University of South Bohemia
Faculty of Science

The role of clonal plants in wetlands

PhD. thesis

Petr Macek

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Annotation

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This thesis is focused on clonal growth traits and their response to changing environment in wetlands. Specifically, plant responses to both abiotic (e.g. waterlogging, salinity) and biotic (surrounding vegetation) stressors were evaluated in field and mesocosm experiments. Trait responses to additions of contrasting concentrations of nutrients and their implications for wetland heterogeneity were studied.

Declaration – Prohlášení

I hereby declare that this PhD. thesis has been fully worked out by myself and the named co-authors, and with the use of the cited references.

I declare that in accordance wit the Czech legal code § 47b law No. 111/1998 in its valid version, I consent to the publication of my PhD. thesis (in an edition made by removing marked parts archived by the Faculty of Science) in an electronic way in the public access to the STAG database run by the University of South Bohemia in České Budějovice on its web pages.

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České Budějovice, 30th November 2008 Petr Macek

Author contribution statement

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I hereby certify that Petr Macek participated the project „Linking ecosystem processes and community structure along salinity and nutrient gradients in tropical marshes" carried out under University of California, Davis, USA. He has been involved in planning, conducting and evaluating the experiment. Further, I agree that Petr includes the paper "Wetland ecosystem changes after three years of phosphorus addition" published in Wetlands (2008, in press) as a part his PhD thesis.

In Třeboň, September 11, 2008
Eliška Rejmánková

To my biological parents — Věra, Eliška, Pavel and Jan

Contents

General introduction

WETLAND CHARACTERIZATION AND VALUE

Swamp, marsh, floodplain, wet meadow, peat bog, fen, tidal marsh, alluvium, lakeshore, flooded forest, prairie pothole etc. Each of these terms represents a special type of environment differing from others in, e.g., physical or chemical properties, vegetation structure or species composition. However, they all could be encapsulated into a single term: wetlands. They share several major features distinguishing them from both aquatic and terrestrial systems (Denny 1995; van der Valk 2006). Contrary to terrestrial systems, water table is elevated, at least temporarily, resulting in anaerobic soils in wetlands. A second feature, distinguishing them from aquatic systems, is a presence of macrophytes, large plants usually emerging from water surface. Wetlands often represent an ecotone or transitional zone between aquatic and terrestrial environment sharing fauna and flora of both systems.

Suitable conditions for plant growth rank some wetland types between the world most productive ecosystems (Armstrong et al. 1994; Roggeri 1999). Besides, large amounts of people, and animals, depend on wetlands as a source of food, and wetlands usually provide further socio-economic benefits to local communities (Silvius et al. 2000; Dixon and Wood 2003). Wetlands are considerable reservoirs of (drinking) water and in the view of ongoing climate change and increasing precipitation variability and irregularity, they can play a crucial role in water management, e.g., sedimentation processes or flood control (Dugan 2005). Wetland biodiversity is another extremely important value and various wetlands are considered as biodiversity hotspots (Rejmánková et al. 2004; van der Valk 2006). Wetland ecosystems play a crucial role in the nutrient cycling. In addition, they typically have a high retention capacity of nutrients, which, under certain circumstances, opens possibility to use some of them for water quality improvement (Hammer and Bastian 1989; Dugan 2005). On the other hand, increased land use and agricultural pressure result in eutrophication and salinization of many natural wetlands (Downing et al. 1999; Jolly et al. 2008).

CLONAL PLANTS IN WETLAND ECOSYSTEMS

In majority of above mentioned processes macrophytes play a significant, if not crucial, role. Since macrophytes are major living constituents of wetlands, it is important to study, among others, their responses to different factors affecting their growth in various wetland systems. Wetland plant growth is frequently constrained by several stressful factors such as prolonged anoxia, elevated salinity or lower nutrient availability frequent under natural conditions (Crawford and Brändle 1996; Noe et al. 2001). To deal with wetland stressors, plants employ various physiological, metabolic or structural adaptations (Brändle 1991; Jackson and Colmer 2005). Vegetative reproduction, i.e. clonal growth, is one of the structural traits helping plants in wetlands (Suzuki and Hutchings 1997; Vartapetian and Jackson 1997), especially if coupled with physiological adaptations (e.g. Lenssen et al. 2000). Some authors regard an increase in clonal modules as a general adaptive response to the stress of waterlogging (Soukupová 1994). Although the presented studies cover only few wetland types, the mechanisms underlying plant growth are most probably similar in other ones. It further fills a gap in our knowledge of species poor systems of clonal plants in highly productive habitats (Herben and Hara 1997).

All studies presented partially focus on different factors affecting plant growth in wetlands. This could be achieved in two dimensions: vertical via changes in plant height and horizontal via clonal spreading, plant vegetative reproduction. The ability to clonally spread is among the most important, crucial, for plant growth in wetlands. Because of large portion of wetland plant tissue consists of aerenchyma (porous tissue full of air space), the gas space continuum between shoots and roots is maintained (Armstrong et al. 1994). Such continuum can be functional in between ramets as well. For example, when flooded, emergent shoots can supply air to submerged shoots, or mother ramet can support (by air and/or nutrients) its vegetative offspring in growth (Allen 1997). The air supply can be functional even between dead broken and living shoots, e.g. venturi-induced pressure flow in *Phragmites australis* (Armstrong et al. 1992).

However, besides the above mentioned advantages of clonal growth for individual plants, the main outcomes of plant clonality in wetlands still require further studies. Herben and Hara (1997) point out the insufficient

attention paid to processes of spatial extension of plants, although they anticipate these processes to be of major importance for community structure. In addition, a spatial pattern of clonal plant communities is specifically affected by growth architecture and the way in which ramets interact and replace each other (Herben and Hara 1997). Studies of plasticity of clonal growth traits, physiological integration between ramets, foraging behaviour and vertical ramet competition form a backbone of this thesis because they represent the critical factors affecting spatial structure of plants.

Asking a general question, such as what is the role of clonal plants in wetlands, leads to more complex or large scale ecosystem studies. However, such questions usually cannot be directly solved without a more detailed knowledge of separate parts of the system. Therefore, I believe, a combination of studies of different levels resulting eventually in a multidimensional approach is extremely valuable for understanding processes at the ecosystem level. Hence, in this thesis, I focus on different mechanisms underlying plant functioning at an individual level, in interactions between plants and also in interactions between plants and animals.

AIMS AND OUTLINES OF THIS THESIS

I focused on different aspects of macrophyte ecology in two contrasting wetland systems: neotropical freshwater marshes of Belize and temperate wetlands (from wet meadows to fens) of Czech Republic. In this series of studies the first aim was to characterize plant ability to deal with stressful environment of seasonally flooded marshes and especially cover the extreme water fluctuations (Chapter 2). Later on, I examined the combined effects of salinity and nutrient (nitrogen and/or phosphorus) enrichment on growth of emergent macrophytes. This was studied in a mesocosm experiment with three different dominant species of Belizean wetlands, *Cladium jamaicense*, *Typha domingensis* and *Eleocharis cellulosa* s.l. (Chapter 3). In the two former studies, only the influence of abiotic factors was investigated. However, to estimate the relative importance of both biotic and abiotic factors, I differentiate them in a large field study of *Potentilla palustris* growth characteristics survey (Chapter 4). When changing the perspective from a small scale to a larger scale and estimating more

realistically marsh responses to various factors, large field experiments are needed. Results from such experiment are discussed in the next chapter allowing comparisons with earlier similar experiments under more controlled conditions (Chapter 5). Previous experiments imply that some plant growth traits are influenced by a combination of different factors. In following chapter I asked, whether a change in conditions and consequently in growth traits can also be reflected in species coexistence. Furthermore, I compared growth dynamics of populations with and without changes in clonal growth traits (Chapter 6). In the last chapter, I looked for the natural causes of vegetation pattern emergence. A combination of field experiments with animal behavioral studies resulted in multidimensional study overlapping from plant – plant interaction to larger ecological study including various trophic levels (Chapter 7). Such consecutive change of perspective enabled me to fully appreciate a phenomenon of plant clonality in wetland ecosystems (see also Herben and Hara 1997). Additional aim of this thesis was to demonstrate a wide range of methods and approaches which can be used for studying plant clonality in wetland ecosystems.

More specifically, in the studies presented I asked following questions:

1. How does *Eleocharis cellulosa* investment into vegetative growth change under conditions of prolonged submergence?
2. What are the effects of increased salinity and nutrients on clonal growth traits of three emergent macrophytes dominating Belizean wetlands?
3. Are there any differences in abiotic and biotic factors in terms of their effect on clonal growth traits? Which of them are better predictors?
4. What is the effect of elevated nutrients on marsh community structure formed mainly by clonal plants? Is it reasonable to expect only negative effect? In other words: could nutrient enrichment increase local heterogeneity and diversity?

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New growth of *Eleocharis cellulosa* almost seven months after a tropical storm Chantal (20th August 2001). This storm raised the water level in a large marsh system called Doubloon from 21 cm to more than 190 cm within four days. However, the opposite process of water level recession to the same level lasted until 11th March 2002, when this picture was taken.

The effect of long-term submergence on functional properties of *Eleocharis cellulosa* Torr.

Macek P., Rejmánková E. and Houdková K.

Aquatic Botany 84, 251–258, 2006

ABSTRACT

Eleocharis cellulosa Torr., a macrophyte dominating marshes of northern Belize, often experiences great water level fluctuations varying from dry conditions to prolonged submergence. We investigated morphological and ecophysiological responses (shoot length, biomass, CO_2 exchange, chlorophyll content and regeneration) to partial and complete submergence followed by emergence in two field experiments. Submergence greatly enhanced shoot elongation, but it also resulted in a low number of viable shoots, lower biomass and consequently in lower plant fitness. The decline in live shoot length started after three months of submergence. The shoots produced by submerged plants were thin and would break easily if the water level decreased fast. Photosynthetic activity, as well as respiration rate, was highly reduced in shoots just emerged from complete submergence. The ability of *E. cellulosa* to retain some level of photosynthesis after emergence is undoubtedly a useful trait in coping with seasonal floods. Submerged plants produced chlorophyll, especially *Chl a*, for at least a period of three months. Shoot regeneration was significantly slower in the case of plants submerged for a longer time, probably due to depleted energy reserves, but there were no significant differences in the total shoot length among treatments after two

months following the emergence. *Eleocharis cellulosa* demonstrated high tolerance to long term (more than 4 months) complete submergence and resulting anoxic conditions and showed rather fast recovery after emergence. This can be viewed as an advantageous trait in habitats of rapid and prolonged increases of water level and also after water recedes, when vegetation starts to colonize newly opened space.

Keywords
Chlorophyll - Flood tolerance - Growth response - Photosynthetic activity - Regeneration

INTRODUCTION

Water is essential for plant life; its surplus, however, may create stressful conditions that most plants are not able to tolerate. Seasonally flooded wetlands represent a habitat of the two extremes of water availability (Kirkman and Sharitz 1993; Santos and Esteves 2004). During high water levels, these wetlands become harsh environments where only plants able to adapt to low oxygen supply and to protect their tissues against phytotoxins can survive (Brändle et al. 1996). Marshes of northern Belize, where water level can increase rapidly by well over 1.5 m (pers. obs.) and then drop close to zero over a period of several months, represent an excellent system to study this phenomenon. We investigated the responses of the dominant emergent macrophyte, *Eleocharis cellulosa*, to submergence and consequent emergence.

To cope with water stress, plants developed various adaptive strategies of either avoidance in time or space or of tolerance by metabolic changes. The most common morphological responses to flooding are shoot elongation, either by cell growth or by cell division (Kende et al. 1998; Cooling et al. 2001), and formation of aerenchyma, in both roots and shoots (Justin and Armstrong 1987; Kende et al. 1998). Anoxic conditions may eventually result in the reduction of growth and total biomass (Mauchamp et al. 2001; Sorrell et al. 2002; Edwards et al. 2003). An important strategy is conservation of energy, e.g. death of older shoots and their replacement by new ones (Cooling et al. 2001) or a switch to anaerobic respiration (Armstrong et al. 1994). When submerged, plants usually produce thinner shoots or

leaves, which are more susceptible to mechanical failure (Sorrell et al. 2002; Edwards et al. 2003). For more details on plant adaptations to flooding see Pfister-Sieber and Brändle (1994), Blom and Voesenek (1996) and Crawford (2003).

Reemergence after flooding represents additional problems because oxygen radicals and acetaldehyde formed under the submergence start oxydative chain reactions leading to a lipidic membrane destruction and consequent death of plant tissues in a process called "post-anoxic injury" (Wollenweber-Ratzer and Crawford 1994; Crawford 1996).

The effect of elevated water level either on plant morphology (Grace 1989; Santos and Esteves 2002; Busch et al. 2004) or on community composition was investigated in a number of studies (McKee and Mendelssohn 1989; Weiher and Keddy 1995). However, studies investigating the response of typical emergent macrophytes to complete submergence are rather scarce (Crawford 1996). Furthermore, while tolerance to flooding is expected in the case of emergent macrophytes, there has not been any report of tolerance to long term complete submergence for *E. cellulosa* yet.

Field observations of *E. cellulosa* surviving prolonged submergence have raised several questions that we try to answer in this paper: 1) What are the morphological responses of *E. cellulosa* to flooding? 2) How long can *E. cellulosa* survive complete submergence? 3) What are the morphological and physiological responses of *E. cellulosa* to re-aeration after prolonged submergence? 4) How fast does it regenerate after emergence? Two consecutive field experiments were established to answer these questions.

We predicted that *E. cellulosa* will invest into shoot elongation rather than shoot number in an effort to reach the water table and maintain air contact. The survival of plants under water will probably be no longer than two months. The submergence will lead to production of weak shoots that will die after emergence. Photosynthetic activity will decrease under submergence, but will reach the pre-submergence values in regenerated shoots. We expected an increased amount of photosynthetic pigments in the shoots grown under the submergence. The overall regeneration of plants will decrease with the length of submergence. Shoot length and number were recorded as morphological responses, aboveground biomass was assessed as a growth response. Physiological responses were measured by CO_2

exchange and chlorophyll content. These characteristics also serve to illustrate plant fitness under submergence and after emergence.

MATERIAL AND METHODS

Study species

Eleocharis cellulosa is a perennial rhizomatous sedge usually from 30 to 80 cm tall. Its terete shoots are formed by one elongated green spike-like terminal internode and undeveloped leaves. The shoots have no septa and are filled with spongy aerenchyma. The species is distributed from the southern part of the USA, throughout Central America and the West Indies. It usually grows in fresh to brackish marshes forming large, often monospecific stands (Godfrey and Wooten 1979).

Study site

Both experiments were conducted in a small marsh located 20 km east of Orange Walk, northern Belize, CA. The location is a part of a larger complex of seasonally flooded wetlands on alluvial sand deposits covered by a moderate layer of peaty marl. The water level fluctuates according to precipitation and consequently the water salinity also varies (at the time of the experiments, the conductivity was ~600 μS cm^{-1}). The species poor communities are dominated by *Eleocharis cellulosa* and *E. interstincta* (Vahl) Roemer & J.A. Schultes and submerged *Utricularia* species. An important component of these marshes are species rich cyanobacterial communities dominated by *Leptolyngbya* spp. that form benthic and floating mats or periphyton on shoots of higher plants. Sediments at the study site are peaty marls with total N and P content of 7.87 mg g^{-1} and 0.18 mg g^{-1} respectively, indicating a strong P limitation. For a more detailed description of soil, hydrology, climate and vegetation of marshes of northern Belize see Rejmánková et al. (1996).

Experimental design and sampling

In the first experiment (April - July 2002) we transplanted 72 young ramets of *E. cellulosa* into pots filled with a soil mixture consisting of the equal parts of peat, marl and clay. Uniformly-looking plants consisting of two shoots were selected (shoot length 22.3 ± 5.5 cm; shoot number 2 ± 0.6 shoots).

The plants in pots were gradually submerged into three different water levels: Low (L), water at the soil surface; Medium (M), the water level 50 cm above soil surface; and High (H), the water level 90 cm above the soil surface. Each treatment was replicated 24 times. Twelve plants of each treatment were harvested 42 days after the start of the experiment, the remaining plants were harvested after 119 days of experiment. The water level had risen during the course of the experiment by ~25 cm. At both harvest days, we recorded the number and length of shoots of each plant. Then the aboveground biomass was harvested, dried at 60 °C and weighed. The number of newly produced shoots (plant regeneration) was also recorded two months after harvest.

In the second experiment (December 2002 - May 2003) we transplanted 140 *E. cellulosa* ramets (shoot length 16.8 ± 3.7 cm; shoot number 2.5 ± 0.5 shoots) into the pots filled with the same soil mixture. Plants were completely submerged. During the experiment, the water level was always kept above the experimental plants to avoid any shoot contact with the air. Plants were grown under complete submergence in anoxic soil for 46, 60, 73, 88, 101 and 130 days (treatment A, B, C, D, E and F respectively). Plants in the saturated soil served as control. The mean values of redox potential in the soil at the time of the emergence were: A 133 mV; B 188 mV; C 184 mV; D 202 mV; E –52 mV; F –65 mV.

We recorded shoot lengths and their conditions (estimated as dead and live shoot lengths), photosynthesis and respiration of shoots on each emergence day (Day 1 for each treatment) and then on Days 4, 14 and 28 after emergence, each time for 4–6 replicates of each treatment. On each of these days we also measured 2–3 control plants.

We used Infra Red Gas Analyzer (Qubit Systems Inc., Ontario, Canada) for measuring the photosynthetic and respiration activity (the exchange of CO_2 in light and dark conditions). At the beginning of each daily measurement, IRGA was calibrated on the air without CO_2, i.e. air pumped through a column containing soda lime. The leaf chamber size was 3 x 3 cm; the mean values ± S.E. of flow rate, irradiance and temperature were 3.533 ± 0.011 ml s^{-1}, 1337 ± 42 μmol m^{-2} s^{-1} and 37.5 ± 0.4 °C, respectively, during the experiment. A sub-apical shoot parts (~15 cm) of just emerged plants were cut into 3 cm segments placed into the horizontally oriented leaf chamber and the photosynthetic activity was recorded each 30 s for about 4 min. The photosynthetic measurements were done in about 5–10 minutes

after the plants were emerged from water. Subsequently the respiration data were recorded in the same way in the darkened chamber. Only data showing three or four stable consecutive measurements were used for the analyses. Sample shoot diameter and length were also measured and an approximate half-shoot area was calculated by their multiplication.

After the measurement, the shoot segments were kept refrigerated until analysis for chlorophyll *a* and *b* contents. For chlorophyll measurement, 0.1 g of plant tissue was extracted with 80% acetone and the absorbance was read at 645 and 663 nm at Shimadzu spectrophotometer. The chlorophyll concentrations were calculated using the following equations: *Chl a* = (12.7 x A663) – (2.69 x A645); *Chl b* = (22.9 x A645) – (4.68 x A663) (Lichtenthaler 1987).

The remaining aboveground biomass was removed after the measurements of the photosynthetic activity. The stage of plant regeneration (number and length of newly produced shoots) was recorded one month after the emergence date.

Data analyses

Analysis of variance was used for data analyses of both experiments. In the second experiment, two factors were differentiated: length of submergence (treatments A - F) and time after the plant emergence (Day 1, 4, 14 and 28). There were no obvious trends between photosynthetic and respiratory activities of the control plant during the experiment, therefore only treatment plants were included in the ANOVA. The statistical analyses were conducted for the first five treatments only (A - E), because the data set for treatment F (submerged for 130 days) was not complete due to low number of living plants on Day 14. Part of the samples for chlorophyll content analysis was lost during transport, thus we analyzed treatments A, B, C and D for Days 1, 4 and 14 and treatment E for Day 1 only.

RESULTS

Effect of submergence on shoot growth

In the first experiment, the elevated water level affected negatively the total shoot length of plants on both harvest days ($F > 8.46$; $P < 0.001$; Fig. 1A). The plants grown under low water level (L) invested more into

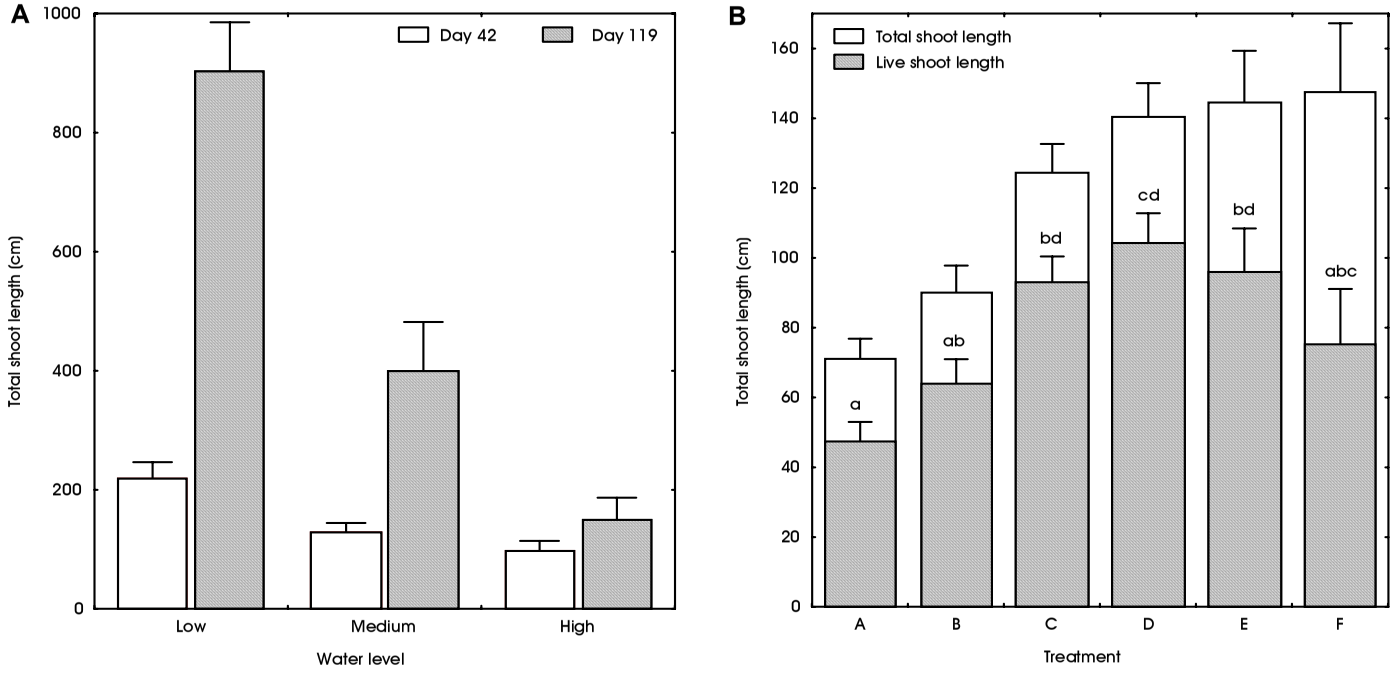

Figure 1

Shoot lengths of A: plants grown under various water levels in the first experiment and B: plants submerged for different time periods in the second experiment. A: the length was recorded at two harvest times, day 42 and day 119. Column, mean; Error bar, mean + S.E. B: both live and total shoot length were measured on the day of emergence. Treatments A, B, C, D, E and F were submerged for 46, 60, 73, 88, 101 and 130 days respectively. Different letters indicate a significant difference between treatments in live shoot length. Column, mean; Error bar, mean + S.E.

Table 1. Means ± S.E. of *E. cellulosa* shoot parameters in three water depths (L, M, H) at two harvest times (42 and 119 days). Figures in bolt are significant ($P < 0.01$). Same letters indicate no significant difference among the treatments.

	L 42	M 42	H 42	L 119	M 119	H 119
Total shoot length (cm)	**217±29^{a}**	**128±16^{b}**	**99±16^{b}**	**903±82^{a}**	**398±84^{b}**	**151±36^{c}**
Number of shoots	**12.2±2.2^{a}**	**3.2±0.3^{b}**	**2.6±0.3^{b}**	**18.9±1.6^{a}**	**6.5±1.2^{b}**	**2.9±0.4^{b}**
Number of daughter ramet	**1.67±0.4^{a}**	**0.00±0.0^{b}**	**0.00±0.0^{b}**	**2.58±0.4^{a}**	**0.33±0.1^{b}**	**0.00±0.0^{b}**
Sum of mother ramet shoots length (cm)	154±16	128±16	99±16	**256±38^{ab}**	**338±62^{a}**	**151±36^{b}**
Mean mother ramet shoot length (cm)	**24.5±1.3^{a}**	**41.1±2.6^{b}**	**34.1±4.3^{ab}**	45.0±4.7	60.6±4.7	47.1±9.5
Dry biomass per plant (g)	**0.91±0.2^{a}**	**0.33±0.0^{b}**	**0.26±0.1^{b}**	**4.26±0.5^{a}**	**1.73±0.5^{b}**	**0.31±0.1^{b}**
Shoot length /dry biomass (cm g^{-1})	278±29	422±39	491±109	**227±19^{ab}**	**452±72^{a}**	**663±102^{b}**
Survival (1/0)	0.50±0.2	0.33±0.1	0.17±0.1	0.75±0.1	0.33±0.1	0.33±0.1

production of new shoots ($F > 16.85$; $P < 0.001$) and their total shoot length was higher compared to either of the treatments with the elevated water level (M and H). The effect was even more pronounced in the case of plants harvested after 119 days because of a higher production of daughter ramets in treatment L ($F = 3.33$; $P = 0.048$). The plants of treatment L invested more resources not only to vegetative, but also to generative reproduction, as documented by higher number of flowering shoots ($F = 17.76$; $P < 0.001$). Considering mother ramets only, there was no significant difference in mother ramet shoot mean lengths among the treatments on the second harvest day ($F = 1.60$; $P = 0.217$). The plant shoots of treatment L were thicker and self-supporting, while the shoots of treatment H were thin and long with a tendency to break down without the water support after emergence. This was well described by differences in shoot length to biomass ratio (cm g^{-1}) that increased with the water level ($F = 8.94$; $P < 0.001$). The mean values of plant characteristics obtained in the first experiment are listed in Table 1.

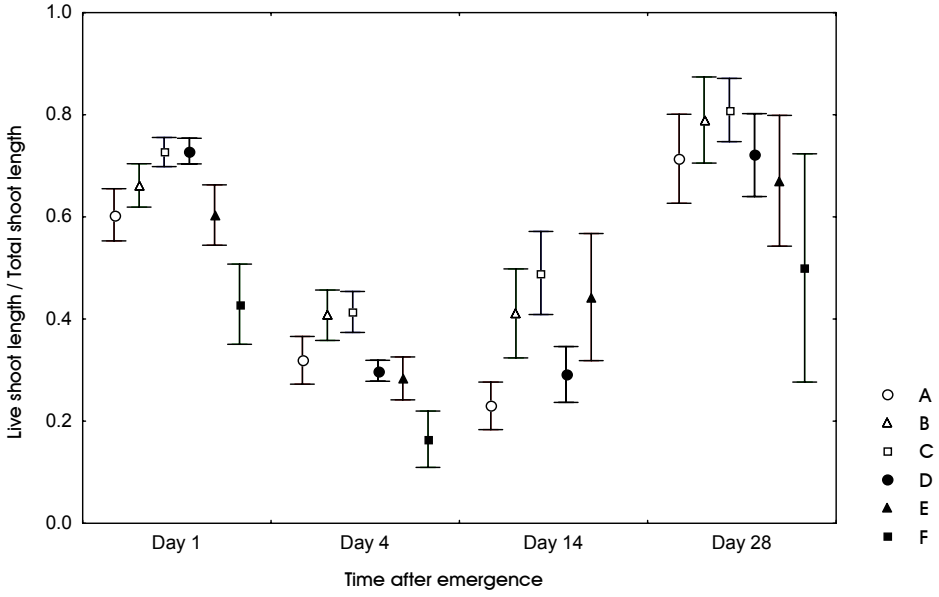

Figure 2

The ratio of live shoot length to total shoot length on the day of emergence (Day 1) and three successive measurements on Day 4, Day 14 and Day 28. Treatments A, B, C, D, E and F were submerged for 46, 60, 73, 88, 101 and 130 days, respectively. Symbols represent the means of recorded values. Error bars, mean ± S.E.

The impact of the complete submergence was explored in the second experiment. The duration of the complete plant submergence affected the ratio of live to total shoot length immediately after emergence ($F = 3.96$; $P = 0.002$). The total shoot length increased with the duration of plant submergence. In contrast, the live shoot length increased until the 3rd month of submergence only (treatment D), but then began to decrease (Fig. 1B). The number of shoots produced under submergence differed with submergence duration ($F = 2.41$; $P = 0.040$). The plants submerged for a longer time produced more shoots. The means ± S.E. for treatments A, B, C, D, E and F were 1.9 ± 0.2, 2.3 ± 0.2, 2.6 ± 0.2, 2.6 ± 0.1, 2.6 ± 0.3 and 2.0 ± 0.4, respectively. After the emergence, the shoots were continuously dying and usually were replaced by newly produced shoots (Fig. 2). The ratio of live to total shoot length differed among treatments ($F = 3.05$; $P = 0.017$), but the interaction treatment x time was not significant ($F = 1.03$; $P = 0.423$; Fig. 2). The younger one of the two planted shoots elongated faster during

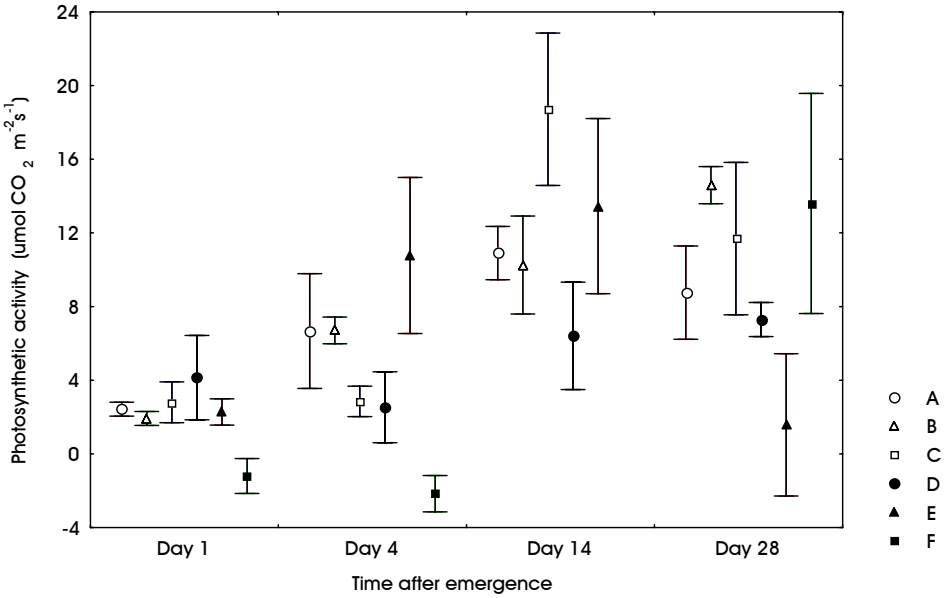

Figure 3

The mean values of photosynthetic activity of *E. cellulosa* following the emergence (Days 1–28). Treatments A, B, C, D, E and F were submerged for 46, 60, 73, 88, 101 and 130 days respectively. Error bars, mean ± S.E.

submergence than the more developed older shoots in all the treatments ($F = 4.06$; $P < 0.001$).

Photosynthesis and respiration

The photosynthetic activity of plants immediately after the emergence was not significantly affected by the submergence duration ($F = 1.97$; $P = 0.108$). The photosynthesis was however very low, the mean C uptake was about 2.1 μmol CO_2 m^{-2} s^{-1} only. During the days following emergence, the photosynthetic activity gradually increased reaching the mean value of 9.7 μmol CO_2 m^{-2} s^{-1} one month after plant emergence. This effect of time was highly significant ($F = 14.30$; $P < 0.001$) and the interaction treatment x time was also significant ($F = 2.39$; $P = 0.010$; Fig. 3). The respiration followed the similar, gradually increasing trend in time in all treatments (treatment x time: $F = 2.26$; $P = 0.016$; data not shown). The mean ± S.E. values of the photosynthesis and respiration for 37 control plants were 9.7 ± 0.9 and 13.3 ± 0.8, respectively.

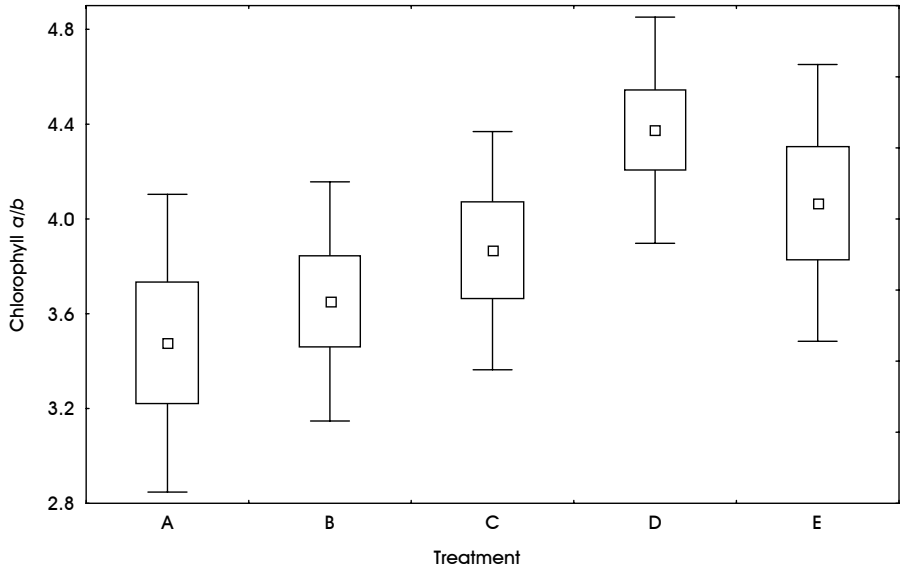

Figure 4

Chlorophyll *a*/*b* ratio for plants of different treatments on the day of emergence (Day 1). Treatments A, B, C, D and E were submerged for 46, 60, 73, 88 and 101 days, respectively. Symbol, mean; Box, mean ± S.E.; Error bar, mean ± S.D.

Chlorophyll content

Both chlorophyll *a* and *b* did not differ significantly among treatments after emergence ($F < 1.47$; $P > 0.2$), nor did the sum of chlorophyll *a* and *b*. The ratio of chlorophyll *a* to chlorophyll *b* at the time of emergence differed among the treatments ($F = 3.02$; $P = 0.034$); the ratio increased with the duration of submergence (Fig. 4). Table 2 shows the mean values of chlorophyll *a* and *b* content in shoots of different treatments at four times after emergence. There was a positive correlation between chlorophyll *a* content and photosynthetic activity of the plants just emerged (for all treatments together, $R^2 = 0.47$; $P < 0.001$; Fig. 5).

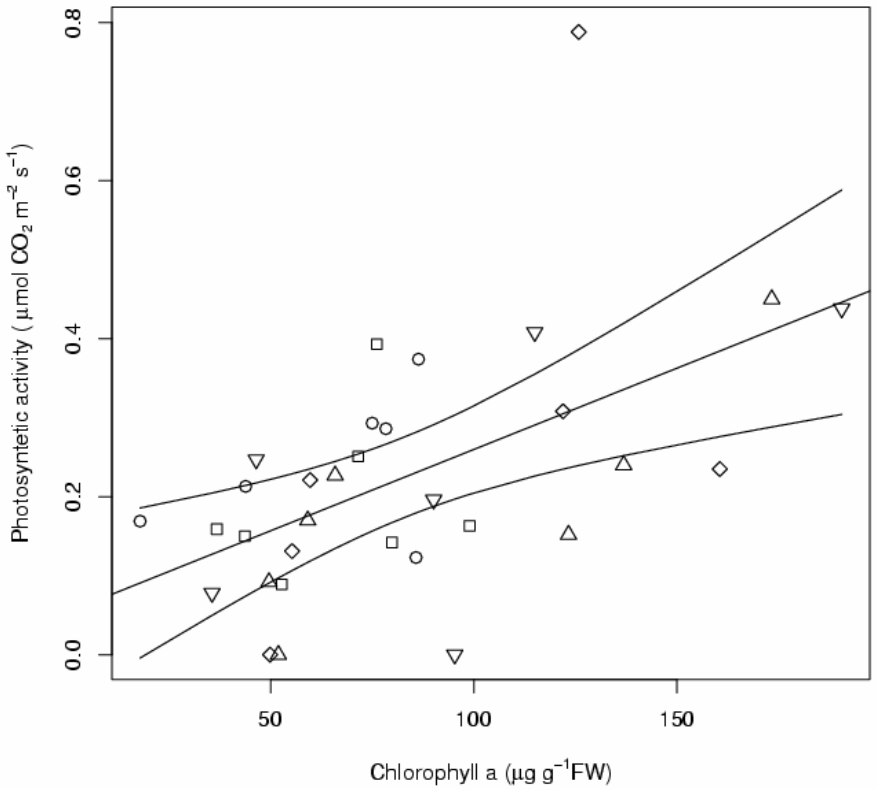

Figure 5

The regression model (with 95% confidence band) relating the chlorophyll *a* content (μg g^{-1} FW) on the photosynthetic activity of *Eleocharis cellulosa* (μmol CO_2 m^{-2} s^{-1}) on the day of emergence (Day 1). Circle, square, diamond, triangle up and triangle down symbols correspond to treatments A, B, C, D and E, respectively.

