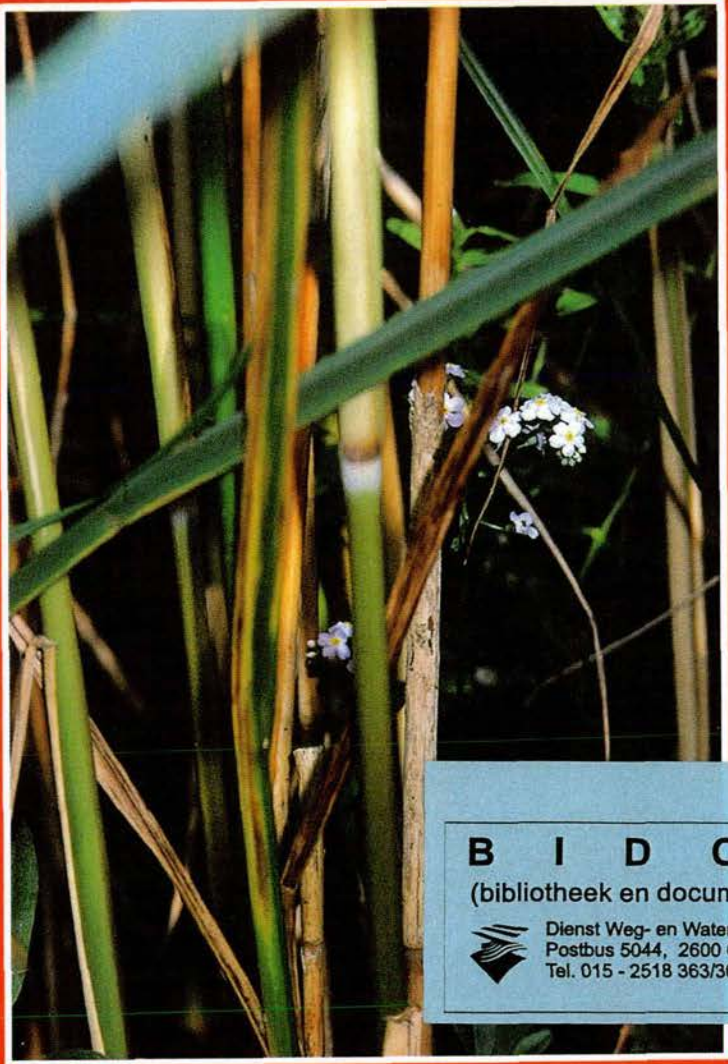
SPECIES RICHNESS IN REED MARSHES



John Lenssen

SPECIES RICHNESS IN REED MARSHES

Een wetenschappelijke proeve op het gebied van de Natuurwetenschappen

Proefschrift

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'Jij studeert,' zei Frits, toen ze weer waren gaan zitten, 'maar wat is wetenschap? Het is volstrekt niets.' Viktor glimlachte. 'Denk je eens in,' zei Frits, 'denk je eens in, dat ze met al hun wetenschap nog niet eens een doodgewone zandkorrel kunnen maken.' Hij maakte het gebaar, of hij zand tussen de vingertoppen wreef. 'Dat kunnen ze niet.'

Gerard Reve- De Avonden

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Contents

1. INTRODUCTION

Scope of this thesis

Ecological values of many reed marshes in North and Middle Europe have strongly decreased in the course of the twentieth century. Water table regulation, use of artificial substrates, deteriorated water quality, intensive ship-traffic and cessation of traditional management practices are generally held responsible for this decline (Den Hartog *et al* 1989). As a consequence, present shoreline vegetation barely resembles the natural vegetation on land-water gradients.

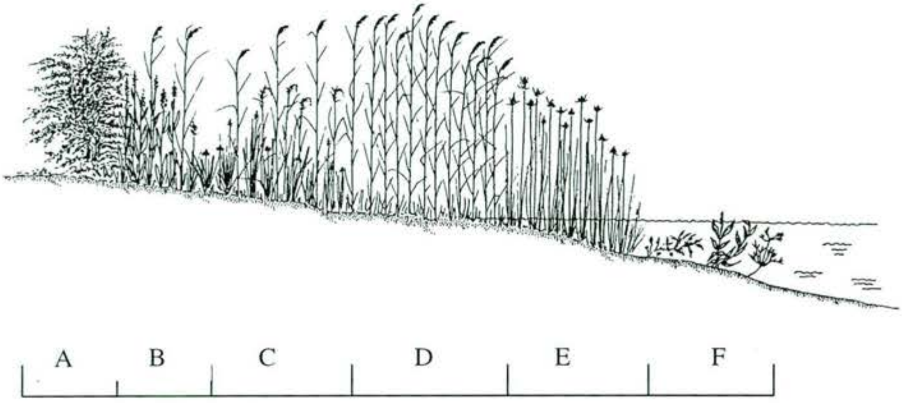

Figure 1: Zonation of vegetation types along a natural water-depth gradient of a eutrophic waterbody with moderate water level fluctuations. A= shrub zone (*Salix* spec., *Rubus caesius*); B= tall forb zone with, among others, *Epilobium hirsutum*, *Urtica dioica*, *Eupatorium cannabinum*, *Cirsium arvense* and marginal presence of *Phragmites australis*; C= species-rich reed: dominant *P. australis* is accompanied by interstitial marsh species such as *Mentha aquatica*, *Myosotis scorpioides* and *Lycopus europaeus*; D= monospecific reed; E= bulrushes (*Scirpus* spec.); F= submerged macrophytes (*Potamogeton* spec.). (Drawing by W. Verholt)

Ecologically impoverished shorelines and marshes lack the presence of extensive reed belts at the waterside. Often, only a small belt of *P. australis* is left, which is immediately succeeded by tall forb vegetation at higher elevations. Hence, the zone where many different wetland species coexist is absent. An additional problem in many anthropogenically-influenced wetlands is that reed marshes have been replaced by tall forb vegetation (De Boois 1982, Gigon and Bocherens 1985, Gryseels 1989, Fojt and Harding 1995).

Conservation and restoration of species-rich reed marshes is an important objective of management because creating opportunities for species richness may, to a large extent, encourage rare marsh species (Wheeler 1988). Diversity of plant species will also enhance food-availability of many invertebrates, which in turn favours species in higher orders of the food chain. In fact, typical reed marsh birds such as the bearded tit (*Panurus biarmicus*) and Savi's warbler (*Locustella luscinoides*) are strongly favoured by floristic diversity of reed marshes (Bibby and Lunn 1982).

Management measures will only be successful if they are based on a thorough insight into the ecosystem mechanism, i.e. the causal relationships between environmental and biotic factors. Solving practical problems with a scientific approach has also been successful in assessing constraints and opportunities for restoration of other wetland vegetation types, such as floodplain forests (Siebel 1998, Van Splunder 1998) and stands of helophytes (Clevering 1995*a*, Coops 1996, Van der Putten 1997).

Aim of this study

This thesis aims at clarifying the mechanism underlying the zonation of monospecific reed, species-rich reed and tall forb vegetation. I have tried to elucidate this mechanism by adressing the following research questions:

1.- Which relationships exist between environmental factors, performance of *P. australis* and the species richness of *P. australis* dominated vegetation?

2.- Under which environmental conditions can representatives from the species-rich reed marsh zone succesfully pass all phases of their life-history?

3.- Which (interacting) environmental factors discriminate between species respresenting the species-rich reed marsh vegetation and representatives from the tall forb vegetation?

4.- Does competition play a role in the replacement of reed marsh vegetation by tall forb vegetation?

From the mechanistic insight, management tools will be derived which increase the species richness of *P. australis* dominated vegetation and prevent replacement of reed marsh vegetation by tall forb vegetation. The results will be applicable for all reed marshes along eutrophic waterbodies such as lakes, canals and downstream parts of rivers.

Species selection

Best opportunities for species richness exist if weak competitive species are favoured and strong competitors are inhibited (Grime 1987, Palmer 1994). Otherwise, strong competitors will be able to intercept most, or even all, of the necessary resources (light and nutrients) and thereby exclude other, weak competitive species. The ability to intercept resources, or the functioning of plant species within the vegetation, is related to the morphology and life history of species. These traits determine to which extent and for how long resources can be captured (Smith and Huston 1989, Shipley *et al* 1989, Boutin and Keddy 1993, Hills *et al* 1994, Grime *et al* 1997). In eutrophic systems, competition for light is very important and maximum height is therefore an important trait determining the capability for high rates of resource capture (Gaudet and

Keddy 1988, Hills *et al* 1994). Another important trait of dominants is the rhizome apparatus, which is used for vegetative expansion and long term occupancy of sites (Shipley *et al* 1989, Boutin and Keddy 1993, Grime *et al* 1997). Both traits are necessary to dominate vegetation in eutrophic, relatively undisturbed vegetation (Boutin and Keddy 1993). Species that lack the combination of both traits will not be able to intercept the majority of resources for a prolonged period of time and will have a subordinate or interstitial position within the vegetation. Throughout this thesis, species with the potential for dominance will be referred to as **clonal dominants** (or simply **dominants**) and species lacking this potential, **interstitials** (cf. Boutin and Keddy 1993). Boutin and Keddy (1993) also recognized obligate and facultative annuals as important functional groups in eutrophic wetlands. However, these groups are not further considered in this thesis since they only play a limited role in relatively undisturbed ecosystems, such as reed marshes.

Table 1: Species classification used in this thesis. Combinations of plant traits (functional groups) and zonation of adults (zonation groups) resulted in four different classes.

Functional group		Zonation group	
		Tall forbs - adults in tall forb zone	Reed marsh -adults in the reed marsh zone
Interstitials	-small and/or -limited lateral spread	tall forb insterstitials	reed marsh interstitials
Clonal dominants	-tall and -vigorous lateral spread	tall forb dominants	reed marsh dominants

Species richness in reed marshes depends upon the factors which discriminate between interstitials and dominants of reed marsh vegetation (*P. australis*), as well as on the balance between reed marsh interstitials and dominants from the succeeding vegetation type, the tall forb zone. If tall forbs are able to expand, the eventual vegetation will be very species-poor (Wheeler and Giller 1982, Wheeler and Shaw 1991) and *P. australis* will only be marginally present (Gigon and Bocherens 1985, Fojt and Harding 1995). Therefore, shoreline species were distinguished on the basis of both functional group, clonal dominants or interstitials, and their position as adults in the zonation, i.e. in **reed marsh** or **tall forb** vegetation (Table 1). In order to answer research questions 2 and 3, I compared responses of reed marsh interstitials and tall forb dominants to environmental conditions considered being relevant for eutrophic wetlands.

Selection of relevant environmental conditions

Flooding

The zonation of species-rich reed marshes and tall forb stands is related to the water depth gradient (De Boois 1982, Klosowski 1988, Gryseels 1989, Coops 1996, Fojt and Harding 1995, Van de Rijt *et al* 1996). However, experiments are required to establish causal relationships between the species zonation and water level, as well as to determine the precise mechanism by which it may determine zonation (Blom and Voesenek 1996).

Position of species on water depth gradients is mainly determined by their flood tolerance. This tolerance may change during life history (Van der Valk 1981, Van der Sman *et al* 1993, Voesenek *et al* 1993*a*). A species can therefore only persist if the flooding regime, i.e. the seasonal pattern and amplitude of water level fluctuations, allows it to pass all stages of the life cycle. This is especially important for species with limited clonal spread, among which are many marsh interstitials. Otherwise, species may alter their position on the land-water gradient by means of vegetative expansion (Welling *et al* 1988, Clevering 1998*b*).

In reed marshes, water level fluctuations in the growing season are moderate (Brock *et al* 1987, Breen *et al* 1988, Blom *et al* 1996). Because marsh interstitials and tall forbs inhabit the shallow flooded and dry parts of the shoreline, total inundation will be restricted to germination, seedling establishment and emergence of shoots in spring. Different tolerances to submergence in these phases of the life history may determine the zonation of marsh interstitials and tall forbs. Zonation may however also be caused by different tolerance of seedlings and adults to waterlogging and partial flooding.

Germination is often inhibited by submergence and because many amphibious species are unable to germinate under water (Van der Valk 1981, Coops and Van der Velde 1995) regular drawdowns may be an essential requirement for long-term persistence of populations. Although germination requirements are usually similar, species may strongly differ in flood tolerance as seedlings (Welling *et al* 1988, Coops and Van der Velde 1995, Clevering *et al* 1996). In addition, zonation patterns may also be the result of different impacts of water levels on vegetative reproduction, since this often determines the lower limits of emergent macrophytes on the land-water gradient (Grace and Wetzel 1982, Grace 1989, Coops *et al* 1996*b*, Clevering 1998*b*).

Substrate conditions

Accumulation of organic matter is an important factor for species distribution in wetland vegetation (Barko and Smart 1983, Johnson *et al* 1985, Klosowski 1988, Smits *et al* 1990, White 1993). When flooded, organic matter may provide adverse conditions for plant growth because the high oxygen demand of the substrate (Brinson *et al* 1981) constrains the plant's ability to oxidize roots and rhizosphere (Armstrong 1970, Van Wijck *et al* 1992, Brix and Sorrell 1996). As a consequence, species with limited rhizosphere oxidizing ability are replaced by better-adapted species when organic matter accumulates (Barko and Smart 1983, Smits *et al* 1990, Van der Putten *et al* 1997). Experimental studies on the effects of flooded organic substrates on growth of emergent macrophytes have yielded contradictory results: both growth stimulating effects (Van den Brink *et al* 1995) and growth reducing effects (Clevering and Van der Putten 1995, Armstrong *et al* 1996*a*, Van der Putten *et al* 1997) have been found. A possible reason for this contradiction is the use of different origins of organic matter.

In shorelines, organic matter may originate from algae, submerged macrophytes or from emergent macrophytes (Pieczynska 1993, Boschker *et al* 1995). The latter type contains a high amount of cellulose and lignin and releases phytotoxic organic acids such as acetic acid and butyric acid during anaerobic decomposition (Drew and Lynch 1980, Kilham and Alexander 1984, Kovacs *et al* 1989).

Several authors have suggested that this accumulation of litter-derived organic matter plays an important role in decreased productivity of *P. australis* (Buttery *et al* 1965, Schröder 1987, Van der Putten *et al* 1997, Clevering 1998*a*). Organic matter may therefore be beneficial for species richness, but only if it inhibits *P. australis* and other clonal dominants more than interstitials.

Species interactions

Due to the eutrophic conditions of the studied system, competition is an important process structuring vegetation composition (Twolan-Strutt and Keddy 1996, Keddy *et al* 1997). Competition may restrict the range of a species on the flooding gradient. Especially weak competitors are often displaced to habitats where conditions are close to their physiological limits because they are outcompeted from positions with optimal environmental conditions (Grace and Wetzel 1981, Wilson and Keddy 1986*a,b*, Austin 1990).

The number of species (species richness) within a certain vegetation zone may also be influenced by competition. Especially in eutrophic environments, many interstitials may be excluded under the dense and tall canopy of the dominant species (Armesto and Pickett 1985, Palmer 1994, Falinska 1995). Decreased performance of the dominant *P. australis* will therefore improve opportunities for other species (Graneli 1989). The relationship between species richness and performance of *P. australis* may, however, not be straightforward. Firstly, because adverse environmental conditions such as flooding and wave exposure may directly constrain the number of species (Nilsson and Grelsson 1990, Gough *et al* 1994, Pollock *et al*

1998) and secondly because many factors which decrease performance of *P. australis* also inhibit other species. Organic matter accumulation in the substrate (Van der Putten *et al* 1997, Clevering 1998*a*) and litter layers have been shown to inhibit growth of *P. australis* (Van der Toorn and Mook 1982). As argued above, organic matter may also adversely affect other species and litter layers inhibit germination, seedling establishment (Van der Valk 1986, Cowie *et al* 1992) and adults of small statured species (Nilsson and Grelsson 1990, Campbell *et al* 1992).

Content of this thesis

The relationships between management tools, environmental factors, plant interactions and their influence on vegetation development of reed marshes are presented in a preliminary model (Fig 2), which was used to formulate the relevant research questions. Initially, a descriptive field study of the correlations between species richness, substrate organic matter and performance of *P. australis* is presented (chapter 2). Consequently, an experiment is described in which abiotic factors and biotic factors were distinguished (chapter 3). This was done to investigate the impact of plant interactions on zonation of marsh interstitials and tall forbs and to study the impact of plant interactions on species richness at different elevations. A comparison of marsh interstitial and tall forb species in their response to environmental factors is the subject of the next four chapters. Firstly the impact of water level on germination and seedling establishment is investigated (chapter 4). Consequently it was tested whether marsh interstitials and tall forb species respond differently to interactions between organic matter (using different types) and water level (chapter 5) and interactions between canopy shade and water level (chapter 6). The impact of water level on vegetative reproduction of a marsh interstitial, *Mentha aquatica*, and a tall forb, *Epilobium hirsutum*, is reported in chapter 7. Finally, interactions between the tall forb *E. hirsutum* and *P. australis* were studied to investigate the role of competition in the replacement of reed by tall forbs (Chapter 8). On the basis of the results presented in this thesis, the mechanism which produces the zonation of species-rich reed and tall forb stands will be discussed and a final qualitative model will be presented. From this model, management tools for increasing species richness of reed marshes will be derived.

