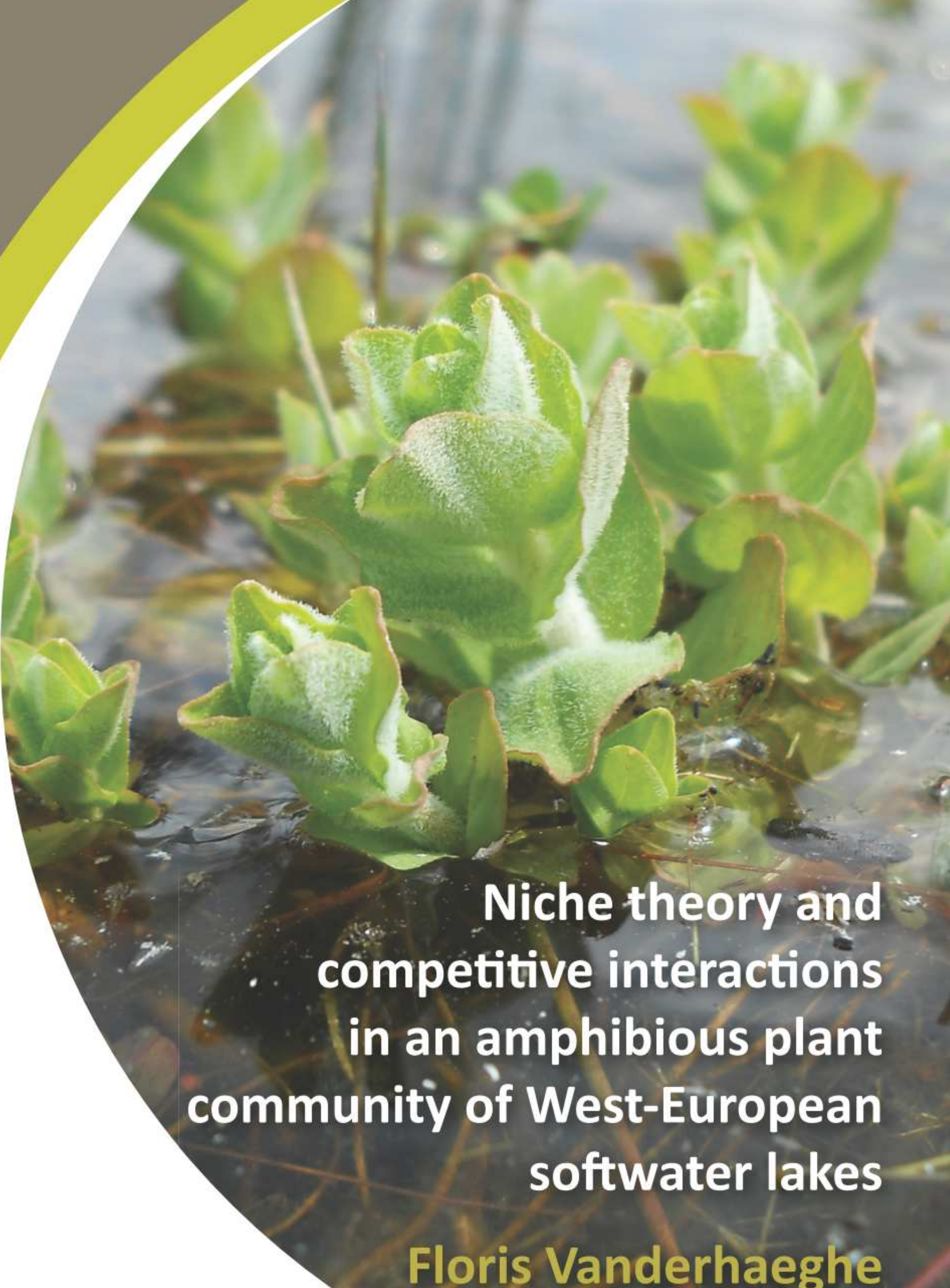

Niche theory and competitive interactions in an amphibious plant community of West-European softwater lakes

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NICHE THEORY AND COMPETITIVE INTERACTIONS IN AN AMPHIBIOUS PLANT COMMUNITY OF WEST-EUROPEAN SOFTWATER LAKES

NICHETHEORIE EN COMPETITIEVE INTERACTIES IN EEN AMFIBISCHE PLANTENGEMEENSCHAP VAN WEST-EUROPESE ZACHTE WATEREN

Floris Vanderhaeghe

Thesis submitted in fulfilment of the requirements for the degree of Doctor (PhD) in Sciences

Proefschrift voorgedragen tot het bekomen van de graad van Doctor in de Wetenschappen

Ghent, 23rd of November 2012

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The author received a 4-year Ph.D. fellowship of the Research Foundation – Flanders (FWO) from 2000 to 2004. Finalization of this thesis was partly accomplished in the course of appointments at Ghent University (2010) and the Research Institute for Nature and Forest (since 2010).

To Willem, Rik and Hanne

The blending of science and natural history has made ecology unnecessarily complex. The beauty of nature, the richness and diversity of species, the comprehensive lists of possible natural arrangements, scarcely leave us time to notice the absence of prediction. Until we have general ecological theories we must try harder to learn and to teach the real state of ecology.
Perhaps then, less burdened and confused by precept,
we will produce a truly scientific ecology.

Peters (1980)

PREFACE

In the absence of generality, ecological research becomes natural history (Keddy 1989, citing Peters 1980). This statement calls for finding, testing and applying general rules in ecology, as in other natural sciences, although it is debated to what extent this is possible and necessary for the field of ecology (Berryman 2003, O'Hara 2005, Lange 2005, Driscoll & Lindenmayer 2012). A theory must be consistent with observations from real systems; therefore several well-known theories originate from empirical research. Both careful observation of natural systems and the quest for generally applicable rules should be regarded as honourable activities of conservation biologists. Ultimately, theory can be used to make predictions of ecological change and support management decisions. While many examples exist of successful *local* application of theory up to the species or population level, much existing ecological theory has very limited *general* and predictive capacity, because of the idiosyncrasies of many particular ecosystems (Driscoll & Lindenmayer 2012). I agree with Driscoll & Lindenmayer (2012) that theory should be developed contingent on circumstances, though as general as possible, and that central coordination and sharing of these theories and systematic reviews is needed by an international institution. These authors also suggest a list of questions that should be asked before embarking on research in management and conservation (table 2 in Driscoll & Lindenmayer 2012). These questions force the researcher to think about the relevance of different phenomena and drivers for their system, in order to formulate focused and relevant research questions. Or, as Keddy (2001) stated (p. 199), “because ecologists usually genuinely enjoy field work, it is all too tempting to rush into data collection without first taking the time to think”.

Having some field background in oligotrophic wetlands and associated macrofauna, I was very fortunate to be allowed to study the amphibious plant communities of softwater lakes and their physicochemical environment, during my master thesis in biology. It was done in the area ‘Turnhouts Vennengebied’ in the north of Belgium, with excellent guidance by Dirk Boeye and Geert De Blust (Vanderhaeghe 2000; see also De Blust 1977 and Boeye *et al.* 2000). Several of these plant species are rare and endangered, mainly because of the deterioration of their specific habitat. As they were once much more common in the vast nutrient-poor regions of Western Europe, they have been protected by European legislation (European Habitat Directive). A further will to do research in these plant communities was a starting ground for my PhD.

Having interests in both natural history and mathematical-physical sciences, it was natural for me to be attracted by ecological theory and its potential predictive applications in nature conservation. Consequently, the emphasis in this thesis is not on phytosociological releve data and syntaxonomic analyses, nor on descriptions of the specific lakes that I investigated. Rather, I opted to verify conceptual frameworks from plant population and community ecology in the considered softwater plant community (ecological theory), while on the other hand I document environmental requirements of some specific amphibious plant species (natural history). Research in ecological theory and research in natural history are two different things, and each has its own virtues. Regrettably, all too often many biologists promote only one of these, leading to human and intellectual boundaries between theory and application. Ecological theory and natural history are two sides of the same coin. Natural historians should hope for better theories that explain and predict their observed patterns; meanwhile they could try to perceive nature in terms of life strategies, population, community and ecosystem functioning. Theoreticians on the other hand, should walk more in nature and observe species more often. Myers *et al.* (2000) started their paper with the observation that "conservationists are far from able to assist all species under threat, if only for lack of funding." Therefore, as (European or global) conservation ecologists, we must hope that ecological theory will evolve in such a way that we are not forced to collect data "for each case study because best-guess management decisions based on theory do not exist" (Driscoll & Lindenmayer 2012).

I did my PhD research within distinct time frames since October 2000, and it has involved many people. Therefore I may forget to mention some people here unintentionally; to them I offer my thanks as well. I am very grateful to the many scientists with whom I had enlightening discussions during my research, and I guess this is one of the major stimuli for every PhD student. In the first place, I wish to thank my first supervisor, Maurice Hoffmann. He made it possible to start my PhD at Ghent University (UGent). Although his expertise is on terrestrial plant communities and ecosystems, he has always encouraged my semi-aquatic research topic and he was of great help through revisions of the manuscripts. I am grateful to have a supervisor who is both a fundamental plant ecologist and a pragmatic conservation ecologist. I also thank Geert De Blust and Dirk Boeye for their role as co-founder of the PhD topic. Initially, the PhD research was funded by the Research Foundation – Flanders (FWO), and it received additional financial support from both UGent and Radboud University Nijmegen. Thanks to a temporary appointment of three months as Maurice's assistant in 2010, I could work closer to the finalization of my PhD research, at the Terrestrial Ecology Unit (TEREC) of UGent. I want to thank the many colleagues at UGent, both from former and recent times, for their kindness and unique atmosphere. I especially mention Eric Cosyns, Nele Somers, Indra Lamoot, Beatrijs Bossuyt, Dries Bonte, Luc Lens, Eduardo de la Peña, Bram D'hondt and Frederik Hendrickx.

Sofie Ruysschaert collected an important part of the field data that are analyzed in this PhD, doing her master thesis in biology at UGent during 2001-2002. She was very devoted to the topic and I gratefully acknowledge her for her enthusiasm and endurance.

The major part of the adopted methodology to collect field data and to perform experiments, originated from an intense cooperation with several staff members of the Department of Aquatic Ecology & Environmental Biology of the Radboud University Nijmegen (The Netherlands). This group has done an enormous amount of scientific research on the softwater lake environment and its restoration during the past decades, under the coordination of Jan Roelofs.

During my research activities in Nijmegen, the department was further led by Jan van Groenendael. I much appreciate the hospitality of both research coordinators to have allowed me to do experiments and learn techniques at the Radboud University. Most of all, I want to thank Fons Smolders. He is the researcher at Nijmegen who has helped most with conceptualizing, techniques, lab analyses and revisions of my manuscripts. He is one of the most enthusiastic researchers I have ever met. Eventually he became my co-supervisor, for which I am very grateful. It was basically through the belief of my two supervisors in this PhD that, two years ago, the opportunity had arisen to go on with it. The team spirit of the group of Jan Roelofs was amazing. I especially mention the following staff members of the department, and of the spin-off research centre B-ware, who have helped in some important way or just created the right atmosphere: Leon van den Berg, Esther Lucassen, Martin Versteeg, Germa Verheggen, Roy Peters, Leon Lamers, Emiel Brouwer, Roland Bobbink, Maaike Weijters, Philippine Vergeer, Jelle Eygensteyn, Hilde Tomassen, Dries Boxman, Ger Boedeltje, Roos Loeb, Jeroen Geurts and Marlies van der Welle.

Also, I wish to thank my former employer Haskoning Belgium, to have allowed me to take a temporary appointment at UGent in 2010 to work on my PhD, and for their respect for my departure to my current employer INBO. I especially thank Bart Vercoutere and Kurt Gutschoven for supporting these changes, and many other ex-colleagues from that period for their kindness and great team spirit, among which Guy Geudens, Filip Waumans, Annemie Pals, Sander Belmans, David Van Eykeren, Karolien Herbos, Jan Stuckens, Tom Carron, Ward Verhaeghe and Wouter Vandekerkhove.

During my research, I was fortunate to receive practical support from the Research Institute for Nature and Forest (INBO), my current employer. I am very grateful to the former and current management for their support, and to all current and former colleagues who were interested in the topic or helped through practical advice. I also thank INBO for the publication of this thesis as a book. I mention Eckhart Kuijken, Maarten Hens, Wouter Van Landuyt, Roald Steeno, Jurgen Bernaerts, Alexander Van Braeckel, Sam Provoost, Thierry Onkelinx, Bruno De Vos, Luc Denys, Rein Brys, Arne Verstraeten, Stijn Vanacker, Piet De Becker, Gert Van Spaendonk, Carine Wils, Nico De Regge and Nicole De Groof (who designed front and back cover), but this list is undoubtedly too short. Since 2010 I have the pleasure to work in the research group of Environment and Climate, led by Maarten Hens. He stimulated me to process a final dataset and to write down the last manuscript on niche models. I wish to express my gratitude to all colleagues of the research group, for their enthusiasm, open mind, kindness and support – the closest colleagues being Maud Raman, Andy Van Kerckvoorde and Sophie Vermeersch.

The West-European dimension of this thesis would not have been accomplished without many persons in Europe who are familiar with the plant community of interest. They have helped a lot by listing softwater lakes in very diverse locations across Western Europe. I gratefully acknowledge the following people (with most affiliations listed according to the period of their involvement): Frédéric Bioret (University of Western Brittany, France), François Pinet (Brenne National Park, France), Klaus Kaplan (Biological Institute of Metelen, Germany), Raimund Kesel and Katharina Urban (University of Bremen, Germany), Carlos

Aguiar (Polytechnic Institute of Bragança, Portugal), Jan Jansen (Institute for Nature Conservation and Biodiversity, Portugal & Radboud University Nijmegen, The Netherlands), Javier Amigo (University of Santiago de Compostela, Spain), José Antonio Moliná (Complutense University of Madrid, Spain), Angela Darwell (Hertfordshire University, United Kingdom), Iwan Edgar & family (Wales), Andy Jones (Countryside Council for Wales, United Kingdom), Kevin Murphy (University of Glasgow, United Kingdom), Gertie Arts (Alterra, Wageningen University and Research Centre, The Netherlands), Jos Berends, René Gerats and Margriet Clabbers (Stichting het Limburgs Landschap, The Netherlands), Wilton de Dooij (Natuurmonumenten, The Netherlands), Jan Meesters (Staatsbosbeheer, The Netherlands), Peter van Beers (Waterschap Veluwe, The Netherlands), Herman van Dam (Consultant Water and Nature, The Netherlands), Ciska van der Genugten (Geldersch Landschap, The Netherlands), Loekie van Tweel-Groot (Landschap Overijssel, The Netherlands), Peter Voorn (Waterschap De Dommel, The Netherlands), Alex Ballet, Mario De Block, Eddy Feyen, Wilfried Godderis, Lily Gora, Jos Gorissen, Karel Molenberghs, Kris Rombouts, Stephan Swinnen, Koen Van Bavel, Frans Van Nevel, Tom Verschraegen and Jan Wellekens (Nature and Forest Agency, Belgium), Jan Dirkx, Marc Smets, Danny Vanderveken and Christine Verscheure (Natuurpunt, Belgium), Erna Vandoren (Orchis, Belgium) and Frans Verstraeten (Stichting Limburgs Landschap, Belgium).

I might never have come to a deep interest in nature, or to studying biology, without the much appreciated influence of many nature lovers during former times. I thank Tim Adriaens, Kobe Boussauw, Piet Desmet, Bram Janssens, Bob Vandendriessche, Bart Vanelslander, Ward Vercruysse, Filip Waumans and all others for those good times. Also, I had the privilege to study plant biology at UGent with some 20 very passionate students. I wish to thank them all for those splendid years of our lives.

It is through the endless and unconditional support and belief of both my parents that I have been able to pursue my interests, study biology and eventually do a PhD. Where there's a will, there's a way. Thank you very much, mom and dad.

As a husband and father, I live for my family in the first place. Liesje, you have shown so much the patience and understanding that I needed to be able to finish this. I dedicate this book to my children: Hanne, Rik and Willem. I thank them for being so stimulating at all times, and I wish them good luck in their life and all the good things they may wish for.

Floris Vanderhaeghe

Lebbeke, Belgium
June 2012

Perhaps ecological societies will one day host conferences where no data are permitted. At such conferences researchers will justify their question, their model system and their research strategy.

Keddy (2001)

Hypericum elodes, Hasselt, Belgium

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General Introduction

Some ecologists might criticize our emphasis […] as overly phenomenological,
however no mechanism for the law of gravity is yet known,
yet its predictive ability enables us to send interplanetary probes
to predetermined locations over a billion kilometers away.

McGill et al. (2006)

Lake with Baldellia ranunculoides ssp. repens, Hasselt, Belgium

CHAPTER 1

GENERAL INTRODUCTION

Once a plant propagule has reached and become established at a site, its PERSISTENCE [1] is controlled by interaction with the physical and chemical environment (abiotic interactions), and by interactions with other organisms present, like plants, animals and fungi (biotic interactions). These basic processes at the plant level translate to the relative abundances of plant species, ultimately giving rise to the COMMUNITY of plants. These linkages have received my interest in early days as a biology student, and therefore shaped the framework and drove the questions of my research.

In this thesis, I present the results of my research into an endangered PLANT COMMUNITY, the *Eleocharition multicaulis* Vanden Berghen 1969, which occurs in and along shallow softwater lakes. It mostly consists of amphibious perennial macrophytes. My research was both descriptive and experimental:

- in the **descriptive** part (chapters 2 and 3)**,** I essentially measured and modelled the natural environmental range within which plant species of the VEGETATION TYPE survive. I started with a detailed environmental survey, in order to test for both expected and previously unknown relationships between one species' abundance and its environment (chapter 2). Then, I selected a narrower subset of variables in order to construct predictive statistical models of the NICHE of several species, based on their biogeographical range (chapter 3).
- in the **experimental** part (chapter 4), starting from both previous results and expectations from literature, I investigated the relative importance of several abiotic and biotic factors in a simulated *Eleocharition* community, by applying a controlled laboratory experiment with four species of this plant community.

As explained above, I was much interested to interpret the findings within a broader plant ecological framework. Therefore, I compared the resulting patterns with the expectations, predicted by theoretical concepts. While my approach does not allow definite tests of these concepts, I discuss the applicability of several paradigms to the *Eleocharition* in the light of my results. Both the actual **observations and analyses** that I carried out, and the discussion in the context of broader plant ecological **frameworks**, are central themes in my thesis.

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[1] Several terms in this thesis are defined in the Glossary. Their first occurrence is formatted as SMALL CAPITALS.

Consequently, I start this introduction by presenting a theoretical plant ecological framework in paragraph 1. Aspects of aquatic macrophyte ecology that are relevant to my research, are explained in paragraph 2. In paragraph 3, I expand on the specific plant community studied. Finally, paragraph 4 lists the specific objectives of my research, and how these are linked to respective chapters.

1. Ecological concepts of plant individuals, populations and communities

The above-mentioned connection between plants and plant communities centres on the **population(s)** of one or several species, and on how they respond to the abiotic environment and biotic neighbourhood. This focus on PLANT POPULATION ecology will be found throughout the remainder of this work, with a particular emphasis on the abiotic environment [2]. However, in order to understand population PERFORMANCE and to underpin questions that pertain to populations, it is important to consider the hierarchical levels below and above the population level, that is, to appreciate both ecology of plant individuals and plant community ecology. Therefore, a concise framework is presented below, that explicitly considers three hierarchical levels: individual, population and community. It should be kept in mind that this framework does not comprise all current knowledge of these system levels. Rather, it highlights those theoretical insights that are necessary to understand the objectives and approaches of my research, i.e. understanding the ecology and ecological constraints of the typical constituent plant species of the *Eleocharition multicaulis*.

Before dealing with plant ecological theory, I first provide a framework to classify the varying abiotic factors that affect plants. It should make the reader at least realize the complexity of the subject ‘environment’ when dealing with plants.

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[2] In this thesis, the use of the simple term ‘environment’ refers to the *abiotic* environment. Biological interactions are mentioned explicitly, e.g. as ‘biotic neighbourhood’.

Box: Some contributions by Mike P. Austin to niche theory and community ecology

From the viewpoint of my research aims and applied methods, it would be ignorant not to highlight the contributions of one specific author, Mike P. Austin (CSIRO, Australia). Austin's scientific work on plant responses to their environment, since the 1980s up to now, has had an ever-increasing influence on the way scientists model and interpret the relationships between plant species and environmental factors. It has also had an equally increasing impact on my own research. His work is unique in several ways. Firstly, he clearly interprets modelled species patterns as a community ecologist (e.g., among his reviews, Austin 1985, Austin 2005). Secondly, not only has he been one of the first to rigorously model species' niches (e.g. Austin *et al.* 1984; see further for a definition of the niche concept), he also has increasingly advocated the use of a clear theoretical niche and community framework for interpretation (e.g. Austin 1999, Austin 2002, Austin 2007). Thirdly, he uses an explicit conceptual model to predict how competition shapes species' responses and organizes the community (see Austin (1990) for a framework devoted to competition). Finally, he has been the only one who has proposed both a framework to classify environmental factors, and expectations as to how plant species should respond to these, both physiologically and ecologically - first in Austin (1980), and more cited from Austin & Smith (1989), both of which were published in *Vegetatio*. These seemed enough reasons for me to start with his preferred classification of the environment.

The abiotic environment as perceived by a plant: three gradient types

Austin (see BOX) considers three environmental gradient types (classes of measurable environmental factors or variables) when modelling – or reflecting upon – the performance of a plant species:

- **Resource gradients**, or resources in short, are the environmental variables that represent an essential resource consumed by plants. These comprise light, water and nutrients, e.g. available concentrations of nitrogen ions, orthophosphate and carbon dioxide. Physiologically - i.e. without taking into account biotic influences and other limiting factors - plants respond to these according to the limiting response type: gradually increasing with the availability of the resource, reaching a plateau-shaped maximum, and steeply decreasing once a non-viable level is reached.
- **Direct environmental gradients**, which directly influence plant physiology. These are non-resource, 'proximal' factors that influence plant performance in a direct and causal manner. Examples include pH and temperature. Different species can be expected to have different optima along these gradients.

- **Indirect environmental gradients**, which have no direct influence on the plant's physiology ('distal' factors), but are potentially related to its performance through a related, more proximal (direct or resource) gradient. An example in softwater lakes is the thickness of an organic soil layer, which affects the availability of nutrients and oxygen to plant roots as well as the accumulation of toxic compounds. Another, more classical one in vegetation science, is the altitudinal gradient, having complex and location-specific relations with rainfall and temperature.

It can be added that a simpler classification of environmental gradients is often made (e.g. in Begon *et al.* 1996), between conditions (not consumable) and resources (consumable). The merit of distinguishing, within conditions, between proximal direct gradients and distal indirect gradients, lies in the different predictive capacity of these factors. Direct gradients can be expected to be more strongly correlated to plant performance, as they are more closely linked to physiology than indirect gradients (Mac Nally 2000, Austin 2002, Ginzburg & Jensen 2004).

Next, I introduce some fundamentals of plant ecology.

1.1 The plant individual

A plant individual can be characterized in many ways. We could *measure* morphological attributes like its height, leaf area, rooting depth, number of flowers and seed mass; life history characteristics such as longevity of the established phase and reproductive investment; as well as physiological traits like maximum growth rate. These are all continuous variables, and their value is determined both by genetic factors and by epigenetic (external) factors, like the abiotic environment and the biotic neighbourhood. In functional plant ecology, it has been shown that several of these 'TRAITS' or 'attributes' confer advantages or disadvantages to the plant during several of its life stages (*Fig. 1.1*), depending on the specific state of each attribute. Weiher *et al.* (1999) nicely categorized those '**FUNCTIONAL PLANT TRAITS**' according to the common challenges faced by plants. For example, seed mass and shape are traits capable of predicting a plant's success to disperse in space or time (Westoby *et al.* 2002), with prediction success depending on the type of dispersal (e.g. Cosyns & Hoffmann 2005, D'hondt & Hoffmann 2011). The challenges of both establishment and persistence are related to leaf traits (Wright *et al.* 2004, Westoby & Wright 2006, Curtis & Ackerly 2008, Ordonez *et al.* 2009). Persistence, and especially its component of COMPETITIVE ABILITY (the degree by which a plant can resist to the COMPETITIVE EFFECTS of its neighbours or suppress them), can be inferred from the plant traits biomass and height (Gaudet & Keddy 1988, Goldberg 1996, Violle *et al.* 2009). In this thesis, focus is primarily on the life cycle phase in which plant **persistence** has the dominant role; therefore, I treat this part of the life cycle in more detail below.

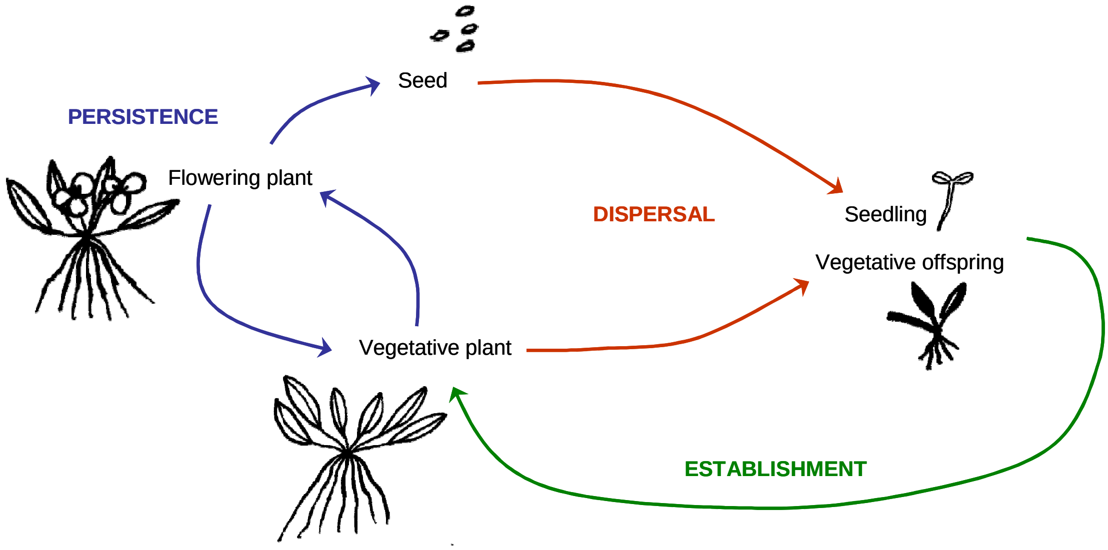

Fig. 1.1. The plant's life cycle, and the challenges it faces (adapted from Grime 2001).

Obviously, the environmental state itself will largely determine to what degree a plant with certain attributes can survive there. Attributes are known to vary within species and individuals, often referred to as 'trait plasticity', especially in relation to the environment (de Kroon *et al.* 2005, Ackerly & Cornwell 2007, Verheyen *et al.* 2009, De Frenne *et al.* 2009). However, attributes often vary more *between* species. This means that species can be ranked, or classified, according to one or several of their functional traits. It follows that these functional groups of species relate to certain types of abiotic environment or biotic neighbourhood where they potentially occur. Functional traits relate to the way these plants cope with these environments and neighbourhoods, commonly referred to as '**plant strategies**'. In order to achieve a functional classification of species and to enhance our understanding of population, community and ecosystem functioning, several initiatives have been undertaken to quantify species traits (Grime 1988, Thompson *et al.* 1997, Poschlod *et al.* 2003, Kleyer *et al.* 2008).

The virtue of functional plant ecology is that it opens a path to find general rules. Research results on an investigated taxon or taxa can potentially be extrapolated to all taxa that are similar in traits. A main challenge in functional plant ecology is therefore to **predict plant performance** from a standardized set of traits (Westoby 1998, Weiher *et al.* 1999, Westoby *et al.* 2002, Lavorel & Garnier 2002, McGill *et al.* 2006, Westoby & Wright 2006, Webb *et al.* 2010).

Many authors explored the relation between **environment and species traits** such as competitive ability (e.g. Wilson & Keddy 1985, Kenkel *et al.* 1991, Dyer *et al.* 2001, De Frenne *et al.* 2011). Many species can be observed to persist over environmental ranges or with various sets of coexisting species. This implies that their competitive ability and other life history traits may differ among different environments in order to cope with the specific abiotic and biotic neighbourhood (Lavorel & Garnier 2002). On the other hand, traits can be thought of as being coined for optimal survival and reproduction in a species' primary habitat, thus being informative on how the species will perform in varying conditions (Grime 1979, Weiher *et al.* 1999). It is therefore compelling to try to predict how communities will be structured in various environments (Keddy *et al.* 2000, Webb *et al.* 2010), starting from essential information on the species' traits.

Several frameworks of plant strategies, called **plant ecology strategy schemes** (PESS), have been elaborated by plant ecologists. Probably most influential was the PESS of Grime (1979), which places species along two rather theoretical environmental axes, visualized in a triangular CSR-diagram. It recognizes three 'primary' strategies: competitive (C), stress-tolerant (S) and ruderal (R), and most species are found somewhere in between these extremes (Grime 1988). Still less well-known, the PESS of Westoby aimed at more consistency with new insights in functional plant ecology (Westoby 1998, Westoby *et al.* 2002, Westoby & Wright 2006). It especially aimed at a feasible way of placing species (or individuals) in the scheme, as it is directly defined by traits related to the leaf, plant height and seed (LHS-scheme). For example, plasticity between individuals as a consequence of different environmental conditions, can easily be allocated and interpreted within the LHS scheme (De Frenne *et al.* 2011).

As functional traits are not the primary subject of my research, I will only focus on trait differences *between* species by considering their different strategies. As I deal with aquatic macrophyte species, which cope with the - for plants - rather 'atypical' aquatic environment, I prefer to use the PESS of Kautsky (1988) for aquatic macrophytes, which is a rectangular extension of the triangle of Grime. As in Grime's scheme, stress and disturbance are the two environmental axes along which species and individuals can be arranged, according to their traits. **STRESS** comprises possible environmental factors that limit growth, through low availability of plant resources (light, water, nutrients) or through adverse conditions like high salinity, extreme pH-values etc. **DISTURBANCE** comprises environmental factors that cause partial or total destruction of biomass, such as wave erosion and water-level fluctuations. Disturbance can also be denoted as 'predictability of the environment', in an opposite sense. Kautsky (1988) links plants' primary production (growth rate) to the stress axis, and plant size (both vertical and lateral) to the disturbance axis. For low-stress environments, she retains the two strategies of Grime: ruderal (R) for high disturbance and competitive (C) for low disturbance (*Fig. 1.2*). Instead of the one stress-tolerant strategy of Grime, two strategies are defined that occur in stressful environments: the biomass storer strategy (B) for low disturbance and the stunted strategy (S) in highly disturbed sites. Note that, rather inconveniently, 'S' has not the same meaning in both PESS's. In my research, I apply Kautsky's plant strategies to the investigated species, in order to verify whether population performance and niche patterns of these species are linked with their plant strategy.

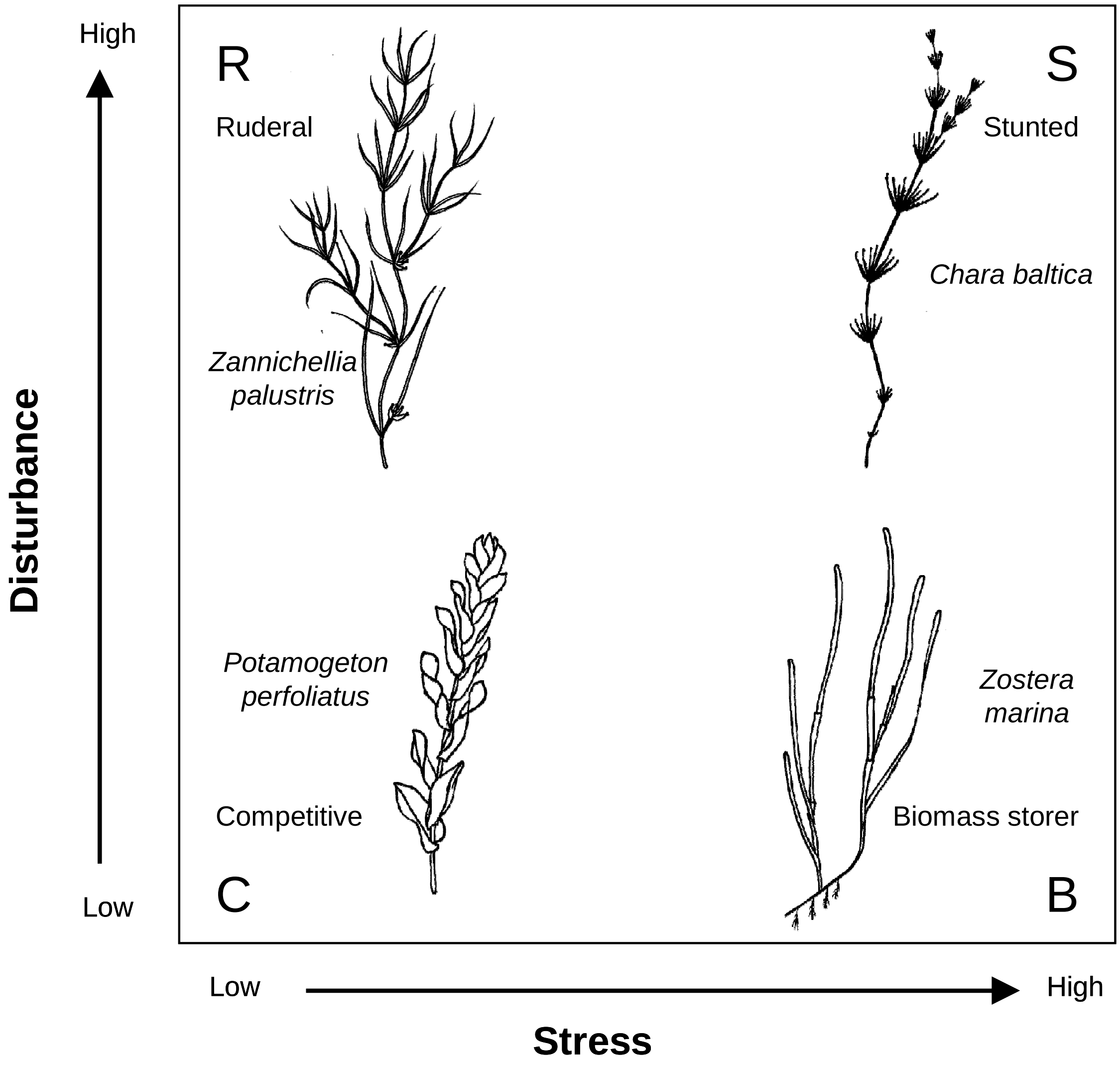

Fig. 1.2. Kautsky's strategy scheme for aquatic plants (adapted from Kautsky 1988).

1.2 The plant population

A strict separation of plant population ecology and plant community ecology is not possible. Most plant community research deals with how populations of different plant species give rise to a community, in a more or less interactive way with each other, with their abiotic environment and with other possible factors. Accordingly, much population ecological research is performed in the context of a community (sometimes simplified, e.g. two-species interactions). Nevertheless, species-specific aspects such as (meta)population dynamics, dispersal, population genetics and reproductive mechanisms are typically investigated at the level of populations of one species. Here, I will discuss under 'plant population ecology' those concepts that focus either on the performance of a population in the context of its abiotic environment, or on the specific mechanisms that operate between populations of different plant species. The former can be synthesized under the concept of the **NICHE**, while I will specifically focus on **COMPETITION** between different plant species for the latter. To typical 'community ecology theory' belong those concepts that try to incorporate all relevant mechanisms that structure the plant community, or that try to provide generally applicable rules for observed patterns (e.g. to predict plant diversity). I will mention biotic interactions other than plant competition, as well as some other factors, only under 'the plant community' heading, as these are not the focus of my work. Niche and competition are central themes among the possible theories of species **coexistence**. In this thesis, I interpret my findings in the light of some of these paradigms, and therefore, I will present an overview of those that are discussed most often in literature.

Silvertown & Charlesworth (2001) define the plant population as the collection of individuals of the same species that live in the same area (e.g. in a lake, or as represented by a sampled plot) [3]. Several *measures* can be taken from a population. One way is to aggregate measurements made at the individual plant level, which can seamlessly be interpreted at the population level. Further, population-level specific measures of performance can be collected such as density, abundance or (ground) cover. Functional plant ecology, basically pertaining to individuals, will clearly be of use to understand population performance and FITNESS of different species, as, in equilibrium situations, population performance is the result of different individuals of the same species coping with their abiotic environment, each other (intraspecific interactions) and other organisms (interspecific interactions). Equally, the biotic interactions that ecologists most often investigate at the population level (or at least for a group of individuals), such as competition, facilitation, herbivory and so on, basically operate at the level of individual plants.

Competition and the niche – local species coexistence at equilibrium

The definition of **competition** between plants that I use in this thesis is "the tendency of neighbouring plants to utilise the same quantum of light, ion of mineral nutrient, molecule of water, or volume of space" (Grime 2001). It can thus be viewed as the negative plant-plant interaction that occurs by consuming a resource (Keddy 1989). In plants, limited resources can be nutrients, light and water, and consequently competition can occur belowground or aboveground. Competition occurs both within a population (intraspecific competition) and between populations of different species (interspecific competition). Competitive ability of a species, which refers to the degree it can suppress 'subordinate' species and resist to the competitive effects of 'dominant' species, is closely linked to several functional traits like typical height and biomass of the species, as first demonstrated by Gaudet & Keddy (1988). For several decades, experimental research on interspecific plant competition has focused on pairwise interactions, i.e. between two species. Studying multispecies interactions in experiments has not often been addressed, although this approach has more potential to reveal results that are relevant to real communities (Gibson *et al.* 1999). Austin *et al.* (1985) – again – were one of the

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[3] Note that they do not explicitly include criteria on genetic interaction in their definition.

first to undertake such experiments. In my research, I also apply a multispecies interaction experiment.

To learn more about further details of the many theoretical competition models, possible mechanisms, research approaches and associated terminology, the reader is referred to the huge amount of literature on this topic (see for example Goldberg 1996, Aarssen & Keogh 2002). From here I will narrow the focus to the way **interspecific** competition influences the performance of populations **along environmental gradients**. In this research area the *competitive hierarchy model* is very promising. It was first made explicit by Keddy (1989). It is matched by many communities (see e.g. McGill *et al.* 2006). Before going into this model, it is necessary however to first define the concept of the niche.

Following the view of Hutchinson (1957), the **niche** can be described as the abstract environmental hypervolume defined by axes of conditions and resources within which a population of the species is able to maintain a long-term average net reproductive rate larger than 1. Hutchinson defined the niche apart from the geographical space to which populations are constrained, which he called *biotope space*. This separation is very powerful as it allows for the logical possibility for parts of a species' niche to be either unavailable (not existing) in the 'biotope', or left unutilized though present in biotope space (Colwell & Rangel 2009); phenomena that are common in nature (*Fig. 1.3*).

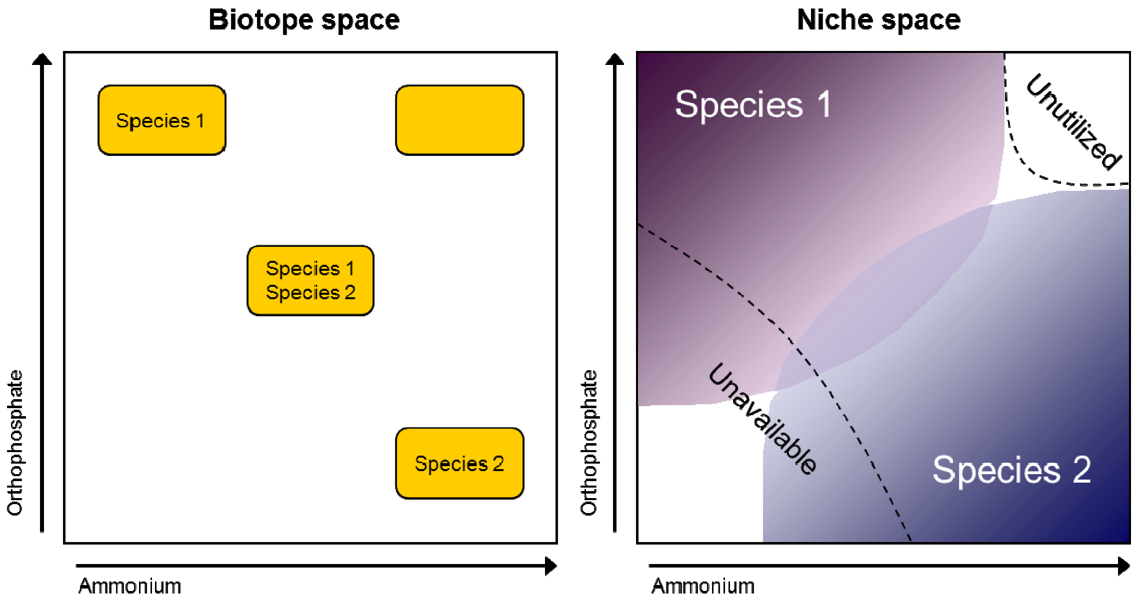


Fig. 1.3. The niche-biotope duality of Hutchinson, applied to softwater plant species along a two-dimensional gradient of ammonium and orthophosphate concentrations in the surface water. Inspired by fig. 1 from Colwell & Rangel (2009). The yellow boxes in biotope space represent lakes or spots within a lake. The niches of two hypothetical aquatic macrophyte species are depicted in niche space, with their performance optima in opposite corners (dark coloured). Areas in niche space that don't exist in the biotope, are called 'unavailable'; areas in niche space that exist in biotope space, but that fall outside a species' niche, are called 'unutilized'. The presence of both species in biotope space is indicated in the yellow boxes.

Another important component of Hutchinson's niche concept is the distinction between the **FUNDAMENTAL NICHE**, which represents the physiological potential of the species, and the **REALIZED NICHE**, which is the part of the fundamental niche that the species occupies in the context of a community, i.e. modified (restricted) by biotic interactions like competition and herbivory. It is important to be aware that the realized niche is still defined here within the abstract niche space and apart from biotope space. Regarding competitive interactions, the reason why species can be the 'winner' only in a *part* of their fundamental niche, is supposed to be linked to a trade-off (Silvertown 2004). For example, a high ability to quickly take advantage of new light and nutrient supplies is not compatible with a high ability to compete in a more resource-limited environment. Particularly, there is growing evidence that species are able to **coexist** in communities through **niche differentiation** (McKane *et al.* 2002, Levine & HilleRisLambers 2009), in the case of resource gradients often referred to as **resource partitioning** (MacArthur 1972, Tilman 1982). In a strict sense, the conceptual model of resource partitioning assumes that fundamental niches are already (largely) separated through evolution, but in a more relaxed sense it refers only to the separation of realized niches along resource gradients.

For coexistence to occur in a stable way, intraspecific competition should exceed interspecific competition (Silvertown 2004). This requires a **stabilizing mechanism**. Niche differentiation (resource partitioning) is the most invoked fluctuation-independent, stabilizing mechanism of coexistence (Chesson 2000). 'Fluctuation-independent' refers to the fact that the mechanism can operate in an unchanging environment, and is therefore related to an **equilibrium** situation. With decreasing niche differentiation, hence with increasing resource use overlap, interspecific competition increases and the best competitor is expected to exclude other species, i.e. in those environments where it achieves highest performance (Tilman 1982, Chesson 2000, Ryabov & Blasius 2011). Fluctuation-independent mechanisms can function in fluctuating environments as well. Another fluctuation-independent, stabilizing mechanism of coexistence is frequency-dependent predation or herbivory (Chesson 2000, Kneitel & Chase 2004). For example, several specialist herbivores can control the density of several plant species independently, thereby stabilizing the community. In the investigated plant community, I do not look at interactions with other trophic levels but specifically address the presence of niche differentiation between plant species. In the next section, I will discuss a fluctuation-dependent stabilizing mechanism.

Now that the niche concept has been touched, we are ready to explain the **competitive hierarchy model** that Keddy (1989) has formalized. It is a conceptual model that predicts a ranking pattern (hierarchy) of several plant species' responses (realized niches) along a resource gradient. Hence, it is compatible with the framework of resource partitioning. Particularly, the competitive hierarchy model provides a **mechanism** that generates the ranking pattern. The mechanism is the whole of its three assumptions (*Fig. 1.4*):

- the species' fundamental niches along resource gradients are inclusive, i.e. all species have best performance at the same preferred end of the gradient. This was also one of the propositions in Austin & Smith (1989), and it has been shown to match many plant communities;
- competitive ability is a species-specific trait, which varies primarily between species;
- because of trade-offs, species with a higher competitive ability have a smaller fundamental niche width than species with a lower competitive ability.

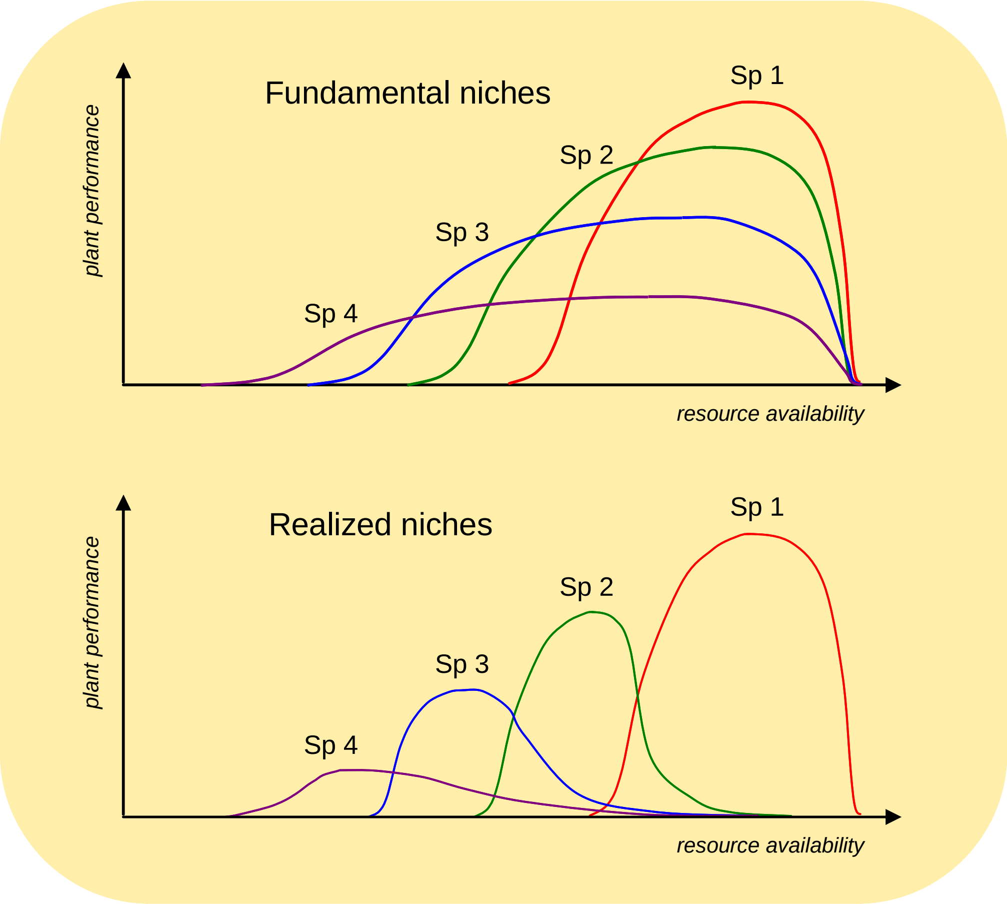


Fig. 1.4. A graphical presentation of Keddy's competitive hierarchy model (Keddy 1989) for four hypothetical species, and supplemented by response shapes, inspired by Austin & Smith (1989) and Austin (1990). It is assumed that the competitive ability of species is ranked as Sp 1 > Sp 2 > Sp 3 > Sp 4, leading to an observed competitive hierarchy of species along the resource gradient.

The model outcome is a hierarchical species ranking along the resource gradient, in which **competitive dominants** have outcompeted their **subordinates** from the 'preferred' part of their fundamental niche. Empirical examples of such a competitive ranking are abundant, e.g. Keddy *et al.* (1994), Rösch *et al.* (1997) and Kotowski *et al.* (2006). The ranking along the gradient is directly determined by the rank order of competitive ability of the species.

Coexistence and fluctuations: the temporal scale

Although often assumed otherwise, fluctuating environmental conditions do not necessarily lead to improved coexistence of species (Chesson & Huntly 1997). To do so, the fluctuations must lead to niche opportunities for species in the community. Accordingly, Shea *et al.* (2004) defined a 'disturbance' as an event that alters the niche opportunities available to species in a system. This definition includes many possible alterations of the environment, and not only those that destroy biomass. In this thesis however, I will reserve the term 'disturbance' to its classical use (an environmental factor that causes partial or total destruction of biomass), and use 'environmental fluctuation' otherwise.

While fluctuation-independent mechanisms of coexistence such as niche differentiation can also function in a fluctuating environment, another well-known stabilizing mechanism is truly fluctuation-dependent: the **temporal storage effect** (often called the 'storage effect'). Here, species are temporally differentiated, while they do not (necessarily) differ in resource usage. A critical feature of the storage effect is that species can have buffered population growth. This applies to a period where the impact of an adverse environment and competitive suppression is bridged during a more or less 'immune' life stage (Chesson 2000). In plants, this can be accomplished by a seed bank, or by a surviving population of long-lived perennials (Silvertown 2004). Different species in the community can then coexist in a fluctuating environment, each of them having recruitment and access to resources at different times.

Instead of being abiotic, temporal fluctuations may also be biotic, sometimes referred to as 'stochastic demography'. **Stochastic demography** as a mechanism of coexistence is one of the key aspects of **neutral theory** (Hubbell 2001, Alonso *et al.* 2006), and refers to random variation in birth, death and migration (and in the longer term, also speciation). In neutral theory, all species have competitive equivalence and they show random drift; therefore the theory as a whole will often not hold in real communities (Abrams 2001). However, in an attempt to reconcile aspects of classical and neutral niche theory, Tilman (2004) presented a '**stochastic niche theory**'. It integrates both resource competition theory (including trait-environment relationships and trade-offs) and demographic stochasticity (with an emphasis on the stochastic entry of propagules). In Tilman's theory, a species can coexist with other species as a resident, when it obtains access to enough resources (through competition), or as an invader - a not yet established species - through the stochastic entry of propagules. Some of the propagules subsequently grow to maturity on resources left *unconsumed* by the established species. This puts a limit to niche similarity (resource use overlap) between invaders and established species, so that highly diverse communities are predicted to have a low invasibility. However, **resource pulses and disturbances** allow new invaders in the community, leading to the coexistence of many more species. The paradigm is also called ‘stochastic competitive assembly’, and clearly requires both a spatial component (see further) and a temporal component.

In a succession of shrub assemblages, Stokes & Archer (2010) found support of these phenomena. First, stochastic dispersal processes governed the community outcome, while later positive and negative interactions progressively structured the community. Hence, they found a shift from **neutral processes** towards more deterministic, **niche-based** processes. The continuum in nature, between neutral and niche-based processes, was also formulated in a model by Gravel *et al.* (2006). Implicit in stochastic niche theory is that the first few species that establish in an empty location may largely determine the community outcome later on, a mechanism sometimes referred to as the ‘priority effect’ (Shulman *et al.* 1983, Wilbur & Alford 1985, Shorrocks & Bingley 1994, De Meester *et al.* 2002, Louette & De Meester 2007). As the order of species entry is important, also time-lags in their reaction to abiotic factors and biological interactions will influence succession in the community (Farrer *et al.* 2010). Under stochastic competitive assembly, the realized niche of a species may change depending on the species that are present. It may potentially equal the fundamental niche when disturbances are such that the species does not experience competitive effects, because disturbances can generate vacant sites which become available to new plants (Chesson 2000).

According to classical resource competition theory (MacArthur 1972, Tilman 1982), simple resource partitioning without other stabilizing mechanisms, such as spatial or temporal heterogeneity or frequency-dependent predation, leads to a deterministic equilibrium where at most k species can coexist on k limiting resources. In well-mixed aquatic systems, these conditions may be met; however limnologists find many more plankton species than the number of limiting resources. This has been referred to as ‘**the plankton paradox**’ (Hutchinson 1961). Huisman & Weissing (1999) proposed a theoretical solution to this paradox, starting from resource competition models and without invoking external, stabilizing mechanisms. More species will be able to coexist than the number of limiting resources, when at least three resources are limiting and each species is an intermediate (not the best) competitor for the resources that most limit its growth rate. The model explicitly considers the time scale over which changes take place in resource levels and species abundances. When one species reaches its upper abundance limit due to limitation by its most limiting resource, another species (a better competitor for that resource) takes over, increases its own abundance and reduces that of the previous species, then a third species takes over, and so on. This generates oscillations of species abundances and resource levels. In their simulations, the authors showed that these oscillations allow more species to be sustained than the number of limiting resources. Depending on the species-specific properties, regular or irregular oscillations result, with shorter or longer periodicities (typically short and unnoticeable in plankton systems). With an increasing number of limiting resources and of species, oscillations become chaotic and don’t repeat themselves; this is referred to as **competitive chaos**. With this model, these authors demonstrated that competition can be a highly dynamical process by its own, which may be an important property in the structuring of communities. These ideas underline that ‘temporal fluctuations’ are also an inherent property of species interactions alone.

