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Original article

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

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A B S T R A C T

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 (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. Competition intensity was 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 conclude that hydrological state was the only important environmental factor. The identity of the species that were present—implying species interactions—largely determined community outcome.

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1. Introduction

A major challenge in plant ecology is to understand how communities are structured and composed in various environments. Our understanding starts with essential information on the species' environmental niche and life history traits (Keddy et al., 2000; McGill et al., 2006; Webb et al., 2010; Westoby and Wright, 2006). 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) (Bornette and Puijalon, 2011; Lacoul and Freedman, 2006; Wetzel, 2001). 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 (Gaudet and Keddy, 1988; Grime, 2001; Kautsky, 1988; Weiher et al., 1999; Westoby and Wright, 2006; Willby et al., 2000).

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. Netten et al., 2010; Spencer and Rejmanek, 2010; Spierenburg et al., 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 (Madsen et al., 2002; Smolders 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 (Arts, 2002; Bobbink et al., 1998; Roelofs et al., 1996; Smolders et al., 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 (Kennedy et al., 2006; Lacoul and Freedman, 2006; Thomaz 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 identify intrinsic species properties, including competitive ability, as '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.

The hypotheses we were testing were: (1) different combinations of nutrients (carbon dioxide and ammonium) drive the performance of the community more than water level fluctuation and species identity, and (2) competition between species for nutrients has a marked role in this community.

2. Methods

2.1. 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 (Vanderhaeghe et al., 2005; unpublished 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 (Vanderhaeghe et al., 2005). 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 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 species traits, 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*.

2.2. 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* was collected from a rivulet 'De Run' (51° 23′ 34.58″ N, 5° 24′ 13.98″ E), *Eleocharis* from a small softwater lake 'Langeven' (51° 47′ 9.92″ N, 5° 48′ 18.49″ E), *Hydrocotyle* from a small softwater lake 'Meeuwenven' (51° 46′ 50.44″ N, 5° 48′ 9.80″ E) and *Baldellia* 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* 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).

2.3. 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. 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. Therefore, the influence of the water level drop was reflected by the factor time, or more explicitly by 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 restraints prevented us from extending the competition experiment to all species

Table 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 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, 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: Knevel et al., 2003).
[d] Further references for *Eleocharis multicaulis* and other Eleocharis spp.: Arts, 2002; Busch et al., 2004; dos Santos and Esteves, 2002; Edwards et al., 2003; Maillette and Keddy, 1989; Pietsch, 1985; Sorrell et al., 2002.
[e] Further references for *Luronium natans* traits: Bazydlo, 2004; Bazydlo and Szmeja, 2004; Greulich et al., 2000a, 2001; Greulich and Bornette, 2003, 1999; Hyldgaard and Brix, 2011; Nielsen et al., 2006; Szmeja et al., 2008.
[f] Further references for *Baldellia ranunculoides* traits: Kozlowski et al., 2008, 2009; Kozlowski and Matthies, 2009; Kozlowski and Vallelian, 2009.
[g] Further references for *Hydrocotyle vulgaris* traits: Evans, 1991, 1992; Grime, 1988; Leeflang et al., 1998.

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.

Each community or monoculture of plants was grown in glass aquaria with dimensions (cm) $30 \times 30 \times 60$ (L × W × H). Every combination of ammonium and carbon dioxide levels, as well as the monocultures, were replicated four times and assigned at random to the aquaria. 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

Fig. 1. Simulated seasonal changes during the experiment.

illumination, providing an average PAR photon flux of $145 \, \mu mol \, s^{-1} \, m^{-2}$ 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. 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 that in the community, with plants positioned as in the community.