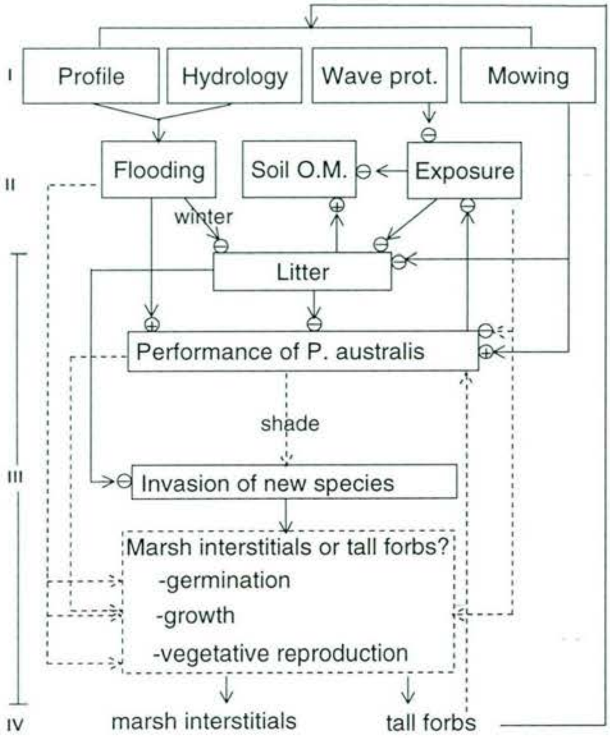

Figure 2: Conceptual framework of the research reported in this thesis. Symbols: + stimulating effect; - inhibitory effect. Management tools (**I**) are used to influence the environmental conditions (**II**). **Profile** refers to the slope and shape of the shoreline, both determining the area positioned at a certain elevation relative to the water level. **Hydrology** refers to both the degree and time of water level fluctuations that are allowed to occur. Together with profile, hydrology determines the depth, duration and frequency of **flooding** that may be experienced by species inhabiting the shoreline. **Wave prot.** (protection against waves) refers to dams before the actual shoreline erected to reduce the wave **exposure**. **Soil O.M.** indicates the amount and type of soil organic matter. **Mowing** in this context refers to the cutting and mowing of dead reed culms in winter or early spring, carried out either once a year or less frequent. Environmental conditions in turn determine the vegetation processes (**III**) although the management tool 'winter mowing' directly affects the **performance of P. australis** (either referring to peak standing crop or stem density). The vegetation processes as influenced by environmental conditions eventually determine the management results (**IV**). These results may be that both marsh interstitials and tall forbs, or only tall forbs, are present in shoreline vegetation. On the basis of these results, it may be decided to either continue or change the use of management tools (as indicated by the solid arrow in the right hand side of the framework). The influence of environmental factors on vegetation processes are either known (solid lines and arrows) or not known (dashed lines and arrows). The latter category indicates the research topics of this thesis.

2. PERFORMANCE OF *PHRAGMITES AUSTRALIS* IN RELATION TO SPECIES DISTRIBUTION AND SPECIES RICHNESS

Abstract

We used performance of *Phragmites australis* as a biotic predictor variable and investigated its relationships with species distribution and species richness in eutrophic shoreline vegetation dominated by *Phragmites australis*, other grasses or tall forbs. In 116 relevés we measured soil organic matter content, litter cover and elevation as well as standing crop, stem density and stem sizes of *Phragmites australis* in addition to plant species composition. Stem density and standing crop of *Phragmites australis* were negatively related to litter cover, elevation and soil organic matter. Ordination of all relevés, across different plant communities, identified elevation as the most important factor determining species distribution. Elevation and litter cover were the only factors significantly related to species richness. However, when relevés dominated by *Phragmites australis* were analyzed separately, stem density or standing crop, in combination with litter cover emerged as the most important factors determining species distribution. Litter cover and standing crop of the dominant explained 64% of the variation in species richness within the *Phragmites australis*-dominated community. This indicates that, at least in eutrophic *Phragmites australis*-dominated shorelines, within-community variation in species richness can be succesfully predicted by focussing on performance of the dominant species.

Nomenclature: Van der Meijden (1996)

Introduction

Many European freshwater shorelines are dominated by *Phragmites australis* (Cav.) Trin. ex Steudel which may form monospecific stands at parts of the shoreline that are flooded during the largest part of the year. At higher elevations productivity of *Phragmites australis* may be decreased due to factors such as insect herbivory (Van der Toorn and Mook 1982, Mook and Van der Toorn 1985, Tscharntke 1989), anaerobic decomposition of reed litter (Van der Putten *et al* 1997, Clevering 1998*a*) and competition (Buttery and Lambert 1965). At dry sites litter layers on the ground, rhizome-feeding larvae of the moth *Rhizedra lutosa* (Van der Toorn and Mook 1982) and terrestrial competitive herbs (Gryseels 1989, Fojt and Harding 1995) may further decrease the productivity of *Phragmites australis*. The existing knowledge about factors which affect the performance of *Phragmites australis* is mainly based upon experiments and incidental field observations (Van der Putten 1997). So far there has been no study in which systematically collected field data of *Phragmites australis* performance have been related to the factors that are regarded as responsible for its degeneration. Because *Phragmites australis* is a strong competitor in its optimal stage, its degeneration may be considered as a key process in vegetation change. Decreased performance of *Phragmites australis* will either precede the dominance of other species (Buttery and Lambert 1965, Van der Putten *et al* 1997) or enhance species richness (Graneli 1989). Further insight into factors related to degeneration of *Phragmites australis* under natural conditions is therefore required to enhance the predictability of vegetation development on *Phragmites australis* dominated shorelines.

Many experiments have shown that species richness is partly dependent on conditions that disadvantage the dominant species, thereby preventing competitive exclusion of subordinate species (Armesto and Pickett 1985, Grime *et al* 1987, Palmer 1994, Falinska 1995). However, a negative relationship between performance of *Phragmites australis* and species richness can not a priori be assumed since in dynamic environments such as shorelines species richness may be more determined by environmental factors than by biotic interactions (Gough *et al* 1994, Pollock *et al* 1998).

In this chapter we investigate the relationships between environmental factors, species distribution, species richness and performance of the dominant plant species *Phragmites australis*. Our aim was to identify the environmental factors related to the performance of *Phragmites australis* and to investigate the relationships between *Phragmites australis*, species distribution and species richness in communities dominated by *Phragmites australis* or other species.

Material and Methods

Study area

The study was carried out in the southwestern part of the Netherlands along the River Nieuwe Merwede and Haringvliet (51°42'N, 4°25'E; 51°49'N, 5°1'E; most western and most eastern sampling site respectively). These waters are part of the estuary of the river Rhine and Meuse, which used to be a freshwater tidal area before it was isolated from the North Sea in 1970 by construction of a barrier dam. As a consequence the daily tidal amplitude was reduced from 2 metres to 30 centimeter and the mean high water levels were lowered. Therefore the area subjected to daily alternating cycles of inundation and drainage was strongly reduced (Ferguson and Wolff 1983). These changes in hydrological conditions caused great changes in the composition of the shoreline vegetation of the former estuaries and connected rivers. Large parts of the vegetation formerly dominated by *Phragmites australis* changed into communities dominated by tall forbs such as *Urtica dioica* L. and *Epilobium hirsutum* L. but *Phragmites australis* remained dominant at frequently inundated sites (De Boois 1982, Van de Rijt *et al* 1996). In the study area, water level fluctuates daily due to a remaining small tidal amplitude and seasonally because of high river discharges in winter and early spring and low water levels in the growing season.

Selection of sites

By means of aerial photographs and field visits a selection was made of shorelines with gradual slopes, not grazed by cattle and not vegetated by scrubs and/or trees. Sites with a substrate consisting of stones, gravel or artificial materials used for bank protection were excluded. Because our main interest was the relation of *Phragmites australis* with species distribution and species richness we included a broad range of *Phragmites australis* performance as was recommended by Mohler (1983).

In total eighteen sites were selected and in each site a maximum of three line transects perpendicular to the shoreline were laid out and a minimum distance of 25 m was kept between neighbouring transects. Each transect consisted of three relevés, i.e. at the open water-fringe, landward but still dominated by *Phragmites australis* and in vegetation dominated by tall forbs. Each relevé was 3 metres wide and 2 metres long.

Sampling procedure

Between 2 July 1996 and 3 September 1996 vegetation data were collected from 116 relevés. In each relevé all higher plant species were recorded and cover/abundance of each species was recorded using the scale of Van der Maarel (1979). Percentage area covered by litter was estimated visually.

Performance of *Phragmites australis* was assessed between 21 August and 3 September, when *Phragmites australis* is at peak aboveground biomass (Kvet and Husak 1978, Van der Toorn and Mook 1982). Within each relevé the performance of *Phragmites australis* was determined in a randomly chosen subplot of 0.4 m x 0.4 m. After measuring the diameter of each stem we calculated the mean stem diameter and the coefficient of variation of stem diameter (as a measure of shoot size variability) for each plot. All *Phragmites australis*-stems within the subplot were clipped off at ground level and total aboveground dry weight in the subplot was determined after drying at 70°C for at least 48 hours. At the same time soil samples were collected by taking ten cores at random positions within the relevé. Coarse (organic) material on the soil surface was removed before the cores were collected. Each soil core was subdivided into a top layer of 0-5 cm and a lower layer of 5-15 cm. Per soil layer samples were pooled and thoroughly mixed to obtain one homogenuous soil sample per relevé. Organic matter content of each sample was determined by the weight loss of ground samples after 24h at 430 °C.

Elevation of each relevé was derived from a Digital Elevation Model, using the Geographical Information system GRASS4.0. The DEMs were constructed using local trend interpolation from aerial photographs (see Van de Rijt *et al* (1996) for further details). Elevation data were consequently expressed as height above mean heigh water level. The mean heigh water level of each site was calculated with records of long-term automated measurements of water level (every 10 minutes over 15-20 yr). Since elevation data derived from DEMs may be unreliable (Van de Rijt *et al* 1996) we determined a second measure of elevation by calculating the weighted average of Ellenberg (1979) moisture indicator values. These indicator values were weighted by the cover of each species and thus provide an acceptable tool for the exploration of species-environment relationships in ordination studies (Persson 1981). This alternative parameter of elevation will hereafter be referred to as 'moisture'.

Data analysis

Vegetation data were analysed by direct gradient analysis using Canonical Correspondence Analysis (CCA) with CANOCO version 3.12 (Ter Braak 1991). Because CCA results may be influenced by multicollinearity of environmental variables we only included variables that best explained the variance in the dataset. This was determined by using forward selection of environmental variables and comparing inflation factors of environmental factors, provided by the output of CANOCO (Ter Braak 1991). We considered inflation factors exceeding 2 as too strongly intercorrelated with other variables. From a highly intercorrelated set, variables that added least to explanation of variance in the forward selection procedure were excluded until inflation factors were below 2.

Detrended Correspondence Analysis (DCA) was applied to check if the variables reflected the real environmental gradients underlying the species distribution (Ter Braak 1987). Both the whole dataset and a subset with relevés in which *Phragmites australis* was dominant or co-dominant were analysed. The latter dataset excluded all relevés which were dominated by tall forbs (n=28), *Rubus caesius* (n=5), *Carex acutiformis* (n=1) and *Phalaris arundinacea* (n=4).

In both data sets rare species were downweighted. Species data in the complete dataset were squareroot-transformed because this results in lower weight of the dominant species (Ter Braak 1986). Since it was our objective to detect relationships between the performance of *Phragmites australis* and species distribution we treated parameters expressing the performance of *Phragmites australis* as environmental variables and analysed its species score passively. Samples with missing values of environmental variables (five in the complete dataset and four in the dataset with *Phragmites australis*-dominated relevés) were also analysed passively. Prior to analysis environmental variables (except moisture) were log-transformed and all variables were standardized to zero mean and unit variance.

Relationships between environmental variables, performance of *Phragmites australis* and species richness were investigated by multiple regression on linear and quadratic terms (Sokal and Rohlf 1995). To avoid autocorrelation we excluded the parameter 'moisture' from the multiple regression analysis. Prior to regression analysis we checked for normality of distribution and applied ln- or log transformation for highly skewed variables.

Results

In both analyses results of DCA and CCA resembled each other closely. Only CCA results are presented because they are the best illustration of relationships between species and environmental variables. The first two axes of the CCA biplot explained 55 % of the variance in species environment correlations (Table 1). Variability in stem diameter and organic matter content in the 5-15 cm soil layer were not included in the analysis because they were highly correlated with other environmental variables and added little to the explanation of variance in the dataset. The horizontal axis in the biplot (Fig. 1) represents an elevational gradient, which is demonstrated by the strong correlation between this axis and moisture. The dry end of the elevational gradient was characterised by relatively high litter cover and high abundance of *Rubus caesius* and tall forb species such as *Urtica dioica*, *Epilobium hirsutum* and *Solidago gigantea* (Fig. 1). Both organic matter content of the surface soil layer and mean diameter of *Phragmites australis* were correlated with the vertical axis, but these correlations were not as strong as the correlation between moisture and the horizontal axis of the CCA biplot (Fig. 1). Hence, relationships of *Phragmites australis* performance and species distribution within the entire dataset were relatively weak and not unambiguous.

Table 1: Summary of ordination results from CCA with the complete dataset (111 active samples and 5 passive samples).

	Axis 1	Axis 2	Axis 3	Axis 4	Total inertia
Eigenvalues	0.208	0.166	0.093	0.068	3.927
Species-environment correlations	0.785	0.782	0.704	0.639	
Cumulative percentage variance:					
of species data	5.3	9.5	11.9	13.6	
of species-environment correlations	30.6	55.0	68.7	78.7	
Sum of all unconstrained eigenvalues					3.927
Sum of all canonical eigenvalues					0.679

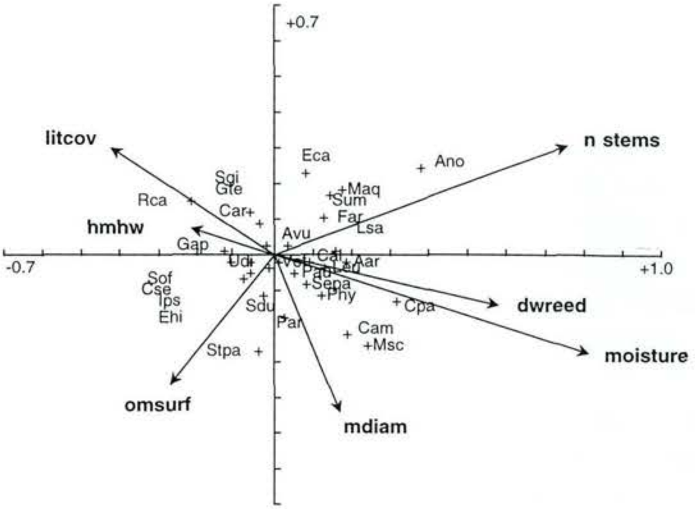

Figure 1: First two axes of the CCA biplot of the complete dataset, based upon 111 active samples and 5 passive samples. Only species present in more than 5% of the samples are indicated. Abbreviations of environmental variables: dwreed= aboveground dry weight of *Phragmites australis*, hmhw= height above mean heigh water, litcov= litter cover, moisture= weighted average of Ellenberg indicator values, mdiam= mean stem diameter of *Phragmites australis*, nstems= stem density of *Phragmites australis*, omsurf= organic matter in soil surface layer (0-5 cm). Abbreviations of species names: Aar= *Angelica archangelica*, Ano= *Apium nodiflorum*, Avu= *Artemisia vulgare*, Cpa= *Caltha palustris*, Cse= *Calystegia sepium*, Cam= *Cardamine amara*, Cal= *Chenopodium album*, Car= *Cirsium arvense*, Ehi= *Epilobium hirsutum*, Eca= *Eupatorium cannabinum*, Far= *Festuca arundinacea*, Gte= *Galeopsis tetrahit*, Gap= *Galium aparine*, Ips= *Iris pseudacorus*, Leu= *Lycopus europaeus*, Lsa= *Lythrum salicaria*, Maq= *Mentha aquatica*, Msc= *Myosotis scorpioides*, Par= *Phalaris arundinacea*, Pau= *Phragmites australis*, Phy= *Persicaria hydropiper*, Rca= *Rubus caesius*, Sum= *Scrophularia umbrosa*, Sepa= *Senecio paludosus*, Sdu= *Solanum dulcamara*, Sgi= *Solidago gigantea*, Stpa= *Stachys palustris*, Sof= *Symphytum officinale*, Udi= *Urtica dioica*, Vof= *Valeriana officinalis.*

Within the *Phragmites australis*-dominated communities, species distribution was clearly related to performance of the dominant species. The first two axes of the CCA biplot explained 57% of the variance in species environment correlations (Table 2). Height above mean heigh water and variability of *Phragmites australis* stem diameter were excluded from the CCA because they were strongly correlated with other variables and added little to the explanation of variance in the dataset. Number of stems was the variable most strongly related to the horizontal axis of the CCA biplot, whereas the vertical axis represent a litter gradient and in the opposite direction a gradient of increasing *Phragmites australis* standing crop (Fig. 2). As already suggested by the different directions of arrows in the biplot (Fig. 2) stem density and standing crop of *Phragmites australis* were weakly correlated (regression equation: Aboveground dryweight= 1.39*Stem density-(Stem density)^2; n=71, R^2_{adj}= 0.23, P<0.001). In contrast to the complete dataset, moisture contributed relatively little to ordination of species within the dataset of *Phragmites australis*-dominated relevés.

Apium nodiflorum, *Mentha aquatica* and *Eupatorium cannabinum* had their highest abundance at high *Phragmites australis* stem density, as indicated by their relatively high score on the horizontal axis. Position of *Myosotis scorpioides*, *Cardamine amara*, *Caltha palustris* and *Persicaria hydropiper* in the biplot indicates that they occcurred at relatively high levels of *Phragmites australis* standing crop.

Although relationships between environmental variables and performance of *Phragmites australis* can be derived from the CCA biplots they are best illustrated by the multiple regression results. In the whole dataset litter cover, organic matter of the surface soil layer and elevation were negatively related to both aboveground dry weight and stem density of *Phragmites australis*, explaining 36% of the variance (i.e. R^2_{adj}=0.36) in both measures of *Phragmites australis* performance (Table 3). Stem diameter was negatively related to litter cover and variability in stem diameter was not related to any measured environmental variable (Table 3). Within the *Phragmites australis*-dominated relevés aboveground dry weight and number of stems were weakly correlated with litter cover only. Mean stem diameter and variability in stem diameter were not significantly correlated with any measured variable (Table 3). No parameter of *Phragmites australis* performance was related to species richness in the complete dataset. Species richness was explained for 38% by a positive, linear term and a negative, quadratic term of litter cover in combination with a negative linear relationship with height above mean heigh water (Table 4). Within the *Phragmites australis* community a model incorporating both litter cover and standing crop of *Phragmites australis* fitted 64% of species richness (Table 4). The relationship between litter cover and aboveground biomass of *Phragmites australis* is illustrated by the distance weighted least squares surface plot (Fig. 3). Species richness was low at both low and high degrees of litter cover. Species richness peaked at relatively low values of aboveground biomass (ca. 200g.0.16m^{-2}) and was slightly decreased at lower biomasses and strongly decreased at higher values (Fig. 3).