Coexistence and the niche in a spatial context

The study of patterns requires analyses at multiple spatial scales in order to capture the relevant mechanisms and to achieve generality (Levin 1992, Kunin 1998, Crawley & Harral 2001, Hartley & Kunin 2003, Rahbek 2005, Kohyani *et al.* 2008). For example, niche characteristics may depend on the spatial scale at which they are measured. This spatial scale dependence differs according to species, scale of variation of the considered environmental factor and the measured plant traits (Sanz *et al.* 2009, De Frenne *et al.* 2009, De Frenne *et al.* 2011). Especially at large spatial scales, trait values can change within the same species (De Frenne *et al.* 2009, De Frenne *et al.* 2011).

The presence of plant populations of one species in distinct areas with different environmental conditions, potentially leads to different evolutionary adaptation between the populations, which are then referred to as ecotypes (Hereford 2009). This implies **local adaptation** of both the fundamental and the realized niche. Adaptation may be for abiotic factors, but also for biotic factors such as competition, pathogens, parasitoids and mutualists (Becker *et al.* 2006). Also, local adaptation may vary among functional traits (e.g. leading to different plant strategies in two ecotypes of *Plantago lanceolata* L.; van Groenendael 1985). While local adaptation does occur, it seems to be not that common (Leimu & Fischer 2008). From a literature survey, Hereford (2009) concluded that three **conditions** should be fulfilled: (1) environmental differentiation must be present, (2) gene flow between populations is limited and (3) genetic drift within populations is limited. Condition 3 will be more easily satisfied in large populations (Leimu & Fischer 2008), while condition 2 depends on dispersal and establishment traits of the species and on spatial distance. Due to these reasons, local adaptation in plants is not so common (Leimu & Fischer 2008) and populations often don’t reach adaptive optima (Hereford 2009). The spatiotemporal scales at which adaptation occurs, can depend on species and external circumstances. For example, small-scale genetic adaptation was found between lakeside and landside clones of the amphibious plant *Ranunculus reptans* L. (Lenssen *et al.* 2004b). Local adaptation was found in *Carlina vulgaris* L. at the European scale, the degree of which was correlated with between-population distance at this scale (Becker *et al.* 2006). On a biogeographical and evolutionary scale, species’ fundamental and realized niches may evolve, e.g. in the context of climate change or continental invasions (Pearman *et al.* 2008). However a sound theoretical framework is still lacking in order to predict the amount of change or stasis of the niche; examples exist of both (Pearman *et al.* 2008). On the temporal scale however, niche conservatism seems predominant (Wiens *et al.* 2010, Giehl & Jarenkow 2012).

While often ignored in literature, **coexistence** theory has been developed that explicitly incorporates **local versus regional scales** (Leibold *et al.* 2004, Kneitel & Chase 2004, Chase 2005). The regional scale considers the metacommunity: the set of local communities that are linked by dispersal of multiple potentially interacting species (Wilson 1992). Several paradigms exist, regarding which mechanism is important to explain the relation between local and regional coexistence of species. Empirical evidence lags behind though, likely because of the logistical difficulties to test the alternatives in nature (Amarasekare 2003). I shortly explain two paradigms here. Several investigations support either the **species sorting** paradigm or the **mass-effects** paradigm, suggesting that an integration of both may be a good framework (Leibold *et al.* 2004). The species sorting paradigm considers sites at the larger (i.e. regional) scale that differ environmentally, and it assumes niche differentiation between species. As a consequence of niche differentiation, in each site another species is best adapted and outcompetes the other species, resulting in regional coexistence only (the so-called spatial storage effect). This paradigm assumes a separation of time scales between local dynamics and colonization-extinction dynamics (Leibold *et al.* 2004). On the other hand, the mass-effects paradigm assumes that dispersal events are more important, so that they affect local population dynamics. It assumes source-sink relations: dispersal from (source) patches that are more favourable for a species, to (sink) patches where the species is at physiological or competitive disadvantage. Local coexistence then results from the continuous inflow of individuals of different species through dispersal. However, the absolute scale to which ‘local’ and ‘regional’ refer in these paradigms, is very dependent on the community and environment studied (Mouquet & Loreau 2002). For example, when **local environmental heterogeneity** is present, the species sorting mechanism predicts local coexistence, applying the mechanism of niche differentiation alone. This is in accordance with the **resource-ratio theory** of Tilman (1982), which predicts that species dominances vary with the ratio of resource availabilities (Miller *et al.* 2005). This enables coexistence when the ratio varies within the habitat and when species are differently limited by those resources (Silvertown 2004). In this thesis, I will look at niche differentiation within a vegetation type at the biogeographical scale.

After several decades of reduced attention to the niche concept, it has regained an attention never seen before, albeit often in an implicit way: since the late 1990s we have seen a rapid increase of publications with results of **species distribution models** (SDM), also known as habitat suitability models or niche models. Their use has become extremely popular in conservation ecology (Guisan & Zimmerman 2000, Austin 2007, Elith & Leathwick 2009). As these techniques are used to approach some of my research objectives, it is useful to clarify some theoretical concepts of SDMs. These models comprise many different statistical approaches, but they are typically of a correlative (descriptive) nature and have the common purpose of predicting a species’ occurrence or abundance in niche and (mostly) biotope space. ‘Predictive’ in this context does at least mean: able to accurately simulate the occurrence or abundance of the species under the environmental circumstances and within the geographical area where the model was trained. The SDMs are mostly used to project the modelled niche on the biotope space, resulting in a predicted species distribution, at least when geographic maps of the environmental predictor variables are available. This is often done without even mentioning the term ‘niche’. Because of theoretical and methodological difficulties to connect Hutchinson’s concept of ‘realized niche’ to the abstract niche entities that are modelled by SDMs (Soberon & Nakamura 2009), several authors refrain from making the connection (Kearney 2006, Jimenez-Valverde *et al.* 2008). A more pragmatic approach is that of Colwell & Rangel (2009), which I will follow here. They **redefined ‘the realized niche’** of the species as the result of all mechanisms that have made the species to be found in one place and not in another. Beside the characteristics of the fundamental niche itself and the biotic interactions working upon it, also the parts of the niche space that are not occupied (absences) for other reasons define this biogeographical concept of ‘the realized niche’. Those other reasons comprise dispersal limitations, local extinctions or the simple absence of those environmental parts in the real world or biotope space (see also Pulliam 2000, Ozinga *et al.* 2005). Furthermore, this realized niche can be larger than the fundamental niche as a consequence of source-sink dynamics (mass effects; Pulliam 2000). At least when the training data to build a SDM include a substantial amount of absence records, there is growing agreement that the modelled niche object is the aforementioned, operationally redefined ‘realized niche’. It should be noted however, that the approach does not take into account spatial or temporal niche shifts: it assumes niche conservatism sensu Pearman *et al.* (2008).

Consequently, SDMs have brought a whole new set of instruments to biogeography and niche research. Some authors found that incorporating drivers of biotic interactions, e.g. the presence of a competitor, can greatly enhance the accuracy of the models (e.g. Leathwick & Austin 2001). Austin (2007) advocated the use of an explicit conceptual framework when building SDMs, one that considers ecological theory, study design and selection of predictors, and choice of the statistical technique. These and other aspects of the application of SDMs, along with many examples, have been extensively reviewed by Franklin & Miller (2009).

1.3 The plant community

Insights from plant community ecology (vegetation ecology) serve as a wider context for valuing and understanding plant ecological research. The below theory will allow readers to assess the relevance of my research objectives for the state of the plant communities as we perceive them in nature.

Much of descriptive vegetation science (phytosociology) is based on a few early 20th century paradigms that regard variation in VEGETATION as a collection of discrete ‘units’ that can be distinguished from each other, based on characteristics such as species composition, species cover and abundance and vegetation structure (Clements 1916, Braun-Blanquet 1921). These paradigms are designated as the **community concept**. Several classification methods were developed that allowed to reveal these units (vegetation types) and a whole terminology and a set of rules were invented to classify, name and describe them (e.g. leading to me using the term *Eleocharition* in paragraph 3). On the other hand, Gleason (1926) developed another paradigm to perceive vegetation: the **individualistic concept**, later called the **continuum concept**.

Here, species are regarded as independent of each other, and each species responds to the environment in a unique way. Both of these extreme concepts seem to be able, to some extent, to predict the complexity and variation of vegetation composition. Neither of them has proven radically wrong, although we now know they are inherently limited in scope and applications. In fact, almost a century later, “plant community ecologists have yet to specify the properties of either the community or continuum concepts in sufficient detail for any variant to be statistically distinguished from another” (Austin 2005).

Austin has come up with a reformulation of continuum theory, which is more consistent with insights from plant population ecology, regarding the concepts of the niche and competitive interactions (Austin & Smith 1989). The model basically incorporates the niche- and competition-based *equilibrium* aspects of plant population ecology that I explained earlier, so I will not go deeper into the model. Furthermore, by making use of the aforementioned concepts of **niche space** and **biotope space** (although he called them ‘environmental space’ and ‘geographical space’), Austin had found an elegant way to **reconcile** the apparently opposed community and continuum concepts: both concepts hold, but the **community concept** is an abstraction based on geographical (biotope) space, while the **continuum concept** is an abstraction based on environmental (niche) space (Austin 2005). A landscape is mostly composed of a limited set of environmental states that are well represented in terms of area, with each environmental state representing a particular position in niche space. While continuum theory predicts continuous vegetation in niche space, only those snapshots from niche space that are abundant enough in a particular landscape, are perceived as discrete vegetation units. Consequently, community units are a property of a particular geographical area, and need to be revised and changed when enlarging or changing the geographical area of interest. Therefore, the community concept is preferred for communication and ecological management, while the continuum concept is preferred for investigation of vegetation-environment relationships (Austin 2005).

Since the late 1990s, ever more complex views have arisen of how communities are organized. This should not surprise, as much more insight had become available, and a lot more debate had taken place at the level of plant population ecology and biotic interactions, compared to several decades before. These views of plant communities are different from the few discussed before, in that they do not simply depart from a generally expected pattern (like how species are clumped into different vegetation units, or not at all). Instead, they provide a framework of all known **mechanisms** that operate between plant populations and their environment, each other and other organisms, taking into account the limitations of geographical space. As such, they provide a nicely integrated view of current mechanistic knowledge: they explain how plant communities *become* organized, not necessarily making predictions about eventual community patterns. Hence, a shift has occurred in the approach of plant communities, from static and pattern-driven towards dynamic and process-driven. Here I shortly discuss this mechanistic approach, in order to place the earlier explained population ecological mechanisms of environmental relationships and competition among all known processes that shape communities.

The ‘**integrated community**’ concept of Lortie *et al.* (2004) is a clear and explicit example of the mechanistic view, illustrated in *Fig. 1.5*. It views a plant community at a specific site as the result of a cascade of hierarchical processes (‘filters’) that work upon the global species pool, i.e. all known plant species of the world. Although Lortie *et al.* (2004) did not mention the term, this falls under the general concept of **assembly rules**. These are rules that dictate how communities are assembled from species pools - an area to which much research effort is currently being devoted (see Weiher & Keddy 2001, Guisan & Rahbek 2011, Götzenberger *et al.* 2012). Lortie’s integrated community concept proposes that “four processes can be important in determining the extant plant community at a given site, but that the relative importance of each process will **vary** in space and time”, and hence between different plant communities. These processes are:

1. ***stochastic biogeographical events***: these include, among other mechanisms, **dispersal** capacity and the **geographical** configuration of dispersal corridors. They determine which species reach a certain site at a certain moment;
2. ***local environmental conditions***: these determine which of the species that have arrived, will potentially be able to survive and reproduce, depending on their fundamental **niche**;
3. ***plant interactions***: these may comprise both negative interactions (**competition**) and positive interactions (**facilitation**), depending on the properties of species pool and environment. Moreover, the degree to which this niche-driven equilibrium is reached will depend on the relative importance of niche and neutral processes, and on the role of temporal environmental fluctuations;
4. ***interactions with other organisms***, especially **herbivory** and **pollination** (see BOX).

Ultimately, both functional plant traits and explicit consideration of different environmental states must be incorporated in mechanistic community models (McGill *et al.* 2006, Ackerly & Cornwell 2007, Webb *et al.* 2010), if they are to evolve **from conceptual to predictive**. Furthermore, biological interactions need to be explicitly incorporated, as these impact on the species’ niches (McGill *et al.* 2006). Only then, more specific expectations can be made as to how a community assembles from species. In research on assembly rules, several rules have indeed been proposed that start from the importance of functional traits, but at this moment there are still methodological problems and problems of comparability between different case studies, which hinder reliable general conclusions (Götzenberger *et al.* 2012). An ultimate dream of plant community ecologists is to be able to predict the assembly of communities in biotope space, under current and changed environmental conditions. However, this involves yet to be established knowledge on the fundamental niches of species, community assembly rules and how macro-ecological principles interact with the local species pool (Guisan & Rahbek 2011). Also niche conservatism versus niche shifts must be considered across spatial and temporal scales (Pearman *et al.* 2008). McGill *et al.* (2006) further write: “Statements about traits give generality and predictability, whereas nomenclatural ecology tends towards highly contingent rules and special cases. […] To prioritize [community organizing mechanisms] is to hypothesize about which fundamental and realized niche processes are most important in a *given* system. Progress of the research program can be thought of as testing and improving those hypotheses by revising or confirming the ranking list".

Box: The plant community as a fraction of the whole community

The fourth process in the integrated community concept (Lortie *et al.* 2004) considers biological interactions other than plant-plant interactions. Although this aspect is not investigated in my thesis, it emphasizes the importance of other trophic levels in the community, hence of **multitrophic interactions**. Plants **affect** and are **affected** both by the below-ground and above-ground communities of pathogens, herbivores, mycorrhizal fungi and decomposers. They act as an interface between the below- and above-ground community, with both positive and negative feedback loops (Wardle *et al.* 2004). The nature of the feedback mechanisms depends not only on the composition of the whole community (Bezemer *et al.* 2005, Eisenhauer *et al.* 2011) but also on the environment itself (Wardle *et al.* 2004). Stability of the plant community over time has been shown to depend on multitrophic interactions and on plant community composition, in a grassland field experiment in which the densities of earthworms, below-ground and above-ground insects were controlled (Eisenhauer *et al.* 2011).

In their review, Wardle *et al.* (2004) state that decomposers (like collemboles and earthworms) may positively or negatively affect plant diversity in a quite unpredictable way, while the effects of the herbivore, root pathogen and mycorrhizal fungi community on **plant diversity** depend on the plant species that are targeted by these organisms. When rare plants are favoured by mycorrhizal fungi (or avoided by root pathogens and herbivores) relative to dominant plants, plant community diversity – hence plant **coexistence** – is enhanced, and vice-versa. Similar results have been obtained in a meta-analysis by Hillebrand *et al.* (2007), in which a specific comparison was made between aquatic and terrestrial systems. Herbivory is expected to lower the diversity of aquatic producers (especially phytoplankton), as evenness in these systems is higher and selective preference by herbivores is lower. The opposite is expected in terrestrial systems. In both cases, evenness is enhanced by herbivory. It could be questioned whether this aquatic-terrestrial distinction is valid for aquatic macrophyte communities (next paragraph), as these are observed in uneven compositions, much like in terrestrial plant communities.

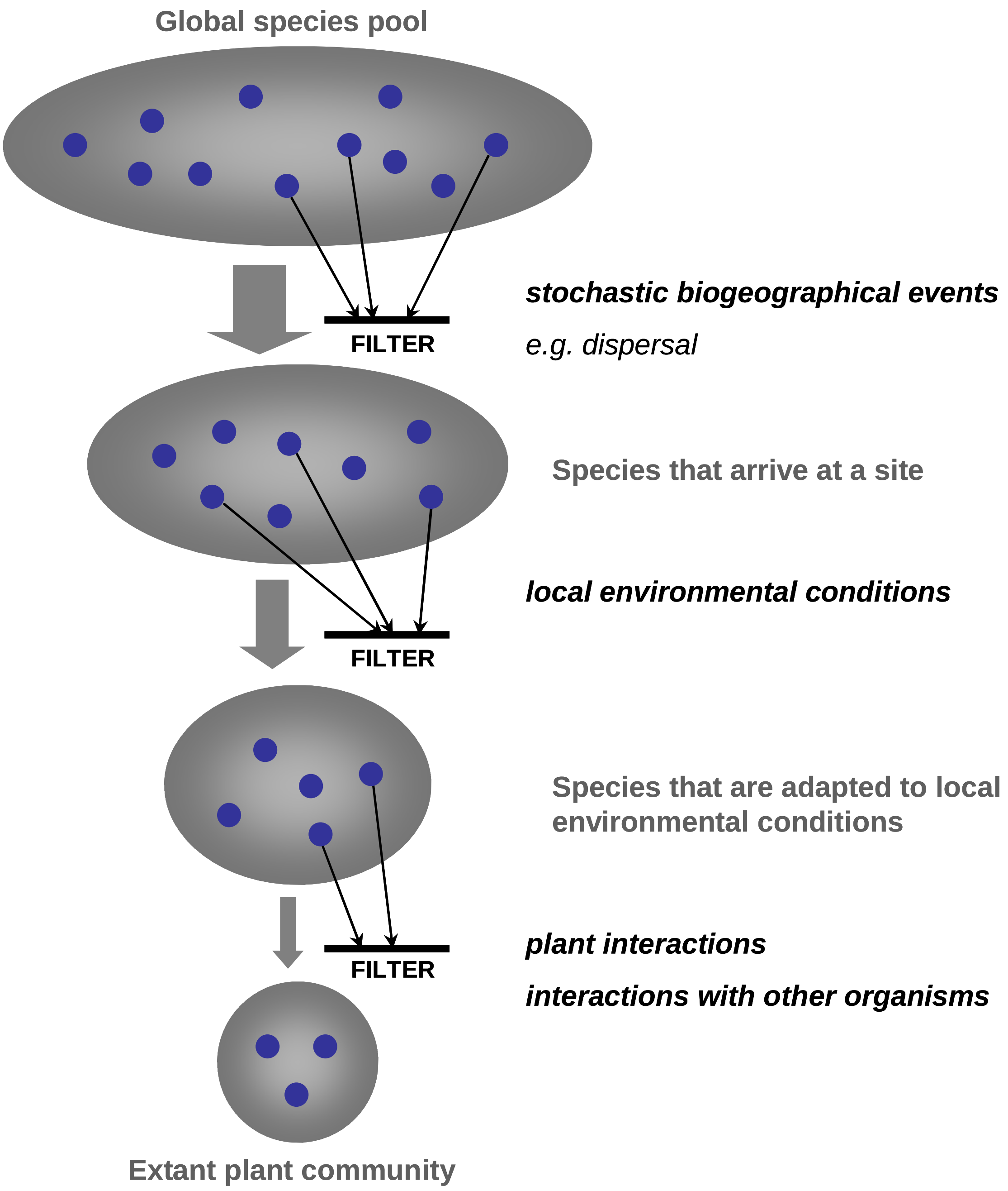


Fig. 1.5. The integrated community concept, after Lortie et al. (2004). Blue dots represent a few highlighted species, to show how some species migrate through the community assembly flow while others are stopped. See text for explanation.

2. Aquatic macrophytes and their specific environment

Aquatic macrophytes (Chambers *et al.* 2008) are a not-so-clearly defined group of aquatic and semi-aquatic plants. Especially the degree to which amphibious plant species and helophytes are included or not, depends on author's preferences. I will stick to lake macrophytes and include 'emergent macrophytes' that become at least partially submerged in the wet season. Rather consistent definitions of some terms can be found in Wetzel (2001).

Studies of aquatic macrophytes often pay attention to the environment itself, in my perception much more frequently than studies on terrestrial plants do. This is undoubtedly because aquatic macrophytes show an impressive variety of specific morphologies, physiologies and life histories that refer to their ways of coping with the aquatic environment (e.g. Den Hartog 1983, Boedeltje 2005). As research in this environment is radically different from terrestrial plant research and requires rather specific equipment, not to mention researcher's devotion to a basically human-unfriendly environment, it should not surprise to see that many papers are published in specialized journals that focus exclusively on freshwater habitat - with a risk of mutual neglect of terrestrial and aquatic research results.

Indeed, compared to the terrestrial situation in which much of plant ecological theory has been developed (paragraph 1), several aspects make **the lake environment** more complex to plants (Roelofs 1983, Maberly & Spence 1983, Roelofs *et al.* 1984, Bloemendaal & Roelofs 1988, Madsen & Sandjensen 1991, Wetzel 2001, Smolders *et al.* 2002, Brouwer *et al.* 2002, Madsen *et al.* 2002, Maberly & Madsen 2002, Lacoul & Freedman 2006, Bornette & Puijalon 2011, Pulido *et al.* 2011, Pedersen *et al.* 2011):

- **nutrients** may be acquired from the sediment (lake bottom), the surface water and, in macrophytes with floating or emergent leaves, from air;
- both sediment and surface water may have **various macro-ionic compositions**, which leads to a variety of potential stress gradients, either by direct gradients such as pH, or through interaction with resource gradients, e.g. calcium supply by upwelling groundwater lowers the availability of orthophosphate. Multiple biogeochemical processes have been described that include exchanges between sediment and surface water compounds (e.g. Lamers *et al.* 2002, Smolders *et al.* 2006, Geurts *et al.* 2008). These processes shape the plant's environment in complex ways;
- **gases** diffuse much slower in water than in air. This limits the availability of CO_2 and O_2 to aquatic macrophytes, which poses serious challenges towards photosynthetic and respiratory efficiency. Various morphological and physiological **adaptations** deal with this aspect. I give a few examples. Many aquatic macrophytes possess finer and thinner leaves, in order to increase CO_2 acquisition from water. Those species that also have floating or emergent leaves, as well as amphibious species, mostly show heterophylly: aerial leaves are thicker and not necessarily linear-shaped. Some aquatic plant species show extensive plasticity, e.g. *Luronium natans* (L.) Rafin. (Greulich *et al.* 2001) and *Juncus bulbosus* L. Several species have aerenchyma bundles, connecting roots and

leaves, that enable gas redistribution between different plant parts. In this way, the plants can utilize CO_2 from respiration and from the sediment in photosynthesis and obtain more O_2 for root respiration. This is well developed in isoetid growth forms, which live in softwater lakes poor in inorganic carbon. They possess an extensive root system in order to obtain CO_2 from the sediment, and have a CAM metabolism that enables them to fixate CO_2 at night. Several photosynthetic pathways have been described in aquatic plants, and photosynthetic efficiency is often high compared to terrestrial plants. In base-rich waters, aquatic macrophyte species occur that can assimilate bicarbonate as their primary source of inorganic carbon;

- submerged **organic sediment** is frequently anoxic, which leads to oxygen loss from the roots. Also, toxic compounds (such as sulphide or reduced iron) frequently accumulate in these layers. Seed germination is often low in organic layers. These stress factors require specific morphological and physiological adaptations of the plants;
- **light** availability under water depends on water turbidity, on depth and on shade from competing plants. It is therefore the most important limiting resource in nutrient-rich, turbid lakes;
- an often encountered disturbance in lakes is **wave erosion**, leading to the risk of breaking of plant parts or uprooting.

While in terrestrial plant ecology there is consensus that a few environmental variables drive plant physiological performance (in many situations most variability is explained by soil pH, temperature, nitrogen, phosphorus and water availability), aquatic macrophyte literature varies greatly in **investigated environmental variables**. Often many, i.e. >20 environmental variables are measured from surface water and soil, e.g. ion concentrations of surface water, soil water or soil derivates, beside physical characteristics such as lake depth, altitude, etc. (e.g. Geurts *et al.* 2008, Hrivnak *et al.* 2010). Further, some examples exist in lake macrophyte studies that used **ionic ratios**, i.e. the quotient of certain ionic concentrations, and that point at a potentially high explanatory value of these ratios (e.g. Seddon 1972, de Lyon & Roelofs 1986, Maberly & Madsen 2002). There is **no consensus** about a more limited list of environmental variables that would suffice to explain variation in (semi-) aquatic macrophyte response; rather it is common to discuss relatively long lists of factors that affect these plants (Spence 1982, Lacoul & Freedman 2006, Bornette & Puijalon 2011). This may in part be the consequence of the large chemical variability among lakes (Nõges *et al.* 2003), and because the major biogeochemical cycles of nitrogen, phosphorus, carbon and sulphur are differentially expressed in different lake types (Bloemendaal & Roelofs 1988, Stumm & Morgan 1996, Wetzel 2001, Lamers *et al.* 2002, Smolders *et al.* 2010). **It might therefore be questioned** whether (semi-)aquatic macrophyte performance and abundance can be predicted from a very restricted set of environmental variables, or whether it cannot.

3. The '*Eleocharition multicaulis*' vegetation alliance – aspects of synecology, syntaxonomy and synchorology

The macrophyte community I have focused on, resides in softwater lakes. These are defined by their low alkalinity, one of the major factors in chemical lake classification (Nõges *et al.* 2003). Lacoul & Freedman (2006) define the maximum alkalinity of softwater lakes at 0.2 meq L^{-1}. Nutrient-poor (oligotrophic) lakes typically represent the majority of the softwater lakes. One of their most typical aquatic vegetation types are isoetid communities, which mainly consist of perennial amphibious species (Murphy 2002). They have been studied since decades for their peculiar physiology and ecology, especially in *Isoetes* spp., *Littorella uniflora* (L.) Aschers. and *Lobelia dortmanna* L. These communities have a very low primary production. In Europe they have been denoted as the vegetation alliance *Littorellion uniflorae* Koch ex Tüxen 1937. They are largely confined to mineral sediments, very low levels of orthophosphate and weakly buffered, nutrient-poor surface water with an ammonium/nitrate ratio below one (nitrate as the dominant nitrogen form) and a low inorganic carbon concentration (Smolders *et al.* 2002, Brouwer *et al.* 2002). This alliance resides in the larger vegetation class of *Littorelletea* Braun-Blanquet & Tüxen 1943, which is typical of softwater lakes and endangered (Arts 2002). These amphibious communities require enough **stress** (nutrient limitation) and/or **disturbance** (e.g. waves, water-level fluctuations or sod-cutting management) in order not to become replaced by more productive macrophytes (Urban 2005). A global review of plant communities of 'ephemeral freshwater ecosystems' is given by Deil (2005).

A vegetation alliance of the *Littorelletea* that received little attention, though endangered, is the ***Eleocharition multicaulis*** **Vanden Berghen 1969**. This vegetation alliance is characterized by *Eleocharis multicaulis* (Smith) Desv., *Hypericum elodes* L. and *Scirpus fluitans* L. It occurs higher on the softwater shores than the *Littorellion uniflorae* and is therefore emergent for a larger part of the year. The *Eleocharition multicaulis* has by some authors been equated to, or diffusely incorporated into the *Hydrocotylo-Baldellion* Tüxen & Dierssen 1972, *Helodo-Sparganion* Braun-Blanquet & Tüxen 1943, *Hyperico elodis-Sparganion* Braun-Blanquet & Tüxen ex Oberdorfer 1957, or *Hyperico-Juncion bulbosi* (Segal 1968) Pietsch 1971. I prefer to use the syntaxon name *Eleocharition multicaulis*, which Pietsch (1977) described extensively in his European account on softwater plant communities and the description of which fits best with the plant community that I investigated.

Pietsch (1977, 1978) and Dierssen (1975) stressed the broad tolerance of the *Eleocharition multicaulis* towards pH (4,3 to 8,1), total salt concentration, calcium and sulphate and several other variables. More recent accounts describe the synecology of one of the associations within the alliance, the *Eleocharitetum multicaulis* Allorge ex Tüxen 1937, in which *Eleocharis multicaulis* has its highest frequency (Pietsch 1985, Schaminée *et al.* 1992, Schaminée *et al.* 1995a, Arts 2002). Synecologically, they describe a preference for acid situations with relatively high levels of ammonium and CO_2, nutrient-poor water and soil and a mineral substrate devoid of organic material (sapropelium). Quantitative information can be found in chapter 2 (*Table 2.1*). Other associations within the *Eleocharition* are denoted as various syntaxa, but overall they are richer in *Scirpus fluitans*. They are said to occur in less acid softwater lakes than the *Eleocharitetum multicaulis*, slightly deeper but equally poor in orthophosphate (Dierssen 1975, Pietsch 1977, Schaminée *et al.* 1995a). *Hypericum elodes* is more broadly characteristic of the *Eleocharition multicaulis*. Some other species of nutrient-poor softwater lakes are more often found within the *Eleocharition*: *Baldellia ranunculoides* (L.) Parl., *Ranunculus ololeucos* Lloyd, *Pilularia globulifera* L., *Luronium natans* (L.) Rafin. (protected in the European Habitat Directive), *Deschampsia setacea* (Huds.) Hack. and *Apium inundatum* (L.) Reichenb. Several species may also be present that are not confined to the *Eleocharition*, such as *Juncus effusus* L., *Hydrocotyle vulgaris* L., *Agrostis canina* L. and *Phragmites australis* (Cav.) Steud. Their abundance seems to increase with increasing nutrient levels or reduced intensity of disturbance. When these species are abundant, CHARACTERISTIC SPECIES often become rare. It would therefore be useful to know whether this is due to competitive effects or because of physiological limitations of characteristic species.

As the synecological statements above were solely based on univariate relationships between the presence of characteristic species and their environment, it remains an unanswered question whether the same relationships are found when modelling the niche of the characteristic species in a multivariate way, respecting the multidimensional nature of the niche.

The vegetation type has an Atlantic distribution, concordant with the distribution of most of its characteristic species (see e.g. Schoof-van Pelt 1973, Dierssen 1975, Pietsch 1977, Pietsch 1985, Arts & Den Hartog 1990, Szmeja & Clément 1990, Schaminée *et al.* 1992, Rodriguez-Oubina *et al.* 1997, Pinto-Cruz *et al.* 2009, Jansen 2011). The community's distribution thus comprises Portugal to North-Germany, including the British Isles.

The typical environment of *Littorelletea* communities has declined dramatically during the 20th century, due to acidification, eutrophication or alkalinisation (Arts 1990b, Roelofs *et al.* 1996, Arts 2002). As a consequence of agricultural activities, atmospheric ammonium deposition has led to massive acidification of weakly buffered soils and waters in NW-Europe (Bobbink *et al.* 1998, Bobbink *et al.* 2010). Softwater lakes that have been acidified by ammonium deposition are characterized by a high ammonium/nitrate ratio, high ammonium concentrations (> 20 µmol L^{-1}), low pH (< 4.2) and zero alkalinity of the surface water, and an accumulated organic and anaerobic soil layer due to a decreased microbial activity. Sites that are eutrophied by the inflow of alkaline and nutrient-rich water typically have a phosphorus-rich, turbid and alkaline water layer, and an accumulated organic and anaerobic layer due to the higher biomass production. These effects are strongest in the case of low water-level dynamics, which promote a lower redox state and accumulation of ammonium in the sediment. Depending on the sulphate supply to the system, sulphide can accumulate in the organic layer and promote internal eutrophication (Smolders *et al.* 2002). Since the end of the 20th century, many projects have been carried out to restore acidified and/or eutrophied softwater lakes, most of them in the Netherlands (Bellemakers 2000, Brouwer *et al.* 2002, Roelofs *et al.* 2002, Rhazi *et al.* 2005, Dorland *et al.* 2005, Van Wichelen *et al.* 2007). These measures basically restored the environmental requirements of the well-studied *Littorellion* alliance. Brouwer *et al.* (2002) observed that several species of the *Eleocharition* profited from these restoration measures as well, indicating that their environmental requirements seem to overlap with those of the *Littorellion*.

4. Objectives and outline

As clarified at the beginning of this General Introduction, the foregoing paragraphs (on plant ecology, aquatic macrophytes and the *Eleocharition*) form the broader framework within which my research took place. From these theoretical concepts, specific patterns are expected, and I will interpret the findings of my research in the light of these concepts by comparing observed and theoretical patterns. Hence, I will return to these frameworks in the discussions of the following chapters, and in a wider sense in the General Discussion. To do my research, I derived *objectives and hypotheses* from the theoretical background on **niche differentiation and competitive hierarchies in a spatial context**, applied within the ***Eleocharition***. Consequently, my objectives are specific and should not be regarded as if they were designed to test theoretical frameworks as such. Later on, I will discuss the potential applicability of these frameworks (mechanisms) to the *Eleocharition* in the light of my results.

The rationale of my research is as follows. I have dealt with an amphibious plant community in which (1) species' niches are not well known and (2) the existence of competitive hierarchies and niche differentiation is poorly identified. My quest has therefore been to determine patterns and possible mechanisms of population performance and abundance, in order to take away some of the aforementioned uncertainties that exist for this plant community. My research is composed of **three steps**:

- because niche characterization of species is critical to exploring niche differentiation, and because much may yet have to be discovered about the investigated species, I start with an elaborate characterization of the **realized niche of *Eleocharis multicaulis***, the most characteristic species of the plant community, testing a broad array of environmental factors;
- starting both from significant variables from the previous step and from some variables that are expected to be generally relevant to plants, I model the **realized niches of five species** and look at niche differentiation and indications of competitive hierarchy;
- using selected environmental drivers from the previous descriptive steps and from expectations in literature, I present the results of an **experiment** that controlled for effects of environmental drivers and competitive interactions.

These three steps are dealt with in chapters 2, 3 and 4, respectively, and chapter 5 is a methodological chapter. Below, I present an outline of chapters and corresponding hypotheses.

In **chapter 2**, I question which **environmental factors** define the **realized niche** of *Eleocharis multicaulis* within Belgium and The Netherlands, the most characteristic species of the *Eleocharition multicaulis*. More specifically, **hypothesis A** from literature is tested that

- A. *Eleocharis multicaulis* is confined to an acid situation rich in ammonium and carbon dioxide, nutrient-poor water and soil and a mineral substrate devoid of organic material (Pietsch 1985, de Lyon & Roelofs 1986, Schaminée *et al.* 1992, Schaminée *et al.* 1995a, Arts *et al.* 2002).

Furthermore, it is questioned whether other, yet unknown factors, such as ionic ratios (Seddon 1972, de Lyon & Roelofs 1986), influence the abundance of this species. In this chapter, discriminant analysis is used to distinguish between broad species' cover classes. The findings are further interpreted in a biogeochemical sense.

Chapter 3 presents the modelled realized niches of five selected species within the *Eleocharition* (two of which are characteristic species), in terms of occurrence and ground cover. In this chapter, I compare the realized niches of these species at the geographical scale of the *Eleocharition*. While competition promotes niche separation at the local scale of a community (Keddy 1989, Silvertown 2004), it is not clear whether realized niches, measured at a large spatial scale, still fit the equilibrium pattern from **coexistence** theory and the **competitive hierarchy model**. I verified **hypothesis B**, that

- B. within one vegetation type, the realized niches of species are still separated at the biogeographical scale of the vegetation type.

To test this hypothesis, a dataset was collected from the West-European distribution range of this vegetation type. To select environmental predictors, I started from a shortlist of predictor variables, mainly consisting of resource and direct variables, many of which were taken from the results in chapter 2. This list was then further reduced based on an analysis of multicollinearity. The modelling framework used here is 1) generalized linear models (GLM) to model presence/absence responses, and 2) proportional odds models (POM) to model cover responses.

Chapter 4 deals with testing possible mechanisms that lead to the realized niches that we perceive. While both preceding chapters concern observational field studies, I have approached this question with a manipulative experiment under controlled conditions. I questioned the **relative importance** of the following factors in the organization of a simplified simulated *Eleocharition* community, as expressed by the population performances of the different species:

- availability of **nutrients** (stress gradient);
- fluctuations of the **water level** (disturbance by a water level drawdown);
- the **identity** of specific species (their functional characteristics), including **competitive** effect and response.

This simplified, multispecies *Eleocharition* community was followed in time during one growing season. It was subjected to two ammonium treatments and two carbon dioxide treatments in order to test for the effect of nutrients, that are known from previous research to significantly influence plant species of softwater lakes (previous chapters; Roelofs *et al.* 1996, Bobbink *et al.* 1998, Smolders *et al.* 2002, Arts 2002). At the high nutrient combination, I tested for diffuse competition by measuring the COMPETITIVE RESPONSE of two species grown without neighbours. During the growing season, the communities underwent a change from submerged to emerged. We used the general assumption that performance would increase for all species at a higher nutrient level, regardless of the species. As we aimed to reveal the way species differed in their response to these factors, we regarded species (SPECIES IDENTITY) as an explicit factor. Hence, **hypothesis C** was that

- C. nutrients (carbon dioxide and ammonium) drive population performance and community composition more than water level drawdown and species identity.

As a second, related **hypothesis D**, we expected that

- D. competition between species has a marked role in this plant community.

In **chapter 5**, several approaches are compared to select relevant predictor variables from an extensive physicochemical survey, which yielded numerous environmental variables. This is done within the statistical framework of discriminant analysis, thereby using the data of chapter 2. Hence, this chapter provides a **methodological foundation** for chapter 2. It applies to those cases where a researcher doubts that all important predictor variables to a phenomenon are already known and therefore starts with a much wider set of potentially relevant variables.

Hence, four hypotheses on the *Eleocharition* are tested in this thesis (*Table 1.1*).

Table 1.1. Hypotheses that are tested in this thesis.

Chapter	Hypotheses
Chapter 2	A. *Eleocharis multicaulis* is confined to an acid situation rich in ammonium and carbon dioxide, nutrient-poor water and soil and a mineral substrate devoid of organic material.
Chapter 3	B. Within one vegetation type, the realized niches of species are still separated at the biogeographical scale of the vegetation type.
Chapter 4	C. Nutrients (carbon dioxide and ammonium) drive population performance and community composition more than water level drawdown and species identity.
	D. Competition between species has a marked role in the *Eleocharition*.

Extending on the above questions and hypotheses, I will further discuss on three related topics, regarding my results. These aspects are dealt with in the **General Discussion**:

- To what degree do the observed response patterns of different species agree with their position in the plant ecological **strategy** scheme of Kautsky (1988)?
- Are the best explanatory variables those expected from general plant ecological concepts (resource and direct variables), or does starting from **many more chemical variables** reveal other significant gradients that improve discriminative power?
- What is the potential of **ionic ratios** as a predictor of plant performance and abundance?

2

Understanding the realized niche of an amphibious softwater plant, *Eleocharis multicaulis*

with Fons Smolders, Sofie Ruysschaert, Jan Roelofs and Maurice Hoffmann

adapted from Vanderhaeghe *et al.* (2005)

Despite two centuries of exploration, our understanding of factors determining the distribution of life on Earth is in many ways still in its infancy.

Rahbek (2005)

Eleocharis multicaulis, Herentals, Belgium

CHAPTER 2

Understanding the realized niche of an amphibious softwater plant, *Eleocharis multicaulis*

Abstract

The West-European amphibious plant *Eleocharis multicaulis*, characteristic of softwater lakes, is rare and endangered in many regions. The present study aimed to evaluate several presumed niche features suggested in syntaxonomic studies, to reveal other important niche variables affecting the cover of this species and to quantify these responses. A dataset of 724 environmental and vegetation variables was built from a survey of 59 plots in The Netherlands and Belgium. Discriminant analysis, Pearson goodness-of-fit calculations, weighted averages and presence profiles were used. We found that *Eleocharis multicaulis* performs best in base-poor environments with an organic top layer and an established vegetation. The species is apparently N limited and profits from N input as long as NO_3^- is dominant over NH_4^+. A high atmospheric N deposition is detrimental, because the resulting strong acidification leads to dominance of NH_4^+ over NO_3^-. Intense acidification in agricultural areas has probably been an important extinction factor in the recent past. We hypothesize that current variability of soil Si, a very good predictor for the species' performance, might reflect these acidification processes. This hypothesis is supported by the fact that Si is correlated with trophic status, which is likely higher in agricultural regions. Nowadays, *Eleocharis multicaulis* suffers from agricultural P, K and alkalinity inputs as well as reduced oxygen supply (e.g. by reduced water-level dynamics), all leading to a lower redox state. These factors could bring about competitive suppression by other species, a high NH_4^+/NO_3^- ratio and P mobilisation.

Introduction

Because of their international decline since the 20th century, several plant species of the syntaxonomic class *Littorelletea* have been studied for their relation with environmental processes. Especially the macrophytes *Littorella uniflora*, *Lobelia dortmanna*, *Isoetes spp.*, *Juncus bulbosus* and *Sphagnum spp.* have been considered, resulting in an extensive literature record (e.g. Schuurkes *et al.* 1986, Farmer & Spence 1987, Rørslett & Brettum 1989, Paffen & Roelofs 1991, van Dam & Buskens 1993, Szmeja 1994, Lucassen *et al.* 1999, Arts 2002, Smolders *et al.* 2002).

An important plant community of the *Littorelletea*, the *Eleocharition multicaulis* Vanden Berghen 1969 alliance, has not been subject to focused research programs as yet. It can be found higher up sandy lake shores and is characterized by *Eleocharis multicaulis*, *Hypericum elodes* and *Scirpus fluitans*. In the present study, we consider *Eleocharis multicaulis* (Many-stalked Spike-rush), an uncommon perennial with an Atlantic distribution area from North-West Africa to North-West and Central Europe. Several authors have commented on its rarity and potentially or actually endangered state (Schoof-van Pelt 1973, Dierssen 1975, Pietsch 1978, Dierssen 1983, Kaplan 1993, Weeda *et al.* 2000).

Table 2.1 gives an overview of environmental characteristics of *Eleocharis multicaulis*, found previously by Pietsch (1978) and de Lyon & Roelofs (1986). The work by de Lyon & Roelofs (1986) in The Netherlands is the only autecological niche study we have encountered. Dierssen (1975) and Pietsch (1977, 1978) stressed the broad tolerance of the plant towards pH (4.3 to 8.1), total salt concentration, Ca^{2+} and SO_4^{2-} and various other variables. More recent accounts (mostly phytosociological) make specific statements: preference for an acid situation rich in NH_4^+ and CO_2 (Pietsch 1985, de Lyon & Roelofs 1986, Arts *et al.* 2002), nutrient-poor water and soil (most authors) and a mineral substrate devoid of organic material (sapropelium) (Schaminée *et al.* 1992, Schaminée *et al.* 1995a).

In Western Europe, it has been suggested that the plant can sustain higher intensities of acidification than other characteristic species (Kaplan 1992, Kaplan 1993, Kaplan 1998, Weeda *et al.* 2000). Where the *Eleocharition multicaulis* alliance disappeared, *Eleocharis multicaulis* often persisted the longest (Schoof-van Pelt 1973, Weeda *et al.* 2000), which leads to the hypothesis that it might be a competitive dominant under these conditions.

It can be assumed that still other environmental variables are influential to the performance of *Eleocharis multicaulis*. In particular, ratios of ion concentrations (ionic ratios) could be meaningful. Some authors used ionic ratios for chemical classification of ecosystems, without reference to plants. Several others used them in respect to the requirements of plants. In the latter case, ratios are often chosen to reflect the intensity of ecologically relevant biogeochemical processes, such as acidification. Examples include Al/Ca, NH_4^+/NO_3^-, S/(Ca+Mg), (Ca+Mg)/(Na+K), Ca/(Ca+Cl) and N/P (Bloemendaal & Roelofs 1988, van Wirdum 1991, de Graaf *et al.* 1998b, Lamers *et al.* 2001, Lucassen *et* *al.* 2002). Macro-ionic concentrations, available from standard chemical analyses, might be important as well.

The hypotheses we wanted to test in this study are: 1. that the species is confined to an acid situation rich in NH_4^+ and CO_2, nutrient-poor water and soil and a mineral substrate devoid of organic material, 2. that the species is a competitive dominant under certain conditions, and 3. that other factors, such as ionic ratios, influence the performance of this species.

To achieve our aims, we conducted a field survey in The Netherlands and Belgium (Ruysschaert 2002). To reveal potentially important variables for the performance of the species, we used discriminant analysis and univariate screening methods. The response to these variables was quantified using presence profiles, percentiles and cover-weighted averages.

Materials and methods

Study design

Fifty-nine plots of 2 m² were sampled in 33 softwater lakes of Pleistocene, sandy areas in Belgium and The Netherlands (*Fig. 2.1*), in which *Eleocharis multicaulis* was either present or absent, but potentially present based on the presence of other characteristic species of the *Eleocharition* alliance. Some lake properties are listed in *Table 2.2*. One to three plots were selected per lake. In this way, the plots were not completely independent. We assumed we could ignore this effect, because plots within one lake were chosen on the basis of obvious ecological differences. The response variable of interest was the cover of *Eleocharis multicaulis*, which was split up into three ecologically relevant classes: absent (cover = 0; 18 plots), low cover ($0 < \text{cover} \leq 10\%$; 26 plots) and high cover ($\text{cover} > 10\%$; 15 plots). For each plot, 35 vegetation variables were measured (including cover of the plant species occurring in at least five plots), as well as 24 physical variables regarding lake, plot, water layer and soil, 27 chemical variables of the surface water, and 31 chemical variables of both mineral (sandy) soil and organic top layer, the latter only if present. 79 derived chemical variables were constructed from equivalent concentrations, mostly ionic ratios. A list of all measured and derived variables can be obtained from the corresponding author. Most chemical variables were sampled once in summer (2001) and once in winter (2002). The difference between winter and summer was a measure of seasonality. Winter nutrient values were an estimate of availability for the next growing season. The full dataset comprised 724 compound predictor variables (variable x season).

Table 2.1. Literature-derived averages of environmental variables, with respect to the presence of Eleocharis multicaulis. Cover-weighted averages (WA) from the current study are presented for summer and winter. For the formula of WA, see Materials and methods. Soil data of the present study apply to the mineral soil layer. The WA of de Lyon & Roelofs (1986) is weighted by presence of Eleocharis multicaulis. + = positive effect on Eleocharis multicaulis, - = negative effect, ^ = optimum at intermediate values; / = not tested. The number of signs is a measure for the indication value, calculated with a modified chi-square statistic.

A. Chemical conditions	Pietsch (1978)	de Lyon & Roelofs (1986)		This study	
Study area	Central Europe	The Netherlands		The Netherlands & Belgium	
Sites with *Eleocharis multicaulis* / total number of sites	109/109	18/ca. 600		41/59	
Variable	Mean	WA	Indication value	Summer WA	Winter WA
Water					
Alkalinity (µeq L^{-1})	510	200	- - -	58.6	78.7
NH_4^+ (µmol L^{-1})	52	33.4		12.5	13.0
HCO_3^- (µmol L^{-1})	510			53.9	71.2
HCO_3^- proportion	0.18		- - -	0.04	0.05
Ca^{2+} (µmol L^{-1})	963	130	- - -	76.5	93.1
CO_2 (µmol L^{-1})	166	high	/	52.2	79.6
Cl^- (µmol L^{-1})	513	420	- - -	251.6	336.1
Cl^- proportion	0.26		/	0.43	0.54
Fe (µmol L^{-1})	11.5	11.3	^	10.7	4.3
Mg^{2+} (µmol L^{-1})	274	80	- - -	28.0	36.2
Mn (µmol L^{-1})	3.5	1.3	- -	0.73	0.51
NO_3^- (µmol L^{-1})	66.9	2.9	-	56.8	27.5
PO_4^{3-} (µmol L^{-1})	7.8	2.6	+	0.2	0.1
pH	6.3	4.5	- -	5.0	5.0
K^+ (µmol L^{-1})		65	-	52.2	35.6
Salinity (µeq L^{-1})		1600	- - -	1024	1106
Si (µmol L^{-1})	108			6.2	29.2
Na^+ (µmol L^{-1})		260	- - -	184.7	236.9
SO_4^{2-} (µmol L^{-1})	967	200	- - -	176.0	125.8
SO_4^{2-} proportion	0.56		/	0.52	0.39
Soil					
Ca digestion (µmol kg DW^{-1})		16000	- -	3923	3497
Fe digestion (µmol kg DW^{-1})		38000	- -	18626	15820
Loss on ignition (LOI) (%)		25	+	2.8	3.0
Mg digestion (µmol kg DW^{-1})		9000	- - -	6494	6133
Mn digestion (µmol kg DW^{-1})		1000	- - -	95.6	85.7
Total N content (mmol kg DW^{-1})		127	^	65	125
P digestion (µmol kg DW^{-1})		4200	- -	1455	1589
K digestion (µmol kg DW^{-1})		12000	- -	6028	6671

Table 2.1, continued.

B. Granulometry (In %)

de Lyon & Roelofs (1986)			This study	
Class (µm)	WA	Indication value	Class (µm)	WA
< 2	2	- -	< 2	2.4
2-50	13	-	2-16	10.1
50-200	51		16-63	10.3
200-2000	31	/	63-125	11.1
			125-250	33.8
			250-500	28.5
			500-1000	2.6
			> 1000	0.0

Table 2.2. Some characteristics of the sampled lakes. IQR = interquartile range (75th minus 25th percentile).

	Minimum	Median	Maximum	IQR/range
Surface class	< 0.1 a	0.5 - 1 ha	> 10 ha	
pH	4.1	5.0	7.6	0.53
Alkalinity (µeq L^{-1})	-81	29	1508	0.23
Electric conductivity EC_{25} (µS cm^{-1})	24	79	455	0.18
Median granule size of mineral layer (µm)	22	167	254	0.48

Chemical sampling and analyses

In each plot, a surface water sample was taken, part of which was used for the determination of pH (WTW Sentix electrode), electric conductivity (WTW Tetracon 325 electrode), redox potential (WTW Sentix ORP electrode) and alkalinity (titration with sulphuric acid down to pH 4.2, using bromcresol green-methyl red indicator, by means of a Hach Digital Titrator, model 16900). The rest of the sample was filtered through a Whatman GF/C filter (pore diameter 0.45 µm). A subsample was prepared for inorganic carbon analysis through injection in a Venoject Glass Vacuum Tube, stored directly at 2-6 °C. The remaining part of the filtered water was divided over two iodated 50 mL polyethylene tubes, one with 1% of 65% HNO_3 solution, the other with 0.8% of 31.24 g L^{-1} citric acid solution, and immediately frozen below –10 °C using a transportable freezer.

Sediment samples up to 0.5 L were taken from the upper 20 cm of soil, separating mineral layer and organic top layer (threshold at 10% organic matter content). The samples were immediately stored air-tight at 2-6 °C. One part (approximately 50 g) was oven-dried at 65 °C for 24 h to determine moisture content. About 10 mg of dried sediment was burnt with a Flash EA 1112 NC Soil analyser (ThermoQuest, Interscience), to determine total C and N content. Granulometric composition of fresh sediment, between 0.4 µm and 2000 µm, was measured volumetrically with a Malvern Mastersizer (S Long Bench) by means of laser diffraction.