Table 2
The mean (± S.E.) chlorophyll *a* and *b* content in *Eleocharis cellulosa* shoots emerged at different times (treatments A, B, C, D and E submerged for 46, 60, 73, 88, and 101 days respectively) and from different times after emergence (Day1–28). For further explanation see text.

Day	Treatment	Chlorophyll *a* (μg g^{-1} FW)	Chlorophyll *b* (μg g^{-1} FW)
1	A	64.5 ± 11	19.4 ± 4
1	B	65.6 ± 8	17.6 ± 2
1	C	95.5 ± 19	24.3 ± 5
1	D	111.7 ± 24	25.3 ± 5
1	E	95.5 ± 23	23.9 ± 6
4	A	79.6 ± 24	22.2 ± 6
4	B	97.9 ± 16	27.7 ± 5
4	C	99.1 ± 13	28.8 ± 4
4	D	87.7 ± 5	24.3 ± 3
14	A	108.9 ± 25	27.9 ± 6
14	B	107.7 ± 12	26.9 ± 3
14	C	88.8 ± 19	16.6 ± 4
14	D	85.0 ± 8	19.3 ± 3
28	A	87.6 ± 22	21.4 ± 5
28	B	116.3 ± 22	25.7 ± 7

Regeneration after submergence

In the first experiment, the variability in water level had no effect on plant regeneration two months after each harvest day ($F < 0.84$; $P > 0.44$). The effect of experiment duration on plant regeneration was not significant either ($F = 0.50$; $P = 0.80$). A different situation was found in the second experiment, where the regeneration was the lowest for plants submerged for

the longest time ($F = 3.12$; $P = 0.012$; Fig. 6). Best regeneration, in terms of shoot length was found for plants submerged for 73 and 88 days. This trend was similar also for the shoot number ($F = 3.47$; $P = 0.006$).

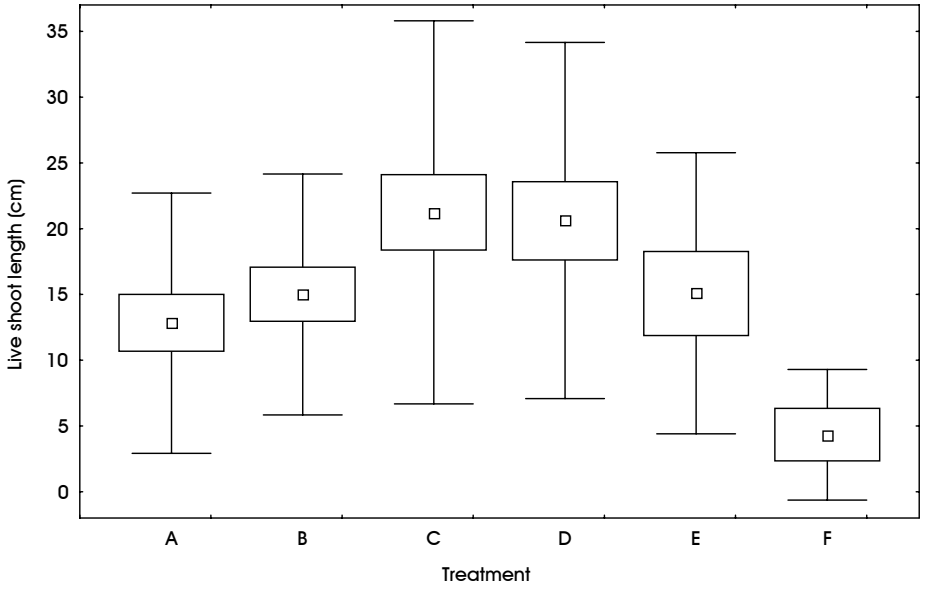

Figure 6
The regeneration of plants one month after the emergence. Treatments A, B, C, D, E and F were submerged for 46, 60, 73, 88, 101 and 130 days, respectively. Symbol, mean; Box, mean ± S.E.; Error bar, mean ± S.D.

DISCUSSION

Shoot elongation is the usual and well documented plant response to flooding (Van der Sman et al. 1993; Coops et al. 1996; Voesenek et al. 1996; Blanch et al. 1999), although some authors suggest no response in shoot length (Kirkman and Sharitz 1993) or even a negative growth response (Setter et al. 1989). We documented increased shoot elongation for *E. cellulosa* in both experiments. Some authors (Van der Sman et al. 1993; Cooling et al. 2001) have linked the ability to elongate with the ontogenic stage of a plant and consequently with energy conservation. Older leaves are usually less responsive to the rapid water rise than younger leaves and this results in higher mortality of older leaves (Mauchamp et al. 2001). The response of

E. cellulosa in our experiment was similar. Of the two shoots at the beginning of the experiment, the younger one elongated more, while the older one remained viable, but did not grow too much under submergence. It seems that the shoot morphological constitution is determined at an early growth stage and switching to another growth pattern at a later stage is more difficult.

While the increase in shoot length, as a response to rising water level, has been widely documented, the gradient of water depth in many of these studies was relatively small (see Lentz 1998; Sorrell et al. 2002). In other experiments investigating the growth response of *E. cellulosa* to varying water levels (Edwards et al. 2003; Busch et al. 2004), the extreme water level were not included. This resulted in the conclusion that "*E. cellulosa* performed best under flooded conditions" (Busch et al. 2004), which we do not regard as specific enough. In our experiments, we found *E. cellulosa* growing best in deeper water (73 cm) considering the total length of mother ramet and stolon number, but in terms of total aboveground biomass, it performed better in rather shallower water of about 40 cm deep. This is caused by the higher vegetative reproduction in shallower water rather than the performance of mother ramet. A lower production of ramets in flooded conditions was reported for many species (Hultgren 1988; 1989; Grace 1989; Vretare et al. 2001). When submerged, *E. cellulosa* suppresses not only vegetative reproduction, but also generative reproduction, similar to other species (Grace 1989; Kirkman and Sharitz 1993; Van der Sman et al. 1993). There is a strong trade-off between investing into reproduction and shoot elongation, which is determined by water level.

Even within the genus *Eleocharis*, there is a variation in biomass production under submergence. Both *E. sphacelata* and *E. interstincta* respond to elevated water level by increasing their biomass (Sorrell et al. 2002; Santos and Esteves 2004), while *E. cellulosa*, consistently with our results, was found to reduce its biomass while flooded (Edwards et al. 2003). Busch et al. (2004) reported an increase in biomass of *E. cellulosa* as a response to flooding, but since their maximum flooding depth was 40 cm only, it does not contradict our results. The differences in optimal flooding depth are given by the differences in shoot morphology within genus *Eleocharis*. For example, *E. sphacelata* changes its morphology under flooded conditions by reducing its pith cavity diameter and increasing the basal diameter, thus making the

basal part of the shoot stronger (Sorrell et al. 2002). Data from various studies on response of *E. cellulosa* to water level changes define a growth optimum for this species to be at water depth of around 50 cm, when the shoots are tall enough and both vegetative and generative reproduction are not yet limited.

The effect of submergence is greatly accented when the submergence is of a long duration. Under such conditions, the plants usually have only one long thin shoot aiming to reach the air (see also Van der Sman et al. 1993; Kende et al. 1998). Contrary to *E. sphacelata*, *E. cellulosa* lacks the structural support in the basal region and cell walls, which results in shoot collapse when water level falls (Crawford 1996; Edwards et al. 2003). Fortunately, under natural conditions, water level recedes rather slowly, which allows bending of the shoot on the water surface and staying alive until collapse occurs.

Emergent macrophytes, contrary to aquatic species, usually lack the ability to sustain photosynthetic activity when completely submerged in water. This is, among others, caused by their inability to utilize CO_2 in the form of bicarbonate (Sand-Jensen et al. 1992; Edwards et al. 2003). Nevertheless, some limited gas exchange between plants and water is documented, e.g. for *Eleocharis sphacelata* or *Phragmites australis* (Sorrell and Tanner 2000; Mauchamp et al. 2001). Furthermore, submerged tissues can also produce O_2 by recycling the internal CO_2 (Sorrell and Tanner 2000). We documented renewal of photosynthetic and respiratory gas exchange of *E. cellulosa* shoots after prolonged submergence and we also documented an increase in absolute photosynthetic activity during two weeks after emergence for shoots submerged for a shorter time (until 101 days). This trait is essential for *E. cellulosa* regeneration as the exhausted plants can restore their energy reserves to some extent and thus assure the growth of new shoots. Also other species, e.g. *Phragmites australis*, were reported to recover their photosynthetic activity after submergence, but their leaves were not viable for a longer period after emergence (Mauchamp et al. 2001). After emergence, the photosynthesis gradually reached the pre-submergence values, which were up to five times higher than the values just after emergence (see Mauchamp et al. 2001). Our values, recorded 2 weeks and 1 month after emergence, were comparable to the values of control plants. It seems, that

the main problem for *E. cellulosa* shoot survival after re-aeration lies in their fragility as discussed above.

Mauchamp et al. (2001) reported subjectively observed an increase in photosynthetic pigments (greener shoots) of submerged leaves of *Phragmites australis*. We did not find any difference in the chlorophyll content among the treatments. There are two probable causes of non significant response of chlorophyll content in our study: 1. low number of replicates and 2. scatter in data because we analyzed even the shoots that possibly experienced some pigment degradation, i.e. they were slowly senescing. The second possibility is also supported by the significant positive correlation between chlorophyll content and the photosynthetic activity in newly emerged plants indicating decreased viability of some shoots. Nevertheless, the highest chlorophyll *a*+*b* contents were found in individuals submerged for a longer time (101, 73 and 88 days, in descending order) and they were all absolutely higher than those of control plants. When we compared three mean values of control plants with the three maximal values in each treatment (A-E), we obtained mostly significant differences (data not shown). We did not expect the chlorophyll production to be limited by nitrogen availability (Lippert et al. 2001) because the N concentrations in water at our study site are usually relatively high contrary to those of P, which is a limiting nutrient (Rejmánková et al. 2004).

Drought or water logging affects the chlorophyll *a*/*b* ratio in plants (Busch 2001). For *E. cellulosa*, this ratio increased with the duration of submergence. It was caused mainly by a faster increase of chlorophyll *a* content during the submergence. Our values for treatment A (46 days) are more close to the ratios reported by Busch (2001) for numerous *Carex* species, while at the later date (88 days) these values became much higher. It seems that a short to medium duration of submergence supports the chlorophyll synthesis and the production of ATP is concentrated into the Photosystem I, while Light Harvesting Center II is slowly degrading (Šantrůček, pers. comm.; Lippert et al. 2001). The extreme submergence seems to affect the reaction centers resulting in protein degradation, but this conclusion is rather speculative, because this likely trend is not supported by further data.

Flood tolerant species usually establish highly conservative resource consumption under submergence (Schlüter et al. 1996). The death of too expensive shoots is also a part of this energy conservation (Cooling et al.

2001). A successful strategy of some wetland species is to replace the expensive shoots, rather than to sustain them under water. Such species usually have rapid shoot recruitment (Rea and Ganf 1994; Cooling et al. 2001). *Eleocharis cellulosa* retain only one or two living shoots under water, while the others eventually die. Supporting of these shoots for an extremely long time period (more than 4 months) resulted in reserves exhaustion and lower regeneration rate. However, a reduced number of plants was able to regenerate. Information on plant regeneration is, according to Crawford (1996), more useful than the time when a flood sensitive organ dies. The plant ability to survive for long periods under extremely high water is however related to the storage organs able to survive and to regenerate new shoots and roots after the water level decreases (Crawford 1996). Therefore, in mature and healthy natural stands of *E. cellulosa*, survival and regeneration rate can be even higher than our observation, because we used only young individuals without substantial energy reserves.

It is evident, that *E. cellulosa* showed extremely high flood tolerance with the capacity to regenerate after 130 days under submergence. We conclude that the morphological response to complete submergence, i.e. structural support of *E. cellulosa* shoots, is more important for plant survival and regeneration than shoot functional changes. The conservation of energy is, most probably, the key to the flooding tolerance of this species. These features give *E. cellulosa* an advantage in competition for space with more productive but less tolerant wetland species.

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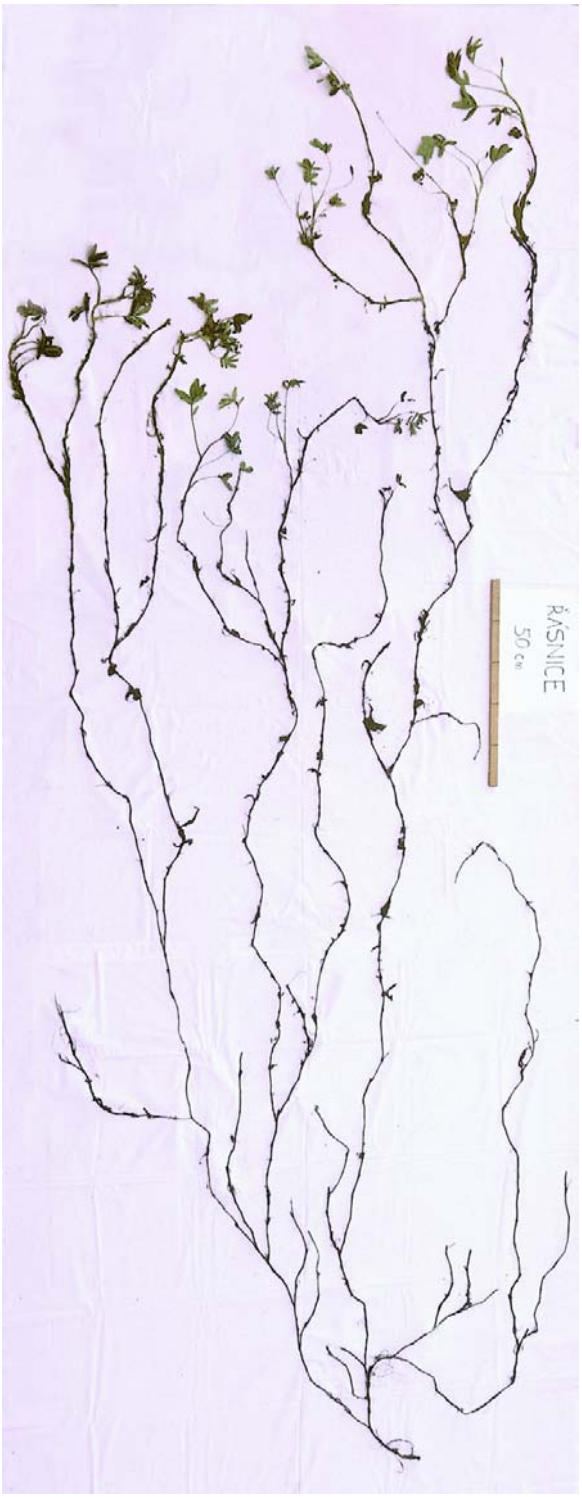

A part of a large clone of *Potentilla palustris* excavated at location Řásnice in July 2003. The clone is not complete, of course, due to unfortunate lost of its previous parts during digging. However, the stolons were all in rather good shape with no obvious mark of decay. The orientation of rhizomes does not correspond to the original position in the field.

Environmental correlates of growth traits of the stoloniferous plant *Potentilla palustris*

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Evolutionary Ecology, 22, 419-435, 2008

ABSTRACT

Growth form is one of the important life history traits ultimately influencing plant fitness. *Potentilla palustris* is a stoloniferous plant growing in a range of habitats from densely vegetated wet meadows to acidic transitional fens, and its growth form varies according to habitat. In a four year multi-site comparative study, we investigated which biotic and abiotic characteristics influence most its growth traits. Vegetation composition and physiognomy, as well as numerous abiotic environmental variables, were recorded at 32 study sites located on an altitudinal gradient. Growth traits of *P. palustris* were best explained by the surrounding vegetation physiognomy and not by abiotic conditions, although the latter obviously represents the factors indirectly influencing its growth. Stolon length traits and branching were positively correlated with vegetation density and height, and negatively with altitude. Plants flowered more in taller vegetation, and leaf area was greater in wetter sites with lower vegetation cover. *Potentilla palustris* appeared to be well adapted to transitional fens, but its vegetative growth was fastest in wet meadows and alluvial habitats on highly organic humid soils. It produced more branches and larger leaves in alluvial habitats with open water, while it had enhanced generative reproduction in wet meadows.

Species composition was less important than vegetation physiognomy. In less favorable habitat types, *P. palustris* prefers an escape strategy of linear growth. Internode length exhibited pronounced plasticity, increasing particularly in tall dense vegetation of lower altitude, whereas internode number remained fairly constant over various habitats. It is evident that both plastic low cost growth traits (internode elongation), and constant high cost traits (internode number) contribute to the *P. palustris* escape strategy under tall dense vegetation. Phenotypic plasticity enhances the potential of *P. palustris* to grow in a wide range of habitats and so increases plant fitness on regional scale.

Keywords
Constrained ordinations - Internode length - Multifactorial multisite study - Partitioning direct-indirect environmental effects - Plastic and constant growth traits - Vegetation composition

INTRODUCTION

Plant growth form is one of the most important life history traits (Fischer et al. 2004). It has important consequences for the survival and reproductive output of clonal plants (i.e. fitness; van Kleunen et al. 2005). Clonal plant fitness is influenced by success in sexual reproduction, clone spreading via branching and clone persistence via horizontal growth (Watson et al. 1997; Winkler and Fischer 1999). As resources are rarely abundant, there is a trade-off between their allocation to sexual or vegetative reproduction (Reekie and Bazzaz 1987; Huber 1995). In many clonal plant species, however, seedling recruitment is observed only when establishing a population, often after some disturbance (Carlsson and Callaghan 1990; Eriksson 1993), while vegetative reproduction and growth are more important later on for success over other species.

Both clone persistence and spreading largely depend on growth architecture, i.e. on plant ontogenetic stage, structural blueprint and phenotypic plasticity. Phenotypic plasticity is a property of the individual meristem triggered by local environmental conditions (de Kroon et al. 2005). While the structural blueprint limits the degree to which a species can respond to environmental conditions, phenotypic plasticity allows a plant to adjust its

form and function to the actual environment (Huber et al. 1999). Phenotypic plasticity is, hence, operating within the architectural limits set by the structural blueprint. Numerous plant organs or growth traits exhibit phenotypic plasticity, e.g. branching, flowering, internode or petiole length (D'Hertefeldt and Jónsdóttir 1994; Huber 1995).

Among the factors influencing plant growth pattern, apical dominance regulates branching. Lateral buds remain often inactive, until some factor prevents apical meristem growth (Phillips 1975). Internal factors include e.g. branch formation after flowering (Svensson and Callaghan, 1988). External factors include both abiotic ones, e.g. low temperatures, seasonal flooding, or biotic ones, e.g. herbivory (Salemaa et al. 1999; Huhta et al. 2000). Strong apical dominance is frequently observed in dense monocultures (Eriksson 1993; Kenkel 1995). Linear growth seems to be an adaptation to crowded conditions or resource poor habitats, i.e. unfavorable environments from which plants tend to escape (Eriksson 1986; Routledge 1990; Salemaa and Sievanen 2002). Favorable light or nutrient conditions, on the other hand, might also enhance branch formation (Huber et al. 1999; Salemaa and Sievanen 2002; Sammul et al. 2003).

It is not clear whether plasticity in internode length may enhance accumulation of plant resource acquiring structures in resource rich sites and therefore be considered as a foraging response. While some studies support the theory that internode shortening potentially results in concentration of resource acquiring structures in rich patches (Oborny 1994), others suggest this behavior to be rather exceptional and insignificant among the plants (Sutherland and Stillman 1988; Cain 1994; de Kroon and Hutchings 1995). Internode elongation can be presented as a foraging response to locally disadvantageous conditions. Nevertheless, successful ramet placing and its remaining in a suitable patch rely on the number and size of favorable patches and their spatio-temporal variability (Oborny and Cain 1997; Piqueras et al. 1999; Kun and Oborny 2003).

There is still a relative scarcity of studies concerning spatial patterns of plant growth. Furthermore, only a few studies investigated the influence of multiple factors on plant growth; experimental studies of a single or a few factors effect are more exact, but also more narrow and simplify vegetation interactions, i.e. they are less realistic. Alternatively, spatial growth

characteristics of a species can be studied in a comparative manner in a set of contrasting natural habitats.

Potentilla palustris (L.) Scop. is found in a variety of wet habitats; in some habitat types, it grows individually, intermingled with other plants, whereas in others it forms large almost monospecific stands. These habitat types can be found geographically quite close to each other. In this study, sites with a high abundance of *P. palustris* were chosen to analyze the species growth and spatial patterns across several environmental gradients, including both abiotic (e.g. pH, water depth or nutrient availability) and biotic variables.

In large scale field comparative studies, the studied characteristics (growth traits in this case) are affected through a rather complicated causal chain of direct and indirect effects. Site abiotic characteristics are probably the primary causal agent; they affect not only the target species, but also the surrounding vegetation, which might exert a much more direct effect on the target species. We expect that the vegetation physiognomy should be a better predictor of target species growth traits than the species composition of the vegetation. One of our aims was, thus, to disentangle the direct and indirect effects of abiotic environments, and of the vegetation; the latter one we distinguish into physiognomy and species composition. More specifically, we asked which of the growth traits are plastic (i.e. change according to the measured environmental characteristics) and which are not and which are the environmental correlates of the plastic traits. Specifically, we wanted to investigate the species plasticity in stolon length. This plasticity is co-determined by variation in internode length and internode number, and we aimed to determine which of these two components was more responsive to environmental variation.

MATERIAL AND METHODS

Study species

Potentilla palustris is a creeping plant with long lignifying stolons, which often forms large monospecific stands with a dense overlaying stolon system. The stolon length can increase by as much as 10 mm day^{-1}, although it is generally less (pers. obs.). Plants grow sympodially with a terminal inflorescence. If not flowering, the annual increments are separated

by short internodes and reduced leaves. The oldest parts of the stolon decay, which results in clone splitting and subsequent total independence of ramets (Irmisch 1861). *P. palustris* has a boreal circumpolar distribution. Its stolons and achenes are found as macrofossils in soil cores of peatlands and shallow lakes from the whole postglacial period (Lavoie and Payette 1995; Saarinen 1996). Water dispersed achenes are a component of a persistent soil seed bank in alluvial meadows (Jutila 2002). The species plays an important role in early successional stages in open water wetlands where it can form floating vegetation (Pietsch 1991; Jasinski et al. 1998). In Central Europe, it occurs in different vegetation types: peat bogs, fens, wet meadows, river alluvia, and water body edges. *P. palustris* is mainly associated with vegetation of Scheuchzerio-Caricetea fuscae and Phragmiti-Magnocaricetea (Soják 1995).

Study site characteristics

A large area of the Šumava Mts. (southwestern part of the Czech Republic), with altitude ranging from 725 to1240 m a.s.l. was selected for this study: a belt about 72 km long and no more than 10 km wide between 48°40'N, 13°20'E and 49°08'N, 14°03'E. Within this area, 32 locations with sufficiently large populations of *P. palustris* (more than 25 m^2 of homogeneous growth) were selected to evenly cover the area on an altitudinal gradient and include a variety of edaphic and hydrological conditions. A vegetation survey using 5 x 5 m relevés (relevé is a quantified vascular species list in a plot) of the 32 locations with *P. palustris* was conducted in June 2000.

A number of abiotic variables and vegetation characteristics were recorded at each location in June 2002 (see Table 1a for the complete list): water level and soil water pH; two soil cores in the root layer (upper 10 cm) were analyzed for soil water and organic matter contents (loss on ignition, LOI).

Soil samples were then analyzed for content of available phosphorus (PO_4^{3-}) by water extraction (Olesen and Sommers 1982) and available nitrogen by KCl extraction (2M KCl, extractant:soil, 2:1, v/w, 1 hour and then filtered through 0,45 μm glass fiber filter and analyzed for NO_3^- and NH_4^+ by flow injection analyzer [Foss Tecator 5042]). Soil samples were analyzed for total N and C content on a Carlo-Erba series 5000 CHNS analyzer. Total P was measured spectrophotometrically after combustion and consequent acid digestion (McNamara and Hill 2000).

Vegetation height was measured, and biomass was collected from three randomly selected 0.3 x 0.3 m subplots, sorted to *P. palustris* and other vascular plants, dried to a constant weight at 60 °C and weighed. Light penetration was determined as a ratio of light intensity inside the vegetation (5 cm above the soil) to total light intensity above the vegetation using Volcraft LX-1108 luxmeter device (as this variable characterizes the intensity of light competition, it is considered a vegetation characteristic in the analyses). The range and median values of the recorded characteristics are given in Table 1a.

Table 1
(a) Recorded explanatory variables at the 32 sampling locations, A denotes abiotic variables and B denotes vegetation physiognomy variables. (b) Growth traits and nutrient contents of *P. palustris*. Recorded variable values (range, median) are listed. Traits labeled by 1 are one year records only, others are sum of all 4 years growth.

		Explanation	Unit	Min	Median	Max
a)	Altitude [A]		m a.s.l.	725	788	1240
	Available N [A]	KCl extraction	μg cm^{-3}	1.78	7.99	16.24
	Available P [A]	Water extraction (Olesen and Sommers 1982)	μg cm^{-3}	0.01	0.19	1.4
	H_2O [A]	Soil H_2O content	%	63	90	95
	LOI [A]	Loss on ignition	%	16	80	94
	pH [A]	Soil water pH	-	3.5	4.9	6.1
	Soil N/P [A]	Total N to total P ratio	-	3.3	10.3	29.9
	Total N [A]	Total soil N content	ppm	4968	15089	25317
	Total P [A]	Total soil P content	ppm	492	1477	3953
	WD [A]	Water level in June 2002 (soil surface=0 cm)	cm	-50	-1	40
	Biomass [B]	Biomass of vegetation (excl. *P. palustris*)	g m^{-2}	58	152	713
	Light penetration [B]	Light intensity 5 cm above soil / light intensity above vegetation	%	1.4	13.1	58.2
	Sphagnum cover [B]	Cover of *Sphagnum* species	%	0	23	95
	Vegetation cover [B]	Cover of vascular plants (excl. *P. palustris*)	%	15	37	85

		Explanation	Unit	Min	Median	Max
a)	Vegetation height [B]	Average vegetation height	cm	30	48	90
b)	Branch p	Proportion of growing stolons	%	5	44	74
	Branches	Total number of produced branches	#	0.7	2.8	10.7
	Dead p	Proportion of dead stolons	%	0	31	64
	Flow p	Proportion of flowering stolons	%	0	3	23
	Flowers	Number of flowering stolons	#	0	0.1	1.1
	Herb_L p	Proportion of herbivore grazed leaves	%	0	0	25
	Herb_S p	Proportion of grazed stolons	%	0	17	48
	Increment[1]	Increase of total plant length	mm	0	163	612
	Internode #	Number of internodes on main stolon	#	11.8	23.2	29.7
	Leaf area[1]	Leaf area of an average leaf	cm^2	7.3	20.8	61.9
	Leaves[1]	Number of leaves on main stolon	#	0.5	2.5	8.3
	Main st. LA[1]	Leaf area of all leaves on main stolon	cm^2	8.1	45.1	236.4
	Main st. length	Distance of the main stolon tip from the tag (tagged 2001)	mm	169	420	747
	Max. int. length	Maximal internode length on main stolon	mm	18	44	76
	Mean int	Mean internode length on main stolon	mm	8.4	16.9	26.3
	Tot length	Total plant length from the tag (including all branches)	mm	189	608	1650
	Plant C[1]	C content in *P. palustris* leaves	ppm	442890	451265	486184
	Plant N[1]	N content in *P. palustris* leaves	ppm	14626	19625	31380
	Plant P[1]	P content in *P. palustris* leaves	ppm	1706	2935	5114
	Ash[1]	Ash content in *P. palustris* leaves	%	4.8	6.2	8.7

Growth traits

Fourteen randomly selected, apparently unconnected ramets of *P. palustris* were tagged 5 cm from the stolon tip at each location in May 2001 and their growth monitored over four seasons (2001–2004). Leaf area of *P. palustris* (6 per location) was measured using a portable leaf area meter (LI-3000A). Leaves were then analyzed for C, N and P contents in the same

way as the soil samples. Whole sequences of internode lengths, flowering and branching incidence were recorded on a main stolon and all branches yearly (June and September). Therefore, some traits are the sum of four years growth (e.g. total length), while others are only for separate years (e.g. increment; Table 1b). The increment can be even null due to heavy grazing of shoots at some locations. The status of each branch (alive, flowering, grazed or dead) and each leaf (alive or grazed) was distinguished. See Table 1b for the range and median values of the plant growth traits.

Data analyses

To differentiate the main vegetation types in which *P. palustris* grows, all available relevés (n = 1229; 690 species) with *P. palustris* from the Czech national phytosociological database (Chytrý and Rafajová 2003) were analyzed with Detrended Correspondence Analysis (DCA) with the effect of rare species reduced by downweighting. Detrending by segments with Hill's re-scaling was used. This means that the DCA axes are scaled in the "SD-units", i.e. in units of tolerance of an average species (see Lepš and Šmilauer 2003 for further explanation). A similar DCA of the species composition in our plots was performed (total of 90 species except *P. palustris*). The first four DCA axes were considered as composite characteristics of species composition, and used subsequently as explanatory variables for growth traits of *P. palustris*.

Data from three successive years for each of the measured growth traits were first analyzed together. As the individual traits between years were almost always well correlated, only data from 2004 were used in further multivariate analyses. Note that for some traits, the 2004 data summarize measurements from all previous years (Table 1b).

Principal Component Analysis (PCA) was used to show correlations among 1) environmental characteristics and 2) growth traits of *P. palustris*. Because the variables were recorded on different scales, they were standardized to their *z*-score prior to the analyses (option center and standardize corresponds to the PCA on the correlation matrix). As in the second analysis, we were mainly interested in the correlation structure of growth traits, only those were used in the PCA. Then, however, the relation to the chemical composition of plant tissues was visualized by passive projection of the chemical composition characteristics to the ordination plane.

Table 2
List of species abbreviations and full names used in the ordination diagrams. Nomenclature follows Flora Europaea (Tutin et al. 1964–1980).

abbrev.	Scientific name	abbrev.	Scientific name
Agrcan	*Agrostis canina*	Holmol	*Holcus mollis*
Agrcap	*Agrostis capillaris*	Juneff	*Juncus effusus*
Alnglu	*Alnus glutinosa*	Junfil	*Juncus filiformis*
Alopra	*Alopecurus pratensis*	Lysthy	*Lysimachia thyrsiflora*
Angsyl	*Angelica sylvestris*	Lysvul	*Lysimachia vulgaris*
Betpen	*Betula pendula*	Mentri	*Menyanthes trifoliata*
Betpub	*Betula pubescens*	Molcae	*Molinia caerulea*
Calcan	*Calamagrostis canescens*	Myonem	*Myosotis nemorosa*
Calcus	*Calliergonella cuspidata*	Peupal	*Peucedanum palustre*
Calpal	*Caltha palustris*	Phaaru	*Phalaris arundinacea*
Caracu	*Carex acutiformis*	Phraus	*Phragmites australis*
Carbri	*Carex brizoides*	Picabi	*Picea abies*
Carbue	*Carex buekee*	Poatri	*Poa trivialis*
Carcur	*Carex curta*	Polbis	*Polygonum bistorta*
Cardia	*Carex diandra*	Potere	*Potentilla erecta*
Carech	*Carex echinata*	Potpal	*Potentilla palustris*
Carlas	*Carex lasiocarpa*	Ranacr	*Ranunculus acris*
Carlim	*Carex limosa*	Rumace	*Rumex acetosa*
Carnig	*Carex nigra*	Salaur	*Salix aurita*
Carpan	*Carex panicea*	Salcin	*Salix cinerea*
Carros	*Carex rostrata*	Sanoff	*Sanguisorba officinalis*
Carves	*Carex vesicaria*	Scisyl	*Scirpus sylvaticus*
Cirarv	*Cirsium arvense*	Senriv	*Senecio rivularis*
Cirhel	*Cirsium helenioides*	Sph_sp	*Sphagnum* sp.
Cirpal	*Cirsium palustre*	Sphfle	*Sphagnum flexuosum*
Desces	*Deschampsia cespitosa*	Sphpal	*Sphagnum palustre*
Equflu	*Equisetum fluviatile*	Sphter	*Sphagnum terres*
Eriang	*Eriophorum angustifolium*	Trieur	*Trientalis europaea*
Fesrub	*Festuca rubra*	Vacoxy	*Vaccinium oxycoccos*
Filulm	*Filipendula ulmaria*	Valdio	*Valeriana dioica*
Galpal	*Galium palustre*	Viopal	*Viola palustris*
Galuli	*Galium uliginosum*		

To relate growth traits to environmental variables, Redundancy Analysis (RDA, again with standardized variables) was used. RDA can be considered an extension of multivariate linear regression for a multivariate response variable (Lepš and Šmilauer 2003), with the parametric test replaced by the Monte Carlo permutation test to overcome problems with distributional characteristics (999 permutations used in all the cases). First, we reduced the number of predictors within each group (abiotic variables, vegetation physiognomy, species composition represented by the four DCA axes) by performing three separate RDAs with growth traits being the response variables and predictors selected using the forward selection procedure from each of the above mentioned groups. With respect to the exploratory character of the study, the variable was accepted if $P < 0.1$. (The system is very complicated and heterogeneous, number of plots is strongly limited by the necessity to carry out repeated measurements in all the locations and so the power of the test is relatively low. As we wanted to include in our model all the useful predictors, i.e. see the Type II error as rather harmful for the model building, we decided for this rather liberal *P*-value.) The predictors were thus selected separately from nine (correlated) abiotic predictors, five (correlated) vegetation physiognomy predictors, and four (nearly uncorrelated) DCA axes. Subsequently, the significant explanatory variables were combined together in one RDA and their relative effects on the growth traits were estimated using variation partitioning (Borcard et al. 1992; see also Lepš and Šmilauer 2003).

The ordination methods (DCA, PCA and RDA) and visualization of their results were carried out using the Canoco and CanoDraw programs (ter Braak and Šmilauer 2002). Species shown in DCA have the highest weight. The species abbreviations and full names used in the ordination diagrams are listed in Table 2. Nomenclature of vascular plants follows Flora Europaea (Tutin et al. 1964–1980).

The effects of significant environmental variables (both abiotic and vegetation) selected in the RDA analyses on internode traits (i.e. number of produced internodes, their mean and maximal lengths) were estimated using linear regression analyses. Similarly, linear regression was used to evaluate relationships between each of the growth traits and selected environmental variables clustered into three groups.

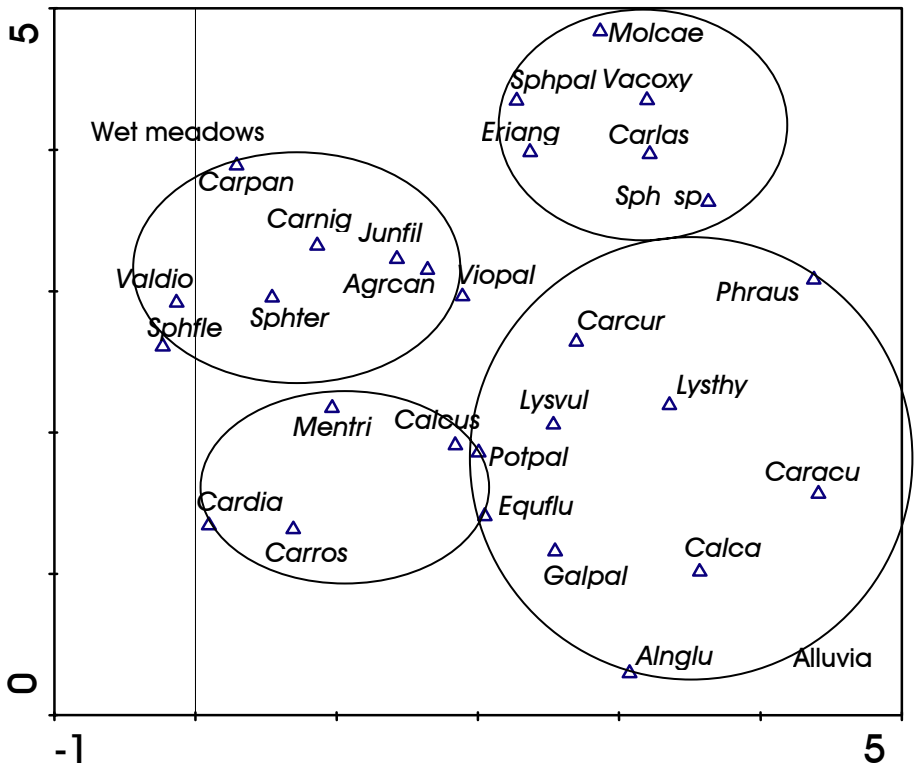

Figure 1

The DCA results of all relevés (n = 1229; 690 species) containing *P. palustris* from the national database of the Czech Republic. The circles and adjacent labels represent four main vegetation types (for further explanation see the text). The eigenvalues of the first and second ordination axes were 0.71 and 0.45 respectively, (total inertia = 18.76). Shown species have the highest weight. Full species names are listed in Table 2.