Mineral sandy sediment was collected from Beuven (upper 20 cm; containing $0.54 \, \mu mol \, g^{-1}$ DW NH_4^+ (NaCl-extraction), $0.03 \, \mu mol \, g^{-1}$ DW NO_3^- (water-extraction) and 1.0% organic matter). It was mixed with $0.25 \, \mu mol \, g^{-1}$ 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 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, $50 \, \mu mol \, L^{-1}$ NH$_4$Cl was added to the medium stock and in the +C treatment the media contained $800 \, \mu mol \, L^{-1}$ CO$_2$. All other ion concentrations were kept the same and pH = 5 was maintained by adding HCl, NaOH and NaCl where necessary. Cyanoguanidine ($0.5 \, \mu mol \, L^{-1}$) was added to prevent nitrification of
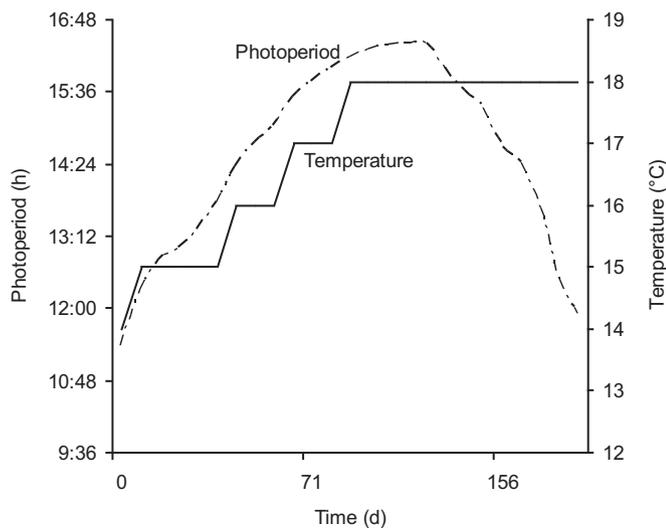
Table 2
Chemical composition of culture mediums and salt dosage in rainwater.

Ion	Basic culture medium −N ($\mu mol\ L^{-1}$)	Medium +N ($\mu mol\ L^{-1}$)	Salt	Rainwater +N ($kg\ ha^{-1}\ y^{-1}$)	Rainwater −N ($kg\ ha^{-1}\ y^{-1}$)
NH_4^+		50	NH_4Cl	25.45	2.55
Na^+	386	336	NH_4NO_3	38.09	3.81
K^+	50	50	KCl	15.13	15.13
Mg^{2+}	60	60	$CaCl_2.2H2O$	9.94	9.94
Ca^{2+}	100	100	Sea salt	33.82	33.82
Cl^-	496	496			
SO_4^{2-}	125	125			
NO_3^-	10	10			

ammonium (Smolders et al., 1996). All ion concentrations were chosen to be representative of the chemical gradients observed in softwater lakes in Western Europe (Vanderhaeghe et al., 2005; unpublished 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 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 N ha^{-1} y^{-1} and for the +N treatment was 20 kg N ha^{-1} y^{-1}.

2.4. 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 × 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).
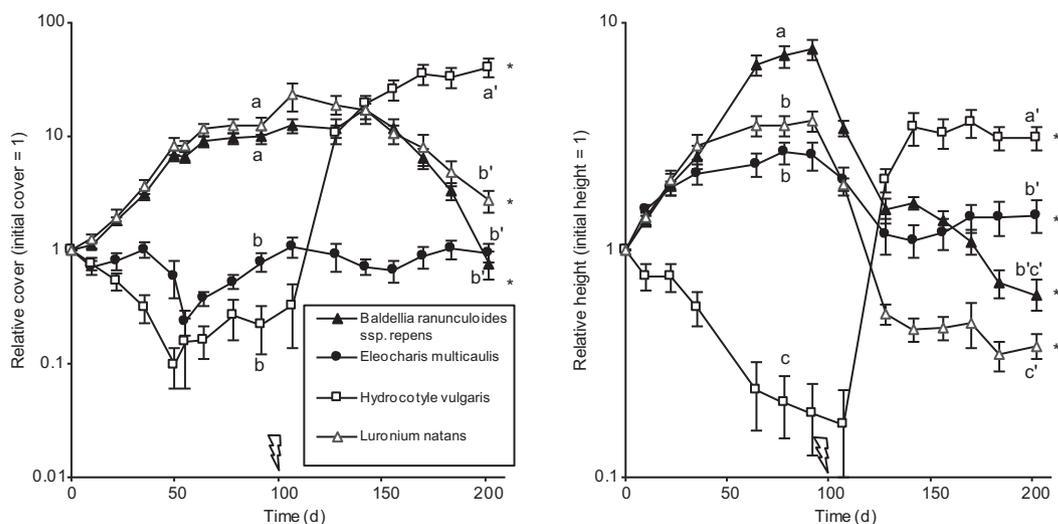
For comparison of species, cover, height and number of leaves or culms were standardized relative to the value at $t = 0$ (Gibson et al., 1999). From the data on number of flowers, the non-repeated measure 'onset of flowering' was derived (days to flowering). For populations that never flowered, the artificial value of 400 days was assigned for 'onset of flowering'.