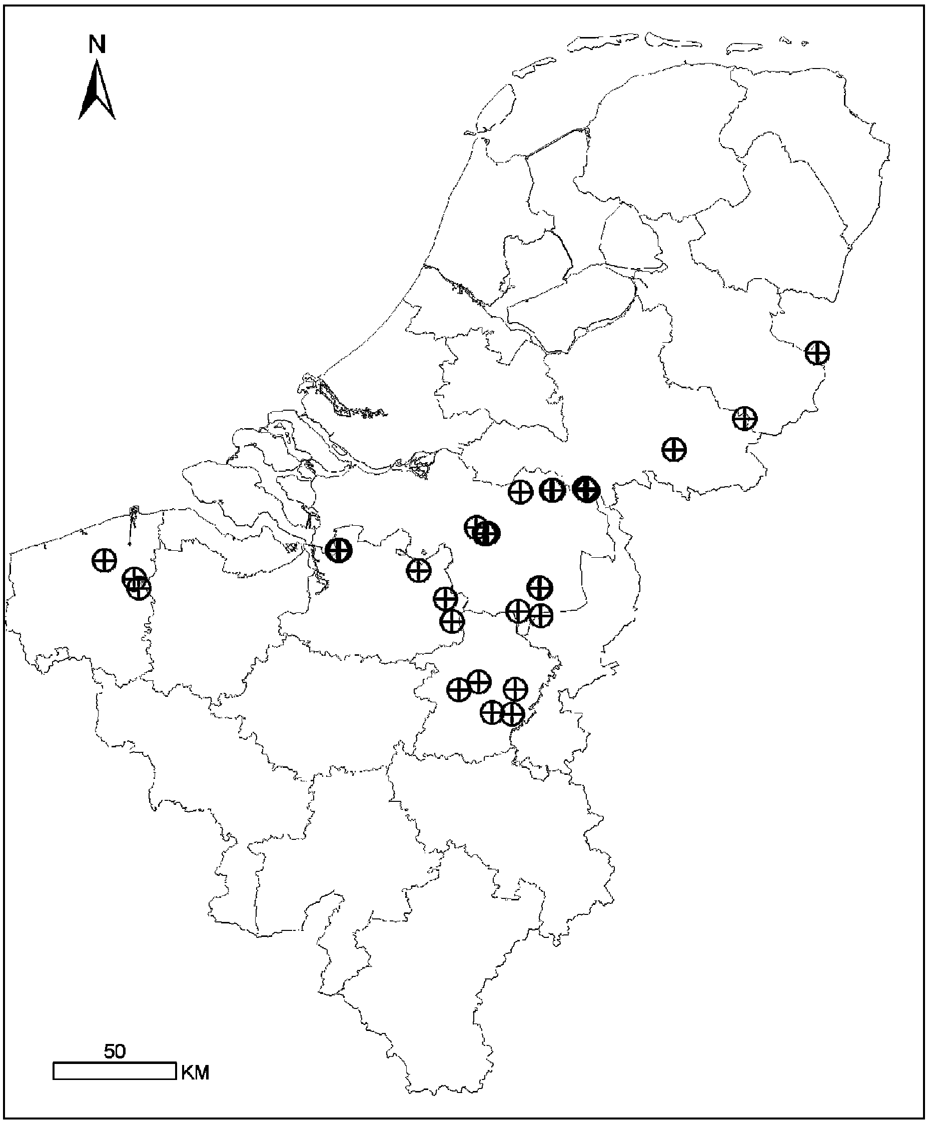


Fig. 2.1. Map of sampling locations (Belgium and The Netherlands).

Digestates of dry sediment were prepared according to Smolders *et al.* (2002), water- and NaCl-extracts of fresh sediments according to Brouwer & Roelofs (2002) and ammonium lactate extracts according to Egnér *et al.* (1960). They were stored in a iodated polyethylene tube at –20 °C. Water- and NaCl-extracts were acidified with citric acid (like the water samples). Lucassen *et al.* (2004) was followed for determination of organic matter content (loss on ignition) of the sediment and inorganic carbon analysis of water samples. Macro-ionic concentrations of all samples and derivates were measured according to Lamers *et al.* (1998).

Statistical analyses

Different types of analysis can be expected to provide different results, which complement each other. Therefore, we carried out three sets of analyses. To reveal previously unknown major predictor variables, these analyses were done with the maximum available number of variables, though each time a different subset of the data was used because of missing values. None of the analyses had specific distributional requirements.

First, discriminant analysis (DA) (Manly 1994) was performed on a data-subset of 30 plots and 392 predictor variables. For reliable application of DA (Williams & Titus 1988), variables were (further) discarded in two steps (see chapter II.2). First, all predictor variables were categorized into three adjacent classes of equal frequency. The association between the predictor variables and the response variable was then evaluated with Pearson chi-square calculations by means of 3 x 3 contingency tables. A p-level of 0.05 was used as a threshold to select a preliminary subset of response-related variables. Secondly, stepwise DA selected those variables (continuous scale) from this subset that, acting together, most successfully separated the three response classes of *Eleocharis multicaulis*. With three response classes, DA yields two discriminant functions. As 46 plots had no missing values for the selected variables, DA scores were obtained for 46 plots. Homogeneity of variances among groups was subjectively evaluated on the basis of the ordination diagram, as proposed by Quinn & Keough (2002).

Next, Spearman correlations were calculated between the variables and the first discriminant function. This univariate screening was done for the 46 plots and all variables. The third analysis consisted of Pearson chi-square calculations like the ones above for the total dataset (see also de Lyon & Roelofs (1986) and Bloemendaal & Roelofs (1988)). Variables that yielded p-values < 0.1 in either of these univariate screening analyses were considered as relevant. P-levels in these procedures are merely criteria to delimit a subset of variables. They have no value for multiple statistical inference, since global type-I error is not under control. Care must be taken in presuming causal effects of these variables on the response because of multicollinearity (Mac Nally 2000, Graham 2003). Therefore, intercorrelations were investigated using non-metric multidimensional scaling (MDS), complemented by cluster analysis with Ward's method. For both multivariate analyses, $1 - r^2$ was used as a distance measure between two variables. Analyses were done with the statistical package SPSS 11.0 for Windows (SPSS Inc. 2001).

Presence profiles were constructed for the most influential predictor variables, in order to directly visualise the presence of *Eleocharis multicaulis* (between 0 and 1). For each predictor variable, presence was calculated for each of five adjacent classes of equal frequency, e.g. for the five classes of water pH. The presence was calculated as the ratio of the number of plots in which *Eleocharis* *multicaulis* was present to the total number of plots belonging to the predictor variable class. Weighted averages of predictor variables (*WA*) were calculated in a similar way as by de Lyon & Roelofs (1986), in order to make comparisons. The medians of five adjacent classes of equal frequency were calculated for each predictor variable, in order to eliminate outliers (M_1 to M_5). Sometimes only two or three classes were distinguished because of insufficient different values. For each class, the mean cover was calculated arithmetically, including absence data (C_1 to C_5). We then used the following formula to obtain the weighted average:

$$WA = \Sigma (M_i \cdot C_i) / \Sigma C_i$$

Results

Discriminant analysis

Discriminant analysis selected five variables which, acting together, separated the three response groups (absent / low cover / high cover) of *Eleocharis multicaulis* (*Fig. 2.2*). There was no significant intercorrelation among these variables ($P > 0.1$) and group variances were of comparable magnitude (visual evaluation of the ordination diagram). The first discriminant function represented a gradient from absent to high cover, while the second separated the low cover group. The first discriminant function explained 76% of between-group variance (eigenvalue 2.705); the second 24% (eigenvalue 0.852). Three abiotic variables were important to the first discriminant function: NH_4^+/NO_3^- ratio of the water in summer (-), seasonality of the N/K ratio of the mineral layer (NaCl-extraction) (+) and seasonality of divalent/monovalent cation ratio of the water (+) (DMR = (Ca+Mg)/(Na+K)), with the +/- signs indicating the response direction of *Eleocharis multicaulis*. Seasonality of N/K had a strong positive correlation with its winter values ($r = 0.93$; $P < 0.001$), while the seasonality of DMR was negatively related with its summer values ($r = -0.37$, $P = 0.006$). The remaining two variables were the cover of the neighbouring species *Hydrocotyle vulgaris* and *Mentha aquatica* (-). The cover of *Hydrocotyle vulgaris* was related to the low cover response of *Eleocharis multicaulis*.

Univariate screening

Table 2.3 shows a selection of significant variables ($P < 0.1$ in at least one test). A full list of results is available from the corresponding author. A first cluster of interrelated variables contained redox potential of the surface water (+), both Si measurements of the mineral soil in winter (digestion (+) and NaCl-extraction (-)) and the NH_4^+/NO_3^- ratio of the water in summer (-) (*Table 2.3*).

Eleocharis multicaulis performed best under the most acidic circumstances. These acidity variables were correlated with variables of the redox cluster and primarily consisted of surface water variables. Among these, the strongest univariate predictor of the species' performance was the so-called 'ion ratio' (-)

(IR = Ca/(Ca+Cl); *Fig. 2.3A*). The acidity cluster had moderate correlations with base cation concentrations of the mineral or organic soil layer (-). Winter replenishment of Mg and K (mineral soil) and Ca (organic top layer) was positively related to the species' performance. Also the presence of an organic top layer was positively related with the cover of the species. Such a layer mostly occurred at lower pH values. Digestion-K content of the mineral soil was negatively correlated with latitude and longitude (*P* < 0.001), which both had a positive relation with *Eleocharis multicaulis* (not shown).

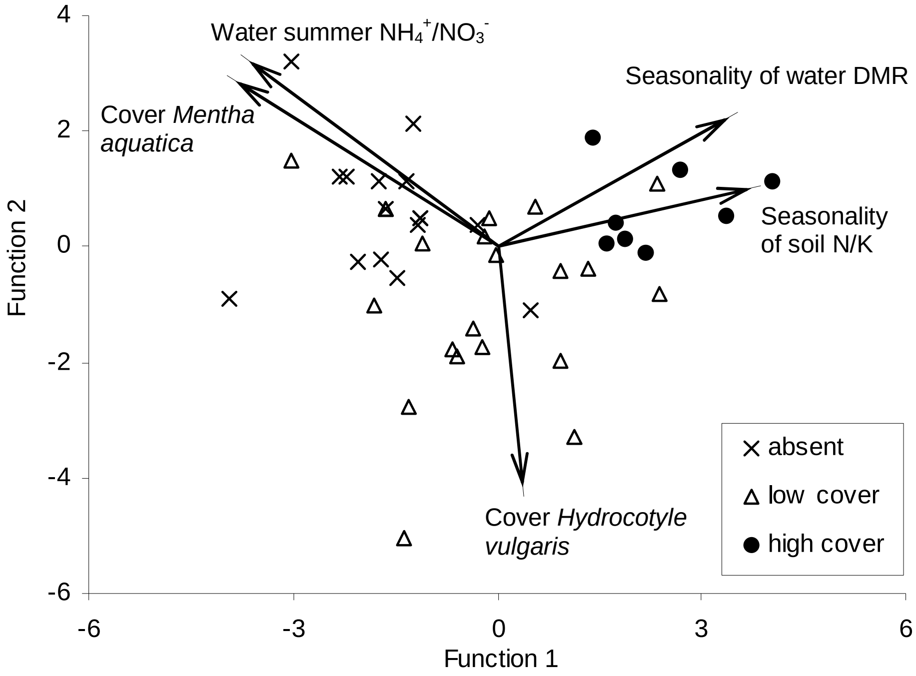


Fig. 2.2. The two discriminant functions separate the response classes of Eleocharis multicaulis. Vectors represent the standardized discriminant function coefficients of the transformed original variables. They have been scaled with a fixed factor to fit properly in the graph.

Table 2.3. The response of Eleocharis multicaulis to some significant variables of univariate screening tests ($P < 0.1$), involving Pearson chi-square tests as well as Spearman correlations with the first discriminant function. Variables are grouped according to intercorrelation structure. Weighted averages are presented, as well as the 10th and 90th percentiles for the plots where Eleocharis multicaulis was present (range). MIN = mineral soil layer; ORG = organic top soil layer; S = summer; W = winter; D = winter minus summer (seasonality); S/W = significant effect in summer and winter, with summer values shown in table; + = *positive effect on Eleocharis multicaulis,* - = *negative effect; WA = weighted average (weighted by mean cover, see Materials and Methods).*

Com-part-ment	Sea-son	Variable	Re-spon-se	WA	Range
Vegetation					
	S	Cover *Drosera intermedia* (%)	+		0-3.6
	S	Cover *Molinia caerulea* (%)	+		0-12
	S	Cover *Juncus bulbosus* (%)	+		0-20
	S	Cover *Agrostis canina* (%)	-		0-8.8
	S	Cover *Juncus effusus* (%)	-		0-4
	S	Cover *Hypericum elodes* (%)	-		0-18.4
	S	Cover *Galium palustre* (%)	-		0-0.8
	S	Cover *Lysimachia vulgaris* (%)	-		0-1.8
	S	Cover *Mentha aquatica* (%)	-		0-0
	S	Total plant cover (%)	+	75.9	32-100
Variables correlated with redox potential of surface water (winter)					
Water	W	Redox potential (mV)	+	265.3	178.2-315.4
ORG	S	Presence of organic top layer	+		0-1
MIN	S/W	Si digestion (µmol kg DW^{-1})	+	27241	2887-50216
Water	S	NH_4^+/NO_3^-	-	0.66	0.07-2.81
MIN	W	Si NaCl-extraction (µmol kg DW^{-1})	-	523.2	18.1-4278.7
Acidity & base richness					
Water	S/W	Alkalinity (µeq L^{-1})	-	58.6	-66.8-410
Water	S/W	pH	-	5	4.2-7.5
Water	S/W	Ion Ratio (IR) = Ca/(Ca+Cl)	-	26.4	12.2-64.9
Water	S/W	Divalent / monovalent cation ratio (DMR)	-	0.75	0.21-2.27

Compartment	Season	Variable	Response	WA	Range
Winter replenishment of base cations in the soil: moderate correlation with acidity complex					
MIN	D	Mg digestion (µmol kg DW^{-1})	+	-1130.43	-6550.65-1620.55
MIN	D	K digestion (µmol kg DW^{-1})	+	1082.07	-2706.67-4402.52
ORG	D	Ca digestion (µmol kg DW^{-1})	+	-9133.73	-53162.41-15950.38
Variables correlated with NH_4^+/NO_3^- ratio of surface water (summer)					
ORG	S	Cover of organic layer (%)	+	85.4	99.6-100
Water	S	NO_3^- (µmol L^{-1})	+	56.8	4.6-220.1
Water	S	NO_3^-/PO_4^{3-}	+	536.5	25.2-2320.1
Water	S	NO_3^-/K^+	+	1.33	0.11-3.70
Nitrogen availability in mineral layer (winter)					
MIN	W	N/K (NaCl-extraction)	+	714	0-2155
MIN	W	Total N content (mmol kg DW^{-1})	+	125	0-186
MIN	W	N/P (NaCl-extraction)	+	17844	0-28930
Phosphorus availability in mineral layer: correlated with silicon in mineral layer (winter) - digestion					
MIN	W	P digestion (µmol kg DW^{-1})	+	1589	119-2881
MIN	W	P NaCl-extraction / digestion ratio	-	0.006	0.002-0.021
Organic layer nutrient richness: correlated with silicon in mineral layer (winter) - digestion					
ORG	S	S digestion (µmol kg DW^{-1})	-	69088	12113-174221
ORG	W	Total N content (mmol kg DW^{-1})	-	482	131-1146
ORG	W	Loss on ignition (LOI) (%)	-	22.8	8.0-56.9
ORG	W	NO_3^- water-extraction (µmol kg DW^{-1})	-	46.4	8.2-212.8

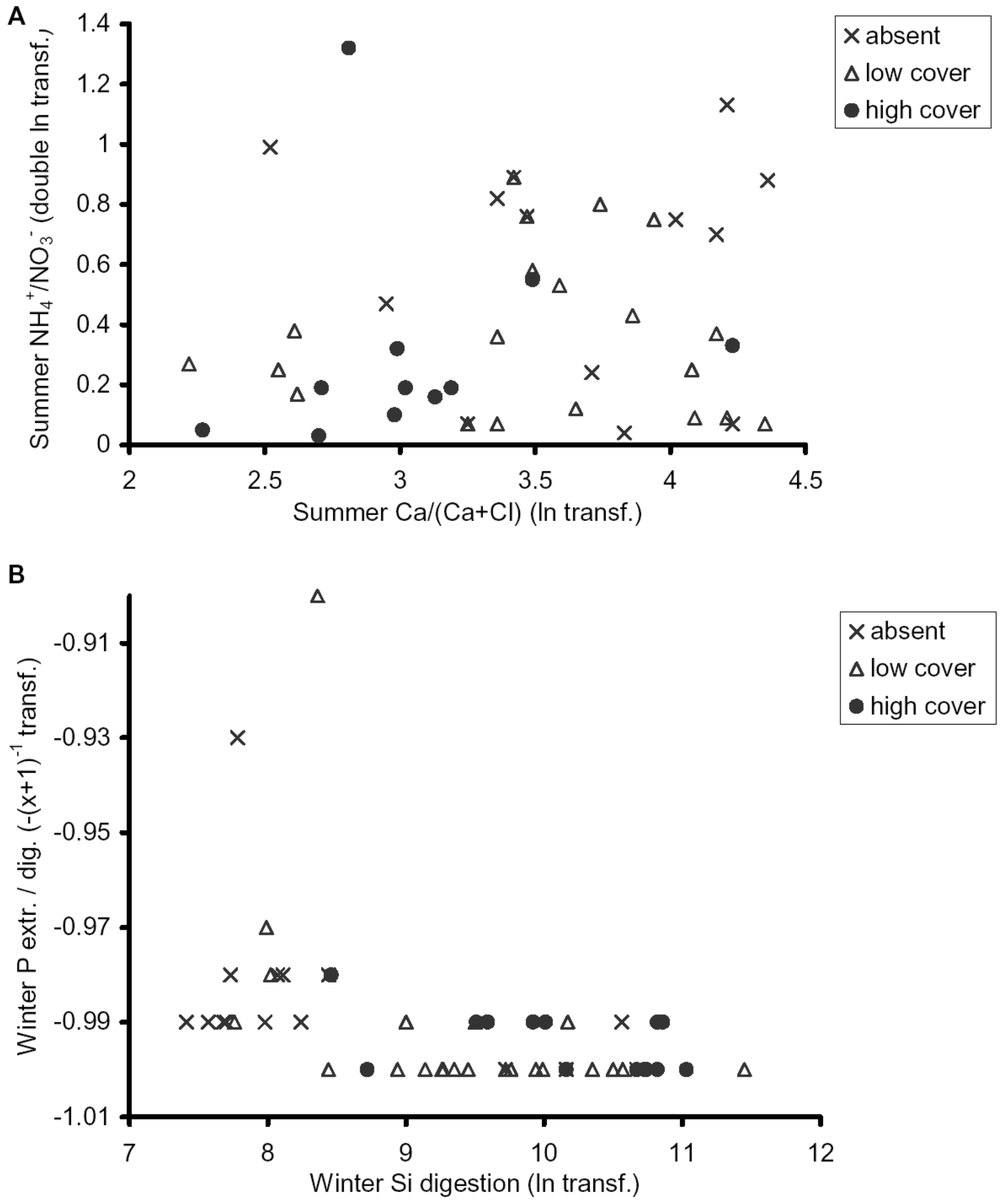


Fig. 2.3. Some influential variables are interrelated, others are not. A. The NH_4^+/NO_3^- concentration has no significant relationship with the ion ratio Ca/(Ca + Cl) (surface water, summer; P = 0.124). B. Digestion-Si and the extraction/digestion ratio of P have an inverse relationship (mineral soil, winter; r = -0.45, P = 0.001).

CO_2 had a slightly negative correlation with the first discriminant function in winter (not shown). It was not associated with acidity variables.

NO_3^- (or N) dominance in the water, in absolute terms or relative to NH_4^+, PO_4^{3-} or K^+, had a significant positive relation with the performance of *Eleocharis multicaulis*. These relations only existed in summer. N in the mineral soil (winter), absolute and also relative to P or K, had a positive relation as well.

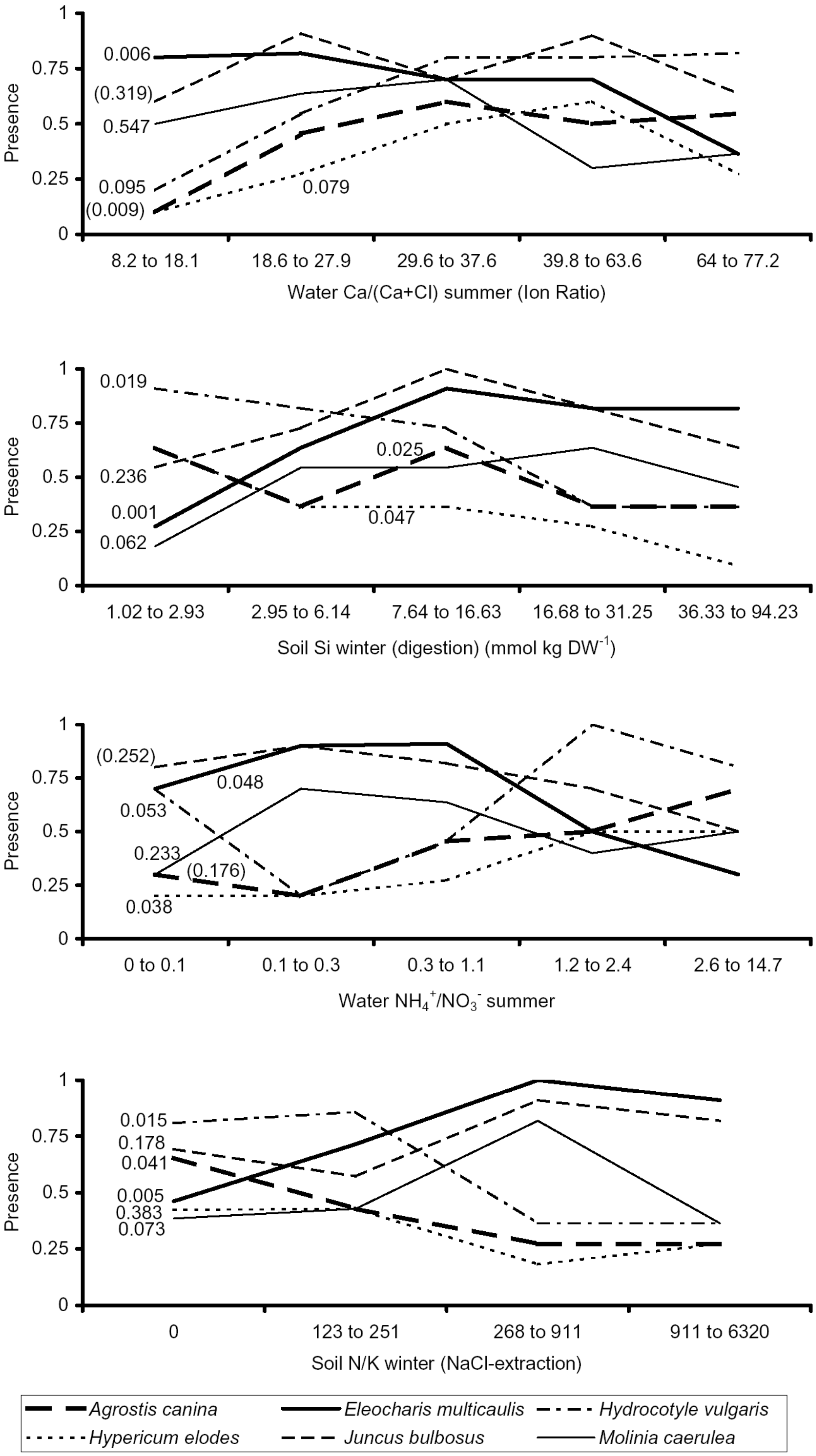
The digestion-Si of the mineral soil in winter (+) was positively correlated with mineral soil ratios that have digestion-P in the numerator (relative to base cations), as well as with P itself (+). Especially the digestion / extraction ratio of P had a positive effect on *Eleocharis multicaulis* (*Fig. 2.3B*). Si was negatively correlated with organic matter content (loss on ignition, LOI) (-), S concentration (-), NO_3^- and N content (-) of the organic top layer. Organic layers with LOI > 20% generally occurred when they were thinner than 5 cm.

Eleocharis multicaulis responded positively to total plant cover (weighted average: 76%). Positively associated species were *Drosera intermedia*, *Molinia caerulea* and *Juncus bulbosus*. Negatively associated species were *Juncus effusus*, *Agrostis canina*, *Hypericum elodes*, *Galium palustre*, *Lysimachia vulgaris* and *Mentha aquatica*. For *Hydrocotyle vulgaris*, no clear association was found.

Presence profiles

The species presence profiles for ion ratio (IR) and digestion-Si were concordant with the species associations (*Fig. 2.4*). The profiles for the NH_4^+/NO_3^- ratio of the surface water (summer), similar to those for extraction-Si (not shown), showed that *Eleocharis multicaulis* and positively associated species reached their optimum within the ratio interval 0.1 to 1 (where NO_3^- is dominant). *Hydrocotyle vulgaris* had a low presence in this region, while it attained a high presence at both very low (0 to 0.1) and higher (> 1) values. For the N/K ratio of the mineral layer in winter (NaCl-extraction), *Eleocharis multicaulis* and companions attained a maximum response at higher values, while the inverse pattern was true for the other species. These presence profiles were similar to those for the N/P ratio of the mineral layer in winter (NaCl-extraction) and the NO_3^-/K^+ and NO_3^-/PO_4^{3-} ratio of the surface water during summer (not shown).

Fig. 2.4 (next page). Presence profiles of Eleocharis multicaulis and some positively or negatively associated species in relation to selected predictor variables. For each profile, the P-value of the corresponding Pearson chi-square test is shown. Low P-values indicate a significantly changing presence profile. P-values in brackets refer to tests in which more than 50% of the expected frequencies in the chi-square test were lower than 5, which makes test results less reliable.


Presence
1
0.75
0.5
0.25
0
0.006
(0.319)
0.547
0.095
(0.009)
0.079
8.2 to 18.1
18.6 to 27.9
29.6 to 37.6
39.8 to 63.6
64 to 77.2
Water Ca/(Ca+Cl) summer (Ion Ratio)
Presence
1
0.75
0.5
0.25
0
0.019
0.236
0.001
0.062
0.025
0.047
1.02 to 2.93
2.95 to 6.14
7.64 to 16.63
16.68 to 31.25
36.33 to 94.23
Soil Si winter (digestion) (mmol kg DW^{-1})
Presence
1
0.75
0.5
0.25
0
(0.252)
0.048
0.053
0.233
(0.176)
0.038
0 to 0.1
0.1 to 0.3
0.3 to 1.1
1.2 to 2.4
2.6 to 14.7
Water NH_4^+/NO_3^- summer
Presence
1
0.75
0.5
0.25
0
0.015
0.178
0.041
0.005
0.383
0.073
0
123 to 251
268 to 911
911 to 6320
Soil N/K winter (NaCl-extraction)
Agrostis canina
Eleocharis multicaulis
Hydrocotyle vulgaris
Hypericum elodes
Juncus bulbosus
Molinia caerulea

Discussion

The most important predictor variables for the cover of *Eleocharis multicaulis* were the digestion-Si content of the mineral layer (+), the NH_4^+/NO_3^- ratio of the surface water (-), N measures of the water and mineral soil layer (+) and base cation richness variables such as ion ratio (IR) and DMR (-), with the signs (+/-) indicating the response direction. Our study thus confirms the species' presumed optimum in acidic and oligotrophic conditions, taking notice that oligotrophy concerns low P or K levels of the soil. In contradiction to earlier studies, we did not find significant results for NH_4^+ or CO_2; neither were they correlated with acidity variables. Also the preference for a mineral soil could not be supported, as we primarily found the species on organic substrate.

Nutrient limitation

Weighted averages of N, K and P (in water and soil) were lower than those measured by Pietsch (1978) and de Lyon & Roelofs (1986). The positive response to soil N and water NO_3^-, at least within the measured environmental range, indicates that *Eleocharis multicaulis* is favoured in times of atmospheric N deposition or other ways of N input. This is true on condition that NH_4^+ does not become the dominant N form in the water and P or K do not increase more than N. As atmospheric N deposition eventually leads to NH_4^+ dominance (Schuurkes *et al.* 1988), this form of N input should remain moderate, however. Probably, excess of soil N is depleted by the vegetation during the growing season, as relationships could not be established for summer data. Soils in the southern and western part of the investigated area were richer in K. This could be an explanation for lower performance of *Eleocharis multicaulis* in these areas, reflected by its response to latitude and longitude.

The extraction / digestion ratio of soil P indicates its availability to plants. The more tightly P is bound to the sediment (digestion-P) and thus unavailable to the extract and the vegetation, the better the performance of *Eleocharis multicaulis*, which is in agreement with observations on the N/P ratio.

It seems that CO_2 was not a limiting nutrient for the plant. Potentially it is only limiting in inundated conditions (Ruysschaert 2002). Neither did we find a relation with absolute NH_4^+, in contradiction to de Lyon & Roelofs (1986). At the time of their study (1970s and 1980s), intense acidification occurred in Dutch and Belgian softwater lakes, driven by strong atmospheric deposition of $(NH_4)_2SO_4$ and H_2SO_4 (Schuurkes *et al.* 1987a, Schuurkes *et al.* 1988). NH_4^+ deposition was strongest in agricultural areas (Schuurkes *et al.* 1987b) and led to acidification through nitrification. A temporary increase of CO_2 often occurred due to protonation of soil CO_3^{2-} and water-dissolved HCO_3^- (Roelofs 1983, Roelofs *et al.* 1995, Smolders *et al.* 2002). Yet before this period (mid 20th century), it is possible that a moderate N-enrichment and subsequent acidification (e.g. to pH 4.5-6) in agricultural areas made these lakes optimal for *Eleocharis multicaulis*. This is a possible explanation why Pietsch (1985) and de Lyon & Roelofs (1986) found a significant effect of CO_2. The NH_4^+/NO_3^- ratio became high as acidification got more intense (1970s and 1980s). Our survey revealed that the high NH_4^+/NO_3^- ratio is detrimental to the species, from which we hypothesize that de Lyon & Roelofs (1986) made their measurements during a transitional stage. It could be that *Eleocharis multicaulis* disappeared soon afterwards from these sites, as was observed by van Beers (1994) for the South of The Netherlands.

Acidification may have caused fixed Si to dissolve in the interstitial water, and leach from the system. Extraction-Si mainly includes interstitial Si, while digestion-Si is the amount yielded by extreme weathering, as can be obtained by intense acidification. We presume that we measured the lowest digestion-levels and the highest extraction-levels in the historically most acidified places. As Si is an accurate predictor of the performance of *Eleocharis multicaulis*, the intensity of historical acidification would be of utmost importance to understand the species' current distribution.

In more recent times, N deposition levels have become lower as a consequence of better fertilization practice and reduced emissions from the energy and transport sector (Erisman & Bobbink 1997, Van Laer & Van Steertegem 2003). Agricultural eutrophication is nowadays often linked with an increase of alkalinity, due to the inlet of buffered water (e.g. Vanderhaeghe 2000). The fact that acidification was most intense in agricultural areas, could explain why Si is now a general measure of agricultural eutrophication and water alkalinity.

Redox state

In the lakes we studied, we found a positive correlation between the NH_4^+/NO_3^- ratio and pH ($r = 0.36$; $P = 0.01$), indicating that a high ratio is not an indication of an acid state anymore. We believe that high values are nowadays caused by a low redox potential and subsequent reduced nitrification. The system can turn into a low redox state through several processes. The first is oxygen consumption, caused by a high decomposition rate of organic substrates under supply of mostly agricultural P, K and buffer capacity (Lamers *et al.* 1998, Smolders *et al.* 2002). Secondly, reduced oxygen-rich shallow groundwater flows can lower the redox state. Flow reduction is caused by the drainage of catchment areas. Finally, damped water level dynamics also induce a lower redox potential. For *Eleocharis multicaulis*, we did indeed find an optimal seasonal water level change of ca. 25 cm ($P < 0.1$) and an optimal alkalinity of only 60 to 80 μeq L^{-1}. Also Dierssen (1975), Schaminée *et al.* (1992) and Runge (1996) stress that water level dynamics are important. Furthermore, oxic summer conditions will immobilize PO_4^{3-} as insoluble $FePO_4$ due to the oxidation of Fe^{2+} (Smolders *et al.* 2002, Lucassen *et al.* 2004). They will also prevent reduction of sulphate and consequential accumulation of phytotoxic sulphide in organic top layers (Smolders *et al.* 2003).

Base richness

Despite the species' negative reaction towards base cation richness, it seems to prefer sites with base cation levels in winter that are not much lower than in summer, and preferably higher, as expressed by its positive reaction to seasonality variables of base cations. An increase of base cations during winter probably prevents extreme acidification of the system. In sandy lake shores these base cations can be provided by discharge of shallow, moderately base-rich groundwater. This is in agreement with Schaminée *et al.* (1992, 1995a), who mention mixed influence of base-poor rainwater and base-rich groundwater as an optimal situation.

On average, we found lower macro-ionic values than de Lyon & Roelofs (1986) and especially Pietsch (1978) (*Table 2.1*). We presume that this is the consequence of leaching of base cations from the system in times of strong acidification. Probably, acidification first caused an increase of most ions in the surface water, due to the protonation of soil particles. Being dissolved, these ions were subject to gradual removal from the system. Also, data from a softwater lake district at Turnhout (northern Belgium) show a continuous decline of ion concentrations from 1973 to 2000 (Vanderhaeghe 2000). Beside this, it is unclear which analytical methods Pietsch (1978) used, so that comparison of some concentrations may be unreliable.

Organic top layer

The species' predominant occurrence on organic sediment was also mentioned by Dierssen (1975), Pietsch (1978), Szmeja & Clément (1990), Vahle (1990), Kaplan (1992), Drengemann *et al.* (1995) and Urban (1999). The opposite view of Schaminée *et al.* (1992, 1995a) may have arisen because they considered this species together with other species of the *Eleocharitetum multicaulis* association like *Deschampsia setacea*, which may be more intolerant of an organic top layer. However, growing on an organic top layer has the risk of living in an NH_4^+ and PO_4^{3-} rich environment if this layer is decomposed under anoxic conditions. Oxygenation of the organic substrate, limited P and K supplies or restriction of buffer capacity might therefore be prerequisites for *Eleocharis multicaulis*.

Competition

In contrast to statements in vegetation science (Pietsch 1978, Schaminée *et al.* 1995a), *Eleocharis multicaulis* is not a real pioneer species; it has its optimum in a succession stage with an established vegetation under which an organic layer has already developed. Our study indicates that *Eleocharis multicaulis* can be a competitive dominant. The optimum of *Eleocharis multicaulis* at NH_4^+/NO_3^- ratios between 0.1 and 1 and at higher NO_3^- levels, and the coinciding retreat of *Hydrocotyle vulgaris*, may indicate that the former is a competitive dominant over the latter under these circumstances. Such an environment will exist as long as acidification is not too strong. The negatively associated species *Hydrocotyle vulgaris*, *Agrostis canina* and *Hypericum elodes* are apparently limited by P or K, as their presence profiles for N/P and N/K (NaCl-extraction) have the inverse form of that of *Eleocharis multicaulis*. For all these species, the regions of low presence are probably due to competitive suppression.

Conclusions

The main results and ideas in this paper are:

- *Eleocharis multicaulis* performs best in an established vegetation where an organic top layer has formed;
- *Eleocharis multicaulis* profits from N input as long as NO_3^- dominates over NH_4^+ and P and K remain poorly available;
- the dominance of NH_4^+ over NO_3^- in the water is negatively related to the species' performance, and can be caused by insufficient water level dynamics, rapid oxygen consumption in organic top layers due to P-K eutrophication in buffered conditions, or by intense acidification through strong atmospheric N deposition;
- *Eleocharis multicaulis* seems to profit from replenishment with base cations by shallow groundwater flows during winter, although the resulting level should remain low in order to maintain an acidic environment;
- intense historical acidification was probably detrimental to the plant and may have strongly influenced its current distribution;
- both Si-determinations of the mineral soil (digestion and NaCl-extraction) may reflect this historical acidification;
- *Eleocharis multicaulis* seems to gain competitive dominance at higher NO_3^- levels in the water, when NO_3^- dominates over NH_4^+, as reflected by its reponse to the NH_4^+/NO_3^- ratio;
- *Eleocharis multicaulis* seems to suffer from competition with *Hydrocotyle vulgaris*, *Agrostis canina* and *Hypericum elodes* when (agricultural) supply of P or K is too high or when redox potential is not high enough to immobilize soil P;
- both Si-determinations of the mineral layer (digestion and NaCl-extraction) may reflect this agricultural influence. This correlation is supposed to be an inheritance of former intense acidification in the same areas.

Experiments could be carried out to confirm hypotheses on nutrient limitation and competition. In order to establish the link with agriculture, a field investigation should be done, relating site parameters like Si, N, P and K levels to landscape variables such as distance to agricultural land and inlet of eutrophied water.

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3

Coexistence and niche differentiation at large spatial scale in a West-European softwater plant community

with Sofie Ruysschaert, Leon van den Berg, Jan Roelofs,
Fons Smolders and Maurice Hoffmann

Submitted

Without tests to determine whether the model usefully describes reality,
theoretical models, like outdated video games, tend to accumulate.

Keddy (2005)

Lake shore at Cilan, Abersoch, Wales

CHAPTER 3

Coexistence and niche differentiation at large spatial scale in a West-European softwater plant community

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Abstract

Aim: The niche concept is used at two very different spatial scales, each representing an ecological discipline on its own. It is rarely questioned in literature whether the results of species distribution models at the biogeographical scale fit with the expectations of niche differentiation at the community and metacommunity level. It is rarely questioned in literature whether at these scales, competitive trade-offs drive niche separation between species of the same vegetation type. We aimed at testing the hypothesis of niche differentiation at a larger scale with a dataset of semi-aquatic plant species of softwater lakes.

Location: Western Europe.

Methods: We collected plant cover data and environmental variables from 79 plots ranging over Atlantic Europe, to which most of the characteristic species of the investigated plant community are confined. Five species were selected for their relative frequency and wide geographical distribution within the dataset. Their niches were modelled both from presence-absence data and from ordinal abundance data, using mixed regression techniques (generalized linear mixed models and proportional odds mixed models, respectively).

Results: The modelled realized niches differed among the species on the West-European scale, although strict separation was not shown. Important parts of the modelled realized niches were provided by the large geographical extent of the sampling scheme. Opposite dominance hierarchies were observed depending on the resource gradient considered. A possible explanation is that competitive trade-offs exist for access to these different resources, conferring competitive advantage of one species over another for one resource, whilst the opposite is true for another resource. Plant strategy characterization of the species supported the assumption that functional traits underpin niche differentiation among species through fitness trade-offs.

Main conclusions: Overall, the paradigm of coexistence through niche differentiation was supported by the results. Mechanistic experimental research on a large spatial scale is needed to further test our propositions. We suggest that this research path would greatly benefit insights in community biogeography.

Introduction

Understanding composition and spatial heterogeneity of plant communities requires a fundamental knowledge of the concept of a species' niche (Mueller-Dombois & Ellenberg 1974, Austin & Smith 1989, Amarasekare 2003, Silvertown 2004). Following the view of Hutchinson (1957) and Silvertown (2004) the niche is described as the abstract hypervolume defined by axes of conditions and resources within which populations of a species can maintain a long-term average net reproductive rate ≥ 1. Presently, the niche concept is used at two very different spatial scales, each representing an ecological discipline, each with its own interests.

At the local scale, niches are a convenient instrument to describe conditions under which species survive, reproduce and coexist in a community. The concept is especially used to visualize how biotic interactions shape the species' realized niche, in relation to its physiological capabilities, also known as its fundamental niche (Austin 1990, Keddy 2001). Why species can be the 'winner' only in a *part* of their fundamental niche, is supposed to be linked to an inherent trade-off (Silvertown 2004, Kneitel & Chase 2004), e.g. a high ability to quickly take advantage of new light and nutrient supplies would not be compatible with a high ability to compete in a more resource-limited environment. Particularly, there is growing evidence that species are able to coexist in communities through niche separation (McKane *et al.* 2002, Levine & HilleRisLambers 2009), in the case of resource gradients often referred to as resource partitioning (Tilman 1982).

At a much larger spatial scale, the realized niche concept has been broadened (Colwell & Rangel 2009) in order to apply to the niches that are modelled by species distribution models at biogeographical scale (also referred to as habitat suitability models or ecological niche models; Guisan & Zimmerman 2000). This concept allows for dispersal limitations and local extinctions. As long as the degree of regional or large-scale dispersal of species between communities is not very high, local sites will not hold all species to which the environment is suitable. Hence, the competitive forces that shape the species' realized niches will differ from site to site, resulting in wider realized niches at large scale compared to the local scale. It can therefore be expected that modelled realized niches of species from the same community may be quite similar at a large scale, as opposed to the local-scale niche differentiation imposed by competition.

However, another view exists that contrasts with this expectation, though. In recent times, theory has been developed for metacommunity ecology, but has hardly been tested (Leibold *et al.* 2004, Chase 2005). A metacommunity is the set of local communities that are linked by dispersal of multiple potentially interacting species (Wilson 1992), and therefore applies to many vegetation types at their range scale. The few investigations that have been done to test for mechanisms of regional coexistence of species, mostly found evidence for niche differentiation (Kneitel & Chase 2004), suggesting that species still show realized niche differences at larger spatial scale. Those cases represent the species-sorting paradigm through the spatial storage effect. Here, local patches only hold the best competitive species for that environment, but regional diversity and coexistence emerge from the environmental differences between the patches (Amarasekare 2003, Leibold *et al.* 2004). Competitive trade-offs between species that force them to specialize into different parts of the multivariate niche space, then become visible at larger scale only. Local coexistence can then still be possible though, as a consequence of fine-scale environmental heterogeneity (Mouquet & Loreau 2002), or because of source-sink dynamics, when continuous species exchange takes place between local patches through dispersal (mass-effects paradigm; Mouquet & Loreau 2002, Amarasekare 2003).

To our knowledge, it is not questioned whether the results of species niche modelling at biogeographical scale, fit with one of the above theories of niche differentiation in both local and regional scale community ecology. Similarly, it is not questioned whether competitive trade-offs are still responsible for niche separation at this scale. We therefore verified whether niche differentiation is still present at a biogeographical scale, within a community of semi-aquatic plant species. As competitive trade-offs are related to functional trait trade-offs (Kneitel & Chase 2004, Westoby & Wright 2006), we also tested whether large scale niche differentiation coincides with functional species classification. Several approaches are available for the functional classification of macrophytes: Grime’s (1979) triangular strategy scheme (Rørslett 1989, Murphy *et al.* 1990), a rectangular modification for macrophytes (Kautsky 1988) or starting from numerical classification of an elaborate trait dataset (Willby *et al.* 2000).

We collected a West-European dataset of a characteristic vegetation type of nutrient-poor softwater lake shores: the *Eleocharition multicaulis* Vanden Berghen 1969 alliance. This vegetation type is often brought into synonymy with the somewhat broader *Hydrocotylo-Baldellion* Tüxen & Dierssen 1972, and is part of the European habitat 3130 of Atlantic softwater lakes (European Commission 2007). The characteristic, amphibious species of this alliance are restricted to Atlantic Europe (Schoof-van Pelt 1973, Dierssen 1975, Pietsch 1977, Arts & Den Hartog 1990, Schaminée *et al.* 1992). The niches of these species have not been subject to much quantitative research (but see chapter 2 on the realized niche of *Eleocharis multicaulis,* the most characteristic species of the community). Several niche axes should be taken into account for characterization. Plants compete for a limited set of resources: light, nutrients and water. An additional important factor limiting the metabolism is temperature. In rooted (semi-)aquatic plants, a distinction must be made between nutrients acquired from the sediment (by root uptake) and from the surrounding water (by the leaves, including carbon dioxide) (Lacoul & Freedman 2006, Bornette & Puijalon 2011). They are further influenced by specific factors of the aquatic environment, especially by the presence and characteristics of an organic mud layer, and by pH and macro-ionic composition of the water layer. In amphibious species, also the length of the period during which they are emerged can play an important role (Bloemendaal & Roelofs 1988).

In the current study, we 1) determine the realized niche, in terms of occurrence and ground cover, of five selected amphibious perennials, within the geographical range of one vegetation type, the *Eleocharition multicaulis*; 2) seek for the existence of niche differentiation and resource separation at this large scale; 3) relate niche differentiation to strategy types, in order to infer a competitive trade-off for resource acquisition between neighbouring species.

Methods

Overview

To describe the realized niche of an entire species, the ideal approach is to sample along the whole biogeographical and environmental distribution range (Austin 2007, Franklin & Miller 2009). However, to verify niche differentiation within one vegetation type, it is necessary to focus only on sites and areas where this community is present. Therefore, we collected plant cover data and environmental variables from 79 plots ranging over Atlantic Europe, to which the characteristic species of the *Eleocharition multicaulis* are confined. Plots were kept small (2 m²) in order to grasp the possibly fine interdependences between environment, plant abundance and plant interactions. The five species were selected for their relative frequency and wide geographical distribution within the dataset. For the objective of niche comparison, we selected 39 environmental variables (*Table 3.1*) out of all initially measured environmental variables (see chapter 2). This selection was based both on the assumed primary importance of several variables for the physiology and ultimately, fitness of plant species (Austin & Smith 1989, Austin 2007, Franklin & Miller 2009), and on additional variables that had previously been shown to predict the cover of one characteristic species (chapter 2). As a proxy of diffuse competition, we also included total cover of the neighbouring species.

Comparison between species needs to be done in the same niche hyperspace. Therefore, the fitted niche models of different species contained the same predictors (species distribution models or habitat models, see Austin 2007, Thuiller *et al.* 2008, Elith & Leathwick 2009). Models were fitted to the data with as few predictors as possible, in order to minimize the degree of overfitting, using the Akaike Information Criterion (AIC) as the main criterion for model selection (Ginzburg & Jensen 2004, Johnson & Omland 2004, Zuur *et al.* 2009). We used generalized linear mixed models (GLMM, also known as mixed logistic regression; Raudenbusch & Bryk 2002, Bolker *et al.* 2009) for the presence-absence response as well as proportional odds mixed models (POMM; Guisan & Harrell 2000, Harrell 2001) to distinguish between cover classes. We considered the use of cover classes as ecologically more appealing as, under the assumption of equilibrium with the environment (Guisan & Zimmerman 2000), it reflects performance, and therefore fitness, a property preferred to describe a species' niche (Austin 1999, Thuiller *et al.* 2010). The models' predictive accuracies were evaluated and predictions for each niche axis were plotted in order to assess niche differentiation.

Table 3.1. The 39 environmental variables that were originally selected for analysis. C = chemical; P = physical; S = summer; W = winter.

Compartment	Type	Season	Variable	Abbreviation
Resource gradients				
Soil	C	S	Ammonium NaCl-extraction (µmol/kg DW)	S_AMMS
Soil	C	S	Nitrate water-extraction (µmol/kg DW)	S_NO3W
Soil	C	S	Phosphorus digestion (µmol/kg DW)	S_PD
Soil	C	S	Orthophosphate lactate-extraction (µmol/kg DW)	S_PO4L
Soil	C	S	Phosphorus NaCl-extraction (µmol/kg DW)	S_PS
Soil	C	S	Total N content (µmol/kg DW)	S_TOTN
Soil	C	W	Ammonium NaCl-extraction (µmol/kg DW)	W_AMMS
Soil	C	W	Nitrate water-extraction (µmol/kg DW)	W_NO3W
Soil	C	W	Phosphorus digestion (µmol/kg DW)	W_PD
Soil	C	W	Orthophosphate lactate-extraction (µmol/kg DW)	W_PO4L
Soil	C	W	Phosphorus NaCl-extraction (µmol/kg DW)	W_PS
Soil	C	W	Total N content (µmol/kg DW)	W_TOTN
Soil	P	S	Moisture (%)	S_MOIS
Surface water	C	W	Ammonium (µmol/L)	AMM
Surface water	C	W	Carbon dioxide (µmol/L)	CO2
Surface water	C	W	Nitrate (µmol/L)	NO3
Surface water	C	W	Orthophosphate (µmol/L)	PO4
Direct gradients				
Air	P	S	Max. Temperature of Warmest Month	BIO5
Air	P	W	Min. Temperature of Coldest Month	BIO6
Air	P	S	Mean Temperature of Warmest Quarter	BIO10
Air	P	W	Mean Temperature of Coldest Quarter	BIO11
Soil	C	S	Calcium NaCl-extraction (µmol/kg DW)	S_CAS
Soil	C	W	Calcium NaCl-extraction (µmol/kg DW)	W_CAS
Soil	C	S	N/P ratio (NaCl extraction)	S_NP
Soil	C	W	N/P ratio (NaCl extraction)	W_NP
Surface water	C	W	Alkalinity (µeq/L)	ALK
Surface water	C	W	Calcium (µmol/L)	CA
Surface water	C	W	pH	PH
Surface water	C	W	NH4/NO3 ratio	ANI

Compartment	Type	Season	Variable	Abbreviation
Indirect gradients				
Soil	C	S	Iron NaCl-extraction (µmol/kg DW)	S_FES
Soil	C	S	Loss on ignition (LOI) (%)	S_LOI
Soil	C	S	Silicon digestion (µmol/kg DW)	S_SID
Soil	C	W	Iron NaCl-extraction (µmol/kg DW)	W_FES
Soil	C	W	Loss on ignition (LOI) (%)	W_LOI
Soil	C	W	Silicon digestion (µmol/kg DW)	W_SID
Soil	P	-	Thickness of organic soil layer (cm)	OS_THIC
Surface water	C	W	Ion Ratio (Ca/(Ca+Cl))	IR
Surface water	P	S	Mean thickness of water layer (cm)	WATLATHS
Surface water	P	W	Mean thickness of water layer (cm)	WATLATHW

Studied community and location of plots

46 softwater lakes that harboured characteristic species of the *Eleocharition multicaulis* alliance, were selected within the geographical range of the plant community. This vegetation alliance is characterized by *Eleocharis multicaulis* (Smith) Desv., *Hypericum elodes* L. and *Scirpus fluitans* L. It belongs to the *Littorelletea* Koch ex Tüxen 1937, which consists of amphibious plant communities along oligotrophic to mesotrophic softwater lakes.

The vegetation type has an Atlantic distribution, concordant with the distribution of its characteristic species (see e.g. Schoof-van Pelt 1973, Dierssen 1975, Pietsch 1977, Pietsch 1985, Arts & Den Hartog 1990, Szmeja & Clément 1990, Schaminée *et al.* 1992, Rodriguez-Oubina *et al.* 1997). To sample the whole biogeographical range of the *Eleocharition multicaulis*, lakes were selected from Portugal to northwest Germany, including Portugal, Spain, the United Kingdom, France, Belgium, The Netherlands and northwest Germany (*Fig. 3.1*). Within each lake, mostly one or two plots of 2 m² were investigated. Their location was chosen based on the on-site variation in species composition. One ('pristine') plot represented the average species composition of *Eleocharition multicaulis* with minimal presence of highly productive negative indicator species like, e.g., *Agrostis canina* L., *Hydrocotyle vulgaris* L., *Juncus bulbosus* L., *Juncus effusus* L. or *Phragmites australis* (Cav.) Steud. When there was a vegetation gradient within the lake towards (visually) more productive vegetation, a second plot was selected to represent the higher production site, generally dominated by one of the aforementioned negative indicator species. In a few occasions, more than one 'pristine' plot was sampled if high spatial variation existed in the relative abundances of characteristic species. Since these plots were always chosen to represent a larger vegetation unit within the lake, the sampling scheme within a lake can be considered as being randomly stratified, with subjective definition of within-lake strata. Statistical interdependence between plots in a lake has been taken into account (see Model building). Although more independence between plots would have been the ideal design - by sampling only one plot per lake and obtaining a more equal geographic distribution - we were logistically constrained to sample no more lakes than the ones we accessed during one growing season. In this way, data were collected from 79 plots, all representing an *Eleocharition multicaulis* community, with an internal gradient from 'pristine' to more or less 'degraded'.

Data collection

In each plot, ground cover was visually estimated for all plant species during the summer of 2001 using the ordinal Londo cover-abundance scale (Londo 1984). For the current study, we selected five species for further examination: *Eleocharis multicaulis* and *Hypericum elodes*, both characteristic of the *Eleocharition multicaulis* alliance, and *Agrostis canina* L., *Hydrocotyle vulgaris* L. and *Juncus bulbosus* L. The latter three can dominate over characteristic species of the *Littorelletea* (e.g. Lucassen *et al.* 1999, Arts 2002), and are less confined to the environment of softwater lakes alone. The five species were selected for their frequency and geographical spread in the dataset (frequency of 58, 32, 46, 58 and 71 % of the sampled plots, respectively). Consequently, the dataset contains both presences and absences of the targeted species, necessary to define their realized niches. In order to avoid cover classes with too low frequency for analysis with POMM, the original cover classes were merged to: 0% (absent), 0-4.99% and 5-100% ground cover.

Both in summer of 2001 and during the following winter in 2002, surface water and soil samples were collected in each plot. Soil moisture content was determined by drying at 65° for 24 hours. Macro-ionic and nutrient concentrations were determined for water and soil. Soil ionic concentrations were obtained with several extraction methods and a digestion. Lucassen *et al.* (2004) was followed for determination of organic matter content (loss on ignition) of the sediment and inorganic carbon analysis of water samples. Further details of water and soil sample preparation and of chemical analyses are given in chapter 2. Because several lakes had dried out in summer, only winter measurements of surface water were retained for further analysis.

It is recommended to include relevant climatic variables when modelling a species' niche over a large geographical extent (Meyer & Thuiller 2006, Coudun & Gegout 2007, Franklin & Miller 2009). Therefore, we used four bioclimatic variables related to temperature, extracted from the WorldClim 1.3 grid (see *Table 3.1*; Hijmans *et al.* 2005). We did not take into account effects of precipitation because the focus was on semi-aquatic habitat, in which the availability of water is primarily controlled by topographic location and hydrological processes. However, the water layer acts as an indirect variable upon oxygen stress and nutrition of plants (Bornette & Puijalon 2011); therefore, mean height of the water layer above the plot was taken into account.