RESULTS

Habitat type differentiation

The DCA analyses delineated four main vegetation types in which *P. palustris* occurs (Fig. 1, 2). They were characterized by the following species composition (the environmental characteristics were deduced from the known biology of constituent species): 1. Wet meadows located mainly at lower altitudes, with moderate to strong N limitation, without peat formation. Typical species were *Carex panicea*, *Holcus mollis*, *Juncus effusus*,

Ranunculus acris and *Valeriana dioica*. 2. Sedge fens, with stronger peat or organic mud accumulation, higher water levels and favorable light conditions. Flooded conditions can be growth limiting. Typical species were *Carex rostrata* and *Menyanthes trifoliata*. 3. Margins of water bodies and alluvia mostly with denser vegetation and generally poor light conditions. Characteristic species were *Calamagrostis canescens*, *Carex buekee*, *C. acutiformis, Lysimachia thyrsiflora* and *Peucedanum palustre*. 4. Transitional fens with great organic matter accumulation and lower pH. Typical species were *Sphagnum* sp., *Eriophorum angustifolium*, *Carex limosa* and *Vaccinium oxycoccos*. The 32 locations used in this study had a similar habitat differentiation as all the locations of *P. palustris* from the national database: meadows, sedge fens, alluvia and transitional fen types were well represented by similar species as in the analysis of relevés from the database (Fig. 2). Interestingly, the subsequent analyses showed that the growth traits of *P. palustris* were predicted with the second and third axes of the DCA; these two axes are shown, and are overlain by the best predicted trait, number of branches. The second axis might correspond to gradient in water depth and the third axis to available nutrients status. The first axis, although not selected as a predictor in RDA, corresponded most to the altitude and vegetation cover gradients.

Correlating growth and environmental variables

Figure 3A shows correlations among the recorded environmental variables. Generally light penetration, *Sphagnum* cover, soil water content and LOI increased with altitude, while pH, biomass production, vegetation height and vegetation cover decreased. Nutrient content was largely not correlated with other environmental factors. The correlation structure of individual growth traits of *P. palustris* was revealed in the second PCA (Fig. 3B). As expected, stolon length traits (i.e. total and main stolon lengths, mean internode length) were correlated with plant N content. Nutrient (both P and N) richer leaves were grazed more often, while only shoot with high P content were preferentially grazed. The proportion of growing stolons was higher in grazed stolons and was (expectably) lower when flowering or death occurred. Some of the relationships were rather trivial: e.g. total length increased with branch production.

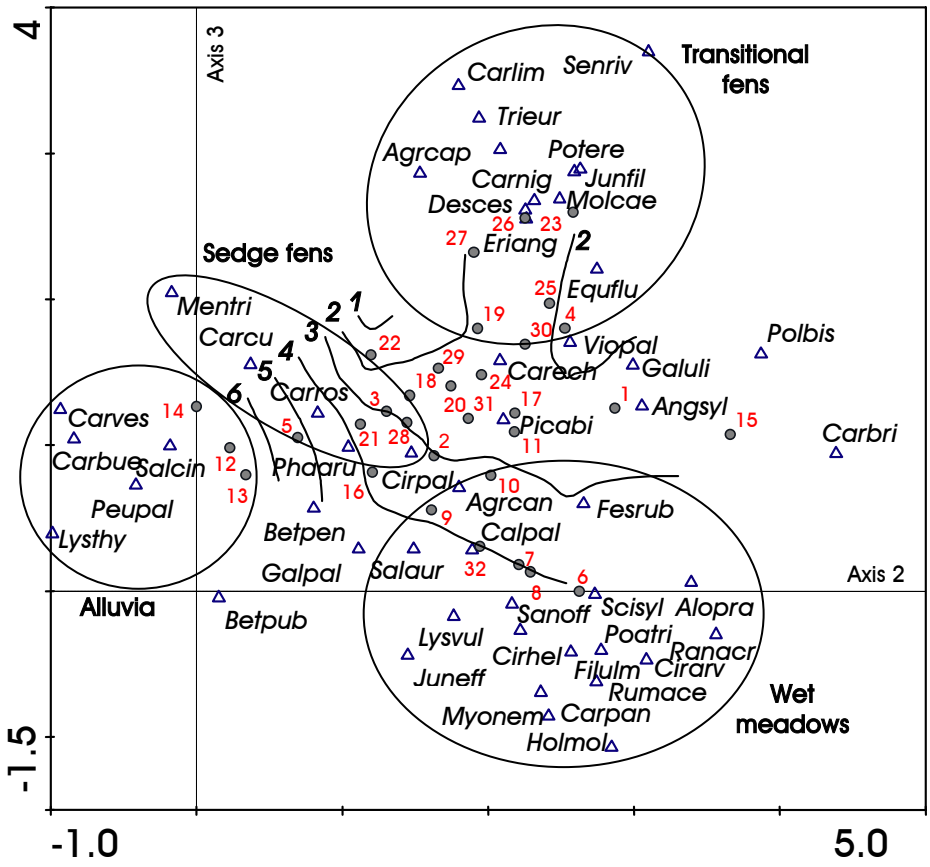

Figure 2

The DCA biplot of sampled locations and recorded species composition. Axes 2 and 3, which were selected in the subsequent RDA as significant predictors of *P. palustris* growth traits, are displayed (see the text). The eigenvalues of the first, second and third ordination axes were 0.55, 0.42 and 0.31 respectively, (total inertia = 5.5). Full species names are listed in Table 2. The numbered contours (***bold italic***) represent value limits of growth trait "branches", which is the trait best correlated with species composition (see Table 4).

These responses of *P. palustris* were then explored in the context of habitat (abiotic characteristics) and vegetation characteristics (vegetation physiognomy and species composition) in three separate analyses. Even though the pool of nine abiotic variables was available for forward selection (in contrast to five vegetation physiognomy variables and four DCA axes),

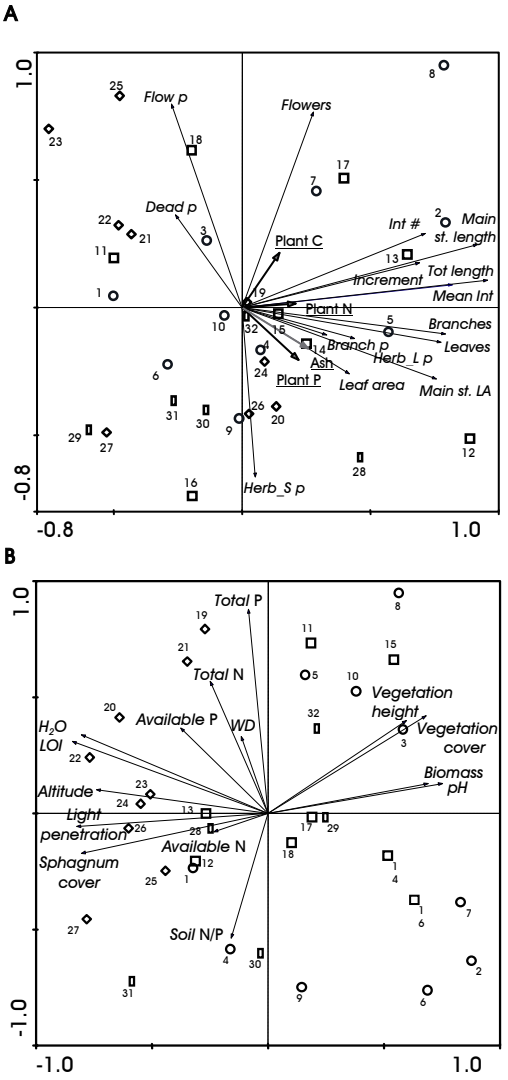

Figure 3

Correlations among A: recorded environmental variables and B: all recorded growth traits of *P. palustris* visualized together with locations (numbered circles) as a PCA biplot. The angles between arrows indicate correlations between variables. Locations were grouped to four groups according to their geographical position, and are represented by different symbols, ranging from the south-east to north-west in order: circle, square, diamond, rectangle. Plant nutrient traits (underlined) were added into B as a supplementary variable without any effect on the analysis. Explanation of abbreviations is given in Table 1.

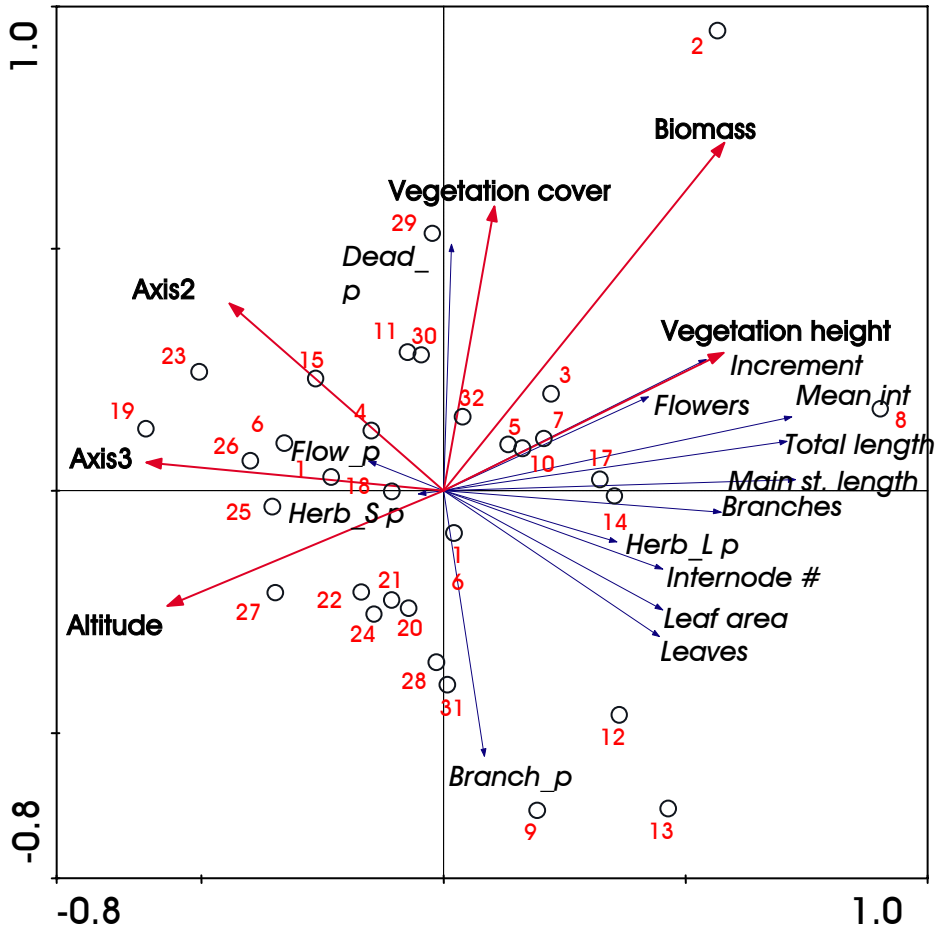

Figure 4

Relations among environmental variables (**bold**) and growth traits of *P. palustris* (*italics*) visualized together with samples (numbered signs) in a RDA triplot. Explanatory variables were selected using forward selection (999 permutations) in three successive RDA analyses with abiotic variables, vegetation physiognomy and species composition respectively. Significances in forward selection: Altitude: $P = 0.012$; Biomass: $P = 0.008$; Vegetation height: $P = 0.099$; Vegetation cover: $P = 0.006$; Axis2: $P = 0.044$; Axis3: $P = 0.026$. The first and second ordination axes explained 42.3% and 15.3% of total variance respectively. For variance partitioning between explanatory variables see Figure 5. For growth traits description see Table 1b.

Table 3
Significance of RDA models and variance partitioning between abiotic variables, vegetation physiognomy and species composition explaining the growth traits of *P. palustris*. The first model includes all variables selected by forward selection, next three include significant abiotic, vegetation physiognomy or species composition variables alone respectively. Abiotic variables: Altitude; vegetation physiognomy: Biomass, Vegetation height, Vegetation cover; species composition: second and third DCA axes. *F* = value of *F*-statistic; *P* = significance level; % = ratio of data variability explained by model.

Variables	Covariables	*F*	*P*	%
Abiotic+ Vegetation +Species	none	2.40	**0.003**	36.6
Abiotic	none	3.01	**0.014**	9.1
Vegetation	none	3.32	**0.001**	26.3
Species	none	2.39	**0.018**	14.1
Abiotic	Vegetation +Species	1.25	0.268	3.2
Vegetation	Abiotic+Species	2.42	**0.002**	18.4
Species	Abiotic+ Vegetation	1.27	0.234	6.4
Abiotic+ Vegetation	Species	2.21	**0.004**	22.4
Abiotic+Species	Vegetation	1.35	0.167	10.3
Vegetation +Species	Abiotic	2.17	**0.006**	27.5

only one of them was selected, whereas three vegetation physiognomy variables and also two axes representing species composition were selected by forward selection: abiotic: altitude: $P = 0.012$; vegetation physiognomy: biomass: $P = 0.008$; vegetation height: $P = 0.099$; vegetation cover: $P = 0.006$; DCA axes: axis2: $P = 0.044$; axis3: $P = 0.026$ and subsequently used as predictors in one common RDA (Fig. 4). The first RDA axis corresponded to stolon length traits with the yearly increment correlating tightly with vegetation height and biomass and negatively with altitude. The gradient in species composition or nutrient availability, represented by DCA axis3, correlated with the stolon length traits: i.e. ramets at sites with higher nutrient content (e.g. wet meadows) had more vigorous growth. Although vegetation cover was uncorrelated to most of the growth traits, it increased RDA explanatory power as it negatively correlated to branching probability

denser vegetation, the shoots had lower branching probability and had a higher probability to die. In taller vegetation, the measured plants tended to flower more as well as invest more into vegetative growth and branching. Ramets of *P. palustris* had larger leaves in sparse vegetation with low cover. Branching was highest in habitats with a high proportion of organic matter: permanently flooded sedge fens and alluvia, while was lowest in transitional fens (Fig. 2).

Variation partitioning (Table 3) demonstrated that vegetation physiognomy is the most important determinant of *P. palustris* growths characteristics. From all of the selected explanatory variables, vegetation physiognomy explained the most data variability (26.3%) with both abiotic variables and species composition explaining much less (9.1% and 14.1% respectively). The difference is even more pronounced when considering the partial effects (i.e. the unique effect, after using the other two groups as covariables): 18.4%, 6.4% and 3.2% for vegetation physiognomy, abiotic and species composition variables, respectively. Furthermore, only the partial effect of vegetation physiognomy was significant (Table 3). The explained

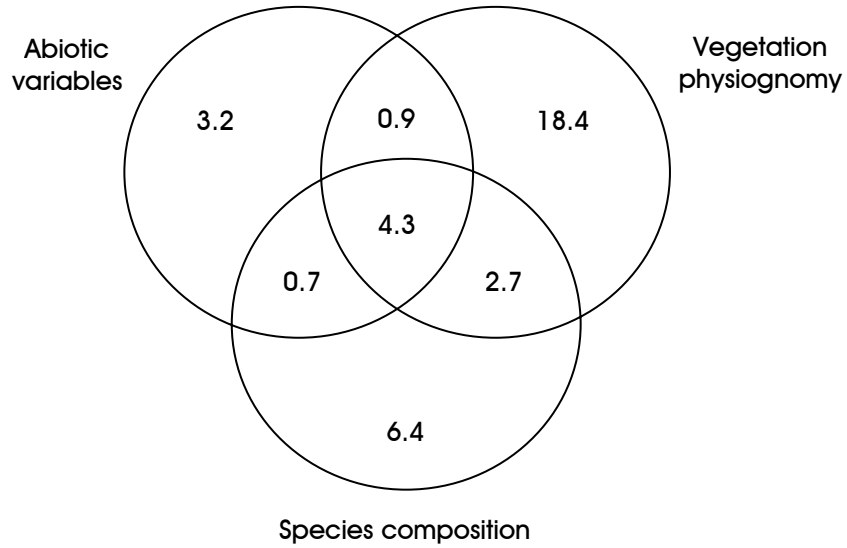

Figure 5

Variance partitioning between abiotic variables, vegetation physiognomy and species composition. Total variance of *P. palustris* growth traits explained by all variables together is 36.6%. Significances are in Table 3.

variation shared by all three groups of explanatory variables is relatively low, 4.3% only; the variation shared by any couple of groups is always less than 7.0% (Fig. 5). Among the growth traits, only leaf herbivory, main stolon length and mean internode length were significantly explained by abiotic variables (altitude). Vegetation physiognomy explained the variation in branching probability, number of branches, flower production, leaf area and the stolon length traits: yearly increment, main stolon length, mean internode length and total stolon length. The variation in the last three stolon length traits, leaf traits (leaf area and number of produced leaves) and number of produced branches were significantly explained by species composition at the study sites (Table 4).

Table 4
Percentage of variability (=100 x R^2) of individual *Potentilla palustris* growth traits explained by the selected abiotic (altitude), vegetation physiognomy (biomass, vegetation height and cover) and species composition (DCA axis 2 and 3) variables. Bold denotes a significant (multiple) regression model ($P < 0.05$). For growth traits description see Table 1b.

Growth trait	Abiotic variables	Vegetation physiognomy	Species composition
Branch_p	3.8	**27.7**	1.5
Branches	8.8	**24.9**	**28.5**
Dead_p	11.5	14.6	1.2
Flow_p	3.4	4.3	2.9
Flowers	2.3	**34.6**	9.8
Herb_S p	1.1	3.1	0.9
Herb_L p	**17.4**	14.9	17.6
Increment	4.0	**41.4**	9.5
Internode #	7.4	14.7	17.5
Leaf area	7.1	**25.0**	**22.9**
Leaves	8.4	15.9	**18.3**
Main st. length	**19.8**	**45.6**	**26.6**
Mean int	**22.1**	**51.0**	**18.5**
Total length	10.6	**50.1**	**22.6**

The effects of the four significant abiotic and vegetation variables on stolon length traits (internode number, mean internode length and maximal internode length) are shown in Fig. 6. The number of internodes was not significantly affected by any of the selected environmental variables. Both mean and maximal internode lengths were significantly higher at lower altitude, taller and denser vegetation. Vegetation cover had no effect on any of the stolon length traits.

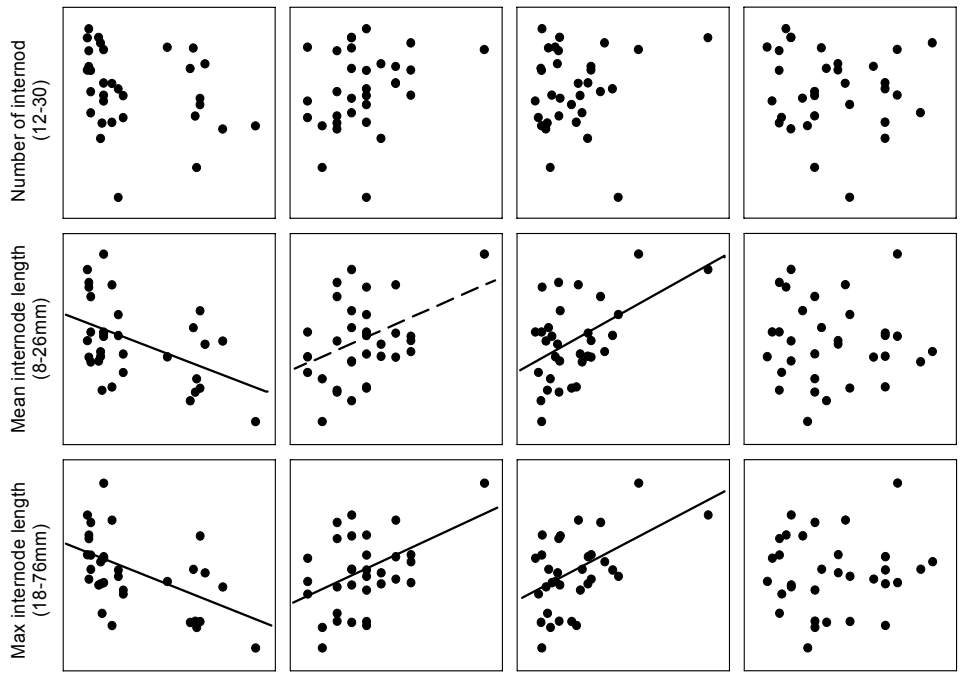

Figure 6
Effects of selected environmental variables (one abiotic and three vegetation) on internode traits estimated using linear regression; insignificant correlation has no line, dashed line ($P < 0.05$), full line ($P < 0.01$). Ranges of values are in parenthesis.

DISCUSSION

The variation partitioning demonstrated that vegetation physiognomy represented the best predictor of *P. palustris* growth characteristics, while the measured abiotic characteristics were the poorest. We are aware that forward selection can be rather unstable and the resulting amount of explained variability depends on the number of selected variables in individual groups. Nevertheless, with the same selection criteria used for all three groups, three out of five physiognomy characteristics were selected, whereas the procedure selected only one out of nine abiotic characteristics. Together with the pronounced differences in explained variability, this clearly demonstrates that the growth of *P. palustris* is mostly affected by the physiognomy of the surrounding vegetation. This does not necessarily imply that the abiotic variables did not have any effect; their effect is indirect, mediated by vegetation physiognomy. This is consistent with the RDA, where vegetation physiognomy characteristics were well explained by abiotic characteristics (not shown, $F = 15.6$, $P = 0.008$). The fact that the originally significant effect of altitude on growth traits, which explained 9.1% of their variability, became non-significant after including the physiognomy characteristics into the model as co-variables also suggests that the physiognomy traits directly affect *P. palustris* growth.

In many wetlands, plant mediated effects overrule direct abiotic effects (Aerts et al. 1999). Numerous studies documented abiotic environmental variables to be modified by ecological interactions with competing species (Fischer et al. 2004). Eventually, vegetation characteristics might also affect site conditions (Lavorel and Garnier 2002). Vegetation physiognomy, such as biomass and height of the surrounding vegetation, and cover of other vascular plants might reflect abiotic characteristics, but over a longer time period than our instantaneous measures of abiotic variables. Therefore, they became more reliable predictors.

Although our pilot nutrient addition experiment (results not shown) and the N/P ratio might suggest N-limitation (N/P values < 12; Koerselman and Meuleman 1996), the total N and P contents in our plants were well above critical values and, thus, nutrient limitation was probably not a major

influence (Rejmánková, pers. comm.). This could be due to an efficient nutrient recycling from senescing leaves, as recorded for fen plants (Aerts 1996; Bedford *et al*. 1999).

Both physiognomy and species composition could serve as a measure of past environmental conditions, and both characteristics could directly influence the growth traits; nevertheless, physiognomy is the better predictor of the growth traits (insignificant species composition effect when physiognomy was used as a covariable; results not shown). This is in agreement with the equivalence of competitors hypothesis (Goldberg and Werner 1983) and the finding of van Kleunen *et al.* (2005) that clonal plants respond mainly to neighborhood density.

If the growth of clonal plants is governed by a trade-off between investment in vegetative and generative reproduction (e.g. Reekie and Bazzaz 1987), we can expect a negative correlation between vegetative growth characteristics and flowering. Foraging theory regards branching to be a response to increased nutrients or light in good patches (de Kroon and Hutchings 1995; Salemaa and Sievanen 2002), whereas linear growth is a means of escape; thus, these two traits should be negatively correlated (comp. Gonzalez and Gianoli 2004; Niva et al. 2006). On the contrary, our results suggest rather neutral or positive relationships. Flowering, internode and stolon length, and branching of *P. palustris* all increased in taller vegetation and, in concordance with other studies (Fischer et al. 2004; Gonzalez and Gianoli 2004; van Kleunen et al. 2005), both flowering and stolon length traits were less responsive to surrounding vegetation biomass or cover. One possible explanation for both enhanced branching and linear growth may be similar to Roff's (1992): vigorous plants do everything well. Whether the concordant response of vegetative growth and generative reproduction to varying environments or if a trade-off between these two traits will prevail depends probably on the range of environmental conditions and intensity of individual species response to this variation. Chaloupecká and Lepš (2004) found that vegetative spread and generative reproduction were positively correlated in *Myosotis nemorosa* (being apparently more affected by environmental heterogeneity), but that there was a negative correlation for *Lychnis floss-cuculi*. In our study, the positive correlation between number of flowers and branching ($r = 0.44$, $P = 0.015$, data not shown) may also be

linked to apical dominance, when flowering leads to apical meristem termination.

The variability in stolon length can be due to both changes in internode number and internode length. Of those two, only internode length exhibited a clear response to the environment. The structural blueprint of *P. palustris* obviously enables quite a large variation in internode length. Numerous other studies report increased internode length as a plant response to various unfavorable conditions such as shading (Gonzalez and Gianoli 2004; Niva et al. 2006), crowding (Fischer et al. 2004; van Kleunen et al. 2005; Griffith and Sultan 2006) or flooding (Lenssen et al. 2004). Studies reporting a change in internode number are rather scarce (but see Griffith and Sultan 2006). Therefore, it is likely that internode length is a plastic trait, while internode number is a more constant trait, of horizontal stolon growth in *P. palustris*.

Internode production comes at a high cost, because a leaf and roots are equally formed. Such biomass formation is also time demanding. The increase in internode number thus results in a lower linear growth rate compared to the production of a small number of longer internodes. The formation of longer stolons was mainly by internode elongation, thus enhancing mobility. Consequently, if stolon length should be a means of escape from unfavorable conditions, it has to be achieved by the plastic response of internode length, keeping the number of internodes constant.

The growth of creeping *P. palustris* was expected to be light limited inside the dense vegetation of wet meadows (e.g. sites #2, 3, 7, 8; Fig. 3A). However, the ramets in these sites showed relatively high linear stolon growth and increased internode lengths. Flowering was also promoted, but, unexpectedly, lower light did not lead to increased leaf area, as would be assumed based on the results of other studies (Gonzalez and Gianoli 2004; Griffith and Sultan 2005; Weijschede et al. 2006). Light levels are lower in meadows, while other abiotic variables (e.g. temperature, pH) are less limiting. Competitively poorer *P. palustris* likely invests energy into both means of escape strategy rather than acclimation by increased leaf area (comp. Hutchings and Bradbury 1986; Routledge 1990). Vigorous vegetative growth was also documented from physiognomically similar alluvial habitats. However, as groundwater is usually close to the soil surface, *P. palustris* encountering open water exhibit a distinct growth form: intensively branched

ramets occasionally form a dense layer of overlaying interweaved stolons (e.g. sites #5, 12, 13, 14). A large leaf area is common at these sites when there is ample light. Together with branching, the longer internodes enable *P. palustris* to quickly colonize open areas.

Both neutral peaty swamps and acidic transitional fens are characterized by higher light and organic matter content. Water is more abundant in peaty swamps (#4, 18, 19), where *P. palustris* had moderate leaf area, moderate stolon and internode lengths, and sufficient branching and flowering. Its growth might be limited by intraspecific competition (size independent and symmetric; de Kroon et al. 1992), thus limiting vegetation height. Intermediate growth is an expected feature at such sites (Hara 1994). On the contrary, plants from transitional fens (#25, 26, 27) displayed rather limited growth in a majority of the measured traits. The resource levels of nutrients, light and water were moderate to high, thus, the major growth limitations were high altitude (i.e. lower mean temperatures) and extreme acidity and toxicity (Aerts et al. 1999).

The experiments under controlled conditions and with a limited number of manipulated factors are undoubtedly a more straightforward means of uncovering direct effects on clonal growth. Nevertheless, our study demonstrated that, in nature, the effects of abiotic conditions are modified and often overruled by the direct effects of the surrounding vegetation, thereby forming a complicated network of causal relationships, which are often difficult to predict from simple cause-effect experiments under controlled conditions. These multiple effects of abiotic and biotic factors on plant growth can be studied in comparative studies like this one. However, to the best of our knowledge, the study presented here is one of the most extensive multi-site comparative studies of clonal growth to date, and even so, the multiple effects and causal chains are difficult to disentangle.

P. palustris seems to be a species well adapted to harsh conditions, although, in terms of the studied area, it prefers sparsely vegetated sites at intermediate altitude; sedge fens on highly organic soils could be considered an optimal habitat. Its growth traits correlate better with vegetation physiognomy than with abiotic characteristics or exact species composition. Although abiotic conditions are surely the ultimate factors having an indirect effect on plant growth, physiognomy, as a proximate factor, has a higher explanatory potential. Being a poorer competitor, the *P. palustris* strategy

under tall dense vegetation is escape by means of plastic, low cost growth traits (internode elongation), keeping the high cost traits (internode number) constant. As this species is able to alter its phenotype, it has the potential to grow in a wide range of habitats (comp. Griffith and Sultan 2006).

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The beggining of the mesocosm experiment at our platform on the outskirts of Orange Walk town, Belize. Plants were located in the vicinity of water in order to provide the most similar microclimate to the natural marshes.

Response of emergent macrophytes to experimental nutrient and salinity additions

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ABSTRACT

Nutrient additions often result in species dominance/compositional changes in wetland ecosystems, but the impact of nutrients may be constrained by different salinity levels. Wetlands of northern Belize, distributed along a salinity gradient, are strongly phosphorus limited and dominated largely by three species of emergent macrophytes: *Eleocharis cellulosa*, *Cladium jamaicense*, and *Typha domingensis*.

We conducted a mesocosm experiment to assess changes in growth characteristics (biomass allocation, plant height, relative growth rate [RGR], rhizome length) and nutrient uptake of these three species in response to simultaneous changes in nutrient levels (nitrogen, phosphorus) and salinity.

The growth characteristics of *Typha* and *Eleocharis* responded positively to N and especially P addition, whereas the growth response of *Cladium* was largely insignificant. The RGR of *Typha* increased under P additions, while RGR of *Eleocharis* increased with N and decreased with salinity additions. Nutrient addition increased the rhizome number of both *Typha* and *Eleocharis*. However, plasticity in rhizome length was observed only in *Typha*, which showed increased rhizome length at medium and high P.

Salinity decreased plant height and shoot and root biomass of *Cladium* and *Eleocharis*, while in *Typha* it reduced only height. Rhizome number and length were decreased only in *Eleocharis*.

Both medium and high P additions increased tissue P content in all three species, but *Eleocharis* accumulated significantly more P than *Cladium* and *Typha*. N additions increased tissue N content in *Cladium* and *Eleocharis*, but not in *Typha*.

Cladium exhibited strong morphological constraint and behaved as a stress-tolerator that was well adapted to low nutrients. *Typha* – characterized by its plastic, opportunistic guerrilla growth strategy, fast and efficient space occupancy and rather wasteful nutrient management, behaved as a typical competitor. *Eleocharis* responded rapidly to nutrients but displayed limited rhizome plasticity, and its growth was affected at higher salinity.

According to recorded traits, we hypothesize that P input into wetlands will result in expansion of *Typha*, leading to competitive exclusion of both co-occurring species. The only conditions allowing coexistence of all three species are those limiting vertical and horizontal growth of *Typha*: low P and higher salinity. To ensure the stability of Belizean wetlands, the maintenance of oligotrophic status is therefore crucial.

Keywords
Biomass allocation - Eutrophication - Oligotrophic tropical wetlands - Plasticity - Rhizomes

INTRODUCTION

In tropical countries, agricultural systems are often extended to meet the needs of rapidly growing populations, and crop yields are improved by increasing fertilizer application. Expansion of agriculture towards natural aquatic ecosystems leads to agricultural runoff, causing eutrophication (Downing et al. 1999). Oligotrophic tropical and subtropical wetlands are particularly sensitive to nutrient addition, and the numerous rivers and wetlands in a country such as Belize (Central America) are therefore under serious threat.

The herbaceous wetlands of northern Belize are part of a group of phytogeographically related, limestone-based marshes that also cover extensive areas on the Yucatan Peninsula and Caribbean islands, and reach up to Florida (Estrada-Loera 1988). These wetlands range in size from small, <1-ha marshes to large, shallow inland lagoons, with salinity ranging from 0.1 to 6 ppt, and they are generally phosphorus-limited (Rejmánková 2001). Increased P input leads to the expansion of emergent macrophytes, resulting in elimination of species-rich cyanobacterial mats (Rejmánková et al. 2004).

As a part of a research project focusing on the changes in ecosystem processes and community structure that result from nutrient addition, we conducted a mesocosm experiment to evaluate the responses of three macrophyte species to factorial combinations of different levels of low, medium and high salinity, phosphorus and nitrogen. Our primary goal was to assess how nutrient availability, constrained by salinity, affects the growth and nutrient uptake of the dominant macrophytes (*Eleocharis cellulosa* Torr., *Cladium jamaicense* Crantz and *Typha domingensis* Pers.), in terms of biomass and nutrient allocation. The individual effects of salinity, N and P have been evaluated in numerous studies of wetland plants, but few studies have examined plant responses, especially below-ground, to combined salinity and nutrient treatments (Morris and Ganf 2001). We manipulated all three variables simultaneously and examined above- and below-ground growth responses.

All three species are clonal macrophytes, but differ in terms of life strategies (*Typha* is a competitor while *Eleocharis* and *Cladium* are stress tolerators; Rejmánková et al. 1996). In the Florida Everglades, the competitive ability of *Typha* increases with P availability (Noe et al. 2001; Miao 2004) and Belizean wetlands adjacent to potential P inputs (sugar cane fields, pastures) have *T. domingensis* as the dominant species (Johnson and Rejmánková 2005; Pope et al. 2005). In previous field experiments in these wetlands, the response of dominant species to P enrichment was rapid, resulting in high above-ground biomass accumulation (Rejmánková 2001).

Numerous studies have shown the impact of elevated nutrients on ecosystem function (e.g. Aerts and Chapin 2000; Daoust and Childers 2004). The short-term response of vegetation to increased nutrients is usually to increase primary production (Lepš 1999), although this may not occur (Güsewell et al. 2003). Ultimately, nutrient loading leads to a switch in species

dominance, to changes in plant species composition and, often, to the reduction of species richness (Bedford et al. 1999; Chiang et al. 2000; Sammul et al. 2003). Species also differ in energy-allocation strategy (Daoust and Childers 2004; Weisner and Miao 2004). Shoot growth generally increases more than root growth (Chen et al. 2005) so that increased nutrient levels often lead to the extensive development of new vegetative and reproductive structures (Li et al. 2000). Consequently, nutrient availability affects not only plant biomass, but also the architecture of clonal plants (Piqueras et al. 1999), mostly by (1) variation in spacer length (Cain 1994) and (2) changes in branching frequency and branching angle (Dong and de Kroon 1994). However, the most important change affecting plant foraging is due to the morphological plasticity of resource-acquiring structures, which allows resource acquisition to be maximised (de Kroon and Hutchings 1995).

Tropical inland wetlands can vary widely in salinity level, with high salinity posing a potential stress to plant growth and ion uptake becoming energy-demanding (Lambers et al. 1998). Strategies to keep internal water potential at appropriate levels at high salinities include increased uptake of mineral salts, formation of organic osmolytica, and dehydration (Adam 1990). As the production of organic osmloytica requires nutrients (especially N), higher N supply could help plants cope better with salinity stress (Mansour 2000).

In our mesocosm experiment, we aimed to answer the following questions. 1. How does the biomass allocation of each species change with increasing nutrient levels? Specifically: (a) Does the allocation to above-ground organs increase compared with the allocation to below-ground organs? (b) Does plant architecture respond by maximizing the area covered by vegetative growth to the same extent for all species?

2. How much does the elevated salinity constrain horizontal and vertical growth? Does N addition alleviate salinity stress?

3. How does nutrient uptake change under the experimental treatment? Are the species taking up nutrients in accordance with their ecological strategies?

We predicted that: (1) All species will grow better under elevated P levels. However, we expected the growth of *Typha* to respond more vigorously than the growth of *Cladium* and *Eleocharis*. (2) In all three species there will be higher biomass allocation to the leaves/shoots than to roots if there is elevated N and P, but the response will be reduced at higher salinity

levels. (3) Ramet production will increase under elevated N and P for all three species. (4) The addition of N under high salinity will improve plant growth because of potential alleviation of salinity stress. (5) Addition of P will increase rhizome length, especially in *Typha*. (6) Growth of *Typha* will be more constrained by elevated salinity than that of *Cladium* and *Eleocharis*. (7) All species will increase the uptake of added nutrients, especially in the case of P.

MATERIALS AND METHODS

Plant and soil material

Experimental plants of the three species, *Cladium jamaicense*, *Typha domingensis* and *Eleocharis cellulosa*, originated in the Buena Vista marsh, northern Belize. The marsh is characterized by medium-salinity (~1 ppt), low sediment P (50 μg cm^{-3}) and sufficient sediment N (3 mg cm^{-3}) (Rejmánková 2001). Cuttings of new ramets were collected in mid-December 2001 and pre-planted in the crates located in the marsh. In mid-January, plants were trimmed, leaving only the youngest 3–4 leaves (*Cladium*, *Typha*) or 2–3 shoots (*Eleocharis*), and planted in 4-l plastic pots. Average initial plant height ± SE was 31 ± 3.6, 30 ± 2.6 and 35 ± 1.4 cm for *Cladium*, *Typha* and *Eleocharis* respectively. The soil, which originated in the same location, is typical of many northern Belizean marshes and consisted of equal parts of peat, clay and marl. Pots were placed in mesocosms (88 x 88 x 30 cm), and flooded by rain water to reach a level of ~3 cm above the soil surface.

Experimental design

A full factorial design of three salinities, three P levels and three N levels was used. Twenty-seven mesocosms were located in a grassland area adjacent to a pond and a small area of a swamp forest on the outskirts of the town of Orange Walk, Belize. Four replicates of each of the three macrophyte species and of a cyanobacterial mat were in a separate pots so that there were 16 pots for each mesocosm. Results of the response of cyanobacteria to N, P and salinity have been published elsewhere (Rejmánková and Komárková 2005).

A mix of salts that reflects the average ionic composition of marsh water in the region (Rejmánková, unpublished data) was used for the salinity

treatment. The salinity was increased gradually over two weeks to reach the final values of low (0.2–0.5 ppt), medium (1–1.5 ppt) and high (4–5 ppt). Salts dissolved in water were added to each tank at 2-day intervals. Salinity was adjusted bi-weekly or after heavy rainfall, as needed.

