden, og var længe vurderet som uddød i Sverige, men er i de senere år genfundet på få lokaliteter. Arten er enarig og har typisk en meteorisk forekomst, hvor den pludselig dukker op, ofte i nydannede udtørende habitater. Den er fundet i et oversvømmet hjulspor i Sverige.
- Ifølge Olsen (1944) var arten observeret på 6 lokaliteter før år 1900, hvorefter arten ikke er blevet observeret. De periodiske oversvømmede habitater, som arten foretrækker, er blevet meget sjældne herhjemme i det drænede danske landskab. Det er derfor overvejende sandsynligt, at arten er nationalt uddød.

**Chara filiformis** er udbredt i Midt- og Østeuropa og voksende indtil 1939 i Furesø. Arten er i dag i Skandinavien kun kendt fra kalksøen, Levrasøen i det østlige Skåne. Den har en særegen morfologi og risikoen for fejlbestemmelse er derfor lille.

- *Chara horrida* var tidligere hyppig i farvandene omkring Fyn og Nordtyskland, men her er undersvandsvegetationen gået voldsom tilbage. I nyere tid (1990-

**Fig. 1.** Procentvis forekomst af arter i studier før 70 år siden (x-aksen, Olsen 1944) og i dag (y-aksen). Tallene er afbildet som logaritmen til den procentvise forekomst plus 1, så arter med muligt tilstedeværelse har logaritmeværdien 0 i diagrammet.
Arten kun kendt fra det indre af Østersøen og fra norske fjorde. Ifølge de nyeste DNA studier kan arten ikke skelnes genetisk fra *C. baltica*, ligesom overgangsformer mellem *C. horrida* og *C. baltica* også kendes fra Østersøen (Boegle et al. 2010). Uanset om *C. horrida* og *C. baltica* er separate arter, er ingen af dem observeret i *C. horrida*’s tidligere kerneområde i det sydfynske øhav i nyere tid. Vi kan ikke med stor sikkerhed konkludere at arten er forsvundet.


*Nitella opaca* er kun genfundet meget få gange Danmark i de seneste århundre. Den er ikke truet i Sverige og Norge, men er truet i Nordtyskland. Vegetativt kan arten ikke adskilles fra *Nitella flexilis*. Men da *N. flexilis* er almindelig, kan *N. opaca* fortsat skjule sig bag de mange ikke artsbestemte fund af *Nitella*.

*antallet er en kombination af private observatørers observationer og voksesteder registreret i miljøovervågningen.

**antallet er en kombination af observationer fra Statens Naturhistoriske Museum og voksesteder registreret i miljøovervågningen i perioden (1997-nu).
Nye arter
To nye arter optræder i de seneste opgørelser, én af dem anser vi for nyindvandrede. Chara cornuncens lever primært i lav-salint brakvand og alkalint ferskvand. Arten er sjælden i det meste af Østersøen, men er dog fundet i stort antal ved Estland. I Danmark er den fundet på mindst 6 lokaliteter af forskellige personer.

Chara virgata optræder i de nye undersøgelser, men er i Olsens studier inkluderet som et synonym af Chara globularis (for kaldet C. fragilis). C. virgata er derfor med stor sandsynlighed ikke ny for Danmark, men har blot været inkluderet i C. globularis, som den ligner meget.


Et revideret nationalt arts- tal og forslag til rødliste

Som bekendt rummer en rødliste seks kategorier af arter, som vurderes som: ikke truede (LC), næsten truede (NT), sårbare (VU), moderat truede (EN), kritisk truede (CR) eller uddøde (RE). En arts placering på listen kan begrundes ud fra flere internationale fastlagte kriterier, primært med fokus på tilbagegang af artens geografiske udbredelsesområde eller bestandsstørrelsen. En art optræder altså som Regel ikke på rødlisten, hvis den ”bare” er sjælden, men der bliver selvfølgelig lagt vægt på sjældne arter i tilbagegang.