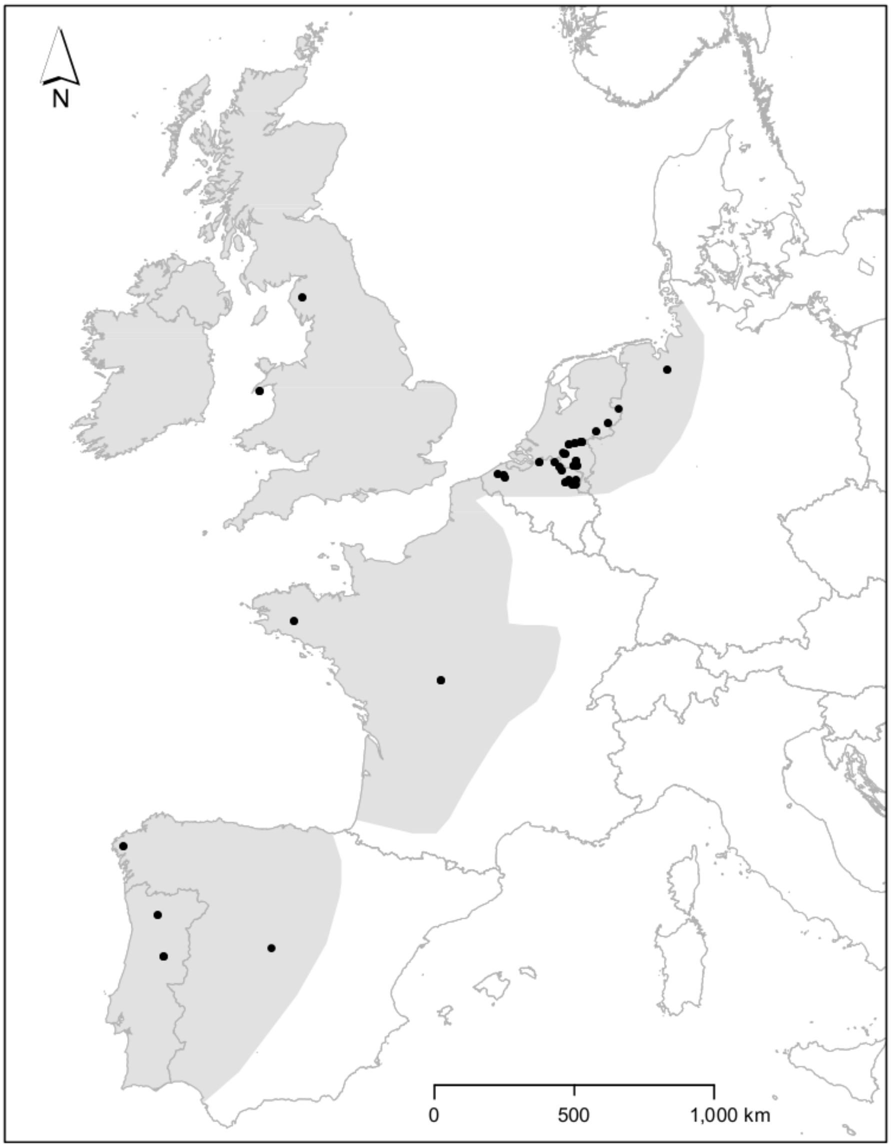


Fig. 3.1. Geographical location of the sampled softwater lakes (see Appendix 1 for more details). The shaded zone is the area where all three characteristic species of the Eleocharition multicaulis occur: Eleocharis multicaulis, Hypericum elodes and Scirpus fluitans (after Fitter 1978, Hultén & Fries 1986 and the GBIF data portal, data.gbif.org).

Species association

In order to assess local (plot) scale coexistence between the selected species in the dataset, gamma was used as a measure of ordinal association between species (Gonzalez & Nelson 1996; see below), applied both to presence-absence and to ordinal cover responses.

Variable selection

39 abiotic variables were selected, based both on the assumed primary importance of several variables for the physiology and fitness of plant species (Austin & Smith 1989, Austin 2007, Franklin & Miller 2009), and on additional variables that had previously been shown to predict the cover of one characteristic species (chapter 2). They can be classified according to their relation to plant performance, as shown in *Table 3.1*: 1) resource gradients, which reflect an essential resource for plants (e.g., nitrogen ions, orthophosphate, carbon dioxide); 2) direct environmental gradients, which directly influence plant physiology (e.g. pH, temperature); 3) indirect environmental gradients, which have no direct influence on the plant's physiology, but are potentially related to its performance through a related, more proximal (direct or resource) environmental variable (e.g. the thickness of an organic soil layer presents an indirect gradient, which affects the availability of nutrients and oxygen to plant roots, as well as the accumulation of toxic compounds).

Chemical concentration variables from water and soil, except pH, were log-transformed to give more weight to differences in the more abundant, lower concentration classes. A preliminary correlation analysis of the 39 variables of interest led to the exclusion of redundant variables. Only those redundant variables were discarded that showed 1) no univariate response (cf. chapter 5) and were correlated with a more direct or resource variable or 2) were strongly correlated with the same variable in the other season (some soil parameters). The remaining 20 predictors are shown in *Table 3.3*, supplemented by four of the 19 discarded variables (from the previous step), of which the collinearity with one or several of the selected variables can be useful for later interpretation. Eight predictors were further removed by an analysis of the variance inflation factors (VIF), in order to minimize multicollinearity among predictors for the regression models (Zuur *et al.* 2010). This was done in an iterative cycle in which the predictor with the highest VIF was eliminated and the VIFs for the remaining predictors recalculated each time, until all VIFs had dropped below 2. The remaining 12 predictors, supplemented by the cover of neighbouring species as a biotic factor, were used in model fitting. The cover of neighbouring species, which differs per target species, was not correlated with the other predictors, and had a VIF below 2 as well.

Model building

Plots within the same lake are not statistically independent. Therefore a random factor 'lake' was incorporated in the models. In the binomial GLMM models, several types of spatial covariance structure were tested (Zuur *et al.* 2009: chapter 4) and evaluated using AIC. These covariance structures fit spatial correlation between residuals. The results showed that the simpler models without the spatial interdependence factors best fitted the data, which can be explained by the hydrological independence between the sampled lakes, and because we sampled within one vegetation type. Hence, the models shown in this study do not include spatial interdependence factors.

Both binomial GLMM (also known as mixed logistic regression) and POMM models were built, using a logit link function. We evaluated the statistical assumptions of both full and final (reduced) models (Harrell 2001, Zuur *et al.* 2009). This entailed the modelling of non-linear relationships with polynomial terms. An examination of spatial interdependence among residuals for the models was done by inspection of the spatial residual pattern (Franklin & Miller 2009), but the earlier findings of no spatial intercorrelation were confirmed. The models were fitted in a backwards elimination procedure, starting from the set of 1 biotic + 12 abiotic predictors. Model reduction was controlled stepwise by evaluation of AIC values (Zuur *et al.* 2009) and by a likelihood ratio test (LRT) between the model with and without the predictor in question (predictor eliminated when $P > 0.05$ for all species). Comparison between species needs to be done in the same niche hyperspace (see also Guisan & Harrell 2000). Therefore, for each model type (GLMM / POMM), backwards elimination was done simultaneously for the five species, and in each step the predictor was chosen that gave the best average result for AIC and LRT. The procedure was stopped when eliminating a predictor significantly reduced the fit of at least one species' model.

In this way, for each species one GLMM and one POMM model was obtained, each with the same predictors for the five species. The results were plotted as probabilities of occurrence (GLMM) or of cover classes (POMM), separately for each predictor, setting the other predictors at constant values. We opted for the use of the outer and central border (Heegaard 2002) to set the lower limits of the visualized species niche. For probabilities of species occurrence (GLMM), we used the central border ($e^{-0.5}$). As the probabilities of the cover classes 5-100, 0-5 and 0 in POMM sum to one for each state of the environmental predictors, probabilities for each cover class are lower than probabilities of occurrence. Therefore, the outer border (e^{-2}) has been used to truncate the graphed niche calculated by POMMs.

Model evaluation

Assessment of predictive accuracy can best be done using independent test data (Franklin & Miller 2009). However this requires large datasets, which come at costs, time and effort. For our data, it was therefore better to use all data to train the models and use a resampling procedure to test the degree of overfitting (Vaughan & Ormerod 2005). We applied a bootstrap resampling procedure using 200 independent bootstrap samples (of size 79) for each model, independently between models (2000 bootstrap samples in total). To each bootstrap sample, a model was fitted from the already selected polynomial combination of predictors. We followed Verbyla & Litvaitis (1989) and Steyerberg *et al.* (2003) for the calculation of the results. First, the accuracy statistics (see below) were calculated for the original dataset. These are denoted as *apparent performance*, because they involve an unknown degree of overfitting. Then the same statistics were calculated for each bootstrap model, evaluated both from the corresponding bootstrap data (*bootstrap performance*) and the original data (*test performance*). The difference between the bootstrap performance and the test performance, averaged over the 200 bootstrap models for each original model, is a measure of overfit of the originally calculated accuracy statistic (apparent performance). Here we report apparent performance, test performance and overfit for each accuracy statistic.

An accuracy assessment should measure two aspects (Vaughan & Ormerod 2005): discriminatory ability, the degree to which the model correctly ranks the responses, and calibration, the numerical accuracy of the predictions. We will use the prefix ‘predictive’ to these terms when we refer to the test performance of the corresponding statistics. We only used threshold-independent accuracy statistics which can be used both in binomial GLMM and in POMM. Therefore, they are comparable between all models.

As a discrimination statistic, we selected the concordance index, which is non-parametric (c-index; Harrell 2001). It is calculated from an ordinal association measure δ as 0.5 + δ/2. Values close to one represent accurate discrimination, a value of 0.5 means the prediction ranks do not in any way relate to the observed response rank. Several ways are available to calculate δ, depending on the way ties are taken into account in the fitted and the observed response, respectively (see Gonzalez & Nelson 1996). We used gamma as the association measure, which doesn’t penalize ties in either type of response variable, because this would lead to undesired artefacts in the bootstrap performance calculation. Gamma is defined as (C-D)/(C+D), where C is the number of concordances and D the number of discordances, when comparing couples of rank numbers between the observed and fitted response. Gamma is the preferred ordinal association measure here, as it does not take into account couples with ties in either the observed or fitted response. Ties that are the consequence of methodological aspects must be regarded as artificially introduced, and should therefore be excluded from the association measure. More information on gamma, the concordance index and other association measures can be found in Gonzalez & Nelson (1996), Guisan & Harrell (2000), Harrell (2001), Vaughan & Ormerod (2005) and Franklin & Miller (2009). It should be noted that the concordance index for a logistic regression model is identical to the area under the ROC curve (AUC), an often used accuracy statistic in presence-absence species distribution models.

As a calibration statistic, we calculated mean calibration, which we defined as the mean predicted response divided by the prevalence of the species. Values close to one correspond to an accurate calibration. The mean predicted response is the mean probability of occurrence in GLMM and the cumulative mean probability for each of both cover classes higher than zero abundance, in POMM. The prevalence is the ratio of observed presences to sample size; for POMM this was applied accordingly to the cover classes in a cumulative way. Furthermore, for GLMM and for each cover threshold in POMM, we inspected calibration plots (Harrell 2001, Vaughan & Ormerod 2005) to assess calibration conditional on the predicted response. This was done for the original models and dataset only.

Species' functional characterization

We applied the functional classification system for macrophytes by Kautsky (1988). It is an extension of the triangular scheme of Grime (1979) with a fourth 'stunted' strategy, occurring in an environment that combines disturbance *and* stress. The strategies are competitive (C), ruderal (R), biomass storer (B) and stunted (S), in which biomass storer is the equivalent of the stress-tolerant strategy of Grime (1979). Several functional traits of the considered species were determined using the LEDA-database (Knevel *et al.* 2003, Kleyer *et al.* 2008) and literature (Grime 1988, Willby *et al.* 2000). The species were then assigned to one or more strategies applying Table 2 of Kautsky (1988).

Software

All data exploration, variable selection and modelling steps were written in the free and open source statistical package *R version 2.14.0* (R Development Core Team 2011), additionally making use of functions from the packages *lme4* for GLMM (Bates *et al.* 2011), *ordinal* for POMM (Christensen 2012), *boot* for bootstrap resampling (Davison & Hinkley 1997, Canty & Ripley 2012) and *Hmisc* for the calculation of the c-index (Harrell & with contributions from many other users 2012). For graphical presentation, the *ggplot2*-package was used (Wickham 2009).

Results

The species association analysis indicated that *Hypericum*, *Agrostis* and *Hydrocotyle* tend to grow together on the plot scale. They showed a positive intercorrelation for their cover response (*Table 3.2*). *Eleocharis* had a weaker, but positive plot-scale association with *Juncus*. No further association was found between both groups, so sometimes species from both groups co-occur, and sometimes they don't.

Both the GLMM and POMM final models ended with the same variable subset, consisting of five variables: the four surface water variables ammonium, calcium (second degree), orthophosphate (second degree) and ammonium/nitrate ratio, plus the total soil silicon content in winter. Several predictors did not significantly contribute to our models and were discarded: cover of neighbouring species, carbon dioxide (surface water), soil ammonium, soil nitrate and soil orthophosphate, both bioclimatic temperature variables and the height of the water layer in winter. From *Table 3.3*, it is clear that most correlations exist within the group of surface water variables and within the group of soil chemical variables, and rarely between both groups. In the surface water, calcium correlates with pH, alkalinity and ion ratio. This represents most of the higher correlations among the surface water variables. On the other hand, soil chemical variables showed strong intercorrelation, but the one retained soil variable, silicon content, was only correlated with water pH. The spatial variation of the selected predictors was considerable, with high ammonium and silicon values mainly occurring in Belgium and The Netherlands.

Table 3.2. Association analysis between the five species within the plant community, using the gamma coefficient. Top-right represents gamma for presence-absence response; bottom-left for cover response. Dark shaded: P < 0.05, light shaded: P < 0.10, in Pearson correlation tests.

Eleocharis	0.05	-0.20	0.13	0.50
-0.06	***Hypericum***	0.59	0.71	0.19
-0.19	0.51	***Agrostis***	0.66	0.30
0.13	0.60	0.53	***Hydrocotyle***	0.17
0.47	0.05	0.07	-0.07	***Juncus***

Table 3.4 shows the predictive accuracy results for these models. The predictive discriminatory ability of the models was moderate (though rather poor for *Juncus*) and comparable between GLMM and POMM. The predictive calibration as well as the calibration plots (not shown) were very good for the POMM models and slightly less for the GLMM models (however poor for *Hypericum* GLMM). The degree of overfitting was much less in the POMM models than in the GLMM models, both for the concordance index and the calibration index. For the concordance index this was due to the higher apparent performance in the GLMM models, which is, as such, misleading.

Table 3.3 (next page). Pearson correlation between 24 environmental covariates of ecological interest (after transformation). Boldface coefficients are higher than 0.5 (as absolute values); for abbreviations of variables, see Table 3.1. To get this subset, several variables were removed from the list in Table 3.1, based on correlations between summer and winter variables and chosen according to strongest univariate response pattern. The variables are ordered according to environmental compartment and plant ecological role (R: resource gradients, D: direct gradients, I: indirect gradients). Further predictor selection steps are explained in the legend.

Swatch	Description
1	Further removal based on correlation
2	Removed by an iterative procedure based on the variance inflation factors (VIF)
3	Removed in the model building process, based on AIC and LRT
4	Predictors in the final models

Air →		Surface water →										Soil →											
D →		R →				D →				I →		R →						D →	I →				
BIO10																							
0.37	BIO11																						
-0.08	-0.19	AMM																					
0.05	-0.11	0.04	CO2																				
-0.07	0.10	0.45	-0.12	NO3																			
-0.08	-0.08	0.26	0.04	0.22	PO4																		
0.24	0.10	-0.22	**0.51**	-0.08	0.07	ALK																	
0.18	-0.02	-0.06	0.45	0.00	-0.04	**0.64**	CA																
0.11	0.24	-0.30	0.25	0.03	-0.01	**0.85**	**0.52**	PH															
0.01	-0.26	0.33	0.16	**-0.69**	-0.02	-0.09	-0.06	-0.28	ANI														
0.33	-0.17	-0.15	0.42	-0.06	0.01	**0.74**	**0.84**	**0.58**	-0.06	IR													
0.16	0.12	0.24	-0.09	0.40	0.12	-0.13	-0.01	-0.12	-0.22	-0.03	WATLATHW												
0.02	0.17	0.19	-0.01	0.00	-0.26	-0.13	-0.04	-0.17	0.15	-0.21	-0.12	S_AMMS											
0.00	0.15	0.03	-0.04	-0.13	-0.10	-0.12	-0.10	-0.10	0.16	-0.16	0.03	0.39	S_NO3W										
-0.19	0.27	0.13	-0.02	0.03	-0.08	-0.12	-0.25	0.00	0.07	-0.40	-0.05	**0.54**	0.42	S_PD									
-0.10	-0.08	0.39	-0.09	0.21	0.22	-0.26	-0.15	-0.24	0.09	-0.17	0.07	0.44	0.39	0.50	S_PO4L								
-0.09	0.08	0.09	-0.05	0.08	-0.10	-0.05	0.03	0.11	-0.01	-0.03	-0.04	**0.57**	0.45	0.48	**0.62**	W_PO4L							
0.02	0.00	0.17	0.17	0.07	-0.18	0.10	0.21	0.10	0.06	0.15	-0.03	**0.67**	0.27	**0.57**	0.46	**0.58**	W_PS						
0.20	0.07	0.00	0.25	-0.06	-0.29	0.24	0.44	0.20	0.06	0.34	0.04	**0.55**	0.22	0.39	0.24	0.43	**0.76**	S_CAS					
-0.08	-0.21	0.22	0.02	0.01	0.04	-0.41	-0.28	**-0.58**	0.16	-0.33	-0.01	0.12	0.14	0.24	0.20	-0.21	0.07	-0.01	W_SID				
-0.13	0.16	0.19	0.01	-0.01	-0.11	-0.26	-0.24	-0.21	0.16	-0.41	-0.10	**0.70**	0.42	**0.85**	0.47	0.49	**0.67**	**0.53**	0.37	S_LOI			
-0.15	0.17	0.16	-0.07	0.08	-0.11	-0.28	-0.29	-0.18	0.05	-0.41	-0.07	**0.62**	0.34	**0.86**	0.46	0.46	**0.72**	0.46	0.37	**0.92**	W_LOI		
-0.08	0.11	0.26	0.09	0.15	-0.18	-0.16	-0.03	-0.16	0.06	-0.21	-0.04	**0.76**	0.40	**0.78**	**0.60**	**0.53**	**0.78**	**0.63**	0.33	**0.85**	**0.81**	S_MOIS	
-0.31	0.16	0.11	0.00	0.20	0.01	-0.23	-0.24	-0.07	-0.12	-0.38	-0.01	0.41	0.21	**0.66**	0.28	0.25	0.47	0.25	0.23	**0.65**	**0.69**	**0.56**	OS_THIC

Table 3.4. Predictive accuracy evaluation of the binomial mixed GLM models (GLMM) and the proportional odds mixed models (POMM). Values are presented in the following order: apparent performance / test performance / overfit. For a definition of concordance index and mean calibration, see text. Apparent performance compares the original data with the fitted model (how well does the model perform for the data?), test performance compares the original data with the bootstrap model (how well do the bootstrap models perform for the data on average?), and overfit compares the difference between bootstrap performance and test performance (the degree to which the original model is overfitted according to the bootstrap method; see text for more details). The model's performance is to be assessed by its test performance (Verbyla & Litvaitis 1989).

GLMM [a]	*Concordance index*	*Mean calibration*
Eleocharis multicaulis	0.885 / 0.766 / 0.210	1.006 / 1.020 / -0.016
Hypericum elodes	0.967 / 0.768 / 0.222	0.944 / 0.614 / 0.359
Agrostis canina	0.848 / 0.770 / 0.209	1.000 / 1.028 / -0.028
Hydrocotyle vulgaris	0.974 / 0.888 / 0.105	1.012 / 1.028 / -0.024
Juncus bulbosus	0.707 / 0.663 / 0.310	1.000 / 1.144 / -0.127

POMM [b]	*Concordance index*	*Mean calibration of first threshold (high cover class)*	*Mean calibration of second threshold (high + low cover class)*
Eleocharis multicaulis	0.776 / 0.753 / 0.051	1.013 / 0.993 / 0.018	1.015 / 1.011 / 0.000
Hypericum elodes	0.803 / 0.763 / 0.078	0.984 / 1.001 / -0.013	0.986 / 0.988 / -0.001
Agrostis canina	0.764 / 0.738 / 0.068	1.021 / 1.044 / -0.013	0.994 / 0.987 / 0.012
Hydrocotyle vulgaris	0.860 / 0.832 / 0.049	0.968 / 1.018 / -0.032	0.994 / 1.000 / -0.003
Juncus bulbosus	0.643 / 0.631 / 0.096	1.016 / 1.039 / -0.023	1.005 / 0.993 / 0.011

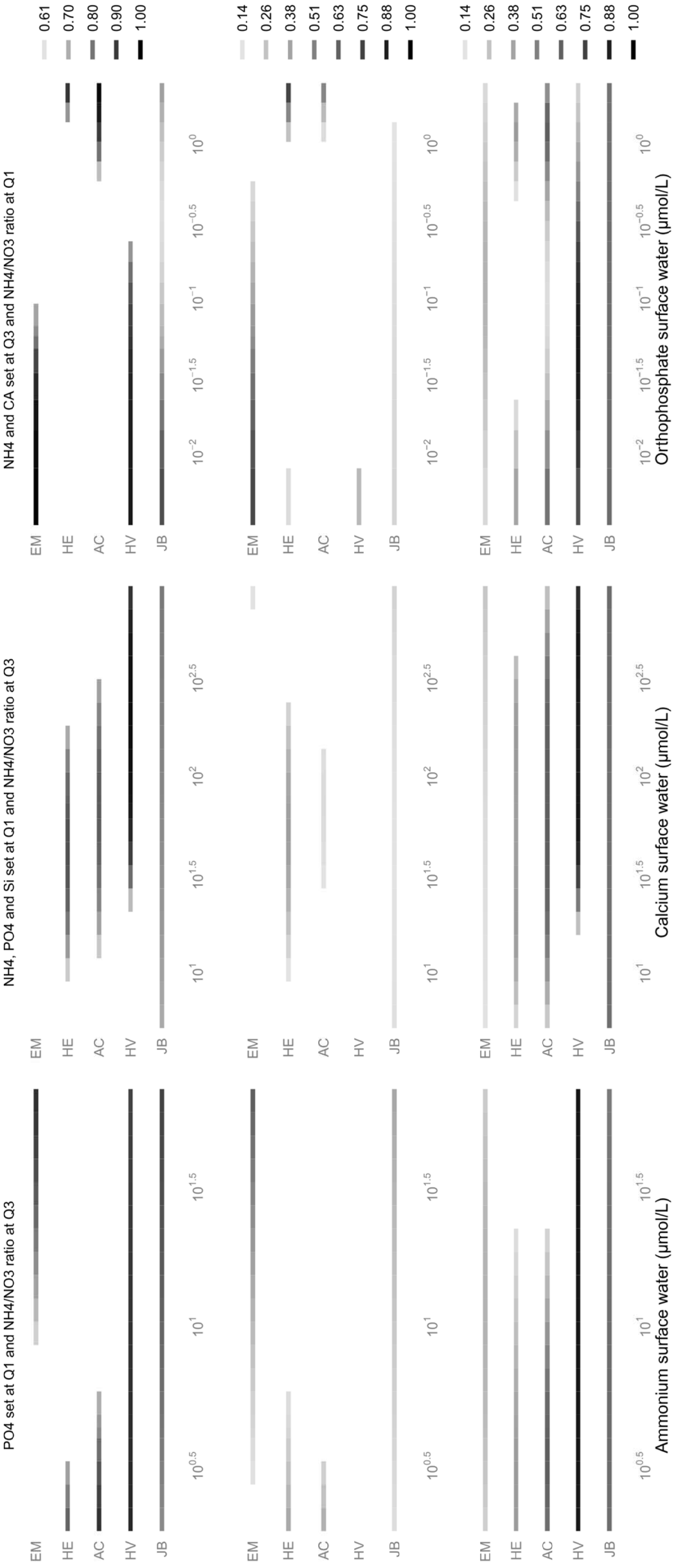
[a] *Generalized linear mixed models for a binomial response*

[b] *Proportional odds mixed models*

Fig. 3.2 shows the modelled realized niches for each predictor, at selected cross-sections defined by the other covariates. *Juncus* didn’t respond to most variables, except to the ammonium/nitrate ratio to which it had a positive presence response. On the contrary, *Eleocharis* was the most ecologically separated species compared to the remaining three species, and performed better at high ammonium concentrations but at a low ammonium/nitrate ratio (< 1), and at low orthophosphate concentrations and high silicon content. This species showed these responses both for its presence as for its high cover class, while the low cover class was a-specific.

Hypericum and *Agrostis* showed a negative response to ammonium and a positive response to ammonium/nitrate ratio and orthophosphate, at least in their response based on presence and high cover class, and with *Agrostis* always having a larger niche width for these variables. *Hypericum*, *Agrostis* and *Hydrocotyle* all showed a negative response to silicon for their presence response and low cover class, with *Hypericum* having the smallest niche width and *Hydrocotyle* the largest. All three showed an optimum for calcium, for which they can be ranked as *Hypericum* < *Agrostis* < *Hydrocotyle* (both for presence response and low cover class). Contrary to *Hypericum* and *Agrostis*, *Hydrocotyle* responded to orthophosphate in a negative way for all response types.

Fig. 3.2 (next two pages). The modelled realized niche of the five species. Predicted probabilities of occurrence (GLMM; first row), and of high and low cover classes (POMM; second and third row, respectively) are shown separately for each predictor. The probabilities are shown above a specific value (see Methods for further details). For each predictor, all other covariates are kept constant at their median observed value, unless stated otherwise. Because for several variables, working with median values results in predictions outside the niche of some species (near-zero probabilities), some graphs are for other than median values of the other covariates, i.e. at their first (Q1) or third quartile (Q3). EM = Eleocharis multicaulis, HE = Hypericum elodes, AC = Agrostis canina, HV = Hydrocotyle vulgaris, JB = Juncus bulbosus.


PO4 set at Q1 and NH4/NO3 ratio at Q3
NH4, PO4 and Si set at Q1 and NH4/NO3 ratio at Q3
NH4 and CA set at Q3 and NH4/NO3 ratio at Q1
EM
HE
AC
HV
JB
0.61
0.70
0.80
0.90
1.00
0.14
0.26
0.38
0.51
0.63
0.75
0.88
1.00
$10^{0.5}$
10^{1}
$10^{1.5}$
10^{2}
$10^{2.5}$
10^{-2}
$10^{-1.5}$
10^{-1}
$10^{-0.5}$
10^{0}
Ammonium surface water (µmol/L)
Calcium surface water (µmol/L)
Orthophosphate surface water (µmol/L)

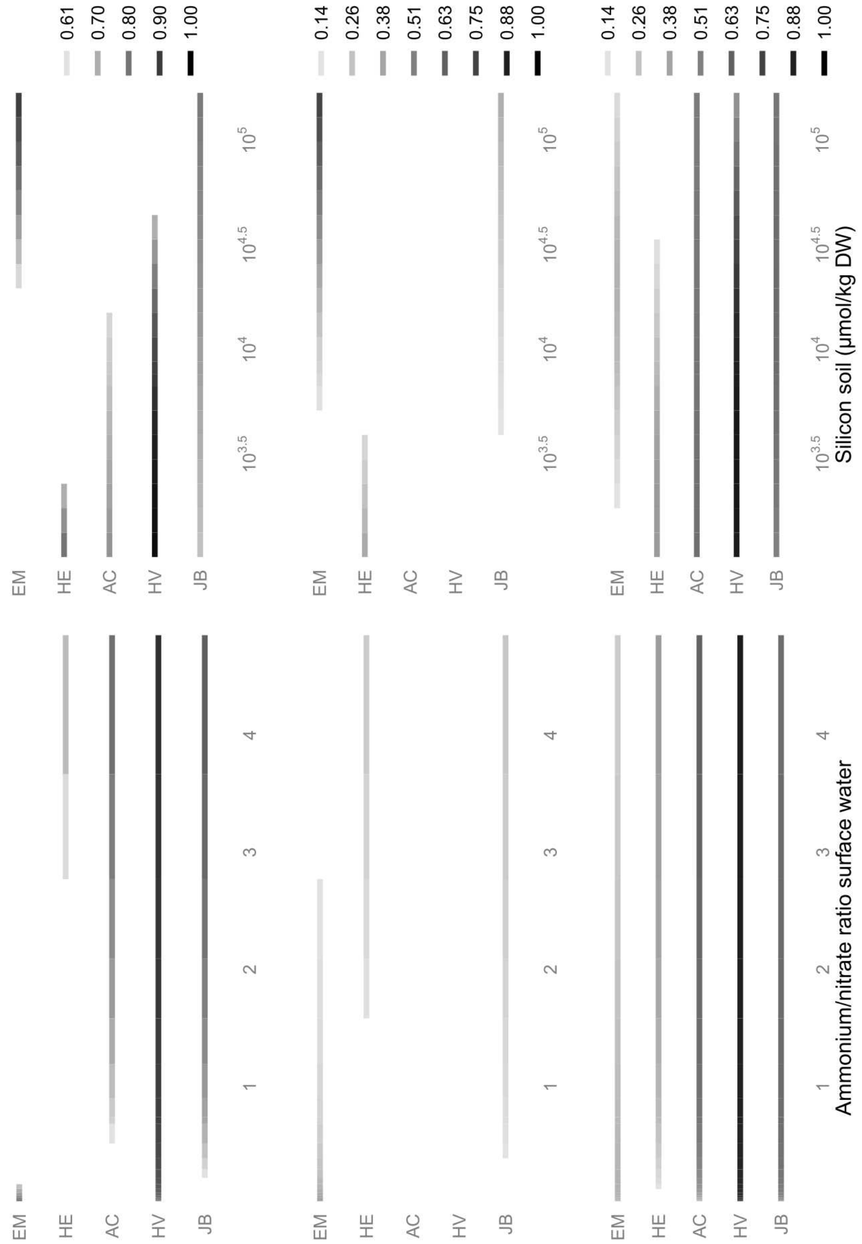


Fig. 3.2, continued.

Table 3.5 shows the functional characterization of the five species. All species have a mixed strategy. *Eleocharis* is functionally distinct from the other species, especially because it has no lateral spread, nor is vegetative reproduction important in the species. *Hypericum*, *Agrostis* and *Hydrocotyle* share many functional traits. Finally, *Juncus* has the widest functional characterization.

Table 3.5. Traits and strategies of the investigated plant species. All species are perennials with anchored leaves.

Plant trait	Corresponding challenge [a]	*Eleocharis multicaulis*	*Hypericum elodes*	*Agrostis canina*	*Hydrocotyle vulgaris*	*Juncus bulbosus*	'+' meaning:	Interpretation of '+' in PESS context [b]
Plant morphology								
Lateral spread	Persistence (space acquisition)		+	+	+	+	Present to extensive	Competitive / Biomass storer
Height	Persistence (competitive ability)	+	+	+	+	+	Higher than 15 cm	Competitive
Specific Leaf Area	Establishment (relative growth rate) & Persistence (competitive ability)		+				Higher than 40 mm^2/mg	Competitive
Heterophylly	Persistence (plasticity)					+	Present	Competitive
Carbon capture from air	Persistence (competitive ability)	+	+	+	+		Prevailing	Competitive
Life history								
Evergreen leaves	Persistence (stress tolerance)					+	Present	Biomass storer / Stunted
Onset of flowering	Persistence (disturbance avoidance)						Early	Ruderal
Seed investment [c]	Dispersal, establishment, persistance (disturbance avoidance)			+		+	Higher than 100 mm^3 of seeds per shoot	Ruderal
Vegetative reproduction	Persistence (disturbance tolerance)		+	+	+	+	Important	Ruderal
Life strategy (Kautsky, 1988)		**CS**	**CR**	**CR**	**CR**	**CBRS**		

[a] Gaudet and Keddy, 1988; Knevel et al., 2003; Tilman, 1988; Weiher et al., 1999; Westoby, 1998

[b] PESS = Plant Ecology Strategy Scheme. Interpretation after Grime, 1988; Grime, 2001; Kautsky, 1988; Maillette and Keddy, 1989; Murphy et al., 1990; Rørslett, 1989; Westoby, 1998; Willby et al., 2000, and using terminology of Kautsky, 1988

[c] Based on the product of estimated seed number per shoot and seed volume (LEDA-database)

Discussion

Species patterns

The realized niches for the tested species differ on the large (West-European) scale, although strict separation was not shown as *Juncus bulbosus* showed no clear pattern, and *Agrostis canina* and *Hypericum elodes* showed overlaps for every predictor. The species often coexisted, as no negative correlations were revealed (*Table 3.2*). Three species showed positive association, coinciding with a similarity of their modelled niches. Interestingly, the two species entitled 'characteristic' of the vegetation alliance (*Eleocharis multicaulis* and *Hypericum elodes*) showed an opposite response to several predictors. This had also been noted in a univariate analysis of a subset of the same data (Vanderhaeghe *et al.* 2005; see chapter 2).

Juncus bulbosus clearly had a wider niche width than the other species within the sampled environmental range. It was present in 71% of the plots. Therefore, the rather poor predictive performance of this species' model is not surprising, as the higher the presence of a species in an investigated sample or area, the more difficult it becomes to model its niche due to less absence records (Jimenez-Valverde *et al.* 2008, Franklin & Miller 2009).

From the spatial variation of the predictors (not shown) it is derived that important parts of the modelled realized niches were provided by the large geographical extent of the sampling scheme. Sampling smaller subsets of this extent only would have revealed a smaller part of the niche. This indicates that realized niches may become more similar among the species when larger areas are sampled. We expect that the degree of niche overlap between species in a vegetation type will become larger when more environmental states have been sampled in which the plant community is present. From our species-level approach, we cannot evaluate the possibility of locally adapted ecotypes on the West-European scale, the niches of which we would have aggregated. More sampling points would allow the modelling of regionally different realized niches. However, local adaptation of the niche can only be tested by growing or transplanting individuals into another environment.

It is obvious that the relatively 'rare' environmental conditions in the dataset might be at least equally important in modelling the niche as those that were measured more often, in Belgium and The Netherlands. On the other hand, rare points on the edges of more common environmental states receive proportionally greater importance in the models, as they have greater influence on parameter estimation. Hence, the applied techniques can be considered quite robust to unequal environmental stratification, and can be used to infer niches from the data. Apart from these general expectations, exploration of the dataset indicated no outliers for the retained environmental predictors. However, as a consequence of relative undersampling in large areas, it is possible that parts of the realized niche are still missed. Consequently, more data could enhance predictive accuracy, and hence, improve the niche models. The models should therefore not be regarded as definitive results for the species. However, we were logistically constrained to sample no more lakes than the ones we accessed during one growing season. Further, the vegetation in plots can be assumed to be independent between lakes, as dispersal between lakes is expected to be low for these plant species (Brouwer *et al.* 2002). The absence of residual spatial autocorrelation confirms this expectation.

Species distribution models are often used to project the biogeographically modelled niche to new places or times. This implicitly assumes that the whole of the modelled niche is an attribute of the species in an area, regardless of the geographical location. Further, an equilibrium is assumed between species response and environment, as measured at a specific time, often without taking into account dispersal constraints and other time-dependent processes (Guisan & Zimmerman 2000, Guisan & Rahbek 2011). It is also assumed that every part of the niche would be expressed at a site if the corresponding environmental state was there. This assumption requires this realized niche to be consistent with theory on coexistence and competition in local communities, i.e. to take into account how the presence of other species shapes the realized niche locally. Also, local adaptation of the niche cannot be considered in species distribution models when no specific experiments have been done. Consequently, it must be taken into account that these modelled realized niches hold a risk of overestimation when projecting to new places. Therefore, evaluation of model projections with complementary field data, whenever possible, remains critical.

Interpretation of the predictors

Considering the predictors retained in the models, we notice that both ammonium and the ammonium/nitrate ratio were selected, meaning that nitrate would have been equally informative in the model as the ammonium/nitrate ratio, in the presence of the predictor ammonium. Orthophosphate was a third resource that was effective in defining the realized niches.

Eleocharis multicaulis preferred a low ammonium/nitrate ratio. Ammonium is typically provided atmospherically in agricultural areas, and eventually leads to high ammonium/nitrate ratios in weakly buffered systems (Schuurkes *et al.* 1987b, Schuurkes *et al.* 1988, Bobbink & Roelofs 1995, Roelofs *et al.* 1996, Bobbink *et al.* 2010). It has become the most important acidifying substance in shallow softwater lakes in recent decades. High ammonium/nitrate ratios are detrimental to several plant species of nutrient-poor ecosystems (de Graaf *et al.* 1994, van den Berg *et al.* 2005, Stevens *et al.* 2011). *Eleocharis multicaulis* seems to be such a sensitive species, requiring at least as much nitrate as ammonium. On the other hand, *Hypericum elodes* and *Agrostis canina* preferred low surface water nitrogen concentrations. Their response to the ammonium/nitrate ratio is supposed to be less relevant, as it may be an artefact considering the low nitrogen concentrations at which they occurred. In literature, we did not find much quantitative information on the niche of the characteristic species *Hypericum elodes*, except in de Lyon & Roelofs (1986) where environmental data of this species are presented. These data coincide with the ranges we measured, but give less indication of the direction of trends.

It may surprise that only resource variables of the surface water were retained, and none of the soil compartment. It is known that rooted macrophytes obtain their nutrients both from water and soil. Moreover, the investigated amphibious species often grow in terrestrial circumstances during part of the year. It is therefore remarkable that water dissolved resources were better predictors than those available from the soil. It is possible that resource concentrations of soil pore water would have been good predictors. However not enough pore water data were available from the plots to incorporate this compartment in our research. It may be expected though, that these would correlate to some extent with the soil measurements in our study. Another possible explanation is that a significant positive nutrient flux through the upper soil was present and also determined plant performance. Fluxes have not been measured and are not necessarily correlated with one-time concentration measurements; so it is possible that we miss an important and independent resource supply dimension, among which species potentially separate. Further, soil organic matter content (percentage loss on ignition) or thickness of the organic soil layer were not selected either. These variables have been found to be important to other softwater lake species though, both negatively or positively, mainly depending on the chemical lability of the organic sediment and plant species (Raun *et al.* 2010, Pulido *et al.* 2011). We regard the presence of organic matter mainly as an indirect variable. In our data, it integrated two soil resources which were not significant to the species' responses: water (moisture) and total phosphorus content.

Probably because of these plants' access to aerial carbon dioxide, CO_2 content of the surface water was not retained in the model building process. In the case of *Juncus bulbosus* however, this is quite unexpected, as the species is known for its strong response to aqueous carbon dioxide (Svedäng 1990, Svedäng 1992, Roelofs *et al.* 1995, Lucassen *et al.* 1999). These investigations considered aquatic forms of the species, though. Hence it appears that the effect is not that strong at higher shore positions. Another possibility is that an effect would only be detected for a cover threshold higher than the 5% we used. However this would have compromised model accuracy in our case, because of the few observations with high cover.

Although we deliberately selected predictors to which a response might be expected, it is important to realize that the relationships are not necessarily causal. It could be that other correlated environmental variables, discarded in an earlier stage or not even measured, have an actual causal relationship to the species' response. This may for example be the case for surface water calcium, which strongly reflects pH and alkalinity in our dataset. Apart from nutrients, alkalinity and pH are known to explain much variation when considering the diversity among aquatic macrophyte communities (Bloemendaal & Roelofs 1988, Wetzel 2001 Smolders *et al.* 2002, Lacoul & Freedman 2006).

Total soil silicon content was the only soil variable in the niche models. It is the silicon concentration made available after digestion with a HNO_3-H_2O_2 solution. This variable is not an expected explaining variable according to macrophyte ecology literature (see, e.g., Smolders *et al.* 2002, Lacoul & Freedman 2006, Bornette & Puijalon 2011). Soil silicon, however, was negatively correlated with surface water pH. It is possible that pH is an actual causal variable, and that soil silicon better integrates past pH variability and was therefore more informative to the models than a one-time pH measurement. Soil silicon was highest in countries with more agricultural activity. In such areas, acidification is more likely to have occurred in isolated lakes due to atmospheric nitrogen deposition (Roelofs *et al.* 1996, Bobbink *et al.* 2010).

Incorporating plant interactions in species distribution models can be done either in a correlative manner by including the presence of other species (e.g. Leathwick & Austin 2001), or by including a mechanistic model component (Ferrier & Guisan 2006, Elith *et al.* 2010, Buckley *et al.* 2010). We incorporated cover of neighbouring species as a measure of the amount of diffuse competition. Remarkably, it was not a meaningful variable to delineate the realized niches of the five species. This is in agreement with what Austin & Nicholls (1988) found. These authors studied variation in the herbaceous layer of eucalypt forest in Australia. On a small plot scale, they found that all differences in species associations depended on environmental differences; hence the environmentally dictated pattern implicitly contained the results of potential species interactions. On the other hand, Leathwick & Austin (2001), in modelling the occurrence of *Eucalyptus* species, demonstrated that incorporating the modelled presence of a competitive dominant as a predictor significantly improved the niche models of some other species. Hence, it seems that adding other species' abundances sometimes does and sometimes does not result in more accurate predictions, perhaps depending on the environmental variables chosen in a species distribution model.

Mechanisms for niche separation

It has been demonstrated many times that occurrence and performance of species in a community most often is influenced by the presence of other species, so it may be assumed this process is at work in the studied (meta-)community. While species interactions will differ over the investigated range, an overall pattern of (partial or complete) separation of realized niches emerges in our case, showing a consistency with the paradigm of coexistence through niche differentiation, at least at regional level (Amarasekare 2003, Silvertown 2004, Kneitel & Chase 2004). Overlapping niches between the species may be due to the effect of the large sampling extent, potentially reducing the role of competitive restrictions on the large-scale niche. However, apparent niche overlap does not exclude the possibility of separation along other unmeasured environmental variables (see below).

As we measured the environment at plot-scale, it is unclear what was the mechanism for local-scale coexistence, as several species were associated within plots. Different mechanisms may be responsible, and one possibility does not exclude another. A first plausible mechanism is fine-scale resource heterogeneity of the environment regarding the niche axes considered in our study, where (slightly) different conditions in space promote coexistence of (slightly) differing specialists (Mouquet & Loreau 2002). Another possibility, compatible with the previous one, is that niche separation between partially overlapping species is actually complete along one or more unmeasured gradients, including functional ones such as spatial resource partitioning (e.g. rooting depth) or partitioning in time (see McKane *et al.* 2002). For example, it is not clear from our data along which environmental niche axis *Juncus bulbosus* would separate from the other four species. Still another mechanism is that moderate local-scale dispersal allows species to be present in different parts of the same environment in a stochastic manner. This is a mechanism often invoked for environmentally homogeneous environments (Kneitel & Chase 2004), and it has been proposed in neutral niche theory (Hubbell 2001). A combination of stochastic and deterministic processes is the most general case, and has been proposed as ‘stochastic niche theory’ (Tilman 2004). It also incorporates disturbances and time-lag responses of species, allowing more species to potentially coexist. Also species interactions by their own can generate dynamics through cyclic patterns in resource consumption, thereby promoting coexistence of more species (Huisman & Weissing 1999). Finally, a theoretical possibility is that regional dispersal frequently occurs between different communities, resulting in source-sink relationships from patches that are more favourable for a species to patches where the species is at physiological or competitive disadvantage. However, between-lake dispersal is probably unimportant, as this habitat is highly fragmented in space and several softwater macrophytes do not succeed to quickly recolonize sites after habitat restoration (Brouwer *et al.* 2002).

It is very remarkable that opposite dominance hierarchies were observed depending on the resource gradient considered (e.g. ammonium compared to orthophosphate). A possible explanation is that competitive trade-offs exist for access to these different resources, conferring competitive advantage of one species over another for one resource, whilst the opposite is true for another resource. A similar trade-off between different resources was recently shown for two grass species, in a competition experiment by Venterink & Güsewell (2010). They found that one species was a stronger competitor under phosphorus limitation while the other species was strongest under nitrogen limitation. The patterns we found within most gradients are compatible with assumptions of the competitive hierarchy model (Keddy 1989, Keddy 2001), in which dominant species with higher competitive ability outcompete subordinant species from a part of their fundamental niche, resulting in a competitive ranking along the resource gradient. In contrast with earlier studies however (Gaudet & Keddy 1988, Keddy *et al.* 1994, Rösch *et al.* 1997, Keddy *et al.* 2000) we found that the observed ranking can change and even reverse depending on the resource.

The mixed ecological strategy of the five species makes hypothesizing a ranking from traits not at all straightforward. The distinct strategy of *Eleocharis multicaulis* coincides with its observed pattern of niche separation. On the other hand, the three species that had a rather similar niche pattern also share many functional traits. Further, the broad characterization of *Juncus bulbosus* in terms of functional traits coincides with its wider realized niche. These findings support the assumption that functional characteristics underpin, at least in part, niche differentiation among species through fitness trade-offs.

Ideally, the study of patterns is done at multiple spatial scales in order to capture relevant mechanisms and to achieve generality (Levin 1992, Kunin 1998, Crawley & Harral 2001, Hartley & Kunin 2003, Rahbek 2005). Moreover, it must be kept in mind that multiple mechanisms may lead to a similar observed pattern. Experiments are the ultimate means of testing the relevance of a proposed mechanism. The attention given to competition for example, stems from a historical view that it is the most important biotic factor that structures communities. However, there is more and more evidence that the relative importance of several structuring mechanisms within a plant community varies, depending on the community (Lortie *et al.* 2004). Among biotic interactions, facilitation has increasingly received attention and is often encountered when looked for (Bruno *et al.* 2003, Brooker *et al.* 2008). Obviously, competitive trade-offs are not the only possible mechanism that leads to the observed pattern of niche differentiation and coexistence.

Conclusions

We found that the realized niches of five amphibious perennial species of the West-European softwater plant community *Eleocharition multicaulis* are best characterized by four surface-water variables - calcium and the resource variables ammonium, nitrate and orthophosphate - and by the soil silicon content, which is related to surface water pH. For four species, their realized niches were completely or partially separated, depending on species and gradient. One species (*Juncus bulbosus*) was rather insensitive to the measured gradients, but possibly separates along an unmeasured environmental or functional axis. Findings on a biogeographical scale were consistent with coexistence and niche theory within one vegetation type.

The observed niche differentiation can be seen as a competitive species ranking. We had indications of two types of trade-off that explain the patterns. First, we see a different, even reversed species ranking depending on the resource gradient, which may be the result of different abilities in obtaining the resource. Secondly, regarding functional characterization of the species, there was a consistency between functional species similarity and the similarity of the species' realized niches as perceived from the graphed model results.

How plant species can coexist in a community is a central theme in both theoretical biology and conservation ecology. For more than a century now, plant and vegetation scientists have sought for generally applicable rules about patterns and mechanisms of species coexistence and their absolute and relative performance. These are much needed if we want to advance predictive plant and vegetation ecology. Knowledge of natural history of specific plant species or vegetation types is inherently limited, and difficult if not impossible to accurately extrapolate to new geographical areas, future climatic scenarios or an altered environment. Hence, the integration of local-scale plant community research on mechanisms of coexistence and large-scale biogeographical research would be a great step forward in our understanding of the current spatial patterns of vegetation composition, and in the reliability of our predictions towards new situations. Our example shows that a reconciliation of both scales must be taken under consideration in community ecology, and thereby supports the views of Levin (1992) and Kunin (1998) that no single spatial scale is sufficient in the study of ecological patterns. Mechanistic experimental research on a large spatial scale is needed to further test alternative hypotheses on biodiversity of metacommunities. We suggest that this research path would greatly benefit insights in community biogeography.

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Supporting information

Additional supporting information may be found in the appendix:

Appendix 1. Location details of the sampled softwater lakes.

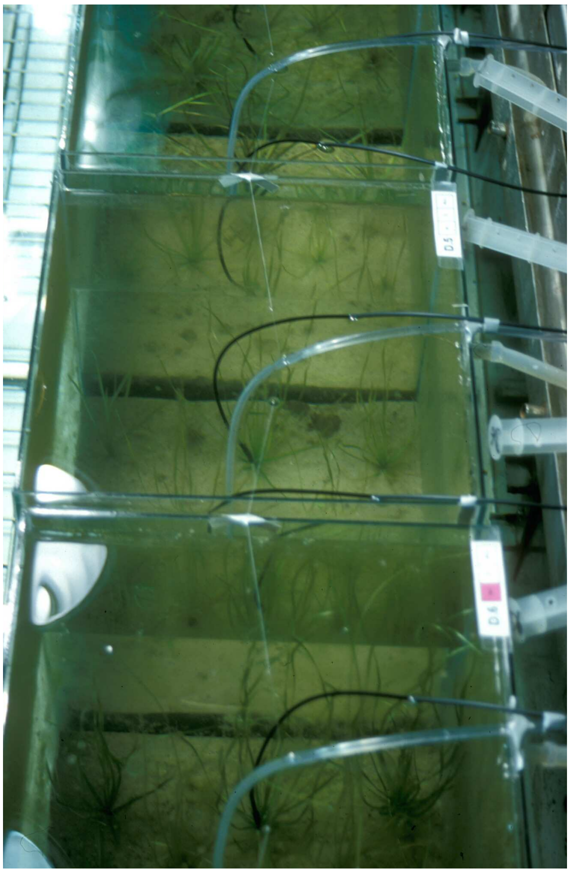
4

Water table and species identity outweigh carbon and nitrogen availability in a softwater plant community

with Fons Smolders, Jan Roelofs and Maurice Hoffmann

Acta Oecologica, in press

> *Applied ecologists most often report research as though theory*
> *does not exist. […] For ecologists to provide information and guidance at the*
> *pace demanded, we first need to have theory that can make reasonable*
> *predictions about the consequences of management interventions.*
>
> *Driscoll & Lindenmayer (2012)*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CHAPTER 4

Water table and species identity outweigh carbon and nitrogen availability in a softwater plant community

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Abstract

Performance of aquatic macrophytes is driven by many environmental factors, and a major challenge is to understand how aquatic macrophyte communities are structured in various environments. In softwater lakes in Western Europe, hydrological state (submersed/emersed), carbon dioxide and ammonium levels and species interactions are considered as driving forces in structuring amphibious plant communities. In this study we aimed at evaluating the relative importance of these factors for four species in a competitive neighbourhood. Softwater lake habitat was simulated during one growing season in laboratory conditions, mimicking water level fluctuation, photoperiod and temperature. Artificial communities consisted of small populations of four softwater macrophyte species: *Luronium natans*, *Baldellia ranunculoides* ssp. *repens*, *Eleocharis multicaulis* and *Hydrocotyle vulgaris*. These communities were subjected to two levels of carbon dioxide and ammonium. Additionally, monocultures of *Baldellia* and *Eleocharis* were grown at a higher nutrient level combination in order to measure their COMPETITIVE RESPONSE in a community. Time (correlated with hydrological state) and species identity turned out to be the only consistently significant factors determining community composition. Plant performance was clearly species-dependent, while carbon dioxide and ammonium did not have major effects. The competitive response was significant in both *Eleocharis* and *Baldellia*. These competitive responses were highest in the emersed state. Carbon dioxide had a supplementary effect on the within-species performance in *Luronium*, *Baldellia* and *Eleocharis*, with high carbon dioxide level mainly resulting in more flowers and more stolons. Community outcomes and competitive responses in aquatic macrophytes appear difficult to predict because of mixed life strategies and morphological and functional plasticity. We presume that hydrological state was the only important environmental factor. The identity of the species that were present – implying possible species interactions – largely determined community outcome.

Introduction

A major challenge in plant ecology is to understand how communities are structured in various environments. To this aim, essential information is needed on the species' environmental niche and life history traits (Keddy *et al.* 2000, McGill *et al.* 2006, Westoby & Wright 2006, Webb *et al.* 2010). Performance of aquatic macrophytes is driven by many environmental factors such as light, temperature, substrate texture, wind and water level fluctuations, chemical composition (e.g. pH) and nutrient content of sediment and water (especially carbon, nitrogen and phosphorus) (Bloemendaal & Roelofs 1988, Wetzel 2001, Lacoul & Freedman 2006, Bornette & Puijalon 2011). Willby *et al.* (2000) showed that temporal variation (disturbance) and resource conditions (stress) were the main distinguishing environmental factors between functional groups of aquatic macrophytes. In order to survive and reproduce in various environments, aquatic macrophytes display a large array of life history traits that enable them to cope with a specific environment. Plant traits like height, biomass and plasticity (e.g. Greulich *et al.* 2001) are generally considered to relate to competitive ability (Kautsky 1988, Gaudet & Keddy 1988, Weiher *et al.* 1999, Willby *et al.* 2000, Grime 2001, Westoby & Wright 2006).