Phosphorus was added as KH_2PO_4 and nitrogen as NH_4NO_3. Once salinity levels had been established, nutrients were injected into the containers with a syringe at 6-day intervals (10 additions in total). Additions of nutrients over the duration of the experiment corresponded to an annual equivalent of 1, 10 and 20 g m^{-2} $year^{-1}$, for low, medium and high N, respectively, and to 0.5, 5 and 10 g m^{-2} $year^{-1}$, for low, medium and high P, respectively. These additions were selected to correspond to an ongoing long-term field nutrient-addition experiment (Rejmánková, Macek and Epps, unpublished data). The experiment began with the first addition of nutrients and lasted from 30th January to 30th March, 2002.

Morphological measurements

At the end of the experiment, the biomass of each plant was separated into living leaves/shoots, roots, and rhizomes (including shoot bases). A leaf or shoot was considered dead if more than two thirds of its length was dry. Leaf length was measured for all leaves. Their sum is described here as "total leaf length", and the average length of the three longest mature leaves is called "plant height". Leaf area of three randomly selected mature leaves/shoots was measured using a portable leaf area meter (LI-3000A, Li-Cor, Lincoln, NE, USA). Relative growth rate (RGR) of leaf/shoot length was calculated as:

$$RGR = (\ln Length_{end} - \ln Length_{beginning})/time$$

The length of each rhizome was measured, and summed to give "total rhizome length". Lengths of rhizomes bearing secondary ramets were averaged to give "ramet distance". None of the species studied forms branched rhizomes, therefore we considered rhizomes as internodes and ramets as branching points. Finally, leaves, roots and rhizomes were dried (70 °C) and weighed.

Plant chemistry

Dry leaf/shoot tissue was ground and assayed for total N with a Perkin Elmer HCN analyzer (Perkin Elmer, Waltham, MA, USA) and for ash content. Total P was measured spectrophotometricaly using ascorbic acid reduction of phosphomolybdate complex after combustion and consequent acid digestion (McNamara and Hill 2000). Nutrient-use efficiency (NUE, PUE) was calculated as production divided by canopy N or P according to Harrington et al. (2001).

Data analyses

To evaluate several responses of all three species together, we used the redundancy analysis procedure (RDA) in the CANOCO package (ter Braak and Šmilauer 2002). We used RDA because it enables good visualization of the main trends in the data in addition to the non-parametric testing of effects. RDA can be considered an extension of multivariate regression for a multivariate response variable (Lepš and Šmilauer 2003). The parametric test is replaced by the Monte Carlo permutation test to overcome problems with distributional characteristics. Our tests were based on 499 random permutations. Phosphorus, nitrogen, and salinity were considered as explanatory variables, with values 1, 2 and 3 assigned to low, medium and high levels. To avoid losing information on the order of the categories (medium lies between low and high), these variables were treated as continuous rather than indicator variables. We first assessed the global significance of the model. Then, using partial analyses (when a tested factor is the only explanatory variable and the other two are covariables), we obtained variability explained by each of the factors and corresponding significance values. Note that, because the factors are orthogonal, the size of the marginal and partial effects is the same (but the significance differs). The option "center and standardize by species" was used in the RDA because variables were measured on different scales. We used the sequential Bonferroni procedure (Quinn and Keough 2002) to express the statistical significance of the correlations between response and explanatory variables.

Data were analyzed using multifactorial ANOVA with species, salinity, N and P as factors. In all analyses, most of the interactions of species x factor were significant, which means that species responded differently to the treatments. Therefore we used only three factors (salinity, N, P) in succeeding

analyses and evaluated data for each species separately. For *post-hoc* comparisons we used Tukey's Honestly Significant Difference (HSD) test. To satisfy ANOVA model assumptions, we log-transformed some of the variables (above-ground biomass, root biomass, rhizome biomass, P content, N/P), which improved data normality and homoscedasticity, but also switched the effects from multiplicative to additive.

RESULTS

Ordination analysis

We conducted the redundancy analysis to visualise the relationship between response and explanatory variables (N, P and salinity) (Fig. 1). For an easier orientation, the response variables are differentiated graphically into variables related to plant growth and morphology (for variables and abbreviations see Figure 1). The Monte Carlo test with the forward selection of variables indicated highly significant conditional effects of all explanatory variables ($P = 0.002$), with P showing the strongest effect, followed by salinity and N ($F = 9.15$, 4.49 and 4.07, respectively). Although there were several positive or negative trends in the relationships between the growth-related and explanatory variables (Fig. 1), not all were significant after Bonferroni correction. Total biomass was positively correlated with both P and N in *Typha* ($P < 0.01$ and $P < 0.05$, respectively), and with N in *Eleocharis* ($P < 0.05$), while there was no correlation between total biomass and either of the two nutrients in *Cladium*. The leaf/shoot length to area ratio (L/A) of *Typha* and *Eleocharis* responded negatively to P: both species formed more robust leaves/shoots under elevated levels of P ($P < 0.01$ and $P < 0.05$, respectively). Although the shoot to root (S/R) ratio of all three species appeared to be positively correlated with increasing nutrient levels, the correlation was not significant. The root/rhizome (Ro/Rh) ratio of *Eleocharis* and *Cladium* was not correlated to either N or P, but there was a significant positive correlation between P and Ro/Rh in *Typha* ($P < 0.01$). In *Typha*, we did not find any significant correlation between the growth-related variables and salinity, except for plant height, which decreased with increasing salinity ($P < 0.05$). On the other hand, shoot height, root/rhizome ratio and RGR of *Eleocharis* were all negatively correlated to salinity ($P < 0.05$) and L/A of *Cladium* responded positively to increasing salinity ($P < 0.05$).

Not surprisingly, variables related to nutrient uptake were tightly correlated to N and especially to P. Tissue P and ash content of all three species increased with increasing P addition ($P < 0.01$), while PUE decreased. With increasing N additions, *Eleocharis* and *Cladium* tissue N increased and NUE decreased ($P < 0.01$ and $P < 0.05$, respectively), but there was no response in *Typha*.

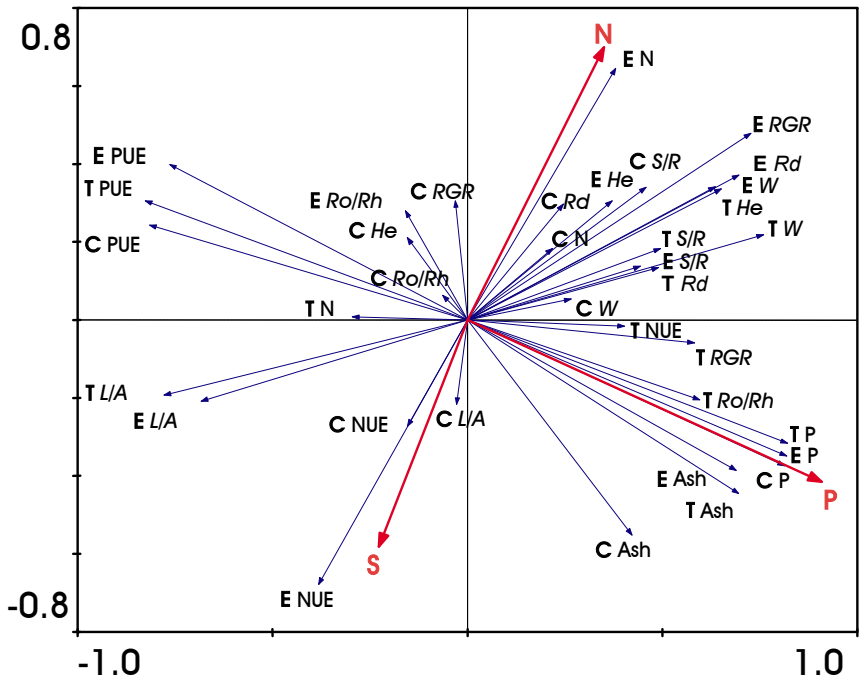

Figure 1

Response variables – environmental variables biplot of the redundancy analysis procedure. Response variables are grouped into variables related to growth (in italics: total biomass, *W*; leaf (shoot) length/leaf (shoot) area, *L/A*; shoot/root ratio, *S/R*; root/rhizome ratio, *Ro/Rh*; rhizome distance, *Rd*; plant height, *He*; relative growth rate, *RGR*) and response variables related to nutrient uptake (non-italic: tissue phosphorus, P; tissue nitrogen, N; ash content, Ash; phosphorus-use efficiency, PUE; nitrogen-use efficiency, NUE). Species codes: **C**, *Cladium jamaicense*, **T**, *Typha domingensis*, **E**, *Eleocharis cellulosa*; environmental variables: **N**, nitrogen; **P**, phosphorus; **S**, salinity. Partial effects of all explanatory variables were highly significant; P, N and salinity explained 27, 12 and 10% of data variability, respectively.

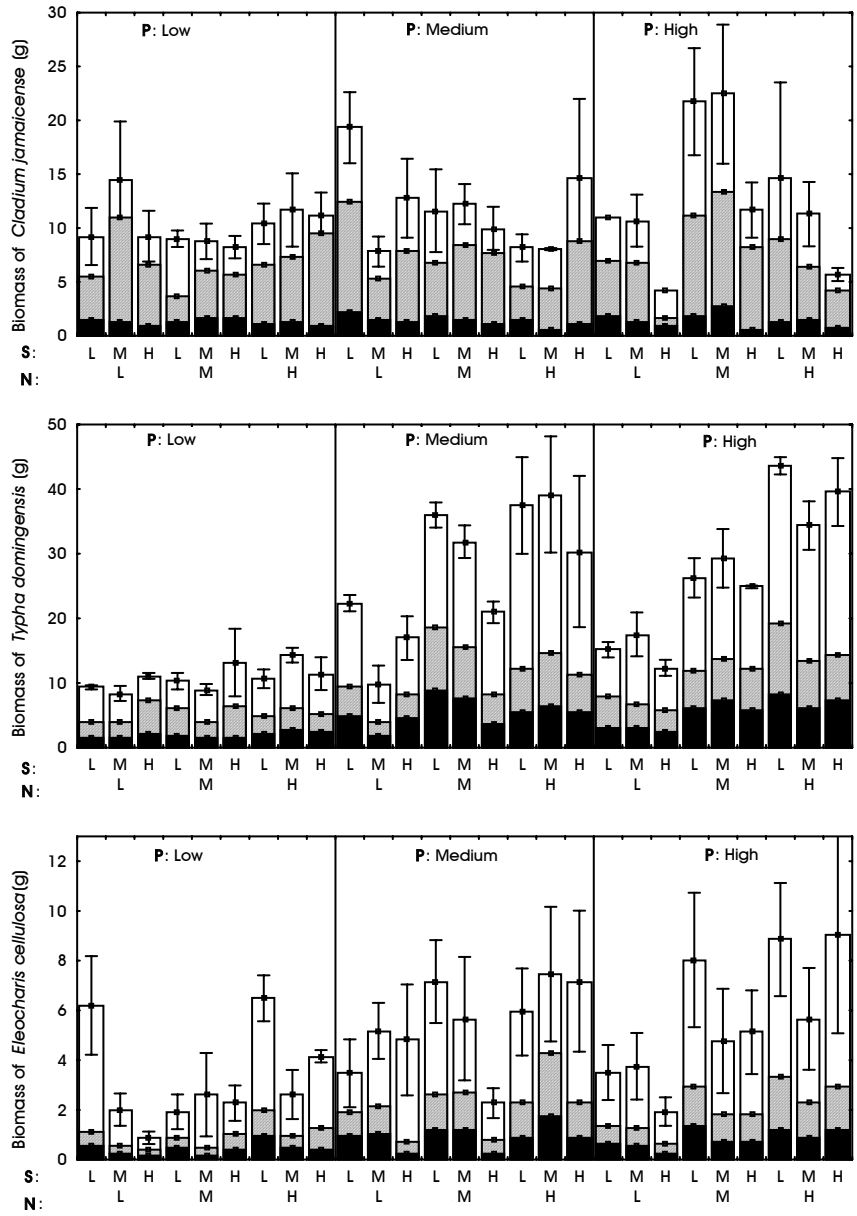

Figure 2

Biomass allocation into leaves/shoots, roots and rhizomes in all experimental treatments. Factors: S, salinity; N, nitrogen; P, phosphorus; levels: L, low; M, medium; H, high. Open bars, leaf/shoot; hatched bars, rhizome; black bars, root biomass. Error bars, ± S.E. for total biomass. Note different scales for each species.

Biomass production and allocation

Both total biomass and biomass of individual plant parts (shoots, rhizomes and roots), generally increased with increasing N and P and decreased with increasing salinity (Fig. 2; Table 1). The above-ground biomass of *Typha* and *Eleocharis* increased significantly in response to both N and P, with both species responding differently to each of the three levels of N (Tukey, $P < 0.005$), while only distinguishing low from medium and high P levels (Tukey, $P < 0.001$). A synergistic reaction to both nutrients was demonstrated by *Typha*, while the above-ground biomass of *Cladium* did not respond to nutrients. Biomass of *Cladium* and *Eleocharis*, but not *Typha*, was negatively affected by salinity addition. Only high salinity (for both *Cladium* and *Eleocharis*) differed significantly from other levels (Tukey, $P < 0.02$). For all species, both total and above-ground biomass were tightly correlated with total leaf length ($R^2 = 0.80$, 0.68, and 0.24 for *Typha, Eleocharis*, and *Cladium,* respectively). In *Typha* and *Eleocharis*, total and above-ground biomass was additionally correlated with the number of shoots, that with the production of secondary ramets ($R^2 = 0.42$ and 0.78 respectively).

Below-ground biomass allocation to roots and rhizomes of *Cladium* did not change after P addition. High salinity addition (compared to low salinity) significantly decreased *Cladium* root biomass (Tukey, $P = 0.02$). The rhizomes were unresponsive to increases in either nutrients or salinity. The response of roots and rhizomes of *Typha* and *Eleocharis* followed the same pattern as their above-ground biomass.

Leaf/shoot growth

Higher salinity levels constrained leaf or shoot growth of all three species, as represented by plant height (Table 1) and, except for *Typha,* by total leaf length (Fig. 3; Table 1). For *Cladium*, only the high salinity level was sufficiently stressful to constrain both plant height and total leaf length (Tukey, $P < 0.002$). The plant height of *Typha* responded negatively to high salinity (Tukey, $P = 0.003$), and increased at high N or medium and high P levels (Tukey, $P < 0.013$). The total leaf length of *Typha* was closely related to leaf number (secondary ramet production) and also increased after nutrient addition. Finally, both plant height and total shoot length of *Eleocharis* decreased with salinity, the high salinity levels differing from both medium and low salinity levels (Tukey, $P < 0.001$). Plant height of *Eleocharis* increased

Table 1

Results of ANOVA comparing effects of salinity, N, and P addition on growth characteristics. *F*-values and factor significance are shown separately for *Cj*, *Cladium jamaicense*; *Td*, *Typha domingensis*; *Ec*, *Eleocharis cellulosa*. Factors: S, salinity, N, nitrogen; P, phosphorus; asterisk between factors signifies interaction.

	Above-ground biomass			Root biomass			Rhizome biomass		
	Cj	*Td*	*Ec*	*Cj*	*Td*	*Ec*	*Cj*	*Td*	*Ec*
S	8.5***	1.61	13.2***	4.55*	0.82	20.2***	0.51	1.32	5.62**
N	1.42	24.8***	25.0***	4.02*	4.12*	13.5***	0.20	6.19**	15.8***
P	1.08	51.3***	28.7***	0.24	15.3***	19.8***	0.25	8.1***	21.3***
S x N	0.24	0.61	1.39	0.63	1.09	2.23	1.56	1.21	0.95
S x P	1.02	0.88	5.28**	2.62*	0.59	5.6***	2.37	0.91	4.42**
N x P	1.55	3.08*	1.02	0.75	2.58*	1.63	4.03**	1.96	1.24
S x N x P	0.65	1.51	0.93	0.81	0.94	0.59	0.84	0.90	0.44
	Plant height			Total leaf length			Total rhizome length		
	Cj	*Td*	*Ec*	*Cj*	*Td*	*Ec*	*Cj*	*Td*	*Ec*
S	8.29***	5.76**	23.7***	8.0***	2.26	23.2***	2.85	5.72**	4.74*
N	0.80	4.24*	21.6***	0.47	16.5***	25.4***	0.41	7.59**	18.7***
P	0.23	10.1***	37.8***	1.13	31.8***	32.7***	0.56	27.6***	19.1***
S x N	1.63	0.46	5.82***	1.44	0.14	2.86*	0.29	1.55	0.24
S x P	0.92	0.66	5.01**	0.47	0.66	5.07**	0.32	1.95	3.50*
N x P	0.59	1.75	1.87	0.39	3.79**	2.93*	3.24*	3.58**	1.66
S x N x P	1.01	0.79	1.00	1.55	0.88	0.55	1.56	0.81	1.08

df = 2 for main effects, df = 4 for first-order interactions, df = 8 for second-order interaction. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

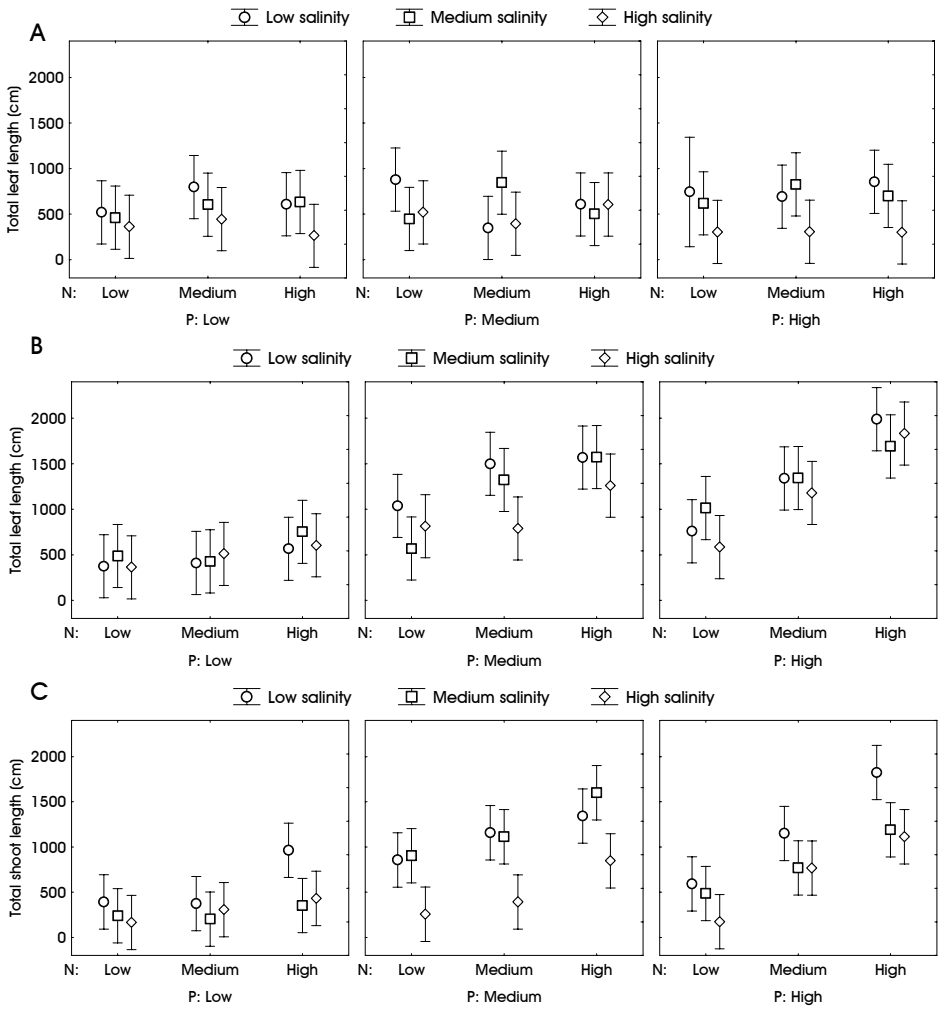

Figure 3

Mean values of total leaf/shoot length for *Cladium jamaicense* (A); *Typha domingensis* (B); *Eleocharis cellulosa* (C) showing the response to three factors (salinity, N, P). Error bars: mean ± S.E.

after elevated N or P additions (Tukey, $P < 0.001$). The total shoot length of *Eleocharis* increased in response to N and/or P additions and salinity x N, salinity x P and N x P were also significant. On the other hand, salinity affected RGR only in *Eleocharis* (low < medium < high level; Tukey, $P < 0.01$). In *Typha* and *Eleocharis*, RGR increased after P additions; in *Eleocharis* RGR was also positively affected by N additions (Table 2).

Table 2
Results of ANOVA comparing the effects of salinity, N, and P addition on tissue nutrient content and RGR. *F*-values and factor significance are shown separately for *Cj*, *Cladium jamaicense*; *Td*, *Typha domingensis*; *Ec*, *Eleocharis cellulosa*. Factors: S, salinity, N, nitrogen; P, phosphorus; asterisk between factors signifies interaction.

	N content			P content		
	Cj	*Td*	*Ec*	*Cj*	*Td*	*Ec*
S	5.86**	5.72**	7.8***	1.97	1.65	1.14
N	14.2***	0.57	13.6***	0.01	1.36	1.34
P	1.85	2.53	1.39	30.0***	184***	334***
S x N	0.68	0.54	2.57*	0.22	2.89*	0.92
S x P	2.11	0.66	1.59	0.66	0.84	2.99*
N x P	4.97**	1.72	2.55*	0.26	5.9***	2.58*
S x N x P	0.56	1.52	0.62	0.69	1.04	1.23
	N/P			RGR		
	Cj	*Td*	*Ec*	*Cj*	*Td*	*Ec*
S	4.33*	15.4***	0.17	0.66	1.12	18.5***
N	5.16**	2.53	10.9***	2.77	1.44	18.9***
P	121***	381***	375***	1.10	5.81**	19.8***
S x N	1.57	3.22**	0.24	1.31	1.18	0.49
S x P	1.21	2.96**	2.49	0.30	0.37	2.19
N x P	3.98**	5.17**	3.42*	2.32	1.82	1.40
S x N x P	2.33*	0.55	1.38	0.33	0.95	0.40

df = 2 for main effects, df = 4 for first-order interactions, df = 8 for second-order interaction. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Rhizome length

Cladium showed no significant variability in ramet distance (Fig. 4); total rhizome length of *Cladium* was also rather unresponsive to the treatments, except for the interaction N x P (Fig. 5). Ramet distance of *Typha* increased only after P additions (Fig. 4; $F = 6.78$, $P = 0.003$), with both medium and high P levels resulting in a longer distance than low P level (Tukey, $P < 0.01$). Its total rhizome length increased under nutrient-enriched levels (high N or elevated P levels compared to low N or low P; Tukey, $P < 0.001$), but decreased under high salinity (Fig. 5; Tukey, $P = 0.003$).

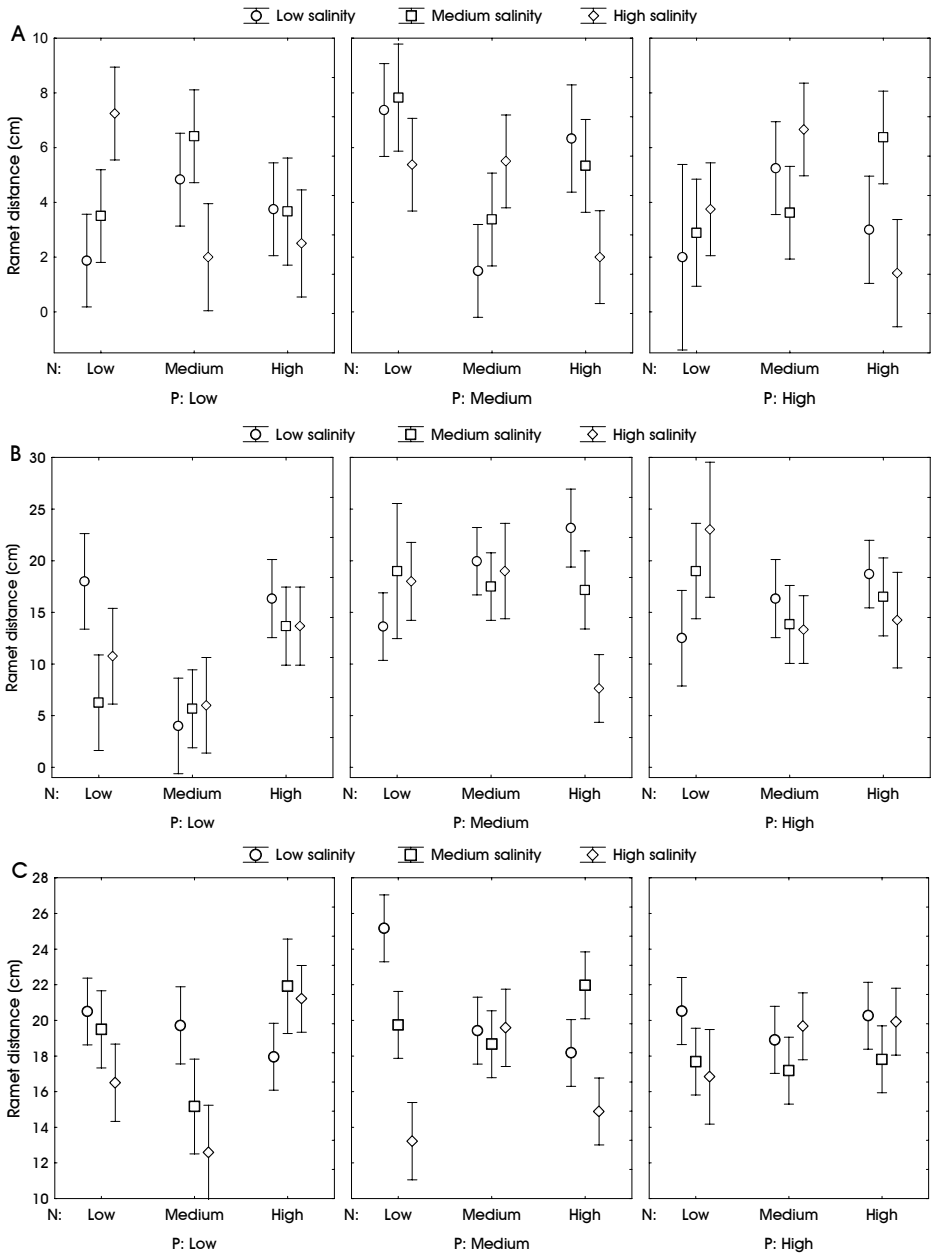

Figure 4

Mean values of ramet distance for *Cladium jamaicense* (A); *Typha domingensis* (B); *Eleocharis cellulosa* (C) in all experimental treatments. Error bars, mean ± S.E. Note different scales for each species.

The total *Eleocharis* rhizome length (Fig. 5) was related to ramet production, with a significant negative effect of high salinity (Tukey, $P = 0.009$), and positive effects of high N (compared with medium and low N levels; Tukey, $P < 0.001$) and medium and high P (Tukey, $P < 0.001$). Ramet distance of *Eleocharis* responded only to salinity with, salinity x N interaction also significant (Fig. 4; salinity, $F = 4.58$, $P = 0.014$; salinity x N: $F = 2.53$, $P = 0.048$), although the individual salinity levels were not significantly different from each other (Tukey, $P > 0.05$). The results of ANOVA of total rhizome length are shown in Table 1.

Nutrients

Nitrogen addition resulted in elevated tissue N content in *Cladium* and *Eleocharis*, but not in *Typha*. The effect of salinity on N content was also significant. Elevated salinity increased N content in *Cladium* and *Typha* leaves, but decreased it in *Eleocharis* tissue (Table 2; Appendix 1). Both medium and high P significantly increased tissue P content in all three species, but the magnitude of the increase in P content differed between species ($F = 26.0$, $P < 0.001$). Both *Cladium* and *Typha* accumulated less P in both P-enriched treatments than *Eleocharis* (Tukey, $P < 0.001$).

The N/P ratio was high for the low P level for each species, and dropped significantly at the medium P level. For *Cladium* and *Typha*, the N/P ratio was also slightly increased by salinity, mostly because of higher N uptake under higher salinity loading; salinity x N and salinity x P interactions were significant for *Typha* only. The effect of N was significant for *Cladium* and *Eleocharis.* The interactive N x P effect was significant for all three species (Table 2).

The PUE decreased significantly with medium and high P addition in all three species, while NUE decreased with N addition only in *Eleocharis* and *Cladium*.

We did not include a treatment with no N or P addition in the data analyses, to avoid problems due to unbalanced design. However, when comparing the lowest nutrient-addition treatment with the no-addition treatment, there was a positive effect of low N and P on most growth characteristics of *Eleocharis* and *Typha*, and belowground biomass only of *Cladium*. Despite the significant increase in growth, tissue nutrient content remained unchanged (results not shown).

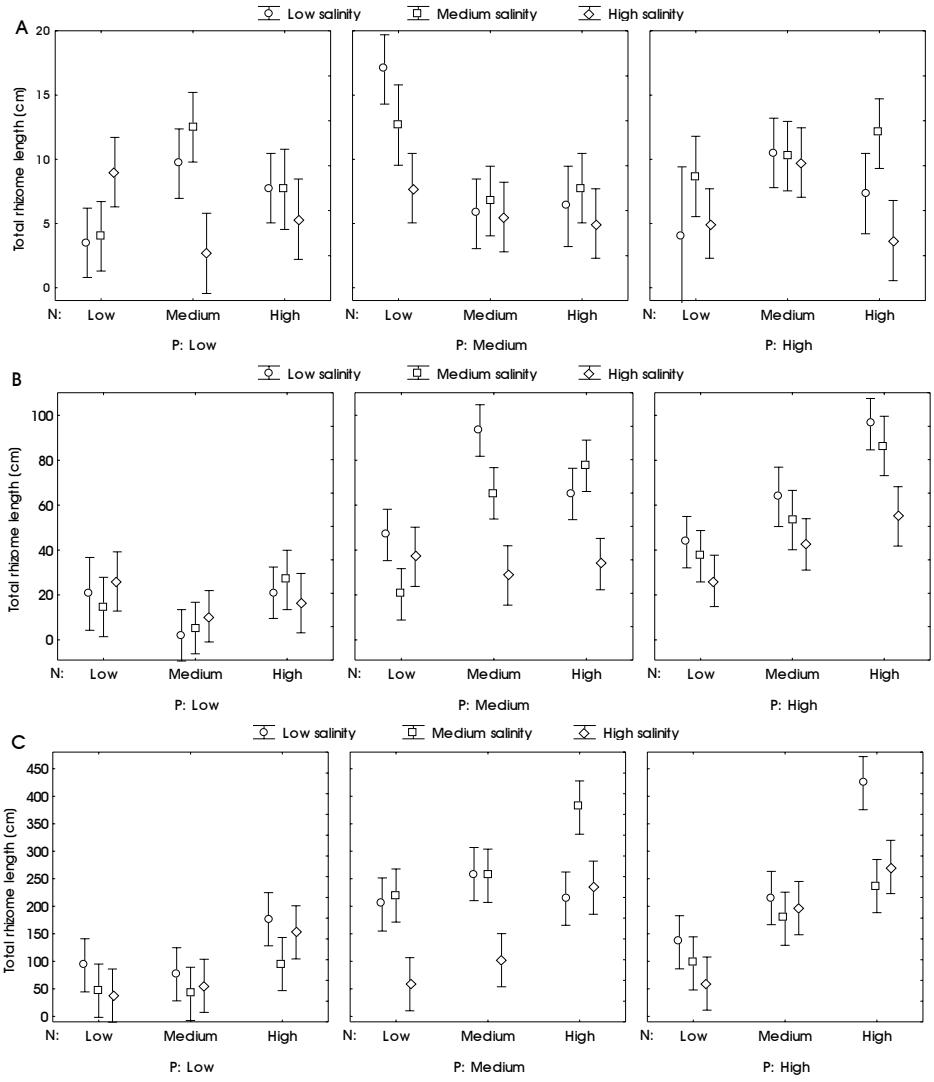

Figure 5

Mean values of total rhizome length for *Cladium jamaicense* (A); *Typha domingensis* (B); *Eleocharis cellulosa* (C) in all experimental treatments. Error bars, mean ± S.E. Note different scales for each species.

DISCUSSION

Higher availability of a limiting resource usually leads to an increase in the biomass of resource-acquiring structures (Alpert and Stuefer 1997). Root responses to nutrient addition in each of macrophytes studied were very different: while *Cladium* was little affected, *Eleocharis* and *Typha* invested heavily in roots. A limited response of *Cladium* to added nutrients can be considered typical behaviour of a plant adapted to low nutrients (Berendse and Elberse 1990). Our results differ from those of Daoust and Childers (2004), who suggested a possible quick response of *C. jamaicense* root production to low P enrichment (0.4 g m^{-2} $year^{-1}$). In their field experiment this response was significant after the first year; however, root production of *Cladium* remained unchanged after the second year of nutrient addition. The positive P effect on above- and below-ground biomass of *Cladium* has also been reported by Lissner et al. (2003); Miao (2004). Limited response of root biomass to nutrient increase is not unusual among wetland plants (Richards and Ivey 2004; Wolfer and Straile 2004). It can be argued that, for the slow growing macrophytes such as *Cladium*, nutrient uptake by their less extended root system is still high enough to supply their growth.

The conservative growth responses of *Cladium* contrasted with the more opportunistic behaviour of *Eleocharis* and *Typha*. The positive effect of nutrients on root, rhizome and above-ground biomass observed in our mesocosms has also been documented in numerous studies with *T. domingensis* (Chiang et al. 2000; Miao et al. 2000; Lorenzen et al. 2001) and *E. cellulosa* (Daoust and Childers 2004; Chen et al. 2005). An interesting feature that we observed, and that has also been reported by Lorenzen et al. (2001) in the Everglades, was the increase in fine root lateral production in *Typha* after P enrichment. By contrast, plentiful roots of *Eleocharis* remained of 'normal' diameter. Considering that the mobility of soil P is low (Verhoeven et al. 1996), we suggest that the quantitative response of root biomass is less important than that of root length or root area, increased mainly by fine root laterals. Therefore the production of fine roots should be quite advantageous for *Typha* nutrient acquisition.

Balancing biomass allocation between roots and shoots leads to an optimal strategy with respect to environmental conditions. All species in our

experiment invested more in shoots in response to nutrients. While *Typha* and *Eleocharis* reacted to additions of both N and P, *Cladium* responded to N only. These responses are in line with previous studies: the lack of a response of *Cladium* after P addition (an unaltered shoot/root ratio) was also reported by Lorenzen et al. (2001; but cf. Lissner et al. 2003), whereas numerous other studies with *E. cellulosa* and *T. domingensis* documented higher shoot/root ratios when enriched by P (Lorenzen et al. 2001; Chen et al. 2005). Although nutrient additions are unlikely to enhance root growth once nutrient supply is sufficient for leaf growth, it is perhaps surprising that *Cladium* did not increase its height (cf. Lissner et al. 2003). Furthermore, the average height of *Typha* and *Eleocharis* in elevated P levels increased by only 21 and 10 cm, respectively, which is minor compared with responses in the field. We hypothesize that the constant height of *Cladium* and relatively small height increase of *Eleocharis* and *Typha* were caused by the lack of competition for light – an artefact of the mesocosm experiment as compared with natural stands of these macrophytes. Although ramet production was supported by nutrient addition, shoot densities in pots were too low to cause light limitation. Competition for light would occurred as our long-term field experiment (Rejmánková, unpublished data) and would probably have occurred here, had our experiment run longer. Furthermore, we recorded enhanced structural support of *Typha* leaves and *Eleocharis* shoots (increased leaf width or shoot diameter) after P addition, showing that there is potential for these plants to grow higher when light limitation occurs. Although vertical growth of all cohabiting species will surely be promoted, we predict the plastic growth of *Typha* might lead to competitive exclusion of both *Eleocharis* and *Cladium*, as has happened to other subdominant species (Bret-Harte et al. 2001). To assess the biomass allocation of these species in experiments with controlled conditions we suggest using total leaf length and ramet production rather than simple plant height.

The species studied vary in their ability to monopolize their surroundings. In agreement with other studies (Brewer 1996; Lorenzen et al. 2001; Miao 2004), we found that *Cladium* was morphologically constrained and changed neither rhizome length nor branching frequency as a response to increased nutrients. Such architectural constraint (or fixity of rhizome growth) might be caused partly by the short duration of our experiment and by meristem determination early in ontogeny (Watson et al. 1997). However,

we believe the fixity in the case of *Cladium* rhizomes is better explained by its acclimation to a rather predictable and nutrient-poor environment, where a plastic response is not favoured (Alpert and Simms 2002). Branching is considered the most consistent plant response to higher nutrients (de Kroon and Hutchings 1995). Both *Typha* and *Eleocharis* increased branching when P was available, but only *Typha* increased internode length (rhizome length). The longest rhizomes of *Typha* were more than four times longer in high nutrients compared with those in low nutrient levels (data not shown). While *Eleocharis* and *Cladium* need to invest energy in regular ramet formation when growing horizontally, *Typha* produces fewer ramets and conserves energy. This strategy allows *Typha* to spread into unoccupied areas quickly and at relatively low cost, as well as to establish a net of new ramets by branching.