Listerne udarbejdes af Dansk Center for Miljø og Energi (DCE) og Naturstyrelsen, men det er ikke sket for kransnålalgernes vedkommende, sikkert pga. manglende kendskab til arternes hyppighed og udvikling.

Da vi har samlet den tilgængelige viden omkring denne oversatte gruppe, foreslår vi her en rødlistede status for de danske arter af kransnålalger (Tabel 1). Vurderingen er primært begrundet i
antallet af kendte voksesteder for den enkelt art samt vores kvantitative undersøgelse af historiske ændringer for arten (Baastrup-Spohr et al. 2013). Antallet af voksesteder er vurderet til at være det maksimale antal lokaler observeret i en af de tre benyttede kilder (Tabel 1). For de fleste arter er det højeste antal lokaliteter i et meget begrænset område. Kun 4 af 26 arter (15%) vurderes at have en gunstig status mens de resterende 85% er truede i mere eller mindre grad. I gruppen af truede arter finder vi de nævnte formodede regionalt uddøde arter (4), de to kritisk truede *Chara polyacantha* og *Chara rudis* som hver kun har et observeret voksested i Danmark, og som begge har været i tilbagegang siden Olsens studier i 1940'erne. Den resterende gruppe (14 arter) har ligeledes et meget begrænset antal voksesteder og de fleste er gået tilbage i løbet af det 20. århundrede.

**Botanisk notitser...**

**Rettelse til URT nr. 1, 2013**


**Havens Rødder**

Havens Rødder er en netværks­ gruppe, hvis formål det er at inddrage Botanisk Have i den gymnasiale naturvidenskabelige undervisning, specielt i biologi men også i tværfaglige projekter. Vi vil gerne så et slag for, at botaniske emner og problemstillinger udgør en større og vigtigere del af den gymnasiale undervisning end de gør i dag. Det er også i denne sammenhæng vigtigt at pege på Botanisk Havens potentiale.

Vi er ca. 20 personer i grup­ pen. Den består af

- forskere fra Botanisk Have, Biologisk institut, LIFE og Pharma både nuværende og tidligere,
- gymnasielærere med fagene biologi, kemi, geografi og matematik,
- andre der interesser sig for Botanisk Have.

Læs evt. mere om formålet med Røddernes virke på hjemmesiden på brughaven.dk

Fra hjemmesiden kan man også downloade hæftet Brug Botanisk Have i Undervisningen som indeholder artikler clever kan læse før et besøg i Haven. Det kan i øvrigt købes i Havens butik for 65 kr.

Vi mangler botanisk vidende og interesserede personer, der har lyst til at assistere gymnasieklasser, -grupper eller applyskrivere ved besøg i haven og ved arbejde med forskellige projekter, der indeholder botaniske problemløsninger. Vi har også brug for faglige oplæg, når rødderne mødes.

Kontakt os, hvis I er interesse­ ret og fortæl evt. samtidig, hvil­ ket emne/emner I gerne vil give oplæg om. Så sætter vi jer i for­ bindelse med dem, der har brug for netop jeres specielle eksperti­ se.

Kontakt: Dorte Hammelev, dorte@centrum.dk

**Litteratur**


*Charophytes* 2: 53-58.


**Forfatternes adresse:**

Københavns Universitet, Ferskvandsvi­ dogisk Laboratorium, Helsingørsgade 51, 3400 Hillerød
Appendix II
Ett kalkbrott på Ölands alvar – en stenöken med knivskarpa miljögränser

Photo: Hans Henrik Bruun
Extrema miljöer pressar växterna till det yttersta och skapar ofta tydliga fördelningsmönster mellan arterna beroende på deras olika härdighet och konkurrensförmåga. Så förhåller det sig också i övergången från uttorkade kalkstenshällar via tillfälliga till permanenta vattensamlingar i ett kalkbrott på Räpplinge alvar.

En forskargrupp från Köpenhamns universitet besökte Öland i maj 2009 för att undersöka den speciella miljön.