2.5. Statistical design and analysis

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*. Experimental units for repeated observations were either populations (the individuals per species taken together) or individuals (nested within plot and species; not shown).

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. An 'unstructured' covariance structure was specified in order to cope with interdependencies of repeated measurements and for observations from the same aquarium.

For the endstage of both the submersed and emersed phases, multiple comparison tests (applying the Bonferroni adjustment) and mixed model subanalyses were done. A comparison between the submersed and emersed endstage was done using mixed model subanalyses. For the analysis of competitive response, the effects of neighbourhood and time were tested separately for each species, and the effects of species identity and time on competitive responses were tested simultaneously.

3. Results

3.1. Experimental outcome of the communities

Performance of *Luronium* and *Baldellia* steadily increased in the submersed phase (Fig. 2, left half of diagram) and these species became dominant (with respect to height and cover). Performance

Fig. 2. Changes in species performance (mean ± SE) with time (from observations at population level, i.e. species × aquarium). Vertical axes are log-scaled. The lightning symbol indicates the point in time when water level was decreased. At the submersed and emersed endstage 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 3
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) | | | | | | | | Observed competitive response in +C+N treatment | |
| | Submersed phase | | | | Emersed phase | | | | Submersed 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	-	-	-	-	+++	+++	+++	+++		

+++ = Excellent performance; ++ = Good performance; + = Moderate performance; − = Low to bad 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).

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. 3), 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

Table 4
ANOVA-table 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

| | | Number of fruits | | | Onset of flowering[b] | | | Relative height[c] | | | Relative number of leaves or culms[c] | | |
Effect	Num DF	Den DF	F Value	P	Den DF	F Value	P	Den DF	F Value	P	Den DF	F Value	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