Increased salinity represented a significant stress to all three species and decreased their growth. But contrary to our expectations, while *Cladium* and *Eleocharis* were salinity-affected in most measured traits, *Typha* was responsive in only a few. Both above- and below-ground biomass of *Cladium* and *Eleocharis* decreased in high salinity, in agreement with studies on other wetland species (Morris and Ganf 2001). Plant height decreased equally in all species studied, constrained only by high salinity levels. In all three species, leaves (shoots) were also narrower in elevated salinity, suggesting that the loss of structural support in narrower leaves or shoots might contribute to lesser height growth. Rhizome growth was suppressed by salinity in *Typha* and *Eleocharis*, but not *Cladium*, again demonstrating its low rhizome length plasticity. Salinity did not affect ramet distance in *Typha*, but reduced it in *Eleocharis*. This might limit the rate of horizontal spread of *Eleocharis,* as the costs per unit length of ramet production increase.

Plants growing at higher salinity usually increase their growth when N is added (Mansour 2000). In a large number of our morphological variables we did not observe any significant response of salinity x N interaction, with the exception of *Eleocharis* height and leaf length. When salinity was elevated, there was no positive N effect on *Cladium* or *Typha*. Therefore for all three species, N addition, surprisingly, is not alleviating salinity stress in most cases.

Clonal growth is considered one of the means of achieving genetic longevity (Watson et al. 1997). In this way, all three species separately are successful, but their optimal ecological strategies differ as well as their

ecological preferences. Our results confirmed the good adaptation of *Cladium* to low nutrients (Lissner et al. 2003; Weisner and Miao 2004); *Cladium* can be characterized as a stress tolerator, relying more on acclimation to stress (Grime and Mackey 2002). Nevertheless, *Cladium* performance is reduced at high salinity. At the other end of the spectrum, *Typha* demonstrated its morphological plasticity in several traits in our experiment, and also in other studies (Lorenzen et al. 2001; Miao 2004). Its growth can be characterized as an opportunistic guerrilla strategy: in suitable conditions, it spreads fast and far. At the same time, it occupies space efficiently by branching and exploits resources through root production. *Typha* prefers elevated nutrient levels, and under high P levels, can also tolerate higher salinities. Finally, *Eleocharis* also shows rapid growth response to P, but with limited rhizome plasticity. Nevertheless, *Eleocharis* occupies space regularly and also exploits available resources efficiently. *Eleocharis* growth, however, is optimal under intermediate salinity levels.

In all three species, extreme increases in tissue P following P addition are in accordance with numerous results of other studies (Richardson et al. 1999; Lissner et al. 2003; Johnson and Rejmánková 2005), but very low P addition had little effect on *C. jamaicense* and *E. cellulosa* (Daoust and Childers 2004). However, we documented differences between species in P management: *Eleocharis* P tissue content was much higher when compared with both *Cladium* and *Typha*, suggesting that *Eleocharis* is capable of a large luxury uptake. Nitrogen is not a limiting resource in Belizean marshes (Rejmánková 2001). However, N addition resulted in higher plant uptake, probably as extra storage, for both *Cladium* and *Eleocharis*.

A mass ratio for wetland plants of N/P > 16 (indicating P limitation) and N/P < 14 (indicating N limitation) was established by Koerselman and Meuleman (1996). In our experiment, plants of all three species in low P were P-limited, but P limitation was removed by medium P addition. Similar results were documented for *E. cellulosa* and *T. domingensis* by Noe et al. (2001) and Daoust and Childers (2004). Finally, ash content correlated with P additions in all three species, which might be the result of their rapid growth and transpiration. These species manage their nutrients corresponding to their ecological strategies: from rather wasteful in *Typha* to more conservative in *Cladium* (N and P) and *Eleocharis* (P).

It has been reported that, for temperate North American wetlands, competitive exclusion occurs where nutrient limitation is missing (Bedford et al. 1999). Competitive exclusion also occurred widely in Everglades after P addition and consequent *T. domingensis* expansion (Noe et al. 2001). Although *Eleocharis* manages nutrients better than *Typha*, the success of *Eleocharis* in competition is unlikely under elevated P levels. When nutrients are abundant, competition for light becomes more important (Lepš 1999). Due to size asymmetry of light competition and lack of sufficient structural support of *Eleocharis* shoots, the species is overgrown by the strong, high and dense *Typha* monoculture (Johnson and Rejmánková 2005). A similar scenario is likely with *Cladium*, which is a rather good competitor under low P.

Numerous studies demonstrate that other factors (e.g. hydrology or fire) are also quite inefficient in suppressing *T. domingensis* (Grace 1988; Newman et al. 1998; Johnson and Rejmánková 2005). Hence we conclude that the only conditions that will allow the coexistence of all three studied species and ensure the stability of the whole ecosystem are those limiting *Typha*'s vertical and horizontal growth: low P and higher salinity. Under these conditions, there is a lack of strong competition for light, as plants are lower and vegetative spread is very limited. The maintenance of the oligotrophic status of the Belizean wetlands is therefore crucial.

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Appendix 1

The mean values ± S.E. of tissue N and P content (mg g^{-1}) under different levels of P, N and salinity for *Cladium jamaicense*, *Typha domingensis*, and *Eleocharis cellulosa*.

P	N	Salinity	*Cladium*		*Typha*		*Eleocharis*	
			N	P	N	P	N	P
		Low	10.9±0.1	0.52±0.01	9.7±0.9	0.59±0.11	13.9±1.3	0.83±0.20
	Low	Med	11.1±1.0	0.64±0.09	12.2±1.5	0.63±0.09	13.1±2.1	0.77±0.23
		High	13.8±2.0	0.61±0.11	12.6±1.5	0.32±0.05	13.3±2.2	0.79±0.26
		Low	12.0±1.7	0.73±0.03	11.9±0.9	0.72±0.06	15.1±1.2	0.65±0.10
Low	Med	Med	16.8±3.0	0.88±0.25	11.6±0.7	0.56±0.02	11.0±0.1	0.47±0.08
		High	15.3±1.6	0.82±0.10	12.5±1.7	0.61±0.07	13.7±0.9	0.77±0.12
		Low	13.2±1.0	0.69±0.05	11.9±1.1	0.57±0.09	17.0±0.6	0.53±0.03
	High	Med	12.3±0.4	0.71±0.03	10.8±0.8	0.55±0.12	13.4±0.7	0.59±0.14
		High	12.0±0.4	0.64±0.08	11.4±1.8	0.57±0.10	13.9±0.7	0.55±0.03
		Low	10.6±0.2	1.50±0.08	9.6±0.8	1.63±0.47	11.5±0.1	4.48±0.32
	Low	Med	17.1±2.8	2.22±0.35	9.4±0.7	1.25±0.14	14.3±1.5	5.84±0.96
		High	11.7±0.4	1.23±0.10	10.5±1.2	1.62±0.30	10.2±0.7	3.95±0.56
		Low	10.8±0.5	1.64±0.09	14.3±3.4	1.81±0.60	16.0±1.6	4.00±0.72
Med	Med	Med	11.3±0.6	1.34±0.05	13.7±1.5	1.26±0.22	14.6±0.8	4.91±1.30
		High	12.2±0.2	1.60±0.10	13.9±2.3	1.32±0.38	14.0±0.9	2.06±0.41
		Low	11.7±1.8	1.36±0.10	13.7±0.8	1.40±0.28	21.8±0.5	5.16±0.71

Appendix 1 (continue)

P	N	Salinity	*Cladium*		*Typha*		*Eleocharis*	
			N	P	N	P	N	P
Med	High	Med	11.9±0.8	1.10±0.11	15.1±0.4	1.40±0.19	16.5±1.0	3.21±0.39
		High	13.8±2.7	1.17±0.17	17.0±2.4	1.14±0.19	15.1±0.9	2.65±0.01
		Low	10.1±1.8	1.85±0.18	8.3±0.4	2.45±0.11	13.2±1.6	3.81±0.79
	Low	Med	12.6±1.8	2.02±0.19	8.7±1.5	1.45±0.23	13.2±1.9	4.90±0.64
		High	11.1±0.8	1.42±0.11	11.9±0.9	2.68±0.55	11.4±1.8	5.23±0.73
		Low	9.4±0.3	1.83±0.12	12.0±1.1	1.94±0.27	15.0±0.3	5.71±1.15
High	Med	Med	11.1±2.0	1.34±0.09	10.0±1.5	2.20±0.45	16.3±2.4	5.86±1.18
		High	12.5±0.6	1.72±0.15	13.5±1.1	2.30±0.58	13.9±2.1	6.94±0.86
		Low	10.4±0.9	1.91±0.11	13.3±1.0	2.04±0.20	17.6±2.4	6.47±0.63
	High	Med	16.9±3.2	2.30±0.45	13.5±1.1	1.83±0.28	15.4±0.2	4.92±0.52
		High	11.6±1.5	1.76±0.21	20.2±1.6	2.99±1.07	13.2±0.4	4.78±1.14

Second year of the fertilization of 10 x 10 m plots in a large field experiment. During fertilization and within next 48 hours, the plastic walls were installed to prevent nutrient leak from the plots. The picture was taken from NP plot and shows the N, P and Control plots behinds respectively. Location New, August 2002.

Wetland ecosystem changes after three years of phosphorus addition

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ABSTRACT

We used oligotrophic, P-limited herbaceous wetlands of northern Belize to assess how changes in nutrient availability impact species composition and ecosystem processes. The P, N, and NP enrichment plots were established in replicated marshes of three salinity levels to document potential salinity constraints. Addition of P or combination of N and P resulted in rapid switch from a microphyte (cyanobacterial mats, CBM) to macrophyte *(Eleocharis* spp., *Typha domingensis*) domination, while N addition did not have any impact. The switch was caused by significant changes in *Eleocharis* stem density and height, and consequently, the aboveground biomass, which increased from an average 120 g m^{-2} in control and N plots to > 500 g m^{-2} in P and NP plots. Decreased light under the dense canopy of *Eleocharis* in P and NP plots caused significant reduction in CBM growth. Biomass of *Eleocharis* in P and NP plots decreased with increasing salinity, but salinity did not affect biomass production in control and N plots. Tissue P of *Eleocharis* from P and NP plots increased 4- to 5-fold compared to P content in plants from control and N plots. Tissue P remained high due to internal nutrient recycling even after P addition ceased. *Typha* transplanted into plots grew exponentially in P and NP plots, while in control and N plots it grew slowly or did not survive. There were significant differences in NH_4-N both in soil extracts and in the interstitial water with soil and water NH_4-N being significantly lower in P-addition plots. The elimination of N_2-fixing CBM is

a potential reason for a decrease in available sediment N as documented by a negative correlation between CBM cover and interstitial NH_4-N.

Keywords
Belize - Cyanobacterial mats - *Eleocharis* - Eutrophication - Marsh - Nitrogen - Nutrient addition experiment - *Typha domingensis*

INTRODUCTION

Accelerated agricultural development in the tropics is increasing nutrient loading to aquatic ecosystems (Downing et al. 1999). This increased nutrient input is resulting in species composition changes, which are known to alter ecosystem function, such as primary production and nutrient cycling (Elser et al. 1988; Shaver et al. 2001; Vitousek 2004; Kerkhoff and Enquist 2006). Using results from a field nutrient addition experiment we document the shift in microphyte and macrophyte ratio leading potentially to a switch from P to N limitation. Changes in nutrient limitation resulting from shifts in land-use have been reported for a variety of ecosystems, although most are temperate (Vitousek et al. 1997; Downing et al. 1999). Whether N or P is the limiting nutrient depends on their availability relative to needs and on several internal processes including resorption from senescing tissues, organic material mineralization, and N_2-fixation (Aerts and Chapin 2000). A shift from N to P limitation caused by increased N loading have been reported more frequently than shifts from P to N limitation (Morris 1991; Jassby et al. 1994; Aerts et al. 1995), partially due to the widespread increase in atmospheric N deposition (Bobbink 1998).

Oligotrophic, P-limited marshes of Belize provide an ideal opportunity to study the ecosystem-level effects of combined nutrient and salinity changes. The limestone aquifer allows intrusion of saline water far inland, and as a result, the salinity of the inland wetlands spans a wide range. Wetland vegetation is similar across the current salinity gradient: several species of macrophytes co-occur with benthic and floating mats of cyanobacteria (CBM) that include a significant proportion of N_2-fixers (Rejmánková and Komárková 2000). Both macrophytes and CBM are strongly P-limited, but no N limitation has been detected (Rejmánková 2001; Sirová et al. 2006).

We established a fertilization experiment in replicated sets of marshes of low, medium, and high salinity to study the ecosystem level response to increased nutrient input. Our hypothesis for the long-term outcome of this experiment is: There is a salinity dependent switch between P and N limitation. The overstory/understory (macrophyte/microphyte) competition following P addition in *low* salinity marshes will constrain cyanobacterial N_2-fixation, which eventually will lead to N-limitation. In *high* salinity marshes, two outcomes are possible: A) P-input into the marshes will lead to increased macrophyte growth and consequent N-limitation; or B) P input will not alleviate salinity stress on macrophytes, but may promote the growth of cyanobacterial N_2-fixers and there will be no switch from P- to N-limitation. Additions of N alone will have little effect and additions of N and P will prevent a shift from P to N limitation.

Here we report changes in biomass production and nutrient composition of macrophytes, changes in sediment nutrient, and changes in aerial cover of CBM over the first three years of the duration of the experiment. Special attention is given to the establishment of *Typha domingensis* Persoon. In addition, we assessed changes in decomposition rates, and changes in sediment nutrients and processes (N mineralization rates, APA activity), but these have been or will be reported elsewhere (e.g., Rejmánková and Houdková 2006; Rejmánková and Sirová 2007).

Understanding the expansion of *T. domingensis* is of particular importance for the management of neotropical wetlands. This tall macrophyte is a strong competitor and formation of its monoculture often results in reduction of overall habitat diversity as documented, e.g., from the Palo Verde National Park in Costa Rica (McCoy and Rodrigues 1994). It is also a favorite habitat for an important Central and South American malaria vector, *Anopheles vestitipennis* Dyar and Knab (Pope et al. 2005; Rejmánková et al. 2006). Knowledge of the conditions that favor formation of favorable mosquito habitats helps in planning malaria control strategies.

METHODS

Study site

Our study sites are located in the lowlands of northern Belize. This part of the Yucatan Peninsula is an uplifted marine platform composed of a 2 to 3 km thick sequence of Cretaceous and Tertiary limestones and gypsum

(Weidie 1985). The marshes are mostly oligosaline (see Cowardin et al. 1979). Their hydrology is closely linked to the ground water system, and water levels are controlled primarily by regional precipitation patterns and ground-water discharge. A limestone aquifer allows intrusion of seawater far inland and, furthermore, the non-marine ground waters are nearly saturated with carbonate and sulfate derived from dissolution of the limestone, dolomite, anhydrite, and gypsum in the platform rocks. Due to these factors, the conductivity of the inland wetlands varies by several orders of magnitude and chemical analyses of ion content reveal large differences in sulfate, bicarbonate, and chloride. The climate of the Yucatan peninsula is tropical wet-dry. The majority of wetlands in the study area remain flooded or water saturated year round, although the total flooded area may vary as water levels rise and fall (changes of > 50 cm are typical). The water level changes become more pronounced during extremely wet or dry years. During the first three years of our experiment we experienced both an extremely wet event (tropical storm Chantal in late August 2001) and a series of low precipitation years 2003 and 2004.

The dominant primary producers in these systems are several species of emergent macrophytes (*Eleocharis cellulosa* Torr., *E. interstincta* (Vahl) Roemer & Schultes, *Cladium jamaicense* Crantz, and *T. domingensis*) and species rich communities of microphytes represented mostly by cyanobacteria (Rejmánková et al. 2004).

Until the mid-19th century, agriculture in Belize was rather insignificant (King et al. 1992). Sugar cane cultivation was established in the 1850's, but most expansion has occurred during the last 30 years and it is still rising. Fertilizer runoff from the sugar cane fields and other crops and increased population density contribute to continuing eutrophication of some marshes (Johnson and Rejmánková 2005).

Plot selection and nutrient application

Our experimental plots were placed in marshes that were located far enough from sugar cane fields and pastures to avoid fertilizer runoff. The 15 marshes representative of non-impacted wetlands of northern Belize were *a priori* grouped into low, medium, and high salinity categories (Table 1). The three categories were defined by water conductivity ranges of 0.2–1, 1–3

and 4–7 mS cm^{-1}. Marshes in these categories differed by their prevailing sediment type: peaty clay in low salinity, clay in medium salinity, and marl in high salinity. Before the experiment started, there were no differences among categories in water and sediment P content (Table 1), nor in the cover of CBM and biomass of *Eleocharis*. In each marsh we located four permanent plots (10 x 10 m) in areas with homogeneous vegetation and randomly assigned one of the following treatments: P, N, NP, and control. P, N, and NP addition was done once in August 2001 and the second time in August 2002. N was added as ammonium nitrate and P as triple super phosphate in the amounts corresponding to 20 g N m^{-2} $year^{-1}$ and 10 g P m^{-2} $year^{-1}$. These amounts represent an extreme scenario under which the total amount of fertilizer applied to a sugar cane field would be washed to the downslope marsh during a torrential rain (Johnson and Rejmánková 2005). Alternatively, a similar amount of nutrients can be expected to be delivered by wastewater if marshes are used for wastewater treatment (Vymazal et al. 1998). Before the nutrient application, walls constructed from heavy plastic were installed around each plot. The walls were left in place for 48 hours by which time the applied nutrients were already incorporated in various ecosystem components based on preliminary trials (E. R., unpublished data and see also Havens et al. 2004). Nutrients were applied in neither 2003 because of the decomposition experiment in progress nor in 2004 because of a severe drought.

Typha *planting*

Typha domingensis can establish by seeds or vegetatively by rhizomes. Since there was no *T. domingensis* establishment by seeds in any of the plots during the first 20 months of the experiment, and only one plot was invaded through vegetative spread, we planted one individual plant into each treatment and control plot in all marshes in March 2003. The individuals of uniform size (3–4 leaves, about 70 cm long) were transplanted from rhizome cuttings originated from the Buena Vista marsh. Plant number and size were assessed and tissue samples collected in August 2003 and again in February and August 2004.

Table 1

Characteristics of 15 marshes selected for nutrient addition experiment. Values represent means and standard deviations of four 10 x 10 m plots in each marsh for water conductivity, soluble reactive phosphorus (SRP), and NH_4-N, and mean soil P and N. Water depth column shows means and maximum and minimum values (in parenthesis) over the whole study period.

Marsh Name	Area, ha	Conductivity, mS cm^{-1}	Sediment Type	Water Depth, cm	SRP, ppb	NH_4-N, ppb	Soil P, mg cm^{-3}	Soil N, mg cm^{-3}
LOW SALINITY								
Frank	1.0	0.317 (0.077)	Peaty Clay	44 (140,-104)	2.3 (0.3	101.7 (8.6)	0.09 (0.01)	3.39 (0.20)
Big Snail	39.8	0.325 (0.113)	Peat	48 (135,-28)	3.9 (0.9)	57.5 (6.5)	0.04 (0.02)	5.05 (1.3)
Cane	3.9	0.646 (0.326)	Clay	30 (95,-153)	4.6 (1.6)	184.3 (31.3)	0.08 (0.01)	2.55 (0.43)
Deep	4.7	0.231 (0.068)	Peaty Clay	34 (96,-100)	5.4 (2.1)	226.9 (56.0)	0.09 (0.01)	3.88 (0.68)
Hidden	11.3	0.658 (0.164)	Marly Clay	30 (88,-88)	4.1 (1.7)	192.5 (29.4)	0.09 (0.01)	3.31 (1.01)
MEDIUM SALINITY								
Buena Vista	83.7	1.614 (0.599)	Clay	23 (70.-101)	8.6 (2.3)	48.9 (11.6)	0.04 (0.01)	2.53 (0.48)
New	78.9	2.992 (1.047)	Clay	27 (140,-115)	4.1 (1.6)	28.5 (6.4)	0.07 (0.01)	2.73 (0.32)
Quiet	68.5	2.374 (1.262)	Clay	24 (129,-124)	3.7 (1.1)	70.7 (6.6)	0.09 (0.01)	3.64 (0.37)
Calabash	4.3	2.161 (0.253)	Clay	30 (113,-147)	6.8 (1.5)	126.7 (32.2)	0.13 (0.04)	3.72 (0.90)
Elis	16.8	1.242 (0.253)	Clay	40 (155,-153)	9.3 (3.7)	152.6 (27.7)	0.07 (0.01)	2.40 (0.24)
HIGH SALINITY								
Doubloon	63.4	6.671 (1.479)	Marl	33 (190,-71)	12.5 (4.7)	58.4 (9.1)	0.10 (0.03)	2.16 (0.50)
Little Belize 7	18.2	5.667 (1.328)	Marl	48 (96,-54)	8.5 (1.5)	61.5 (48.9)	0.10 (0.01)	1.54 (0.03)
Little Belize 9	9.6	4.383 (1.040)	Marly Clay	36 (63,-100)	5.6 (1.5)	212.7 (62.1)	0.06 (0.01)	2.10 (0.38)
Littkle Belize 11	66.7	6.703 (0.600)	Marl	22 (34,-62)	7.6 (1.5)	138.9 (14.3)	0.03 (0.001)	2.30 (0.58)
Chan Chen	193.3	3.888 (0.910)	Marl	33 (127,-123)	6.1 (1.2)	153.5 (16.6)	0.11 (0.04)	3.68 (0.16)

Plant, soil, and water sampling and analysis

Plots were monitored and samples collected at the beginning of the experiment and then bi-annually (in the wet and dry season). Water depth was recorded and water samples collected and stored on ice until processing. A suction sampler was used to remove the interstitial water from the 10–20 cm zone of the sediment as described by McKee et al. (1988). Conductivity and pH were measured with Corning combined conductivity and pH meter. Samples for NH_4-N, NO_3-N, and PO_4-P were filtered through a 0.45 μm filter and frozen until analysis. They were later analyzed according to standard procedures (Hunter et al. 1993).

Plant height was measured and shoots were counted in 10 randomly selected 20 x 20 cm subplots in each plot. Live, flowering, and standing dead individuals were counted separately. Ten mature stems from each plot were collected, length measured, dried at 80 °C, and weighed to calculate specific stem weight (g cm^{-1}). The same plant material was ground on a Wiley mill and used for nutrient analysis. Since *Eleocharis* is structurally a very simple plant, leafless, consisting of green photosynthesizing spike-like stems, its biomass was easy to express as a product of average number of stems x average height x specific stem weight. Ten stems in each control and P plots of marshes #1, 4, 5, 6, 7, and 10 were tagged and repeatedly measured to assess the length of growth, mature and senescing phases.

Nutrient-use efficiency (NUE, PUE) was calculated as production divided by biomass N or P according to Harrington et al. (2001). Samples were analyzed for N and C content on a Carlo-Erba series 5000 CHN-S analyzer. Total P was analyzed spectrophotometrically using ascorbic acid reduction of phosphomolybdate complex after combustion and consequent acid digestion (McNamara and Hill 2000). Soils were sampled to a depth of 20 cm before the treatment started and then in 2003 and 2004 the 0–10 cm and 10–20 cm layers were analyzed separately. In each plot, five randomly located samples of the sediment were collected with a 5-cm diameter sharp edge PVC corer and kept refrigerated before analysis. Soil subsamples were oven dried at 105 °C for 24h to determine gravimetric water content. To obtain values of plant available N and P, field-moist soils were extracted with 1M KCl at 1:50 dry-weight to volume ratio (Reddy et al. 1998). PO_4-P and NH_4-N in the extracts were determined colorimetrically by the molybdate blue

method and NH_4-N was determined by a modification of the indophenol blue method by Liddicoat et al. (1975).

Data analysis

Hierarchical (nested) ANOVA with location (marsh) nested within salinity level was used to test the effect of environmental factors on response variables. To meet the ANOVA model assumption, we log transformed some of the variables to normalize the data. Data were further analyzed using the redundancy analysis (RDA) in the CANOCO package (ter Braak and Šmilauer 2002). Redundancy analysis could be considered as an extension of multivariate regression for a multivariate response variable (see Lepš and Šmilauer 2003). To evaluate the effect of environmental factors, we used the Monte Carlo test with 499 random permutations. Isoclines of conductivity and P content were passively projected to the ordination diagrams. The effects of environmental variables on plant biomass and the cover of CBM were evaluated using stepwise multiple regressions carried out in StatView 4.51 (Abacus Concepts 1996).

RESULTS

Response to nutrient addition

Addition of P alone or in combination of N and P resulted in significant changes in *Eleocharis* stem density and height, and consequently, the aboveground biomass (Figure 1). It also led to rapid elimination of cyanobacterial mats. There was no response to N addition alone. We conducted the redundancy analysis, RDA, to relate and visualize the relationship of response variables (plant biomass, plant height and density, plant P and N content, and the surface and bottom cover of CBM) to the explanatory variables, i.e., environmental variables. The major trend in data ran along the soil P gradient (see isoclines of P superimposed on the ordination diagram in Figure 2). It separated samples from control and N addition sites (left side of the diagram, < 0.1 mg P cm^{-3}) and P enriched sites (right side, > 0.16 mg P cm^{-3}). Plant growth characteristics showed a positive relationship with explanatory variables such as sediment and water P content and negative relationship with CBM cover. The Monte Carlo test with

forward selection of variables indicated significant conditional effects of sediment P (P = 0. 02), and interstitial water pH (P = 0.002).

Figure 1
Aerial view of a control and P-addition plot, Buena Vista marsh, March 2003.

To learn more about the response of *Eleocharis* biomass and CBM cover, we conducted the stepwise regression using the same environmental variables as in the RDA ordination. For *Eleocharis* as the dependent variable, stepwise regression eliminated interstitial water pH, NH_4-N, PO_4-P, and plant available P, and kept total soil P (positive effect), water conductivity, and depth (negative effects, Table 2). For CBM the stepwise regression revealed a very strong negative correlation with *Eleocharis* biomass and positive correlation with NH_4-N (Table 2).

Table 2
Results of stepwise regression analysis of dependence of *Eleocharis* plant biomass and cyanobacterial mat cover on environmental variables.

Variable	Standardized partial regression coefficient	*P*
PLANT BIOMASS		
Total Soil Phosphorus	0.754	**<0.001**
Water Depth	-0.380	**<0.001**
Water Conductivity	-0.372	**<0.001**
CYANOBACTERIAL MATS		
Plant Biomass	-0.546	**<0.001**
NH_4-N interstitial water	0.501	**0.020**

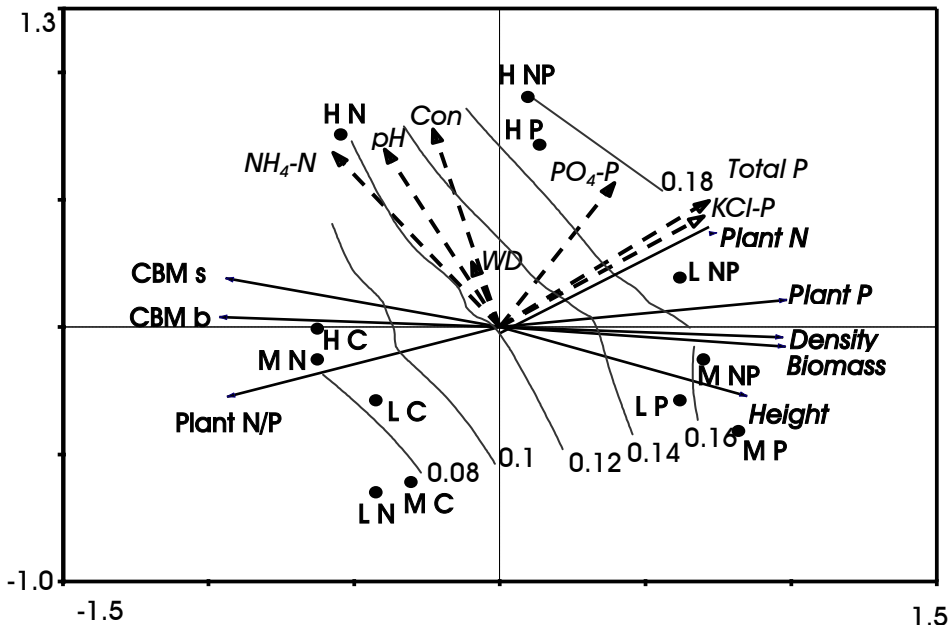

Figure 2

Redundancy analysis, RDA, triplot ordination. Experimental plots are indicated by full circles (labels composed of L, M, and H for low, medium, and high salinity, and C, P, N, and NP for control, P-addition, N-addition, and NP-addition). Response variables are indicated by small arrows and full lines, labeled as: Plant N, tissue nitrogen; Plant P, tissue phosphorus; Density, stems number m^{-2}; Biomass, dry mass m^{-2}; Height, average plant height; and CBM b and CBM s, cover of benthic and floating cyanobacterial mats, respectively). The environmental variables are indicated by large arrows and dashed lines labeled as: PO_4-P_i, interstitial water phosphate P; Total P and KCL-P, soil total and extractable P, respectively; NH_4-N, interstitial water ammonium N; pH, interstitial water pH; Con, interstitial water conductivity; WD, water depth). Isoclines indicate sediment P content 0.08 through 0.18 mg P cm^{-3}.

Biomass of *Eleocharis* over the three years (Figure 3) gradually increased in P and NP plots until August 2003 and then decreased in subsequent sampling periods. Hierarchical repeated measures ANOVA evaluating effects of P, N, salinity level, and time on biomass showed strongly significant effects of P and time, and a marginally significant effect of salinity

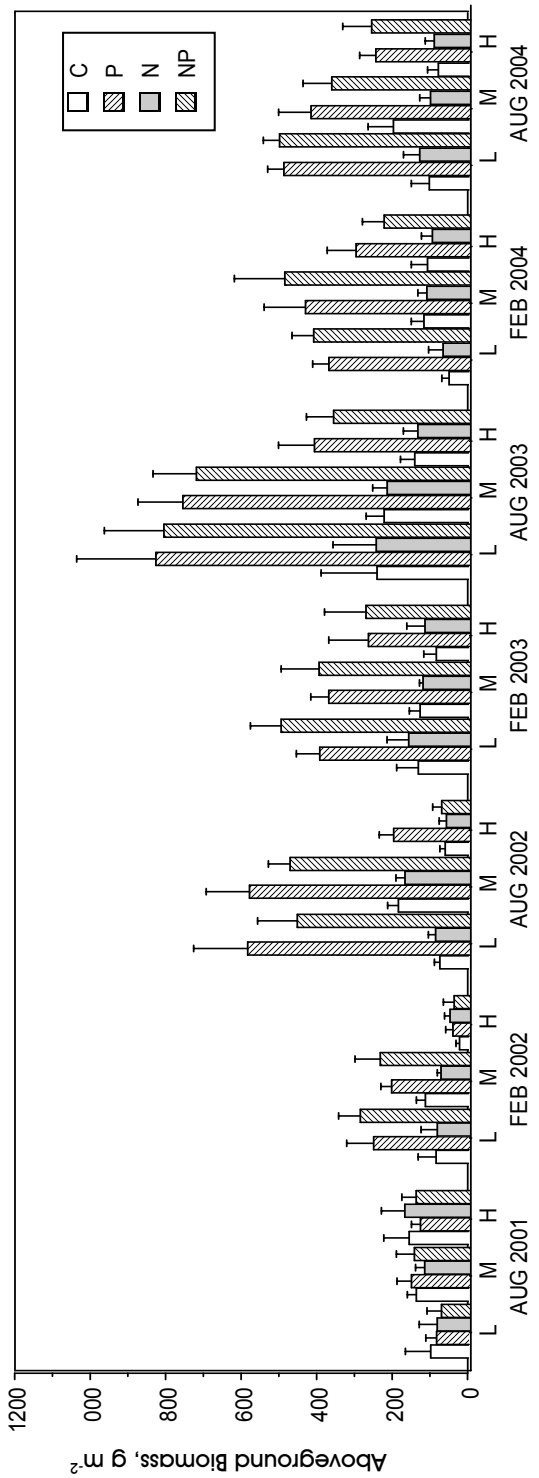

Figure 3
Aboveground biomass of *Eleocharis* (g DW m^{-2}) in plots of low (L), medium (M), and high (H) salinity before the treatment (August 2001) and following the nutrient addition. Vertical bars, mean + S.E.

(Table 3). The effect of salinity was highest in the sampling period following the first nutrient addition. Caution should be taken when interpreting this though, because the biomass in high salinity marshes was lower even in controls compared to pre-treatment levels (compare August 2001 and February 2002 in Fig. 3). A plausible explanation is that a tropical storm that hit Belize in late August of 2001 resulted in rapid increase in water levels that

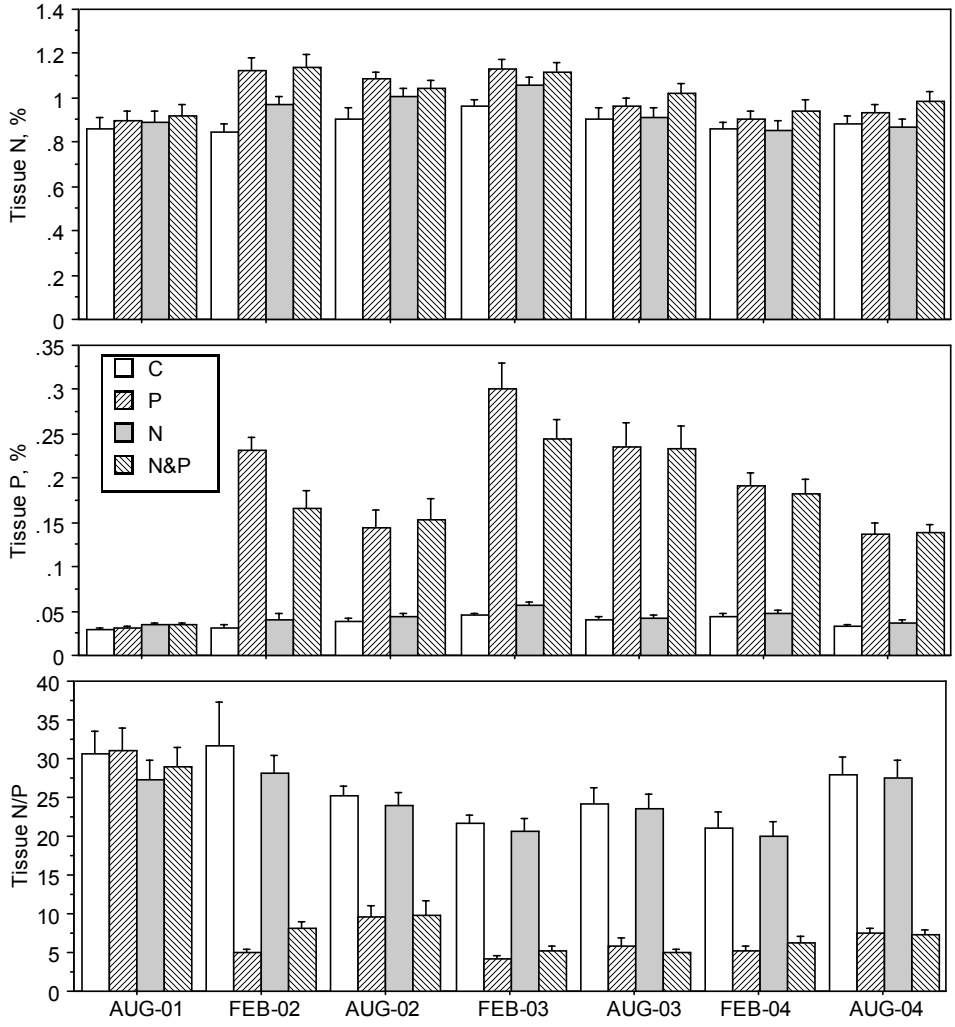

Figure 4

Tissue N and P and mass N/P ratio of *Eleocharis* before the treatment (August 2001) and following the nutrient addition. Values for individual treatments are averaged over all salinity categories. Vertical bars, mean + S.E.

flooded two of the high salinity marshes so rapidly that *Eleocharis* growth was not able to keep up with rising water levels. Interestingly, when we did the regression of *Eleocharis* biomass on salinity separately for plots that received P versus those that did not, the negative effect of salinity was apparent in P-addition plots only ($R^2 = 0.34$, $P = 0.001$) while control and N plots did not show any relationship between salinity and biomass.

Stem density, height, and consequently the plant biomass were strongly influenced by P addition, while there was no significant difference in specific stem weight. Stem density and stem height were also influenced by water depth. Stem height increased with increasing water levels ($R^2 = 0.421$, $P < 0.001$) while stem density decreased ($R^2 = 0.624$, $P < 0.001$). These two effects canceled one another when expressing the plant biomass. P addition significantly promoted flowering that increased from an average 8.9% and 8.6% in control and N plots, respectively, to 24.2% and 20.5% in P and NP plots, respectively (data not shown). There were only minor differences in the stem longevity among the treatments. On average, the growth phase (from stem emergence to reaching maturity) lasted 60 days, mature phase (before stems start dying off at the tips) lasted 30 days, and senescing phase (until 95% of a stem was dead) lasted 45 days. This means that stems in P addition plots grew faster because they were, on average, 22% taller.

The growth of CBM was significantly reduced in P and NP plots (Figure 1). Mean cover of CBM in C and N plots was 67.2% and 62.5%, respectively, and 6.9% and 7.8% in P and NP plots, respectively.