Kaj Sand-Jensen, Lars Baastrup-Spohr, Anders Winkel, Claus Lindskov Møller, Jens Borum, Klaus Peter Brodersen, Torbjørn Lindell & Peter Anton Stæhr

Ad som vid första påseendet verkade vara ett öde och ointressant landskap i ett stenbrott på Räpplinge alvar bjuder i själva verket på ett fascinerande utbud av växter med utomordentlig anpassningsförmåga. Inom några få meter skifter miljön från vänlig och tillmötesgående med god vattentillgång, ordentligt fäste för växternas rötter och små temperatursvängningar, till den mest fientliga, där en sjö förvandlas till en öken på några få dagar och där temperaturen varierar från bitande kyla till stekhet solvärme.

Fascinationen förstärks av att man här finner både den utpräglade torrmarksväxten vit fetknopp, som lever av nästan ingenting under torrperioder och överlever vinterns översvämningar, och vattenväxten skyfallsalg, som överlever torkan genom att ”spela död” men omedelbart återupptarstå från de döda när vattnet kommer tillbaka.

Miljögraderienter och växtfördelningsmönster

Tydliga fördelningsmönster mellan växter finns man längs gradienter för resurser som ljus, fuktighet och närskal (Vestergaard 2007). Gradienter av andra miljöfaktorer som vind, temperatur, salthalt och pH skapar också tydliga fördelningsmönster (Tyler 1971, Crain m.fl. 2004). I regel förekommer många arter längs gradienten, och arternas utbredningar går ofta gradvis in i varan-

Figur 1. Räpplinge stenbrott. Mellan de 5–10 cm tjocka skiktet av hård kalksten finns mer lättvittrat material. Foto: Lars Baastrup-Spohr.

The lime quarry at Räpplinge alvar. Profile of 5–10 cm thick layers of hard limestone with thinner and more easily disintegrating layers inbetween.
Negativa eller positiva interaktioner mellan olika arter påverkar också fördelningen (Maestre m.fl. 2009), och då i synnerhet konkurrens. Om det finns få arter blir gränserna särskilt påtagliga och om flera av miljöfaktorerna förstärker varandra blir växlingen mellan arterna ännu tydligare. Man kan till exempel se en tydlig zonering i sjöar med ökande djup där hela bälten med enartsbestånd avlöser varandra beroende på skillnader i belysningskrav, tolerans mot olika vattendjup och konkurrensförmåga (Sand-Jensen & Søndergaard 1997).

Ett liknande fenomen kan man se i avrinningsområden från brunkolsbrott, där extremt surt vatten (pH 2–4) med höga koncentrationer av metaller bara medger att en enda blomväxt förmår överleva (löktåg Juncus bulbosus), men där fler arter ansluter i takt med att pH-värdet stiger och metallkoncentrationen sjunker när allt renare vatten från omgivningen tillförs (Sand-Jensen & Rasmussen 1977).

I miljöer där det finns få arter och där graderterna är påtagliga och fördelningen mellan arterna tydlig, blir det möjligt att testa om det är speciella fysiologiska och ekologiska anpassningar hos arterna eller om det är skillnader i konkurrensförmåga som förklarar varför de växer där de växer längs gradienten.

En sådan mycket lämplig situation hittar vi längs gradienten från land till vatten i stenbrottet vid Räpplinge alvar på Öland. Här ändrar sig vatten- och näringstillgången snabbt och påtagligt och när förhållandena är som mest extrema finner man endast några få arter.