Table 2: Summary of ordination results from CCA with the complete dataset (111 active samples and 5 passive samples).

	Axis 1	Axis 2	Axis 3	Axis 4	Total inertia
Eigenvalues	0.309	0.169	0.114	0.085	4.047
Species-environment correlations	0.797	0.845	0.773	0.746	
Cumulative percentage variance:					
of species data	7.6	11.8	14.6	16.7	
of species-environment correlations	36.7	56.8	70.2	80.4	
Sum of all unconstrained eigenvalues					4.047
Sum of all canonical eigenvalues					0.843

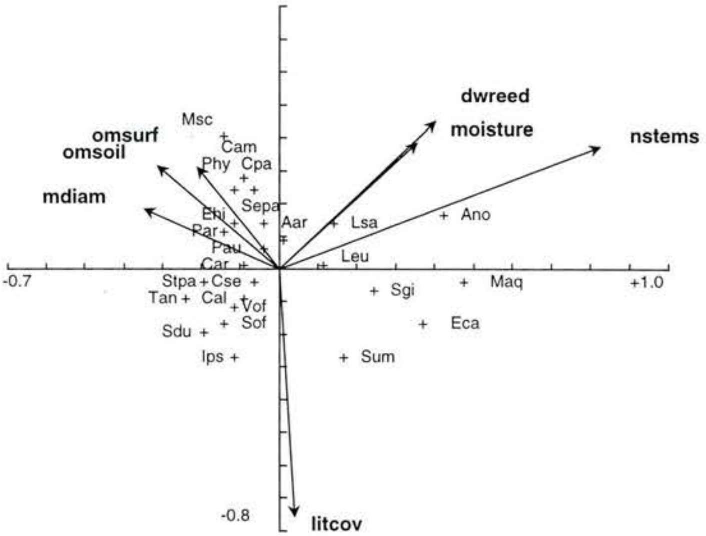

Figure 2: First two axes of the CCA biplot of relevés with dominance of *Phragmites australis*, based on 78 active samples and 4 passive samples. Only species that were present in more than 5% of the samples are indicated. Abbreviations according to Fig. 1, except omsoil= organic matter in deep soil layer (5-15 cm).

Table 3: Multiple regression analysis of performance of *Phragmites australis* across all relevés and in *Phragmites australis*-dominated communities (relevés with (co-)dominance of *Phragmites australis*). Only variables that significantly contributed to the model fit are presented. Cumulative R^2_{adj} cumulates from top to bottom and indicates the total proportion of variation of a given dependent variable explained by the listed predictor variables. Species richness and litter cover refer to ln- and log-transformed data respectively. Abbreviations: CV= Coefficient of variance, DW= dry weight, n.s.= no significant regressions with any environmental parameter, OM= organic matter, HMHW= height above mean high water.

Dependent variable	Predictor variables	Coefficient	Cumulative R^2_{adj}
All relevés			
- DW aboveground	- litter cover	-0.41	0.29
	- OM 0-5cm	-0.20	0.33
	- HMHW	-0.19	0.36
- Stem density	- (litter cover)^2	-0.35	0.26
	- (OM 0-5cm)^2	-0.26	0.34
	- HMHW	-0.20	0.36
- Stem diameter	- (litter cover)^2	-0.36	0.12
- CV Stem diameter		n.s.	
***Phragmites australis*-dominated relevés**			
- DW aboveground	-litter cover	-0.47	0.21
- Stem density	-(litter cover)^2	-0.29	0.08
- Stem diameter		n.s.	
- Litter cover		n.s.	

Discussion

Within the entire data set both standing crop and stem density of *Phragmites australis* were negatively related to litter cover, organic matter content and elevation. Litter cover was negatively related to *Phragmites australis* performance because litter layers offer mechanical resistance to emerging shoots (Jordan *et al* 1990, Campbell *et al* 1992) and because *Rhizedra lutosa* overwinters in the litter layer (Van der Toorn and Mook 1982). The significant negative relationship with elevation may also be related to these moths, since they can only complete their life cycle in dry, i.e. high elevated, parts of the shoreline (Van der Toorn and Mook 1982).

Table 4: Multiple regression analysis of species richness across all relevés and in *Phragmites australis* dominated relevés. Only variables that significantly contributed to the model fit are presented. Cumulative R^2_{adj} cumulates from top to bottom and indicates the total proportion of variation of a given dependent variable explained by the listed predictor variables. Species richness and litter cover refer to ln- and log-transformed data respectively. Abbreviations: CV= Coefficient of variance, DW= dry weight, n.s.= no significant regressions with any environmental parameter, OM= organic matter, HMHW= height above mean high water.

Dependent variable	Predictor variables	Coefficient	Cumulative R^2_{adj}
- **All relevés**	- litter cover	2.66	0.19
	- (litter cover)^2	-2.20	0.36
	- HMHW	-0.18	0.38
- ***Phragmites australis*-dominated relevés**	-litter cover	2.12	0.48
	-(litter cover)^2	-1.5	0.57
	-(DW aboveground)^2	-0.22	0.64

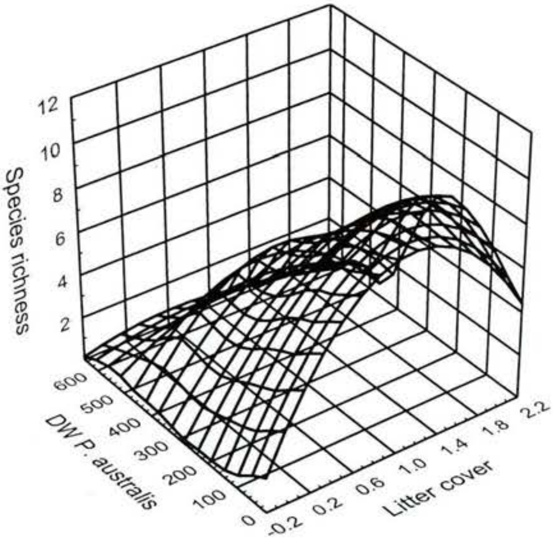

Figure 3: Visualization of relationship between aboveground biomass of *Phragmites australis* (DW *P. australis*; g/0.16m^2), litter cover (log-transformed data) and species richness (number of species/6m^2) using a surface plot of distance weighted least squares.

The detrimental effects of litter-derived organic matter on *Phragmites australis* growth have been shown experimentally (Armstrong *et al* 1996*a*, Van der Putten *et al* 1997, Clevering 1998*a*). Field data to support the ecological importance of litter-derived organic matter for the decrease in vigour of *Phragmites australis* result from our study. However, only a small fraction of variance in *Phragmites australis* performance was explained by organic matter content, which may be inherent to the study area. Here, most *Phragmites australis* stands reach their maximum water depth at frequently but not permanently flooded parts of the shoreline (Coops 1996). The regular drawdowns (most of our relevés were above the water level throughout the growing season) will have allowed aeration of the soil thereby ameliorating substrate conditions (Blom and Voesenek 1996, Clevering 1998*a*).

A large fraction of variance in aboveground dry weight, stem density, stem diameter and variability in stem diameter of *Phragmites australis* was unexplained by any measured environmental variable. This suggests a strong role of other factors not covered by the present study, such as insect herbivory by *Archanara gemipuncta* in the previous year (Mook and Van der Toorn 1985), frost damage (Van der Toorn and Mook 1982) and shading of outgrowing culms by dead standing litter (Graneli 1989). The weak relationships between stem density and standing crop indicates that stands with many small shoots can reach standing crops comparable to stands with a few, but large shoots (Mook and van der Toorn 1982).

This study has provided clear indications that *Phragmites australis* determines the species distribution and species richness within the *Phragmites australis*-dominated plant communities. Across all relevés however, elevation was the major environmental variable. In accordance with other studies we found that *Rubus caesius* and tall forb species such as *Urtica dioica*, *Epilobium hirsutum* and *Solidago gigantea* increased in abundance at dry parts of the shoreline (Fojt and Harding 1995, Coops 1996, Van de Rijt *et al* 1996). These competitive species might, in addition to elevation-related environmental factors mentioned above, directly affect *Phragmites australis*. The ordination of the complete dataset (Fig. 1) did not provide a clear second environmental gradient independent of elevation. Probably because factors determining species abundance and species richness along gradients parallel to the water line vary with elevation (Brewer *et al* 1997).

In contrast to the complete dataset, the impact of elevation on species distribution within the *Phragmites australis*- dominated plant communities was limited and litter cover, *Phragmites australis* stem density and standing crop emerged as the most important variables determining species distribution. Interstitial marsh species were either related to *Phragmites australis* standing crop or to *Phragmites australis* stem density, which reflects two different strategies for coexistence with *Phragmites australis*. *Apium nodiflorum* and *Mentha aquatica* were most abundant at relatively high stem densities and low stem diameters. These species will have benefitted from insect herbivory of *Phragmites australis* stems which leads to formation of an extra number of thin stems and to a delay in the closure of the canopy (Mook and Van der Toorn 1982). In stands where *Phragmites australis* reaches high standing crops with a few stems, indicating an absence of insect damage or frost damage (Van der Toorn and Mook 1982, Mook and Van der Toorn 1982) *Caltha palustris*, *Cardamine amara* and *Myosotis scorpioides* had their highest abundance. This may be due to a different phenology which allows them to grow and flower before the shoots of *Phragmites australis* have reached their maximum height (Zonneveld 1960, Haraguchi 1993) or to morphological adaptations to shade in case of *Myosotis scorpioides* (chapter 6). Measures of *Phragmites australis* stem diameter (i.e. both mean and variability in diameters) offered little explanation for the distribution of other species. This may be due to the fact that diameters are fixed throughout the growing season and are more related to the potential than to the actual stem height and -biomass (Hara *et al* 1993).

Apart from performance of *Phragmites australis*, litter cover was the most important variable in the CCA ordination of the *Phragmites australis* community. Species found at relatively high litter cover (i.e. negative scores on the y-axis in Fig. 2) were either climbing species (*Solanum dulcamara*) or tall erect herbs (*Scrophularia umbrosa*, *Symphytum officinale* and *Valeriana officinalis*). Litter layers inhibit species of small stature and with weak stems (Nilsson and Grelsson 1990, Campbell *et al* 1992) and representatives of these growth forms (*Myosotis scorpioides*, *Cardamine amara*, *Persicaria hydropiper*) all had their centroids at the opposite end of the litter arrow (Fig. 2).

The adverse effects of litter on small species, combined with its inhibition of germination and seedling emergence (Van der Valk 1986, Cowie *et al* 1992) also explain the negative relationship between the quadratic term of litter cover and species richness (Table 4, Fig. 3). However, species richness was also low in relevés with low litter cover (Fig. 3). To our knowledge there is no plausible explanation for beneficial effects of litter on species richness in eutrophic shorelines. It is more likely that relevés with low litter cover represent sites exposed to waves and current water because these water movements remove litter (Nilsson and Grelsson 1990). Most species are very susceptible to exposure because they are superficially rooted and have rather fragile stems (Haslam 1978). Species richness will therefore be low at exposed sites, even if performance of *Phragmites australis* is decreased. *Phragmites australis* can withstand considerable degrees of exposure (Coops *et al* 1996*a*) and this explains why its performance had no positive relationship with litter cover (Table 3).

In wetlands, environmental factors tend to be more determining for species richness than biotic factors such as standing crop (Gough *et al* 1994, Pollock *et al* 1998). This was confirmed for the entire dataset in which only litter cover and elevation (which determines the degree and depth of flooding) were significantly related to species richness. At the within community level, Pollock *et al* (1998) could only explain 36% of the variation in species richness with the environmental factor flooding frequency. Attempts to relate species richness within a single plant community to the biotic factor total standing crop have often yielded insignificant results (Wheeler and Giller 1982, Verhoeven *et al* 1988, Moore and Keddy 1989, Wheeler and Shaw 1991).

However, within the *Phragmites australis*-dominated communities, we were able to explain 64% of the variation in species richness. This was done by using the performance of the dominant species instead of total standing crop as the biotic predictor variable in combination with environmental factors (litter cover, which partly reflected the amount of wave exposure). Studies in grasslands and old fields have already acknowledged the large impact of the dominant species on species richness (Armesto and Pickett 1985, Grime *et al* 1987, Falinska 1995). Our study has shown that also in dynamic environments such as shorelines focus on the status of the dominant species may enhance the predictability of species richness at the within community level.

interactions determine zonation of all species and whether plant interactions exert a similar effect at all positions of the flooding gradient.

In a wide range of habitats it has been established that in particular the distribution of interstitial species, i.e weak competitors with limited ability for clonal spread (Boutin and Keddy 1993), is determined by dominant species (Armesto and Pickett 1985, Grime 1987, Keddy 1989). From many zonation studies it has been concluded that weak competitors grow at positions which are closer to their physiological limit than their physiological optimum because they are excluded from their optimum position by stronger competitors (reviewed by Smith and Huston 1989, Austin 1990). Along flooding gradients experimental results have both confirmed (Grace and Wetzel 1981) and rejected (Shipley *et al* 1991*a*) an important role for plant interactions in producing zonation patterns. Both studies however have focused on a limited number of morphologically similar species. A comparison of the impact of plant interactions on species widely differing in growth form (and hence in competitive abilities) may further clarify i) whether plant interactions determine the position of different species in flooding gradients and ii) if so, whether the impact of plant interactions on zonation differs among species.

Another important question with regard to the role of plant competition is when it plays a role. Recently, several authors have suggested that even in highly productive vegetation competition may not be the only mechanism determining the number of species (Gough *et al* 1994, Abrams 1995, Oksanen 1996). In one of the few experiments where effects of vegetation canopy were tested directly, Brewer *et al* (1997) found that plant interactions only play an important role at rarely flooded parts of the shoreline, because at lower parts extreme abiotic conditions already rule out many species. Since this study has been carried out in salt marshes where the influence of vegetation canopies on recruitment also depends on salinity (Bertness and Shumway 1993), conclusions from these studies may not be applicable to freshwater shoreline vegetation.

In this study we investigate the role of canopy removal on the zonation of interstitials and clonal dominants and on species richness at two elevations of a freshwater shoreline. Canopy removal allowed us to distinguish between biotic and abiotic factors (cf. Silander and Antonovics 1982). We chose a riparian wetland with a fluctuating water level and highly productive vegetation as our study area, so that substantial abiotic and biotic differences could be included in the field trial. To investigate to which extent differences in recruitment between elevations could be attributed to zonation patterns in the seed bank (Rabinowitz 1978, Schneider and Sharitz 1988) we determined seed bank content at both elevations and added a seed mixture to a subset of plots.

3. CONTROL OF PLANT SPECIES RICHNESS AND ZONATION OF FUNCTIONAL GROUPS ALONG A FRESHWATER FLOODING GRADIENT

Abstract

We investigated the role of plant interactions in producing the zonation of strong competitors, i.e. clonal dominants, and weak competitors, i.e. interstitials. In addition, it was tested whether the effect of plant interactions on species richness depends on the position at the flooding gradient. During one growing season vegetation canopy was removed at a low elevation, dominated by *Phragmites australis* and at a high one, dominated by tall forbs, mainly *Urtica dioica*. The seed bank was examined and in half of the plots seeds of clonal dominants from high elevations and interstitials from low elevations were sown to ensure that seeds of both groups were present at both zones.

At both elevations, removal of vegetation canopy resulted in a strong increase of interstitial species, but interstitials from low elevations failed to establish in cleared plots at the tall forb zone. This could not be attributed to the absence of seeds and we concluded that conditions, unfavourable for germination, rather than plant interactions determine the zonation of interstitials from the *P. australis* zone. Many seedlings of tall forb dominants emerged in cleared plots at the low elevation. However, number of seedlings rapidly declined during the first year. Hence, abiotic conditions, most probably flooding, prevented seedling establishment of tall forb dominants at zones dominated by *P. australis*.

Canopy removal increased species richness at the low elevation in the first year only, whereas at the higher elevation species richness in cleared plots remained higher throughout the second year when the canopy was no longer removed. We therefore concluded that species richness of freshwater shorelines is mainly controlled by abiotic factors in the frequently flooded zone and by plant interactions at higher elevations.

Introduction

Species distribution and species richness along environmental gradients are supposed to be determined by both abiotic and biotic factors (Grime 1979, Austin 1990). Along flooding gradients depth and duration of flooding are the main environmental variables determining zonation in vegetation (Blom and Voesenek 1996). Individual plant species may be affected directly due to their germination requirements with regard to soil moisture (Keddy and Ellis 1985, Coops and Van der Velde 1995) or due to different tolerances to flooding as seedlings and adults (Welling *et al* 1988, Squires and Van der Valk 1992, Blom *et al* 1994, Clevering *et al* 1996). Biotic interactions, especially competition, are also considered as a determining factor for the zonation of species along flooding gradients (Buttery and Lambert 1965, Grace and Wetzel 1981, Snow and Vince 1984, Austin 1990). It is, however, far from clear whether plant

Material and Methods

Description of study site

The field experiment was carried out on a shoreline of the river Nieuwe Merwede, situated in the southwestern part of the Netherlands (51°45'N, 4°45'E). This river passes the former freshwater tidal area of the Rhine and Meuse estuaries. Before the closure of the Haringvliet in 1970, many reedbeds in the estuary were used for winter harvesting of reed culms. The closure caused a reduction in tidal difference from 1.5 to 0.30 m and a lowering of the mean high and low water tables (Ferguson and Wolff 1983). These hydrological changes led to an end of the commercial use of the reedbeds, which have been left unmanaged since then. The study site was in one of these derelict reedbeds. Along the land-water gradient two different vegetation types could be recognized. The lower elevation was still dominated by *Phragmites australis* (Cav.) Trin. ex Steudel, which was accompanied by interstitial species such as *Caltha palustris* L. and *Lycopus europaeus* L. This *Phragmites australis* dominated vegetation type has its upper range around the mean high water level and is frequently flooded throughout the year. At the higher elevation tall forbs, in particular *Urtica dioica* L., were dominant and *Phragmites australis* was only marginally present. Flooding of the tall forb zone is usually restricted to winter and early spring. Low and high elevations will hereafter be referred to as reed marsh and tall forb zone, respectively.