[a] Unless otherwise stated, performance measures refer to observations at population level (species × aquarium). P values < 0.10 appear shaded. Num DF = numerator degrees of freedom, Den DF = denominator degrees of freedom.
[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.
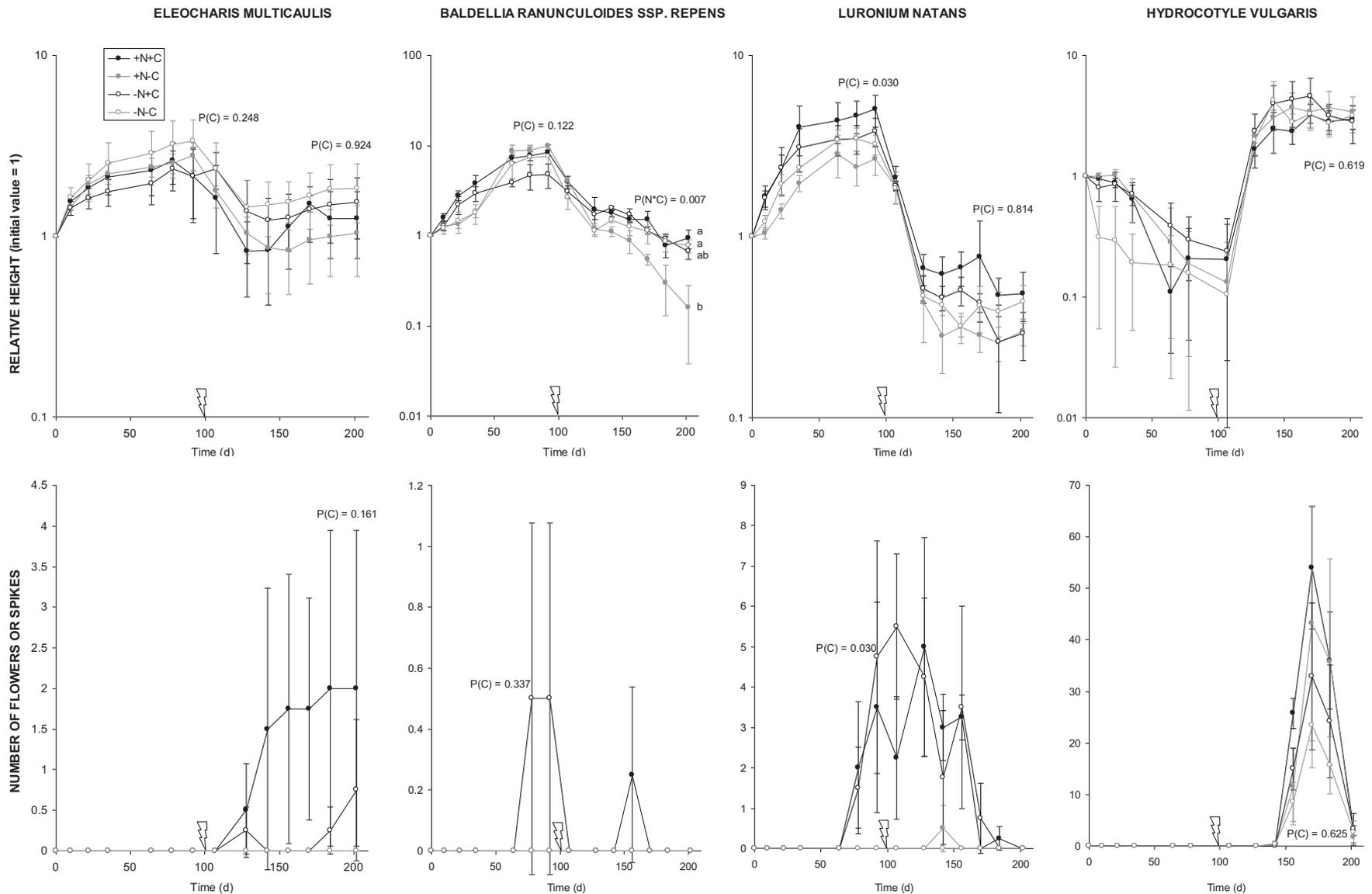
Fig. 3. Effects (mean ± SE) of carbon dioxide (black versus grey) and ammonium supply (closed versus open circles) on several performance measures (population as measurement unit except for relative number of leaves or culms). Relative number of leaves or culms was determined at individual plant level, 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 lightning symbol indicates the point in time 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.
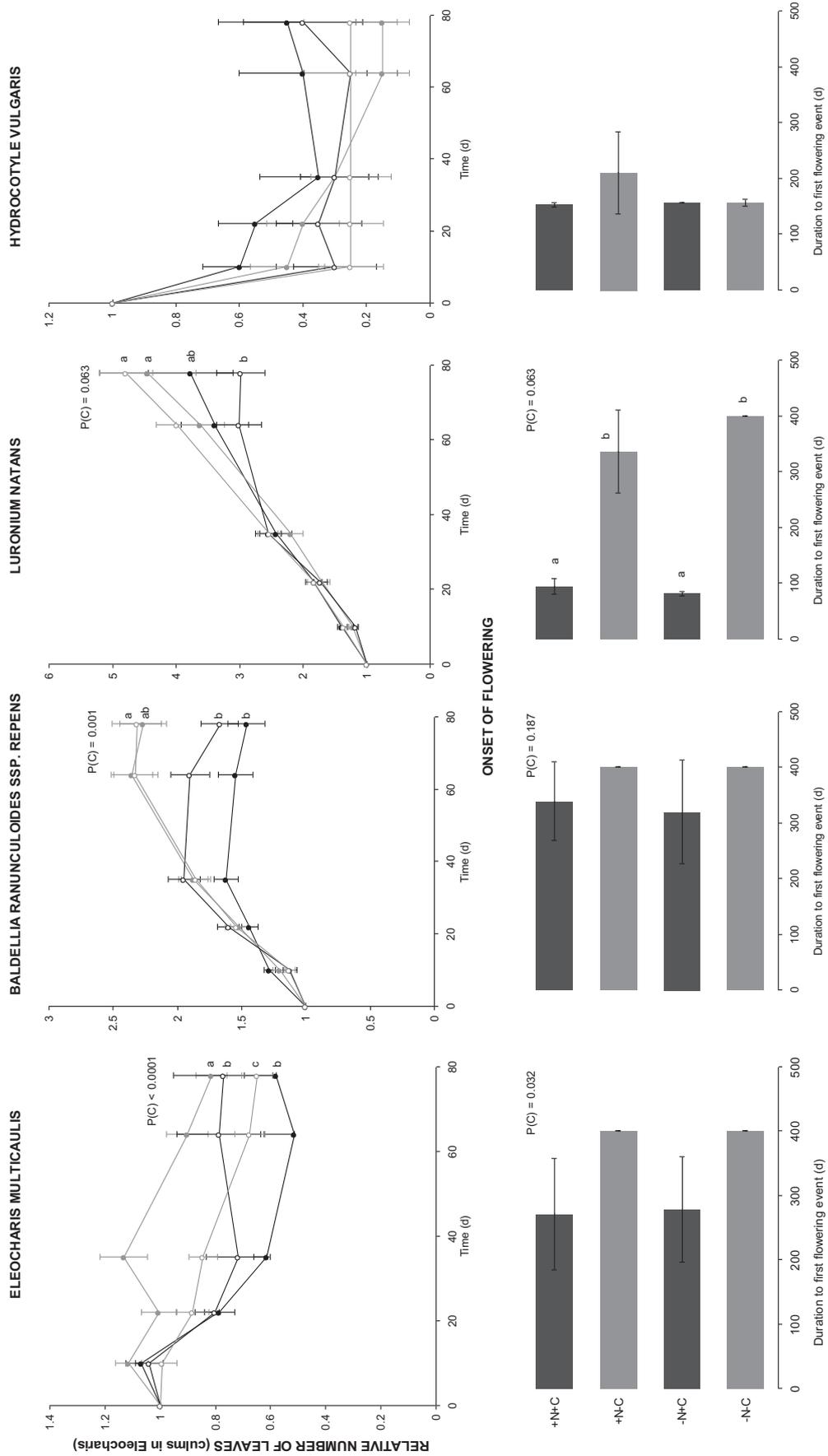
Fig. 3. *(continued)*