Dried out limestone slabs covered by reddish Sedum album and black, dry crusts of Nostoc commune.
En varierande miljö i stenbrottet

Den hårda kalkstenen är bruten i horisontella skikt (figur 1). Mellan de 5–10 cm tjocka kalkflisorna finns mer lättvittrat material som tillsammans med sönderbrutna kalkflisor samlats i avfallshögar av 2–3 meters höjd. Dessa avfallshögar är bevuxna med buskar och ett stort antal olika örter, inklusive johannesnycklar *Orchis militaris* och ängsnycklar *Dactylorhiza incarnata*. Denna vegetation har vi inte närmare undersökt. Däremot har vi undersökt de endast några få centimeter höga övergångszonerna mellan kalkstensytorna, där damm och jordpartiklar har samlats och så småningom bildat ett 1–4 cm tjockt jordlager, som ger växterna rotfäste. Jorden är något fuktig vilket gynnar växtlivet, som kan omfatta mer än trettio olika arter.

På de helt plana kalkstensytorna växer endast vit fetknopp *Sedum album* tillsammans med gul fetknopp *S. acre* och tåliga mossor (figur 2). Här finns endast ett mycket tunt jordlager som inte kan bevara någon fuktighet, så här krävs en extrem anpassning till frost på vintern, stekande hetta på sommaren, bitande vind, samt växlingar mellan översvämning och torka för att kunna överleva.


Dry crusts of *Nostoc commune* (left) can rapidly absorb water, turn metabolically active (middle) and gradually become even thicker and greener within a couple of days (right).
aktiva, geléartade kolonierna – därav förmodligen namnet skyfallsalg.


De grunda vattensamlingarna domineras av skyfallsalger samt av mattor av trådalger i de delar som har sediment. Fram på eftermiddagen kan här uppmätas ett pH på närmare 10, vilket innebär att koldioxidkonsentrationerna är försumbara och hundra gånger lägre än i luften. Det höga pH-värdet och underskottet av koldioxid beror på den intensiva fotosyntesen samt växternas förmåga att aktivt utnyttja vätekarbonat ($\text{HCO}_3^-$). pH nådde inte riktigt upp till samma nivå (ca 9,3) i de djupare vattensamlingarna. Här dämpas pH-vari-

Olika växter i vattensamlingarna

Skyfallsalg i små uttorkade vattensamlingar

Skyfallsalg och vit fetknopp utgör de dominerande arterna på kalkstenshällarna. De torra kalkhällarna – som en månad tidigare stod under vatten – har nu i maj svarta, intorkade skorpor av skyfallsalg mellan blodröda partier med vit fetknopp (figur 6, område 2). Det är tänkbart, men inte närmare undersökt, om skyfallsalgen tack vare sin förmåga att binda atmosfäriskt kväve gynnar etableringen av vit och gul fetknopp på den nakna kalkstenen. Det skulle i så fall vara ett exempel på ett gynnsamt samspel i ett område som präglas av brist på resurser.

I de mest temporära vattensamlingarna på den nakna kalkstenshällen kan skyfallsalger täcka 75 procent av botten, medan resten består av vit fetknopp och naken kalksten (figur 6, område 3). Om vattensamlingarna är lite djupare och har ett några millimeter tjockt sediment etablerar sig kransalger, medan man i strandkanten återfinner spridda exemplar av kärrkavle Alopecurus geniculatus och krypven Agrostis stolonifera.

Vattenmöja i större uttorkade vattensamlingar

I djupare, men ändå uttorkade vattensamlingar med ett 1–2 cm tjockt sediment ovanpå kalkhällen växer vattenmöja Ranunculus aquatilis, härsärv Zannichellia palustris och en filt av trådalg på sedimentet medan skyfallsalger återfinns på den nakna kalkstenshällen vid de allra lägsta vattennivåerna (figur 6, område 4–7). På något djupare vatten dominerar kransalger. I de delar av vattensamlingarna som nyligen har blivit torr-
lagda, är kransalgerna döda men har satt oosporer som kan gro när vattnet återkommer.

Vattenmöja tål inte heller att torka ut, men tack vare fuktigheten i sedimentet kan de klara sig lite längre här än på den bara kalkhällen, där endast skyfallsalgen kan överleva.