Experimental design

Sixteen plots of 1 m^2 each were laid out in the reed marsh as well as in the tall forb zone. Four treatments, with four replicates each, were applied at each elevation: 1) plots containing aboveground vegetation (hereafter referred to as 'closed'), 2) plots containing aboveground vegetation but with addition of a seed mixture, 3) plots with removal of aboveground vegetation (hereafter referred to as 'open'), 4) plots with removal of aboveground vegetation but with addition of a seed mixture. Treatments were assigned to plots at random. On the 22nd March 1994, standing litter and litter on the ground were removed from all plots. Throughout the growing season of 1994, in the open plots shoots with vegetative origin were clipped at ground level and removed every second week.

Seed addition

Representatives of reed marsh interstitials and tall forb dominants were selected for the seed mixture. The content of the seed mixture and maximum germination of each species in the seed mixture is shown in Table 1. Seeds were sown at the high elevation on 20th April 1994 and, because of temporary high water, on 29th April at the low elevation. Seeds of species to be sown were collected in 1993 along the river Nieuwe Merwede from a single population of each species. After collection, seeds were stored moist and dark at 4°C. Maximum germination of each species in the seed mixture was determined on moist filter paper in Petri dishes with 50 seeds per dish and six replicate dishes per species. Germination trials were carried out in a germination chamber with a day/night regime of 18h/6h at 25°C/10°C at the same time as the seeds were sown in the plots.

Table 1: Content of seed mixture added to both open and closed plots. Listed are the numbers of seeds per m^2 (N), maximum germination in the germination trial (%, mean ± SD), functional group and elevation where adults occur most frequently. Reed marsh = elevation with dominance of *P. australis*; tall forb= elevation with dominance of *E. hirsutum* and *U. dioica*.

Species	N	Max. germination	Functional group	Zone
Epilobium hirsutum	800	69.4±12.9	Clonal dominant	Tall forb[1,2]
Filipendula ulmaria	400	2.3±3.0	Clonal dominant	Tall forb[1]
Eupatorium cannabinum	800	11.6±3.7	Interstitial	Tall forb[1,2]
Lycopus europaeus	800	85.8±4.8	Interstitial	Reed marsh[1]
Lythrum salicaria	800	90.8±2.9	Interstitial	Reed marsh[1]
Sium latifolium	800	2.0±1.3	Interstitial	Reed marsh[1]

Source: 1: De Boois (1982); 2: Van de Rijt *et al* (1996).

Counting of seedlings and monitoring of vegetation development

We counted seedlings within subplots due to the high numbers of seedlings in the open plots and in order to avoid severe disturbance of aboveground vegetation in closed plots. A subplot consisted of an iron ring of 8 cm diameter. On 11th May 1994, twelve of these subplots were installed at random in each plot. From this date onwards, numbers of seedlings in the subplots were counted every week until August and every second week until 22nd September 1994. This resulted in a total of 16 counting days for the tall forb zone. Due to occasional floodings, the numbers of counting days at the reed marsh zone was restricted to 14. Subplots which were covered by litter after flooding or disturbed by animals were omitted from the moment disturbance was first observed. Species composition and relative abundance per species were determined on 14th-15th June 1994, 9th May 1995 and at the end of the growing season in 1995 (13th September for the reed marsh zone and 11th October 1995 for the tall forb zone). Numbers of seedlings were counted in open plots during June 1994. In May 1995, cover at the reed marsh zone was too low due to the onset of the growing season and therefore counts were made. In all other circumstances cover per species was determined because this was considered to be more informative than number of plants. Counts and cover values were standardized by expressing the value of each species as its proportional contribution to the total vegetation (cf. Greig-Smith 1983, p. 212).

Seed bank analysis

Species composition and numbers of viable seeds per species in the seed bank were determined by taking 59 soil samples (29 at the reed marsh zone and 30 at the tall forb zone) with a 3.8 cm diameter soil corer. Samples were taken from the soil surface under the litter layer to a depth of 7 cm and separated into top layer (0-2 cm) and the remaining layer of 2-7 cm below the soil surface. At 14 July 1994 samples were collected from undisturbed parts of the vegetation in the immediate neighbourhood of the plots. No new seed fall had taken place before this date and sampling sites covered by litter were chosen to minimise the possibility of change in seed bank composition due to spring germination. Seed bank samples were stored moist and dark at 5 °C until further processing.

Fine and coarse soil material was removed from seedbank samples and seedling emergence was assessed according to Ter Heerdt *et al* (1996), except that concentrated samples were placed in a climate chamber with a day/night regime of 18h/6h at 25°C/10°C. No attempt was made to check for ungerminated viable seeds afterwards.

Monitoring of environmental parameters

In 1994 and 1995, daily minimum and maximum water levels at the study site were determined using data from neighbouring gauge stations. Light extinction in open and closed plots (regardless of seed addition) was determined at both elevations by measuring light intensity at ground level and above the undisturbed vegetation canopy by a photosynthetic radiometer RA 200 (Botteman Weather Instruments). Light extinction was measured in June, July and August 1994. At each date three plots were randomly chosen from all open and closed plots. Three readings were made per plot and the within-plot means were consequently treated as one observation.

Distinction of zonation- and functional groups

Each species was assigned to a zonation group based upon the occurrence of adult plants in the reed marsh or in the tall forb zone. Within each zonation group, species were further divided into functional groups.

Information about zonation of the species was obtained from De Boois (1982) and Van de Rijt *et al* (1996), who carried out extensive field surveys in the study area. Species were classified into functional groups on the basis of plant traits according to the system of Boutin and Keddy (1993). Information on plant traits was obtained from literature data, but not all values considered to be relevant by Boutin and Keddy (1993) were available. Therefore, some modifications to the classification system had to be made. Species were classified into two functional groups: interstitials and clonal dominants. Obligate annuals were ignored. Woody species (in this case *Solanum dulcamara* L. and *Rubus caesius* L.) and climbing species (*Calystegia sepium* L.) were also excluded from classification because they have adopted different strategies to interact with other species (Grime 1979, Den Dubbelden and Oosterbeek 1995). Cover of these species never exceeded five percent in any plot.

Interstitial species and clonal dominants were distinguished from each other on the basis of their height and their ability to dominate the vegetation by clonal spread. Data about these plant traits were derived from Van der Meijden (1996; maximum height) and Grime et al. (1988; 'lateral spread' and 'gregariousness'). The group of interstitials includes all species with a maximum height less than 100 cm or species with a maximum height greater than 100 cm but with a lateral spread less than 25 cm or with an 'intermediate' gregariousness (sensu Grime *et al* 1988).

Species whose maximum height exceeded 100 cm and whose lateral spread exceeded 25 cm or with gregariousness called 'stand forming' (by Grime *et al* 1988) were classified as clonal dominants. The maximum height treshold was based upon Boutin and Keddy (1993) who found a height of 138±39 cm (mean and 95% confidence interval) for clonal dominants. The phytosociological literature (Wheeler 1980; Schaminée *et al* 1995) was consulted for species not mentioned by Grime *et al* (1988). If a species was taller than 100 cm and reported to be dominant under any circumstance it was classified as a clonal dominant. Wheeler (1980), Schaminée *et al* (1995) and Van de Rijt *et al* (1996) were used to check the classification of species mentioned in Grime *et al* (1988).

All species were thus classified as reed marsh interstials, reed marsh dominants, tall forb interstitials and tall forb dominants. A full list of species and the functional group to which they were assigned is given in the Appendix. Proportion of each group was obtained by summation of proportional contribution of all species belonging to the same group.

Data analysis

Because the volume of the seed bank sample of 2-7 cm soil layer was 2.5 fold larger than the 0-2 cm soil layer the numbers of seeds in the upper soil layer were multiplied by 2.5 to allow direct comparison with the 2-7 cm soil layer. For the most abundant species in the seed bank the mean number of seeds per sample were compared by the Mann-Whitney U-test (Sokal and Rohlf 1995).

Counts of seedlings in subplots were used to evaluate the effects of aboveground vegetation and elevation in the first year. For each date and plot a mean number of seedlings per subplot was calculated by dividing the total number of seedlings by the total number of (undisturbed) subplots. This was only done for the four most abundant species. For each plot the maximum number of seedlings per subplot and number of seedlings per subplot at the end of the first growing season (September 22^{nd} 1994 for both elevations) were determined. Effects of elevation, seed addition and vegetation removal on numbers in subplots during the first year (1994) and numbers in May of the second year (1995) were tested by log-linear analysis for goodness of fit against a Poisson distribution (Sokal and Rohlf 1995) using type 3 analysis in SAS procedure GENMOD (SAS Institute 1993). Effects of vegetation removal, seed addition and elevation on the proportion of each zonation-/functional group were tested against a binomial distribution (Sokal and Rohlf 1995), using SAS procedure GENMOD (SAS Institute 1993). In all cases where log-linear analysis for goodness of fit was applied fully saturated models were fitted first. If these models could not be fitted due to treatments with zero values, model fitting was restricted to main effects and possible second order interactions. Means of proportions were compared by Kruskal-Wallis. In each case where means were compared the significance level was adjusted according to the method described in Sokal and Rohlf (1995), keeping the overall experiment-wise error rate at 0.05.

Effects of seed addition, vegetation removal and elevation on the number of species were tested by ANOVA, with elevation as an unreplicated factor in which vegetation removal, seed addition and vegetation removal*seed addition were confounded (Steel and Torrie 1980). Per survey date means were compared with LSD.

Results

Environmental parameters

In both 1994 and 1995, plots at the reed marsh zone were frequently flooded during the growing season whereas plots at the tall forb zone were only flooded during autumn and winter (Fig. 1). In June 1994, more light was intercepted by canopies in closed plots of the tall forb zone than by those of the reed marsh zone (Fig. 2), but later differences between elevations disappeared. In June and July 1994, at both elevations light interception in open plots was significantly lower than in closed plots. In August 1994, the established seedlings in the open plots had formed a dense canopy and light interception had reached the same level as in closed plots (Fig 2).

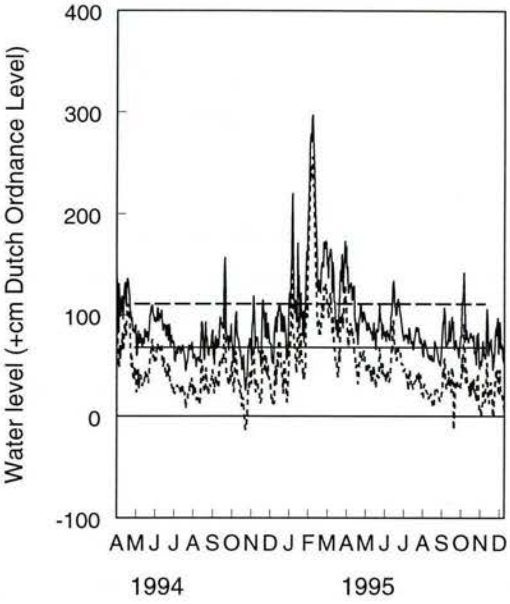

Figure 1: Water level fluctuations from April 1994 until November 1995 at the study site and mean elevation of plots at the reed marsh zone (————) and tall forb zone (------).

Seed distribution

Seed densities of the tall forb dominants *U. dioica* and *Epilobium hirsutum* and the reed marsh interstitials *Lycopus europaeus* and *Lythrum salicaria* L. did not differ between elevations, whereas significantly more seeds of the reed marsh interstitial, *Mentha aquatica* L. were found at the tall forb zone (Table 2). However, seed densities of *Phragmites australis*, *Cardamine amara* L. and *Caltha palustris* were higher at the reed marsh zone, whereas more seeds of *Thalictrum flavum* L. were found at the tall forb zone.

Addition of a seed mixture neither influenced the number of species (Table 3) nor the proportions of groups to which species in the seed mixture belonged (Table 4) and the number of seedlings of the unsown species *U. dioica* (Table 5). *Epilobium hirsutum*, *Lycopus europaeus* and *Lythrum salicaria* were the only species from the seed mixture whose emergence was high enough to be analysed further. At both elevations maximum numbers of *Epilobium hirsutum* were significantly increased by seed addition (Table 5 and Fig.3), but at the end of the growing season the effect of sowing was no longer significant.

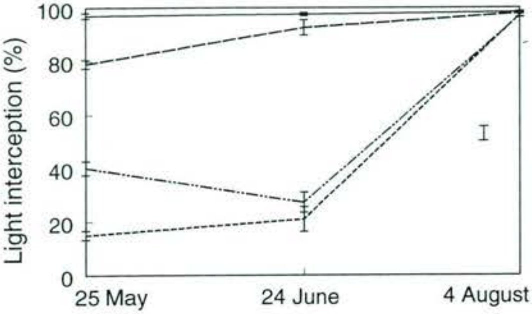

Figure 2:Light extinction (means±SE, n=3) during 1994 in closed plots at the tall forb zone (———), open plots at the tall forb zone (-------), closed plots at the reed marsh zone (----) and open plots at the reed marsh zone (—··—). Light interception represents the proportion of light intensity intercepted between the top of the canopy and the ground level.

The maximum number of seedlings of *Lycopus europaeus* was significantly influenced by interactions between vegetation removal, elevation and seed addition (Table 5). Seed addition only increased the number of seedlings in open plots at the reed marsh zone but not at the tall forb zone (Fig. 3). The significant effect of seed addition on number of *Lycopus europaeus* seedlings had disappeared at the end of the first year (Table 5). Sowing significantly increased numbers of *Lythrum salicaria*, but this effect was strongest in the reed marsh zone (Table 5, Fig. 3).

Effect of elevation and vegetation treatment

Interactions between elevation and vegetation removal significantly influenced the number of species throughout the study period (Table 3). In the first year, vegetation removal increased the number of species at both elevations, but increase of species richness seemed strongest at the tall forb zone. In the second year the effect of vegetation removal in the reed marsh zone had disappeared, whereas the number of species in the tall forb zone remained significantly higher in open plots (Table 6).

Table 2: Seed densities (mean per soil sample (=56.7 cm^3) ± SE, n=29-30) of most abundant species in the seed bank of tall forb zone and reed marsh zone. Significant differences between means in the same row are indicated by different letters (Mann-Whitney U-test, $P<0.05$). Abbreviations: CD- clonal dominant, FG- Functional group, I- interstitial, RM- reed marsh zone, TF- Tall forb zone, Z- Zonation, - no seeds found.

			Tall forb zone		Reed marsh zone	
	Z	FG	0-2 cm	2-7 cm	0-2 cm	2-7 cm
Epilobium hirsutum	TF	CD	0.2±0.1 b	0.2±0.1 ab	0.07±0.05 b	0.4±0.1 a
U. dioica	TF	CD	19.9±2.3 a	14.0±1.6 a	18.6±3.9 a	25.7±5.0 a
T. flavum	TF	I	2.5±0.7 a	1.0±0.2 a	0.1±0.1 b	0.4±0.4 b
Phragmites australis	RM	CD	0.6±0.3 b	0.1±0.05 b	8.9±2.4 a	3.1±0.9 a
Lycopus europaeus	RM	I	1.7±0.6 a	0.4±0.2 a	0.6±0.2 a	0.3±0.1 a
Lythrum salicaria	RM	I	0.04±0.04 ab	0.1±0.06 ab	0.2±0.1 b	0.6±0.2 a
Cardamine amara	RM	I	-	0.05±0.05 b	1.6±0.6 a	0.9±0.3 a
Mentha aquatica	RM	I	0.1±0.05 a	0.1±0.06 a	0.07±0.05 a	0.05±0.03 a
Caltha palustris	RM	I	-	-	0.2±0.1 a	0.1±0.04 a

Table 3: F-values for number of species under influence of elevation (E), vegetation removal (VEG) and seed addition (S) in June 1994 and May and September/October 1995. Degrees of freedom are listed in parentheses. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

	June 1994	May 1995	Sept/Oct 1995
VEG (1)	5.33*	2.12	0.94
S (1)	0.07	0.04	0.00
E (1)	0.92	0.20	3.28
VEG*S(1)	0.25	1.26	2.61
Error (MS)	30.37	49.62	17.59
VEG*E (1)	10.56**	12.67*	5.11*
S*E (1)	1.56	0.00	1.40
VEG*S*E (1)	3.06	1.26	0.01
residual (MS)	2.00	3.56	2.70

Table 4: G-values for proportion of functional groups under influence of elevation (E), vegetation removal (VEG) and seed addition (S) in June 1994 and May and September/October 1995. Degrees of freedom are listed in parentheses. Symbols/abbreviations: -- interaction model could not be fitted; n.d. not determined; $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

	VEG(1)	S(1)	E(1)	VEG*S(1)	VEG*E(1)	S*E(1)	VEG*S*E(1)
Reed marsh dominants							
- June 1994	26.28^{***}	n.d.	154.52^{***}	n.d.	17.87^{***}	n.d.	n.d.
- May 1995	0.15	n.d.	187.13^{***}	n.d.	0.42	n.d.	n.d.
- Sept/Oct 1995	0.29	n.d.	236.60^{***}	n.d.	0.08	n.d.	n.d.
Reed marsh interstitials							
- June 1994	80.39^{***}	1.07	264.35^{***}	--	--	1.27	--
- May 1995	4.82^{*}	0.86	60.56^{***}	--	--	0.00	--
- Sept/Oct 1995	5.77^{*}	3.04	6.37^{*}	--	--	0.26	--
Tall forb dominants							
- June 1994	0.01	0.08	17.66^{***}	0.04	45.13^{***}	0.74	0.43
- May 1995	7.18^{**}	3.55	19.20^{***}	0.04	2.05	0.85	0.08
- Sept/Oct 1995	0.04	0.00	172.74^{***}	0.03	2.12	0.01	1.88
Tall forb interstitials							
- June 1994	6.37^{*}	0.17	13.40^{***}	0.20	0.01	0.04	0.03
- May 1995	5.97^{*}	0.17	61.93^{***}	0.02	0.99	0.04	0.08
- Sept/Oct 1995	4.17^{*}	0.04	6.62^{*}	0.15	0.74	0.03	0.78

Vegetation removal significantly increased the proportion of reed marsh interstitials in the reed marsh zone, but there was only a very small increase in the tall forb zone (Table 6). Here, vegetation removal seemed to be especially beneficial for tall forb interstitials, such as *Arctium lappa* L., *T. flavum* and *Symphytum officinale* L. (Table 6, data on individual species not shown). At the end of the second year, the proportion of reed marsh interstitials had strongly decreased at both elevations and no differences between vegetation treatments were found. Responses of the group of reed marsh interstitials were also reflected by emergence patterns of separate species. Interactions between vegetation and elevation significantly influenced maximum numbers of *Lycopus europaeus* seedlings (Table 5). At both elevations canopy removal increased the number of seedlings, but more seedlings were found in open plots of the reed marsh zone, especially when sown (Fig 3). Emergence of the other common reed marsh interstitial, *Lythrum salicaria*, was also enhanced by vegetation removal and throughout the first year highest numbers were found in open plots at the reed marsh zone (Fig 3).