Tissue nutrients

The most remarkable response of tissue P was in the increase by *Eleocharis* from P and NP plots (Figure 4, Table 3). While P content in plants from controls and N plots averaged 0.041% (range 0.008–0.132%), it was 0.166% (range 0.02–0.505) in P addition plots. The P content was highest in the plants collected six months after the second nutrient addition (February 2003). It slightly decreased in the following sampling periods, but still remained about 4-fold higher than in controls. There was a slight increase in N content in plants from N plots at the 2nd, 3rd and 4th sampling dates, but it was smaller than the increase of tissue N in P addition plots. Higher N content of plants from P-enriched plots probably reflected more vigorous growth of plants in these plots. There was no effect of salinity on the tissue P and

N content (Table 3). The mass N/P ratio decreased from approximately 30 before treatments began to about 7 in P addition plots (Figure 6).

Nitrogen-use efficiency did not change with treatment and stayed in the range of 100 to 119 g biomass g^{-1} N. Phosphorus-use efficiency decreased 5-fold following the P addition, apparently due to the increased P concentration in plant tissue (Figure 5).

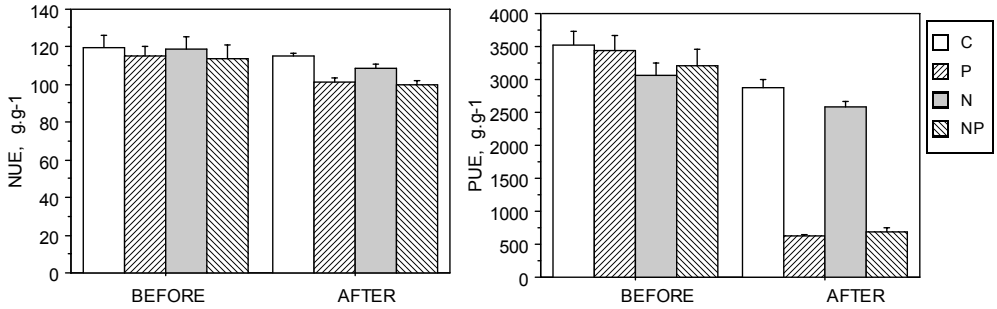

Figure 5

Nitrogen-use efficiency (NUE, g biomass produced per g N) and phosphorus-use efficiency (PUE, g biomass produced per g P) before the treatment (August 2001) and after the treatment. Values for individual treatments are averaged over all salinity categories; after the treatment values are also averaged over all the six sampling periods. Vertical bars, mean + S.E.

Typha *establishment*

With the exception of one plot (F1 NP), there was no spontaneous establishment of *T. domingensis* in any of the experimental plots during the 1.5 years of the experiment. A likely explanation for this is that *T. domingensis* only rarely flowers in the nutrient poor marshes of the study area and therefore potential establishment from seeds was limited. Survival rate of transplanted plants was initially high in all plots. However, plants in control and N plots were not growing very well, and by August 2004 they were surviving in only 30% of all control and N plots. In P and NP plots, on the contrary, *T. domingensis* showed an exponential increase (Figure 6). Individual plants from P and NP plots were significantly taller and produced more leaves and more new shoots than *Typha* in control and N plots.

Table 3

Results of hierarchical repeated measures ANOVA comparing the effects of salinity (S), phosphorus (P), nitrogen (N), and time (T) on biomass of *Eleocharis* spp., the cover of cyanobacterial mats, CBM, plant tissue P, and plant tissue N. Marsh nested within salinity level.

		BIOMASS		CBM			PLANT TISSUE P		PLANT TISSUE N	
Source	DF	*F*-value	*P*	*F*-value	*P*	DF	*F*-value	*P*	*F*-value	*P*
S	2	2.90	0.094	0.851	0.451	2	0.43	0.663	0.03	0.974
P	1	35.74	**<0.001**	352.34	**<0.001**	1	1387.7	**<0.001**	46.83	**<0.001**
N	1	0.31	0.587	2.44	0.143	1	0.05	0.821	10.54	**0.007**
T	6	7.09	**<0.001**	10.26	**<0.001**	5	15.53	**<0.001**	6.26	**<0.001**
S x P	2	2.95	0.091	2.29	0.143	2	19.9	**<0.001**	0.83	0.457
S x N	2	0.99	0.400	6.72	**0.010**	2	0.45	0.646	2.43	0.129
P x N	1	4.98	**0.045**	2.78	0.121	1	10.03	**0.008**	1.92	0.190
P x T	6	9.21	**<0.001**	15.43	**<0.001**	5	4.13	**0.003**	2.45	**0.043**
N x T	6	1.99	0.078	1.72	0.145	5	1.05	0.399	0.42	0.833

Table 4.

Results of hierarchical repeated measures ANOVA comparing the effects of salinity (S), phosphorus (P), nitrogen (N), and time (T) on sediment total P, plant available P, and soil extracted and interstitial water NH_4-N. Marsh nested within salinity level.

		SEDIMENT TOTAL P		PLANT AVAILABLE P			SOIL NH_4-N			WATER NH_4-N	
Source	DF	*F*-value	*P*	*F*-value	*P*	DF	*F*-value	*P*	DF	*F*-value	*P*
S	2	0.92	0.426	2.44	0.129	2	0.03	0.973	2	4.86	**0.028**
P	1	40.06	**<0.001**	11.29	**0.006**	1	6.62	**0.024**	1	22.97	**<0.001**
N	1	0.32	0.584	7.75	**0.017**	1	0.33	0.578	1	0.002	0.961
T	2	9.58	**<0.001**	10.99	**<0.001**	1	0.003	0.985	5	9.80	**<0.001**
S x P	2	0.94	0.416	2.36	0.136	2	0.98	0.403	2	1.62	0.238
S x N	2	0.09	0.915	0.06	0.945	2	1.63	0.236	2	0.183	0.834
P x N	1	0.03	0.872	0.61	0.452	1	0.001	0.998	1	0.12	0.739
P x T	2	5.64	**0.009**	12.31	**<0.001**	1	7.09	**0.020**	5	11.18	**<0.001**
N x T	2	2.45	0.107	0.48	0.624	1	4.75	**0.049**	5	0.07	0.996

Sediments

There were no significant differences in total soil P content among the marshes at the beginning of the experiment. Total N content was slightly higher in low salinity marshes (mean of 3.4 mg cm^{-3} for combined low and medium salinity marshes *vs.* 2.4 mg cm^{-3} for high salinity marshes; $P = 0.051$). This difference did not change over the duration of the experiment. The hierarchical repeated measure ANOVA for total nitrogen did not show any differences between treatment and control plots (data not shown). There was a significant effect of P addition and time on both the total P and plant available P. The salinity effect was not significant (Table 4). The total P in P-addition plots was more than double that of the pre-treatment values in August 2003, but decreased again in 2004 (Figure 7).

While total N did not seem to change significantly among treatments, there were significant differences in NH_4-N both in soil extracts and in the interstitial water (Figure 8, Table 4), with both soil and water NH_4-N being significantly lower in P-addition plots. The decrease of available N can be the result of higher plant or microbial uptake but more probably it was due to the elimination of CBM and its nitrogen fixing activity (see the positive correlation between CBM cover and interstitial NH_4-N, Table 2).

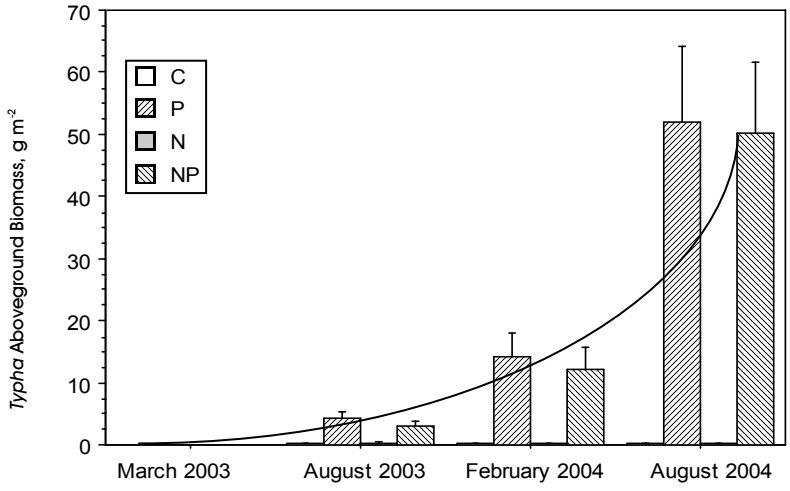

Figure 6

Changes in the biomass of *Typha domingensis*, g DW m^{-2}, planted in each plot in March 2003. Values for individual treatments are averaged over all salinity categories. Vertical bars, mean + S.E.

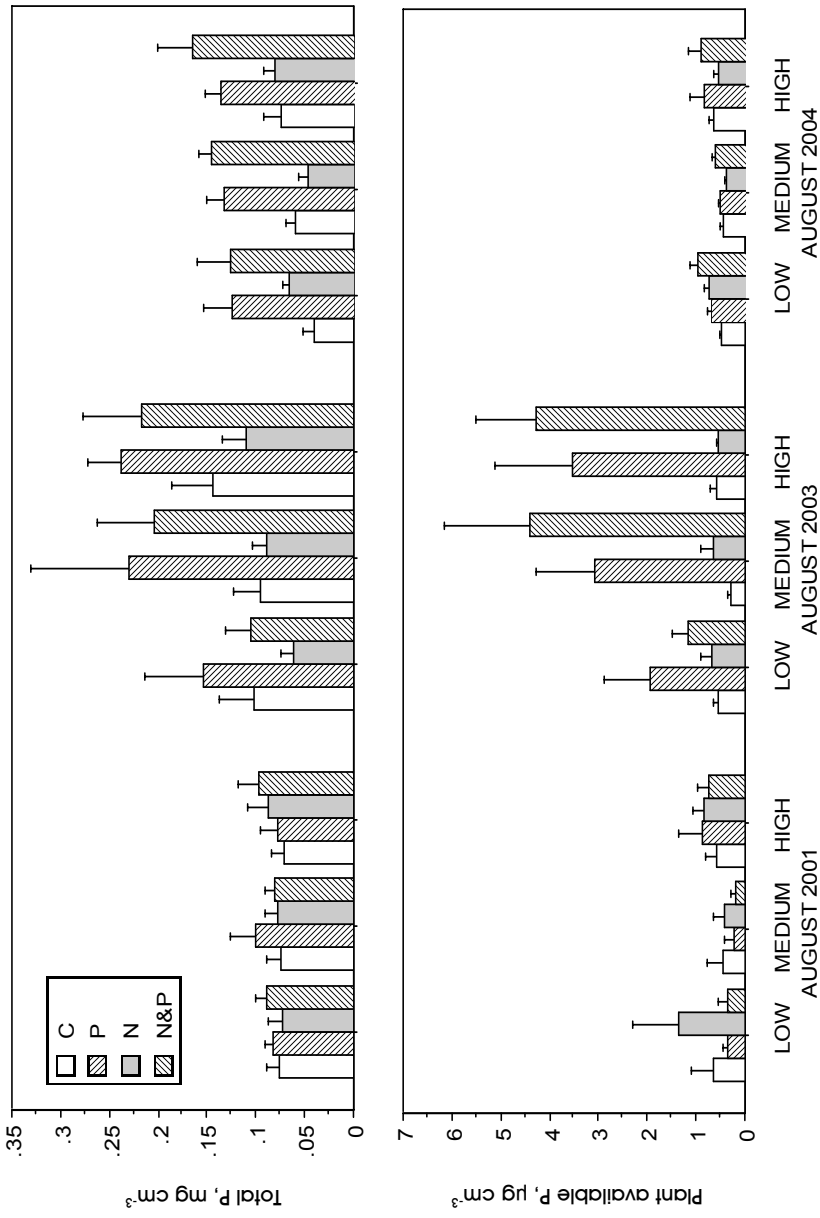

Figure 7

Values of total sediment P (upper figure) and plant available P (lower figure) before the August 2001 treatment and in August 2003 and August 2004 for plots in low, medium and high salinity categories. Vertical bars, mean + S.E.

Phosphorus budget

Phosphorus recovery in the sediments was quite high with no difference in P recovery among P and NP plots. In the two applications (2001, 2002) a total of 20 g m^{-2} P was added. The recovery was 53%, 78%, and 81% in low, medium, and high salinity marshes, respectively. There were no significant salinity related differences among P and NP plots in P recovered in plants, and it averaged 4.8, 3.7, and 2% in low, medium, and high salinity marshes, respectively. Nitrogen recovery was zero. Neither the total N nor NH_4-N content increased in N or NP plots (data not shown). We assume that majority of added N was lost via denitrification or leaching to lower sediment layers.

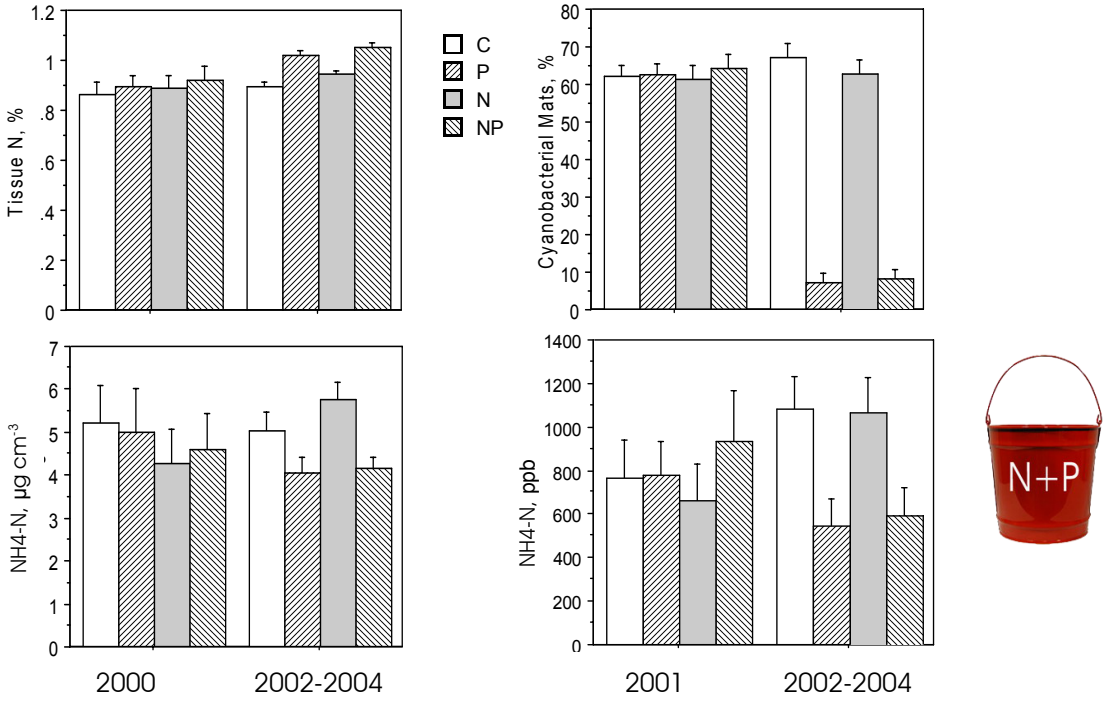

Figure 8.

A) *Eleocharis* tissue N, B) soil NH_4-N, C) cover of cyanobacterial mats, and D) interstitial water NH_4-N before the treatment (August 2001), and following the treatments (2002–2004). Values for individual treatments are averaged over all salinity categories. After the treatment, values are also averaged over all the sampling periods. Vertical bars, mean + S.E.

DISCUSSION

Results over the three year duration of this experiment mostly confirmed our predictions. The addition of P caused the switch in autotrophic communities from microphyte- to macrophyte-dominated. We found that although the macrophyte biomass production was lower in high salinity plots, the macrophyte cover was sufficient to shade out CBM. We found changes in sediment available N that may indicate the beginning of N limitation in P-enriched plots. We also demonstrated that there are important differences between the two potentially dominant macrophytes, *Eleocharis* spp. and *Typha domingensis.* Similar or contrasting responses to nutrient addition from similar wetland systems are discussed below.

Expansion of macrophytes

Eleocharis responded rapidly to P addition by increasing stem density and height and thus the overall aboveground biomass to about 3- to 4-fold of that of control and N plots. Dense cover of *Eleocharis* stems, both live standing and lodged senescent, creates an unfavorable environment for *Typha* establishment by seeds. However, it does not prevent its vegetative spread, once at least one *Typha* individual is established. Without nutrient limitation, *Typha* with its more complex architecture and better light utilization is capable to outcompete *Eleocharis* in 2–3 years.

Expansion of *Typha* in marshes formerly dominated by sparse *Eleocharis* with CBM can have serious consequences for public health in Central and South America throughout the range of *Anopheles vestitipennis* mosquito. This mosquito is a more efficient in malaria transmission than *A. albimanus* Wiedemann – a species commonly found in oligotrophic sparse *Eleocharis* and CBM marshes (Grieco et al. 2002; Rejmánková et al. 2006). A survey of spatial and temporal changes in mosquito larval occurrence found 82% of *A. vestitipennis* larvae in marshes dominated by dense *Typha*. The majority of these marshes were adjacent to sugar cane fields or pastures and have increased P levels in the sediments (Johnson and Rejmánková 2005; Pope et al. 2005). Whether these marshes have always been dominated by *Typha* or whether *Typha* became dominant as a result of P enrichment is unknown, but the second scenario is certainly conceivable.

Elimination of CBM

Both floating and benthic CBM were suppressed to less than 10% of original cover as soon as six months after the first P application. CBM are typical communities of "extreme" environments (Stal 1995) that do not respond favorably to nutrient increase. Decrease of cyanobacteria in the periphyton and the appearance of green filamentous algae have been reported from Florida wetlands (McCormick et al. 1998; Havens et al. 2004; Newman et al. 2004). Similarly, in our mesocosm experiment, the mats from P-enriched treatments were shaded out by a massive growth of phytoplankton, and were impacted by grazing (Rejmánková and Komárková 2005). Marshes of northern Belize are important reservoirs of cyanobacterial diversity with species richness closely related to the unique ecological conditions in Belize and other limestone regions of the Caribbean/Central America (Rejmánková et al. 2004). There is a whole suite of organisms, some already endangered, that are dependent on these marsh habitats (apple snail, snail kite, Morelet's crocodile, etc). Thus eutrophication of these ecosystems will result not only in creating public health problems but it will also pose serious threats to species and habitat.

Switch from P to N limitation

It is too early to determine that the switch of P limitation to N limitation is already happening, but the significant decrease in available mineral N in P-enriched plots dominated by dense *Eleocharis* as compared to CBM dominated controls and N plots provides indirect evidence. There are several potential explanations for this. 1) More N is tied up in the manifold higher biomass of macrophytes in P-enriched plots. 2) As a consequence of increased input of P, the CBM are eliminated due to the shading effect of dense growth of *Eleocharis.* Elimination of cyanobacterial N_2-fixation that contributes on average 5 g N m^{-2} $year^{-1}$ (Rejmánková and Komárková 2000) could be one reason for lower available N in P-addition plots. Unless heterotrophic N_2-fixers replace cyanobacteria in contributing N into the system, N will eventually become limiting. 3) Removing P limitation increases the overall bacterial activity in the sediments and thus more N can be tied up in microbial biomass. Indirect proof of the higher microbial biomass in P-enriched plots comes from our decomposition experiment where the content of phospholipid fatty acids, PLFA, (a reliable index of microbial

biomass) was 2–3x higher in enriched plots than in controls (Rejmánková and Houdková 2006). Newman et al. (2004) found in their long term mesocosm P addition experiment that at the highest P addition of 12.8 g P m^{-2} $year^{-1}$, corresponding to our dose, NH_4-N in the interstitial water was lower than in controls, which is in agreement with our findings.

Tissue nutrients and nutrient use efficiency

While N addition did not have any effect, P addition increased both N and P tissue content of *Eleocharis*. The increase in N was small, on average only 11%, yet significant, and can be explained by higher metabolic activity of *Eleocharis* in plots without P limitation. Similarly, Chen et al. (2005) found increased N concentration in *Eleocharis cellulosa* at higher P availability and Newman et al. (2004) reported an increase in N content in *Nymphaea* sp. at high P addition. Tissue P increase was rapid, rising to > 600% of the control plots value only one month after P addition, which is in agreement with the enhanced uptake of P reported in P-deficient plants exposed to a P pulse (Vance et al. 2003). A similar disproportionably large increase in P uptake after P addition was reported by Harrington et al. (2001) for the *Metrosideros* forest in Hawaii, where the reduction in PUE was much greater than corresponding increase in biomass production. The unusually high increase in tissue P was clearly due to luxury uptake. Plants from P-poor sites are capable of a large internal storage of P when it becomes available (Aerts and Chapin 2000; Güsewell and Koerselman 2002) and it appears that *Eleocharis* maximized this capability. The important point is that *Eleocharis* has a strong capacity for resorption of P before senescence (Rejmánková 2005) and high resorption continues even after P enrichment. Thus once a large amount of P is taken up by plants, it keeps being internally recycled and supports high biomass production even without further P additions. Differences in P content in *Typha* between control and P-addition sites were much smaller (0.08% control, 0.16% P and NP plots), which is more in agreement with published data for wetland macrophytes (McJannet et al. 1995; Svengsouk and Mitsch 2001; Güsewell and Koerselman 2002; Olivares et al. 2002).

There are many studies reporting the response of a similar ecosystem to nutrient addition in the Florida Everglades (Craft et al. 1995; Richardson et al. 1999; Chiang et al. 2000; Miao et al. 2000; Noe et al. 2001; Smith and

McCormick 2001; Daoust and Childers 2004 and others). Of these, Daoust and Childers (2004) provide an interesting comparison with our data, showing that timing and level of nutrient addition may lead to very different results. They reported results on the impact of continuous low-level P addition on characteristics of wet prairie and sawgrass communities. The Florida Everglades "wet prairie" is quite similar in species composition to marshes at our study site. They found decreases in longevity in P addition plots and decreases in tissue P content. Apparently, with a slow P input, the bacterial component of CBM (termed periphyton in Daoust and Childers 2004) outcompetes plants for additional P. When a large single dose of P is applied as in our experiment, microorganisms take up only a fraction of that amount and enough is left for macrophytes to show a rapid response.

Sediment P and P budget

The total P in P-addition plots was more than double the pre-treatment levels in August 2003, but decreased again in 2004. That decrease could have been caused by fire; many marshes burned in the spring of 2004 because of drought. Although inorganic P from ash may become available after burning, some of the ash is moved by wind and lost from the system (Handreck 1997). The P recovery was 53%, 78%, and 81% in low, medium, and high salinity marshes, respectively. The highest P retention in high salinity marshes could be also caused by the highest carbonate content in these marshes. Our budgets included only the upper 20 cm of soil, and some P could have been in deeper layers.

Conclusions

Our research facilitated quantification and improved understanding of changing structure and biogeochemical processes in sensitive marsh ecosystems that are being increasingly exposed to nutrient inputs from human activities. Our findings confirmed that P is the key nutrient influence on the structure and functions of both macrophytes and microphytes from Belizean oligotrophic marshes. Shortly after P addition, *Eleocharis* density and height significantly increased, while cyanobacterial mats were almost eliminated. *Typha* did not establish spontaneously but once transplanted into the P and NP plots, it has been growing exponentially, outcompeting *Eleocharis*. It did not survive or has been growing very slowly in control and N plots. Tissue P of

Eleocharis from P and NP plots increased 4- to 5-fold compared P content in plants from controls and N plots. Tissue P remained high due to internal nutrient recycling even two years after the last P addition. There were indications of a potential switch to N limitation, namely a decrease in interstitial water and sediment NH_4-N. However, study over a longer period of time and more insight into heterotrophic microbial activities are needed to clearly confirm this.

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The growth of *Typha domingensis* clone three years after establishment of a single individual into nitrogen and phosphorus enriched plot. Extension of *Typha* clone is 23 m from left to right. Location Calabash, 12th May 2006.

Dynamics of *Typha domingensis* spread in oligotrophic tropical wetlands following nutrient enrichment

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(Submitted)

ABSTRACT

Accelerated land use in tropical countries has increased nutrient input into wetland ecosystems. Higher nutrients often lead to changes of vegetation structure and, eventually, shifts in species dominance.

We studied a dynamics of species shift in a manipulative nutrient enrichment experiment (N, P, NP) in oligotrophic wetlands of northern Belize distributed along a salinity gradient. We monitored a spread and biomass accumulation of an introduced single individual of *Typha domingensis* within a four years period. We focused on speed of the spreading and the relative importance of neighbouring ramets in this process.

Large differences were found between control (C) and N addition plots versus P and NP addition plots. The ramets planted in C and N plots died or barely survived, while ramets in P and NP plots grew vigorously and almost completely outcompeted original vegetation represented by *Eleocharis* spp. Average numbers of ramets at the end of the experiment were 2 and 576 per 100 m^2 for C and N versus P and NP plots. The filling dynamics of P-enriched plots of differing salinity changed in time. The spreading was delayed in low salinity plots compared to high and medium salinity plots, although it finally reached comparable rates and values. We attribute this delay to originally denser vegetation and less suitable soil conditions in low salinity plots than to a direct salinity effect. Eventually, the

number of ramets stabilized and often even decreased due to self-thinning and insect damage.

Spatiotemporal model extrapolating observed vegetative spread suggested that in P-enriched conditions, a clone originating from a single individual is able to cover 1-ha plot completely within 9 years. We conclude that P-enrichment highly increase the possibility of fast take over of Belizean wetlands by *Typha domingensis*.

Keywords
Eutrophication - Insect damage - Light limitation - Nitrogen - Phosphorus - Rhizomes - Salinity - Self–thinning - Spatiotemporal horizontal growth model - Vegetative reproduction

INTRODUCTION

Oligotrophic wetlands in northern Belize belong to one of the largest wetland system in Central America covering extensive inland areas of the Yucatan peninsula. These marshes are generally dominated by native emergent macrophytes (*Eleocharis cellulosa, E. interstincta, Cladium jamaicense* and *Typha domingensis*) and species rich communities of microphytes represented mostly by cyanobacteria (Rejmánková et al. 2004). Under natural oligotrophic conditions, macrophyte species coexistence as well as coexistence between both macro- and microphytes is possible (Rejmánková et al. 2008).

A majority of Belizean marshes are P-limited and until recently, they have been relatively unaffected by human activities (Johnson and Rejmánková 2005). However, the major increase in agricultural, mainly sugar cane production during the last 30 years has resulted in fertilizer runoff from the fields and consecutive eutrophication of many marshes. In addition, increasing population density will probably exacerbate the situation by waste water discharge into the marshes. Nutrient additions, especially phosphorus (P), have been shown to have a dramatic effect on ecosystem balance of Belizean marshes (Rejmánková et al. 2008). The originally sparse macrophytes increase in height and density which leads to elimination of cyanobacterial communities. While no experimental evidence of macrophyte species switch has been reported from Belize, a switch in

macrophyte species dominance resulting from eutrophication has been repeatedly documented from a comparable system of the Florida Everglades (e.g. Newman et al. 1996; Chiang et al. 2000).

Typha species are generally fast growing when not limited by nutrients, and behave as strong competitors in many natural systems worldwide (Svengsouk and Mitsch 2001; Farnsworth and Meyerson 2003; Konisky and Burdick 2004; Boers and Zedler 2008). Among them, *Typha domingensis* has been shown to be a stronger competitor than both *Cladium jamaicense* and *Eleocharis* spp. due to its superior height and its opportunistic behaviour (Macek and Rejmánková 2007; Newman et al. 1996). The natural invasion of *T. domingensis* into nutrient enriched plots and its rapid spread within the plot was observed previously (Rejmánková et al. 2008). *Typha domingensis* has shown high morphological plasticity (Macek and Rejmánková 2007), which might further facilitate its invasion in nutrient enriched areas (Herr-Turoff and Zedler 2007). Furthermore, elevated nutrient conditions have been repeatedly linked to loss of plant species diversity in wetlands (Svengsouk and Mitsch 2001; Chiang et al. 2000; Boers et al. 2007). Hence, nutrient enrichment of marshes could have dramatic effect on species dominance and eventually also diversity.

In this study, we investigate the spread of *T. domingensis* (from now on *Typha*) in marsh ecosystems under different nitrogen and phosphorus regimes in a long-term field experiment. In particular, we ask how fast could *Typha* spread and what distance can it grow within a year? What is the role of neighbours and when self-thinning starts to occur? Is the salinity an important factor in *Typha* spreading?

Since the studied marshes are known to be P-limited, we expected to find the major differences between P-enriched and P-limited plots, with the effect of N addition being rather weak. We predicted that The P enrichment will strongly enhance *Typha* vegetative reproduction. We hypothesized that the positive effect of closest neighbours will be equally important in enriched and unenriched plots, however, the effect of more distant neighbours will be more pronounced in P-enriched plots only. Consistently, the negative neighbour effect resulting in self-thinning will occur only in P-enriched plots shortly after complete plot overgrowth. We expected the elevated salinity to restrict *Typha* growth.

MATERIAL AND METHODS

Study site

The study sites are located in wetlands of northern Belize, Central America. Marsh hydrology is closely linked to the ground water system, and water levels are controlled primarily by regional precipitation patterns and groundwater discharge with a typical water level variation of < 50 cm yearly. Water is rich in carbonates and sulphates, which in combination with sea water intrusion inland through a porous limestone forms a gradient in water salinity between the marshes. The marshes are rather oligotrophic with average soil P content = 0.358 mg g^{-1} (for specific values for all marshes see Rejmánková and Houdková 2006). An extreme drought event occurred during the time of the study resulting in burn out of all marshes and experimental plots (April 2005).

Plot selection and nutrient application

Fifteen marshes were selected: five in each low, medium and high salinity category, with ranges of conductivity values 0.231–0.658, 1.242-2.992 and 3.888–6.703 mS cm^{-1} respectively. In each marsh four permanent plots (10 x 10 m) were located in areas with homogeneous vegetation (*Eleocharis* spp.) and one of the following treatments: P, N, NP and control was randomly assigned. Nitrogen was added as NH_4NO_3 and phosphorus as KH_2PO_4 in the amounts corresponding to 20 g m^{-2} $year^{-1}$ and 10 g m^{-2} $year^{-1}$, respectively. The experimental plots were fertilized two times before *Typha* establishment (August 2001 and August 2002). No application was done either in 2003 because of the decomposition experiment in progress or in 2004 because of a severe drought. The nutrient addition continued in August 2005 and September 2006. Plastic walls were installed around plots while applying the nutrients; for detailed description see Rejmánková et al. (2008).

Typha *planting*

One *Typha* individual was planted into each treatment and control plot in all marshes in March 2003. Ramets of uniform size (3–4 leaves, about 70 cm long) were transplanted in the centre of each plot from rhizome cuttings originated from the natural stand in the Buena Vista marsh. Each ramet was transplanted into the centre of a plot where the above-ground

vegetation was previously removed from about 1 x 1 m area to prevent immediate competition with *Eleocharis* species. The spatial structure of horizontal growth was monitored in five successive years 2003 to 2007. The growth was monitored yearly using a measuring grid (cell size = 1 m^2) both within the fertilized plot (10 x 10 m) as well as out of the plots (10 m buffer zone).

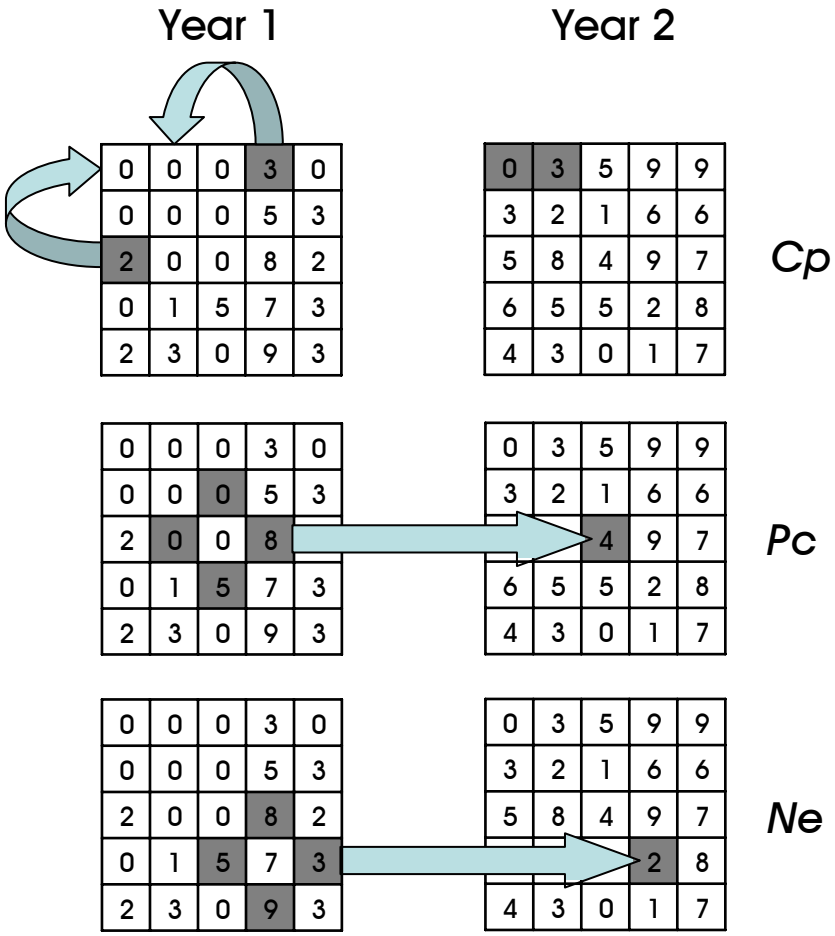

Figure 1

Calculation of indices: 1) *Cp*: The effect of nearest neighbour on "Colonization probability" (for empty cells with nearest neighbour 2 m distant), *Cp* = 0.5; 2) *Pc*: Power of colonization (for the neighbours distant up to 1 m), two values for Pearson correlation are: 13 and 4 in this case; 3) *Ne*: Neighbour effect (for the neighbours distant up to 1 m), two values for Pearson correlation are: 25 and -5. For calculation details see the text.

Indices of spread and data analyses

Several indices were calculated based on the data recorded in a measuring grid. First, a "Quantity change index" (*QI*) was calculated as a difference in ramet number in each cell between two consecutive years. The "Colonization probability" (*Cp*) was calculated as a proportion of newly occupied cells and was related to the distance of nearest neighbour, i.e. distance to the closest occupied cell in the preceding year with range of values from 1 to 7 m. To estimate a magnitude of this colonization, an index "Power of colonization" (*Pc*) was calculated as a Pearson's correlation coefficient of empty cells *QI* to the number of neighbouring ramets in various distances (1–7 m). Finally, to reflect the fate of already colonized cells, an index "Neighbour effect" (*Ne*) was estimated as Pearson's correlation coefficient of occupied cells *QI* to number of neighbouring ramets in various distances (1–7 m). Although its purpose is to reflect the effect of crowding and self-thinning, it is not possible to separate this effect from the Pc for these cells. To avoid an edge effect in indices calculations, these were calculated for cells in fertilized 10 x 10 m plot and the cells in buffer zone were used for neighbour counts only. Calculation of indices is shown in Fig. 1.

Data were first analysed using repeated measures ANOVA with all treatments being a separate factor; location was a factor with random effect nested within the salinity factor. However, since the N addition and all interactions of N with other treatment had always been highly insignificant (for total number of ramets see Table 1; and also Rejmánková et al. 2008), N addition treatments were combined with control and NP treatments were combined with P treatments in the further analyses. Some indices (*Cp*) were analysed with ANOVA for first year only (there was not sufficient number of empty cells in the subsequent years). Due to low number of control plots with surviving *Typha* ramets, the values in all three different salinities were pooled together for analyses of *Pc*. For *Ne* the P treatment was analyzed only. Then, for both *Pc* and *Ne*, repeated measures ANOVA were performed with both neighbour distance and year as separate repeated measures factors.

Calculated Colonization probability values (*Cp*) for different neighbour distances were then used to parameterize a simple stochastic model of clonal spreading. The spatio-temporal extrapolation covered 1-ha plot (100 x 100 m, i.e. a grid of 10 000 basic 1 m^2 cells) and the simulation was run for 10 steps (years). Each cell can be in two states, empty or

occupied. Similarly as in the experiment, the simulation started with empty grid with a single occupied cell in the centre. In each step, for each cell in the grid, the distance to the nearest occupied cell was calculated; this distance determined probability of cell being colonized. Whether the cell will be really colonized was decided by a random process: if a random number with uniform distribution between 0 and 1, obtained from a random number generator, was smaller than the probability of colonization, the cell was considered occupied, otherwise it remained empty. Similar randomization was used to specify cell vacation.

RESULTS

In terms of total ramet numbers, both salinity and P had significant effect, while the N effect was always highly insignificant including all interactions. The effect of time was, obviously, highly significant, but more importantly, so were the interactions of salinity x P x time and P x salinity (Table 1). The number of ramets in P-enriched low salinity sites was considerably lower in the first years of the study and eventually slowly increased, however, stayed behind the values at higher salinity P-enriched plots in the fourth and fifth year after establishment (Table 2). Analogically, the number of ramets in P-poor high salinity sites was higher at the first years and eventually decreased.

The values of *Cp* were significantly different between salinities as well as P treatments (i.e. salinity x P interaction; $F = 5.93$, $P = 0.005$) and the treatment combinations differed in relationships between *Cp* and distance ($F = 3.07$, $P < 0.001$; Fig. 2). In the P-enriched plots, *Typha* was able to spread up to 7 m from an occupied cell within a year, with most pronounced spread in medium salinity plots, where the cells within 3 m distance from an occupied cell had 80% probability to be occupied next year. On the contrary, in the P-poor plots, the spread was negligible, and generally did not exceed 2 m from the parent ramet.