A great deal of research remains to be done in disentangling the mechanisms that structure aquatic macrophyte communities. For example, the importance of competitive interactions to community organization has not often been studied in aquatic macrophytes. The exceptions are usually limited to testing pairwise competitive interactions (e.g. Spierenburg *et al.* 2010, Netten *et al.* 2010, Spencer & Rejmanek 2010). Furthermore, Keddy (1989) formulated a general conceptual model of competitive hierarchy, which predicts a ranking pattern of species' realized niches according to their competitive ability, and in which the fundamental niches of species along a resource gradient all include the 'plenty' end of the gradient. However Keddy *et al.* (2000) stated that reality seems more complex and more work is needed to assess the relative importance of different factors to plant performance in communities.

Oligotrophic softwater lakes represent a specific aquatic habitat. They are generally acidic (mostly with pH around 5) and carbon-limited, with carbon dioxide being the main carbon source (Smolders *et al.* 2002, Madsen *et al.* 2002). Although many cases exist of softwater lake eutrophication by the inlet of nutrient-rich water, most softwater lakes in Northwest Europe are hydrologically isolated and have mainly suffered from atmospheric ammonium deposition, greatly influencing plant community composition of the lakes (Roelofs *et al.* 1996, Bobbink *et al.* 1998, Smolders *et al.* 2002, Arts 2002). Furthermore, water level fluctuations can be a disturbance to plants in the amphibious zone of lake shores, with submergence and emergence being potentially destructive to terrestrial and aquatic life forms respectively (Thomaz *et al.* 2006, Lacoul & Freedman 2006, Kennedy *et al.* 2006; compare to Bornette *et al.* (2008) for fluvial systems). For plant species that survive in an amphibious environment (seasonal submergence and emergence), and perhaps even depend on it, aerial availability of carbon dioxide could be an important seasonal resource.

In this study, we investigated the relative importance of stress, disturbance and species identity for the organization of an amphibious plant community of softwater lakes in a multispecies interaction experiment. Multispecies interaction experiments have the advantage of resembling nature more than classical laboratory experiments with only one or two species (Gibson *et al.* 1999). We considered aqueous carbon dioxide and ammonium levels as stress factors and a water level drop as a potential disturbance factor, in line with the environmental drivers associated with shores of softwater lakes. We consider intrinsic species properties, including competitive ability, disturbance avoidance, seed production etc. (Weiher *et al.* 1999), as being synthesized by the factor 'species identity'. The role of species identity and competition in determining community composition is related to species selection. We used three perennial species that are characteristic of pristine Atlantic softwater lake shores, and one that is characteristic of slightly degraded shores. Each species displays characteristics of several aquatic macrophyte life strategies sensu Kautsky (1988), leading to different expectations regarding the competitive ability of each species. We assume that these different trait combinations drive relative species composition, depending on the environmental state of nutrient and water level.

Consequently, we expected a species-specific effect of both nutrient levels and water level; however the response of the selected species to combinations of these factors and to biotic interactions was unknown beforehand. We therefore used the general assumption that growth would increase at a higher nutrient level, regardless of the species. As we aimed to reveal the way species differed in their response to these factors, we regarded species (species identity) as an explicit factor. Hence, our first working hypothesis was that nutrients (carbon dioxide and ammonium) drive population performance and community composition more than water level drawdown and species identity. As a second hypothesis, we expected that competition between species has a marked role in this plant community.

Methods

Species selection

Three of the species considered are characteristic of pristine conditions: *Baldellia ranunculoides* ssp. *repens* (Lam.) À. Löve and D. Löve, *Eleocharis multicaulis* (Smith) Desv. and *Luronium natans* (L.) Rafin, and co-occur in softwater lakes (chapter 2 and unpubl. data). None of these species are expected to be strong 'competitors' (sensu Grime 1979, Kautsky 1988). *Hydrocotyle vulgaris* L. (Leeflang *et al.* 1998) was included as a fourth species as it is expected to be a better competitor according to our earlier observations (chapter 2). This species is mostly present when at least one of the other three species occurs, and it often dominates as dense mats along borders of slightly enriched lakes.

The life strategies of the species sensu Kautsky (1988) are shown in *Table 4.1*. *Eleocharis* has long, needle-like culms originating from one point and has no stolons and highly reduced leaves. It has characteristics of both competitive and stunted strategies (CS). Based on its needle-like culms, this species is expected to be best adapted to stressful situations and therefore the species with the lowest competitive ability. Among the four species, *Luronium* and *Baldellia* are most alike. Both have evergreen leaves, a moderately high specific leaf area and are able to spread clonally. They also possess more features of reproduction investment than the other two species. They combine aspects of all life strategies (CBRS), presumably making them well adapted to a temporally variable and stressful habitat. *Hydrocotyle* combines vegetative reproduction, lateral spread, tallness and the ability to sequester carbon from the atmosphere and can be described as having a CR strategy. According to this functional characterization, competitive abilities may be ranked as *Hydrocotyle* > *Luronium* + *Baldellia* > *Eleocharis*.

Origin of plant material

Fresh young plants of the four species selected were collected from four locations in The Netherlands. As many plants were needed and several species were considered to be rare, it was only possible to collect plant material from large populations, and therefore not from the same site. *Luronium natans* was collected from a rivulet ‘De Run’ (51°23' 34.58" N , 5°24' 13.98" E), *Eleocharis multicaulis* from a small softwater lake ‘Langeven’ (51° 47' 9. 92" N, 5° 48' 18.49" E), *Hydrocotyle vulgaris* from a small softwater lake ‘Meeuwenven’ (51° 46' 50.44" N, 5° 48' 9.80" E) and *Baldellia ranunculoides* ssp. *repens* from a large softwater lake ‘Beuven’ (51° 24' 8.30" N, 5° 38' 50.72" E). Plants of *Eleocharis* and *Baldellia* had been growing in oligotrophic softwater lakes (acid, low alkalinity, few nutrients), *Hydrocotyle* in a eutrophic softwater lake (plants grew in organic sediment), and *Luronium natans* in a rivulet rich in base cations and with a high alkalinity. The plants were cleaned and incubated in a basic culture medium (see below).

Table 4.1. Traits and strategies of the macrophyte species selected for the experiment.

Plant trait	Corresponding challenge [a]	*Eleocharis multicaulis* [d]	*Luronium natans* [e]	*Baldellia ranunculoides* ssp. *repens* [f]	*Hydrocotyle vulgaris* [g]	'+' meaning:	Interpretation of '+' in PESS context [b]
Plant morphology							
Lateral spread	Persistence (space acquisition)		+	+	+	Present to extensive	Competitive / Biomass storer
Height	Persistence (competitive ability)	+			+	Higher than 15 cm	Competitive
Specific leaf area	Establishment (relative growth rate) & Persistence (competitive ability)		+			Higher than 40 mm²/mg	Competitive
Heterophylly	Persistence (plasticity)		+	+		Present	Competitive
Carbon capture from air	Persistence (competitive ability)	+			+	Prevailing	Competitive
Small or leathery or needle-like leaves	Persistence (longevity)	+				Present	Stunted
Life history							
Evergreen leaves	Persistence (stress tolerance)		+	+		Present	Biomass storer / Stunted
Onset of flowering	Persistence (disturbance avoidance)		+			Early	Ruderal
Seed investment [c]	Dispersal, establishment, persistance (disturbance avoidance)		+	+		Higher than 100 mm³ of seeds per shoot	Ruderal
Vegetative reproduction	Persistence (disturbance tolerance)		+	+	+	Important	Ruderal
Life strategy (Kautsky 1988)		**CS**	**CBRS**	**CBRS**	**CR**		

[a] Gaudet & Keddy 1988, Knevel et al. 2003, Tilman 1988, Weiher et al. 1999, Westoby 1998

[b] PESS = Plant Ecology Strategy Scheme. Interpretation after Grime 1988, Grime 2001, Kautsky 1988, Maillette & Keddy 1989, Murphy et al. 1990, Rørslett 1989, Westoby 1998, Willby et al. 2000, and using terminology of Kautsky 1988

[c] Based on the product of estimated seed number per shoot and seed volume (LEDA-database: Knevel et al. 2003)

[d] Further references for *Eleocharis multicaulis* and other Eleocharis spp.: Arts 2002, Busch et al. 2004, dos Santos & Esteves 2002, Edwards et al. 2003, Maillette & Keddy 1989, Pietsch 1985, Sorrell et al. 2002

[e] Further references for *Luronium natans* traits: Bazydlo 2004, Bazydlo & Szmeja 2004, Greulich et al. 2001, Greulich & Bornette 1999, Greulich & Bornette 2003, Greulich et al. 2000, Hyldgaard & Brix 2011, Nielsen et al. 2006, Szmeja et al. 2008

[f] Further references for *Baldellia ranunculoides* traits: Kozlowski et al. 2008, Kozlowski & Matthies 2009, Kozlowski et al. 2009, Kozlowski & Vallelian 2009

[g] Further references for *Hydrocotyle vulgaris* traits: Evans 1991, Evans 1992, Grime 1988, Leeflang et al. 1998

Experimental set-up

Two ammonium and two carbon dioxide levels were applied to artificial communities of these species in a laboratory experiment (-N/+N and –C/+C treatments). These communities were subjected to a simulated growing season regarding temperature (increasing from 14 to 18 °C), photoperiod and water level (*Fig. 4.1*). The experiment lasted for 202 days in order to simulate one complete growing season. From day 93 to 115, the water level was gradually lowered to attain an emersed state with the groundwater at surface level, which was further maintained with simulated rainwater (see below). Therefore, the water level drop was correlated with the factor time, or more explicitly with the differences between the endstages of each hydrological state (submersed versus emersed).

To assess the relevance of competition, monocultures of *Eleocharis* and *Baldellia* were grown at high levels of ammonium and carbon dioxide, in order to enable comparison with community responses at the same nutrient level. The COMPETITIVE RESPONSE is the difference in performance between both neighbourhood treatments (with/without neighbours). Practical constraints prevented us from extending the competition experiment to all species and all nutrient level combinations. Competition response is expected to be highest in the nutrient-rich situation and for species with the lowest competitive ability if competition is at work in these communities.

The complete experiment consisted of two split-plot designs with repeated measures (Underwood 1997). The community experiment (first design) had ammonium, carbon dioxide (whole-plot factors), species and time (subplot factors) as fixed orthogonal factors, while community served as a random factor (plot), nested within the factorial combinations of ammonium and carbon dioxide. The competition experiment (second design) had neighbourhood as a whole-plot factor and time as the only subplot factor, and was applied separately for *Eleocharis* and *Baldellia*. Linear model diagrams are a convenient way to effectively visualize orthogonal and nested relationships between experimental factors (Vilizzi 2005) and are shown in *Fig. 4.2*. Experimental units for repeated observations were either populations (the individuals per species taken together) or individuals (nested within plot and species; not shown). Ammonium, carbon dioxide and neighbourhood treatment were replicated 4 times and assigned at random to the plots.

A remark must be made concerning the investigation of the factor ‘water level’ (hydrological state) in our experiment. This factor was not applied as an experimental factor for practical reasons. This would imply different, replicated plots receiving different water level regimes, for each nutrient level combination. Instead, hydrological state covaried with time in the same way for all plots (from submersed to emersed). Consequently, significant changes over time can be attributed to hydrological state in a causal manner only as a presumption and with sound ecological argumentation, but not from the design itself.

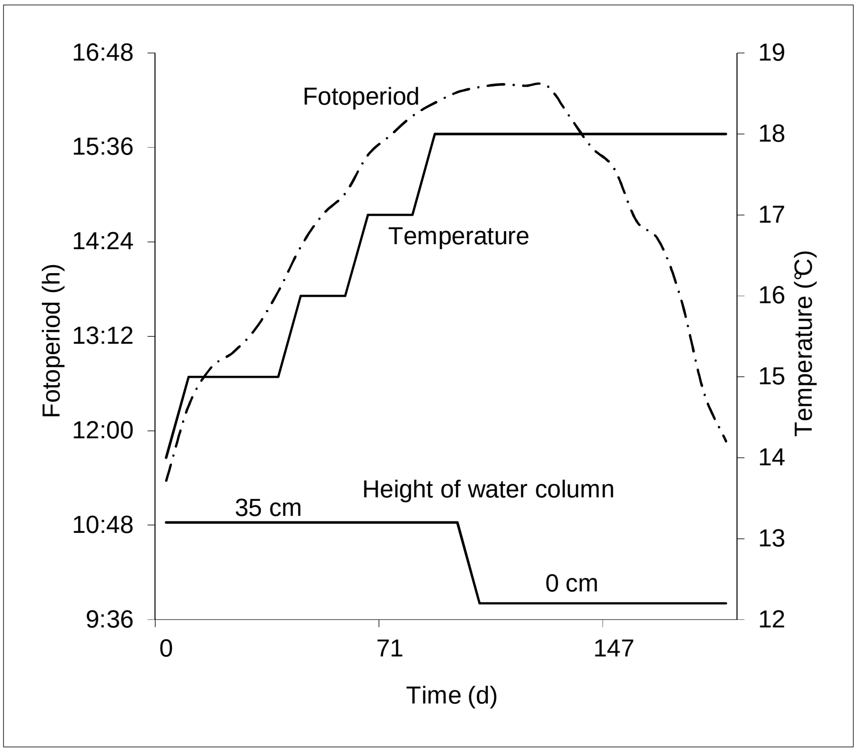


Fig. 4.1. Simulated seasonal changes during the experiment.

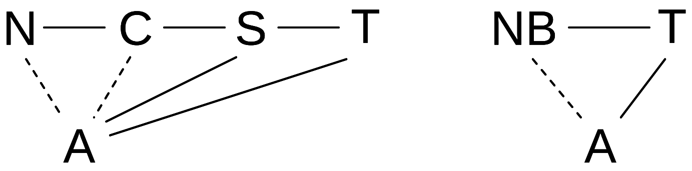


Fig. 4.2. Linear model diagrams of the two statistical designs incorporated in the experiment, showing the relations of orthogonality and nesting between factors. Notation follows Vilizzi (2005): solid lines join orthogonal factors (higher-way interactions are implicit in sequential joining); dashed lines join nested factors (below) with their nesting factor(s) (above). N = nitrogen (ammonium); C = carbon dioxide; S = species; T = time; A = aquarium; NB = neighbourhood.

The statistical plots, containing either a community or a monoculture, were glass aquaria with dimensions (cm) 30 x 30 x 60 (L x W x H). The temperature of the aquaria was regulated by maintaining them in an indoor environment in a water bath. Metal halide lamps (Philips Powertoner HPI-T Plus 400 W) were used for illumination, providing an average PAR photon flux of 145 µmol s^{-1} m^{-2} at surface water level. Each community was composed of five rows of four plants, each row containing one individual of each species. Individuals were spaced equidistant in all directions with 67 mm as the distance between two neighbours (*Fig. 4.3*). Allocation of species and individuals to positions occurred in a systematically randomized way, so that each individual's nearest neighbours belonged to another species, in order to maximize potential interspecific interactions. For monocultures, five individuals of just one target species were planted, keeping its density equal to its density in the community, with plants positioned as in the community. Hence, we applied an additive competition design, which effectively tests the neighbourhood effect alone (Gibson *et al.* 1999).

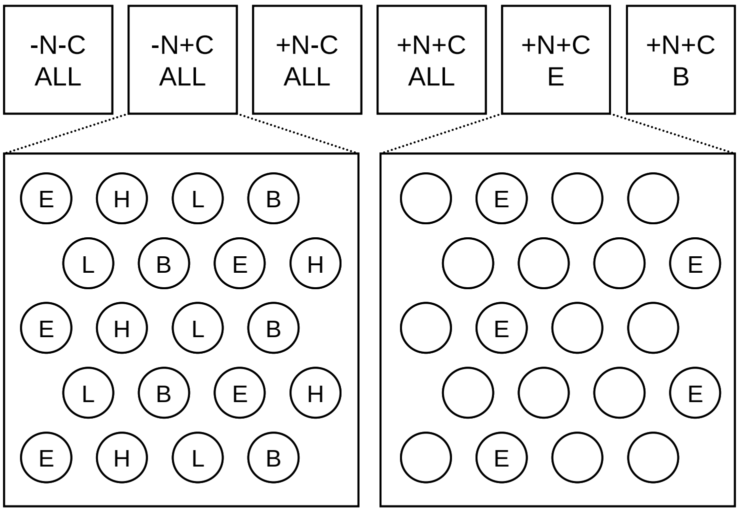


Fig. 4.3. Experimental layout. 1 square = 1 aquarium. Circles are individual plant positions; empty circles contain no individual (in monocultures). Top: one replication series of the aquarium treatments. Bottom: 2 examples of systematically randomized positions for species. ALL = all species present; B = Baldellia ranunculoides ssp. repens; E = Eleocharis multicaulis; H = Hydrocotyle vulgaris; L = Luronium natans, -N/+N = N treatments (ammonium not added/added), -C/+C = C treatments (carbon dioxide not added/added).

Mineral sandy sediment was collected from Beuven (upper 20 cm; *Appendix 2*). It was mixed with 0.25 µmol g^{-1} DW $Ca_3(PO_4)_2$ in order to simulate a softwater lake sediment that is not limited by phosphorus but by nitrogen or carbon. Aquaria contained a water column of 35 cm on top of 20 cm of sediment. The upper 2 cm of the sediment was washed with demineralized water in order to achieve a highly oxidized state, thereby preventing phosphorus from leaching into the surface water. A basic culture medium (*Table 4.2*) served as the baseline for nutrient additions and as the incubation medium for the cleaned plants before insertion into the aquaria. Media were flushed through the aquaria from replicated containers (stocks) using peristaltic pumps at a continuous flow rate of 250 ml h^{-1}. No ammonium or carbon dioxide was present in the basic culture medium (used for -N-C treatment). For the +N treatment, 50 µmol L^{-1} NH_4Cl was added to the medium stock and in the +C treatment the media contained 800 µmol L^{-1} CO_2. All other ion concentrations were kept the same and pH = 5 was maintained by adding HCl, NaOH and NaCl where necessary. Cyanoguanidine (0.5 µmol L^{-1}) was added to prevent nitrification of ammonium (Smolders *et al.* 1996). All ion concentrations were chosen to be representative of the chemical gradients observed in softwater lakes in Western Europe (chapter 2; unpubl. data). Containers of medium stock were replenished on weekly basis.

All aquaria were filled with the basic culture medium and plants were all planted on the same day. After a 3-week settlement period, the treatments were applied to the containers. One week later, the first measurements were taken and considered as t = 0 days. During the emersed state (i.e. from day 115 on), groundwater level was maintained weekly by sprinkling 520 mL of simulated rainwater in each aquarium (*Table 4.2*). Only the ammonium factor was further maintained during this stage. The dose of ammonium applied in the –N treatment was equivalent to 2 kg ha^{-1} y^{-1} and for the +N treatment was 20 kg ha^{-1} y^{-1}.

Table 4.2. Chemical composition of culture mediums and salt dosage in rainwater.

Ion	Basic culture medium -N (µmol L^{-1})	Medium +N (µmol L^{-1})
NH_4^+		50
Na^+	386	336
K^+	50	50
Mg^{2+}	60	60
Ca^{2+}	100	100
Cl^-	496	496
SO_4^{2-}	125	125
NO_3^-	10	10
Salt	**Rainwater –N (kg ha^{-1} y^{-1})**	**Rainwater +N (kg ha^{-1} y^{-1})**
NH_4Cl	2.55	25.45
NH_4NO_3	3.81	38.09
KCl	15.13	15.13
$CaCl_2.2H_2O$	9.94	9.94
Sea salt	33.82	33.82

Data collection

Every two weeks the following performance measures were determined in all treatments: cover (percentage estimation), height (cm), number of flowers (or spikes), number of fruits (or fruiting spikes) as measures per population (aquarium x species). During the submersed phase, performance of individual plants was measured as height and number of leaves (or culms in *Eleocharis).* The mother ramet in stolon-forming species was taken as the 'individual'. Visual impressions of stolon formation were noted. In the emersed phase, lateral spread by leaves or stolons had become so large that only population measurements were done. All measured traits are related to plant persistence (Weiher *et al.* 1999).

For comparison of species, cover, height and number of leaves or culms were standardized relative to the value at t=0, as (value at t)/(value at t=0) (Gibson *et al.* 1999). From the data on number of flowers, the non-repeated measure ‘onset of flowering’ was derived (days to flowering). For populations that never flowered, the artificial value of 400 days was assigned for 'onset of flowering'.

Statistical analysis

The statistical analyses were done using SAS 9.1.3, applying the MIXED procedure (SAS Institute Inc. 2004). Time was considered as a continuous variable, for which a linear trend was fitted. An ‘unstructured’ covariance structure was specified in order to cope with interdependencies of repeated measurements and for observations from the same aquarium. In this way, overall interdependencies between repeated measurements and species were taken into account.

For the endstage of both the submersed and emersed phases, multiple comparison tests (applying the Bonferroni adjustment) and mixed model analyses were done. In order to verify the results, a multivariate analysis of variance (manova) was performed at the community (aquarium) level, in order to overcome the possibly changing species interdependencies between each aquarium. To this end, the *manova* function from the *stats* package was applied in R (R Development Core Team 2012). Further, a comparison between the submersed and emersed endstage was done using mixed models. For the analysis of competitive response, the effects of neighbourhood and time were tested separately for each species, and the effects of species and time on competitive responses were tested simultaneously.

Results

Experimental outcome of the communities

Performance of *Luronium* and *Baldellia* steadily increased in the submersed phase (*Fig. 4.4*, left half of diagram) and these species became dominant (with respect to height and cover). Performance of *Eleocharis* and *Hydrocotyle* decreased during the submersed phase, with *Hydrocotyle* having mortality rates of up to 100 % in some aquaria. Visual inspection revealed that lowering the water table pulled the flexible submerged leaves of *Luronium*, *Baldellia* and *Eleocharis* downwards, resulting in reduced height. In the emersed phase, relative cover and height of *Luronium* and *Baldellia* decreased to levels equal to or below those at the start of the experiment. *Eleocharis* more or less maintained its low height and cover during the emersed phase. *Hydrocotyle* greatly increased its performance during the emersed phase and gained dominance over all other species. Consistent with these observations, interaction between time and species, time itself and the factor 'species' were highly significant for most performance measures (*Table 4.3*). For the ammonium and carbon dioxide treatments only a limited number of overall significant results were obtained.

From more detailed tests at the end of the submersed and emersed phases, significant effects of ammonium or carbon dioxide treatments were not common and restricted to specific time intervals and species. Effects of carbon dioxide treatment were observed at the end of the submersed phase (*Fig. 4.5*), where both *Baldellia* and *Luronium* produced less leaves in the +C treatments. During the experiment it was also evident that in +C treatments, both species produced more stolons with daughter ramets. Furthermore, +C treatments resulted in a greater number of flowers in *Luronium*, during both phases (although not at the *end* of the emersed phase). In *Baldellia*, this effect only occurred in the –N+C treatment and was only weakly significant (*P* = 0.069). For *Luronium* and *Eleocharis*, flowering occurred earlier in +C treatments compared to the –C treatments, moreover flowers in the –C treatments were only observed for *Luronium*, in the +N-C treatment. See *Fig. 4.5* for specific statistical test results. The manova for the submersed and emersed endstages supported all these results: in each case where at least one species showed a significant response to the N/C treatments in the foregoing analyses (and only then), the manova detected this as a community response (not shown).

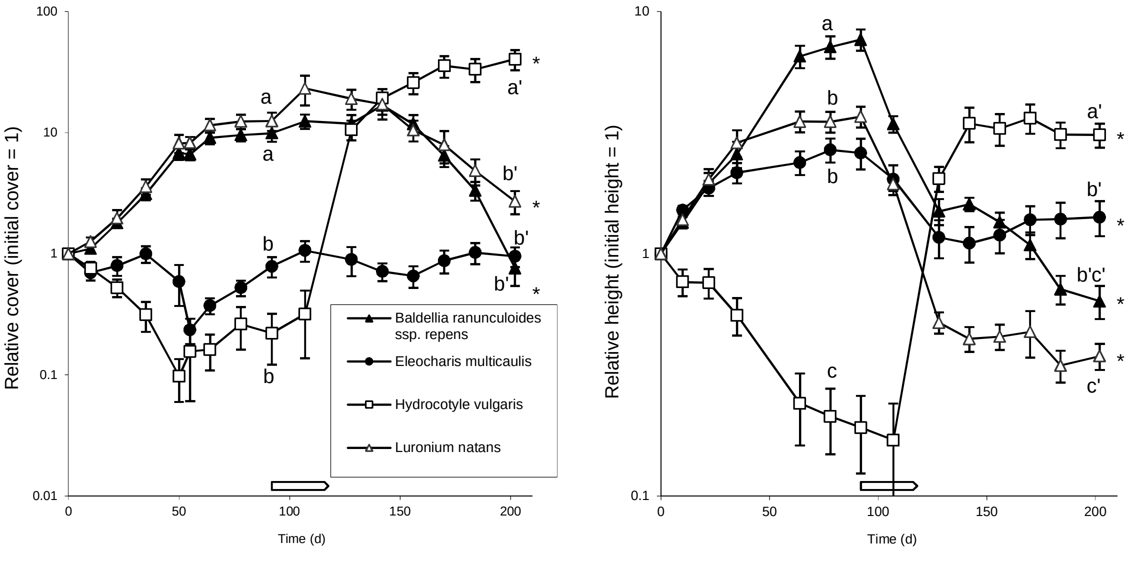


Fig. 4.4. Changes in species performance (mean ± SE) with time (from observations at population level, i.e. species x aquarium). Vertical axes are log-scaled. The horizontal arrow indicates the period when water level was decreased. At the submersed and emersed endstages respectively, means with the same letter are not significantly different (P > 0.05, Bonferroni-adjusted). An asterisk next to the timeline (*) indicates a significant difference between submersed and emersed endstage for the particular species (P < 0.05).

Table 4.3. Statistical results for several measures of performance in community. [a]

		Relative cover			Relative height			Number of flowers		
Effect	Num DF	Den DF	F Value	P	Den DF	F Value	P	Den DF	F Value	P
N	1	25.5	0	0.986	27.8	1.35	0.255	70.8	0.43	0.515
C	1	25.5	0.29	0.597	27.8	0.20	0.662	70.8	0.02	0.889
N*C	1	25.5	0.15	0.700	27.8	0.41	0.528	70.8	0	0.955
Species	3	980	38.66	<0.0001	836	52	<0.0001	836	6.30	0.000
N*Species	3	980	0.34	0.797	836	1.65	0.175	836	0.32	0.808
C*Species	3	980	0.36	0.779	836	1.58	0.192	836	0.19	0.901
N*C*Species	3	980	1.27	0.282	836	0.33	0.804	836	0.01	0.999
Time	1	980	191.71	<0.0001	836	3.39	0.066	836	65.52	<0.0001
Time*N	1	980	0.25	0.617	836	2.36	0.125	836	4.37	0.037
Time*C	1	980	2.75	0.098	836	0.08	0.776	836	2.44	0.119
Time*N*C	1	980	8.09	0.005	836	0.16	0.686	836	0.02	0.882
Time*Species	3	980	97.54	<0.0001	836	49.63	<0.0001	836	55.07	<0.0001
Time*N*Species	3	980	0.33	0.801	836	0.43	0.732	836	3.40	0.018
Time*C*Species	3	980	0.94	0.419	836	0.47	0.704	836	0.87	0.457
Time*N*C*Species	3	980	1.05	0.368	836	0.12	0.946	836	0.03	0.991

[a] Unless otherwise stated, performance measures refer to observations at population level (species x aquarium). *P*-values < 0.10 appear shaded. Num DF = numerator degrees of freedom, Den DF = denominator degrees of freedom

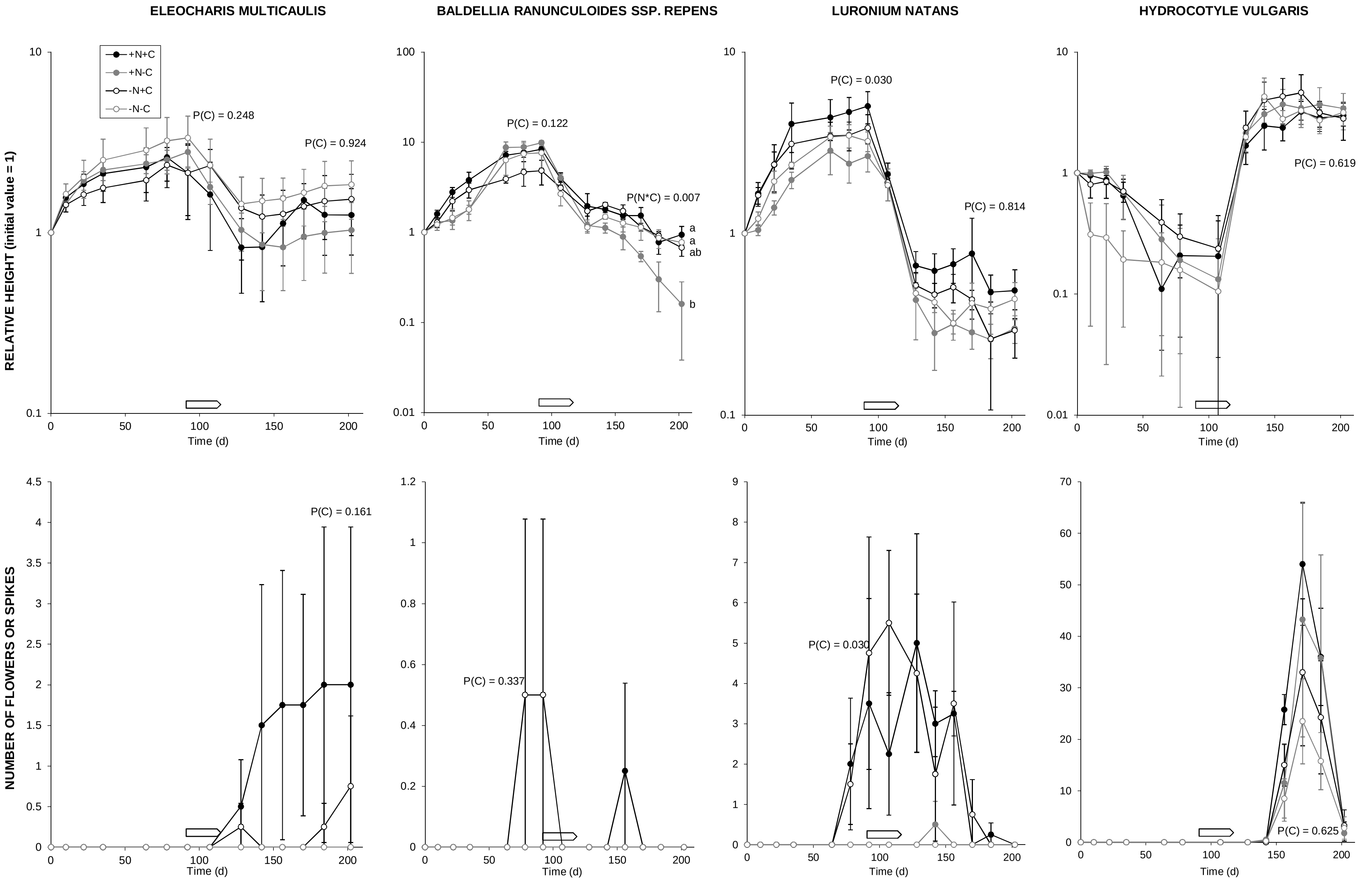
Table 4.3, continued.

Effect	Num DF	Number of fruits Den DF	Number of fruits F Value	Number of fruits P	Onset of flowering [b] Den DF	Onset of flowering [b] F Value	Onset of flowering [b] P	Relative height [c] Den DF	Relative height [c] F Value	Relative height [c] P	Relative number of leaves or culms [c] Den DF	Relative number of leaves or culms [c] F Value	Relative number of leaves or culms [c] P
N	1	130	0.36	0.550	24.3	0.13	0.717	262	2.11	0.147	376	0	0.971
C	1	130	0.22	0.638	24.3	160.92	<0.0001	262	2.27	0.133	376	1.16	0.282
N*C	1	130	0	0.969	24.3	0.37	0.546	262	0.09	0.769	376	0.04	0.834
Species	3	836	11.48	<0.0001	21.3	65.11	<0.0001	262	5.78	0.001	376	12.76	<0.0001
N*Species	3	836	0.34	0.794	21.3	0.71	0.558	262	0.06	0.980	376	0.41	0.749
C*Species	3	836	0.22	0.880	21.3	21.60	<0.0001	262	1.25	0.293	376	1.64	0.181
N*C*Species	3	836	0	1.000	21.3	1.07	0.381	262	0.24	0.869	376	0.10	0.958
Time	1	836	59.38	<0.0001				973	694.26	<0.0001	1222	557.03	<0.0001
Time*N	1	836	1.65	0.200				973	44.18	<0.0001	1222	0.67	0.414
Time*C	1	836	1.28	0.258				973	4.75	0.030	1222	26.19	<0.0001
Time*N*C	1	836	0.01	0.942				973	1.77	0.184	1222	0.39	0.531
Time*Species	3	836	57.76	<0.0001				973	227.61	<0.0001	1222	554.86	<0.0001
Time*N*Species	3	836	1.71	0.163				973	3.80	0.010	1222	0.56	0.644
Time*C*Species	3	836	1.06	0.366				973	23.64	<0.0001	1222	14.36	<0.0001
Time*N*C*Species	3	836	0.01	0.999				973	9.29	<0.0001	1222	5.49	0.001

[b] 'Onset of flowering' is a non-repeated measurement

[c] from observations made at individual plant level, and only at 6 stages during the submersed phase

*Fig. 4.5 (next two pages). Effects (mean ± SE) of carbon dioxide (black versus grey) and ammonium supply (closed versus open circles) on several performance measures (population as measurement unit except for relative number of leaves or culms). Relative number of leaves or culms was determined at individual plant level, and only at six dates during the submersed phase. Onset of flowering: populations that did not flower were given an artificial value of 400 days. The horizontal arrow indicates the period when water level was decreased. Two types of subanalyses are presented: 1) multiple comparisons of the four N/C treatment means at the submersed and at the emersed endstages. Only when a significant difference was detected, letters are shown (means with the same letter are not significantly different; P > 0.05, Bonferroni-adjusted); 2) effect of the carbon dioxide treatment (+C/-C) at the submersed and at the emersed endstages (P-values are shown). In one case, the P-value of an N*C-interaction is shown instead. Where no P-value is presented, submodel estimation was not possible because of infinite likelihood and the results must be considered not significant.*

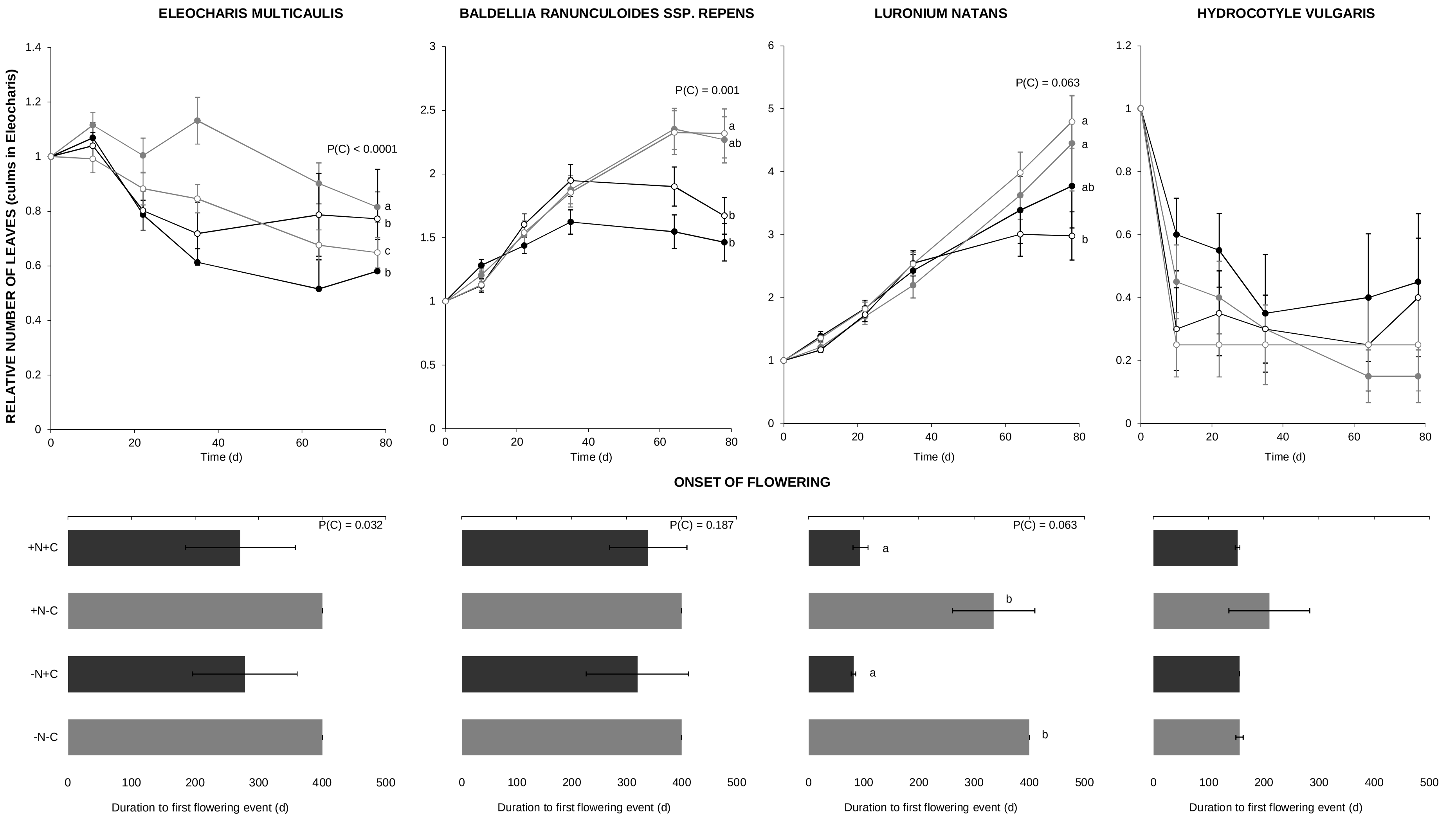


ELEOCHARIS MULTICAULIS

BALDELLIA RANUNCULOIDES SSP. REPENS

LURONIUM NATANS

HYDROCOTYLE VULGARIS


ELEOCHARIS MULTICAULIS
BALDELLIA RANUNCULOIDES SSP. REPENS
LURONIUM NATANS
HYDROCOTYLE VULGARIS
RELATIVE NUMBER OF LEAVES (culms in Eleocharis)
P(C) < 0.0001
a
b
c
b
P(C) = 0.001
a
ab
b
b
P(C) = 0.063
a
a
ab
b
Time (d)
0
20
40
60
80
ONSET OF FLOWERING
+N+C
+N-C
-N+C
-N-C
P(C) = 0.032
P(C) = 0.187
P(C) = 0.063
a
b
a
b
Duration to first flowering event (d)
0
100
200
300
400
500

The patterns described above are summarized in *Table 4.4*. The experimental outcome was governed by the interaction between species identity and time (as reflected by hydrological state: submersed / emersed). As mentioned above, there was an effect of carbon dioxide level within several species, largely independent of ammonium level, but this effect was smaller compared to the species rankings and the change with time.

Table 4.4. Relative performance of the species during and after several experimental treatments. Values can be compared across rows and columns, and are for the end of the submersed phase and for the performance peak of the emersed phase, respectively.

	Observed overall performance (outcome in community) [a]								Observed competitive response in +C+N treatment	
	Submersed phase				Emersed phase				Submer-sed phase	Emersed phase
	+C+N	-C+N	+C-N	-C-N	+C+N	-C+N	+C-N	-C-N		
Eleocharis multicaulis	+	+	+	+	+	+	+	+	moderate	high
Luronium natans	+++	++	+++	++	+	+	+	+		
Baldellia ranunculoides ssp. *repens*	+++	++	+++	++	+	+	+	+	moderate	high
Hydrocotyle vulgaris	-	-	-	-	+++	+++	+++	+++		

[a] *+++ = Excellent performance; ++ = Good performance; + = Moderate performance; - = Low to bad performance*

Competitive response of Eleocharis and Baldellia

Significant competitive responses were found for both species, depending on performance measure and time (*Fig. 4.6*, *Table 4.6*). Competitive response differed significantly between both species, depending on time. As competitive response and the difference between both species were time dependent, significant interactions were found between time and neighbourhood and between time and species (*Table 4.5*). For both species, a competitive response was most obvious in the emersed state (*Fig. 4.6*). Competitive response for relative cover was greater in *Baldellia*, while in *Eleocharis* competitive response was greater for relative height and number of flowers (spikes) and fruits (fruiting spikes). Thus, in the high nutrient level combination, both species were evaluated to have a ‘high’ competitive response in the emersed phase, and a ‘moderate’ competitive response in the submersed phase (*Table 4.4*).

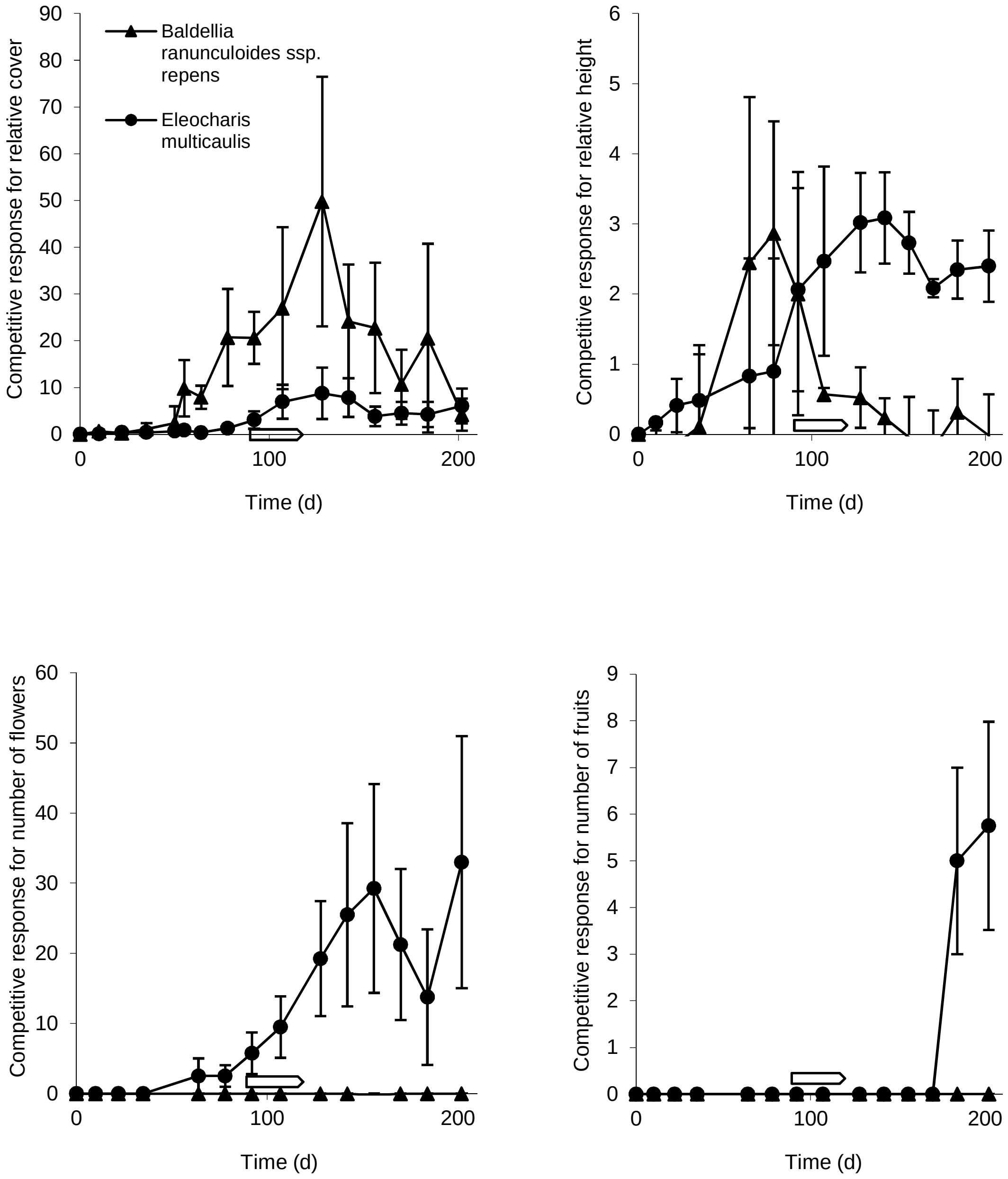


Fig. 4.6. Competitive response of Baldellia ranunculoides ssp. repens and Eleocharis multicaulis at high resource levels (+N+C treatment) (mean ± SE).

Table 4.5 (next page). Statistical results for competitive response of Eleocharis multicaulis and Baldellia ranunculoides ssp. repens for several measures of performance in a community under high nutrient conditions (+N+C treatment). The first analysis tests the presence of a competitive response in each species, while the second analysis tests for (temporary) differences in competitive responses between species. [a]

Analysis per species on neighbourhood													
Baldellia		*Relative cover*			*Relative height*			*Number of flowers*					
Effect	Num DF	Den DF	F Value	P	Den DF	F Value	P	Den DF	F Value	P			
Neighbourhood	1	16.3	0.30	0.5943	50.7	0.48	0.4907	51.3	0.04	0.8491			
Time	1	118	10.01	0.002	102	6.49	0.0123	102	0.77	0.3828			
Time*Neighbourhood	1	118	4.63	0.0334	102	0.04	0.849	102	0.77	0.3828			
Eleocharis		*Relative cover*			*Relative height*			*Number of flowers*			*Number of fruits*		
Effect	Num DF	Den DF	F Value	P	Den DF	F Value	P	Den DF	F Value	P	Den DF	F Value	P
Neighbourhood	1	8.69	0.04	0.842	10.5	0.18	0.6826	9.65	0.71	0.419	52.4	3.96	0.0517
Time	1	118	33.61	<0.0001	102	10.40	0.0017	102	63.24	<0.0001	102	18.15	<0.0001
Time*Neighbourhood	1	118	30.15	<0.0001	102	35.58	<0.0001	102	48.13	<0.0001	102	18.15	<0.0001
Analysis on competitive response													
		Relative cover			*Relative height*			*Number of flowers*			*Number of fruits*		
Effect	Num DF	Den DF	F Value	P	Den DF	F Value	P	Den DF	F Value	P	Den DF	F Value	P
Species	1	13.6	0.60	0.4529	6.16	0.56	0.4827	9.36	0.92	0.3625	105	4.13	0.0447
Time	1	3.74	4.39	0.1089	102	8.53	0.0043	3.11	5.41	0.0995	10.2	12.59	0.0051
Time*Species	1	117	3.70	0.0569	102	14.16	0.0003	99.2	57.75	<0.0001	105	19.05	<0.0001

[a] *P*-values < 0.10 appear shaded.

Table 4.6. P-values of analyses of the competitive response of Eleocharis multicaulis and Baldellia ranunculoides ssp. repens at specific time stages, for several measures of performance in a community under high nutrient conditions (+N+C treatment). These analyses were done for the endstage of the submerse and emerse phases. [a]

Species	Endstage	Relative cover				Relative height				Number of flowers				Number of fruits			
		Estimate	DF	t Value	P	Estimate	DF	t Value	P	Estimate	DF	t Value	P	Estimate	DF	t Value	P
Baldellia ranunculoides ssp. *repens*	Submersed	20.625	5.29	5.75	0.002	2.005	5.94	1.45	0.198	-	-	-	-	-	-	-	-
	Emersed	4.23	6	1.36	0.223	-0.02	6	-0.04	0.968	-	-	-	-	-	-	-	-
Eleocharis multicaulis	Submersed	3.04	5.29	0.85	0.434	2.06	5.94	1.49	0.188	5.75	3	3.17	0.050	-	-	-	-
	Emersed	6.02	6	1.94	0.101	2.3975	6	5.05	0.002	33	3	3	0.058	5.75	3	4.21	0.025
Species difference	Submersed	17.585	3	4.36	0.022	-0.055	3	-0.03	0.978	-5.75	3	-2.24	0.111	-	-	-	-
	Emersed	-1.79	6	-0.41	0.698	-2.4175	6	-3.6	0.011	-33	3	-2.12	0.124	-5.75	3	-2.98	0.059
Baldellia ranunculoides ssp. *repens*	*Both endstages compared*	16.395	3	5.25	0.014	2.025	3	1.63	0.202	-	-	-	-	-	-	-	-
Eleocharis multicaulis	*Both endstages compared*	-2.98	3	-1.3	0.284	-0.3375	3	-0.29	0.792	-27.25	3	-1.87	0.159	-5.75	3	-2.98	0.059

[a] *P-values < 0.10 appear shaded.*

Discussion

Overall patterns

Our experiment tested the effect of carbon dioxide, ammonium and competitive neighbourhood on population performance in communities. We explicitly considered ‘species’ as a factor in order to highlight interspecific differences. As we had collected repeated measurements, we could also test whether patterns changed with time. Remarkably, in the community experiment, species identity and time determined the population response most strongly. Our first hypothesis, that nutrient level combinations drive most of the population performance for a species, generally proved invalid, with the exception of high carbon dioxide levels leading to better performance of several species at specific times. The outcome that different nutrient levels had no dominant effect is quite surprising, given the importance of these nutrients according to literature and the realistic levels that were chosen. Also, effects of ammonium were detected in the observational studies of chapters 2 and 3. It should be added however, that photon influx was much lower under artificial light than under outside conditions (Madsen & Sandjensen 1994), and therefore light was potentially limiting in our experiment, especially under high-nutrient conditions. On the other hand, we observed normal growth in the aquaria compared to plants growing in the field. This may be because of the low light saturation point of many aquatic macrophytes, i.e. often < 500 µmol s^{-1} m^{-2} of PAR (Wetzel *et al.* 1984, Boston *et al.* 1989, Robe & Griffiths 1992, Wetzel 2001). PAR irradiance was probably not saturating in our experiment, although it is not known whether it was actually limiting. It might be that plant responses would have been somewhat higher under higher light intensity, both in mixtures and monocultures. However, potential light limitation did not prevent the observation of significant competitive responses. The competitive responses were mainly caused by nutrient limitation through competition, as plants hardly shaded each other for a large part of the duration of the experiment.

While we did not make independent replicates of different water table treatments (see remark in Methods), we presume that the hydrological state was the actual causal factor leading to the drastic change in time, as reflected by the difference between the submersed and emersed endstages. This is suggested both by the rapid changes in the performance measures at the moment of water level drawdown, and by specific observations of species in relation to the water table (see Results). Our results therefore point at a greater importance of water table and species identity, relative to nutrient levels.

The effect of species on population performances implies that the species selection was of great influence. The effect also includes the resulting potential species interactions, as the species were grown in a mixture. Therefore, we expect that a partial replacement of our species selection with different species might substantially alter the performances, even for species that are common to both selections.

Many species of aquatic macrophytes combine very different life strategy characteristics (i.e. the traits that constitute their identity). These are related to coping with different challenges in their environment, especially gradients of temporal variation (i.e. disturbances such as waves, wind, currents and water level drawdown) and resource conditions (i.e. sources of stress such as availability of light or nutrients) (Willby *et al.* 2000, Thomaz *et al.* 2006, Bornette *et al.* 2008). However, the relationship between such traits and these challenges is not always clear, as Willby *et al.* (2000) showed that there is considerable overlap in the environmental niches occupied by species with different functional groups. These patterns are further complicated by the considerable degree of morphological and physiological plasticity in aquatic macrophytes (e.g. Greulich *et al.* 2001, Puijalon *et al.* 2007, Szmeja *et al.* 2008, Kozlowski *et al.* 2008, Puijalon *et al.* 2008a, Hyldgaard & Brix 2011). Plasticity can both enhance competitive ability and increase an individual’s resistance to disturbance events. The functional variability of aquatic macrophytes may hinder the prediction of community outcomes and competitive interactions without species-specific research. The fact that our first working hypothesis was rejected suggests that the ecology and trait-environment relationships for the species studied are not yet sufficiently established in order to correctly predict the observed behaviour in a community setting.