In 1994 the proportion of tall forb dominants was significantly influenced by an interaction between elevation and vegetation removal (Table 4), because vegetation removal led to increased proportions in the reed marsh zone and decreased proportions in the tall forb zone (Table 6). In the second year, the proportion of tall forb dominants at the reed marsh zone was very low and not different from closed plots in that zone.

Emergence of the tall forb dominant *Urtica dioica* was significantly increased by vegetation removal (Table 5, Fig. 3). Throughout the growing season, most seedlings were found in the reed marsh zone, but numbers strongly declined although differences between elevation were still significant at the end of the first year (Table 4). In May 1995, *Urtica dioica* occurred in three open plots of the reed marsh zone but ground cover never exceeded one percent (data not shown). The tall forb dominant *Epilobium hirsutum* also had its highest number of seedlings in open plots at the reed marsh zone (Fig. 3). Similar to *Urtica dioica*, numbers strongly declined and at the end of the first year no differences between elevations were found (Table 4). In May 1995, *Epilobium hirsutum* was most abundant in open plots of the tall forb zone (data not shown).

Table 5: G-values for number of seedlings in subplots during the first year (1994) as influenced by vegetation removal (VEG), seed addition (S) and elevation (E). Degrees of freedom are listed in parentheses. See text for further details. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

	VEG(1)	S(1)	E(1)	VEG*S(1)	VEG*E(1)	S*E(1)	VEG*S*E(1)
Species in seed mixture							
Epilobium hirsutum							
-max 1994	12.39^{***}	4.63^{*}	11.58^{***}	0.25	4.77^{*}	1.02	2.11
-end 1994	31.77^{***}	1.21	0.63	--	--	1.32	--
Lycopus europaeus							
-max 1994	5.67^{***}	0.02	19.64^{***}	6.30^{*}	0.05	0.00	6.46^{*}
-end 1994	13.79^{***}	2.99	0.01	--	--	0.00	--
Lythrum salicaria							
-max 1994	1.67	7.31^{**}	28.77^{***}	2.11	1.73	0.35	--
-end 1994	6.47^{*}	19.42^{***}	46.48^{***}	0.00	3.37	--	--
Unsown species							
U.dioica							
max 1994	6.06^{***}	0.05	5.06^{*}	0.21	0.23	0.03	1.23
end 1994	0.93	0.11	2.50^{*}	0.07	0.78	0.00	0.00

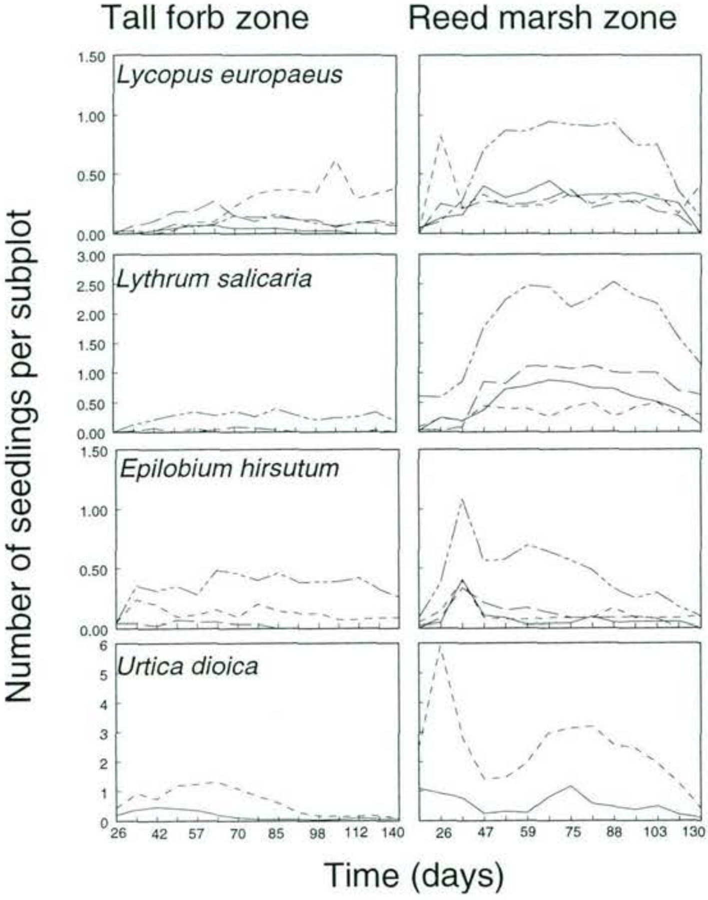

Figure 3: Mean number of seedlings per subplot (mean, n=4) for *L. europaeus*, *L. salicaria*, *E. hirsutum* and *U. dioica* at the tall forb zone and reed marsh zone during 1994 in open plots without added seed mixture (– – – –), open plots plots with added seed mixture (— · · —), closed plots without seed mixture (———) and closed plots with added seed mixture (— — — —). Since no seeds of *U. dioica* were added, means of sown and unsown plots were pooled (n=8).

Table 6: Number of species and proportion of functional groups for open (-VEG) and closed (+VEG) plots at the tall forb zone and the reed marsh zone in June 1994 and May and September/October 1995 (mean ± SE; n=8). Significant differences between means are indicated with different letters (LSD for number of species and Kruskal-Wallis for proportions, P<0.05).

	Tall forb zone		Reed marsh zone	
	-VEG	+VEG	-VEG	+VEG
Number of species				
- June 1994	13.5±0.4 a	7.3±0.6 c	12.7±0.9 a	10.9±0.5 b
- May 1995	13.7±0.7 a	7.7±0.6 cd	10.2±0.6 b	9.0±0.6 bc
- Sept/Oct 1995	9.1±0.7 a	6.4±0.5 b	5.1±0.5 bc	5.0±0.6 bc
Proportion of Reed marsh dominants				
- June 1994	0.9±0.3 c	3.4±0.5 c	14.3±4.9 b	94.7±1.4 a
- May 1995	3.1±0.3 b	2.0±0.5 b	59.8±5.0 a	66.8±6.7 a
- Sept/Oct 1995	11.9±3.7 b	19.7±5.7 b	88.5±1.9 a	90.8±3.8 a
Proportion of Reed marsh interstitials				
- June 1994	0.8±0.2 b	0.0±0.0 b	53.4±6.3 a	1.8±1.1 b
- May 1995	1.3±0.3 c	0.0±0.0 c	31.1±4.3 a	16.6±6.6 b
- Sept/Oct 1995	4.3±1.9 ab	0.0±0.0 b	5.0±1.6 a	5.0±4.0 ab
Proportion of Tall forb dominants				
- June 1994	17.9±2.8 b	73.5±7.0 a	25.3±6.1 b	4.0±1.8 c
- May 1995	10.6±3.2 b	73.9±10.1 a	3.2±2.1 b	5.8±3.9 b
- Sep/Oct 1995	36.9±8.2 b	59.7±7.6 a	2.1±0.9 c	0.5±0.2 c
Proportion of Tall forb interstitials				
- June 1994	70.5±6.1 a	3.4±0.5 b	4.8±2.0 bc	0.3±0.3 c
- May 1995	81.2±3.6 a	19.0±9.9 b	2.0±0.6 bc	0.8±0.3 c
- Sept/Oct 1995	34.0±10.3 a	6.1±2.6 b	3.1±1.1 b	1.7±0.8 b

Discussion

A possible complication in our study may have arisen from the use of a former tidal wetland as the study area. However, the species composition of the present vegetation beared little resemblence to that before the regulation when both zones were dominated by *Phragmites australis* and accompanied by *Caltha palustris* only (De Boois 1982). The present zonation, with *Phragmites australis*-dominated vegetation in frequently flooded parts of the littoral zone and dominance of *U. dioica* in dry parts, arose after the regulation and is common for many eutrophic non-tidal littoral zones (Gryseels 1989, Fojt and Harding 1995). In addition, it is unlikely that remnant seedbanks played a role. Since a strong correspondence between species composition of seedbank and vegetation is common for freshwater tidal wetlands (Parker and Leck 1985, Leck and Simpson 1987) the seedbank before the regulation will mainly have consisted of *Phragmites australis* and *Caltha palustris*. In the present study, emergence of these species was incidentally observed but neither species emerged in great numbers or established succesfully.

There was little correspondence between distribution of seeds in the seedbank and distribution of mature plants in the vegetation. Except *Cardamine amara* and *T. flavum*, the most common seedbank species *Epilobium hirsutum*, *U. dioica*, *Lycopus europaeus*, *Lythrum salicaria* and *Mentha aquatica* were restricted to one elevation but their number of seeds in the seedbank did not differ significantly between elevations (Table 2). In addition, many species that were present in the seedbank (data not shown) were not found in the vegetation. This lack of correspondence between composition of seedbank and vegetation is very common for non-tidal wetlands (Keddy and Reznicek 1982, Schneider and Sharitz 1986, Welling *et al* 1988) and mesocosm studies have shown that this is due to the overruling effect of hydrology on the community development (Stockey and Hunt 1994, Weiher and Keddy 1995).

In the present study functional groups of plant species were distinguished on the basis of plant traits which presumably reflect the role of species in the community (Boutin and Keddy 1993). The relevance of a certain classification system of functional groups depends upon the ecosystem under study (Chapin *et al* 1992). In a eutrophic system such as used for the present study, availability of both light and nutrients is of great importance (Campbell *et al* 1991). Therefore, we chose plant traits that reflect a capability for high rates of resource capture ('maximum height' criterium) and direction of resources to organs necessary for the long term occupancy of sites ('lateral spread' or 'gregariousness' criterium). Both traits are necessary requirements for the potential to dominate vegetation (Grime 1987).

The classification according to Boutin and Keddy (1993) was chosen because their system is based upon inherent properties of the plants rather than their responses to environmental conditions as has been used by many authors (reviewed by Smith and Huston 1989). Since these responses are variable, a classification based upon plant traits is more appropriate (Smith and Huston 1989, Shipley *et al* 1989). At both elevations abundance of interstitials was restricted by clonal dominants since removal of the vegetation canopy, which mainly consisted of clonal dominants, increased the proportion of interstitials. Abundance of interstitials is often controlled by the dominant species (Armesto and Pickett 1985, Grime 1987, Keddy 1989). The increase of interstitials in cleared plots of the reed marsh zone showed that plant interactions also played a role in this frequently flooded zone.

Removal of vegetation canopy however did not lead to a different zonation of reed marsh interstitials along the elevational gradient. This was certainly not related to zonation in the seedbank, since even addition of seeds did not result in a higher abundance of reed marsh interstitials at the tall forb zone. The lack of emergence of reed marsh interstitials after vegetation removal at the tall forb zone will most likely have been due to conditions unfavourable for germination. Even the tall forb dominants *U. dioica* and *Epilobium hirsutum* had a low emergence at the tall forb zone. It seems likely that there was a constraint on germination at this elevation, most probably due to low soil moisture, which often limits recruitment at higher elevations of shorelines (Keddy and Ellis 1985, Van Splunder *et al* 1995). Water availability may vary from year to year and because this was a relatively short-term experiment, it cannot be concluded that establishment of reed marsh interstitials at higher elevations will always be primarily limited by unfavourable germination conditions instead of competitive exclusion. These results however, underline the importance of studying all phases of life history before the development of vegetation zonation in wetlands can be ascribed to species interactions (Van der Valk and Welling 1988).

Vegetation removal only significantly increased species richness at the tall forb zone. At the reed marsh zone cleared plots also had a higher number of species than closed plots, but only in June 1994. In May 1995 there were no differences between open and closed plots anymore. At that time *Phragmites australis* could not have influenced the number of species in open plots because shoots were removed throughout the first season and new ones were just emerging from soil in May 1995. Since the canopy could not have played a role, the decrease in species richness between June 1994 and May 1995 must have been caused by abiotic factors.

Recruitment of tall forb species contributed to the increase of species richness in open reed marsh plots in June 1994. However, at the end of the first growing season a low number of seedlings of *U. dioica* and *Epilobium hirsutum* remained and in the second year the proportion of tall forb dominants even further decreased. Flooding was the most likely abiotic factor which limited species richness at the reed marsh zone by removing tall forb species, which are known to be sensitive to inundation (Klimesova 1994, Stockey and Hunt 1994). The importance of flooding was also indicated by the fact that plots at the reed marsh elevation were, in contrast with those at the tall forb zone, regularly flooded during the growing season of 1994 and almost permanently inundated during winter (Fig.2).

In wetland vegetation, relationships between total standing crop and species richness within a single community are either absent or very weak (Wheeler and Giller 1982, Moore and Keddy 1989, Shipley *et al* 1991*b*, Wheeler and Shaw 1991), which indicates that abiotic factors play an important role (Gough *et al* 1994). To our knowledge this was the first study in freshwater wetlands which experimentally distinguished abiotic from biotic influences on species richness at different elevations. Results have clearly demonstrated that in frequently flooded zones abiotic factors are the main determinant of species richness. Keddy (1989), although not explicitly comparing different elevations, also noticed that removal of the dominant species on a freshwater lakeshore had the strongest impact in the least frequently flooded parts. Our finding is also in accordance with Brewer *et al* (1997) who have demonstrated, using experiments comparable to ours, that in salt marshes abiotic factors limit species richness at low elevations. However, they found that vegetation removal at low elevations even decreased species richness, because the vegation canopy ameliorated effects of high salinity. Salinity did not play a role in our study and therefore we did not find a beneficial effect of canopy on species richness. Contrary to Brewer *et al* (1997) canopy removal at the reed marsh zone enhanced seedling emergence. Hence it may be concluded that amelioration of recruitment by the canopy does not play a role in freshwater wetlands.

This study has shown that in shoreline vegetation the role of plant interactions in determining zonation patterns depends on elevation. At both elevations studied, plant interactions play a role, but at the low elevation they only determined the abundance of those species adapted to the prevailing abiotic conditions. At the high elevation plant interactions exerted a strong influence on both the abundance of species and on species richness.

Appendix: List of species found as seedlings and or adults with autoecological information used for classification into 'zonation group' and 'functional group'. Abbreviations: P- perennial, B-biennial, A-annual, RM- reed marsh zone, TF- tall forb zone, CD- clonal dominant, I- interstitial.

Species	Life cycle[1]	Maximum height (cm)[1]	Lateral spread[2]	Gregariousness	Zone	Func. group
Achillea ptarmica	P	90	3		RM[6]	I
Angelica archangelica	B/P	250	None[7]		TF[5,6]	CD
Anthriscus sylvestris	P	150	2	Intermediate[2]	TF[5,6]	I
Apium nodiflorum	P	100	5	Intermediate[2]	RM[6]	I
Arctium lappa	B	150	1		TF[6]	I
Berula erecta	P	60	5		RM[6]	I
Bidens frondosa	A	100				A
Callitriche stagnalis	P	50	4		RM[6]	I
Caltha pal.ssp.araneosa	P	50	2	Intermediate[2]	RM[6]	I
Cardamine amara	P	45	5	Intermediate[2]	RM[6]	I
Carex riparia	P	120		Stand forming[3,4]	RM[6]	CD
Cirsium arvense	P	120	5	Stand forming[2]	TF[5,6]	CD
Epilobium hirsutum	P	150	5	Stand forming[2,5]	TF[5,6]	CD
Equisetum arvense	P	80		Intermediate[2]	TF[6]	I
Eupatorium cannabinum	P	150	3	Intermediate	TF[5,6]	I
Filipendula ulmaria	P	120		Stand forming[2,4]	TF[6]	CD
Galeopsis tetrahit	A	75				A
Galium aparine	A	120				A
Heracleum sphondylium	P	150	2		TF[6]	I
Impatiens glandulifera	A	200				A
Iris pseudacorus	P	120	5	Dominant[8]	RM[5,6]	CD
Lycopus europaeus	P	90	3		RM[6,8]	I
Lythrum salicaria	P	120	2		RM[6]	I
Mentha aquatica	P	90	5		RM[6]	I

Species	Life cycle[1]	Maximum height (cm)[1]	Lateral spread[2]	Gregariousness	Zone	Func. group
Myosotis scorpioides	P	45	3		RM[6]	I
Phalaris arundinacea	P	200	5	Stand forming[2,3]	RM[6]	CD
Phragmites australis	P	300	4	Stand forming[2,3]	RM[5,6]	CD
Polygonum persicaria	A	100				A
Ranunculus repens	P	50	5	Intermediate[2]	RM[6]	I
Ranunculus sceleratus	A	70				A
Rorippa palustris	A					A
Rumex obtusifolius	P	150	2	Intermediate[2]	TF[6]	I
Scutellaria galericulata	P	45	3		RM[6]	I
Senecio paludosus	P	180		Intermediate[3,4]	RM[6]	I
Sium latifolium	P	120		Intermediate[3,4]	RM[6]	I
Solidago gigantea	P	150		Stand forming[5]	TF[5,6]	CD
Stachys palustris	P	80	4		RM[6]	I
Symphytum officinale	P	100	3		TF[5,6]	I
Thalictrum flavum	P	90			TF[6]	I
Urtica dioica	P	300	4	Stand forming[2,3]	TF[5,6]	CD
Valeriana officinalis	P	120	2	Intermediate[2]	TF[5,6]	I

Data source: [1] Van der Meijden (1996); [2] Grime *et al* (1988); [3] Wheeler (1980); [4] Schaminée *et al* (1995); [5] Van de Rijt *et al* (1996); [6] De Boois (1982); [7] Weeda *et al* (1987); [8] Sutherland (1990).