Vattenmöja kan bilda både undervattens-, flyt- och övervattensblad men arten klarar sig först när det finns ett par centimeter sediment för att säkra näringstillgången (och vattentillgången på land), och landformen växer endast mellan stenar som skyddar mot vindens uttorkande effekt.

Kransalger i de djupaste vattensamlingarna

I de permanenta vattensamlingarna är vattnet djupare och sedimenten upp till 6 cm tjocka. Här kan kransalger täcka ungefär 80 procent av bottnen (figur 6, område 9 och 10). Mellan de täta bestånden av kransalger växer enstaka exemplar av axslinga *Myriophyllum spicatum*, krusnate *Potamogeton crispus* och gäddnate *P. natans*. Sedimenten bildas eftersom vattensamlingarna fungerar som en fälla för jordpartiklar som kommer med vatten och vind från omgivningen. I sedimenten ansamlas också kalk och organiskt material som producerats av växterna.


Takmossan och de övriga mossarna växer därför enbart i ett snävt intervall av den studerade miljögradienten, på torra till halvfuktiga och ytterst tunna jordar där de kan dra nytta av sin förmåga att tåla uttorkning. De upphör då med ämnesomsättningen men återupptar aktiviteten snabbt när de åter får vatten. Alla fyra mossorna bildar tät kuddar som likt svampar suger upp vatten och också förlorar vattnet långsamt, vilket medger en förlängd aktivitetsperiod.


Skyfallsalgen utnyttjar effektivt vätekarbonat i vatten (tabell 1). Den klarar sig också utan tillförsel av nitrat och ammonium tack vare sin förmåga till kvävefixering. Skyfallsalgen är aktiv så länge den får vatten, men när underlaget torkar ut går kolonierna snabbt samma väg, varvid fotosyntes och respiration upphör. Skyfallsalgen besitter således en imponerande anpassning till perioder med omväxlande extrem torka och översvämning.


Kransalgen taggsträfse *Chara hispida* dominerar i de permanenta vattensamlingarna, och den finns också i de djupare delarna av de tillfälliga vattensamlingarna innan de torkar ut (figur 6). Taggsträfse utnyttjar effektivt vätekarbonat som kolkälla för sin fotosyntes (tabell 1) genom att i en aktiv process fälla ut kalk på cellyttorna. För varje utfälld molekyl calciumkarbonat frigörs en vätejon som förvandlar en annan vätekarbonatjon till fri koldioxid som används i fotosyntesen. Tack vare kalkutfällningen kan fotosyntesen fortsätta utan att pH-värde blir extremt högt och hämmar vidare aktivitet.

**Tre mästare i anpassning**

På kalkstenshållarna vid Räpplinge alvar kan man således direkt se vilka arter som kan clara de mest extrema miljöförhållandena.
En mästare i att överleva i denna extrema miljö är vit fetknopp, som upprätthåller sin aktivitet på land trots torka, medan dess fotosyntes går i stå under vatten på grund av brist på organiskt kol. Den andra är takmossan som är aktiv i fuktigt tillstånd på land. Den tredje är skyfallsalgen, som är aktiv både i fuktigt tillstånd på land samt under vatten. Alla tre arterna är endast några få centimeter höga och är vatten- och näringsintillaggen mer riklig konkurreras de ut av en mera artrik och högväxt vegetation med större biomassa.

Man kan fråga sig hur vit fetknopp, takmossa och skyfallsalg får tillräckligt med närsalter ur regnvattnet och den hårdt kalkhällen. Särskilt är det ett problem att få tag i fosfat som är hårt bunken. Skyfallsalgen anlänner sig av aktiva jonpumpar för att kunna utnyttja vättekarbonat till fotosyntesen i vatten med högt pH, och den har som andra cyanobakterier sannolikt specifika proteiner i cellmembranen för att ta upp fosfat (Zhang m.fl. 2009). Det är också tänkbart att skyfallsalgen kan avge organiska syror för att upplösa kalkfosfater, något man funnit hos andra kalkväxter (Ström m.fl. 1984).