Power of colonization (*Pc*) was significantly different between the treatments, neighbour distances and years (interaction treatment x distance x year; $F = 1.56$, $P = 0.016$) and similarly for *Ne* (interaction treatment x distance x year; $F = 1.84$, $P = 0.008$). In other words, *Typha* had much higher

probability to occupy neighbouring cells in P-enriched sites; between P-enriched plots, higher probabilities as well as more important effect of further

Table 1
Results of repeated measures ANOVA comparing the effect of N, P and salinity on total number of ramets in 10 x 10 m experimental plot.

Treatment	df Effect	df Error	*F*	*P*-level
Nitrogen (N)	1	12	0.029	0.868
Phosphorus (P)	1	12	89.09	**0.000**
Salinity (S)	2	12	9.897	**0.003**
Time	4	48	35.38	**0.000**
N x P	1	12	0.039	0.848
N x S	2	12	0.801	0.472
P x S	2	12	10.12	**0.003**
N x Time	4	48	1.675	0.171
P x Time	4	48	35.91	**0.000**
S x Time	8	48	4.956	**0.000**
N x P x S	2	12	0.744	0.496
N x P x Time	4	48	1.626	0.183
N x S x Time	8	48	1.044	0.418
P x S x Time	8	48	4.998	**0.000**
N x P x S x Time	8	48	1.040	0.420

Table 2
Typha domingensis ramets within fertilized plots: median values for different treatments of P enrichment and salinity.

Year /	P-limited			P-enriched		
Salinity	Low	Medium	High	Low	Medium	High
2004	0	0	3	13	429	148
2005	0	0	4	64	346	344
2006	0	0	2	217	1309	846
2007	0	0	4	234	810	644

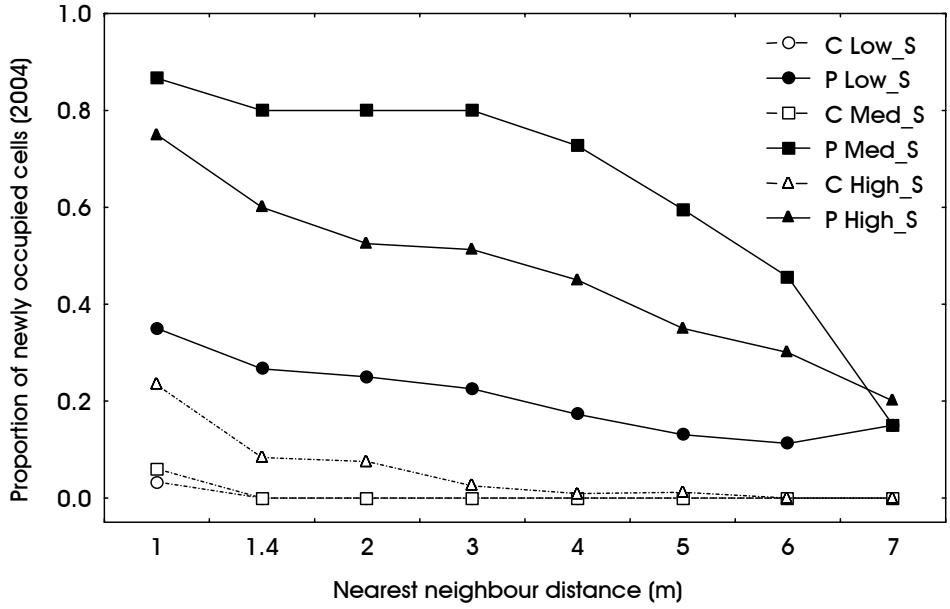

Figure 2
Median value of index *Cp* (Colonization probability, i.e. proportion of newly occupied cells related to the distance of nearest neighbour) of six different treatment combinations of salinity and P enrichment. Empty symbols represent the control P-limited plots, the full symbols represent the enriched plots. Circles, squares and triangles represent low, medium and high salinity plots respectively.

distant neighbours were in elevated salinity plots. Contrariwise, vacation probability was higher in control than P-enriched plots, although almost every P-enriched plot underwent selfthinning event within the study time. Low salinity plots experienced a similar boom in occupation and vacation in the last years of the study. For median values of both indices of different treatments see Fig. 3 and Fig. 4.

The model was able to realistically mimic the spread in corresponding plot; as we do not have any independent data than those used for model parametrization, the model can be considered validized, but not verified. Concerning P-enriched plots, the model resulted in a larger area covered by *Typha* compared to real data, however, this is incomparable since our P enrichment was spatially limited and the growth slowed down outside fertilized plots (Appendix 1). The simulations show three different patterns of

clonal growth spreading. The major difference was between fertilized and unfertilized plots, where the simulation resulted in much lower covered area in P-limited plots compared to P-enriched plots (Fig. 5). However, a certain distinction was also noticed between fertilized plots of low and elevated (i.e. both medium and high) salinity. Low salinity plot was overgrown less densely, but covered roughly the same area as elevated salinity simulation after 10 years of spreading (Fig. 5).

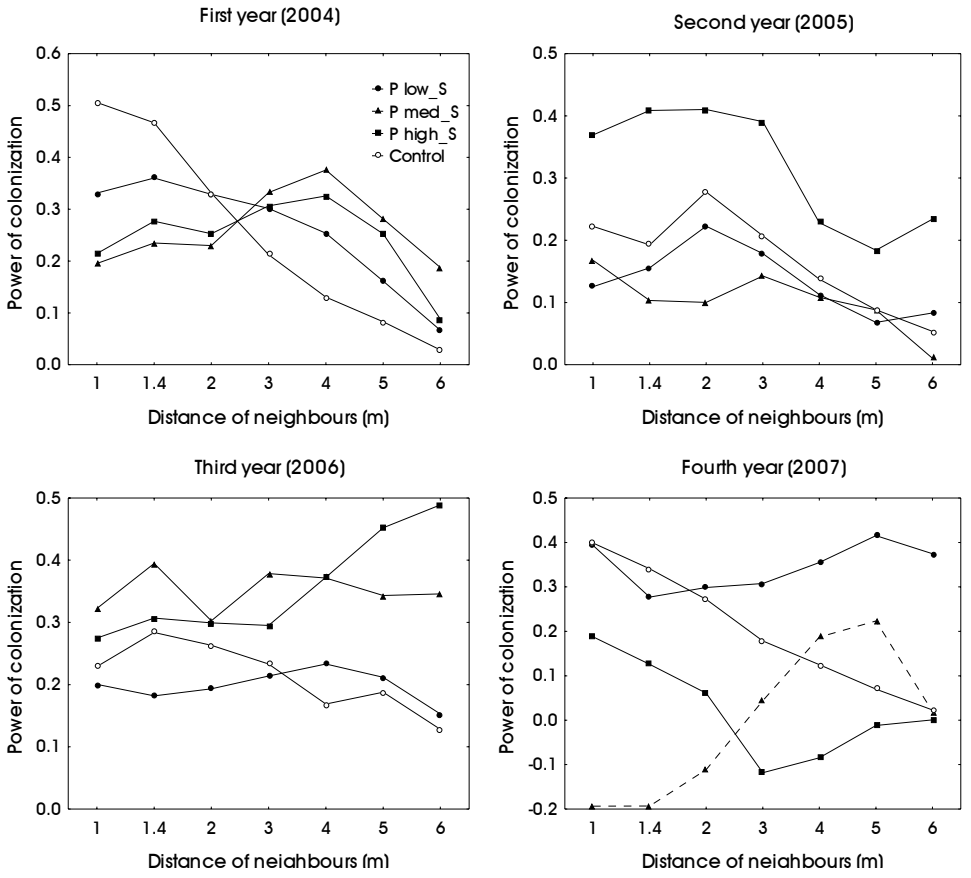

Figure 3

Median values of *Pc* indices (Power of colonization, i.e. Pearson's correlation coefficient of empty cells *QI* to the number of neighbouring ramets in various distances) of P-enriched plots (three salinities) and control plots. The index values in four successive years are shown. Empty circles represent the control (P-poor) plots, the full symbols of circles, squares and triangles represent low, medium and high salinity (P-enriched) plots respectively.

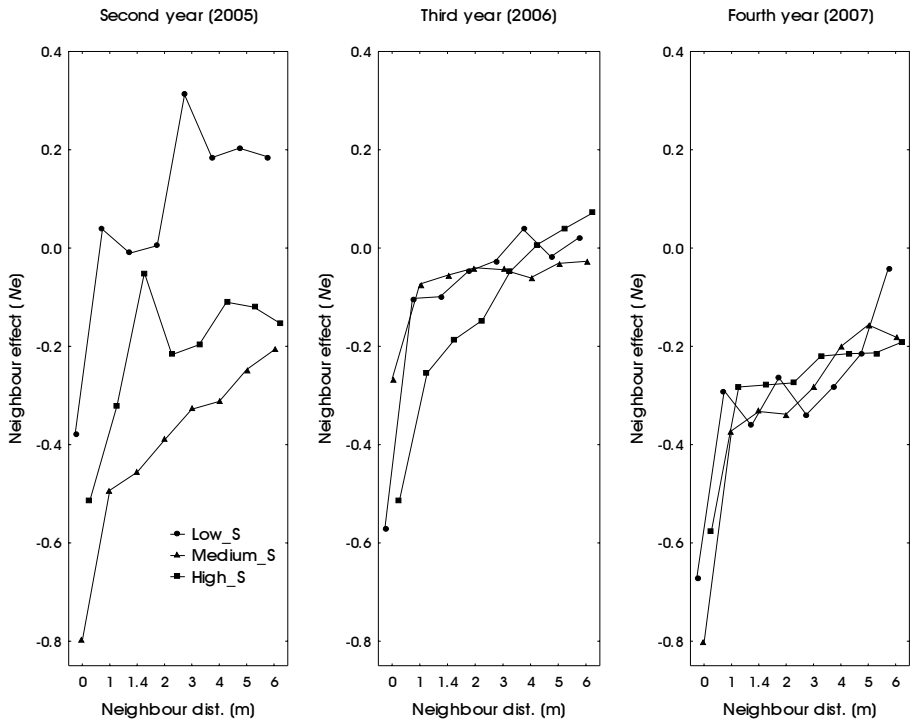

Figure 4

Median values of *Ne* indices (Neighbour effect, i.e. Pearson's correlation coefficient of occupied cells *QI* to number of neighbouring ramets in various distances) of P-enriched plots. The values in three years are shown. Circles, squares and triangles represent low, medium and high salinity plots respectively.

DISCUSSION

Not surprisingly, the most obvious difference in *T. domingensis* spreading dynamics was between controls and P-enriched plots. In P-limited plots a total of only 53 ramets survived in 5 out of 30 plots 5 years after *Typha* establishment. Contrastingly, *Typha* survived and spread vigorously in P-enriched plots and reached the maximum of 1613 ramets in a 10 x 10 m plot. Here, after 5 years, the maximum was 1613 ramets in 10 x 10 m plot. The difference in ramet height followed a similar trend: ramets in P-limited plots

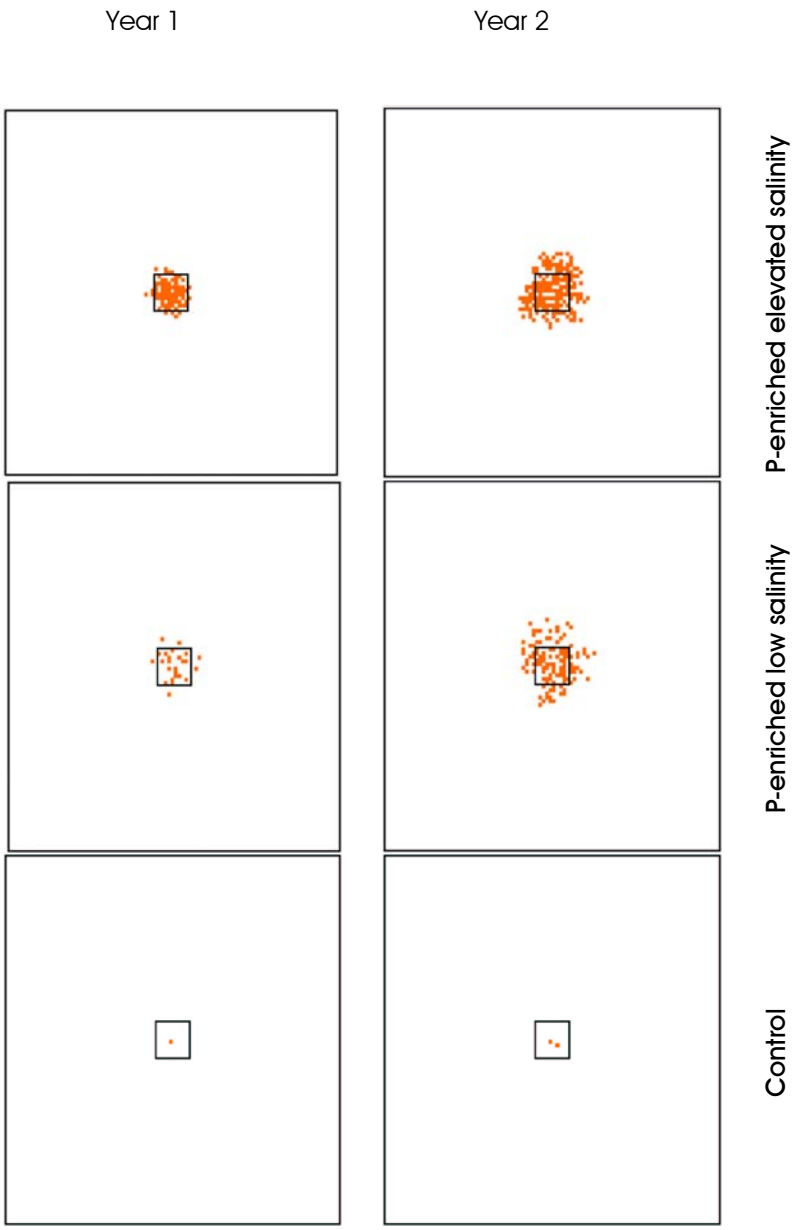

Figure 5 (part 1)

Figure 5 (part 2): Result of 1, 3, 5 and 10 years of clonal spreading of *Typha domingensis* in unfertilized and fertilized marsh (area of 100 x 100 m). The inner square represents a 10 x 10 m plot. The total number of 1 m^2 cells occupied by *Typha* was 37, 8290 and 9734 for control plot, P-enriched plots of low and elevated salinity, respectively.

did not exceed 2 m, while ramets in P-enriched plots reached up to 4.5 m (results not shown).

Based on our linear regression between plant height and dry weight ($R^2 = 0.912$, $P = 0.003$; Macek, unpublished results), and expecting average plant height of 3.6 m in P-enriched plots, we can estimate mean aboveground biomass of 0.47, 2.62 and 1.69 kg m^{-2} (dry weight in 2006) for low, medium and high salinity marshes respectively. Boers et al. (2007) reported even higher ramet densities for *Typha x glauca* stands, i.e. up to 80 ramets m^{-2}, 3 m tall. In our experiment, the maximal yearly production was 23 mature and 6 young individuals per m^2 resulting in approximately 5.0 kg of dry biomass increase per year, with values of 4.0 kg $year^{-1}$ commonly found in P-enriched plots in various other marshes (results not shown). This value is well above the values reported for numerous other freshwater marshes (Vymazal 1995).

When *Typha* individuals establish in P-rich marsh, their spread can be very fast (up to 7–8 m per year, compared to 2 m in P-limited plots). Furthermore, while the ramets in P-enriched marsh persist or establish offspring at the same place, the growth of *Typha* in P-limited plot is usually "rolling", i.e. energy expensive production of young ramet often results in mother ramet senescence and death. The spreading rates in our P-enriched plots are twice as high as values reported for *Typha x glauca* (Boers and Zedler 2008), but this could be also consequence of shorter growing season in temperate zone than in Belize.

The vigorous growth in P-enriched plots can potentially result in rapid overgrowth of large marsh areas. Specifically, a 1-ha plot can be covered by *Typha* within 9 years period especially in elevated salinities. This might affect not only macrophytes, which will be outcompeted by strong *Typha*, but also microphytic and animal communities of oligotrophic marshes. When comparing *Eleocharis* spp. dry biomass production in P-enriched plots with and without *Typha* presence, the differences were more then obvious ($n = 6$, $F = 12.09$, $P = 0.013$; Rejmánková, unpublished results). Comparisons could be made with some parts of Florida Everglades, where aggressive growth of *T. domingensis* outcompeted slow-growing *Cladium jamaicense* and other macrophytes (Newman et al. 1996). Similarly, an aggressive invader *Typha x glauca* can dominate and almost completely suppress native species in temperate wetlands (Boers et al. 2007). Also, the whole system functioning

was altered by different conditions provided by dense *T. domingensis* stands (Chiang et al. 2000). Such changes could affect also the invertebrate communities. Larvae of an efficient malaria vector in Yucatan Peninsula, *Anopheles vestitipennis* Dyar and Knab (Diptera, Culicidae), have been previously shown to be closely connected to *T. domingensis* stands (Grieco et al. 2007). Increase of P load into marshes could hence result in potential increase in malaria incidence.

While P addition resulted in vigorous growth, the N effect was negligible in all cases, which is not very common in wetland species (e.g. Güsewell et al. 2003; Darby and Turner 2008). Such discrepancy could be caused by very low P availability due to alkaline character of Belizean marshes and surplus of N caused e.g. by N fixing cyanobacterial communities (Rejmánková and Komárková 2000).

Although P addition was the most important factor, salinity level was also quite important. The elevated salinities (both our medium and high salinity categories) were always more suitable for *T. domingensis* growth. In the elevated salinity marshes, ramets survived longer (P-limited plots) and grew and spread faster (P-enriched plots). This could be however caused by several different factors and the salinity could play only an indirect role. First, although the plots were established in similar conditions in 2001, the situation was different in 2003 when ramets of *Typha* were introduced in the plots. At that time, the *Eleocharis* biomass in P-enriched plots was higher at low compared to high salinities (Tukey HSD test, $P = 0.016$; see also Rejmánková et al. 2008). Hence, a denser biomass in low salinity plots could have mechanically hindered *Typha* growth both above- and belowground. Outside of the 1 m^2 establishment plot with aboveground vegetation removed, light limitation was probably more severe in dense *Eleocharis* layer and shading further limited *Typha* growth. A second possibility causing variation in *Typha* growth could be a difference in between sediment types at different locations. While soils at low salinity plots usually have higher content of peat, the soils at medium and some high salinity plots contain more clay. Clay soils are typical for *T. domingensis* natural stands in Belize, thus its better growth in these soils is expectable. This explanation could be valid for some medium salinity marshes where *Typha* grows best. Although we also considered climatic factors (i.e. fire regime and water depth), we did not observe any consistent differences between plots of different salinities. Water

level usually rises in June/July or September and is closely related to hurricane season. Partial or complete drying of the marshes occurs usually in April/May.

An opposite effect, a decrease of ramet number in P-enriched plots, started to be noticeable in the second year after enrichment. Again, there are at least two plausible explanations of this self-thinning. Based on its traditional definition, a self-thinning is described as a density dependent mortality occurring in dense plant stands due to resource limitation (White and Harper 1970), in our case most probably by light. This is likely also the case of our P-enriched plots, since the density of mature ramets was often higher than 25 m^{-2}. Similar density limits were reported by Roberts and Ganf (1986) for *Typha orientalis*. Inoue and Tsuchiya (2006) however reported much higher density for this species. In addition to living plant biomass affecting both light availability and R/FR ratio, there is a large amount of standing dead biomass shading strongly the understory. Apart of self-thinning, accumulated biomass can be effectively removed by fire (mostly dead biomass) or by herbivory (mostly live biomass). Fires are usually not efficient in suppressing growth of *Typha* species (cf. Newman et al. 1998), several studies document even opposite effect (Urban et al. 1993). On the contrary the insect damage can effectively suppress *Typha* growth (Penko and Pratt 1986), especially, if the insect selectively eats young leaves and bore holes inside the plant. In our P-enriched plots we found *Bellura obliqua* (Noctuideae), cattail borer, causing this type of damage. This damage was observed since 2005 and resulted most probably in large openings inside dense stands. We did not find any insect damage either in our experimental P-limited plots or in natural dense stands of *Typha*. This could be due to higher palatability of *Typha* leaves from P-enriched plots. Also, the size of the ramet may play an important role: plants in natural stands have much smaller diameter at leaf base, which is possibly too small for last larval instar and hence its life cycle can not be completed. The insect damage could be potentially considered as a tool in *Typha* management and control.

The damage on *Typha* individuals caused by any of these factors and subsequent opening of dense stands was followed by an increase in *Eleocharis* presence (pers. obs.). However, this increase was probably enabled by small size of our experimental plots, surrounded by *Eleocharis* and consequently not limited by propagule availability. Potential of re-establishment of *Eleocharis* species in large P-enriched areas dominated by

Typha would be more complicated due to limited speed of clonal growth and relative distance of available seeds.

Conclusions

P addition greatly promotes *Typha domingensis* spread and its competitive advantage over *Eleocharis* spp., while N addition does not have any effect. *Typha domingensis* behaviour can be characterized as aggressive growth and *Typha* spreads its ramets to more than 7 m per year; this results in a potential overgrowth of a 1-ha marsh in a period of 9 years solely via clonal spreading. Eventually, the high biomass accumulation results in light limitation and/or insect damage and subsequent thinning of dense stands, however, *Typha domingensis* remains the dominant species. To prevent changes in vegetation character of Belizean marshes and therefore to keep suitable environment for numerous endangered species it is important to maintain the low input of nutrients, especially phosphorus.

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A young individual of limpkin, *Aramus guarauna*, caught in the close vicinity of patches of denser and taller *Eleocharis*. These patches and limpkins were at the birth of following chapter, since their occurrence was very obvious within this marsh. Location Quiet, April 2006.

Biological activities as patchiness driving forces in wetlands of northern Belize

Macek P., Rejmánková E. and Fuchs R.

(Submitted)

ABSTRACT

Patchiness in wetlands is a common and well documented phenomenon. Oligotrophic wetlands of northern Belize display noticeable vegetation heterogeneity at both large and small scales. In this paper, we document the small scale patches in herbaceous wetlands, describe differences between patches and surrounding wetland habitats and explain patch formation and sustenance.

We conducted a survey of patches and confirmed their occurrence by spatial analysis. Patches were distinguished from a surrounding wetland by denser and taller vegetation, higher amount of empty snail shells and elevated soil phosphorus (P). Plants in patches had higher tissue nitrogen (N) and P content and there was also higher total N and P per m^2 incorporated in plant biomass. In terms of stable isotopes, plants in patches were enriched in ^{15}N; patch soils were depleted in ^{13}C.

Observations of focal individuals of *Aramus guarauna*, limpkin, a wading bird feeding almost exclusively on snails, revealed the origin of the snail shell piles frequently found in patches. An adult limpkin captured on average 18 snails daily, of these 80% were handled in patches and birds often repeatedly used the same patch.

Experimental patch creation by adding chicken manure or P to 1 m^2 plots resulted in higher and denser vegetation with values increasing in order: control, P, manure plots. The effect was significant at both experimental

locations 6 months after the treatment and at one location even 40 months after the treatment.

We present a simple mechanistic explanation for nutrient redistribution in wetlands and their eventual accumulation in patches. Both nutrient and isotopic differences result from animal input into patches, e.g. bird droppings or prey remnants. Foraging activity of *Aramus guarauna* is most likely responsible for patch formation. A positive feedback (repeated use of a suitable patch) is apparently the mechanism sustaining patches in these marsh environments.

Keywords
Aramus guarauna (limpkin) - Bird dropping - *Eleocharis cellulosa* (rush) - Food webs - Oligotrophic neotropical wetlands - Nutrient redistribution - Phosphorus - *Pomacea flagellata* (apple snail) - Stable isotopes ^{13}C and ^{15}N - Vegetation heterogeneity and pattern

INTRODUCTION

Vegetation heterogeneity called patchiness is displayed in many wetland systems worldwide. In tropical and subtropical regions, among the most prominent examples of wetland patchiness are the Florida Everglades, Pantanal in Brazil, and Okavango Delta in Botswana (Loveless 1959; Prance and Schaller 1982; Diniz de Araujo Neto et al. 1986; Ellery et al. 1990; Wetzel et al. 2005). Both abiotic and biotic mechanisms causing patchiness have been documented, and in many cases the two mechanisms interact. The abiotic causes include raised topography in otherwise flat wetland, groundwater discharge or uneven soil conditions (Ellery et al. 1990; Sklar and van der Valk 2002). The biotic mechanisms include positive plant interactions or facilitation (Callaway 1995) or animal activities (DeOliveira 1992).
Oligotrophic wetlands of northern Belize, Central America are a part of the limestone-based complex of marshes that cover extensive areas of the Yucatan Peninsula. Large-scale patchiness is represented by small tree islands in otherwise macrophyte dominated marshes. In addition, prominent small scale patchiness is also apparent in many marshes. No information is known about causes and processes leading to the formation and sustenance of small scale patchiness in these wetlands. Belizean marshes are dominated

by emergent macrophytes of genus *Eleocharis*, with other species including genera *Cladium*, *Typha* and *Rhynchospora*. Among these, *Eleocharis* forms large homogeneous almost monodominant stands with a noticeable patchiness in form of denser and higher plant clumps conspicuous for their abundance of empty snail shells.

A large (mean ± S.D. aperture size = 27 ± 3 mm; mean ± S.D. shell length = 37 ± 7 mm) opercula apple snail, *Pomacea flagellata* Say, is an abundant inhabitant of Belizean wetlands. It feeds on plant tissue (Lege 2001) and behaves similarly to *Pomacea paludosa*, described from the Everglades (Sharfstein and Steinman 2001; Darby et al. 2002). *Pomacea* is the prey of numerous snail eating animals, including diverse avifauna. Among them, two bird predators are almost exclusive food specialists: *Rostrhamus sociabilis* Lesson, snail-kite and *Aramus guarauna* Vieillot, limpkin (Reed and Janzen 1999). While snail-kite piles the shells under its preferred perch, limpkin creates piles throughout a wetland. The majority of shells in these piles are perforated because, contrary to snail-kite, limpkin extracts snail tissue by a couple of hard blows to the shell (Tanaka et al. 2006), thus making these shells easily distinguishable.

A simple way to reveal different degree of processes happening within a nutrient limited ecosystem is a measure of nutrient concentrations in different system components. A use of ecosystem markers, e.g. stable isotopes, represents another elegant method how to indicate different processes in the ecosystem (Dawson et al. 2002). Although the most important nutrient in our case (P) lacks its stable isotopes, the use of other elements constituting living tissue (carbon, C and N) is feasible to trace the nutrient circulation in the system (Fry 2006). Measures of both ^{13}C and ^{15}N represent a method commonly used in nutrient circulation descriptions in tropical and subtropical wetlands (Fellerhoff 2002; Williams and Trexler 2006).

In this study, we will investigate the degree and scale of patchiness in selected marshes of northern Belize. We will then characterize differences between patch and surrounding wetland habitats and explain patch formation and sustenance. Through both the observations and manipulative experiments, we gathered data to support our working hypothesis: In the dry season, *Pomacea* snails cluster in shrinking water areas and are foraged upon by limpkins. The piles of shells are formed throughout the wetland. Bird droppings and prey remnants increase the nutrient status of these places.

The nutrients are eventually utilized by plants that then form denser and taller stands, which result in patches. We further expect that these bird activities are reflected into nutrient status and isotopic composition of both macrophytes and soils in patches in terms of $\delta^{15}N$ and $\delta^{13}C$ values respectively.

MATERIAL AND METHODS

Study sites

We selected three locations in a large wetland system in northern Belize. We conducted a patchiness survey in large Quiet marsh (58-ha; N18°10', W88°31'). We established manipulative experiments in Quiet marsh and Buena Vista marsh (75-ha, N18°14', W88°31'). None of these two large marshes was conducive to bird observations (for their large surface) and thus we observed the avian predator behaviour in a small Calabash marsh (4.9-ha, N18°27', W88°28').

The selected marshes are characterized by a dominance of *Eleocharis cellulosa* Torr. and *Eleocharis interstincta* (Vahl) Roemer & J.A. Schultes, the water conductivity in the range of 1000-1500 μS, and similar water level fluctuations ranging from completely flooded (up to 1.5 m) to almost completely dry. Sediments are composed of peaty marls with total N and P content of 3.3 mg cm^{-3} and 0.08 mg cm^{-3} respectively, indicating a strong P limitation (Rejmánková 2001). For a more detailed description of soil, hydrology, climate and vegetation of marshes of northern Belize see Rejmánková et al. (1996).

Patchiness survey

In 2002, we measured 60 patches and 12 control plots (2.25 m^2) in about 200 x 50 m area of Quiet marsh. We arbitrary defined a patch as a clump of dense vegetation with minimal size of 1 m^2, while control plots we randomly selected in between patches. We recorded vegetation height, live and dead stem density (averaged from three 20 x 20 cm counts) and collected and counted empty snail shells. Plant material for nutrient analyses originated from 12 randomly selected paired plots.

In 2006, we selected a 50 x 50 m area to quantify the patchiness on a precise scale (unit size: 1 m^2). In each unit, we recorded the average water

level and vegetation height, live and dead stem density per inner 20 x 20 cm and we searched and counted living snails and empty snail shells. We measured patch elevation as a distance between soil and water surface when all patches were flooded.

Limpkin observations

We observed focal individuals of limpkin from a tree observatory at the wetland edge in March - April 2004 and 2006. Total observation time was 63 hours of bird activities (episodic observations shorter than 20 min we excluded from the analyses). We could not avoid repeated observations of same individual; however, we observed a minimum of 14 different bird individuals (sighted simultaneously in the marsh area). We distinguished five main activities such as (1) resting (i.e. passive standing with no moves), (2) moving (i.e. walking through vegetation but without searching for prey), (3) foraging (i.e. active searching for snails in various vegetation types and returning with prey; foraging differs from moving by low speed of the steps and probing moves), (4) eating (including prey handling) and (5) grooming (e.g. preening, feather ruffling, bill cleaning). We recorded limpkin activities, their duration and location in a wetland (i.e. open water, sparse vegetation, dense vegetation, patch and *Cladium* thicket). Sparse vegetation is characterized by scattered *Eleocharis* shoots (< 150 shoots m^{-2}) with large portion of open water. Dense vegetation consists of small portion of open water and regular cover of *Eleocharis* shoots. A patch is a clump of dense vegetation (> 500 shoots m^{-2}) with no open water and *Eleocharis* shoots elevated over surrounding vegetation. *Cladium* thicket represents tall dense vegetation consisting of different species dominated by *Cladium jamaicense*.

Experimental patch creation

We selected two 30 x 10 m areas with homogeneous *Eleocharis* for experimental chicken manure and P addition in two different wetlands in August 2003. The areas consisted of 60 permanent plots (1 m^2) with 1 m buffer at each side. We randomly assigned them one of each treatments, manure, P and control, and treatment had 20 replicates. We installed plastic walls around each plot before treatment application and we left them in place for 48 hours by which time the applied nutrients were already incorporated in various ecosystem components (Rejmánková 2001).

We added chicken manure (250 g fresh weight) simulating bird droppings with the total P content of 1.6 g and 3 g of P (as KH_2PO_4) into plots in a single addition. The amount of chicken manure we chose as an equivalent of 75 g fresh weight of droppings from a rooster nourished by snail meat (Macek pers. obs.). We recorded water depth and vegetation height as well as the number of live and dead shoots per 20 x 20 cm in each plot prior the treatment, 6 and 40 months (in 2004 and 2006) after treatment application. We carried out the last measurement at Buena Vista location only, because fire damaged permanent plots at Quiet marsh.

Nutrient analyses and isotopic composition

Shoot tissue and chicken manure we dried at 70 °C, ground and assayed for total N with the Perkin Elmer HCN analyser. Total P we measured spectrophotometrically using ascorbic acid reduction of phosphomolybdate complex after combustion and consequent acid digestion (McNamara and Hill 2000). For comparisons of PO_4-P content in soil of 12 patches and 12 control plots we used anion exchange membranes (membrane type 204-U-386 Ionics, Watertown, MA; Cooperband and Logan 1994). We exposed the membranes (25 x 25 mm, three replicates) vertically in sediments (10 cm deep) for 11 days. After the exposure, we rinsed the membranes in H_2O and kept moist and refrigerated until processing. We extracted PO_4-P from anion exchange membranes using 1M NaCl, then we analysed it spectrophotometrically by ascorbic acid reduction of phosphomolybdate complex (American Public Health Association 1985).

For isotopic composition we analysed the oven dried samples of four important marsh constituents: emergent macrophytes (*Eleocharis cellulosa*), soils originating from below sparsely vegetated marsh and below patches (upper soil layers without plant remnants, 1–5 cm deep), apple-snail tissue and snail eating bird droppings. We analysed carbon and nitrogen isotope ratio ($\delta^{13}C$ and $\delta^{15}N$) using an elementar analyser (EA 1110, ThermoQuest, Italy) linked to isotope ratio mass spectrometer (Delta^{plus} XL, ThermoFinnigan, Brehmen, Germany). The obtained $^{13}C/^{12}C$ ratios of all samples, Rp, we referenced to $^{13}C/^{12}C$ of standard VPDB (Vienna-Pee-Dee Belemnite), Rs, and expressed as $\delta^{13}C = (Rp/Rs - 1) \times 1000$ in ‰. We expressed $\delta^{15}N$ for $^{15}N/^{14}N$ ratios (referenced to atmospheric N_2) in a similar way. The standard deviation

of $\delta^{13}C$ and $\delta^{15}N$ determination in standard samples was lower than 0.1‰ and 0.2‰, respectively.

Data analyses

For patchiness quantification we used Spatial Analysis by Distance Indices (SADIE) method (Perry 1995). In this method, we counted the clustering index v_i for each unit as a measure of the degree to which the unit contributes to clustering, as a member of a group of donor units that constitute a patch (positive clustering). Similarly, we counted the clustering index v_j to measure the negative clustering, presence of a gap (Perry et al. 1999). We based our tests on 45 random permutations. We considered the absolute indices values greater then 1.5 as contributing to cluster formation and the values close to unity indicating random placement of the unit in relation to others nearby. The other patchiness survey data we analysed using regression and Student's *t*-test. For manipulative experiment evaluation we used ANOVA with *post-hoc* comparisons among treatments (Tukey HSD test). Finally, we used PCA (Principal Component Analysis) in the CANOCO package (ter Braak and Šmilauer 2002) to visualize limpkin activities together with their timing in the day and location in the wetland vegetation. Each focal sample of continuous activity constituted a row in input matrix (when one activity took place in two distinct locations, it was considered as two rows), resulting in total of 382 rows.

RESULTS

Patchiness survey

The number of live *Eleocharis* shoots in the 50 x 50 m plot showed highly significant patch and gap arrangement ($P < 0.001$). Mean clustering indices v_i and v_j were 3.13 and -3.32 respectively. Gaps were present on 48% of the area, patches covered 16.5% of the area, the rest, 35.5%, was randomly covered by live shoots (Fig. 1). Surveyed patches were characterized by denser *Eleocharis* shoots, both live ($t = 7.05$, $P < 0.001$) and dead ($t = 4.87$, $P < 0.001$; Fig. 2). The shoots were also taller than those in control plots ($t = 10.93$, $P < 0.001$). Shoot height and shoot density were positively correlated ($R^2 = 0.53$, $P < 0.001$). The amount of empty snail shells in patches was an order of magnitude higher than in control plots, (means: 21

and 2 shells m^{-2}, respectively; $t = 4.56$, $P < 0.001$). No trends were found in numbers of living snails (data not shown). Average patch size was 2.5 m^2. Patches were slightly elevated (2.9 cm) over control plots ($t = 3.37$, $P = 0.006$). Soil PO_4-P was significantly higher in patches than in control plots (means: 0.231 and 0.024 μg membrane^{-1}, respectively; $t = 4.45$, $P < 0.001$).

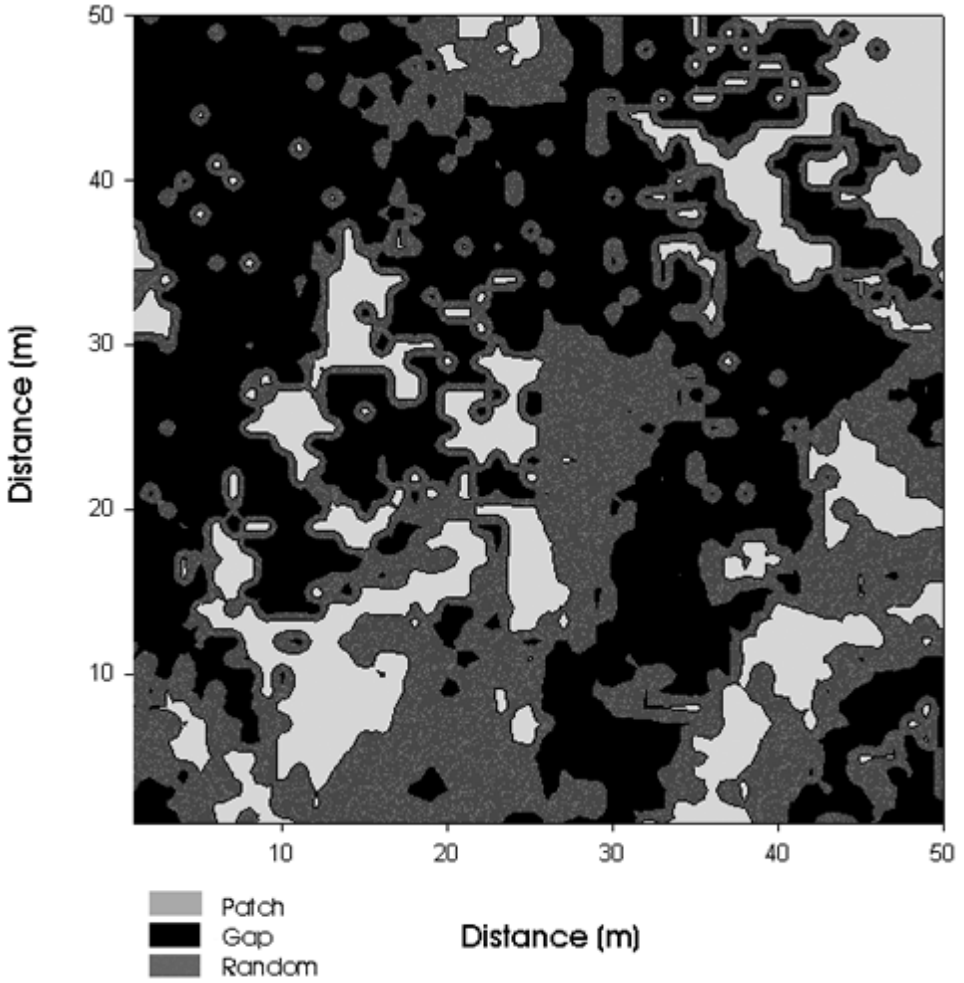

Figure 1

Distribution of gaps and patches on a 50 x 50 m area based on spatial analysis (SADIE) of live *Eleocharis* shoot numbers. Patches (light grey) are places with clumped shoots, gaps (black) are places with very sparse shoots, and places in between, called random (dark grey, dotted), are represented by average shoot numbers.