Presumably, water level was the dominant environmental factor for population performance in our artificial community. This strengthens the notion that water table fluctuations are of utmost importance to community structure in temporally submersed shores as found by Thomaz *et al.* (2006). Furthermore, it should be taken into consideration that for aquatic macrophytes, general rules that are effectively predictive at the site scale (i.e. community scale) may be hard to uncover (cf. Willby *et al.* 2000) because of species-specific mixed strategies and plasticity. For example, in a field experiment with four aquatic macrophytes among which *Luronium natans*, Greulich and Bornette (1999) found that it was not possible to consistently rank species according to competitive ability. Some other examples of unexpected or spurious patterns can be found in Greulich & Bornette (2003), Puijalon & Bornette (2006) and Puijalon *et al.* (2008a). Bornette *et al.* (2008) proposed a more general model on trait-disturbance relationships for plants in and along river systems which has to be tested further.

We demonstrated a competitive response of two species at the high nutrient level combination. We found that *Hydrocotyle vulgaris* was a competitive dominant in nutrient-rich emersed conditions and that other species showed a higher competitive response compared to the submersed state. This result is consistent with Keddy’s (1989) competitive hierarchy model and the theory of Grime (1979), stating that competition is most intense in stable and nutrient-rich situations. Competition may be an important component for determining community structure of the *Eleocharition multicaulis*; however, the significance of competition at other nutrient levels should be a subject of future experimentation. Further, it should be noted that the experiment was carried out at a single density for each species. Therefore, the influence of density cannot be separated from the influence of species presence. In general, the boundaries of the realized niche of a species may be the result of the competitive effect by neighbours outside its realized niche (Keddy 1989), but they can also indicate physiological restrictions of the species. Predicting competitive response will therefore remain difficult if it is not clear beforehand what the relative contribution is to the realized niche.

Patterns in three focal species

According to de Lyon & Roelofs (1986), Arts (2002) and Pietsch (1985), *Eleocharis multicaulis* should have a preference for a habitat rich in ammonium and carbon dioxide. A clear effect of greater availability of carbon dioxide was indeed observed for reproduction-related performance measures. However, no effect of increased ammonium was detected. In an earlier field study, we found that *Eleocharis multicaulis* benefits from nitrogen addition as long as nitrate remains dominant over ammonium (chapter 2). In the case of high atmospheric nitrogen deposition, ammonium concentration increases and becomes dominant because of the resulting acidification, which has probably caused *Eleocharis multicaulis* to disappear from many sites (chapter 2). It may well be that in the current experiment, ammonium was not detrimental because of higher pH levels (pH 5 to 6) compared to acidified situations which can occur in the field (pH < 4).

Baldellia species (Kozlowski & Matthies 2009) and *Luronium natans* (Greulich & Bornette 1999, Szmeja & Bazydlo 2005, Nielsen *et al.* 2006, Hyldgaard & Brix 2011) are considered to be ‘weak competitors’. Applying Keddy’s (1989) competitive hierarchy model, this would mean that they have relatively broad fundamental niches. According to Greulich *et al.* (2000b) in southern France (transplantation experiment), and Szankowski & Klosowski (2001) in Poland (realized niche study), the fundamental niche of *Luronium natans* is rather broad. Both species persisted in the artificial community during the course of the current experiment under all nutrient and hydrological conditions. Although we did not test continuous gradients, our results seem to support the earlier findings of occupation of a broad niche. This is only partly true for hydrological state however, as *Baldellia* suffered from desiccation in the emersed state (see further). Our findings for *Luronium natans* are in agreement with Keddy’s model.

In a field experiment, evergreenness of *Luronium natans* was found to have a competitive advantage over summergreenness (Greulich & Bornette 2003). A considerable degree of plasticity also conferred advantages to the species (Greulich *et al.* 2001, Szmeja *et al.* 2008, Hyldgaard & Brix 2011). Evergreenness, in general, is a trait of biomass storers and stunted species, not of competitive species (Kautsky 1988). Such conflicting evidence indicates the difficulties in disentangling the way this species copes with challenges in different circumstances. More research is needed to be able to predict the long-term response of this species, both under different abiotic and biotic circumstances in a community-context and under different management regimes (cf. Nielsen *et al.* 2006).

From our life strategy analysis, it seems that *Baldellia ranunculoides* ssp. *repens* is most ecologically similar to *Luronium natans*. Few predictions exist in literature regarding the expected response of this subspecies to abiotic and biotic conditions. Kozlowski *et al.* (2008) report that both subspecies of *Baldellia* *ranunculoides* (ssp. *ranunculoides* and ssp. *repens*) suffer from shade and eutrophication. We hypothesize that this is mainly because of the competitive dominance of other species in these circumstances. Water level fluctuations may be important for the persistence of these subspecies in the presence of competitive dominants that prefer either the submersed state (e.g., *Juncus bulbosus*) or the emersed state (e.g., *Hydrocotyle vulgaris*).

Water-level change

Carbon is often a limiting nutrient in the water column of softwater lakes (Brouwer 2001, Smolders *et al.* 2002, Madsen *et al.* 2002). As such, the gradient from submersed to emersed conditions along these softwater lakes can be regarded as a resource gradient, with maximum carbon availability at the emersed end. The expectation of Keddy (1989) that the fundamental niche of a competitive dominant (*Hydrocotyle vulgaris*) is relatively narrow (restricted to the emersed end of the gradient), is confirmed by the poor performance and even mortality of *Hydrocotyle* under submersed conditions. Submergence can indeed be regarded as a disturbance for this species, and this will be true for most plant species that grow best in the terrestrial phase (comparable to flooding in the fluvial model of Bornette *et al.* (2008). As such, the gradient from submersed conditions (aquatic macrophytes) to emersed conditions (terrestrial plants) seems compatible with Keddy's competitive hierarchy model. However, the adaptation of many aquatic macrophytes to submersed conditions with certain degrees of stress and disturbance, may imply physiological limitations to cope with the 'preferred' emersed end of the gradient, the occurrence of which may then act as a disturbance as well (Thomaz *et al.* 2006, Lacoul & Freedman 2006). This seemed to be more the case for *Baldellia* than for *Eleocharis* when grown in monocultures. For *Baldellia*, relative height was substantially lower with the transition to an emersed state, while this change was not measured in *Eleocharis*. Apparently this was the consequence of the linear leaves of *Baldellia* not being adapted to desiccation, so that the transition to the emersed state is primarily a disturbance for this species. Therefore we argue that the assumption of 'inclusive niches' in the competitive hierarchy model of Keddy (1989) may not hold for several amphibious plants when considering competition for aerial carbon dioxide along shorelines.

Eleocharis and *Luronium* had more and/or earlier flowering when grown with addition of carbon dioxide compared to no additional carbon dioxide. In addition, both *Luronium* and *Baldellia* produced more stolons and daughter ramets in the +C treatment. This indicates that these three species are primarily 'ruderals'. If they had a more competitive strategy, we would expect them to quickly allocate the extra resources to per-ramet-biomass (height, leaves). If these species had a stunted or a biomass storer strategy (typical for specialists of nutrient-poor sites), we would not expect them to make use of additional carbon dioxide at all (Grime 1979).

Conclusions

From our one-year experiment, we draw the following conclusions:

- Ammonium and carbon dioxide did not affect relative population performances in an important way; however high carbon dioxide levels led to better performance of several species at specific times.
- The identity of the species that were present – implying possible species interactions – largely determined the community outcome.
- Presumably, hydrological state (which changed from submersed to emersed) was the only important environmental factor that drove relative population performance, and hence, community composition.
- Competition intensity, measured in the high nutrient level combination, was greatest in the emersed state, which is in accordance with plant ecological theory.
- It appears difficult to accurately predict individual species performances in a community because the effect of the species’ trait constitution (their identity) is difficult to estimate due to the mixed life strategies and functional plasticity.

Acknowledgements

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Supporting information

Additional supporting information may be found in the appendix:

Appendix 2. Chemical characteristics of the sediment used for the experiment.

5

Selecting predictors for discriminant analysis of species performance: an example from an amphibious softwater plant

with Fons Smolders, Jan Roelofs and Maurice Hoffmann

adapted from Vanderhaeghe *et al.* (2012)

Classical ecological models treated communities as closed, integrated, deterministic, and homogeneous. Such models are simplifications in real systems, and provide a place to begin analysis. However, each of these assumptions must be relaxed if we are to understand the factors governing the diversity and dynamics of ecosystems.

Levin (1992)

Lake at Cilan, Abersoch, Wales

CHAPTER 5

Selecting predictors for discriminant analysis of species performance: an example from an amphibious softwater plant

Abstract

Selecting an appropriate variable subset in linear multivariate methods is an important methodological issue for ecologists. An interest often exists in obtaining general predictive capacity or in finding causal inferences from predictor variables. In lack of solid knowledge of a studied phenomenon, scientists want to explore sufficient predictor variables in order to find the most meaningful (i.e. discriminating) ones. As an example, we modelled the response of the amphibious sofwater plant *Eleocharis multicaulis* with canonical discriminant function analysis. We asked how variables can be selected, by comparison of several methods: univariate Pearson chi-square screening, principal components analysis (PCA) and stepwise analysis, as well as some combinations. From other author's findings, we expected PCA to perform best. The selection methods were evaluated through fit and stability of the resulting discriminant functions and through the correlations between these functions and the predictor variables. The chi-square subset at $P < 0.05$, followed by a stepwise subselection, gave the best result. In contrast to our expectations, PCA performed bad, and so did stepwise analysis. The different chi-square subset methods all yielded ecologically meaningful variables, while probable noise variables were also selected by PCA and stepwise analysis. We advise against the simple use of PCA or stepwise discriminant analysis to obtain an ecologically meaningful variable subset, the former because it does not take into account the response variable, the latter because noise variables are likely to be selected. We suggest that univariate screening techniques are a worthy alternative for variable selection in ecology.

Introduction

Modelling the response of plant species to environmental factors is important in the methodology of many ecological studies. Several recommendations nowadays exist regarding the choice of predictors. In their reviews on species distribution models, Elith & Leathwick (2009) and Austin (2007) point out that best results are obtained when proximal (causal) predictors are selected on forehand, based on existing knowledge. Austin (2007) explicitly refers to ‘move away from using all possible predictors and use existing knowledge to best advantage’. This view is now largely accepted. Ecologists have been making more use of model-selection criteria in order to model the response of a species to its multivariate environment (Johnson & Omland 2004, Rushton *et al.* 2004). In this approach, only those variables are sampled that are predefined in the models to be evaluated. If the models contain variables that represent the most important causal factors for a species’ response, these models have a larger predictive or explanatory value (Mac Nally 2000, Ginzburg & Jensen 2004).

However, the model-selection method has its limitations (Rushton *et al.* 2004, Ginzburg & Jensen 2004). It is often not possible to know which predictor variables will be most decisive for the response variable, for example by lack of sufficient knowledge of the species’ ecology or the ecosystem (e.g. Van Sickle *et al.* 2006). Next, it can be a challenging goal to find new important variables beside ‘generally accepted’ ones - like pH, nitrogen and phosphorus concentrations in the case of plant species - given a dataset with detailed environmental information (many variables). This is especially the case for (semi-)aquatic plant species, where literature on the species’ environmental niche is often scattered and incomplete. For this reason, Vanderhaeghe *et al.* (2005) sampled many variables during a field survey in order to unravel the most important predictor variables for *Eleocharis multicaulis* (Smith) Desv., an amphibious plant of West-European softwater lakes (see chapter 2).

In such cases, we must turn to preliminary variable selection from a larger dataset of many potentially relevant variables, before model fitting (James & McCullogh 1990, Neter *et al.* 1996, Quinn & Keough 2002). If in this way we obtain a parsimonious model with a good fit to the data, chances are high that causal factors were selected (Mac Nally 2000, Austin 2002). Therefore, ecologists have designed ways to reduce multivariate information (Austin 1985, James & McCullogh 1990, Manly 1994), among which canonical ordinations (ter Braak 1995) are very popular. Through inspection of the coefficients of a canonical function, many authors have interpreted the meaning of the original variables in relation to the observed phenomenon. However, parsimony of these models has often been ignored.

In our analysis of the realized niche of *Eleocharis multicaulis* (chapter 2), we applied canonical discriminant function analysis (discriminant analysis, DA) in order to find the main predictors that distinguish between three performance categories of the species (absent, low and high cover). A major advantage of DA is that no distributional assumptions are made for the predictor variables. In this paper, we explain the backgrounds of the applied variable selection. We specifically ask how variables can be selected for discriminant analysis, and we therefore compared several variable selection methods. While only the best performing variable selection technique was applied and ecologically interpreted in chapter 2, the preceding comparison of variable selection methods is subject of the current chapter. Hence, no new ecological information is provided here. The results of the comparison of variable selection methods can be useful in future explorations of large multivariate datasets in which selecting the right predictors is not self-evident. An important issue in variable selection is to disentangle the web of multicollinearity among the variables, in order to select those that have most potential to be causal (Mac Nally 2000, Graham 2003, Zuur *et al.* 2010). Williams & Titus (1988) recommend a 1:3 ratio of variables to observations as a maximum (after selection), in order to obtain narrow confidence intervals of the canonical coefficients and thus to achieve a reliable interpretation.

Several methods of variable selection have been put forward. One approach is to conduct a principal components analysis (PCA) and select original variables by means of the factor loadings (Jolliffe 1972a, Jolliffe 1972b, Krzanowski 1987, King & Jackson 1999). This results in an effective reduction of multicollinearity among the final predictor variables. In some studies the principal components, which are linear combinations of the original variables, are used as actual predictor variables for the response model (Manel *et al.* 2001, Graham 2003). Stepwise canonical ordinations (in contrast to direct analysis) are an alternative approach. They combine a forward selection procedure and a backward elimination procedure at each intermediate step of model fitting, mostly using *P*-values as criteria for entering and removing variables. This approach has received criticisms as the selected subset is considered highly variable, thus depending on the specific sample (e.g., Flack & Chang 1987, James & McCullogh 1990, Mac Nally 2000, Guisan & Zimmerman 2000, Quinn & Keough 2002, Guisan *et al.* 2002). Univariate screening of predictor variables, e.g. through their partial correlation with the response variable, constitutes another algorithm to obtain a subset of variables, although it has been criticized, exactly because of its univariate nature as well as for its compromised type-I error rates (Mac Nally 2000). Finally, hierarchical partitioning (Chevan & Sutherland 1991, Mac Nally 2000) quantifies the independent effect of each predictor on the response, so that they can be ranked.

In our application of DA, we compared three procedures to reduce the set of variables to enter: stepwise DA, PCA and univariate screening with Pearson chi-square calculations. In stepwise DA, the variable subset is formed during the actual DA procedure, while in the other two methods this is accomplished beforehand. The Pearson chi-square procedure consists of univariate screening of all predictors in relation to the response variable (Garson & Moser 1995). It makes no distributional assumptions regarding the predictor variables. Eventually, we performed a few combinations of the three methods. The aim of the present study was to evaluate these selection procedures. From other author’s findings (see above), we expected the worst result with the stepwise method, while PCA would work best because of its ability to reduce multicollinearity.

Methods

1. The dataset

The same dataset was used as in chapter 2, and stems from a field survey in the summer of 2001 and the winter of 2002. Data were collected from plots of 2 m² on the shores of 26 shallow softwater lakes in sandy areas of Belgium and The Netherlands. One to three plots were sampled per lake. The statistical sample consists of 46 units (plots), 232 predictor variables and one response variable: the cover of *Eleocharis multicaulis*, which is an uncommon species in the investigated region. We selected this species because it is typical of the *Eleocharition multicaulis* Vanden Berghen 1969 alliance, and because this plant community had not been subject to more elaborate research as yet. Both simple and derived variables comprise the predictor dataset (derived variables are typically ratios or summer-winter-differences of simple variables). Although the sampling design implies a limited interdependence among plots, we assume that this effect can be ignored as many lakes were sampled and plots within one lake were chosen to be dissimilar and distant. For a summary of the predictor variables, see *Appendix 3*. The response variable was split up into 3 classes in order to reflect the major variation of the species' response and in order to obtain enough sample units per response class. The following classes were chosen: absent (cover = 0%; 16 cases), low cover (cover 10% or less; 22 cases) and high cover (cover > 10%; 8 cases). To improve the performance of PCA, six possible transformations were applied to different predictors in order to normalize them (monotone functions). 101 (44%) of the 232 predictors attained a normal distribution (Kolmogorov-Smirnov test, $P > 0.05$).

Analyses were done with the statistical package SPSS 11.0 for Windows (SPSS Inc. 2001).

2. Variable selection

2.1. Principal components analysis

For each principal component, the variable with the highest loading was retained (method 'B4' of King & Jackson (1999)). The first p components with eigenvalues larger than those generated by the broken-stick model (Frontier 1976) were taken into account. However, when this led to $p > 15$, only the first 15 principal components were taken, in agreement with Krzanowski (1987). This was done to keep the ratio of variables to observations below 1:3, in order to achieve reliable canonical coefficients in DA.

2.2. Stepwise discriminant analysis

DA will be explained further on. In the stepwise procedure, the probability of the F value in ANOVA was used for inclusion and exclusion of variables, using $P < 0.05$ and $P > 0.10$ as criteria for entry and removal, respectively.

2.3. Pearson chi-square screening

For each variable, the sample was divided into three quantiles of equal frequency. In this way the frequency distribution of the response variable could be compared between the quantiles, by means of a 3 x 3 contingency table, one table per predictor. We acknowledge the fact that there is no control for type-I error when performing so many successive tests. In this case, the use of chosen *P*-levels is merely a criterion to delimit a subset of variables and to measure their association with the response variable; it is not intended for making statistical inference. This aspect typifies variable selection methods in general (Quinn & Keough 2002). We therefore prefer the term 'screening' instead of 'testing'. Two *P*-levels were used for variable selection, 0.01 and 0.05. We did not accept significant results when more than 50% of the contingency table cells contained expected frequencies less than 5. The $P < 0.01$ criterion yielded 10 variables (4.3%), the $P < 0.05$ yielded 31 (13.4%). Only the $P < 0.01$ subset was used for direct DA, as the other subset contained more than 15 variables.

2.4. Combinations

Three combinations of the previously described methods were made, starting from the variable subsets yielded by the Pearson chi-square screening. These were stepwise DA, applied to both subsets (with $P < 0.01$ and $P < 0.05$ respectively), and PCA, applied to the largest subset (with $P < 0.05$).

We thus compared six selection methods, through the results of DA. The three variable subsets in which stepwise analysis was not involved, were used for direct DA.

Because of the high number of variables, we did not perform hierarchical partitioning (Chevan & Sutherland 1991, Mac Nally 2000). This technique may however be effective in datasets with a shorter list of potential predictors.

3. Evaluation with discriminant analysis

In direct DA, all variables entered appear with a coefficient in the discriminant functions (DFs), which are the canonical functions in this type of ordination. In the case of three groups, two orthogonal DFs are constructed, leading to a total of 12 calculated DFs for all scenarios together. The discriminant coefficients are chosen to maximize the F-ratio of a one-way ANOVA, in which the three cover classes play the role of the grouping factor and the DF is the dependent variable. Homogeneity of variances among groups was subjectively evaluated on the basis of the ordination diagram, as proposed by Quinn & Keough (2002). To interpret the relative contribution of each original variable, standardized discriminant coefficients were used because these correspond to scaled variables with unit variance. The match between the six selected subsets was evaluated with Jaccard's similarity coefficient (Krebs 1999).

We considered three criteria as important for ecologists when using DA. First, the resulting DFs must clearly separate the response groups (model fit). This was verified by their classification success (percentage of correctly classified sample units on the basis of DF scores) and by means of the F-ratios.

Second, the DFs must be stable to small changes in the sample, in order to have a general value. This was verified in two ways. We applied jack-knife classification, in which each sample unit in turn is assigned to one of the categories, based on the DFs calculated from all remaining sample units (Manel *et al.* 2001). Especially if a large drop was observed in the classification success between standard classification and jack-knife classification, the DA is considered unreliable. Beside the jack-knife classification criterion, results were considered suspicious if the variable subset contained more than 15 variables, the maximum allowed for stable coefficients in our case (Williams & Titus 1988).

Third, we considered the result acceptable only if at least one original variable was present in the DF of those that most strongly correlate with the DF. For this purpose, Spearman rank correlations between the 232 predictor variables and the 12 DFs were calculated, and for each DF the predictor variables with significant correlation ($P < 0.05$) were assigned a rank number according to correlation strength. We did not perform any type I error correction when calculating these correlations, because it was our aim to select and rank the predictors, not to make a multiple statistical inference regarding the correlations. Remember that each DF contains p predictor variables. The p predictors with lowest correlation rank (most strongly correlating) of all 232 predictors were considered and it was determined which of them are present in the DF. Furthermore, considering the p predictor variables of a DF, we wish a close relationship between standardized coefficients and Spearman rank correlations. This was verified by the Pearson correlation coefficient between these two measures.

Results

Overall similarity between the subsets of predictor variables is low, largely due to the differing number of variables selected (*Table 5.1*; see *Appendix 4* for Jaccard similarities). The stepwise DA subset has too many variables to yield reliable coefficients, but we will examine the result for other properties. Most subsets originating from the chi-square selections are relatively similar.

The ability of the subsets to distinguish the cover classes can be derived from *Table 5.1*. A clear separation of classes (high F-ratio) with a perfect classification is reached by the stepwise analysis, illustrated by *Fig. 5.1A*. The only other analyses that yield good separation are the stepwise DA of the $P < 0.05$ chi-square subset (*Fig. 5.1B*) and the bare PCA-selection (diagram similar to *Fig. 5.1B*).

The stability of the DFs is derived from the drop in classification success when jack-knife classification is done (*Table 5.1*). At first sight, the stepwise analysis seems the best solution (no drop). However, this result is achieved through incorporation of 24 variables in the DFs, making the coefficients unreliable. So, the most stable solutions in our case are those with a small drop in classification success: the stepwise DAs of the chi-square subset, $P < 0.01$, retaining two variables (drop: 7%), and $P < 0.05$, retaining five (drop: 8%).

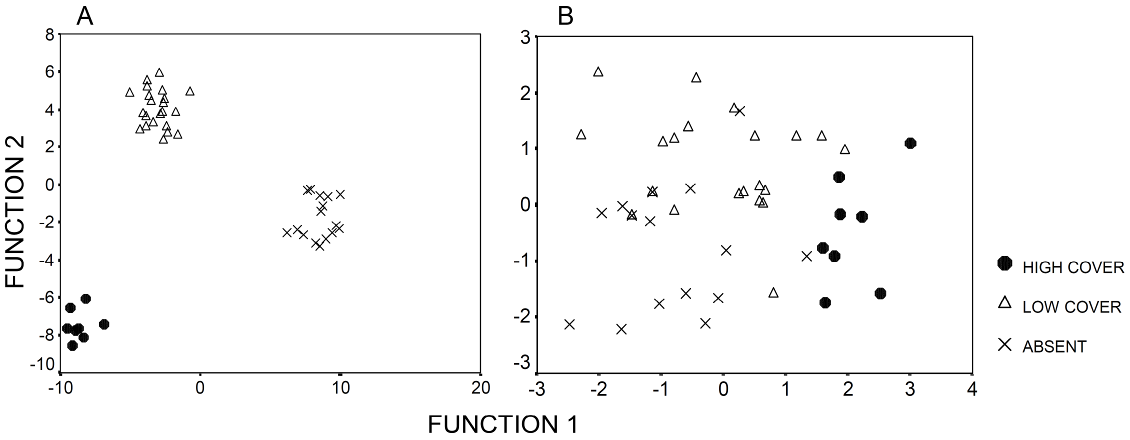

Fig. 5.1. Examples of ordination diagrams for two discriminant analyses. A. Stepwise discriminant analysis starting from all 232 predictors. The cover classes are strongly separated. B. Stepwise discriminant analysis using the P < 0.05 chi-square subset. The cover classes are rather well separated.

Table 5.1. F-ratios for the discriminant functions and classification success of each discriminant analysis.[a] A clear separation is established for the lower three scenarios.

Type of analysis	Number of retained variables	Discriminant function	F(2,43)	*P*	Standard classification success (%)	Jack-knife classification success (%)	Percentage drop [b]
CHI (0.01) DIRECT	10	DF1	18.7	<0.001	61	41	32
		DF2	0.9	0.426			
CHI (0.01) STEPS	2	DF1	12.0	<0.001	61	57	7
		DF2	0.2	0.824			
CHI (0.05) PCA DIRECT	4	DF1	3.1	0.057	46	39	14
		DF2	0.7	0.512			
CHI (0.05) STEPS	5	DF1	22.9	<0.001	78	72	8
		DF2	10.2	<0.001			
PCA DIRECT	15	DF1	29.8	<0.001	65	39	40
		DF2	8.1	0.001			
STEPS	24	DF1	975.0	<0.001	100	100	0
		DF2	427.2	<0.001			

[a] *Abbreviations: DF1, first discriminant function; DF2, second discriminant function; CHI (0.01) DIRECT: direct discriminant analysis with the chi-square subset P < 0.01; CHI (0.01) STEPS: stepwise discriminant analysis with the chi-square subset P < 0.01; CHI (0.05) PCA DIRECT: direct discriminant analysis with the PCA-selected variables from the chi-square subset P < 0.05; CHI (0.05) STEPS: stepwise discriminant analysis with the chi-square subset P < 0.05; PCA DIRECT: direct discriminant analysis with PCA-selected subset; STEPS, stepwise discriminant analysis.*

[b] *Percentage is calculated relative to standard classification success.*

The DFs from the chi-square derived analyses retain variable proportions of the predictors that correlate well with the DF (0 to 100%, see *Appendix 5*), with the lowest proportions occurring in the PCA-selected subset and the stepwise DA. All six analyses retain at least one of the best correlating predictors. However, the standardized DF coefficient is not always reflected by the Spearman correlation with the DF (*Fig. 5.2*). A significant relationship between both parameters is present for the stepwise DA, the analysis of the PCA-selected subset and the stepwise DA of the $P < 0.05$ chi-square subset. The latter analysis is marked by very high Pearson correlation coefficients between both measures (*Table 5.2*). However, for the PCA-selected subset and the stepwise DA, the actual Spearman rank correlation coefficients of the variables retained in the DFs are generally low compared to the other variable subsets, as is apparent from *Fig. 5.2* (see also *Appendix 5*). The smaller variable subsets of the four Pearson chi-square screening approaches are shown in *Table 5.3*.

Table 5.2. Pearson correlation coefficients for each discriminant function, between the standardized function coefficients and the Spearman rank correlation coefficient of the corresponding predictors with the discriminant function.[a] *Only the shaded results are significant ($P < 0.05$).*

	Pearson correlation	
Type of analysis	DF1	DF2
CHI (0.01) DIRECT	0.40	0.57
CHI (0.01) STEPS	1.00	1.00
CHI (0.05) PCA DIRECT	0.90	0.91
CHI (0.05) STEPS	0.99	0.92
PCA DIRECT	0.76	0.72
STEPS	0.57	0.73

[a] *Abbreviations as in Table 5.1.*

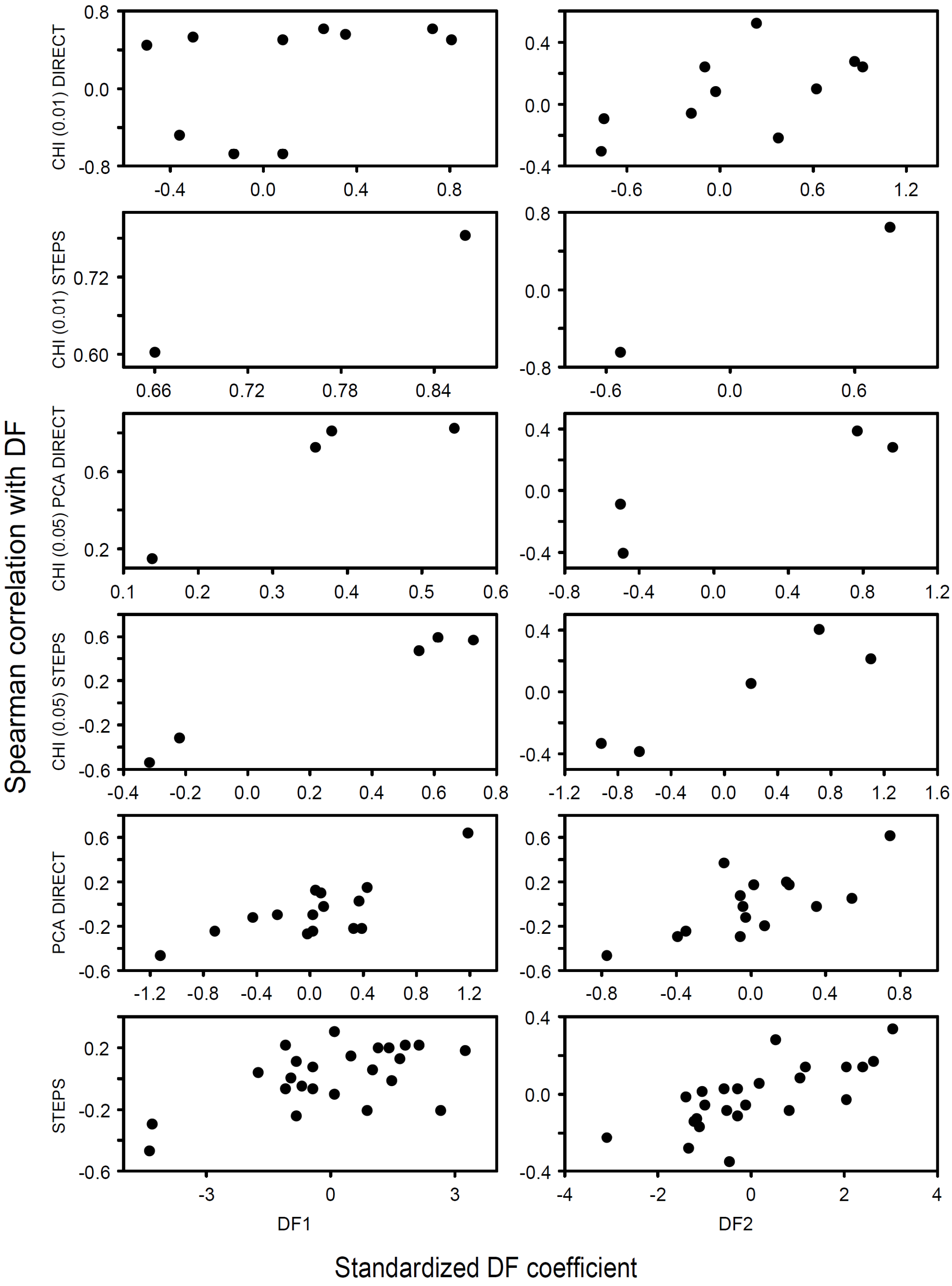

Fig. 5.2. Graphical relationship between standardized discriminant function coefficients and the corresponding Spearman rank correlation between the retained predictors and the discriminant function. Standardized discriminant function coefficients are not strictly associated with the Spearman rank correlation between the retained predictors and the discriminant function. Only for the lowest three analyses (six graphs) there is a significant correlation between both measures (P < 0.05). Abbreviations as in Table 5.1.

Table 5.3. Retained variables of the Pearson chi-square screening approaches, marked with their maximum absolute Spearman rank correlation (with either DF1 or DF2) when they belonged to the most strongly correlating predictors, and otherwise with “X”. The PCA-selected subset and the stepwise DA subset are not shown in full (see Appendix 6). They mainly do not coincide with the chi-square subsets shown here, and they contain only few highly correlating predictors, of which most belong to chi-square subsets.[a]

Covariate [b]	CHI (0.01) DIRECT	CHI (0.01) STEPS	CHI (0.05) PCA DIRECT	CHI (0.05) STEPS	PCA DIRECT	STEPS
Number of retained variables	10	2	4	5	15	24
Seasonality of Mineral soil layer Silicon concentration NaCl-extraction	0.67					
Mineral soil layer Winter Silicon concentration NaCl-extraction	0.65					
Seasonality of Mineral soil layer Total N content	0.52	0.66		X		
Mineral soil layer Winter Total N content	0.55					
Seasonality of Mineral soil layer Potassium concentration	0.58					
Seasonality of Surface water Ammonium / nitrate concentration	0.64	0.79		0.60		0.45
Mineral soil layer Winter Silicon concentration	0.64					
Cover *Juncus bulbosus*	X					
Surface water Summer Ion Ratio (IR)	X					
Surface water Winter Chloride proportion	X					
Surface water Winter Potassium concentration				0.52	0.45	
Seasonality of Surface water Divalent / monovalent cation ratio				0.57	0.65	0.34
Cover *Mentha aquatica*				X	0.44	
Cover *Agrostis canina*			X			
Mineral soil layer Summer Magnesium concentration			X			
Surface water Summer Magnesium concentration			0.81			
Surface water Winter pH			0.82			

[a] *Abbreviations as in Table 5.1.*

[b] *Seasonality variables are the difference between summer and winter values. Other variables are always confined to winter or summer conditions, with cover values belonging to the summer period. See chapter 2 for technical aspects and for ecological interpretation of results.*

Discussion

In our study, the stepwise DA of the $P < 0.05$ chi-square subset combines all desirable properties: it is able to effectively discriminate between the cover classes, the analysis meets the variables to observations ratio condition that should lead to stable DF coefficient estimation, and the interpretation of the coefficients is supported by the pattern of Spearman rank correlations. At least one of these criteria is not accomplished in any of the other methods. In particular, our study suggests failure of the popular stepwise procedure and, in contrast with our expectations, also the PCA-selection procedure.

Moreover, the causal relations of the chi-square selected predictor variables with the performance of the plant species can be ecologically explained (see chapter 2), whereas several variables retained in the stepwise and PCA procedure would be difficult to explain in a plant ecological context (e.g. lake surface, or water colour (absorption at 450 nm)). Thus, the models we compared statistically, also differ in ecological meaningfulness. Austin (2007) encourages the use of this criterion when evaluating models.

James & McCullogh (1990) and Quinn & Keough (2002) summarize the criticisms against the use of stepwise procedures in linear methods. Stepwise procedures attempt to maximize the percentage of variation accounted for by the linear function. However, meaningless variables are likely to be selected to serve this purpose, as shown in a simulation study by Flack & Chang (1987). Our results support these findings. Although the stepwise selection method showed promising results, the rather poor relationship with the Spearman correlations, the low involvement of predictors that correlate well with the DFs and the high number of selected variables make this model unlikely to be generally applicable. Similarly, Van Sickle *et al.* (2006) compared stepwise DA with best-subsets DA, the latter being an approach not considered by us. They have come to similar conclusions regarding stepwise DA and they equally discourage its use.

Multicollinearity is a problem of all methods in which linear combinations of predictor variables are involved (Graham 2003). Especially when scientists are interested in the causal (explanatory) value of a model, rather than in its predictions, multicollinearity brings the risk of retaining non-causal predictor variables that are correlated with a causal, but discarded variable. Multicollinearity is the main reason for several authors to recommend interpretation by means of correlations with the canonical function (linear function) instead of the actual canonical coefficients (e.g., Manly 1994, Quinn & Keough 2002). However, in observational studies an orthogonal design is rarely present, in which one predictor varies while the others remain constant (Johnson & Omland 2004). Hence, the additive effect of each predictor variable can only be interpreted from the canonical coefficients, as suggested by Rencher (1988, 1992), Williams & Titus (1988), Tardif & Hardy (1995) and Mac Nally (2000). We have therefore used the presence of the best correlating predictors in the canonical function and a good relationship between correlations and coefficients, as model eligibility criteria.

Retaining the variables with the highest loading in PCA reduces the multicollinearity problem, because this selection method effectively excludes redundant variables. King & Jackson (1999) selected variables from a climatic dataset using PCA, in order to conduct a canonical correlation analysis between the climatic data and data concerning thermal lake stratification. However, selection by means of PCA does not take into account the direct relevance of the predictor variables to the response variable(s), implying a potential for withholding less meaningful, though less redundant, predictor variables (Graham 2003). A similar reasoning has been made by ter Braak (1995, p. 136), in the context of correspondence analysis. The low jack-knife classification success in our case indicates that PCA did not mark the necessary variables for prediction of the performance of *Eleocharis multicaulis*. The algorithm of PCA subset selection contrasts with that of the chi-square screening approaches, in which the direct relation of each predictor with the response variable is the first criterion to retain or reject a predictor variable.

Univariate chi-square screening before embarking on any multivariate analysis turned out to be a very satisfactory method. Conducting a stepwise procedure with these variables (subset $P < 0.05$) does not run the risk of yielding an ecologically less meaningful variable subset. On the contrary, it indicates their predictive ability by further selection and assigning coefficients. It is probable that the rather low drops of classification success when jack-knife classification is performed (*Table 5.1*), are due to the selection of the ecologically most meaningful variables beforehand. In general however, by excluding variables that are not significant in univariate analysis, there is a risk of losing actually causal variables when their effects cancel out each other in the specific dataset (Mac Nally 2000). Although this is theoretically possible, we expect that the ecologically most significant predictors will in most cases be significant on the univariate level when the sample is not too small.

In conclusion, we agree with other authors that purely stepwise methods are not recommendable for achieving a good explanatory ecological model when starting from many predictor variables. From our results, ecologists should be prudent when using PCA subset selection. PCA can be used if the only purpose is to get a limited dataset that still contains much of the variation of the original data. PCA however, will not necessarily withhold the important variables for the interpretation of an extra phenomenon. In general, we suggest to evaluate any selected variable subset by means of the efficacy and credibility of the obtained results from the analysis of real interest (discriminant analysis in our case), using objective statistical criteria as well as the ecological interpretability and credibility of the models, thereby supporting the view of Austin (2007). The univariate evaluation of variables in relation with the response variable is a method with potential, e.g. chi-square screening in the case of a categorical response variable. Subsequent selection with one of the previous methods (PCA or stepwise) can prove useful. Other methods that we did not consider, like best-subsets comparison and hierarchical partitioning, may also be useful. There clearly is a need for simulation studies on these subjects, so that more generally applicable conclusions can be drawn than presently possible from ecological literature.

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Supporting information

Additional supporting information may be found in the appendices:

Appendix 3. The number of different types of predictors measured.

Appendix 4. Overall characteristics of the six variable subsets.

Appendix 5. Match between the variable subsets and the variables that best correlate with the discriminant functions.

Appendix 6. Retained variables of all approaches, marked with their maximum absolute Spearman rank correlation (with either DF1 or DF2) when they belonged to the most strongly correlating predictors, and otherwise with "X".

6

General Discussion

Failure to examine the basic assumptions of a paradigm
still determines our research agendas.

Austin (1999)

Flower of Baldellia ranunculoides ssp. repens with a hoverfly near to it,
Someren, The Netherlands

CHAPTER 6

GENERAL DISCUSSION

1. Summary of basic results

Hypothesis A:

Eleocharis multicaulis is confined to an acid situation rich in ammonium and carbon dioxide, nutrient-poor water and soil and a mineral substrate devoid of organic material

In the study on *Eleocharis multicaulis* (chapter 2), discriminant analysis and univariate techniques resulted in a rather long list of influential variables that were partially inter-correlated (*Table 2.3*). An explicit further selection among these was not made. They came as the result of an extensive screening of hundreds of resource, direct and indirect physicochemical variables, including seasonality aspects, many ionic ratios and the distinction between soil layers. The main groups of influential variables were those that were related to 1) the redox potential (among which soil silicon and surface water ammonium/nitrate ratio), 2) acidity & base richness, 3) base cation replenishment during winter, 4) soil nitrogen availability (among which several ionic ratios), 5) soil phosphorus availability and 6) nutrient richness of the organic layer. Groups 5 and 6 were also correlated to soil silicon content.

Eleocharis multicaulis primarily reacted to nitrogen ions, in a positive sense, as long as nitrate remained dominant over ammonium (ammonium/nitrate ratio < 1), phosphorus and potassium remained low, the water was acid and had a high redox potential (for weighted averages and ranges, see *Table 2.3*). The species was primarily found on an organic substrate. Silicon of the soil digest was correlated to several of these variables and *Eleocharis multicaulis* showed a clear positive response to it. In this dataset, no significant results of ammonium or carbon dioxide were found.

In conclusion, only the hypothesized acid and nutrient-poor requirements are supported, while the results contradict the hypothesized preference for ammonium, carbon dioxide and mineral layers. Consequently, hypothesis A as a whole does not hold, demonstrating the incompleteness of previous knowledge of the species' ecology.

Hypothesis B:

Within one vegetation type, the realized niches of species are still separated at the biogeographical scale of the vegetation type

In the West-European study (chapter 3), five abiotic predictors were considered important in regression models that included five species: four surface water variables - calcium and the resource variables ammonium, nitrate and orthophosphate - and the soil silicon content, which was related to surface water pH. Nitrate was negatively correlated with the ammonium/nitrate ratio, which was actually used in the regression models. Calcium was related to pH and alkalinity of the surface water. They were selected with statistical criteria from a list of 39 environmental variables from surface water, soil and climate, that mainly focused on direct and resource variables sensu Austin (2005). The selected variables showed no further high correlations ($|r| > 0.5$) with other predictors than the aforementioned.

Four species showed clear patterns in relation to the selected gradients. One species (*Juncus bulbosus*) was very unspecific; its realized niche was not limited within the investigated environmental range and vegetation type. The two species entitled ‘characteristic’ of the vegetation alliance (*Eleocharis multicaulis* and *Hypericum elodes*) showed opposite differences with respect to nutrients and silicon. *Agrostis canina*, *Hypericum elodes* and *Hydrocotyle vulgaris* were similar in their reaction to these predictors but with different positions along the gradient. An exception was that *Hydrocotyle* responded to orthophosphate in a negative way, in contrast to the other two species but similar to *Eleocharis*.

In conclusion, the species’ realized niches were completely or partially separated, depending on species and gradient. One species (*Juncus bulbosus*) was rather insensitive to the measured gradients, but possibly separates along an unmeasured environmental or functional axis, including functional gradients of resource acquisition such as rooting depth. Findings on a biogeographical scale were consistent with coexistence and niche theory within one vegetation type, and hence I mainly found evidence in support of hypothesis B.

Hypothesis C:

Nutrients (carbon dioxide and ammonium) drive population performance and community composition more than water level drawdown and species identity

Distinction between these mechanisms was achieved by using a controlled experimental setup in a laboratory environment (chapter 4). Care was taken to mimic natural situations as close as possible. Species performance generally was not influenced by the ammonium or carbon dioxide nutrient treatments, although high carbon dioxide levels led to better performance of several species at specific times. The results stressed the importance of intrinsic properties of the species (their identity) and as such, their presence, to the community outcome. From the rapid population changes during water level drawdown and specific observations of species in relation to the water table, it was concluded that water level drawdown was presumably very influential on relative species performances and ranking order, most likely through its effect on the availability of aerial carbon dioxide to the plants. The water level drop acted as a disturbance to *Baldellia* at least.

Consequently, hypothesis C does not hold.

Hypothesis D:

Competition between species has a marked role in the Eleocharition

In the experiment (chapter 4), a large competitive response was observed in both *Eleocharis* and *Baldellia*, depending on performance measure. Competition intensity, measured in the high nutrient level combination, was greatest in the emersed state, which is in accordance with plant ecological theory.

Our observations support the marked presence of competition in the *Eleocharition*, and hence support hypothesis D.

2. Revisiting theoretical frameworks on competition and the niche

Now, I will revisit several of the plant ecological frameworks that I explained in the General Introduction (paragraph 1) and discuss my results in the light of these. I follow the same subtitles as in paragraph 1.2. A further discussion relating to the environment of aquatic macrophytes and of the *Eleocharition* follows in paragraphs 3 and 4.

2.1 Competition and the niche – local species coexistence at equilibrium

Plant coexistence has been addressed by numerous authors, and many mechanisms have been proposed to explain coexistence of species. Most evidence exists for plant coexistence through niche differentiation, including separation by resource use strategy (McKane *et al.* 2002, Levine & HilleRisLambers 2009). Some recent examples include Ashton *et al.* (2010), Harrison *et al.* (2010), Siefert (2012) and Douda *et al.* (2012), and for aquatic macrophytes, Stiers *et al.* (2011) and Njambuya *et al.* (2011). Silvertown (2004) listed several testable assumptions of the paradigm: 1) intraspecific competition is greater than interspecific competition, 2) species segregate on a resource or environmental axis, 3) trade-offs exist between traits or in performance on different niche axes, and 4) niche shifts in response to experimental manipulation of competitors. He found only 13 studies published since 1990 that tested at least two of these assumptions. My analyses were in support of assumptions 2 (chapter 3) and 4 (chapter 4) and I found indications for assumption 3 (chapters 3 and 4; based on plant strategies and an indication of a resource-usage trade-off; see below). The assumptions all regard competition as the driving mechanism.

In my one-year experiment (chapter 4), the competitive response was found to be large in characteristic softwater lake species in competition with the CR strategist *Hydrocotyle vulgaris*. Only a modest competitive ability must be attributed to this species, given its limited height. Hence, at least in the amphibious *Eleocharition* and within a certain water level range, it is possible that the interactions between the occurring species are more important than the precise physicochemical characteristics, as long as species are able to survive and grow in the given environment. Put otherwise, this means that it could be that their fundamental niche is a lot larger than their realized niche, which then greatly depends on the presence and densities of species at sites. However, much more long-term experimentation on fundamental niches and interactions is needed to fully test this hypothesis.

On the other hand, this means that realized niches, as observed in the field, can be expected to be smaller. In order to predict relative abundances from a given list of species at a site, the abiotic environment must be taken under consideration, as shown by the realized niche differentiation along different axes in chapter 3. Essential information of how different environments act upon species (via their functional traits) and upon species interactions is needed to quantify the resulting community outcome from species intrinsic properties, as stressed by McGill *et al.* (2006) and Webb *et al.* (2010). This implies that different species will be dominant under different environmental conditions.

The competitive hierarchy model assumes that this dominance is the result of competitive interactions. In the descriptive part of my work, patterns were detected that match the predictions of this model (chapter 2 and 3). The competitive hierarchy model critically links species rankings to competitive ability ranking. According to the paradigm of coexistence through niche differentiation, fitness trade-offs underpin niche separation. The match between niche patterns and functional characterization (chapter 3) provided one indication of this, while another was that dominance hierarchies could reverse depending on the resource gradient considered. This reversal of dominance hierarchy might be the consequence of a physiological trade-off in accessing one versus another resource, and hence in fundamental niche width along these resources. Consequently, the pattern along each single resource gradient was in support of the competitive hierarchy model (except for *Juncus*). However, the constancy of the competitive species ranking as found in some other studies, was contradicted when different resource gradients were considered. However, from our data the mechanisms of the competitive hierarchy model could not be tested but only hypothesized or supported by plausible trade-offs between resources. Hence several of its assumptions remain untested, especially the inclusive nature of species’ fundamental niches, and the rank order along the resource gradient that must be consistent with competitive ability ranking.

Two aspects complicate the relationship between the competitive hierarchy model and coexistence of species within the *Eleocharition*. Firstly, I feel a lot of confusion about the term ‘resource partitioning’, which the competitive hierarchy model is meant to account for. In animal ecology ‘resource partitioning’ is strictly used to denote the fact that different species consume different, discrete resources (e.g. seed eaters vs. insect eaters, or eating insect species 1 vs. species 2 and so on). However in plant ecology, it is often used in a more relaxed sense, especially by authors that stress the fact that all plant species share the same few resources (e.g. Austin 1990, Keddy 2001, Silvertown 2004). These authors focus on resource partitioning along a resource gradient, i.e. essentially for the same resource (e.g., plant-available phosphorus). They often broaden the concept to niche differentiation along one or several axes (not necessarily resources). On the other hand, several authors take the paradigm in its ‘pure form’, i.e. partitioning among discrete resources. Either they contradict the paradigm and propose other mechanisms, e.g. neutral theory (Hubbell 2001), or they demonstrate that discrete resource partitioning also occurs among plants, sometimes allowing different resource acquisition strategies within the paradigm (e.g. McKane *et al.* 2002). Personally, I have focused on the relaxed approach, i.e. differentiation along continuous niche axes.

Secondly, a fixed competitive hierarchy ranking between species may not hold for similar species, or at fine spatial scale, as Keddy and co-workers stated themselves (Keddy *et al.* 2000). They discussed this in the light of the fact that only half of the papers reviewed by Goldberg (1996) found a consistent hierarchy pattern across different environments. Accordingly, my results indicated that hierarchy can reverse according to the environmental state (chapters 2, 3 and 4). Keddy never claimed universal validity of his model (e.g. Karez & Chapman 1998) and, as Keddy *et al.* (2000) continue, “further empirical work is needed to sort out the factors that may be operating at different scales of organization.”

Competition is often loosely mentioned when the presence of high-productive species correlates with lower performance of target species (e.g. Szmeja 1994). Actual testing of competitive exclusion from a part of the fundamental niche, depending on which other species are present, can only be done by larger experiments. These must carefully document the fundamental niches of several species, i.e. by systematically varying several environmental variables. Moreover, the experiments should include many-species interactions, not just pairwise interactions. It has been shown that applying higher levels of species interactions lead to more stable species hierarchies (Perkins *et al.* 2007), although for bacteria and algae, multispecies interactions appeared consistent with pairwise interactions (Zhang & Zhang 2012). Probably due to practical constraints, such experiments have not often been done in (partly) submersed macrophytes. An alternative is to perform field competition experiments along abiotic gradients (e.g. Opdekamp *et al.* 2012). Understandably, competition experiments of aquatic macrophytes have been simplified, mostly using two species (e.g. Mulderij *et al.* 2009, Spierenburg *et al.* 2010, Netten *et al.* 2010, Spencer & Rejmanek 2010, Stiers *et al.* 2011).

2.2 Coexistence and fluctuations: the temporal scale

The integrated community concept of Lortie *et al.* (2004) implies varying possible degrees of importance of different community organizing mechanisms, depending on the community at question. We must therefore accept that competition is not the only possible biotic factor that shapes the investigated plant community and leads to coexistence. For example, as discussed in chapter 3, also facilitation might be important. Moreover, and consistent with Tilman's stochastic niche theory, stochastic processes of species arrival at a site and subsequent (temporary) establishment may be superposed on deterministic processes of abiotic and biotic interactions, allowing more possible patterns of coexistence (e.g. Schamp *et al.* 2008). Dispersal traits appear to be critical in understanding species occurrence and co-existence (Ozinga *et al.* 2005, Ozinga *et al.* 2009). For example, the importance of species' dispersal capabilities through time and space was demonstrated in plant community restoration of wet meadows (Klimkowska *et al.* 2007, Klimkowska *et al.* 2010). This random component is the main tenet of neutral theory, as opposed to classical niche theory. Both can be accommodated in community ecology if one allows for varying degrees of importance of these different mechanisms (Lortie *et al.* 2004, Leibold & McPeek 2006, Gravel *et al.* 2006).

In my research, relatively short time scales were considered: seasonal fluctuations of soil and water chemical characteristics were tested in the observational study in chapter 2, while a water level drop was applied to the simulated communities in the experiment in chapter 4. In both cases, a correlation was found between these fluctuations and the abundance of the investigated plant species, in a community. Softwater lake shores are a dynamical environment, especially because of the water level fluctuations. These can be regarded as a disturbance to several species. Disturbances and time-lags in the reaction of species create space and resource opportunities for new plants to establish. Therefore, it should be expected that water level fluctuations play an important role in community assembly, as the tolerance to inundation differs between species (chapter 4, Lenssen *et al.* 2004a, Sumberova *et al.* 2005, Urban 2005). However, niche- and competition-based processes can be expected to play an important role as well, as plant densities in the *Eleocharition* are somewhat higher compared to exclusively aquatic macrophytes of softwater lakes. Consequently, the stochastic niche theory of Tilman (2004) might be a promising model to the *Eleocharition*, as it encapsulates competitive hierarchy (Keddy 1989) and niche differentiation, and also comprises neutral processes.

The shores of one lake that I have sampled in Germany in 2001, were followed for nine years by Urban (2005). During this period, no clear succession had taken place in the amphibious zone, but PLANT ASSEMBLAGES did however show high short-term dynamics. Disturbances through submergence created gaps in the vegetation, which were recolonized by many different plant species, mainly from vegetative regeneration. Consequently, stochastic demography (neutral processes) seemed to rule in this case, and this was essentially what the author proposed as an overall conceptual model for the encountered plant species assemblages (although she did not refer to it).