4. THE EFFECT OF FLOODING ON THE RECRUITMENT OF REED MARSH AND TALL FORB PLANT SPECIES

Abstract

Recruitment of plant species in wetlands dominated by *Phragmites australis* often results in a zonation of two vegetation types. A development of reed marshes takes place in the shallow flooded parts where the dominant *P. australis* becomes accompanied by interstitial marsh species. The vegetation on higher elevations becomes dominated by tall forbs. We investigated whether this zonation is related to the effects of flooding on recruitment of species from both vegetation types.

All but one species had maximum germination at field capacity and germination was strongly reduced in drier soils and flooded soils. Nearly all seedlings of reed marsh species, irrespective of whether they initially supported two or six true leaves, survived eight weeks of total inundation. Survival of tall forbs was however significantly decreased by inundation. At both seedling stages a considerable number of *Cirsium arvense* lost their leaves when submerged, but all plants were able to recover when drained. *Eupatorium cannabinum* suffered extra mortality when transferred from flooded to drained conditions, whereas only young seedlings of *Epilobium hirsutum* died. Of all investigated species *Epilobium hirsutum* was the only one with a significantly increased stem length after one week of submergence. Root porosities did not differ between reed marsh species and tall forbs but stem porosities of the reed marsh species *Mentha aquatica* and *Lycopus europaeus* were significantly higher than those of the tall forbs *C. arvense* and *Eupatorium cannabinum*. It was concluded that both reed marsh species and tall forbs require lowered water levels for germination. Flooding during the seedling stage will assign species to their position on the elevational gradient. Reed marsh species establish at lower elevations because they are best able to survive flooding and because their higher stem porosities allows them to withstand higher levels of partial submergence.

Introduction

Eutrophic shoreline vegetation is usually dominated by perennial species. The dense canopy inhibits germination and establishment of other plant species. Recruitment can therefore only take place after removal of aboveground vegetation by disturbances (Grubb 1988, Eriksson 1992) or when the dominant species is reduced in vigour (Falinska 1995).

Stands of common reed, *Phragmites australis* (Cav.) Steudel, are invaded when its vigour is reduced. This may be due to standing litter (Graneli 1989), accumulated organic matter (Van der Putten *et al* 1997) or insect damage (Van der Toorn and Mook 1982). Subsequently a zonation pattern along the elevational gradient develops. On the lower, regularly flooded parts, *P. australis* remains dominant but becomes accompanied by a number of interstitial species such as *Mentha aquatica* L. and *Myosotis scorpioides* L. Above these reed marshes tall forb stands develop with marginal presence of *P.australis* and dominance of species such as *Epilobium hirsutum* L. and *Urtica dioia* L. (Gryseels 1989, Fojt and Harding 1995, Van de Rijt *et al* 1996).

The different positions of reed marsh and tall forb stands may be related to differential flooding tolerance of the component species, because duration and depth of flooding are the factors which most strongly vary along elevation gradients (Blom and Voesenek 1996). Position of species on this gradient is often related to the physiological response to flooding during germination and seedling establishment (Parker and Leck 1985, Van der Valk and Welling 1988).

Zonation of reed marsh and tall forb species may develop because germination requirements of plant species restricts recruitment to specific positions on the elevational gradient (Keddy and Ellis 1985, Moore and Keddy 1988, Coops and Van der Velde 1995, Van Splunder *et al* 1995). Successful establishment will also depend on the ability of seedlings to survive flooding and at least the lower limits of shoreline species therefore often correspond to the tolerance to submergence of its seedlings (Welling *et al* 1988, Voesenek *et al* 1993*a*, Clevering *et al* 1996).

When submerged, flood tolerant species are often distinguished from intolerant species by the ability to elongate stems and/or petioles. This enables plants to protrude from the water and thus restore contact with the atmosphere (Armstrong *et al* 1994, Blom and Voesenek 1996). A high content of aerenchyma in stems and roots is required to maintain gas flows through the plant and is a necessary adaptation for both complete and partial submergence (Smirnoff and Crawford 1983, Justin and Armstrong 1987, Beckett *et al* 1988, Armstrong *et al* 1994, Blom and Voesenek 1996).

When flooding is too deep for the plant to reach the surface, survival under total submergence may depend on the ability to photosynthesize in water (Sand-Jensen *et al* 1992), but under turbid water conditions, or during the night, plant tissues easily become anoxic. Therefore submergence tolerance will also be determined by the efficiency of carbohydrate use and the amount of carbohydrates avalaible for anaerobic metabolism (Setter *et al* 1987, Crawford and Brändle 1996). The carbohydrate reserves may change with developmental stage of seedlings and flooding tolerance of species may therefore vary during ontogeny (Van der Sman *et al* 1993, Voesenek *et al* 1993*a*). In addition, plants require adequate mechanisms to prevent post-hypoxic injury when the water levels recede (Hendry and Brocklebank 1985, Armstrong *et al* 1994). Because species have developed many different mechanisms to cope with the wide array of stresses that flooding imposes on the plant, a good evaluation of the flooding tolerance of different species can only be obtained by studying the final effect on the whole plant (Crawford 1996).

In order to find out whether flooding causes the zonation of reed marsh species and tall forbs we compared tolerance to flooding during both germination and seedling establishment stages. We choose a whole plant response, survival, as a measure of flood tolerance of seedlings, but also determined specific adaptations like stem elongation and stem- and root-porosities. We hypothesized that flooding during the recruitment phase assigns reed marsh and tall forb species to their specific position on elevation gradients. Thus, reed marsh species are expected to have better germination in waterlogged and flooded soils than tall forbs. Seedlings of reed marsh species are supposed to survive longer periods of flooding, to elongate their stems when submerged and to have higher porosities of stems and roots than tall forb species.

Methods

Germination: seed collection and storage

Germination response of six plant species from reed marshes (e.g. *Iris pseudacorus* L., *Lycopus europaeus* L., *Lythrum salicaria* L., *Mentha aquatica*, *Myosotis scorpioides* and *Rumex hydrolapathum* Hudson) and six species from tall forb stands (e.g. *Cirsium arvense* (L.) Scop., *Epilobium hirsutum*, *Eupatorium cannabinum* L., *Symphytum officinale* L., *Thalictrum flavum* L.and *Urtica dioica*) were compared in the germination experiment. In 1993 and 1994 seeds were collected from a single population of each species along the River Nieuwe Merwede (51°45'N, 4°45'E). Seeds were stored dry and dark at 20°C, except *I. pseudacorus* seeds, which were stored dark and wet at 5°C. Storage requirements were based on previous pilot experiments on seed germination following different modes of storage.

Germination: experimental design

Seeds were buried at ± 1 mm depth in pots (height 6.4 cm, diameter 7.5 cm) filled with sediment collected from Lake Drontermeer (52°58'N, 5°50'E; 93% of soil particles are between 16 and 212µm in size, organic matter content is 3.5%). Each pot was placed in a bigger outer pot to allow high water tables. Four treatments were applied: submerged, waterlogged, field capacity and half field capacity. In the submerged treatment water level was kept 5 cm above the surface of the inner pot and in the waterlogged treatment equal to the soil surface. Field capacity was defined as the water content of the sediment at which water starts to leak from the bottom of the pots. Half the amount of water necessary to obtain field capacity was used to obtain half field capacity. Every third day the weight of each pot was determined and tap water was added every third day to restore initial soil weight. Moisture content of the soils in the different treatments were (Mean ± SE, n=8): 33.9% ± 1.1% (waterlogged), 7.4% ± 1.0% (field capacity), 2.8% ± 0.3% (half field capacity).

Each combination of species and treatment was replicated four times with 25 seeds per pot, except for *I. pseudacorus* of which 10 seeds per pot were used. The germination experiment was carried out in two climate chambers at 16h/8h day/night temperatures of 25°C/10°C (relative humidity 70%). During daytime 155 mmol m^{-2} s^{-1} light was provided. Two replicates of each species-treatment combination were placed per climate chamber. Within a climate chamber all pots were randomized. The experiment started on 22 November 1995 and germinated seeds were then counted daily and removed. Seeds were considered to have germinated as soon as the cotyledons became visible. After eight weeks no more seeds germinated and the experiment was terminated.

Three germination parameters were determined: lag time, germination rate and maximum germination. Lag time refers to the first day at which a germinated seed was found. Germination rate was estimated as a weighted mean according to the formula used by Shipley *et al* (1989):

$$r_{ij}=S_j(p_{ij}\ x\ j)$$

where p_{ij} is the proportion of all seeds of species *i* germinating on day *j*, relative to the total number of germinated seeds of species *i*. The germination rate *r* thus represents the number of days from the beginning of the experiment at which the average seed of species *i* germinated.

Seedling establishment: plant material

Six species were used to study adaptations to water level fluctuations. *Lycopus europaeus*, *Mentha aquatica* and *Myosotis scorpioides* represented species from the reed marsh zone whereas *C. arvense*, *Epilobium hirsutum* and *Eupatorium cannabinum* were chosen as representatives of the tall forb zone. Seeds were collected from the same populations as used in the germination experiment in 1995 (*Mentha aquatica* and *Eupatorium cannabinum*) and 1996 (other species) and stored dry and dark at 20°C. Seeds were germinated on a substrate of glass beads and tap water in a germination chamber at a day/night of 16h/8h and 25°C/10°C. Seedlings with two cotyledons were transferred to 0.3 l plastic pots on a 1:1 (v/v) mixture of sand and river clay and placed in a greenhouse with a similar daylength and temperatures of 23±2°C during daytime and a minimum of 15°C during nighttime.

Seedling establishment: survival experiment

Survival experiments were carried out in a climate chamber (16h/8h day/night; 25°C/10°C; relative humidity 70%; light intensity 140 mmol m^{-2} s^{-1}). Two stages of seedling development were compared: young seedlings with two cotyledons and two true leaves and older seedlings with six true leaves. In the latter stage *Mentha aquatica*, *Epilobium hirsutum* and *Lycopus europaeus* also showed an onset of stolons.

For each stage sixty plants per species were placed in aquaria of 60 x 40 x 40 cm (l w h) filled with water up to 32 cm above the soil surface of the pots. In order to prevent algal blooms the water was flushed with compressed air and after two, four and six weeks 0.01 g of solid $FeCl_3$ was added to immobilize free phosphate in the water layer. The pH of the water was checked regularly and maintained at pH 7.5-8.2 using H_2SO_4.

Because plants of the old seedling stage needed more time to grow in the greenhouse they were placed in the aquaria four weeks after the young seedlings. For each seedling stage eight aquaria were used. Practical limitations prevented a randomized block design with an equal number of replicates of species-stage in each aquarium. In order to get an impression of the variability among aquaria light extinction between water surface and soil surface was determined halfway through the experiment and at the end with a LI-COR LI-189 light intensity meter. In each aquarium three readings were made at random positions. Light extinction was considered to be most relevant since this may strongly influence seedling survival (Clevering *et al* 1996). Analysis of light extinction data (results not shown) did not reveal a significant change during the experiment or a significant difference between aquaria used for the same stage. In aquaria with young seedlings an average of 61% of the light was intercepted by the water. This was slightly (but significantly) higher than in the aquaria with older seedlings where on average 57% of the light was intercepted.

After two, four, six and eight weeks fifteen pots of each species and stage were collected randomly. Plants, which had lost all green leaves when taken out of the aquaria, were considered to be dead. Consequently all pots, including those with 'dead plants', were transferred to the greenhouse, drained and watered regularly to prevent desiccation. After this recovery, a period of two weeks, the number of plants that were green or showed at least some sign of regrowth, i.e. green tips at the stem indicating intact meristem functioning, were recorded as being alive.

Seedling establishment: elongation responses

In order to study elongation responses to total submergence twelve seedlings per species with six true leaves were placed under water during eight days in the same manner as described for experiment 2, but without addition of $FeCl_3$ or compressed air. Twelve plants of each species were grown in the same climate chamber in pots that were drained and another twelve plants were harvested immediately. These two controls, i.e. the beginning of the experiment and eight days in drained soil, were compared with the submerged plants. Number of internodes, length of stem and length of longest petiole were measured.

Seedling establishment: stem and root porosities

Two-week old seedlings were grown in pots of 6.5 x 7 cm (h x d) filled with a mixture of sand and clay (as before) in a controlled-climate chamber (16h/8h day/night; temperature 25°C/10°C; relative humidity 70%; light intensity 155 mmol m^{-2} s^{-1}). All plants were drained during the first three weeks. Thereafter, eight replicates were subjected to waterlogging by raising the water level to 1 cm above soil surface. Another eight replicates remained drained. Three weeks later roots were washed out by gently running water and both stems and roots were carefully dried with tissues. Stem and root porosities were determined by the pycnometer method according to Jensen *et al* (1969), although gas was not removed by maceration, but by evacuation. For each measurement between 0.5-2 g fresh weight of plant material was used. If individual plants provided insufficient material replicates were pooled.

Statistical analysis

Influence of climate chamber, species, treatments and interactions between these factors on proportion of germinated seeds were tested with maximum likelihood analysis with binomial errors (Sokal and Rohlf 1995), using SAS procedure GENMOD (SAS Institute Inc. 1993). Means of each species were compared with a Tukey test, using arcsin-transformed data for maximum germination and ln-transformed data for lag time and germination rate.

Effects of species and flooding duration were tested on the proportion of plants alive immediately after flooding (hereafter referred to as 'survival-recovery') and the proportion alive after two weeks of recovery under drained conditions ('survival+recovery'). Tests were performed for each stage separately because light extinction in aquaria with young seedlings differed from aquaria with older seedlings. Because light extinction did not differ between aquaria within the same stage, effect of aquarium was not included in the tests. Proportions of living plants were analysed with maximum likelihood analysis using the same methods as for the germination experiment. Because survival was sometimes one hundred percent, tests could not be performed on the original data. Therefore data were analysed using the number of survivors minus one. Survival of the group of reed marsh species and the group of tall forbs and of separate species was compared by non-orthogonal contrasts.

Stem length was analysed with one-way ANOVA after ln-transformation of data and means were compared with Tukey tests. Because of unequal sample size arcsin-transformed root- and stem-porosities were analyzed with type-III Sums of Squares in SAS procedure GLM. Species were compared with non-orthogonal contrasts.

Results

Germination

Effects of treatments on maximum germination were not similar for all species, since there was a significant interaction between species and treatment (Table 1). However, nearly all reed marsh and tall forb species had maximum germination at field capacity and a significantly lower germination at waterlogging and submergence as well as at half field capacity. Only *Lythrum salicaria* had similar germination in waterlogged and submerged soils (Figure 1). *Myosotis* *scorpioides* was the only species whose maximum germination at half field capacity was not significantly lower than at field capacity (Figure 1).

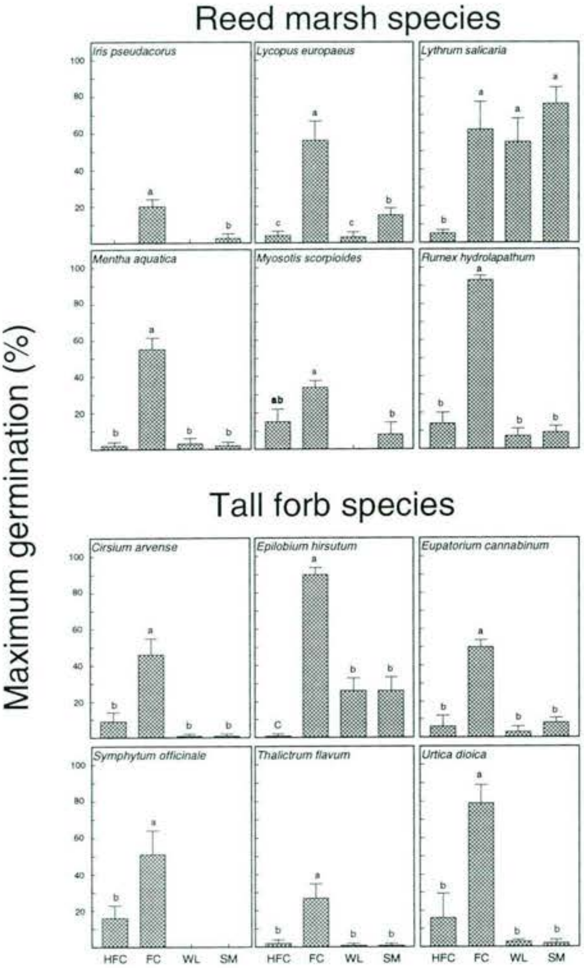

Figure 1: Maximum germination (mean ± SE, n=4) under influence of moisture content. Significant differences between treatments within each species are indicated with different letters. Abbreviations: HFC= half field capacity, FC= field capacity, WL= waterlogged (water level equal to soil surface), SM= submerged (water level 5 cm above soil surface).

Table 1: G-values of maximum germination of twelve species under influence of four moisture regimes (treatment). df= degrees of freedom, $^{*}P<0.05$, $^{***}P<0.001$.

Factor	df	Maximum germination
Climate chamber	1	2.8
Species	11	69.2^{***}
Treatment	3	231.9^{***}
Species x Treatment	33	135.5^{*}

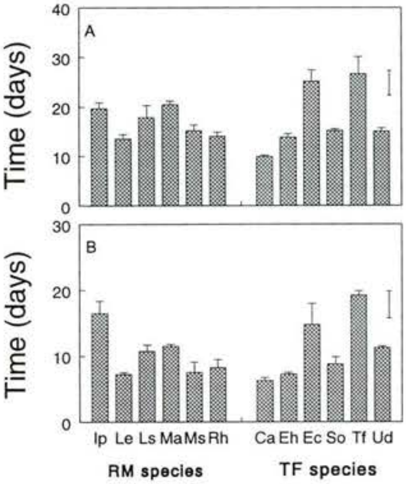

Figure 2: Germination rate (A; day at which average seed germinated) and lag time (B; first day at which germination took place) at field capacity (means ± SE, n=4). Vertical bar represents least significant difference. Abbreviations: Ip=*I. pseudacorus*, Le=*Lycopus europaeus*, Ls=*Lythrum salicaria*, Ma=*Mentha aquatica*, Ms=*Myosotis scorpioides*, Rh=*R. hydrolapathum*, Ca=*C. arvense*, Eh=*Epilobium hirsutum*, Ec=*Eupatorium cannabinum*, So=*S. officinale*, Tf=*T. flavum*, Ud=*U. dioica*, RM= Reed marsh, TF= Tall forb.