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. 3 for specific statistical test results.

The patterns described above are summarized in Table 3. The experimental outcome was governed by the interaction between species identity and hydrological state (submersed/emersed: factor 'time'). 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 effect of time (hydrological state).

3.2. Competitive response of Eleocharis and Baldellia

Significant competitive responses were found for both species and were dependent on performance measure and hydrological state (Fig. 4, Table 6). Competitive response differed significantly between both species, depending on the hydrological state. 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 5).

For both species, a competitive response was most obvious in the emersed state (Fig. 4). Competitive response for relative cover was greater in *Baldellia*, while for *Eleocharis* competitive response was greater for relative height and number of flowers (spikes) and fruits (fruiting spikes). Thus, both species were evaluated to have a 'high' competitive response in the emersed phase, and a 'moderate' competitive response in the submersed phase (Table 3).

4. Discussion

4.1. Overall patterns

Our experiment tested the effect of carbon dioxide and ammonium levels, hydrological state and competitive neighbourhood on population performance in communities. We explicitly considered 'species' as a factor in order to highlight interspecific differences. Remarkably, in the community experiment, species identity and hydrological state 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. In

our study, the results point to greater importance of water table and species identity, including competitive interactions.

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 with 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) (Bornette et al., 2008; Thomaz et al., 2006; Willby et al., 2000). 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; Hyldgaard and Brix, 2011; Kozlowski et al., 2008; Puijalon et al., 2007, 2008; Szmeja et al., 2008). 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.

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, 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 and Bornette (2003), Puijalon et al. (2008) and Puijalon and Bornette (2006). 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 can conclude that competition is an important component for determining community structure, as demonstrated by the competitive response of two species at the high nutrient level combination. We found that *H. vulgaris* was a competitive dominant in nutrient-rich emersed conditions and that other species showed a higher competitive response compared to the submersed
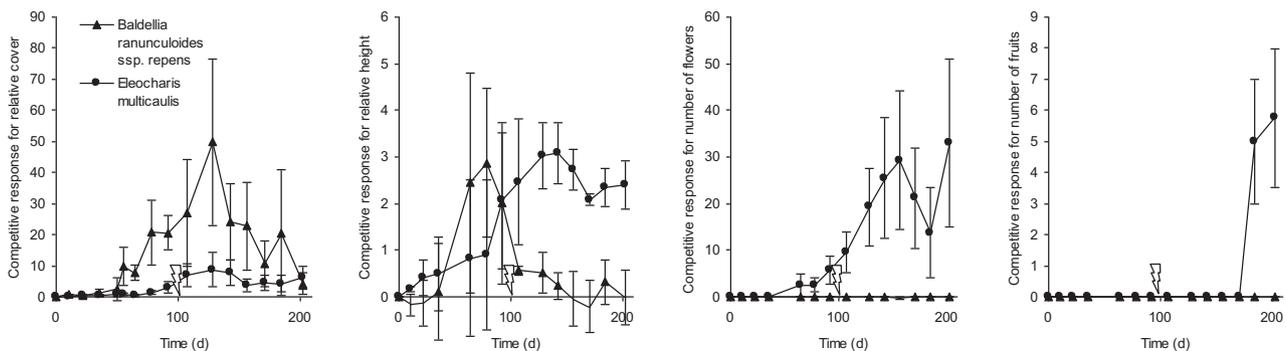
Fig. 4. Competitive response of *Baldellia ranunculoides* ssp. *repens* and *Eleocharis multicaulis* at high resource levels (+N+C treatment) (mean ± SE).