Citing litteratur

Tack till Carlsbergsfonden för ekonomiskt stöd.

ABSTRACT

Extreme environmental gradients generate distinct patterns in species distributions dependent on their stress tolerance and competitive capability. This is the situation along the steep land-water gradient in a stone quarry on Öland’s alvar. Availability of water and nutrients varies profoundly between bare limestone slabs with no soil, via temporary to permanent ponds with thick sediments. Only a few, extremely robust species of plants (Sedum album and S. acre), colonial cyanobacteria (Nostoc commune) and mosses (Syntrichia ruralis and Racomitrium canescens) survive on the bare limestone slabs experiencing prolonged summer drought and winter flooding. Nostoc commune can survive drought for many months and resume photosynthesis and efficient HCO₃⁻ utilization within minutes after water has become available.
Kaj Sand-Jensen är professor i akvatisk ekologi vid universitetet i Köpenhamn och förestår den forskargrupp av lektorer (JB, KP, PAS), doktorander och forskarassistenten (LBS, AW, CLM) som besökte Öland för att studera skyfällsalgens ekologi och fysiologi. Torbjörn Lindell, lektor vid Latinskolan i Växjö, deltog också i projektet.

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E-post: ksandjensen@bio.ku.dk
Supplementary material
Included is models, graphs, and tables supporting paper II and paper III.
Supporting information for:

Fig. S1. Relationships between the logarithm to number of known localities of charophyte species in Sweden (Sw) and Denmark (Dk) and their corresponding Red List category. Red List categories have been translated to a numerical scale appropriate for statistical analysis: 0 = regionally extinct, 1 = critically threatened, 2 = endangered, 3 = vulnerable, 4 = nearly threatened and 6 = not listed. Spearman rank correlation showed highly significant relationships (p< 0.001) while model II regression yielded no significant difference between the slope of the lines for the two countries P= 0.38. National abundance and Red List categories were taken from Baastrup-Spohr et al. (2013), Langangen (2007), and Gärdenfors (2010).

Table S2. Functional traits and Scandinavian abundance of the 38 charophyte species that have been found in Sweden, Norway, Finland and Denmark. The functional traits values were obtained from Langangen (2007) and supplemented with values from Gabka (2009). The assignment of a given trait value to each species are explained in detail in the material and methods section of the paper. Abundance data is based on Langangen (2007), Gärdenfors (2010), Koistinen (2010), (Sjøtun et al. 2010) and (Baastrup-Spohr et al. 2013).
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</tr>
</tbody>
</table>

N/A is indicating that for the given species, the trait value has not been evaluated in the literature
References

Langangen, A. 2007. Charophytes of the Nordic countries. - Saeculum ANS.
Supplementary material: Community assembly Öland

**Water level modeling**

In two of the focal ponds the water level fluctuations were modeled using the known water level trace of pond 1 (one measurement per 30 min from 8th of April to 23rd of May) and the manual recordings of water at four dates during the investigated period in the two ponds of unknown trace. Water level was thus modeled for three periods (one between each of the 4 manual recordings).

The water level in pond 1 was normalized to the range of values measured during the manual depth recordings using the following equation:

\[
NW = \frac{(a - \text{Depth}_{end\ cont})}{(\text{Depth}_{start\ cont} - \text{Depth}_{end\ cont})}
\]

Where NW is the normalized water level, a is the observed depth value while Depth\(_{start\ cont}\) and Depth\(_{end\ cont}\) is in the start and the end of the period respectively.

The modeled water level was calculated as:

\[
MW = \text{Depth}_{end\ manual} \times (NW \times (\text{Depth}_{start\ manual} - \text{Depth}_{end\ manual}))
\]

Where MW is the modelled water level and Depth\(_{start\ manual}\) and Depth\(_{end\ manual}\) is the water manually measures at the beginning and end of the each period.