Because only a small number of seeds germinated at half field capacity and in waterlogged and flooded soils no reliable estimates of germination rate and lag-time could be obtained for these treatments. At field capacity germination rate and lag time did not clearly differ between reed marsh species and tall forb species (Figure 2). *Lycopus europaeus*, *R. hydrolapathum*, *Epilobium hirsutum* and *C. arvense* had the highest germination rate and germinated significantly faster than *Mentha aquatica*, *Eupatorium cannabinum* and *T. flavum*, which were the slowest germinators. *Lycopus europaeus*, *Myosotis scorpioides*, *Epilobium hirsutum* and *C. arvense* started to germinate earliest, whereas *I. pseudacorus* and *T. flavum* had the longest lag time (Figure 2).

Seedling establishment: survival experiment

All plants except *Eupatorium cannabinum* survived a flooding period of four weeks (Figure 3). Only after six and eight weeks mortality was high enough to allow statistical testing and therefore only the latter two flooding durations were included. For both young and older seedlings the survival after six weeks was not significantly different from survival after eight weeks (i.e. no significance of 'duration' in table 2).

Survival of young seedlings was significantly different between reed marsh and tall forb species. All reed marsh species had nearly hundred percent survival, but survival of tall forbs was decreased considerably. *C. arvense* appeared to have suffered the highest losses but after two weeks under drained conditions nearly all plants had recovered. Additional plants of *Epilobium hirsutum* and *Eupatorium cannabinum* died during the recovery period (Figure 3).

Survival of old seedlings was not significantly different between reed marsh and tall forb species (Table 2). *Eupatorium cannabinum* suffered highest mortality and its survival was significantly less than *Epilobium hirsutum* and all reed marsh species. A considerable, though insignificant number of *C. arvense* appeared to be death immediately after flooding, but nearly all plants were able to recover (Figure 3).

Table 2: G-values of survival of young and old seedlings immediately after submergence (-recovery) and after submergence and two weeks recovery (+recovery) of three tall forb species and three reed marsh species under influence of duration (six and eight weeks of flooding). df= degrees of freedom. * P<0.05, ** P<0.01, *** P<0.001.

Factor	df	- recovery	+ recovery
Young seedlings			
Species	5	17.9**	36.5***
Contrast:			
Tall forbs vs. Reed marsh species	1	9.5*	8.6*
Duration	1	3.6	2.1
Species x Duration	5	0.9	3.0
Old seedlings			
Species	6	34.2***	74.5***
Contrast:			
Tall forbs vs. Reed marsh species	1	6.4	4.6
Duration	1	0.9	0.8
Species x Duration	5	1.1	1.2

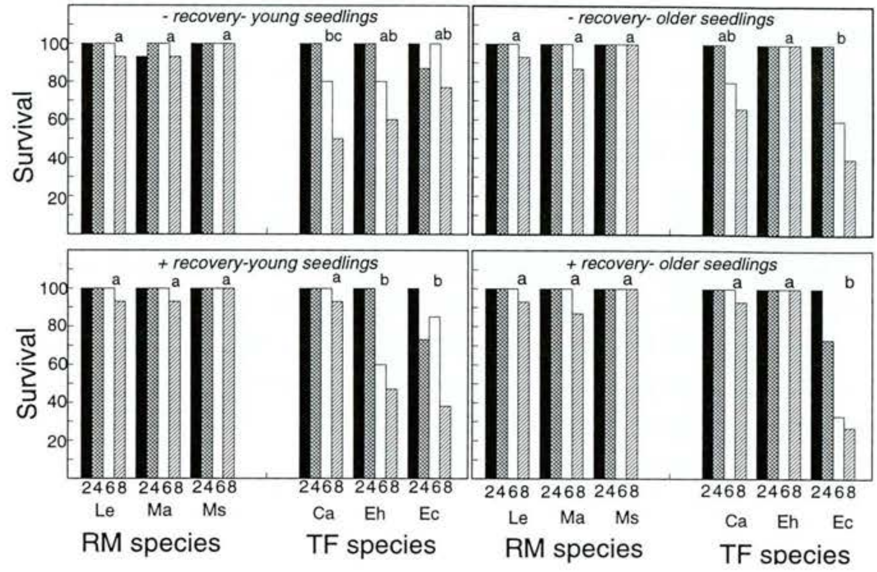

Figure 3: Survival (%; proportion of a total number of 15 plants) of seedlings with two true leaves (young seedlings) and six true leaves (older seedlings) after 2, 4, 6 and 8 weeks of total submergence. Significant differences between species are indicated with different letters. Significance was tested with maximum likelihood analysis with binomial errors on pooled survival data of six and eight weeks. Abbreviations according to Figure 2.

Seedling establishment: elongation responses

All species had significantly longer stems at the end of the experiment than the plants measured at the onset, regardless whether they were grown drained or submerged. After eight days of submergence, only plants of *Epilobium hirsutum* had a significantly longer stem than the drained plants (Figure 4). Stems of plants grown submerged for eight days were on average 3 cm longer than of plants grown drained.

The longest petiole of submerged *Epilobium hirsutum* was also longer than of drained plants and also with regard to petiole elongation this was the only species showing any sign of response to submergence (results not shown). The number of internodes was not increased under submerged conditions for any species (results not shown).

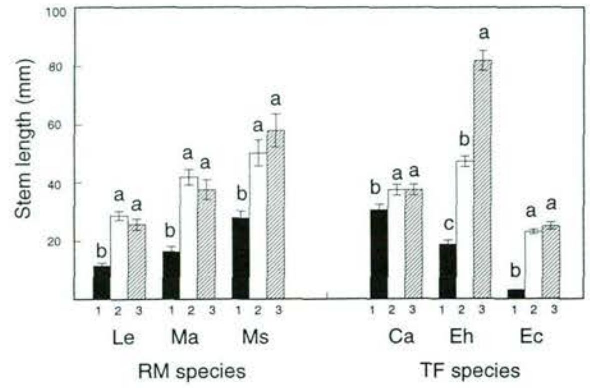

Figure 4: Stem length (mean ± SE, n=12) at the onset of the experiment (1) and after 8 days of growth under drained (2) or submerged conditions (3). Significant differences between treatments within each species are indicated with different letters. Abbreviations according to Figure 2.

Seedling establishment: stem and root porosities

Water level did not influence stem porosities (Table 3). Stem porosities of the reed marsh species *Lycopus europaeus* and *Mentha aquatica* were significantly higher than porosities of the tall forb species *C. arvense* and *Eupatorium cannabinum*, but equal to the tall forb *Epilobium hirsutum*. Stem porosity of the reed marsh species *Myosotis scorpioides* was significantly lower than the other two reed marsh species (Table 4).

Root porosity was significantly increased by waterlogging for *Mentha aquatica*, *Myosotis scorpioides* and *C. arvense*. Values did not show a clear distinction between reed marsh and tall forb species. The tall forb species *Epilobium hirsutum* had the highest root porosity whereas that of the reed marsh species *Myosotis scorpioides* was below the tall forbs *Epilobium hirsutum* and *Eupatorium cannabinum* (Table 5).

Table 3: F-values of stem- and root porosities of six different species grown at drained and waterlogged conditions. df=degrees of freedom, ** P<0.01, *** P<0.001.

	df	Stem porosity	Root porosity
Species	5	4.6^{**}	41.4^{***}
Contrast:			
Tall forbs vs Reed marsh species	1	4.4	0.4
Water level	1	0.8	15.6^{***}
Species x water level	5	1.0	2.3

Table 4: Stem porosity (% vol vol^{-1}, mean ± SE) of species grown in drained (Dr) and waterlogged (Wl) soils with the number of replicates given in parentheses. Significant differences between species are indicated with different letters and are based upon nonorthogonal comparison of species' means (water levels pooled).

Species	Dr		Wl		Significance
Reed marsh species					
Lycopus europaeus	13.8±1.2	(4)	12.8±0.4	(4)	A
Mentha aquatica	17.1±1.0	(4)	14.2±1.4	(6)	A
Myosotis scorpioides	5.6±0.9	(4)	8.9±1.4	(3)	AB
Tall forb species					
Cirsium arvense	7.7±1.4	(4)	7.9±1.2	(2)	B
Epilobium hirsutum	12.2±0.7	(4)	8.8±2.0	(2)	AB
Eupatorium cannabinum	2.2±0.6	(4)	3.8±0.7	(4)	C

Table 5: Root porosity (% vol vol^{-1}, mean ± SE) of species grown in drained (Dr) and waterlogged (Wl) soils with the number of replicates given in parentheses. Asterixes indicate significant difference between drained and waterlogged conditions (comparison per species). Significant differences between species are indicated with different letters and are based upon nonorthogonal comparison of species' means (water levels pooled).

Species	Dr		Wl		Significance
Reed marsh species					
Lycopus europaeus	9.3±0.8	(4)	10.6±1.1	(4)	B
Mentha aquatica	9.1±0.7	(4)	13.7±0.9*	(6)	B
Myosotis scorpioides	4.1±0.8	(4)	6.6±0.9*	(4)	D
Tall forb species					
Cirsium arvense	7.2±0.4	(4)	9.4±0.6*	(6)	C
Epilobium hirsutum	15.5±0.8	(4)	15.5±0.6	(2)	A
Eupatorium cannabinum	4.2±0.3	(4)	5.1±0.4	(4)	E

Discussion

Contrary to our hypothesis reed marsh and tall forb species had the same germination responses with regard to water level. All species except *Lythrum salicaria* were severely hampered at half field capacity and under waterlogged and submerged conditions. Lack of water probably inhibited seed germination of all species at half field capacity (Keddy and Ellis 1985, Moore and Keddy 1988). Germination in the waterlogged and submerged soils was most probably inhibited by low oxygen availability (Frankland *et al* 1987). Ability to germinate under water or at low oxygen tensions has been shown for a number of wetland species like *Typha latifolia*

(Bonnewel *et al* 1983), *Scirpus lacustris* and *Scirpus maritimus* (Clevering 1995*b*) and *Rumex crispus* and *Rumex maritimus* (Voesenek *et al* 1992). All species, except *Lythrum salicaria*, can be considered as drawdown species (*sensu* Van der Valk 1981), i.e. species that require exposed soil with unrestricted availability of oxygen and water in order to germinate. After germination seedlings will start to grow and compete with each other for light and nutrients and the probability of survival is highest for early emerging seedlings (Miller 1987, Stockey and Hunt 1994). This will include reed marsh species, i.e. *Lycopus europaeus*, *R. hydrolapathum* and *Myosotis scorpioides*, and tall forbs like *Epilobium hirsutum* and *C. arvense* since these were the species with the highest germination rate and shortest lag-time.

Flooding during the seedling phase may strongly influence species interactions. Survival data provided some evidence that reed marsh species are more tolerant to flooding than tall forb species. Nearly all reed marsh species survived flooding and only very small effects of developmental stage or flooding duration were found. Available physiological data on these species are in accordance with our findings. *Myosotis scorpioides* and *Mentha aquatica* are able to photosynthesize under water (Maberley and Spence 1983, Sand-Jensen *et al* 1992). *Myosotis scorpioides* has an efficient protection against post hypoxia-injury (Biemelt *et al* 1996), whereas *Mentha aquatica* has a number of metabolic adaptations which enables this species to use its carbohydrates in a conservative way once the plant is subject to hypoxic conditions (Farmer *et al* 1986).

None of the reed marsh species showed an increased elongation of stems and petioles when submerged. A lack of elongation response has also been shown for the flooding-tolerant species *Rumex palustris* (Van der Sman *et al* 1993). Recently it was demonstrated that the energy costs for elongation decreases the ability for maintenance and growth when shoots or leaves can not reach the atmosphere (Setter and Laureles 1996).

A more general adaptation to flooding is a high volume of air space in roots and shoots (Beckett *et al* 1988, Armstrong *et al* 1994, Blom and Voesenek 1996) and all reed marsh species in this study showed these adaptations. Root porosities of the reed marsh species *Lycopus europaeus* and *Mentha aquatica* were above 7- 10%, the treshold between tolerant and intolerant species (Smirnoff and Crawford 1983, Justin and Armstrong 1987) and the species had stem porosities higher than any other species in this study.

Porosity-values of *Myosotis scorpioides* were lower than the other reed marsh species. However, Smirnoff and Crawford (1983) and Justin and Armstrong (1987), also using the pycnometry method, measured higher values of root porosity for *Myosotis scorpioides*: 8.4% and 9.4% respectively. Justin and Armstrong (1987) trimmed roots back before the seedlings were subject to waterlogging. This was not done here and therefore our root systems may have contained a higher proportion of dead roots. This may have led to an underestimation of root porosity (Laan *et al* 1989*b*). The relatively low stem porosity of *Myosotis scorpioides* may be explained by its size. With regard to the maximum stem length it can obtain, *Myosotis scorpioides* was the smallest of all species used in this study. Because diffusion distances within shoots are shorter *Myosotis scorpioides* requires lower volumes of air space (Armstrong and Beckett 1987) than the other species which have tall erect shoots.

The results clearly indicated that tall forbs were less tolerant to flooding than reed marsh species, although large differences between species were found. *Eupatorium cannabinum* was the least tolerant: its survival rates were lowest of all studied species and it also suffered extra mortality from post-anoxic injuries. Its low root- and stem-porosities indicated that *Eupatorium cannabinum* also lacks adaptations to soil-waterlogging and partial submergence.

C. arvense suffered from loss of its leaves during flooding but meristem functioning of most plants was left intact since nearly all plants were still alive after a recovery period (Figure 3). This explains the dominance of *C. arvense* in the lowest, frequently flooded parts of intensely grazed parts of river floodplains (Blom *et al* 1996). In reed marshes however grazing is absent and because competition for light may play an important role in this habitat the loss of leaves may strongly decrease opportunities for persistence (Tilman 1987, Stockey and Hunt 1994). From its relatively high root porosity it may be concluded that *C. arvense* will be able to tolerate waterlogged soils but when shoots are also partially flooded it will experience more difficulties than the reed marsh species or *E. hirsutum*. *C. arvense* has a lower stem porosity and therefore a higher diffusion barrier for gas transport within the stem which limits the water depth that can be tolerated (Yamasaki 1984, Tornbjerg *et al* 1994).

The tall forb *Epilobium hirsutum* was only vulnerable to flooding as young seedlings. Older seedlings had already an onset of rhizomes which may have served as an extra supply of carbohydrates and thus enabled this species to survive hypoxic conditions (Crawford and Brändle 1996). Since *Epilobium hirsutum* is not able to photosynthesize under water (Sand-Jensen *et al* 1992) it will either rely upon fermentation processes during submergence or upon avoidance of flooding by means of stem- and petiole-elongation. Elongation will certainly enhance survival when flooding is shallow but it will be a disadvantage when the waterlayer cannot be protruded (Setter and Laureles 1996). It may be concluded that *Epilobium hirsutum* is less adapted to flooding than reed marsh species because its survival was more dependent upon the carbohydrate status of seedlings, as reflected by developmental stage, than survival of reed marsh species. However, air space volume in roots and stems was not below those of the tall forbs. Therefore the limited depth penetration of *Epilobium hirsutum* in comparison with reed marsh species (Van Donselaar-ten Bokkel Huinink 1961, Brock *et al* 1987) is related to other, not investigated factors.

In conclusion, these results have shown that flooding during the recruitment phase may strongly determine zonation patterns on eutrophic shorelines. Differences in position on the flooding gradient are more related to differential responses during the seedling stage than during germination. Both reed marsh species and tall forbs require well aerated, but humid soils for germination. Because seedlings of reed marsh species survive longer periods of flooding and have a higher proportion of aerenchyma in their stems they are better able to establish succesfully in reed marshes where water table is above soil surface for much of the year.

5. EFFECTS OF ORGANIC MATTER AND WATER LEVEL ON BIOMASS PRODUCTION OF WETLAND PLANT SPECIES

Abstract

We investigated how water level and different substrate types affect the growth of shoreline plant species. Twelve different species were grown in drained and waterlogged substrates, representing types normally encountered in wetlands: a mineral substrate from exposed sites, a substrate from a sheltered site rich in labile organic matter and an organic substrate with decomposing litter of *Phragmites australis*. The tested species included both interstitial and dominant species inhabiting flooded or dry parts of the water-depth gradient.

Due to nutrient limitation biomass production of most species was lowest in the mineral substrate and in this substrate only *Cirsium arvense* and *Eupatorium cannabinum* were inhibited by waterlogging. Most species performed best in the substrate with labile organic matter, where waterlogging did not inhibit plant growth. Compared to this substrate, biomass production of four species was significantly lower in the litter-substrate at both water levels. Six species, including those normally inhabiting flooded soils, were significantly decreased in waterlogged litter substrate. It is argued that the differences in plant performance between both organic substrates are due to the presence of refractory organic matter. The specific responses in the litter-substrate contrasted with the similar response to both other substrates. These results show that accumulation of litter, instead of accumulation of organic matter in general, will be an important factor in determining species composition of littoral zones. Our results have shown that litter does not enhance species richness by favouring interstitials above clonal dominants. At a large scale however, variability in litter accumulation may increase the number of species in shoreline vegetation because species composition of litter-rich sites will differ from mineral sites.

Introduction

In shoreline vegetation hydrology is generally considered to be most important in determining zonation and species composition of wetland plant communities (Spence 1982, Blom and Voesenek 1996). There is however increasing evidence that accumulation of organic matter is also an important factor for species distribution (Barko and Smart 1983, Johnson *et al* 1985, Klosowski 1988, Smits *et al* 1990, White 1993). Above the water level productivity of individual species and standing crop often increase with organic matter content (Wilson and Keddy 1985), but in flooded parts organic matter may provide adverse conditions for plant growth. Oxygen consumption by microbial activity is higher than in mineral soils (Brinson *et al* 1981) which constrains the ability for rhizosphere oxidation (Armstrong 1970, Van Wijck *et al* 1992, Brix and Sorrell 1996). As a consequence species with limited rhizosphere oxidizing ability are replaced by better adapted species when organic matter accumulates (Barko and Smart 1983, Smits *et al* 1990) and emergent macrophytes therefore eventually replace submerged macrophytes (Barko and Smart 1983).