Table 5
ANOVA-table 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]

Effect	Num DF	Relative cover			Relative height			Number of flowers			Number of fruits		
		Den DF	F Value	P	Den DF	F Value	P	Den DF	F Value	P	Den DF	F Value	P
Analysis per species on neighbourhood													
Baldellia													
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													
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													
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.

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. The significance of competition at other nutrient levels should be a subject of future experimentation. 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.

4.2. Patterns in three focal species

According to Arts (2002) and Pietsch (1985), E. 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 E. multicaulis benefits from nitrogen addition as long as nitrate remains dominant over ammonium (Vanderhaeghe et al., 2005). In the case of high atmospheric nitrogen deposition, ammonium concentration increases and becomes dominant because of the resulting acidification, which has probably caused E. multicaulis to disappear from many sites (Vanderhaeghe et al., 2005). It may well be that in the current experiment, ammonium was not detrimental because of higher pH levels (pH 5–6) compared to acidified situations which can occur in the field (pH < 4).

Baldellia species (Kozlowski and Matthies, 2009) and L. natans (Greulich and Bornette, 1999; Hyldgaard and Brix, 2011; Nielsen et al., 2006; Szmeja and Bazydlo, 2005) 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 and Klosowski (2001) in Poland (realized niche study), the fundamental niche of L. 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 B. ranunculoides ssp. repens suffered from desiccation in the emersed state (see further). Our findings for L. natans are in agreement with Keddy's model.

In a field experiment, evergreenness of L. natans was found to have a competitive advantage over summergreenness (Greulich and Bornette, 2003). A considerable degree of plasticity also conferred advantages to the species (Greulich et al., 2001; Hyldgaard and Brix, 2011; Szmeja et al., 2008). 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 B. ranunculoides ssp. repens is most ecologically similar to L. natans. Few predictions exist in literature regarding the expected response of this subspecies to abiotic and biotic conditions. Kozlowski et al. (2008) report that

Table 6
P values of subanalyses of the 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). Subanalyses were done for the endstage of the submerse and emerse phases.

Species	Endstage	Relative cover	Relative height	Number of flowers	Number of fruits
Baldellia ranunculoides ssp. *repens*	Submersed	0.002	0.198	–	–
	Emersed	0.223	0.968	–	–
Eleocharis multicaulis	Submersed	0.434	0.188	0.050	–
	Emersed	0.101	0.002	0.058	0.025
Species difference	Submersed	0.022	0.978	0.111	–
	Emersed	0.698	0.011	0.124	0.059
Baldellia ranunculoides ssp. *repens*	Both endstages compared	0.014	0.202	–	–
Eleocharis multicaulis	Both endstages compared	0.284	0.792	0.159	0.059

P values < 0.10 appear shaded.

both subspecies of *B. 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., *H. vulgaris*).

4.3. Water level change

Carbon is often a limiting nutrient in the water column of softwater lakes (Brouwer, 2001; Madsen et al., 2002; Smolders 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*) 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 well as a disturbance (Lacoul and Freedman, 2006; Thomaz et al., 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).

5. Conclusions

In our one-year experiment, hydrological state and intrinsic species properties were of greatest influence on plant performance. The expected influence of ammonium and carbon dioxide was not confirmed. One possible reason may be a mismatch in the characteristics of the environmental niche of a given species (e.g. ammonium tolerance at higher pH in *E. multicaulis*, and the dominant effect of the water level drawdown). Another reason is the inability to accurately predict individual species performances in a community because the effect of their trait constitution (their identity) is difficult to estimate due to the mixed life strategies and functional plasticity. Hence, water table fluctuations were the

dominant environmental factor in the artificial community. It dramatically changed species dominance, most likely through the effect on availability of aerial carbon dioxide to the plants, but also through dessication. The water level drawdown acted as a disturbance for at least one species. Competition intensity was greatest in the emersed state, which is in accordance with plant ecological theory. Because of the considerable flexibility of aquatic macrophytes to cope with different and changing environments, we suggest that more experimental work is needed on the population ecology of selected macrophyte species in order to achieve better community outcome predictions in real situations.

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