For all the focal ponds, there were no continuous measurements occurring within the last 5 days of the investigation period. This period was sunny and without rain and the water level in this period was thus calculated at the linear interpolation between measurements the 23rd of May and the 28th of May.

Every vegetation plot (local community) could be directly influenced by the water level of one of the focal ponds due to the very even morphology of the landscape. By leveling each plot according to a focal pond we could construct the water level history of each local community. We condensed the continuous water level of each local community to at mean daily water value(cm), where positive values indicated depth under the water surface and negative values indicated height above the water line. From this data set we constructed several indices that could be involved in determining plant abundances (table 1).
Table S1. The abiotic parameters of the local communities were condensed using a principal components analysis. A) the amount of variation explained by the first two principal component axis. B) The eigenvectors of the individual parameters included in the analysis, showing that most variables except bare soil had substantial loadings on the PC1.

A

<table>
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<tr>
<th>PC</th>
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<th>% variation accounted for</th>
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B

<table>
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<th>Variable</th>
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<td>Nostoc</td>
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Table S2. Plant traits measured on specimens sampled over as much as possible of the environmental gradients in the study area. For all species, the mean value, standard deviation and the number of replicates (n) are given. Plant height was obtained from the LEDA database and values are not shown here.
<table>
<thead>
<tr>
<th>Plant species</th>
<th>SLA (cm² g⁻¹)</th>
<th>WLOD (%)</th>
<th>LA (cm²)</th>
<th>LDMC (mg g⁻¹)</th>
<th>Root porosity (%)</th>
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</thead>
<tbody>
<tr>
<td>Agrostis gigantea</td>
<td>152.9 ± 53.3 (34)</td>
<td>35.13 ± 3.34 (3)</td>
<td>0.73 ± 0.24 (34)</td>
<td>327 ± 81.6 (34)</td>
<td>7.6 ± 2.2 (3)</td>
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<tr>
<td>Alisma lanceolatum</td>
<td>278 ± * (7)</td>
<td>25.57 ± 5.46 (3)</td>
<td>21.84 ± 3.26 (7)</td>
<td>147.4 ± 40.9 (3)</td>
<td>24.96 ± 4.21 (3)</td>
</tr>
<tr>
<td>Alisma plantago-aquatile</td>
<td>275.9 ± 33.5 (10)</td>
<td>20.98 ± 3.27 (3)</td>
<td>7.89 ± 2.69 (10)</td>
<td>194.2 ± 37.2 (10)</td>
<td>26.29 ± 2.2 (3)</td>
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<tr>
<td>Alopecurus geniculatus</td>
<td>253.3 ± 133 (60)</td>
<td>24.26 ± 13.65 (3)</td>
<td>0.75 ± 0.48 (60)</td>
<td>242.1 ± 64.7 (60)</td>
<td>34.33 ± 7 (3)</td>
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<tr>
<td>Bromus hordaceus</td>
<td>302.5 ± 41.9 (10)</td>
<td>31.61 ± 4.9 (3)</td>
<td>0.47 ± 0.16 (10)</td>
<td>216.1 ± 22.8 (10)</td>
<td>9.7 ± *** (1)</td>
</tr>
<tr>
<td>Carex nigra</td>
<td>211.2 ± 31.9 (7)</td>
<td>47.59 ± 6.39 (3)</td>
<td>1.83 ± 0.81 (10)</td>
<td>351.4 ± 60.4 (40)</td>
<td>26.3 ± 1.82 (3)</td>
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<td>Carex panicea</td>
<td>170.2 ± 29.6 (29)</td>
<td>28.66 ± 4.95 (3)</td>
<td>1.57 ± 0.75 (29)</td>
<td>351.4 ± 60.4 (40)</td>
<td>26.3 ± 1.82 (3)</td>
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<td>Carex viridula</td>
<td>198.5 ± 55.7 (10)</td>
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<td>331.1 ± 62.1 (10)</td>
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<td>Eleocharis palustris</td>
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<td>161.7 ± 31.9 (50)</td>
<td>25.6 ± 7.46 (3)</td>
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<td>182.7 ± 71.8 (30)</td>
<td>36.18 ± 15.54 (3)</td>
<td>0.43 ± 0.17 (30)</td>
<td>297.8 ± 44.6 (30)</td>
<td>20.8 ± 3.58 (3)</td>
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<td>Equisetum arvense</td>
<td>155.3 ± 20.3 (20)</td>
<td>40.91 ± 3.71 (3)</td>
<td>0.19 ± 0.04 (20)</td>
<td>182.2 ± 15.1 (20)</td>
<td>16.48 ± *** (1)</td>
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<td>Festuca ovina</td>
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<td>0.46 ± 0.8 (10)</td>
<td>409.6 ± 51.8 (10)</td>
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<td>Galium palustre</td>
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<td>186 ± 19.8 (17)</td>
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<td>Juncus articulatus</td>
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<td>182.3 ± 33.6 (20)</td>
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<tr>
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<td>76.94 ± 3.71 (3)</td>
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<td>Prunelle vulgaris</td>
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<td>2.85 ± 4.98 (3)</td>
<td>0.99 ± 0.02 (10)</td>
<td>97.7 ± 27.5 (10)</td>
<td>4.89 ± 5.47 (3)</td>
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<tr>
<td>Sedum acre</td>
<td>112.3 ± 54.9 (50)</td>
<td>6.28 ± 3.08 (3)</td>
<td>0.15 ± 0.08 (50)</td>
<td>94.8 ± 101.5 (50)</td>
<td>-0.93 ± 9.15 (3)</td>
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<tr>
<td>Sedum album</td>
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<td>40.29 ± 4.94 (3)</td>
<td>0.09 ± 0.02 (10)</td>
<td>324 ± 22.1 (10)</td>
<td>5.02 ± 6.37 (3)</td>
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<td>0.03 ± 0.01 (29)</td>
<td>170 ± 107 (5)</td>
<td>27.62 ± 1.35(2)</td>
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</tbody>
</table>