Disturbances have been investigated in lake shore vegetation during an even longer period. This was the case for lake vegetation in West Connemara (Ireland), which was followed during 18 years (van Groenendael *et al.* 1996). Although some changes could be observed in the vegetation composition that were related to a unidirectional trend in orthophosphate levels, the major part of vegetation dynamics was related to water levels, consistent with year-dependent climatic conditions. Furthermore, it seemed that a period of 18 years was still too short, as the vegetation still appeared to be changing in response to orthophosphate supply. Consequently, in order to better grasp time-dependent mechanisms of coexistence and assembly in these communities, longer monitoring periods would be needed. The necessary period of study will depend on the time-lag of the community in the response to unidirectional environmental changes, on the effect of the interaction between environmental trends and the frequency and intensity of recurring disturbances, and on the rate of an investigated monotone environmental change.

While it may be very difficult to demonstrate the possible relevance of competitive chaos in the *Eleocharition* (as discussed by Huisman & Weissing (1999) for plankton communities), the importance of the temporal storage effect is known for this plant community as a whole. Especially under more nutrient-rich conditions, species appear to be competitively excluded by more productive species (Vanderhaeghe 2000, Brouwer 2001). However, several of these species clearly reside for a longer time in the seed bank, as they can recover from it after restoration measures have been taken (Brouwer *et al.* 2002). The same was found in many Dutch softwater lakes, after recovery from decades of acidification (Arts *et al.* 2001). As such, the temporal storage effect may account for cyclic succession on longer time scales.

Multitrophic interactions, and their role in plant coexistence and community assembly, have not been investigated in the *Eleocharition* as far as I know. As these phenomena are expected to vary in space and time, they further add to the random component of the stochastic competitive assembly of Tilman (2004). A well-known phenomenon in the *Littorellion* is that arbuscular mycorrhizal fungi, in the roots of isoetid plants, are more abundant in lakes with a very low phosphate availability (Wigand *et al.* 1998, Smolders *et al.* 2002, Baar *et al.* 2011). These mycorrhizal fungi probably improve the availability of phosphate to the plants. It may be that mycorrhizal fungi also play a role in species of the *Eleocharition*; however this remains to be investigated. Also regarding herbivory, effects are to be expected, since herbivory has been found to have important effects in certain aquatic plant communities. For example, herbivory by weevils altered competitive interactions between aquatic macrophytes (Center *et al.* 2005), and competitive interactions between 3 aquatic macrophytes were found to be differentially affected by herbivory, both aboveground (fish and waterfowl) and belowground (swans) (Hidding *et al.* 2010).

2.3 Coexistence and the niche in a spatial context

I demonstrated partial niche separation by taking measures from 2 m² plots over a relatively large geographical area. Essentially it means that I defined each species' niche from several distinct sites. Niche differentiation emerged as a pattern at the large spatial scale, and, when seen as an intrinsic species property, it allows coexistence according to the aforementioned paradigm. At the local community scale, the species I investigated do at least partly coexist within the plot scale of 2 m² (chapters 2 and 3). However, whether environmental separation occurs at this scale (within a plot) has not been measured. If niche separation through competition is to be responsible for (equilibrium) coexistence at this scale, finer-scale environmental heterogeneity is needed to accommodate the species. So this remains to be tested for the *Eleocharition*. Authors differ a lot in the extent to which they consider this mechanism. Many authors don't focus on fine spatial heterogeneity to allow coexistence by competition; Keddy (1989) even stated that niche overlap indicates that competition is not important in such cases. Although that might be true (e.g. facilitation may be at work), he ignored that separation by competition may still occur along other resource gradients in that case. The first assumption of Silvertown (2004), borrowed from Williamson (1957), provides another way-out by including high intraspecific competition in abundant species in order to prevent competitive exclusion of subordinates. Still, it does not account for different positions in niche space to occur at one site, cf. his second assumption. Although it was not my focus, environmental heterogeneity is an important component of the resource ratio theory of Tilman (1982). In this theory, resource availability also varies through the very consumption by competing species, automatically resulting in fine spatial heterogeneity (Miller *et al.* 2005). The importance of fine-scale heterogeneity in community organization has been addressed by several authors (Levin 1992, Mouquet & Loreau 2002, Amarasekare 2003).

Accepting the concept of niche separation as a consequence of competitive constraints and physiological trade-offs at the local scale, I found it tempting to see whether this holds over different areas with significantly different environments, i.e. at the biogeographical scale of a plant community. At this scale, one might expect that in very different areas, mechanisms that account for the realized niche may differ. This would result in realized niches that are closer to the fundamental niches than when they were measured at a small geographical scale (cf. Colwell & Rangel 2009). Moreover, disturbances and stochastic demography may induce a reduced – or delayed – competition intensity, potentially leading to a larger realized niche. In the case of high dispersal rates, the realized niche can even be larger than the fundamental niche (Pulliam 2000). I still found considerable niche differentiation however, supporting the paradigm of coexistence through niche differentiation (chapter 3). Beside the possibility of a consistently recurring mechanism of competitive coexistence over a large geographical area, at least three other points must be considered, and my observations may be the result of all. First, the assumption of inclusive fundamental niches may not always hold (e.g. chapter 4), suggesting that niche separation may also occur evolutionary, and as such lead to non-overlapping fundamental niches (see Keddy 1989). Second, local adaptation of the niches may be present in the investigated species, which is a spatially dependent and intraspecific version of my previous point. Hence I may have aggregated the niches of different ecotypes of a species on the West-European scale. However, local adaptation of the niche can only be tested by growing or transplanting individuals into another environment, in order to compare their fundamental niches. Third, although 28% of the plots were scattered over a wide West-European region, a majority of 72% was confined to a similar geological setting in Belgium and The Netherlands. Specific tests would be interesting that compare both geographical scales in order to verify the degree to which the modelled niches were affected by this scale aspect; e.g. in the light of the coinciding results for *Eleocharis multicaulis* when comparing chapters 2 and 3. However, this would require more data points outside Belgium and The Netherlands. As the values of some predictors obviously varied with geographical location, realized niche differences could be expected between regions. However, this would not necessarily suggest ecological or evolutionary divergence within species, as it might be a purely environmental effect. Therefore, an ecological interpretation of spatial niche differentiation would be more difficult when not complemented by manipulative experiments, e.g. transplantation.

Regarding plant's niche modelling from environmental factors in general, a wide variety of techniques is available (Elith & Leathwick 2009, Franklin & Miller 2009). Some software packages more easily allow to compare the results of many techniques (e.g. Thuiller *et al.* 2009), but these packages are limited to presence-absence responses. This can be regarded as a drawback, as a community is more than just a list of co-occurring species; it also covers species abundance, other aspects of performance and structure. To that aim, I applied techniques that distinguish between cover classes. As estimated plant cover must be regarded as ordinal (Guisan & Harrell 2000, Austin 2007) - not continuous as many believe - I used ordinal techniques: discriminant analysis and proportional odds models (POM), an ordinal regression method (Harrell 2001, Liu & Agresti 2005). Several authors recently used proportional odds models in ecology (Guisan 2002, Dirnböck *et al.* 2003, Coudun & Gegout 2007, Randin *et al.* 2009, Irvine & Rodhouse 2010), but they are rare compared to the numerous practitioners of linear techniques for continuous or binomial responses (GLM, PCA etc.). My data only allowed to distinguish between two cover classes above zero. This may explain why there was a high similarity in selected predictors and modelled responses between binomial GLMs and POMs. However, the estimated probabilities from POM doubled the information content yielded by GLM, as for each cover class the corresponding probability was modelled. Furthermore, degree of overfit was much lower in POM, making them more robust. With a larger dataset (i.e. more plots), it would have been possible 1) to further enhance predictive accuracy of the models, and 2) to apply non-parametric regression methods in a reliable way, like generalized additive models (GAMs), in order to accommodate complex response shapes. Development of statistical techniques belonging to the regression family moves fast (Yee & Mackenzie 2002, Yee 2008, R Development Core Team 2012). Ultimately, mixed GAMs for ordinal responses will be needed in sound niche modelling of ordinal abundance data that are collected in a hierarchical spatial design with random factors. However, these models have yet to be developed (Simon Wood, pers. comm.).

A point should be raised relating to the syntaxon *Eleocharition multicaulis*. In the West-European survey, I regarded *Agrostis canina*, *Hydrocotyle vulgaris* and *Juncus bulbosus* as non-characteristic of the *Eleocharition*. My analysis suggests that this view is not correct, as both *Hypericum elodes* (characteristic species), *Agrostis canina* and *Hydrocotyle vulgaris* show niche overlap and are positively associated. As such, it can be derived that a clear distinction between ‘characteristic’ and ‘non-characteristic’ does not hold among these species. It was merely a consequence of departing from a syntaxonomical system, originated in static community-unit theory. This demonstrates the risk of departing from community-unit theory to design research in general. Vegetation scientists should be very critical about the framework they use to choose their questions. It is more realistic to investigate vegetation as a continuum, regardless of its discrete patterning in geographical space (see General introduction, 1.3). This approach centres on species ecology and regards the community as a result of assembly mechanisms, which vary in space and time. Predicting species assemblages, then, may be best approached by the combination of species niche models, ecological assembly rules and constraints by macro-ecological theory (Guisan & Rahbek 2011). The most challenging step in this approach will be in defining and implementing ecological assembly rules, which must identify the species or functional groups that can coexist in a place, from those that can reach and physiologically survive in this place. These rules can be based on empirical or experimental data (Guisan & Rahbek 2011).

3. Answers concerning the number of investigated environmental predictors, ionic ratios and plant strategies

Beside the four hypotheses, I posed three additional questions at the end of the General Introduction. Below, I give my answers and ideas to these questions. A more theoretical discussion that extends on these themes, can be found in paragraph 4.

- To what degree do the observed response patterns of different species agree with their position in the plant ecological **strategy** scheme of Kautsky (1988)?

From chapter 3, it is derived that none of the five species is confined to a single strategy type; they all have a mixed strategy: either CS (*Eleocharis*), CR (*Agrostis*, *Hypericum* and *Hydrocotyle*) and CBRS (*Juncus*) (classification according to the PESS of Kautsky 1988). Their functional (dis)similarities coincided with their observed niche overlaps and differences (see above). The relative species dominance ranking could not be inferred from this mixed functional characterization, however. Neither was this the case in the community experiment in chapter 4, in which partly different species were used. Here it was attributed both to the mixed strategies of all species and the high morphological plasticity in *Baldellia ranunculoides* and *Luronium natans*. It is clear that the discrete strategies C, B, R and S are simplifications of the real world, and hence must be applied with caution. In conclusion, response patterns did match functional characterization of the species, though not in a predictive way. I will discuss further on this subject in paragraph 4.1.

- Are the best explanatory variables those expected from general plant ecological concepts (resource and direct variables), or does starting from **many more chemical variables** reveal other significant gradients that improve discriminative power?

I considered two approaches to describe a species' niche. They differed entirely, both in the set of environmental variables to start with, and in their statistical approach to select variables. The approach that started from a reduced list of mainly proximal variables and that resulted in just 5 predictors (chapter 3), has the advantage of simplification in searching for relevant niche differences, as well as robustness for prediction because of model parsimony. To a large extent, the longer list of relevant variables in chapter 2 can be matched to these 5 predictors by correlation. This was true even though the approach in chapter 2 was largely based on univariate relationships, as opposed to chapter 3 (see also chapter 5). Consequently one might prefer the 'short list'. The short list was convenient to test some expected niche patterns from theoretical ecology in a regression framework. However, its scope remains limited when the aim is also to hypothesize on relevant biogeochemical processes that shape the species' niche. In that case, the added explanatory value of several ionic ratios and macro-ionic concentrations becomes apparent, even though they are statistically correlated to other variables with explanatory power. For example, hypotheses on historical and landscape-level processes that link ammonium-nitrate ratio, pH, silicon, redox potential, phosphorus, potassium and alkalinity would not have emerged when working with just a few predictors, even if these suffice to define the plant's niche. Obviously, starting with a long variable list has a drawback of per-observation measurement effort by the researcher, and must be well argued before embarked on. So I conclude that both approaches have their merits, depending on the specific aspects of macrophyte ecology that one wants to investigate. My advice to practitioners of one approach, is to be conscious of the pros and cons of each approach. They should clearly base their field design on explicit questions or hypotheses, and always point out the restrictions of the set-up when chosen for a limited number of explanatory variables.

For some remaining issues on the environment of the *Eleocharition*, I refer to paragraph 4.2.

- What is the potential of **ionic ratios** as a predictor of plant performance and abundance?

In chapter 2, several ionic ratios were found to have a high discriminatory value for the cover response of *Eleocharis multicaulis*. Ionic ratios may have a high potential as proximal predictors to plant performance and abundance, as has been demonstrated by other authors (e.g. Roelofs *et al.* 1985, de Graaf *et al.* 1997, Güsewell *et al.* 2003, Güsewell 2005, van den Berg *et al.* 2005, Stevens *et al.* 2011). However, they are not often applied in plant habitat studies. I will discuss further on this subject in paragraph 4.3.

4. Revisiting the Eleocharition multicaulis and aquatic macrophytes

Below, I extend on the findings that were summarized in the previous paragraph, putting them in the broader perspectives of the *Eleocharition multicaulis*, of aquatic macrophytes, and ultimately, of plants (paragraph 4.3).

4.1 What do we learn from the trait-based approach?

Willby *et al.* (2000) did a numerical classification based on 17 traits of 120 submersed macrophyte species, and they related it to habitat use. The classification led to 20 functional species groups, and multivariate analyses allowed to explain 72% of habitat use variation among the species, based on their traits. Temporal variation (disturbance) and resource conditions proved to be main distinguishing environmental factors along which functional groups were ordered. However the functional groups could not be reordered into a few simple strategies as in terrestrial plant strategies: only the finer classification levels were ecologically meaningful. Furthermore, these authors found considerable overlap in the occupied environmental niches occupied by species with different functional groups. They propose that there may be few robust relationships between macrophyte species, traits and environment. This is further complicated by the large morphological plasticity exhibited by several species, hindering a clear species characterization (except for their degree of plasticity). Possibly the only robust functional relationship between traits and environment is the gradient of small, rosette-like species at low-production sites to tall canopy-forming species at high-production sites. This coincides with Tilman's (1982) model of plant distribution along resource axes. Bornette *et al.* (2008) have proposed a conceptual model of how plant traits in fluvial systems may be driven by the nature and intensity of disturbances; however many of their propositions remain to be tested further. Willby *et al.* (2000) conclude that "hydrophyte attribute groups should be used cautiously for habitat assessment or prediction as confidence limits will often be fairly broad." Of course, this is not a very encouraging conclusion to the advancement of predictive ecology of (semi-)aquatic macrophyte communities, which must surmount 'nomenclatural ecology' (sensu McGill *et al.* 2006); however the authors' warning must be taken into account.

Nevertheless, as the species I investigated were partly terrestrial, it was worth to try to elucidate to what extent species traits are informative to infer their competitive outcome in communities, and to what extent this can be represented by a relatively simple plant ecological strategy scheme. In order not to ignore intrinsic morphological trait differences between aquatic macrophytes and terrestrial plants, I applied the strategy scheme of Kautsky (1988) for aquatic macrophytes, which distinguishes four primary strategies instead of the three traditional Grime-strategies (see General introduction, 1.1). From chapter 3, I conclude that variation in plant strategy effectively matched the degree of niche differentiation between species. It must be realized that this was tested only for the five species under investigation. A generalization of this finding could only be concluded when the niches of more species were modelled, which would require more data. However no simple mechanistic explanation can currently be given why or how the different functional attributes led to the observed pattern, for example by a trait or strategy based rule that predicts species ranking reversals along different resource gradients. This has a superficial resemblance to the complexity that Willby and co-workers found: there is a considerable *observed* linkage between environment and the combination of many traits in multivariate correlation analyses, but a straightforward *predictive* rule cannot easily be given (cf. plant strategies that apply to specific environments). This is also reflected by the fact that all the species I investigated combined traits of at least two strategies of Kautsky (1988), i.e. they had a mixed strategy. Similarly, in the experiment (chapter 4), it was not possible to correctly assess the importance of different community structuring factors beforehand, including species' identity (i.e. their traits). I attributed this finding both to the mixed strategies and to the morphological plasticity of some species.

Although particular trait-based rules may be found for specific aquatic species or communities as a function of the environment, my impression is that these are constructed post-hoc, i.e. after observation of how particular species respond to specific circumstances. Examples can be found in Greulich & Bornette (1999, 2003), Kennedy *et al.* (2006), Puijalon *et al.* (2007) and Puijalon *et al.* (2008b). This has the drawback that these rules may not hold for other species, or for the same species under non-tested environments.

Also within species, trait values can vary in important ways with the environment (see General Introduction: 1.1, and Wilson & Keddy 1985, Kenkel *et al.* 1991, Dyer *et al.* 2001, de Kroon *et al.* 2005, Ackerly & Cornwell 2007, Verheyen *et al.* 2009, De Frenne *et al.* 2009, De Frenne *et al.* 2011), potentially accompanied with local genetic adaptation (van Groenendael 1985). As a connection can be assumed between species' traits and its niche (McGill *et al.* 2006), this implies that responses of a species, both to the environment and to other species, may vary considerably along a large geographic gradient through a shifting set of attributes. As this was not considered in my research, this remains to be tested for the investigated species.

The fact that there is consistency between traits and environment in aquatic macrophytes, makes me conclude that the search for general rules should be continued, although accurate predictions may not be expected. Anyhow, the slow increase of functional insights in aquatic plant ecology contrasts to the quickly advancing science of trait-based assembly rules in the terrestrial environment (e.g. Götzenberger *et al.* 2012), which seems to ignore the findings in freshwater macrophytes. I argue that there is a serious lack of collaboration between both worlds, and that much more attention from functional ecologists is needed towards aquatic plant ecology. Hence more shared interest is needed between terrestrial and aquatic plant ecologists, as well as the ambition to reconcile theories from both.

4.2 The abiotic environment of the *Eleocharition multicaulis*

Regarding important variables in aquatic macrophyte ecology (General introduction, paragraph 2), some questions have not been resolved. For example, why did aqueous CO_2 have no effect on species in the field survey (chapter 2 and 3), and so little in the experiment? It could be that competition regulates community organization more than CO_2 did in the studied plant community. However competition intensity at low CO_2 was not tested in my experiment as monocultures were not grown at different nutrient levels. *Juncus bulbosus* is known for its strong preference for surface waters rich in CO_2, where it can dominate over isoetids (e.g. Lucassen *et al.* 1999); however this was not found from my field data. I presume this is because the *Eleocharition* community grew at higher places along shores and therefore the plants were not limited by CO_2 in the dry season. It can be assumed that the dry season is the most important period for their performance.

Further, wave action and light are considered important to aquatic macrophytes (Lacoul & Freedman 2006, Bornette & Puijalon 2011) but have not been investigated. It can be expected that waves have an influence on *Eleocharition* species, favouring species that are morphologically better adapted to this disturbance. However, this has not been investigated for these species as far as I know. On the other hand, light will probably not be a limitation to plant performance in the field, at least when measured at plot scale. This is because the community mostly occurs in open places with abundant direct sunlight, and never in deep water. However, this does not exclude the expectation that in nutrient-rich situations species will shade each other, and consequently compete for light at the micro-scale.

4.3 The explanatory value of ionic ratios to plant performance

with Leon van den Berg, Fons Smolders and Maurice Hoffmann

Submitted

Abstract

Plant community ecologists study the interaction of plant species with the environment. Here we assess the potential usefulness of ionic ratios in plant habitat modelling. Based on a literature search of habitat studies, we give an overview of ionic ratios that have been used. For most of the ratios there is a physiological explanation how they directly act upon the plant. It can be concluded that several ionic ratios have the potential of being a strong predictor for species performance or occurrence, more than individual ion concentrations. We hope that with our synthesis, niche modellers will carefully consider whether ionic ratios can be incorporated in their models.

Introduction

Plant community ecologists study plant species in communities and their interaction with the environment. A general re-occurring theme in these studies is the modelling of a species' niche, in terms of occurrence and performance, using habitat information such as altitude, rainfall and land use. In recent years new statistical techniques emerged in this area, known as niche models (or habitat models, species distribution models; Guisan & Zimmerman 2000, Elith & Leathwick 2009). Many practitioners of these techniques ignore an important issue though: a plant's performance can only be understood and reliably predicted if we know which environmental variables are critical to the performance of the plant (Austin 2002, Austin 2007).

Ionic ratios are an often overlooked group of variables in determining plant performance and thereby plant distribution. For example, in a literature survey of habitat studies of aquatic macrophytes, we found that only 8.5% (5/59) had considered ionic ratios (*Appendix 8*). Ionic ratios are obtained by making the quotient of specific ion concentrations. As we will discuss further, such ratios can have unique properties in the explanation of plant performance compared with the absolute ion concentrations. This can be linked to the fact that several ionic ratios, instead of individual ion concentrations, tend to govern physiological processes such as hydration, nutrient assimilation and toxicity attenuation. The fact that they are not often used in habitat studies, may be because the combined use of the ratio's constituting ions in a model could be equally explanatory, depending on the data. However, the actual explanatory effect of the ratio as a whole consequently looses attention and is not given any interpretation. Also, in cases where both numerator and denominator are high or low, the ionic ratios may be less conclusive as relative levels will not matter so much anymore. Although ionic ratios remain too often unexploited in habitat models, their use can be very powerful. In chapter 2, for instance, the predictive value of several ionic ratios was tested for the occurrence and cover of the amphibious softwater macrophyte *Eleocharis multicaulis* (Smith) Desv. The species' cover was accurately predicted by ionic ratios of the surface water, especially by NH_4^+/NO_3^- and the divalent / monovalent cation ratio (DMR = $(Ca^{2+} + Mg^{2+})/(K^+ + Na^+)$) [4]. Here we want to assess the potential usefulness of ionic ratios in future plant habitat modelling.

Below we provide an overview of ionic ratios that have been used in earlier studies, guided by the question 'what is the explanatory value of ionic ratios to plant performance?'. We summarize the use of ionic ratios and discuss how they may explain biogeochemical processes and performance of plants.

Use and functional meaning of ionic ratios

Appendix 7 lists several ionic ratios, that were earlier used in literature. The studies can be divided in two groups; one in which the authors' interest lies directly in biogeochemistry and one in which the interest lies in the ecological effects of an ionic ratio to plants. Often, the selected ionic ratios have a known or presumed biogeochemical role and are selected based on ecological arguments. In these cases, biogeochemical processes reflected by these ratios are thought to be important for the state or fate of plants. They have direct effects on the plant's physiology and are therefore called proximal factors. Several other ratios are distal factors, meaning that they have no direct operational effect on the plants, but rather represent biogeochemical properties of an ecosystem.

Here we will discuss some examples of the use of ionic ratios. We pay particular attention to those ratios for which a clear correlation with plant performance has been shown, preferably in a causal (experimental) way. For many more ratios however, a physiological mechanism is at least hypothesized. An overall view of all ionic ratios that we encountered in literature is given in *Appendix 7*.

Several ionic ratios reflect the intensity of current and historical nitrogen deposition or drought and the resulting acidification, by taking into account the relative amount of ions that are influenced by these processes, e.g. S^{2-}, SO_4^{2-}, NH_4^+, NO_3^-, Al^{3+} and base cations. Often, acidification is caused by deposition of acidifying substances like ammonium, or as a result of a drought period. Depending on the local environmental conditions, acidification can be accompanied by an increase of ions like SO_4^{2-}, NH_4^+ and Al^{3+}, and a decrease of other ions like base cations, S^{2-} and NO_3^-. The involved processes include oxidation, inhibition of nitrification, soil cation exchange and leaching. Therefore, several ionic ratios can be used to reflect the degree of acidification or nitrogen deposition. It is well known that acidification is detrimental to many plant species (Maessen *et al.* 1992, Roelofs *et al.* 1996, Bobbink *et al.* 1998, de

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[4] In this paragraph, ion symbols represent concentrations expressed as equivalents.

Graaf *et al.* 1998a). Ecophysiological mechanisms for the effects on plants include toxicity and nutrient deficiency, and from these, proximal ionic ratios have been constructed. In several cases, these proximal ionic ratios are the same as the ones that signify geohydrological processes, as the same ions are involved. Hence most of these ratios have been used to predict plant performance or occurrence.

For example, soil acidification results in increased Al^{3+} concentration in the rhizosphere and in leaching of base cations. Al^{3+} was shown to have toxic effects on the characteristic heath plant *Arnica montana* L., but the toxicity effect was reduced by Ca^{2+} (de Graaf *et al.* 1997). Although the physiological mechanism involved is not fully understood, it is clear that the Al^{3+}/Ca^{2+} ratio expresses the degree of toxicity to the plant much better than the individual ion concentrations. Similarly, soil NH_4^+/cation ratios have been found to be higher in coniferous forests affected by acidification (Houdijk *et al.* 1993). It has been demonstrated that these ratios have more impact on growth and vitality of coniferous trees than the individual ion concentrations (Roelofs *et al.* 1985). Elevated values of both afore-mentioned ratios led to a reduction in root function and mycorrhizal development in Swedish trees (Persson & Majdi 1995). It has been found for trees that Al^{3+} reduces the uptake of N, Ca^{2+}, Mg^{2+} and P, and that higher soil Ca^{2+} concentrations reduce the toxicity effect of Al^{3+} (Gobran *et al.* 1993), which explains the importance of the ionic ratio over the individual ion concentrations, like in the heath plant example. Further examples in forest ecosystems have been reviewed by Cronan & Grigal (1995).

Several plant species of weakly buffered soils are vulnerable to high NH_4^+/NO_3^- ratios when NH_4^+ levels are high (Vanderhaeghe *et al.* 2005, van den Berg *et al.* 2005, Stevens *et al.* 2011). It was found that the relative unavailability of NO_3^- in relation to NH_4^+ causes the toxicity effect, which explains the larger explanatory value of the ionic ratio over the individual ion concentrations. Several mechanisms have been postulated, on which further research is required (Stevens *et al.* 2011). These are that NH_4^+ uptake may reduce base cation uptake; NH_4^+ may be metabolized to N-rich amides and amino acids instead of contributing to plant growth; and NH_4^+ uptake and assimilation require an extra investment of plant resources into the maintenance of cytosol pH.

Several ratios concern the relative proportions of the cations Ca^{2+}, Mg^{2+}, K^+ and Na^+. Divalent / monovalent cation ratio of the surface water (DMR = $(Ca^{2+} + Mg^{2+})/(K^+ + Na^+)$) is supposed to affect aspects of cell membrane transport, and consequently, root and plant (de-)hydration. Larcher (1995) mentions Ca^{2+} on the one hand and K^+ and Mg^{2+} on the other as antagonists for hydration. In a Welsh lake survey by Seddon (1972), DMR was referred to as the ‘hardness ratio’ as it was related to total hardness and conductivity. This ratio was significantly better in explaining differences in macrophyte assemblages between the lakes in floristic ordination diagrams than hardness and electrical conductivity alone. Equally, we found that the ratio was robust in predicting the cover of *Eleocharis multicaulis* (chapter 2).

Most nutrient-based ionic ratios are proximal factors, reflecting the relative availability of different nutrients. CO_2/HCO_3^- of the surface water has a deterministic inverse relationship with pH, as the equilibrium is governed by pH solely. Consequently, the ratio has as much predictive power as pH but it is more proximal since both ions are carbon sources for photosynthesis, depending on the species (Maberly & Madsen 2002). Similarly, soil N/P ratios are used to make inferences about nutrient limitation of plants. For example, Güsewell *et al.* (2003) and Güsewell (2005) experimentally found that N/P ratios were more important than absolute concentrations of N and P for the nutrient retention by five *Carex* species. In addition, N/P ratios are commonly measured in biomass of vegetation (Güsewell & Koerselman 2002) or planted phytometers (Roem & Berendse 2000) to infer the type of nutrient limitation (N-limited versus P-limited). These authors presume that N and P concentrations in biomass better represent the N and P supplies from soil to the plants; hence biomass N/P is also called nutrient supply ratio (Roem & Berendse 2000). However contradicting results have been found that question the capacity of biomass N/P to predict the type of limitation (Drenovsky & Richards 2004, Craine *et al.* 2008).

Some ratios have been constructed for purposes of chemical typology and the inference of geohydrological processes. Mg^{2+}, Cl^- and Na^+ usually originate from the sea and come into the system via precipitation. Their relative proportions are known for seawater. In this way, ratios like Na^+/Cl^- and Mg^{2+}/Cl^- serve to estimate the relative influence of seawater on the ionic composition of water (Proctor 1992, Proctor 1994). The 'Ion Ratio' (IR; van Wirdum 1991) is given by $Ca^{2+}/(Ca^{2+} + Cl^-)$ and represents most of the major ions' relative concentration variability of water in The Netherlands, along the gradient from groundwater to seawater and rainwater. It can therefore be used to connect a water sample to its position in the hydrological cycle. To explain the ionic composition of surface water or groundwater, ionic ratios like IR incorporate ions of primarily non-marine origin. Other examples are Ca^{2+}/Cl^-, Ca^{2+}/Mg^{2+} and K^+/Cl^-. Possible sources for surplus Ca^{2+} in water, compared to precipitation, are soil cation exchange and terrestrial dust, among others (Gorham 1956, Proctor 1994, Kumar *et al.* 2009).

The potential value of ionic ratios

From the above cases, different ionic ratios appear to have the potential of being a good predictor for species performance or occurrence. In several instances it was shown from *experimental* work that a specific ratio was a better predictor than the individual ion concentrations. Which ionic ratios must be considered in a specific case, will depend primarily on the studied environmental gradients, the key biogeochemical processes and the species under study. The species' sensitivity to potentially toxic compounds (NH_4^+, Al^{3+}) and their ability to cope with low relative availability of specific nutrients play an important role in this. Much research remains to be done to establish the applicability of several ratios throughout different ecosystems.

For quite some ratios there is a physiological explanation how they directly act upon the plant. This means these ratios can be considered as proximal factors. The most accurate estimation of plant response to the environment is achieved with proximal factors (Mac Nally 2000, Austin 2002). This might explain the strong predictive value of specific ionic ratios in several studies, like Seddon

(1972), Roelofs *et al.* (1985), de Graaf *et al.* (1997), Vanderhaeghe *et al.* (2005) and van den Berg *et al.* (2005).

The modeller's ability to choose the right ionic ratios will however depend on his/her insight in the relevant biogeochemical and physiological processes for the studied plant species. Furthermore, it should be kept in mind that for many ratios, very low absolute concentrations of the constituting ions will lower the information value of the ratio to the plant. For example, if nitrogen concentrations are very low, the physiological effect of NH_4^+/NO_3^- will be quite irrelevant, and the same will be true for other ratios. Ratios that normally express nutrient limitation, like N/P and CO_2/HCO_3^-, will equally be irrelevant when both the numerator and denominator nutrients are available in excess. In this situation the ratios do not longer express a limitation and therefore the informative value of a ratio will also depend on the absolute ranges of individual ion concentrations. A analogous behaviour has been found for Al^{3+}/Ca^{2+} and $Al^{3+}/(Ca^{2+} + Mg^{2+} + K^+)$ in the case of high sea salt deposition (Hansen *et al.* 2007).

In wetlands, the seasonal lowering of water tables generally leads to higher absolute ion concentrations in the surface water, making temporal comparison of measurements more difficult. However, several ionic proportions will remain the same, like ratios between Cl^-, Na^+ and K^+. Therefore, several surface water ionic ratios don't vary with temporal surface water level fluctuations. Their constancy is an advantage over absolute concentrations.

Perspectives

Many modelled ecosystem types lack a wide geographical chemical characterization, which hinders projection of niche model results in space. As such, many studies with the aim of map-based plant species prediction will continue to restrict themselves to predictor variables that are available as maps in geographic information systems (GIS). However, when using observation points with measured chemical properties, incorporating ionic ratios could serve to better model and grasp the realized niches [5] of plant species, regardless of geographic space. More generally, the obvious value of incorporating chemical properties of a plant's environment in niche modelling stimulates to generate reasonably accurate maps of ion concentrations of the soil or water environment. This should in turn allow to more accurately predict plant species distributions.

Several aspects of ionic ratios deserve further research. By more often incorporating ionic ratios in niche models, further testing can be done on their predictive capacity to plant performance. Beside model testing of ionic ratios with known or presumed direct plant effects (*Appendix 7*), other ionic ratios still largely have to be put to the test. Among these are the ratios that are mostly used for chemical typology (e.g., $Cl^-/(SO_4^{2-} + Cl^- + HCO_3^-)$) or to infer the origin

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[5] Realized niches sensu Colwell & Rangel 2009, i.e. obtained by including observed absences in model training.

of water. Some of them were already used for direct correlation with plant occurrence (e.g. Pietsch 1978, Bloemendaal *et al.* 1988). In those cases, a clear mechanistic understanding of the relation between the ratio and plant species occurrence is lacking. Even when geohydrological factors explain why a certain ecosystem has a specific ionic composition, it does not necessarily explain why this leads to the plant's response. Such ionic ratios are at least intriguing though and are not less valuable for correlation and modelling purposes. Further, ratios that emerge from ecological studies are worth of further experimental physiological research, as several mechanisms are not yet clearly understood (e.g. in the discussed NH_4^+/NO_3^- and Al^{3+}/Ca^{2+} ratios). Knowledge of physiological mechanisms could allow the extension of predictions to non-investigated species, based on functional traits like morphological, physiological or life history properties (McGill *et al.* 2006, Webb *et al.* 2010). Finally, opportunities exist to construct new ionic ratios that are useful in relation to plant performance, from either biogeochemical or ecophysiological knowledge and assumptions.

Ionic ratios may summarize relevant environmental processes and conditions, in certain cases more than simple ion concentrations do. Inclusion of ionic ratios may improve plant habitat models, compared to models which do not take ionic ratios into account. We hope that with our synthesis, niche modellers will carefully consider incorporation of ionic ratios in their models.

Supporting information

Additional supporting information may be found in the appendices:

Appendix 7. Ionic ratios found in literature.

Appendix 8. Examples of studies that did not use ionic ratios in the habitat description of aquatic plants.

5. The research approach in the light of my objectives

Here, I discuss aspects of my research approach that may limit generalizability of certain conclusions, or that may need further clarification from a research designer's point of view.

First, in my descriptive studies, I have used ground cover of plant species to obtain a quantitative measure of a population's performance. Three points could be raised. First, 'abundance' (being related to cover) is sometimes used synonymously in literature to 'density', in cases where abundance of a species varies within a fixed area which physically limits the species. In those cases, it is clear that increased abundance (hence, density) leads to decreased performance of individuals, due to higher intraspecific interactions. Available space generally appeared not to be so restrictive to populations in the investigated plots; however sometimes species densities were high and open space was rare, so this may have affected the performance of individuals in a negative sense, in those cases. This brings me to a second point: when unravelling realized niches, some measure of performance or fitness is ideally used. This might be best achieved in the field by measuring functional plant traits (response traits sensu Webb *et al.* 2010). On the other hand, this poses problems to compare between morphologically very different species (e.g. some are clonal, while others are not). It might therefore be argued that cover better reflects a species' relative success in a community than for example, height, or number of individuals, because cover directly relates to the species' share of physical space. Eventually, it depends on a researcher's objectives whether cover will satisfy. If the goal is to reflect resource use efficiency, or total resource acquisition per species, cover will have different species-specific relationships with the desired species property, and will pose problems. For my objectives however, I consider cover as useful, and not unimportantly, practical. A third point is that using cover as a measure of population performance in relation to the environment rests on an important equilibrium assumption. It is an assumption that also holds for most species distribution models, and that currently is an important challenge to overcome (Austin 2007, Franklin & Miller 2009, Guisan & Rahbek 2011). It is assumed that the measure of abundance (e.g. cover) not only reflects aspects of performance or fitness, but also that it is in equilibrium with the observed environment at the time of measurement. However, because of time-lags (dependent on life history traits), species cover is more the result of an integration of historical environmental circumstances (including disturbance events) to some (often unknown) extent. Also, cover may not reflect the net reproductive rate of a population, which can be considered as the preferred measure of the niche by definition (Pulliam 2000; see Glossary). Consequently, this may be problematic in monotonously changing environments, where a delayed transition of the community is taking place. However, I see no straightforward solution to avoid this problem, in those many cases where only one-time biodiversity records are available. In my view, pragmatic approaches are needed and to be welcomed, if we are to use data in the most profitable way to biodiversity conservation. Literature on species distribution models is full of examples with an applied conservation purpose, which is exactly because they provide a major step forward for conservation, notwithstanding unavoidable assumptions.

Ideally, my spatial sampling design throughout Western Europe (chapter 3) would have been 1) more extensive, and 2) more equally distributed among geographical regions and environmental strata. The relatively ‘rare’ environmental conditions in the dataset might be equally important in modelling the niche to those that I measured more often, in Belgium and The Netherlands. On the other hand, rare points on the edges of more common environmental states have relatively large influence on the fitted model in regression and multivariate analyses, and therefore receive proportionally greater importance. Hence, the applied techniques can be considered quite robust to unequal environmental stratification, and can be used to infer niches from the data. Also, GLMs will translate the low number of observations in a subpart of environmental space into a wider confidence interval. Apart from these general expectations, exploration of the dataset indicated no extreme outliers for the retained environmental predictors. However, as a consequence of relative under-sampling in large areas, it is possible that parts of the realized niche were missed. Consequently, more data could enhance predictive accuracy, and hence, improve the niche models. The models should therefore not be regarded as definitive results for the species. Another design aspect is the dependency among plots that are sampled from the same lake. First, their dependency is taken care of statistically by incorporating a random factor ‘lake’. Secondly, it may be considered as a powerful test of whether or not it is the environmental state that determines the absence of a species in one plot, while it is present in another plot in the same lake. When only neutral dispersal processes would be important to explain this difference, environmental responses should be inconsistent between lakes, and therefore a weak response to the environment would be expected in my analyses – which mostly was not the case. To conclude, although there are the above arguments, the ideal situation is the sampling of more lakes (and consequently, more environments), in a geographically and environmentally stratified way, instead of allocating energy to more plots within fewer lakes. The followed sampling scheme was mainly the consequence of practical restrictions as well as opportunities.

I would probably not collect so many environmental variables if I were to go out to the investigated sites again. However, this would be more because of personal and practical reasons, than for anything else. Virtually every academic researcher is under great pressure to produce scientific results, spending as few costs, time and energy as possible. This alone is what I consider to be the main driver of the *currently* predominant paradigm that very specific research questions (or hypotheses) must be tackled so that each can be solved within a year (or perhaps, within a month). While, for sure, no researcher is personally to blame for this trend in recent decades, this leaves few room for larger *exploratory* research. The reasoning in exploratory surveys is simple and logical: what you don’t look for (measure), you will not find (detect).

Considering the experiment (chapter 4), ideally all possible neighbourhood factor levels would have been applied (hence, by at least growing a monoculture of each species), and applied orthogonal to all four nutrient level combinations, thereby replicating each combination of levels. However, this would lead to a requirement of 5 neighbourhood levels (mixture + 4 monocultures) x 2 N levels x 2 C levels = 20 aquaria for 1 replication series.

When 4 replicates would have been maintained, this gives a total of 80 aquaria to continuously treat and monitor instead of the 24 that I used. This was impossible for many practical reasons. Applying different water level treatments here, would lead to a further multiplication by two at least. Instead, I have opted for a non-full-factorial design with water level as a correlate of time instead of applying it as an experimental factor. I am confident that this approach, with repeated measurements, yielded more results for 24 aquaria than if the option were taken of limiting the questions and the number of factors. The results must be interpreted accordingly: causal effects of nitrogen and carbon can only be derived for the mixture of species, and the neighbourhood treatment is only to be interpreted at high nutrient levels and for two species only. However, this approach gave a rich set of ideas about which factors may be important outside in the field. Several other aspects are idiosyncratic to the specific setup: temperature regime, light influx (see below), water level regime (see below), photoperiods, size of aquaria, species selection, density of species, soil characteristics etc. On the other hand, these factors were kept constant among replicates, and they are well documented compared to a field experiment. From a *design* point of view, the results cannot be extrapolated to field situations insofar conditions differ between the field and the experiment. Consequently, the results must be *interpreted* with common (ecological) sense when making generalizations. I have tried to do so in this thesis. Two specific points are addressed below.

As mentioned above, a remark must be made concerning the investigation of the factor ‘water level’ (hydrological state) in the experiment. This factor was not applied as an experimental factor, because different, replicated plots receiving different water level regimes, for each nutrient level combination, would have required too much research resources. Instead, hydrological state co-varied with time in the same way for all aquaria (from submersed to emersed). Consequently, we could only *presume* that the hydrological state was the actual causal factor leading to the drastic change in time, as reflected by the difference between the submersed and emersed endstages. This was suggested both by the rapid changes in the performance measures at the moment of water level drawdown, and by specific observations of species in relation to the water table.

The outcome that different nutrient levels had no dominant effect is quite surprising, given the importance of these nutrients according to literature and the realistic levels that were chosen. It should be added however, that photon influx was much lower under artificial light than under outside conditions (Madsen & Sandjensen 1994), and therefore light was potentially limiting in our experiment, especially under high-nutrient conditions. On the other hand, we observed normal growth in the aquaria compared to plants growing in the field. This may be because of the low light saturation point of many aquatic macrophytes, i.e. often < 500 µmol s^{-1} m^{-2} of PAR (Wetzel *et al.* 1984, Boston *et al.* 1989, Robe & Griffiths 1992, Wetzel 2001). PAR irradiance was probably not saturating in our experiment, although it is not known whether it was actually limiting. It might be that plant responses would have been somewhat higher under higher light intensity, both in mixtures and monocultures. However, potential light limitation did not prevent the observation of significant competitive responses. The competitive responses were mainly caused by nutrient limitation through competition, as plants hardly shaded each other for a large period of the experiment. As a note, it should be added that I was constrained by the available – and already elaborate – laboratory equipment at the time of the experiment at Nijmegen, so that higher illumination was not an option.

I raised an additional question about the match between the strategy of species and species response to the environment. As explained in the General Introduction (paragraph 1), attributes often vary more between species than within species. This means that species can be classified according to one or several of their functional traits. On the other hand, traits are known to vary within species and individuals, often referred to as 'trait plasticity', especially in relation to the environment. The ideal approach would be to directly measure correlation between trait values and environment. Examples of functional trait values that could be measured, are height, specific leaf area, seed mass and seed investment, amount of lateral spread and onset of flowering, of which the relations to life history challenges are known quite well (see General Introduction, paragraph 1). Several of these measurements would require repeated visits to the same site within a growing season. Hence, measuring such traits of several individuals per species in each plot would have posed new constraints to the feasibility of the West-European survey. It was my choice not to give so much weight to this aspect in my thesis, and I followed a qualitative method by assigning a strategy type to each species. These strategy types are underpinned by existing (qualitative or quantitative) knowledge of the (average) values of species traits. Although I found a consistent pattern, with four to five species, generalizability of the match between strategy type and environmental response should not be inferred from my results. It has only been investigated for these specific species, and in a qualitative way. In order to obtain more detailed results about how niche separation relates to traits, it would be a good alternative to use the quantitative traits (where each species has one value per trait) instead of a strategy category per species. However, to fit sound relationships where each species represents one data point, more species should be taken into consideration, and therefore more species' niches should be modelled, which again would require more plots. This would have been the way to go if species-dependent trait-environment relationships were the primary subject of my thesis.

Hence, depending on the weight a researcher gives to the different questions in my research, the number of plots and the choice of measurements that were made therein pose limits to the generalizability of the results. The plots did however allow to model the realized niche of a few species, and explore relationships between the environment and species abundance, which were the primary aims of the descriptive part of my thesis.

6. *Some notes on the restoration of the Eleocharition multicaulis*

The extensive knowledge of the physiology and biogeochemistry of typical isoetids of the *Littorellion uniflorae*, like *Littorella uniflora* and *Lobelia dortmanna*, have led to a profound understanding of the measures that are needed to restore their former sites. Several decades of research in Western Europe resulted in hundreds of scientific articles on these isoetid species. Target species of the *Eleocharition* are not documented that extensively. Most softwater lake restoration projects focus on the *Littorellion*, but to some extent, they seem to enhance the environment of the *Eleocharition multicaulis* as well (Brouwer *et al.* 2002). Nonetheless, more descriptive and experimental research is needed to better understand the requirements of *Eleocharition* species, if we want to know how to restore their habitat most effectively.

The restoration of the abiotic environment of target species and the enhancement of their successful colonization and establishment in a spatial context, are important keys to success in nature restoration (Brouwer *et al.* 2002, Declerck *et al.* 2005, Van Wichelen *et al.* 2007, Klimkowska *et al.* 2007, Klimkowska *et al.* 2010). Regarding several rare target species (*Eleocharis multicaulis*, *Hypericum elodes*, *Baldellia ranunculoides* spp. *repens*), my thesis and the data therein contribute to the needed restoration knowledge with:

- predictive niche models. They allow the calculation of a probability estimate for the presence (or cover class) of species, given the present or targeted environmental state in a softwater lake. The models may be refined by collecting more data, and several experiments could be set up in order to test expectations on niche and performance of these species, and in order to elucidate physiological mechanisms;
- a nuanced view of the requirements of the characteristic species *Eleocharis multicaulis*. Especially, from the collected data it is clear that this species performs best in base-poor environments with an organic layer, where nitrate dominates over ammonium. While the species responds positively to nitrogen and might therefore profit from nitrogen deposition, it seemed to be sensitive to more extremely acidified situations with dominance of ammonium. It can be hypothesized from our findings that atmospheric ammonium deposition favours *Eleocharis multicaulis* at first, but eventually leads to its decline and disappearance. It can be expected that the species will profit from restoration measures against acidification. After removing a eutrophied organic layer and restoring a supply of nutrient-poor and weakly buffered water, the species can be expected to be present in low numbers, and to gradually increase its abundance in places where organic matter accumulates along lake shores. A lower ammonium/nitrate ratio in the surface water as a consequence of reduced ammonia emissions, may be favourable to *Eleocharis multicaulis*;
- the insight that plant-plant interactions are important to relative population performance, at least in the short term. The experiment in chapter 4 demonstrates how limited our predictive understanding of softwater macrophyte performance is in the presence of competitive

effects. In an applied sense, this means that an established species will potentially become more abundant when not many species have established in a lake. Therefore, more biodiversity in a lake also means smaller or less vigorous populations. This forces us to think clearly about the goals of a specific lake restoration project, and to ask which species are expected to establish after restoration, as assessed from seed bank analysis, spatial connectivity and species' dispersal capacity. The more species are able to establish, the higher the potential biodiversity but the smaller the potential population sizes due to competition. Moreover, it can be expected from theory that competitive effects are stronger at higher nutrient levels. Therefore, in relatively nutrient rich (and spatially homogeneous) situations, competition will lead to the exclusion of many species and dominance of a few stronger competitors.

7. Perspectives

Below, I summarize challenges for further research, several of which were already encountered during the foregoing discussion. Some are specific to the studied species and communities, while others refer to general themes.

7.1 The environment of the *Eleocharition multicaulis*

- Most softwater plant research is directed towards species that are submerged during most of the year, including isoetids. Emergent macrophytes along the borders of oligotrophic shallow lakes, such as *Eleocharis multicaulis* and *Hypericum elodes*, have been rarely studied. Moreover, literature varies considerably regarding their environmental preferences, when compared to isoetids. Starting from the current results, more work remains to be done to further answer questions or to test assumptions on the effects of wave action, CO_2 content of the water, presence and characteristics of organic sediment, water level fluctuations and the currently important biogeochemical processes. For example, it is unknown to which extent these species are vulnerable to anoxic conditions in the rhizosphere and to toxic compounds. I found indications of trade-offs in resource acquisition strategies between several species, as their dominance ranking reversed with the considered resource gradient. Further elucidation of the underlying mechanisms should be a subject of future experimentation. Also, spatially-dependent realized niches could be modelled for the species by collecting more data from each part of their geographical range, in order to enhance a geographically differentiated understanding of the species' niches. Finally, the collection and usage of more data would enhance predictive accuracy of the niche models and accommodate complex response shapes, thereby improving current knowledge of the species' niches and its application in restoration projects.
- Apart from environmental research at plant and lake scale, much benefit will come to conservation ecology when the relationships between local

site biogeochemistry and historical and larger scale spatial processes are investigated or made more explicit when known. For example, in chapter 2 several hypotheses arise on the effect of historical or current land use (like agriculture) and of groundwater processes. It is suggested that these processes are responsible for observed values of pH, ammonium-nitrate ratio, alkalinity, redox potential, silicon and several other chemical variables at *Eleocharition* sites. These hypotheses might be tested for the investigated sites by collecting site-specific information on historical and current land use and on hydrological functioning of the landscape. Remediation experiments can further test the validity of these assumptions, e.g. how restoring a moderately base-rich groundwater supply in winter results in better conditions for species of the *Eleocharition*. It may well be that many aspects of the isoetid environment and its restoration are applicable to species of the *Eleocharition*. Although Brouwer (2001) took into account *Eleocharition* species in his research on softwater lake restoration, few literature addresses the restoration of the *Eleocharition* environment as a primary subject.

7.2 Modelling observed plant abundance in geographical space

- I refer to paragraph 4.3 for an overview of the meaning of certain ionic ratios to plants. Here I repeat that 1) ionic ratios offer a largely unexplored potential to plant habitat modelling and hence should be considered far more often; 2) several ionic ratios, like those used in chemical typology of water, have yet to be tested for their predictive capacity to plant abundance and occurrence; 3) further ecophysiological research is needed to better understand the actual mechanisms reflected by several ionic ratios in different ecosystems.
- Proportional odds modelling is the preferred regression technique to model ordinal species abundance (cover). Visual estimates of abundance according to numerically defined cover or abundance classes are often called “semi-quantitative”, but this term is misleading. What is measured, is abundance along a discrete and ordered scale, and this defines ordinal data. It does not matter for this statement whether those classes are defined in a numerical way. Only when one directly measures abundance, the response can be regarded as numeric (e.g. counts, or by using instrumentation that allows to measure on a continuous scale). Consequently, the use of proportional odds models (POM) must be promoted in ecological research and education.
- When models are to be used along the environmental range within which they were calibrated, they can be built by using predictors that correlate well to plant abundance under current abiotic and biotic circumstances. However, the model is likely not to be as reliable in a changed environment or a different biotic neighbourhood. This is an important aspect in the face of climate change and the establishment of invasive alien species (Pearman *et al.* 2008). For example, Poloczanska *et al.*

(2008) did research on fundamental niches and competition of barnacle species, and predicted major changes in the community structure as a result of climate change by 2050, primarily because of the competitive release of some species. Such examples demonstrate the importance of incorporating mechanisms in models that are useful for prediction to new situations in time or space. Species distribution models are currently evolving in this direction (Ferrier & Guisan 2006, Elith *et al.* 2010, Buckley *et al.* 2010).

7.3 Putting ecological theories to the test

Incorporating mechanisms into models in order to enlarge predictive capacity necessitates rules how organisms affect each other and how they are affected by their environment. At best, it can be hoped from ecological research that general rules emerge that can be applied to all species or their traits and to all communities, as is the case in other natural sciences (Berryman 2003, Lange 2005, Webb *et al.* 2010). It is in predictive model applications that the greatest benefit from such rules can be gained, either qualitatively or quantitatively. However, regarding debates in scientific literature, more directed research effort will be necessary and more meta-analyses of earlier studies need to be done. Furthermore, the primary interest of the scientific community in short-term scientific output reduces the opportunities to undertake the needed larger experiments and make a concerted effort to rigorously test the assumptions of current theories in ecology. Below I shortly draw attention to three points:

- the assumptions of the competitive hierarchy model must be examined much more often. This especially applies to the assumed inverse relationship between fundamental niche width and competitive ability. It is one of the key aspects that will be needed in predictive models of how plant communities change depending on species (or functional groups) that enter or leave the community, e.g. as a result of environmental change;
- the extent should be tested to which a community organizing mechanism, e.g. the competitive effect of a species, can be spatially extrapolated at a large geographical extent (in varying places of the species' range). For example, in predictive community models it will be necessary to know whether similar competitive processes occur in one environment compared to another within the same community type, and whether these lead to the same niche response patterns in both places;
- a specific area where more concerted effort is needed in order to obtain - if any - general rules, is in reconciling terrestrial and aquatic plant ecology (paragraph 4.1). For example, trait-based rules and plant ecological strategy schemes, which mostly originated in the terrestrial environment, do not necessarily match aquatic plants. Plant ecological theories have to take into account the freshwater habitat in order to achieve better predictions, as this habitat is of utmost importance to global biodiversity (Dudgeon *et al.* 2006).

All of this reinforces the recognition that there is no single correct scale at which to view ecosystems, the individualistic nature of responses to environment means that what we call a community or ecosystem is really just an arbitrary subdivision of a continuous gradation of local species assemblages.