The plants in patches had higher tissue N and P contents than control plots. In combination with higher shoot density, this resulted in a higher amount of both nutrients per m^2 in patches (Table 1). Our mean values of $\delta^{15}N$ and $\delta^{13}C$ in snail and control plant tissue were $\delta^{15}N$ = 4.9‰, $\delta^{13}C$ = -28.8‰ and $\delta^{15}N$ = -3.7‰, $\delta^{13}C$ = -25.0‰ respectively, which are very close to the values from sparsely vegetated sites in Everglades (Williams and Trexler 2006). We documented differences in $\delta^{15}N$ values in plants (*Eleocharis cellulosa*) from patches and controls (Tukey HSD, $P < 0.001$), but not in values of $\delta^{13}C$ (Tukey

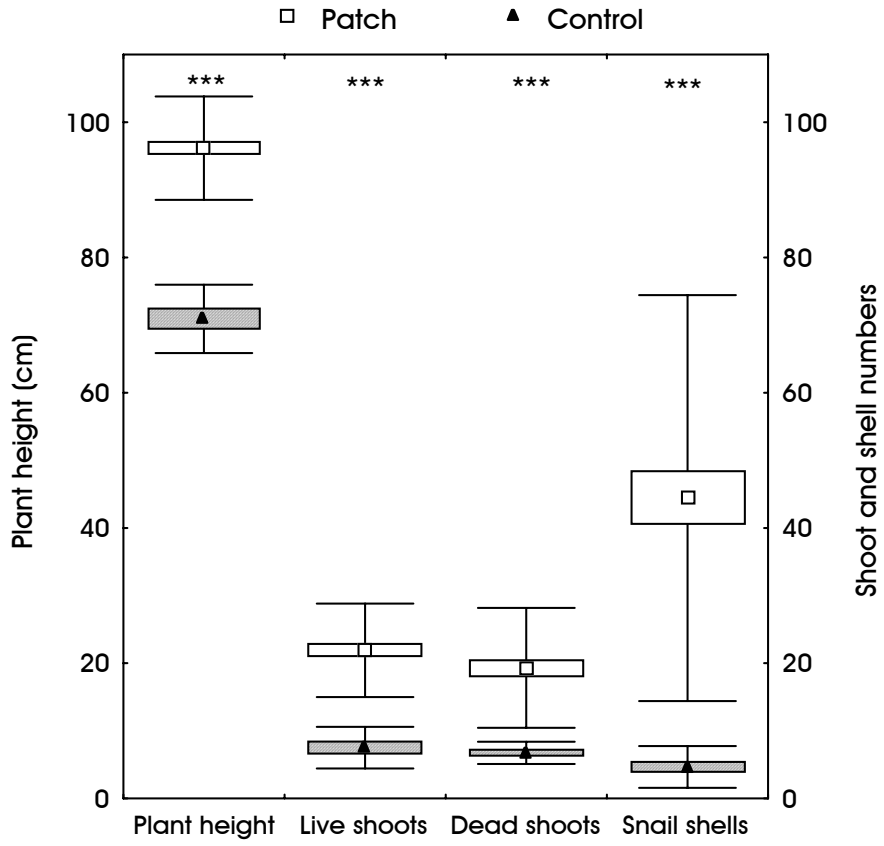

Figure 2

Differences between patch and control plots in terms of vegetation characteristics (plant height, *Eleocharis* live and dead shoot densities) and amount of empty *Pomacea* shells. Symbol, mean; box, mean ± S.E.; error bar, ± S.D.; ***, $P < 0.001$.

Table 1

The mean ± S.E. of total aboveground biomass (g m^{-2}), plant nutrient content (µg g^{-1}) and total nutrients in plant biomass (g m^{-2}) in patches and control plots. The results of Student's *t*-test are shown.

	Patch	Control	*t*	*P*
Total biomass	2248 ± 219	450 ± 52	7.76	**<0.001**
Plant tissue N	9630 ± 150	8650 ± 250	3.31	**0.003**
Plant tissue P	369 ± 12	309 ± 17	2.92	**0.006**
Total aboveground biomass N	21.66 ± 0.34	3.89 ± 0.11	49.68	**<0.001**
Total aboveground biomass P	0.82 ± 0.03	0.15 ± 0.01	21.19	**<0.001**

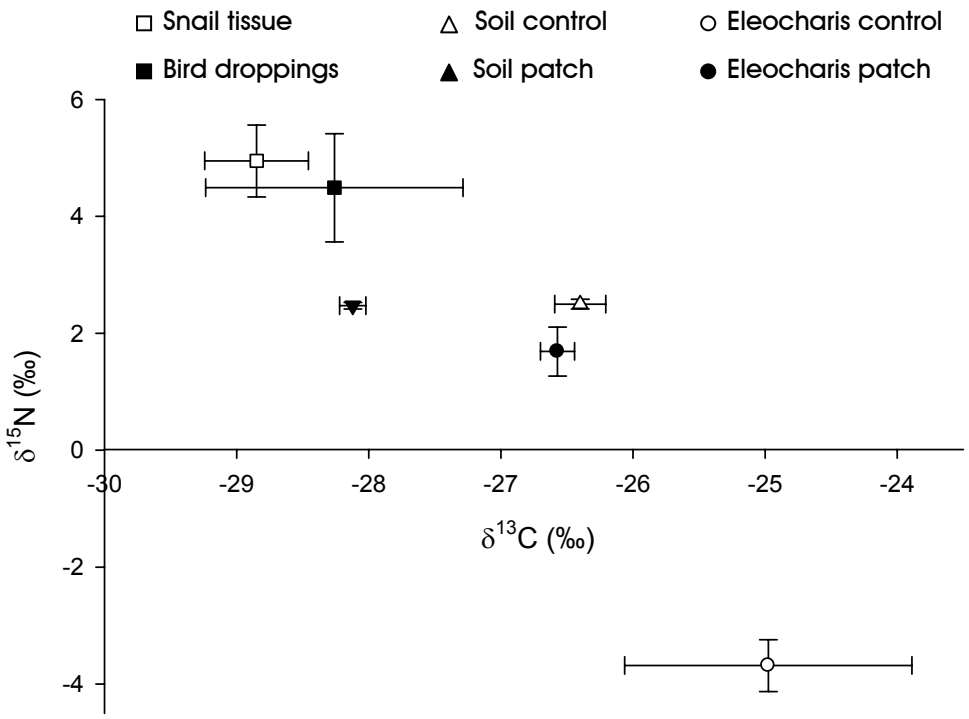

Figure 3

$\delta^{13}C$ and $\delta^{15}N$ composition of emergent macrophytes (*Eleocharis cellulosa*; n = 16), soil originating from below marsh and patch sites (n = 16), snail tissue (n = 10) and snail eating birds droppings (n = 4). Error bars: mean ± S.E.

HSD, $P = 0.286$). In other words, plants in patches were enriched in ^{15}N compared to control marsh plants. The patch and marsh soil did not differ in either ^{13}C signatures (Tukey HSD, $P = 0.210$), and ^{15}N (Tukey HSD, $P = 0.999$). Snail tissue and snail-eating bird droppings were close together both in $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 3).

Limpkin observations

PCA resulted in three main correlated groups of limpkin activities, time of the day and preferred microhabitats: (1) In the early morning (7–9 a.m.), birds were usually resting in dense vegetation or *Cladium* thicket and they started to move toward wetland center and prepare for foraging. (2) Foraging time came in late morning (10–11 a.m.) and it took place predominantly in open water and sparsely vegetated wetland. (3) After snail capture, eating was tightly correlated to patch habitat. The birds waded toward a neighboring patch (wading distance longer than 15 m was not observed) and there they handled and ate the snail tissue leaving the shell. Only a small portion of captured snails was handled at the place of capture. This resulted in significantly higher piling of shells in areas suitable for prey handling, i.e. patches (t=7.0, $P < 0.001$), compared to sparse vegetated marsh. At the same places, the birds had midday (12–2 p.m.) and afternoon (3–6 p.m.) siestas spent by grooming and occasional foraging. The visualization of limpkin activities is in Fig. 4. The correlation coefficients between the limpkin activities and their location in the marsh are shown in Table 2.

Estimation of limpkin impact on vegetation

Limpkin foraging efficiency was 4.6 snails per an hour of foraging. Average daily time devoted to foraging was 4 hours, resulting in 18 snails captured per day. Of these, 80% (14.5 snails) were handled in patches. Average snail shell number found in a patch was 44 and it could potentially be produced by a single bird in 3 days. Patchiness survey revealed the approximate number of patches to be 60 ha^{-1}. The marsh area, where focal observation was conduced (extent of 2.5-ha), had ~150 patches and hosted from 5 to 10 birds. Hence, potentially, these birds can produce piles of shells in this marsh over a ~60 day's period. However, this reasoning assumes that the birds use the patches evenly. Over time, nutrients from bird droppings

and left snail tissue cumulate in the sediment and consequently in the vegetation.

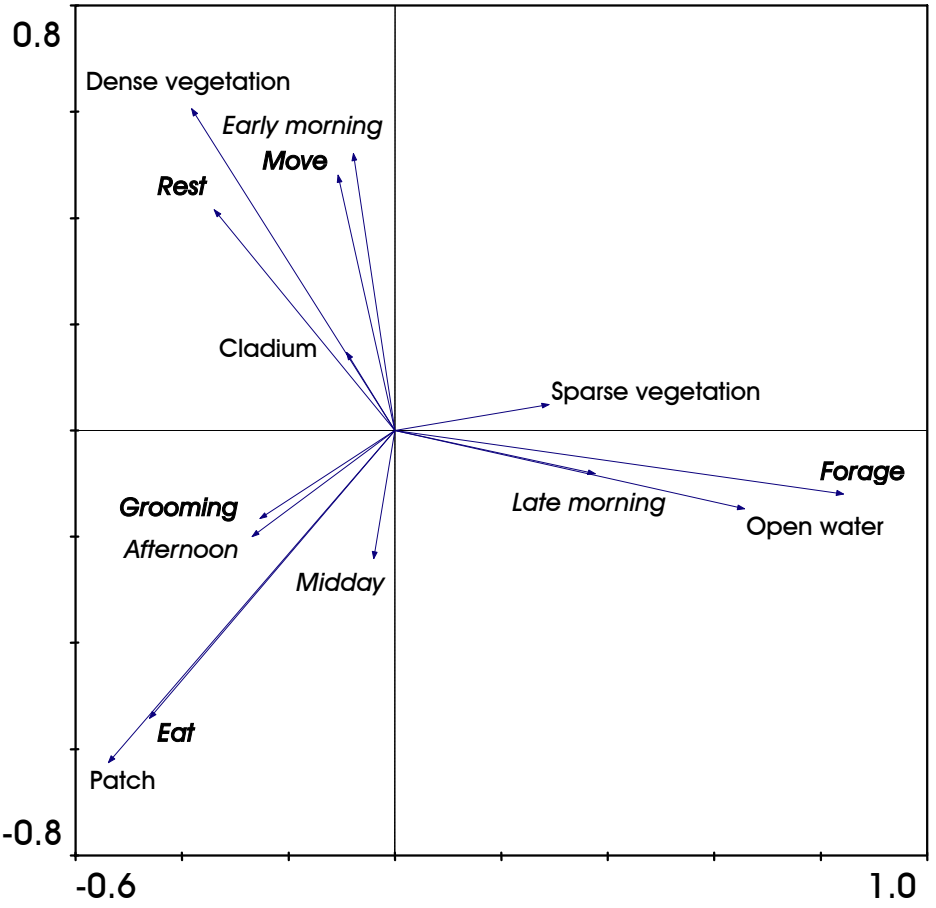

Figure 4
Results of PCA (Principal Component Analysis) of limpkin activities (***bold, italics***) and their spatial location (normal font) and time of the day (*italics*; *Early morning*, 7–9 a.m.; *Late morning*, 10–11 a.m.; *Midday*, 12–2 p.m.; *Afternoon*, 3–6 p.m.). Smaller angles between response and explanatory variables mean stronger positive correlation, perpendicular arrows are not correlated, opposite arrows are negatively correlated. For correlation coefficients between limpkin activities and their locations see Table 2.

Table 2
The correlation coefficients (R) between limpkin activities and their locations within the marsh based on the PCA analysis (Fig. 4). The values in bold represent significant correlation ($P < 0.05$).

	Move	Forage	Eat	Grooming	Rest
Patch	**-0.166**	**-0.322**	**0.419**	**0.206**	-0.014
Dense vegetation	**0.193**	**-0.273**	**-0.126**	0.072	**0.274**
Cladium thicket	**0.132**	**-0.150**	0.065	-0.065	0.037
Sparse vegetation	0.005	**0.243**	**-0.151**	-0.089	**-0.103**
Open water	**-0.115**	**0.417**	**-0.152**	**-0.144**	**-0.179**

Experimental patch creation

None of the locations, chosen for experimental patches creation, had significantly different vegetation characteristics prior to the treatment. At both locations addition of P or chicken manure resulted in significant increase of *Eleocharis* shoot density and height, and consequently higher aboveground biomass. The effects were visible 6 months after treatment and stayed significant 40 months after treatment at Buena Vista location (Table 3). Further, after 40 months, plant height was significantly different between all treatments (Tukey, $P < 0.036$, Fig. 5) and it increased in order: control, P, manure plots. *Eleocharis* live shoot number responded in a similar manner (Tukey, $P < 0.016$, Fig. 5). Dead shoot number increased in manure plots only (Tukey, $P < 0.001$, Fig. 5).

DISCUSSION

We documented a small-scale patchiness in wetlands of northern Belize in terms of plant physiognomy, patch elevation and nutrient content. Both positive and negative indices of clustering in spatial analyses were quite high (see also Perry et al. 1999) documenting significant patchiness in plant height and *Eleocharis* shoot numbers, as well as the presence of the gaps in the vegetation. Patches in our study area cover much smaller area than gaps and regularly spaced vegetation and can thus be considered as a specific vegetation type. Furthermore, a comparison of patches and

controls, revealed small, but significant differences in water depth, probably as a result of increased sedimentation in patches (see also Tomassen et al. 2005). In our case, sedimentation rate will probably increase even more, once other less water tolerant woody species (e.g. *Mimosa* spp.) get established in a patch.

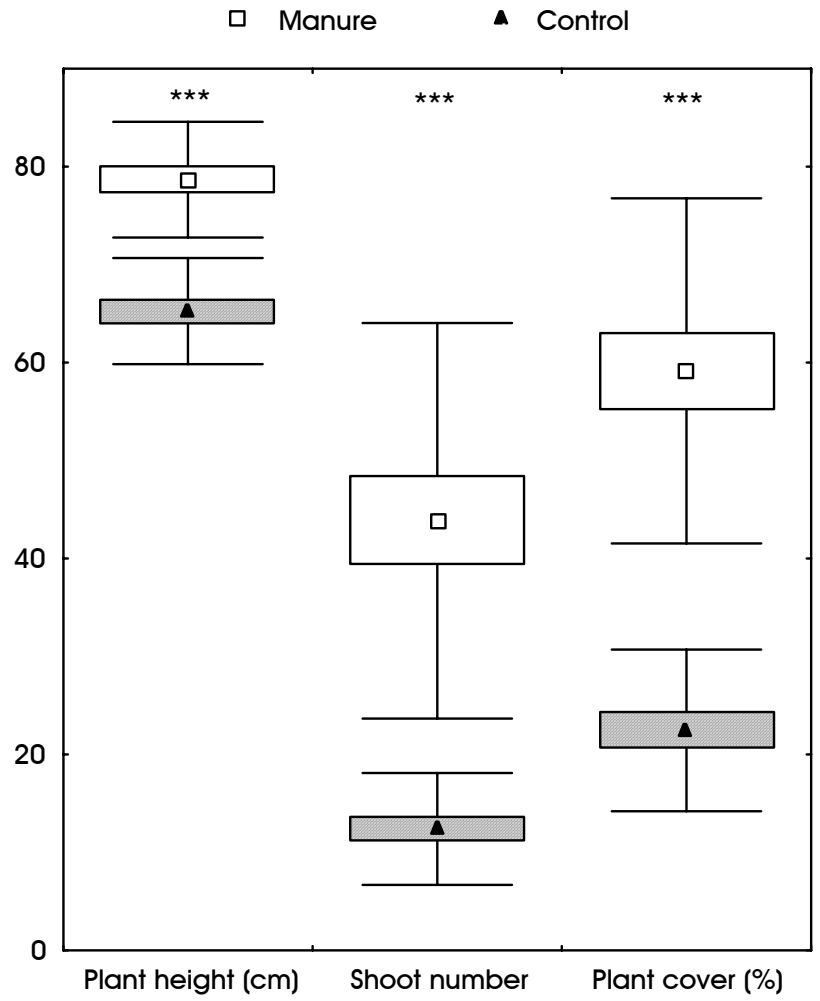

Figure 5
Vegetation characteristics comparison of treated (manure) and control plots 40 months after chicken manure addition at the location Buena Vista. Symbol, mean; box, mean ± S.E.; error bar, ± S.D.; ***; $P < 0.001$.

We proved that limpkins prefer patches with dense vegetation for prey handling and eating. After eating, limpkin usually excretes in a patch. Nutrients are increased not only by bird droppings, but also by the remnants from a messy act of meat extraction from the shells (Snyder and Snyder 1971). The amount of snail shells in patches was substantial (mean 44 shells per patch) and the activities connected with consumption of these snails probably resulted in elevated P contents in patch soil. In concordance, the increased nutrient input was reflected in higher N and P contents in terms of plant tissue nutrients.

Most of the available nutrients were rapidly incorporated in plant tissue; hence, macrophytes in patches were enriched in ^{15}N compared to surrounding marsh vegetation. This is concordant to elevated ^{15}N values of emergent macrophytes leaves from nutrient enriched sites of Florida Everglades (Inglett and Reddy 2006). Since wetland macrophytes respond to P addition by a decrease of discrimination during N uptake (Inglet et al. 2007), recorded difference in ^{15}N between patch and marsh plants is a strong support for selective P enrichment of patches. Such enrichment could be an indirect evidence of the animal origin of patches. Similar conclusions were applied in several other studies of bird – plant system (e.g. Erskine et al. 1998). As expected, there were no differences in plant $\delta^{13}C$ values as plant carbon uptake was always from the same source regardless of plant location (and water availability did not limit plant growth there). Nevertheless, the animal impact in patches was reflected in patch soils, which were depleted in ^{13}C and their values of $\delta^{13}C$ were much closer to the animal than plant tissue. However, we are aware that the ^{13}C values in soils can be influenced by other factors, e.g. microbial processes (Šantrůčková et al. 2000).

Changes in plant cover in response to bird droppings are relatively common (e.g. Anderson and Polis 1999; Dean et al. 1999), some studies reported changes in species composition as well (Tomassen et al. 2005). Similarly to our results, other authors reported an increase in soil nutrient content as a response to bird dropping (Anderson and Polis 1999; Ligeza and Smal 2003; Hobara et al. 2005). However, to our best knowledge, all these studies focused on perching birds, and the impact of a wading bird, *Aramus guarauna,* limpkin, is therefore quite unique.

According to nutrient status in patches, bird activities clearly have an impact on nutrient cycling there. The limpkin is a solitary bird with little

difficulties in prey capturing (Bennetts and Dreitz 1997). In our sites, its average daily intake was 18 snails [comparable to *Rostrhamus sociabilis* snail tissue daily intake; (Beissinger 1983)] of which 14.5 were handled in patches. An average amount of shells in a patch can be potentially gathered in three days of limpkin activity. It can even be sooner, as we did not observe night foraging, which is reported as not uncommon to limpkins (Bryan 1996). Our observation confirmed that limpkins usually forage throughout a smaller area very rigorously (see also Snyder and Snyder 1971). In several cases, individual limpkins were rather conservative and returned toward the same patch repeatedly. These observations are also supported by numerous findings of piled fresh shells found in P enriched plots that were part of another experiment (Rejmánková and Macek unpublished data). When birds are present under prolonged stable conditions of water level, their nutrient input increases plant height and density in patches. These in turn represent better conditions for snail handling and therefore are more suitable for further bird eating visit. Such positive feedback can guarantee the patch sustenance for a long period. Both limpkin observations and results from the manipulative experiment confirmed that this avian predator plays a major role in patch formation. However, other animals, e.g. shelter seeking turtles, cichlids or eels (*Ophisternon aenigmaticum*, whose holes were found in many patches) can further impact patch nutrient status. Yet, we believe, the presence and impact of other animals is rather small compared to the effect of limpkin activities (see also Dean et al. 1999). Similarly to Anderson et al. (1999), we can conclude, that allochthonous nutrient input via bird dropping might be an acceptable explanation of system dynamics.

However, only a small number of such "fortunate" patches can be sustained. Patch retrogression can have several reasons ranging from nutrient output by environmental factors (i.e. fires and extreme flooding) to decrease of predator activity. Patches that are not repeatedly used slowly degrade and the nutrients are redistributed by clonal growth of macrophytes to the surrounding wetland. In our patchiness survey, we documented that observed wetland patterns consist of the whole scale of patches ranging from growing to disintegrating ones.

Limpkin occurrence and feeding in a larger geographical scale are rather scattered, site and time unpredictable and irregular (Macek and Rejmánková pers. obs.). A similar unpredictable movements between

wetlands were also documented for *Rostrhamus sociabilis* (Bennetts and Kitchens 2000), and both might result from snail density and water level fluctuations. We did not document high density of living snails in our patchiness survey, probably due to low water level and snail ability to aestivate (Kushlan 1975). However, the snail abundance can be temporarily quite high (3–4 m^{-2}; Lege 2001) in suitable locations (see also Bennetts et al. 2006). Snail densities are reported to be higher in sparsely vegetated marsh (Karunaratne et al. 2006), where bird visual orientation is better, which also explains, why are foraging activities performed there.

As for the further destiny of well utilized patches with continuous nutrient supply we predict that the litter decomposition rates will increase (Tomassen et al. 2005; Rejmánková and Houdková 2006), higher nutrient content will lead to higher microbial activity, and, generally, to increased biogeochemical activity of these patches (see also Van Miegroet et al. 2000). Similarly, in Florida Everglades, patches represented by tree islands were described as hotspots of biogeochemical cycling in the landscape (Troxler Gann et al. 2005; Wetzel et al. 2005). The nutrients will slowly accumulate compared to surrounding wetland and can be stable even over longer periods without any further supply (Facelli and Brock 2000). This may result in creation of a hospitable (e.g. elevated) environment for other species such as *Mimosa* spp., which can extend to previously not accessible habitat (Hacker and Bertness 1995; Zanini and Ganade 2005; Scheffer et al. 2006), although this process is probably quite slow. This might also lead to the succession towards tree islands as described from the Everglades (Wetzel 2002). In this way, patches may play an important role in increasing ecosystem complexity, providing habitat for other plant and animal species. Although tree islands are also present in our system, links between patches and tree islands are not supported by any strong evidence yet.

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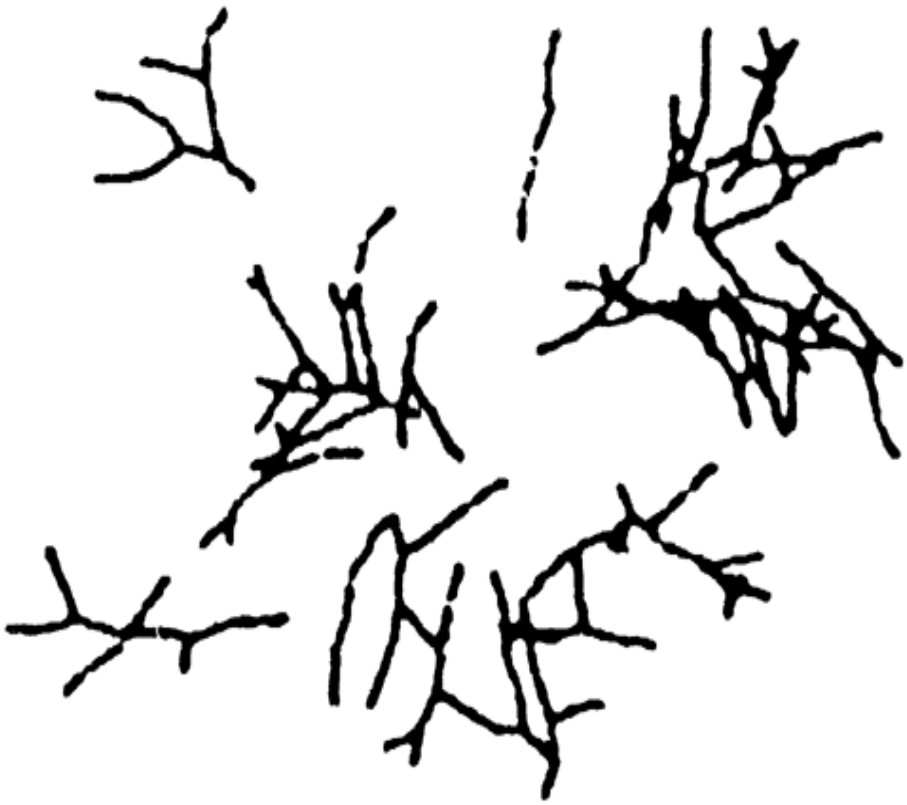

General Discussion

INDIVIDUAL BENEFITS OF CLONALITY AND FACTORS RULING CLONAL GROWTH TRAITS

Across main vegetation types, the wetlands include the largest portion of plants capable of clonal growth (Klimeš et al. 1997). This can have several causes, but the type of substrate is undoubtedly among plausible explanations: wetland soils are often rather soft and thus not constraining rhizome growth as much as other soils. Further, another selection pressure frequently exerted in harsh wetland environment is represented by frequent changes of water level. This phenomenon mainly limits plant success in generative reproduction. Flooded seedlings of emergent plants usually do not survive under prolonged anoxic conditions, which decrease their relevance. Hence, clonality is of major importance for plant survival in wetlands, although it is not overwhelming: for a long term survival there, generative reproduction is often needed (van der Valk 2006).

Examples of clonality enhancing plant survival under stressful conditions as described in the first chapter have been reported quite frequently, I will not report them here (but see e.g. Suzuki and Hutchings 1997). Our results agree with part of these findings: plants with at least some of the shoots emerging above water level can support greater amount of ramets than completely submerged plants (Chapter 2). Under natural conditions, this leads to partial survival within whole population subjected to extreme long-term flooding events. Plants keeping the contact with the atmosphere support the neighbouring connected ramets and enhance their survival. Furthermore, after the water recedes and plants start to reoccupy the newly opened space, surviving young individuals have an advantage, even though the original supporting shoot usually dies due to its tendency to break down without the water support after emergence (see also Suzuki and Hutchings 1997). Similar partial survival has been previously observed in the field with *Eleocharis cellulosa* after an extreme flood caused by tropical storm Chantal in 2001 (Macek and Rejmánková, pers. obs.).

Not only abiotic factors (e.g. water level or nutrient poor patches) can represent stressful conditions affecting clonal growth traits. Biotic factors represented by vegetation physiognomy (e.g. vegetation height or biomass) exert an influence on clonal traits as well. Often, biotic factors have even

better predictive value than abiotic factors ultimately causing them (Chapter 4). However, the mechanism by which clonal plants respond to unsuitable habitat is similar regardless whether habitat unsuitability is represented by biotic of abiotic factor: an escape strategy (Macek and Lepš 2003). Apart from generative reproduction, an escape in clonal plants is effectuated by an increase of distance between two ramets. Importantly, a change in two clonal growth traits with contrasting costs can result in similarly increased distance: these traits are internode length and internode number. If an escape strategy is about to be useful, it should be rather inexpensive for plant. Since the increase of internode number results in additional costs of leaf and/or root production, the only meaningful strategy would be to increase the length of internodes. Similarly I conclude, the efficient escape strategy in competitively poorer species *Potentilla palustris* is keeping stable high cost traits and plastic low cost traits of clonal growth (Chapter 4).

Apart of other factors, both systems studied differ in nutrient limitation: in limestone based Belizean marshes plants are strongly P-limited while in more acidic temperate fens and bogs plants are rather N limited. However, plant response to enrichment by limiting nutrient, i.e., the increased branching, is common to species from both systems: (Chapters 3 and 4). This is consistent with foraging theory in clonal plants which expects concentration of resource acquiring structures in more favorable places (de Kroon and Hutchings 1995; Salemaa and Sievanen 2002). Although increase of branching in taller vegetation might seem to be in contradiction with an escape strategy from less suitable places (plants also increased internode length as response to lower light availability there), it is not mutually exclusive, since taller vegetation often also represents elevated nutrients. A positive effect of nutrients may further be reflected in better space capture. However, this result is valid only for species with high phenotypic plasticity of clonal growth traits. Three macrophyte species of Belizean marshes can serve as a good example of variability in phenotypic plasticity among clonal plants. The most rigid species, *Cladium jamaicense*, shows no change in rhizome length and branching in response to nutrient and salinity changes. An intermediately plastic *Eleocharis cellulosa* alters branching accordingly to conditions, but it does not change its rhizome length. The last species, *Typha domingensis*, shows the most plastic response in both rhizome length and branching (Chapter 3). Such differences in architectural constraint and

plasticity ultimately drive emergency of different patterns in wetlands based on portion occupied by different species: a mixture of tussocks and monodominant stands (Herben and Hara 1997; see below).

INTERACTIONS BETWEEN CLONAL PLANTS

An equally interesting consequence of plant clonality can be noticed in processes of competition between species. In nutrient limited environments, a possibility of competitive exclusion of inferior species is reduced. On the other hand, when nutrient enrichment occurs, fast changes in species abundance can result in dominance of competitively superior species and outcompeting of other species in plant community (de Kroon and Bobbink 1997). Altered conditions may lead to a change in invasiveness of native species: they can take an advantage of changes in environmental conditions and may spread out of their normal range through the means of various dispersal mechanisms. Under this scenario, clonal growth is an efficient and important dispersal mechanism in wetland systems. The rhizomatous expansion can speed up the process of space filling resulting ultimately in a dense and uniform cover, as was reported for the case of both *Eleocharis* spp. and *Typha domingensis* in Belizean marshes (Chapters 4, 5 and 6). While invasive behaviour is less likely in *Eleocharis* spp. due to its limited horizontal spreading, the behaviour of the second species, *Typha domingensis*, under altered nutrient regime should be considered as invasive. The results of simulations in Chapter 6 clearly demonstrate invasive nature of *Typha domingensis* spreading. This is in concordance with "fluctuating resource availability" hypothesis stating that a community is more susceptible to invasion when nutrient surplus occur (Davis et al. 2000).

Compared to other habitats, wetlands host relatively large amount of clonally spreading invaders (Pyšek 1997). A bottleneck in their success at this habitat lies in establishment, which is often difficult. After all, once established, clonal plants seem to be more persistent and competitive, which leads to an effective occupation of the available space. Limitation due to low survival after initial establishment was also observed in our implant experiment: where the original vegetation was too dense, ramets of *Typha* sometimes died (Chapter 6).

Such a contrasting output from plant performance in various nutrient enriched plots is an excellent example of two different strategies in horizontal growth competition: dominance and founder control (Herben and Hara 1997). When original density of *Eleocharis* shoots was lower, *Typha* growth was not limited and consecutively by overtopping of shorter *Eleocharis*, *Typha* succeeded to capture space in both vertical and horizontal dimensions. Hence, this community looked to be dominance controlled. Alternatively, when original stands of *Eleocharis*, due to its extreme density, prevented further establishment of competitively stronger invader - *Typha*, the community was founder controlled (mainly in low salinity locations; Chapter 4 and 6). Nevertheless, in part of founder controlled plots, *Typha* was able to survive and eventually succeed in spite of dense original vegetation, although this success was delayed by several years. What were the reasons of this switch between founder and dominance control? Possible starters were natural disturbances frequent to these wetlands: fire and/or elevated water level. In both cases, aboveground biomass was removed, at least partially. The newly open space enabled a full expression of *Typha* competitive superiority over *Eleocharis* spp. Last but not least, a third operating mechanism leading to ultimate expression of dominance control was a clonal growth trait: a functional connection between ramets enabling a support of small daughter *Typha* ramets competing directly with *Eleocharis* (specifically nutrient transport; Macek and Rejmánková, data not shown). In fact, there was an indispensable necessity of clonal growth in all these processes of species switch (Chapter 6).

CLONALITY AND ECOSYSTEM PROCESSES

Altered nutrient conditions resulting in macrophyte species switch can furthermore lead to changes of the whole system (e.g. Grieco et al. 2007). Spread of invasive species can also modify chemical and physical properties of habitat and also decrease biodiversity (de Kroon and Bobbink 1997; Chiang et al. 2000; Svengsouk and Mitsch 2001; Boers et al. 2007). Furthermore, rather homogeneous soil fertility increase in Belizean marshes (due to nutrient runoff from agricultural fields) would lead to a decrease of spatial heterogeneity, which is originally present there thanks to coexistence of several morphologically different species. Keeping wetland oligotrophic

status should be therefore among primary objectives (Chapters 5 and 6). It is a well known fact, that wetland restoration is much more costly than their protection.

On the other hand, not all nutrient enrichment does necessarily result in loss of species diversity or environmental heterogeneity. Actually, the nutrient enrichment originating from animal activities investigated in the Chapter 7 has a completely opposite effect. Here, the processes involved in the response of plants are the same, i.e. increased branching in nutrient rich patches. However, animal caused nutrient input is very local and also much lower than in our previous experiments. This is far the most important difference, because animal caused nutrient enrichment result in vegetation differentiation, i.e. pattern emergence, due to variation of vegetation density and height. Over time, this pattern reflects in soil elevation. It has been reported several times, that any local increase in topography can increase wetland biodiversity (Vivian-Smith 1997; Sklar and van der Valk 2002; Wetzel 2002). An increase in heterogeneity due to differential growth of emergent macrophytes in response to small scale nutrient enrichment will eventually have an important effect on wetland biodiversity. Although a direct link between small and large scale heterogeneity is not supported by any strong evidence yet, it is likely that a small scale patchiness caused by animal activity is antecedent of large scale tree islands. Plasticity in clonal growth traits is therefore at the very beginning of heterogeneity in wetlands.

While nutrient enrichment is the ultimate cause of above mentioned changes, its effect is mediated through the plasticity of clonal growth traits. In agreement with the intermediate disturbance hypothesis (Huston 1979), I conclude that nutrients can increase heterogeneity at lower doses, while both extremes, i.e. no nutrients or high nutrients often result in lower heterogeneity. Nevertheless, both processes of environmental heterogeneity change similarly result from the changes of plastic clonal growth traits: branching and spacer length.

So far, I focused on the effect of more plastic species on vegetation pattern. However, I believe the species with constant clonal growth traits are of similar importance. In case of *Cladium jamaicense*, a production of relatively short rhizomes with rather constant length frequently results in large tussocks formation. Such tussocks represent a suitable habitat for numerous bird species as a good place for nesting. Larger animals seek for tussocks of

Cladium as well, e.g. *Crocodylus moreletii* use them for prey handling and as platforms for resting above water. Hence, also plants with constant traits may result in vegetation pattern emergence.

SUMMARY AND CONCLUSIONS

Species with plastic clonal growth traits are able to better accommodate to changing wetland conditions in terms of both resource acquiring strategy and/or escape strategy. Clonally growing plants are more efficient in new space occupation after biomass removing events, such as fires, grazing or high water levels. Variable phenotype enables them to occupy a wider range of habitats and eventually to succeed in competition with co-occurring species when growth conditions are changed. In some cases, a plasticity of clonal growth traits mediate loss of heterogeneity, while in others it can be the important property responsible for greater heterogeneity in wetlands: it largely depends on external factors driving these traits, e.g. nutrient availability. On the other hand, rigid clonal growth may also result in pattern eventually increasing wetland heterogeneity and habitat diversity. Clonal plants are extremely important in wetland functioning, although only coexistence of both plastic and rigid species (in terms of clonal growth traits), which is favored particularly under oligotrophic conditions, can be beneficial for wetland diversity.

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