*Value based on the stated n-value but because of small leaf size troublesome morphology they were bulk-weighted not enabling calculation of SD.

**Estimated on the basis of area length relationship based 4 leaf parts and plant height of 16 plants.

***Only enough root material for one replicate could be sampled because of low biomass of roots. Roots were sampled from more than three individuals.
Table S3. Trait clustering index from each trait in each of the 21 investigated local communities. The significance is based on the percentage of null models yielding a higher range in traits than observed. Observed trait ranges smaller than 95% of the null models produced are regarded as significant.

<table>
<thead>
<tr>
<th>Plot</th>
<th>SLA</th>
<th>LDMC</th>
<th>Root porosity</th>
<th>WLOD</th>
<th>Height</th>
<th>LA</th>
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<td>0.88</td>
<td>0.82</td>
<td>0.12</td>
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<td>1.31</td>
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<td>0.69</td>
<td>1.15</td>
<td>0.52</td>
</tr>
<tr>
<td>3</td>
<td>1.06</td>
<td>0.70</td>
<td>1.83</td>
<td>1.76 *</td>
<td>2.30 **</td>
<td>0.03</td>
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<tr>
<td>5</td>
<td>1.70</td>
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<td>0.17</td>
<td>1.13</td>
<td>1.76 *</td>
<td>2.95 **</td>
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<td>-0.59</td>
<td>-1.96</td>
<td>-0.84</td>
</tr>
<tr>
<td>8</td>
<td>1.95 **</td>
<td>0.31</td>
<td>0.50</td>
<td>2.25 **</td>
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* ≥ 95% of null models with a larger range than the observed.
** 100% of the null models with a larger range than the observed.
Fig. S5. λ-values of the analyzed traits along the environmental gradient. The λ-values yielded from the maxent models can be interpreted as indicators of trait importance in community assembly. p-values indicate the significance of the correlation between PC1 and the λ-value of a given trait.