Levin (1992)

Flowering aspect of the Eleocharition, Hasselt, Belgium

SUMMARY

In this thesis, several questions are raised that are related to the niche concept and population ecology. They are within a framework of the amphibious softwater plant community *Eleocharition multicaulis* Vanden Berghen 1969. This community has declined during the 20th century and several of its species are rare or endangered. The community, with typical species like *Eleocharis multicaulis*, *Hypericum elodes* and *Scirpus fluitans*, has not been the subject of much research as yet. Therefore, more research is needed to better understand the requirements of *Eleocharition* species and the mechanisms that rule community structure, if we want to know how to restore their habitat most effectively.

The research questions in this thesis can be broadly summarized as 1) which environmental factors define the realized niche of the species, and what is the realized niche of these species, 2) how important are environmental gradients compared to biotic interactions in determining community structure, 3) to what extent do the realized niches of the species support theoretical models of coexistence, and 4) is relative population performance related to plant strategy in a predictive way?

To document the realized niches, soil and water chemical data were collected from several softwater lakes across Western Europe, both in summer and winter. Two different approaches were used to model the niches, either using a regression framework (chapter 3; generalized linear mixed models and proportional odds models) or by the use of discriminant analysis (chapter 2). The former was applied at a West-European scale for five species, while the latter was applied at the scale of Belgium and The Netherlands for one species, *Eleocharis multicaulis*. Also, two different approaches of predictor selection were applied before analysis. One way (chapter 3) was to make a shortlist of mainly proximal environmental variables (resource gradients and direct gradients sensu Austin 2005). Another was to start with all information from a large environmental dataset, and to screen for potential explanatory variables using a univariate method (chapter 2). The approach that started from a reduced list of mainly proximal variables resulted in five predictors (see below) after model fitting (chapter 3). This method has the advantage of simplification in searching for relevant niche differences, as well as robustness for prediction because of model parsimony. To a large extent, the longer list of relevant variables in chapter 2 could be matched to these five predictors by correlation. Consequently one might prefer the ‘short list’. The short list was convenient to test some expected niche patterns from theoretical ecology in a regression framework. However, its scope remains limited when the aim is also to hypothesize on relevant biogeochemical processes that shape the species’ niche. In that case, the added explanatory value of several ionic ratios and macro-ionic concentrations becomes apparent, even though they are statistically correlated to other variables with explanatory power. It is therefore

suggested that univariate screening techniques are a worthy alternative for variable selection in ecology. The advice to practitioners of either approach of variable selection, is to be conscious of the pros and cons of each approach, and clearly base their field design on explicit questions or hypotheses.

The main groups of influential variables that came out of the analyses in chapter 2, were those that were related to 1) the redox potential (among which soil silicon and surface water ammonium/nitrate ratio), 2) acidity & base richness, 3) base cation replenishment during winter, 4) soil nitrogen availability (among which several ionic ratios), 5) soil phosphorus availability and 6) nutrient richness of the organic layer. Our hypothesis, that *Eleocharis multicaulis* is confined to an acid situation rich in ammonium and carbon dioxide, nutrient-poor water and soil and a mineral substrate devoid of organic material, did not hold as a whole. The five predictors obtained with the methods in chapter 3, were: four surface water variables - calcium and the resource variables ammonium, nitrate and orthophosphate - and the soil silicon content, which was related to surface water pH. Nitrate was negatively correlated with the ammonium/nitrate ratio, which was actually used in the regression models. Calcium was related to pH and alkalinity of the surface water.

Four of the five investigated species showed clear patterns in relation to the finally selected gradients in chapter 3. One species (*Juncus bulbosus*) was very unspecific; its realized niche was not limited within the investigated environmental range and vegetation type. The two species entitled ‘characteristic’ of the vegetation alliance (*Eleocharis multicaulis* and *Hypericum elodes*) showed opposite differences with respect to nutrients and silicon. *Agrostis canina*, *Hypericum elodes* and *Hydrocotyle vulgaris* were similar in their reaction to these predictors but with different positions along the gradient. As *Hypericum elodes* (characteristic of the *Eleocharition*), *Agrostis canina* and *Hydrocotyle vulgaris* (non-characteristic) were positively associated, the distinction between characteristic and non-characteristic species does not hold. *Eleocharis multicaulis* reacted positively to nitrogen ions, as long as nitrate remained dominant over ammonium (ammonium/nitrate ratio < 1), phosphorus and potassium remained low, the water was acid and had a high redox potential.

The importance of ionic ratios as potential predictors of plant performance and abundance received special attention in this thesis. Based on a literature search of habitat studies, we give an overview of ionic ratios that have been used. For most of the ratios there is a physiological explanation how they directly act upon the plant. It can be concluded that several ionic ratios have the potential of being a strong predictor for species abundance or occurrence. Modellers of plant’s niches and performance should more often consider ionic ratios instead of testing only for simple nutrient or macro-ionic gradients.

In order to assess the relative importance of nutrient availability, surface water level fluctuations and species identity in the organization of *Eleocharition* communities, a laboratory experiment was conducted (chapter 4). Softwater lake habitat was simulated during one growing season, mimicking water level fluctuation, photoperiod and temperature. Artificial communities consisted of small populations of four softwater macrophyte species: *Luronium natans*, *Baldellia ranunculoides* ssp. *repens*, *Eleocharis multicaulis* and *Hydrocotyle vulgaris*. These communities were subjected to two levels of aqueous carbon dioxide and ammonium. Additionally, monocultures of *Baldellia ranunculoides* ssp. *repens* and *Eleocharis multicaulis* were grown at the higher nutrient level combination in order to measure their competitive response in community. During one growing season it was observed that water level drawdown was very influential on relative species performances and ranking order, most likely through its effect on the availability of aerial carbon dioxide to the plants. However, the water level drop acted as a disturbance to *Baldellia ranunculoides* ssp. *repens* at least. Species performance generally was not influenced by the ammonium or carbon dioxide nutrient treatments. This stressed the importance of intrinsic properties of the species (their identity) and as such, their presence, to the community outcome. Furthermore, a large competitive response was observed in both *Eleocharis multicaulis* and *Baldellia ranunculoides* ssp. *repens*, depending on performance measure. Hence, at least in the amphibious *Eleocharition* and within a certain water level range, it is possible that the interactions between the occurring species are more important than the precise physicochemical characteristics, as long as species are able to survive and grow in the given environment. Put otherwise, this means that it could be that their fundamental niche is a lot larger than their realized niche, which then greatly depends on the presence and densities of species at sites. However, much more long-term experimentation on fundamental niches and interactions is needed to fully test this hypothesis.

At the local scale, niches are a convenient instrument to describe conditions under which species survive, reproduce and coexist in a community. The concept is especially used to visualize how biotic interactions shape the realized niche of a species, as opposed to its physiological capabilities, also known as its fundamental niche. Why species can be the ‘winner’ only in a *part* of their fundamental niche, is supposed to be linked to a trade-off. For example, a high ability to quickly take advantage of new light and nutrient supplies would not be compatible with a high ability to compete in a more resource-limited environment. This trade-off is made more explicit in the competitive hierarchy model of Keddy (1989). Particularly, there is growing evidence that species are able to coexist in communities through niche separation, in the case of resource gradients often referred to as resource partitioning.

At a much larger spatial scale, the realized niche concept has been broadened in order to apply to the niches that are modelled by species distribution models at biogeographical scale (also referred to as habitat suitability models or ecological niche models). At this scale, one might expect that in very different areas, mechanisms that account for the realized niche may differ. This would result in realized niches that are closer to the fundamental niches than when they were measured at a small geographical scale. Moreover, disturbances and stochastic demography may induce a reduced – or delayed – competition intensity, potentially leading to a larger realized niche.

However, in this study, considerable niche differentiation between species was still found at the investigated West-European scale (chapter 3). Some other researchers tested for mechanisms of regional coexistence of species in a metacommunity, and also found evidence for niche differentiation. Results from the current study supported the expectations from classical coexistence theory by niche differentiation, at least on the regional scale, as subplot environmental heterogeneity was not assessed. If niche separation through competition is to be responsible for coexistence at the subplot scale, fine-scale environmental heterogeneity is needed to accommodate the species. Furthermore, we must accept that competition is not the only possible biotic factor that shapes the investigated plant community and leads to coexistence. For example, also facilitation might be important. Moreover, stochastic processes of species arrival at a site and subsequent (temporary) establishment may be superposed on deterministic processes of abiotic and biotic interactions, allowing more possible patterns of coexistence.

The functional (dis)similarities between the five species coincided with their observed niche overlaps and differences (chapter 3). The relative species ranking could not be inferred from their strategies, however. Neither was this the case in the community experiment in chapter 4, in which partly different species were used. Here it was attributed both to the mixed strategies of all species and the high morphological plasticity of some. According to the paradigm of coexistence through niche differentiation, fitness trade-offs underpin niche separation. The match between niche patterns and plant strategy provided one indication of this, while another was that dominance hierarchies could reverse depending on the resource gradient considered. This reversal of dominance hierarchy might be the consequence of a physiological trade-off in accessing one versus another resource, and hence in fundamental niche width along these resources. The pattern along each single resource gradient was in support of the competitive hierarchy model.

The restoration of the abiotic environment of target species and the enhancement of their successful colonization and establishment in a spatial context, are important keys to success in nature restoration. This thesis contributes to the knowledge that is needed to restore the *Eleocharition multicaulis*, with: 1) predictive niche models of five species, 2) a biogeochemical and ecosystem-wide interpretation of the requirements of the characteristic species *Eleocharis multicaulis*, and 3) the insight that plant-plant interactions are important to relative population performance, at least in the short term, thereby forcing the conservation biologist to consider aspects of colonization (in a spatial and temporal context), and not only environmental constraints.

The thesis concludes with opportunities for further research on the *Eleocharition multicaulis*, suggestions in order to improve niche modelling habits by ecologists, and challenges and obstacles in the testing of ecological theory.

SAMENVATTING

In dit proefschrift worden verschillende vragen gesteld met betrekking tot het nicheconcept en populatie-ecologie. Dit gebeurt binnen het kader van een amfibische plantengemeenschap van zachte wateren (vennen), het *Eleocharition multicaulis* Vanden Berghen 1969 (verbond van veelstengelige waterbies). Deze plantengemeenschap is achteruitgegaan gedurende de 20ste eeuw, en verscheidene typische plantensoorten zijn dan ook zeldzaam of bedreigd geworden. Deze plantengemeenschap, met als kenmerkende soorten *Eleocharis multicaulis* (veelstengelige waterbies), *Hypericum elodes* (moerashertshooi) en *Scirpus fluitans* (vlottende bies), werd nog maar weinig bestudeerd. Om te weten hoe het milieu van deze gemeenschappen het meest effectief wordt hersteld, is er meer onderzoek nodig naar de vereisten van *Eleocharition*-soorten en naar de mechanismen achter de structuur van deze gemeenschap.

De onderzoeksvragen in dit proefschrift kunnen in brede zin worden samengevat als: 1) welke milieufactoren bepalen de gerealiseerde niche van de soorten, en wat is de gerealiseerde niche van deze soorten, 2) hoe belangrijk zijn milieugradiënten in vergelijking met biotische interacties voor het bepalen van de gemeenschapsstructuur, 3) in welke mate worden theoretische modellen van co-existentie ondersteund door de gerealiseerde niches van de soorten, en 4) is de relatieve performantie van populaties op een voorspelbare manier gerelateerd met plantstrategie?

Om de gerealiseerde niches te documenteren, werden chemische gegevens verzameld van bodem en water in verschillende zachte wateren in West-Europa, zowel ’s zomers als ’s winters. Twee verschillende benaderingen werden gebruikt om de niches te modelleren: enerzijds met behulp van regressietechnieken (hoofdstuk 3; veralgemeende lineaire modellen en proportional odds modellen), en anderzijds met behulp van discriminantanalyse (hoofdstuk 2). De regressiebenadering werd toegepast op West-Europese schaal voor vijf soorten, terwijl de discriminantanalyse werd toegepast op Belgisch-Nederlandse schaal voor één soort, *Eleocharis multicaulis*. Tevens zijn twee verschillende methoden van predictorselectie gebruikt voorafgaand aan de analyse. Eén manier (hoofdstuk 3) was om een beperkte lijst op te stellen van voornamelijk proximale milieuvariabelen (bronnen en directe gradiënten sensu Austin 2005). Een andere manier was om te starten met alle informatie uit de verzamelde milieudataset, en te screenen voor potentiële verklarende variabelen met behulp van een univariate methode (hoofdstuk 2). De benadering die startte met een gereduceerde lijst van voornamelijk proximale variabelen, resulteerde in vijf predictoren (zie verder) na het fitten van de modellen (hoofdstuk 3). Deze methode heeft het voordeel van eenvoud bij het zoeken naar nichedifferentiatie, alsmede robuustheid bij voorspellingen omwille van de spaarzaamheid van de modellen. Voor een groot deel kon de langere variabelenlijst in hoofdstuk 2 in verband worden gebracht met deze vijf

predictoren door correlatie. Bijgevolg zou men deze ‘shortlist’ kunnen verkiezen. De shortlist was handig om enkele theoretisch verwachte nichepatronen te kunnen testen met regressietechnieken. De scope ervan blijft evenwel beperkt wanneer het doel is om biogeochemische processen aan het licht te brengen die relevant zijn om de niche van soorten te begrijpen. In dat geval wordt de bijkomende verklarende waarde duidelijk van verschillende ionenratio’s en concentraties van macro-ionen, ook al zijn ze statistisch gecorreleerd met andere verklarende variabelen. Er wordt dan ook geopperd dat univariate screening een waardig alternatief is voor variabelenselectie in de ecologie. Het advies voor gebruikers van de ene of de andere methode van variabelenselectie, is om zich bewust te zijn van de voor- en nadelen van elke benadering, en om de veldopzet duidelijk te baseren op expliciete vragen of hypothesen.

De belangrijkste groepen van bepalende variabelen uit de analysen in hoofdstuk 2, waren gerelateerd met 1) de redoxpotentiaal (waaronder bodemsilicium en de ammonium/nitraat-ratio van het oppervlaktewater), 2) zuurtegraad en basenrijkdom, 3) basekationenaanvulling in de winter, 4) beschikbaarheid van bodemstikstof (waaronder verschillende ionenratio’s), 5) beschikbaarheid van bodemfosfor en 6) nutriëntenrijkdom van de organische laag. Onze hypothese, dat *Eleocharis multicaulis* gebonden is aan een zure situatie met relatief veel ammonium en koolstofdioxide, nutriëntenarm water en bodem en een mineraal substraat zonder organisch materiaal, werd ten dele verworpen. De vijf predictoren die bekomen werden met de methoden in hoofdstuk 3, waren vier oppervlaktewatervariabelen – calcium en de bronnen ammonium, nitraat en orthofosfaat – alsook het siliciumgehalte van de bodem, dat gerelateerd was met de pH van het oppervlaktewater. Nitraat was negatief gecorreleerd met de ammonium/nitraat-ratio, die feitelijk in de regressiemodellen werd gebruikt. Calcium was gecorreleerd met pH en alkaliniteit van het oppervlaktewater.

Vier van de vijf onderzochte soorten vertoonden duidelijke patronen ten opzichte van de finaal geselecteerde milieuvariabelen in hoofdstuk 3. Eén soort, *Juncus bulbosus* (knolrus) was zeer aspecifiek; zijn gerealiseerde niche vertoonde geen grenzen binnen de onderzochte milieurange en gemeenschap. De twee soorten die als ‘karakteristiek’ worden aanzien voor het verbond (*Eleocharis multicaulis* en *Hypericum elodes*), vertoonden tegenovergestelde responsen voor nutriënten en silicium. *Agrostis canina* (moerasstruisgras), *Hypericum elodes* en *Hydrocotyle vulgaris* (waternavel) waren gelijkaardig voor hun reactie ten aanzien van deze predictoren, maar met verschillende posities langs deze gradiënten. Aangezien *Hypericum elodes* (karakteristiek voor het *Eleocharition*), *Agrostis canina* en *Hydrocotyle vulgaris* (niet-karakteristiek) positief met elkaar geassocieerd waren, blijkt dat het onderscheid tussen karakteristieke en niet-karakteristieke soorten niet opgaat. *Eleocharis multicaulis* reageerde positief op stikstofionen, zolang nitraat domineerde over ammonium (ammonium/nitraat-ratio < 1), fosfor en kalium laag bleven, het water zuur was en een hoge redoxpotentiaal vertoonde.

Het belang van ionenratio’s als potentiële predictoren voor de performantie en abundantie van planten, kreeg bijzondere aandacht in dit proefschrift. Op basis van een literatuuronderzoek over habitatstudies wordt een overzicht gegeven van ionenratio’s die gebruikt worden. Voor de meeste ratio’s is er een fysiologische verklaring hoe ze rechtstreeks inwerken op de plant. Er kan besloten worden dat verschillende ionenratio’s potentieel sterke voorspellers zijn van abundantie of voorkomen van planten. Modelleurs van niches en performantie van planten zouden vaker ionenratio’s in overweging moeten nemen, in plaats van alleen te toetsen voor enkelvoudige gradiënten van nutriënten of macro-ionen.

Om het relatieve belang van nutriëntenbeschikbaarheid, peilfluctuaties en soortidentiteit vast te stellen voor de organisatie van *Eleocharition* gemeenschappen, werd een laboratoriumexperiment uitgevoerd (hoofdstuk 4). Een venhabitat werd gedurende één groeiseizoen nagebootst op vlak van peilfluctuatie, fotoperiode en temperatuur. De artificiële gemeenschappen bestonden uit kleine populaties van vier venplanten: *Luronium natans* (drijvende waterweegbree), *Baldellia ranunculoides* ssp. *repens* (kruipende moerasweegbree), *Eleocharis multicaulis* en *Hydrocotyle vulgaris*. Deze gemeenschappen werden onderworpen aan twee niveaus van opgelost koolstofdioxide en ammonium. Bijkomend werden ook monoculturen van *Baldellia ranunculoides* ssp. *repens* en van *Eleocharis multicaulis* opgevolgd voor de hoog-niveau-combinatie van beide nutriënten, om hun competitieve respons in de plantengemeenschap te kunnen meten. Op basis van één groeiseizoen werd waargenomen dat het waterpeil zeer bepalend was voor de relatieve performantie en hiërarchische rangschikking van de soorten, waarschijnlijk wegens het effect op de beschikbaarheid van koolstofdioxide voor de planten. Tenminste voor *Baldellia ranunculoides* ssp. *repens* betekende de waterpeildaling een verstoring. De soortperformantie was in het algemeen niet beïnvloed door het ammonium- of koolstofdioxideniveau. Dit benadrukte het belang van intrinsieke eigenschappen van de soorten (hun identiteit), en dus van hun aanwezigheid, op de uitkomst voor de gemeenschap. Voorts werd een hoge competitieve respons vastgesteld voor zowel *Baldellia ranunculoides* ssp. *repens* als *Eleocharis multicaulis*, afhankelijk van de gekozen maat van performantie. Bijgevolg is het voor het *Eleocharition*, tussen bepaalde waterniveaus mogelijk dat interacties tussen de voorkomende soorten belangrijker zijn dan de precieze fysicochemische kenmerken, zolang het abiotisch milieu overleving en groei toelaat. Met andere woorden is het mogelijk dat de fundamentele niche van deze soorten veel breder is dan de gerealiseerde niche, die dan grotendeels afhangt van de aanwezigheid en densiteit van andere soorten in een gebied. Om deze hypothese ten volle te kunnen testen is echter veel meer experimenteel lange-termijnonderzoek nodig naar fundamentele niches en interacties.

Op lokale schaal zijn niches een handig concept om de condities te beschrijven waarbij soorten overleven, reproduceren en samen voorkomen in een gemeenschap. Het concept wordt in het bijzonder gebruikt om te visualiseren hoe biotische interacties de gerealiseerde niche van een soort vorm geven, ten opzichte van zijn fysiologische mogelijkheden, aangeduid als de fundamentele niche. Waarom soorten alleen in een *deel* van hun fundamentele niche de ‘winnaar’ kunnen zijn, wordt verondersteld het gevolg te zijn van een ‘trade-off’. Een voorbeeld hiervan, is dat een hoog vermogen om snel gebruik te maken van de beschikbaarheid van licht en nutriënten, niet compatibel zou zijn met een hoog vermogen om te competiteren in een licht- of nutriëntengelimiteerd milieu. Deze trade-off is verder duidelijk gemaakt in het model van competitieve hiërarchie van Keddy (1989). Bijzonder hierbij op te merken, is dat er steeds meer aanwijzingen zijn dat soorten samen kunnen voorkomen als gevolg van nicheseparatie (nichedifferentiatie), in het geval van bronnen vaak aangeduid als 'resource partitioning'.

Op een veel grotere ruimtelijke schaal heeft men het concept van de gerealiseerde niche verruimd zodat het van toepassing is op de niches zoals gemodelleerd door soortverspreidingsmodellen op biogeografische schaal ('species distribution models', ook gekend als 'habitat suitability models' of 'ecological niche models'). Op deze schaal kan men verwachten dat de mechanismen die aanleiding geven tot de gerealiseerde niche, verschillen van gebied tot gebied. Dit zou kunnen resulteren in gerealiseerde niches die nauwer aanleunen bij de fundamentele niches dan wanneer ze op een kleinere geografische schaal zijn bemeten. Bovendien kunnen verstoringen en stochastische demografie leiden tot een verminderde – of uitgestelde – competitie-intensiteit, wat potentieel resulteert in een bredere gerealiseerde niche.

In deze studie werd echter nog een behoorlijke nichedifferentiatie vastgesteld tussen soorten, op de onderzochte West-Europese schaal (hoofdstuk 3). Enkele andere onderzoekers hebben mechanismen getoetst voor de regionale co-existentie van soorten in een metagemeenschap, en ze vonden eveneens aanwijzingen voor nichedifferentiatie. De resultaten van de huidige studie ondersteunden de verwachtingen van de klassieke co-existentietheorie door nichedifferentiatie, althans op regionale schaal aangezien milieuheterogeniteit op subplot-schaal niet werd bemeten. Indien nicheseparatie door competitie verantwoordelijk is voor co-existentie op de subplot-schaal, dan veronderstelt dit fijnschalige milieuheterogeniteit om de soorten naast elkaar te laten bestaan. Daarnaast moeten we beseffen dat competitie niet de enige mogelijke biotische factor is die de onderzochte plantengemeenschap structureert en die tot co-existentie leidt. Bijvoorbeeld kan ook facilitatie belangrijk zijn. Bovendien kunnen stochastische processen van aankomst en (tijdelijke) vestiging van soorten op een plaats, werken bovenop de deterministische processen van abiotische en biotische interacties. Dit laat meer mogelijke patronen toe van co-existentie.

De functionele overeenkomsten en verschillen tussen de vijf soorten, kwamen overeen met hun geobserveerde niche-overlappingen en –verschillen (hoofdstuk 3). De relatieve rangschikking van de soorten kon echter niet worden voorspeld vanuit hun plantenstrategie. Dit was evenmin het geval in het gemeenschapsexperiment (hoofdstuk 4), waarin gedeeltelijk andere soorten werden gebruikt. Hier werd dit toegeschreven aan zowel de gemengde plantenstrategie van de soorten en de hoge morfologische plasticiteit van enkele soorten. Volgens het paradigma van co-existentie door nichedifferentiatie zorgen fitness trade-offs voor nicheseparatie. De overeenkomst tusen nichepatronen en strategie van de soorten was één aanwijzing hiervoor; een andere was dat dominantiehiërarchieën konden omkeren afhankelijk van de beschouwde bron. Het patroon langsheen elke brongradiënt afzonderlijk was in overeenkomst met het model van competitieve hiërarchie.

Het herstel van het abiotische milieu van doelsoorten en de verhoging van hun succes in kolonisatie en vestiging binnen een ruimtelijke context, zijn belangrijke sleutels voor een succesvol natuurherstel. Dit proefschrift draagt bij aan de kennis, nodig om het *Eleocharition multicaulis* te herstellen, met: 1) predictieve nichemodellen van vijf soorten, 2) een biogeochemische en ecosysteembrede interpretatie van de vereisten van de karakteristieke soort *Eleocharis multicaulis*, en 3) het inzicht dat plant-plant-interacties belangrijk zijn voor relatieve performantie van populaties, tenminste op korte termijn, wat voor de natuurbehoudsbioloog betekent dat hij/zij aspecten van kolonisatie moet beschouwen (binnen een ruimtelijke en temporele context), en niet alleen milieu-implicaties.

Dit proefschrift sluit af met kansen voor verder onderzoek naar het *Eleocharition multicaulis*, suggesties om de manier te verbeteren waarop ecologen niches modelleren, en de uitdagingen en hindernissen bij het testen van ecologische theorie.

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Free Software Foundation (1991)

Flowering of Baldellia ranunculoides ssp. repens,
Mézières-en-Brenne, France

APPENDICES

Appendix 1. Location details of the sampled softwater lakes.

Appendix 2. Chemical characteristics of the sediment used for the experiment.

Appendix 3. The number of different types of predictors measured.

Appendix 4. Overall characteristics of the six variable subsets.

Appendix 5. Match between the variable subsets and the variables that best correlate with the discriminant functions.

Appendix 6. Retained variables of all approaches, marked with their maximum absolute Spearman rank correlation (with either DF1 or DF2) when they belonged to the most strongly correlating predictors, and otherwise with "X".

Appendix 7. Ionic ratios found in literature.

Appendix 8. Examples of studies that did not use ionic ratios in the habitat description of aquatic plants.

Appendix 1. Location details of the sampled softwater lakes. (PT = Portugal; ES= Spain; F = France; B = Belgium; NL = The Netherlands; D = Germany; UK = United Kingdom).

Country	Lake	Number of plots	Latitude	Longitude
PT	Small Ranunculus ololeucos lake near road Serra da Estrela	1	40°19' 57" N	7°36' 55" W
PT	Tiny dug lake near road in Serra da Estrela	1	40°19' 57" N	7°36' 55" W
PT	Ranunculus ololeucos Lake Serra da Estrela	1	40°20' 25" N	7°36' 35" W
PT	Small lake 1 near Rio Olo	1	41°21' 25" N	7°48' 20" W
PT	Small lake 2 near Rio Olo	1	41°21' 25" N	7°48' 20" W
ES	Laguna de Castrejón	1	40°32' 24" N	4°8' 47" W
ES	Lake near Rio Jallas	2	43°0' 24" N	8°55' 20" W
F	Etang de Bellebouche	3	46°47' 30" N	1°18' 20" E
F	Etang de Bel-Air	2	48°3' 35" N	3°25' 10" W
B	De Kuil	2	50°55' 15" N	5°31' 0" E
B	Lake in Neerharenheide	1	50°55' 50" N	5°37' 50" E
B	Stalvijver (Zonderik - Kolberg)	3	50°58' 35" N	5 °18' 0" E
B	Lobelia Lake in De Teut	2	51°1' 10" N	5°24' 15" E
B	Ruwmortelven	2	51°1' 20" N	5°37' 20" E
B	Gulke Putten 1	1	51°4' 50" N	3°20' 45" E
B	Gulke Putten 2	1	51°4' 50" N	3°20' 45" E
B	Eendeput 1	2	51°6' 30" N	3°18' 30" E
B	Eendeput 2	1	51°6' 30" N	3°18' 30" E
B	Lake in Vloethemveld	2	51°9' 0" N	3°6' 40" E
B	Buitengoor 1	1	51°13' 0" N	5°10' 52" E
B	Buitengoor 2	1	51°13' 0" N	5°10' 52" E
B	Goorvijver	2	51°17' 30" N	5°6' 50" E
B	Pasture Lake Kasteeltjes	2	51°22' 20" N	4°55' 4 5" E
B	Small lake near Putse Moer	1	51°22' 50" N	4°26' 10" E
B	Putse Moer	1	51°22' 50" N	4°26' 10" E
B	Ganzeven	1	51°23' 0" N	4°26' 45" E
NL	Soerendonks Goor Zuidwest	3	51°18' 8" N	5°32' 53" E
NL	Koolespeelke	2	51°18' 12" N	5°41' 0" E
NL	Beuven	5	51°24' 10" N	5°38' 55" E
NL	Winkelsven West	2	51°33' 15" N	5°15' 40" E
NL	Bank of rivulet Heiloop	2	51°33' 22" N	5°16' 3 0" E
NL	Van Esschenven	1	51°34' 10" N	5°12' 25" E
NL	Karregat	3	51°43' 53" N	5°25' 10" E
NL	Groot Ganzenven	2	51°45' 40" N	5°35' 53" E
NL	Lake south of Klompven	1	51°45' 40" N	5°36' 35 " E
NL	Botersnijdersven	2	51°47' 5" N	5°48' 35" E
NL	Langeven	2	51°47' 10" N	5°48' 15" E
NL	Kersjesven	1	51°47' 25" N	5°47' 30" E
NL	Talingenven	2	51°47' 30" N	5°47' 55" E

Country	Lake	Number of plots	Latitude	Longitude
NL	Lake in the 'Kruisbergsche Bosschen' (Landgoed Hagen)	2	51°59' 40" N	6°16' 0 " E
NL	Teeselinkven	2	52°9' 25" N	6°38' 52" E
NL	Lake in the Brecklekampse Veld	1	52°26' 30" N	7°0' 20" E
D	Eispohl Bremen	2	53°12' 7" N	8°33' 0" E
UK	Lake 1 at Cilan	1	52°47' 35" N	4°31' 57" W
UK	Lake 2 at Cilan	2	52°47' 35" N	4°31' 55" W
UK	Derwentwater Lake	2	54°34' 0" N	3°9' 15" W

Appendix 2. Chemical characteristics of the sediment used for the experiment.

Parameter	Value
Aluminium concentration NaCl-extraction (µmol/g DW)	0.22
Ammonium concentration NaCl-extraction (µmol/g DW)	0.54
Calcium concentration NaCl-extraction (µmol/g DW)	1.33
Granulometric proportion < 2 µm (ln %)	1
Granulometric proportion 2-16 µm (ln %)	2
Granulometric proportion 16-63 µm (ln %)	3
Granulometric proportion 63-125 µm (ln %)	11
Granulometric proportion 125-250 µm (ln %)	50
Granulometric proportion 250-500 µm (ln %)	33
Granulometric proportion 500-1000 µm (ln %)	2
Granulometric proportion > 1000 µm (ln %)	0
Iron concentration NaCl-extraction (µmol/g DW)	0.14
Loss on ignition (LOI) (%)	1.0
Magnesium concentration NaCl-extraction (µmol/g DW)	0.22
Manganese concentration NaCl-extraction (µmol/g DW)	0.02
Median granule size (µm)	210
Moisture (%)	19.9
Nitrate concentration water-extraction (µmol/g DW)	0.03
Orthophosphate concentration lactate-extraction (µmol/g DW)	0.03
pH NaCl-extraction	4.2
pH water-extraction	5.2
Phosphorus concentration NaCl-extraction (µmol/g DW)	0.01
Potassium concentration NaCl-extraction (µmol/g DW)	0.39
Silicon concentration NaCl-extraction (µmol/g DW)	1.88
Sulfur concentration digestion (µmol/g DW)	1.45
Sulfur concentration NaCl-extraction (µmol/g DW)	0.10
Zinc concentration NaCl-extraction (µmol/g DW)	0.01

Appendix 3. The number of different types of predictors measured.

	Summer [a]	Winter	Seasonality [b]	Totals
Geographical situation	2	-	-	2
Lake surface	1	-	-	1
Vegetation				
Species cover	28	-	-	28
Vegetation structure	6	-	-	6
Surface water layer				
Physical properties	1	2	1	4
Chemical properties	22	32	22	76
Soil				
Physical properties	11	-	-	11
Chemical properties	35	36	33	104
Totals	106	70	56	232

[a] Some variables that do not depend on the season are included in this column (e.g. lake surface).

[b] Seasonality variables are the difference between summer and winter values, primarily for chemical data.

Appendix 4. Overall characteristics of the six variable subsets. Jaccard similarities of interest are underlined. [a]

Variable subset acquisition	Number of retained variables	Jaccard similarities					
CHI (0.01) DIRECT	10	1					
CHI (0.01) STEPS	2	0.20	1				
CHI (0.05) PCA DIRECT	4	0	0	1			
CHI (0.05) STEPS	5	0.15	0.40	0	1		
PCA DIRECT	15	0	0	0	0.18	1	
STEPS	24	0.03	0.04	0	0.07	0.03	1
		CHI (0.01) DIRECT	CHI (0.01) STEPS	CHI (0.05) PCA DIRECT	CHI (0.05) STEPS	PCA DIRECT	STEPS

---

[a] *Abbreviations: CHI (0.01) DIRECT: direct discriminant analysis with the chi-square subset P < 0.01; CHI (0.01) STEPS: stepwise discriminant analysis with the chi-square subset P < 0.01; CHI (0.05) PCA DIRECT: direct discriminant analysis with the PCA-selected variables from the chi-square subset P < 0.05; CHI (0.05) STEPS: stepwise discriminant analysis with the chi-square subset P < 0.05; PCA DIRECT: direct discriminant analysis with PCA-selected subset; STEPS, stepwise discriminant analysis.*

Appendix 5. Match between the variable subsets and the variables that best correlate with the discriminant functions.

Most often at least one of the variables retained in the discriminant function is among the best correlating of the 232 initial predictors with this discriminant function. [a]

			the predictors that correlate best with DF (*p* are considered)			
				the predictor with maximum correlation with the DF *and* retained in the DF		
Type of analysis	DF	Total number of variables retained in DF (*p*)	Number that are retained in DF (% of *p*)	Variable name	Correlation rank	Spearman R
CHI (0.01) DIRECT	DF1	10	7 (70%)	Seasonality of Soil Silicon concentration NaCl-extraction	1	-0.67
	DF2	10	1 (10%)	Seasonality of Surface water Ammonium / nitrate concentration	4	0.52
CHI (0.01) STEPS	DF1	2	1 (50%)	Seasonality of Surface water Ammonium / nitrate concentration	1	0.79
	DF2	2	2 (100%)	Seasonality of Soil Total N content	1	0.66
CHI (0.05) PCA DIRECT	DF1	4	2 (50%)	Surface water Winter pH	3	0.82
	DF2	4	0 (0%)	-	-	-
CHI (0.05) STEPS	DF1	5	3 (60%)	Seasonality of Surface water Ammonium / nitrate concentration	2	0.60
	DF2	5	1 (20%)	Seasonality of Surface water Ammonium / nitrate concentration	5	0.42
PCA DIRECT	DF1	15	2 (13%)	Seasonality of Surface water Divalent / monovalent cation ratio	1	0.65
	DF2	15	2 (13%)	Seasonality of Soil Phosphorus concentration	1	0.64
STEPS	DF1	24	1 (4%)	Seasonality of Surface water Ammonium / nitrate concentration	1	-0.45
	DF2	24	2 (8%)	Seasonality of Surface water Divalent / monovalent cation ratio	2	-0.34

[a] *Abbreviations: DF1, first discriminant function; DF2, second discriminant function; CHI (0.01) DIRECT: direct discriminant analysis with the chi-square subset $P < 0.01$; CHI (0.01) STEPS: stepwise discriminant analysis with the chi-square subset $P < 0.01$; CHI (0.05) PCA DIRECT: direct discriminant analysis with the PCA-selected variables from the chi-square subset $P < 0.05$; CHI (0.05) STEPS: stepwise discriminant analysis with the chi-square subset $P < 0.05$; PCA DIRECT: direct discriminant analysis with PCA-selected subset; STEPS, stepwise discriminant analysis.*

Appendix 6. Retained variables of all approaches, marked with their maximum absolute Spearman rank correlation (with either DF1 or DF2) when they belonged to the most strongly correlating predictors, and otherwise with "X".

This table is the extended version of Table 5.3. Abbreviations as in Table 5.1.

Covariate [a]	CHI (0.01) DIRECT	CHI (0.01) STEPS	CHI (0.05) PCA DIRECT	CHI (0.05) STEPS	PCA DIRECT	STEPS
Number of retained variables	10	2	4	5	15	24
Seasonality of Mineral soil layer Silicon concentration NaCl-extraction	-0.67					
Mineral soil layer Winter Silicon concentration NaCl-extraction	-0.65					
Seasonality of Mineral soil layer Total N content	0.52	0.66		X		
Mineral soil layer Winter Total N content	0.55					
Seasonality of Mineral soil layer Potassium concentration	0.58					
Seasonality of Surface water Ammonium / nitrate concentration	0.64	0.79		0.60		-0.45
Mineral soil layer Winter Silicon concentration	0.64					
Cover *Juncus bulbosus*	X					
Surface water Summer Ion Ratio	X					
Surface water Winter Chloride proportion	X					
Surface water Winter Potassium concentration				-0.52	-0.45	
Seasonality of Surface water Divalent / monovalent cation ratio				0.57	0.65	-0.34
Cover *Mentha aquatica*				X	-0.44	
Cover *Agrostis canina*			X			
Mineral soil layer Summer Magnesium concentration (µmol/kg DW)			X			
Surface water Summer Magnesium concentration (µM)			0.81			
Surface water Winter pH			0.82			
Cover *Carex nigra*						X
Cover *Eleocharis palustris*					X	
Cover *Hydrocotyle vulgaris*						0.34
Cover *Hypericum elodes*						X
Cover *Juncus effusus*						X
Cover *Molinia caerulea*					X	
Cover of litter Summer						X
Cover of water layer Winter					X	
Cover of woody taxocoenose						X
Mineral soil layer Summer Granulometric proportion 16-63 µm					X	
Mineral soil layer Summer Nitrate concentration water-extraction					X	
Mineral soil layer Summer Sodium concentration						X
Mineral soil layer Winter						X

Covariate [a]	CHI (0.01) DIRECT	CHI (0.01) STEPS	CHI (0.05) PCA DIRECT	CHI (0.05) STEPS	PCA DIRECT	STEPS
Number of retained variables	10	2	4	5	15	24
Potassium concentration NaCl-extraction						
Seasonality of Mineral soil layer Alumium / calcium (NaCl-extraction)						X
Seasonality of Mineral soil layer Ammonium / nitrate (water-extraction)						X
Seasonality of Mineral soil layer Ammonium concentration water-extraction					X	
Seasonality of Mineral soil layer Extinction 450 nm lactate-extraction						X
Seasonality of Mineral soil layer pH water-extraction						X
Seasonality of Mineral soil layer Phosphorus concentration					0.64	
Seasonality of Mineral soil layer Total C content					X	
Seasonality of Surface water Chloride concentration						X
Seasonality of Surface water Electric conductivity EC(25)						X
Seasonality of Surface water Extinction 450 nm						X
Seasonality of Surface water Manganese concentration					X	
Seasonality of Surface water Silicon concentration						X
Seasonality of Surface water Sulfur concentration						X
Surface class						X
Surface water Summer Alumium concentration						X
Surface water Summer Orthophosphate concentration						X
Surface water Summer TIC concentration						X
Surface water Winter Calcium concentration					X	
Surface water Winter Silicon concentration					X	
Surface water Winter Sodium concentration						X
Total height of vegetation					X	

[a] *Seasonality variables are the difference between summer and winter values. Other variables are always confined to winter or summer conditions, with cover values belonging to the summer period. See chapter 2 for technical aspects and for ecological interpretation of results.*

Appendix 7. Ionic ratios found in literature.

Abbreviations: G: geohydrological use (the ionic ratio is used to indicate a geohydrological ecosystem class, or is a measure for the intensity of a certain biogeochemical process); GE: geohydrological and ecological use (the ionic ratio is also used to predict the state or fate of plants). Ion symbols represent equivalent concentrations.

Ionic ratios	Compart-ment	Appli-cation	Related with…	Physiological mechanism? [a]	Examples
Acidification and nitrogen deposition					
Al/Ca	Water and soil	GE	N deposition and acidification; Al toxicity to plants; base cation nutrition	Y	Lakes: Maessen *et al.* 1992 Heath: de Graaf *et al.* 1994, de Graaf *et al.* 1997, de Graaf *et al.* 1998b, de Graaf *et al.* 2000 Forest: Houdijk *et al.* 1993, Cronan & Grigal 1995, Hansen *et al.* 2007
Al/cations	Soil	GE	N deposition and acidification; cation deficiency and root function of conifers	Y	Forest: Persson & Majdi 1995, Hansen *et al.* 2007
N/cations	Soil	GE	N deposition and acidification; cation deficiency and root function of conifers	Y	Forest: Persson & Majdi 1995
NH_4/NO_3	Water and soil	GE	N deposition and acidification; N conversions; availability of different N forms to plant; NH4 toxicity to plant; base cation deficiency in plant	Y	Mires: Clymo 1984 Lakes: Schuurkes *et al.* 1988 Precipitation: Schuurkes *et al.* 1987b, Salve *et al.* 2008 Heath: de Graaf *et al.* 1994, de Graaf *et al.* 1998b Crops: Kafkafi 1990 Terrestrial habitats: Stevens *et al.* 2011
NH_4/K, NH_4/Mg	Soil	GE	N deposition and acidification; NH4 toxicity to plant; base cation deficiency in plant; tree dieback	Y	Heath: de Graaf *et al.* 1994 Forest: Houdijk *et al.* 1993
Base saturation	Soil	GE	Sensitivity to acidification		Heath: de Graaf *et al.* 1994

Drought and resulting oxidation and acidification					
SO_4/Cl	Water	G	Acidification by oxidation of sulphides, after drought periods; marine aspect of precipitation		Lakes: Vangenechten 1980 Mires: Proctor 1992, Proctor 1994 Precipitation: Bloemendaal *et al.* 1988 Groundwater: Kumar *et al.* 2009
S/(Ca + Mg)	Soil	GE	Sensitivity of ecosystems to acidification and mobilisation of heavy metals following drought		Mires: Lucassen *et al.* 2002
Base cation status					
K/Na	Water	G	Uptake and release of K by plants	Y	Mires: Proctor 1994
Divalent / monovalent cation ratio (DMR) = (Ca + Mg)/(K + Na) [b]	Water	GE	Distinction between sea water and freshwater; algae and macrophyte presence and community assemblage	Y	Lakes: Pearsall 1922, Provasoli 1958, Seddon 1972, de Lyon & Roelofs 1986, Bloemendaal *et al.* 1988, Wetzel 2001 Mires: Baaijens 1992 Polders: Barendregt & Nieuwenhuis 1993, Barendregt *et al.* 1993
Ca/cations	Soil	GE	Plant-available Ca; salinity-induced Ca deficiency; performance of calcicole species	Y	Prairies: Carter & Webster 1990 Dune-slacks: Ernst & Nelissen 1998
Nutrient limitation					
CO_2/HCO_3	Water	GE	Acidification; carbon assimilation of macrophytes	Y	Lakes: Roelofs *et al.* 1996
N/P	Soil	GE	Nutrient limitation; saturation of *Sphagnum* carpet; nutrient retention by plants	Y	Mires: Bragazza *et al.* 2003, Sorrell *et al.* 2011 Meadows: Venterink & Güsewell 2010 *Carex* spp.: Güsewell 2005

N/P	Plants (either at vegetation, species or phytometer level)	GE	Nutrient limitation; nutrient supply ratio	Y	Non-forested wetlands : Güsewell & Koerselman 2002, Güsewell *et al.* 2003 Heath and nutrient-poor grassland : Roem & Berendse 2000 Grassland : Craine *et al.* 2008 Desert shrubland : Drenovsky & Richards 2004
C/P, C/N, C/K	Soil	GE	Degree of ombrotrophy in peat; nutrient limitation for soil organic matter decomposition	Y	Mires: Hayati & Proctor 1991, Lamers *et al.* 2001
N/Si	Water	GE	Nutrient limitation of diatoms	Y	Sea: Gilpin *et al.* 2004
Fe/PO_4, Fe/P	Soil	GE	Eutrophication (Fe lowers PO4 availability); occurrence of endangered plant species	Y	Fens: Geurts *et al.* 2008

Origin of water

Na/Cl, Ca/Cl, Mg/Cl, K/Cl SO_4/Cl (see also under “drought”) Ca/Mg Ca/(HCO_3+SO_4) (Ca+Mg)/HCO_3	Water	G	Precipitation and sea water; cation exchange and leaching in soil; groundwater origin		Streams: Sutcliffe & Carrick 1983 Mires: Gorham 1956, Proctor 1992, Proctor 1994 Groundwater: Wen *et al.* 2008, Kumar *et al.* 2009
Na/Mg	Water	G	Degree of ombrotrophy or minerotrophy		Mires: Proctor 1994
Ca/Mg	Water	GE	Degree of ombrotrophy or minerotrophy; algae assemblage		Lakes: Provasoli 1958, Wetzel 2001 Mires: Proctor 1994, Bragazza *et al.* 2003, Bendell-Young 2003

Chemical typology

Ion ratio (IR) = Ca/(Ca + Cl)	Water	G	Water type with respect to Ca molar fraction		Mires: van Wirdum 1991

Ca/Mg	Soil	G	Serpentine content	Soil: Murren *et al.* 2006
Relative ionic composition (anions, cations, all major ions)	Water	GE	Water typology; sometimes brought in connection with eutrophication, acidity class, acidification or drought; occurrence of plant species	Lakes: Pietsch 1977, Pietsch 1978, Vangenechten *et al.* 1981a, Vangenechten *et al.* 1981b, de Lyon & Roelofs 1986, Bloemendaal *et al.* 1988, Leuven *et al.* 1992 Mires: van Wirdum 1991, Boeye *et al.* 1991, Boeye *et al.* 1994 Polders: Barendregt & Nieuwenhuis 1993, Barendregt *et al.* 1993 Groundwater: Wen *et al.* 2008

[b] *Y = a physiological mechanism is known or hypothesized.*

[c] *We note that the sometimes used proportion of divalent cations, PRD = (Ca^{2+} + Mg^{2+})/(Ca^{2+} + Mg^{2+} + K^{+} + Na^{+}), e.g. de Lyon & Roelofs (1986), is a function of DMR, as follows: PRD = DMR/(DMR + 1).*

Appendix 8. Examples of studies that did not use ionic ratios in the habitat description of aquatic plants.
These studies contrast with 5 habitat studies of aquatic plants that did consider ionic ratios: Pearsall 1922; Seddon 1972; Pietsch 1977; Pietsch 1978; de Lyon & Roelofs 1986.

Pearsall 1920, Roll 1939, Gies & Lötschert 1973, Sand-Jensen & Søndergaard 1979, Jensen 1979, Rintanen 1982, van Groenendael *et al.* 1982, van Groenendael *et al.* 1983, Wilson & Keddy 1985, Yan *et al.* 1985, Farmer & Spence 1986, Arts & Leuven 1988, Jackson & Charles 1988, Pip 1988, Raven 1988, van Dam 1988, Vöge 1988, Kaplan & Prolingheuer 1989, Rørslett & Brettum 1989, Arts *et al.* 1990, Hellberg & Cordes 1990, Arts 1990a, Boeye *et al.* 1991, Kunii 1991, Rørslett 1991, Slater *et al.* 1991, Vöge 1992, Kaplan 1993, Papastergiadou & Babalonas 1993, Vöge 1993, Gacia *et al.* 1994, Klosowski 1994, Krause & King 1994, Pietsch 1994, Robe & Griffiths 1994, Botineau & Ghestem 1995, Toivonen & Huttunen 1995, Szankowski & Klosowski 1996, van Groenendael *et al.* 1996, Spink *et al.* 1997, Szmeja 1997, Szmeja *et al.* 1997, Riis & Sand-Jensen 1998, Szankowski & Klosowski 1999, Vestergaard & Sand-Jensen 2000, Heegaard *et al.* 2001, Szankowski & Klosowski 2001, Makela *et al.* 2004, Szankowski & Klosowski 2006, Auderset Joye *et al.* 2006, Feldmann & Noges 2007, Kocic *et al.* 2008, Istvanovics *et al.* 2008, Free *et al.* 2009, Hrivnak *et al.* 2010

There are so many possibilities for niche differentiation that one wonders whether general theories are possible, or whether ecologists will become no more than natural historians painstakingly documenting the niches of each species in each community.

Keddy (2001)

Hypericum elodes and Juncus bulbosus, Tielen, Belgium

GLOSSARY

Below, the definitions of some terms are given that are either used in this thesis, or used in subsequent definitions. The definitions reflect the use of these terms in plant ecological literature and in this thesis.

Characteristic species	A species that is characteristic of a certain *plant assemblage* (*vegetation type*), due to its higher frequency and/or mean abundance within the plant assemblage compared to other plant assemblages.
Community	The individuals of all species that potentially interact within a single patch or local area of habitat (Leibold *et al.* 2004). Used either in a concrete or in a conceptual sense. See also: *plant community*.
Competition	The tendency of neighbouring plants to utilise the same quantum of light, ion of mineral nutrient, molecule of water, or volume of space (Grime 2001). Also, the negative plant-plant interaction that occurs by consuming a resource (Keddy 1989). **Remark**: as competition leads to metabolic and energetic costs, components such as *competitive effect* or c*ompetitive response* are generally quantified with measures of *performance*, including growth and/or reproductive measures (Keddy 2001).
Competitive ability	The degree to which a species can suppress subordinate species (*competitive effect*) and resist to the competitive effects of dominant species (*competitive response*) (Goldberg 1996).
Competitive effect	Ability of a plant or *population* to suppress other plants or populations through *competition* (Goldberg 1996, Keddy 2001).
Competitive response	Ability of a target plant or *population* to resist to competitive suppression by neighbouring plants or populations. In experiments (cf. chapter 4), defined as the difference between the performance of a species in monoculture versus mixture (Goldberg 1996, Keddy 2001).
Disturbance	The mechanisms which limit the plant biomass by causing its partial or total destruction (Grime 2001).

Fitness	The contribution made to a population of descendants by an individual relative to the contribution made by others in its present population. The relative contribution that an individual makes to the gene pool of the next generation (Begon *et al.* 1996).
Functional trait	Any trait which impacts *performance* or *fitness* indirectly via its effects on growth, reproduction and survival (McGill *et al.* 2006, Violle *et al.* 2007).
Fundamental niche	The region of its *niche* that a species is able to occupy in the absence of interspecific competition and natural enemies (Silvertown 2004).
Niche	An n-dimensional hypervolume defined by axes of resource use and/or environmental conditions and within which populations of a species are able to maintain a long-term average net reproductive rate that is ≥ 1 (Silvertown 2004).
Performance	State of an individual, regarding survival, growth or reproduction. It is measured by a corresponding trait, such as biomass, size or fecundity (Keddy 2001). It is often used as a proxy for *fitness*. See also the remark under *competition*. The term is also applied to populations or species and then mostly refers to their absolute or relative abundance in a community.
Persistence	Maintenance or enhancement of performance in an individual or a population over time, by tolerating a range of abiotic and biotic conditions (Weiher *et al.* 1999).
Plant assemblage	A specific assemblage of plant species growing together at the local scale, as defined by the combined presences and absences of specific species. Either used in a concrete sense (specifically observed in a location) or in an abstract sense (in that case, synonymous to *vegetation type* and the third meaning of *plant community*). (after Rodwell 1995, Schaminée *et al.* 1995b)
Plant community	Used in three different meanings, dependent on the context (after Rodwell 1995, Schaminée *et al.* 1995b). (1) A concrete *community* of plants, in a specific place. (2) The concept of a local *community* of plants. (3) One abstract class from a hierarchical framework of different possible *plant assemblages*, that applies to a certain geographical area; synonym to *vegetation type*.

Plant population	The collection of plant individuals of the same species that live in the same area (Silvertown & Charlesworth 2001).
Realized niche	(1) The region of its niche that a species is able to occupy in the presence of interspecific competition and natural enemies (Silvertown 2004). (2) The projection in niche space of the observed geographical distribution of a species (McGill *et al.* 2006 and also Colwell & Rangel 2009, who refer to this as “a practice dating at least to Austin et al.’s early work” (Austin *et al.* 1984)). This meaning extends the first meaning with other phenomena that define the observed niche, especially dispersal limitation.
Species identity	The species a population belongs to. It integrates intrinsic species properties, including competitive ability, disturbance avoidance, seed production etc. (Weiher *et al.* 1999).
Stress	The external constraints that limit the rate of dry matter production of all or part of the vegetation (Grime 2001).
Trait	Any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organization (Violle *et al.* 2007). Usually measured at the individual level and used comparatively across species (McGill *et al.* 2006).
Vegetation	All plants that grow together (after Schaminée *et al.* 1995b). Applicable to different spatial scales.
Vegetation type	One abstract class from a hierarchical classification of different possible *plant assemblages*, that applies to a certain geographical area. The term *plant community* is used more often, however. (after Rodwell 1995, Schaminée *et al.* 1995b)

The goal of community ecology (as of all science) is to find general rules.

MacArthur (1972)

Eleocharis multicaulis and Drosera intermedia, Turnhout, Belgium

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Thus, resource partitioning can result in temporally constant spatially non-uniform patterns, or spatially constant temporally non-uniform ones, or spatiotemporal mosaics.

Levin (1992)

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