Emergent macrophytes, however, may also be susceptible to organic matter accumulation. Although Van den Brink *et al* (1995) found a stimulating effect of organic matter on the growth of eight waterlogged helophytes, many studies have found growth reduction in flooded organic substrates where the organic matter originated from emergent macrophytes (Clevering and Van der Putten 1995, Armstrong *et al* 1996*a,b*, Van der Putten *et al* 1997). This refractory organic matter differs from the labile organic matter originating from algae and submerged macrophytes because it contains a high amount of structural carbohydrates like lignin and cellulose. During the anaerobic decomposition of these compounds phytotoxic organic acids such as acetic acid and butyric acid are released (Drew and Lynch 1980, Kilham and Alexander 1984, Kovacs *et al* 1989). Refractory organic matter is the common type in stands of emergent macrophytes where the vegetation itself, through the production of litter, is the main source of organic matter (Pieczynska 1993, Boschker *et al* 1995).

In order to gain further insight into the role of separate and interactive effects of substrate type and water level we measured biomass production in a mineral and two different types of organic substrates, one with and one without decomposing reed-litter. We compared twelve shoreline species to investigate how general the responses to substrate type and water level are. While selecting species we took care to include species from different functional groups and from different positions on the water level gradient.

Material and methods

Species selection

The twelve species used for the experiment are all common for nutrient-rich wetlands in the Netherlands. Table 1 lists their full names and provides information on their positions on the land-water gradients, obtained by screening literature reports of field surveys in the Dutch river area. Marsh species, i.e. species from flooded parts of the shoreline, were further divided into interstitial species and clonal dominants on the basis of their height and ability to dominate the vegetation by clonal spread, criteria which were also mentioned by Grime (1987) and Boutin and Keddy (1993). Data on these plant traits were derived from literature data mentioned by Van der Meijden (1996; data on maximum height) and Grime *et al* (1988; data on 'lateral spread' and 'gregariousness'). Phytosociological literature (Wheeler 1980, Schaminée *et al* 1995) was consulted to check whether the classification of clonal dominants and interstitials was in agreement with the relative abundance of the various species in plant communities.

Table 1: List of species used for the experiment (in alphabetical order), the functional group to which marsh species belong as well as their position on the elevation gradient. Series number refers to experimental run in which the species was tested (see 'Material and Methods' for further details). Abbreviations: A- Annual, CD- clonal dominant, I- interstitial.

Species	Functional group	Zonation	Series
Tall forbs			
Cirsium arvense L.		mainly above water line [5,6,9]	3
Epilobium hirsutum L.		mainly above water line [5,6,9]	1
Eupatorium cannabinum L.		above water line [6]	1
Marsh species			
Iris pseudacorus L.	CD[1,7]	shallow water and above water line[5,6,8]	1
Lycopus europaeus L.	I[1,2]	shallow water and above water line[6,8]	2
Mentha aquatica L.	I[1,2]	shallow water and above water line[6,8,9]	2
Myosotis scorpioides L.	I[1,2]	shallow water and above water line[6,8,9]	2
Phragmites australis (Cav.) Steudel	CD[1,2,3]	deep and shallow water [5,6,8]	3
Rorippa amphibia L.	I [1,10]	deep and shallow water [6,8,9]	2
Sium latifolium L.	I[1,4,10]	shallow water and above water line[6,8]	1
Typha angustifolia L.	CD[1,2,3,4]	deep and shallow water [6,8]	3
Typha latifolia L.	CD[1,2,3,4]	deep and shallow water [6,8]	1

Data source: [1] Van der Meijden (1996), [2] Grime *et al* (1988), [3] Wheeler (1980), [4] Schaminée *et al* (1995), [5] Van de Rijt *et al* (1996), [6] De Boois (1982), [7] Sutherland (1990), [8] Van Donselaar-ten Bokkink Huinink (1961), [9] Brock *et al* (1987), [10] Gryseels (1989).

Substrate types

In February 1994, the mineral and organic substrates were collected from an exposed and sheltered site of the littoral zone of the Canal Zuid-Willemsvaart (51°37'N, 5°32'E). These substrate types will hereafter be referred to as EXPOS- and SHELT-substrate, respectively. In the field these substrates were constantly submerged and the SHELT- substrate was vegetated by the submerged macrophyte *Myriophyllum spicatum*. The reed-litter substrate (hereafter referred to as REEDL) was collected from the upper 20 cm of a reedbed at the shore of lake Gooimeer (52°19'N, 5°10'E), which was waterlogged during autumn and winter, and submerged during spring and summer. After collection, all substrates were stored under water in the dark until further use.

Experimental procedures

Seeds were collected from reed beds along the rivers Nieuwe Merwede and Oude Maas. One single population of each species was used for seed collection. Depending on what gave highest germination in previous germination tests seeds were either stratified or stored dry. Before the start of the experiment, seeds were germinated in a germination chamber with a light/dark regime of 16h/8h and corresponding temperatures of 25^0C/10^0C. Two-week old seedlings were transplanted to black pots of 1.5 L (diameter 13.5 cm, height 12 cm) each filled with one of the three substrate types. These pots were placed in pots with a diameter of 15 cm and a height of 14.5 cm with a hole at either 7 cm under the top of the inner pot or at 1 cm above the top of the inner pot to mark the water level. Drained and waterlogged conditions were obtained by filling the inner and outer pot with tap water.

Because a considerable number of young seedlings died in previous pilot experiments six seedlings were planted per pot initially. For three weeks all plants were grown drained. Thereafter the number of seedlings was reduced at random to four for *E. cannabinum* and *E. hirsutum* and, due to a higher mortality, to one for *M. scorpioides* and to two for all other species. At the same time water level in the waterlogged treatments was raised to 1 cm above the soil surface and all plants were grown for another four weeks. The water level was maintained manually by supplying tap water every second day.

Between February 21 1994 and October 20 1994, three series of experiments were carried out with different species in each series (see Table 1). The plants were grown in a greenhouse with a minimal day length of 16h obtained by illumination with Philips HPI-T lamps (100 PAR) and 23±2°C, and a dark period of maximally 8h with a minimum temperature of 15 ^{0}C.

For each species six replicates were used per treatment. To avoid effects of heterogeneity in greenhouse conditions all pots were randomized over six different tables and the position of tables was changed every week.

Soil analyses

Total N was determined colorimetrically following digestion of ground samples with a mixture of H_2SO_4-Se and salicylic acid (Novozamsky *et al* 1984). Percentage organic matter was estimated as weight loss of ground samples after 24h at 430 ^{0}C. Particle-size analysis was performed on the mineral soil-fraction by sieving (coarser fractions: Gee and Bauder 1986) and sedimentation (Houba *et al* 1985).

Available P, Mn and Fe were determined in unground soil samples that were air-dried in a 30 ^{0}C climate chamber. Fresh-/dry weight ratios were determined on a subset of soil samples. P-availability was measured in a 0.5M $NaHCO_3$ soil-extract. This Olsen-P includes all inorganic P and a small fraction of the pool of labile organic-P (Bowman and Cole 1978). We chose this method because inorganic P is the major P-form which is absorbed by plants (Anderson 1980) and because many studies have shown strong correlations between Olsen-P in the soil and the amount of P absorbed by plants (Fixen and Grove 1990). Mn- and Fe-concentrations were measured in a $CaCl_2$ soil extract according to Houba *et al* (1985).

In order to investigate the N-availability pots with the three substrates (without plants) were placed under the same conditions as used for biomass production study between October-December 1994. After three weeks, waterlogging was realised in half of the pots. At the start of the experiment and after two, three, five, seven and eight weeks, three replicate pots were harvested and NH_4^+ and NO_3^- were measured in 2 M KCl-extract of a fresh soil sample. Extracts were stored in a freezer until all concentrations of NH_4^+ and NO_3- were determined by Technicon auto-analyser (TRAACS 800). Concentrations of NH_4^+ and NO_3^- were expressed on a soil volume basis to enable comparison between substrates.

Soil redox potentials

The effects of substrate type on soil redox potential was determined every week in waterlogged substrates at a depth of 7.5 cm. Pots without plants were used as well as pots with *P. australis*, *T. angustifolia* and *C. arvense*. For each treatment, single measurements were made in two or three replicate pots. Redox potentials were measured with platinum electrodes and a calomel reference cell. Actual redox potentials were calculated by adding the potential of the calomel reference cell (250 mV) and correcting for soil-pH.

Harvesting and plant analyses

After eight weeks of growth, roots were washed out under gently running water and dry weights of roots and shoots were determined after drying for at least 48h at 70 ^{0}C. Root weight ratio (RWR) was calculated as the ratio of root dry weight to total dry weight.

N-, P-, and K- concentrations were determined in shoots of two replicate pots. Fe-concentration in the shoot was determined in one sample, obtained by pooling all replicates, because the available shoot biomass per pot was insufficient for replicate analyses. N and P were determined colorimetrically (indophenol-blue method and molybdenum blue, respectively), and Fe by atomic absorption in a sulphuric acid/salicylic acid digest.

Statistical analyses

For each plant species a two-way ANOVA with three substrate types and two water levels was performed on total dry weight and RWR. Prior to ANOVA homogeneity of variances was tested by Cochran's Q. If necessary, total dry weight was ln(x) and RWR arcs-sqrt transformed. Differences among means were compared by a Tukey test (Sokal and Rohlf 1995). Effects of substrate type, plant species and time on redox potentials were analyzed with ANOVA repeated measures using week as a within-pot repeated factor (SPSS-procedure MANOVA, Norusis

1986). Means of redox potentials were compared with the least significant difference according to the Tukey-Kramer method (Sokal and Rohlf 1995).

Results

Soil analyses

Organic matter content was highest in the REEDL-substrate, intermediate in the SHELT-substrate and lowest in the EXPOS-substrate (Table 2). Soil bulk density significantly differed between substrate types and was lowest in the REEDL, intermediate in the SHELT-substrate and highest in the EXPOS-substrate. These values corresponded with the proportion of sand (soil particles> 53μ) which was highest in EXPOS and lowest in REEDL.

Olsen-P was highest in the SHELT-substrate, intermediate in the REEDL-substrate and lowest in the EXPOS- substrate. The available nitrogen was mainly in the form of NH_4-N (Fig.1). NO_3-N was found in low concentrations under all circumstances (data not shown). Throughout the experimental period, concentrations of NH_4-N in the REEDL- and SHELT-substrate were similar and in both substrates concentrations were higher when waterlogged. At both water levels NH_4-N concentration in the EXPOS-substrate was much lower than in the other two substrates (Fig. 1).

Table 2: Physicochemical characteristics of substrates used in the experiment (means ± SE, n=6; n=8 for soil bulk density; n=3 for Olsen-P and pH). All values are given for size distribution of particle sizes. Significant differences (P<0.05) between means in the same row are indicated with different letters.

	REEDL	SHELT	EXPOS
Organic matter (%)	35.48±0.07 a	9.67 ± 0.11 b	0.67±0.03 c
Soil bulk density (kg.l^{-1})	0.83±0.01 c	1.19±0.02 b	1.84±0.01 a
Ntot (mg/kg dry soil)	14700±697 a	1261±251 b	348±41 c
Olsen-P (mg/kg dry soil)	58±1.2 b	102±4.1 a	20±0.5 c
Mn (mg/kg dry soil)	0.17±0.06 a	0.09±0.03 a	0.13±0.027 a
Fe (mg/kg dry soil)	0.48±0.15 ab	0.18±0.082 b	0.68±0.084 a
pH (range)	6.7-7.3	6.8-7.2	8.1-9.4
Size distribution of soil-particles (% of mineral fraction)			
>53 μ	7.1-8.3	71.1-71.1	93.3-93.7
16-53 μ	26.1-26.9	14.5-14.5	2.4-2.7
2 –16 μ	44.5-49.6	5.1-5.3	0.6-0.6
< 2 μ	17.3-20.3	9.1-9.3	3.3-3.4

Mn-concentrations did not differ between substrates. The concentration of Fe was significantly higher in the EXPOS-substrate than in the SHELT-substrate, whereas the concentration of Fe in the REEDL-substrate was intermediate.

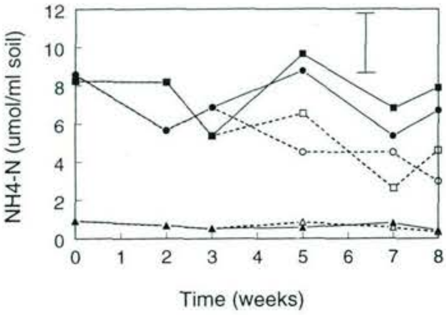

Figure 1: Concentration of NH4$^+$ on a soil volume basis (product of mean soil bulk density and mean NH4-N concentration on mass basis) in drained REEDL-substrate (--o--), waterlogged REEDL-substrate (—●—), drained SHELT-substrate (--□--), waterlogged SHELT-substrate (—■—), drained EXPOS-substrate (--Δ--) and waterlogged EXPOS-substrate (—▲—). Waterlogging was imposed three weeks after the onset of incubation. Vertical bar indicates least significant difference.

Soil redox potential

Soil redox potentials were significantly affected by an interaction between all factors (Table 3). In the EXPOS-substrate values remained relatively stable within the range of 120 mV to 220 mV (Fig.2), which is indicative for manganese reduction (Ponnamperuma 1984). In the EXPOS-substrate root biomass of all species was very low and redox potentials did not increase during the experiment (Fig. 2). Soil redox potentials of the SHELT- and REEDL-substrate tended to be lower than in the EXPOS-substrate. They fell for most instances within the range 0 mV to 200 mV, which is indicative for iron reduction (Ponnamperuma 1984). The marsh clonal dominant *T. angustifolia* increased soil redox potential in both organic substrates. *C. arvense* only showed a clear increase when grown in REEDL-substrate. *P. australis* had the lowest root biomass of the three species used for redox potential measurements and did not significantly increase redox potential in any substrate.

Biomass production and allocation to roots

Two-way analysis of variance revealed no interactive effect of substrate type and water level on biomass production of *T. angustifolia, M. aquatica* and *R. amphibia* (Table 4). These species were strongly affected by substrate type, but less or not significantly by water level. For all other species the effect of water level depended on substrate type (Table 4). In the EXPOS-substrate waterlogging only decreased biomass production of the tall forbs *C.arvense* and *E. cannabinum* (Fig. 3). In the SHELT-substrate no species was decreased by waterlogging, but in REEDL-substrate the high water level negatively affected productivity of the marsh clonal dominant *I. pseudacorus*, the marsh interstitials *M. scorpioides*, *R. amphibia* and *S. latifolium* and the tall forbs *Eupatorium cannabinum* and *Epilobium hirsutum* (Fig. 3). Even in drained REEDL-substrate *I. pseudacorus*, *P. australis*, *T. latifolia*, *M. aquatica*, *S. latifolium* and *C. arvense* did not produce as much biomass as in the waterlogged SHELT-substrate.

Table 3: ANOVA of redox potentials in three different waterlogged substrates (mineral different plants (without plants and with *Phragmites australis*, *Typha angustifolia* or *Cirsium arvense*). The weekly measured redox potentials (R) were measured as a within pot repeated factor. n.s.= not significant, * = P<0.05; *** = P<0.001.

Factor	Df	F-values
Substrate (S)	2/15	79.50^{***}
Plant (P)	3/15	13.10^{***}
S * P	6/15	3.35^{*}
Redox (R)	3/45	68.83^{***}
S * R	6/45	6.19^{***}
P * R	9/45	$1.90^{n.s.}$
S * P * R	18/45	2.20^{*}

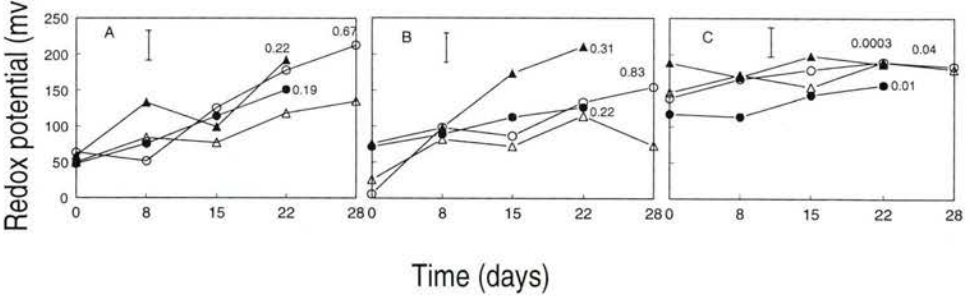

Figure 2: Redox potentials (mV; means, n=3) of waterlogged REEDL-substrate (A), SHELT-substrate (B) and EXPOS-substrate (C) without plants (Δ) and with *P. australis* (●), *T. angustifolia* (▲) and *C. arvense* (O). Vertical bars represent the least significant difference (P<0.05). Total root biomass is indicated at the end of each line.

Table 4: F-values of biomass from twelve different plant species grown in three different substrate types (S) at two water levels (W). Data of *Typha latifolia*, *Cirsium arvense*, *Eupatorium cannabinum* and *Epilobium hirsutum* based on ln-transformed data. Abbreviations: MCD= Marsh clonal dominants; MI= Marsh interstitials; TF= Tall forbs. *P<0.05, **P<0.01, ***P<0.001. Degrees of freedom in parentheses.

Species	Group	S(2)	W(1)	S*W(2)	MS (error) (30)
I. pseudacorus	MCD	78.26***	8.05***	30.66***	0.045
P. australis	MCD	579.11***	0.20	6.25**	0.838
T. angustifolia	MCD	566.55***	7.85**	3.14	0.131
T. latifolia	MCD	37.61***	2.55	3.80*	0.124
L. europaeus	MI	178.97***	28.64***	17.16***	0.122
M. aquatica	MI	116.06***	0.99	0.24	0.340
M. scorpioides	MI	73.97***	27.61***	8.23***	0.424
R. amphibia	MI	178.35***	16.98***	2.86	0.184
S. latifolium	MI	97.92***	10.17***	23.63***	0.057
C. arvense	TF	443.95***	53.16***	14.11***	0.072
E. cannabinum	TF	53.62***	59.47***	27.47***	0.089
E. hirsutum	TF	36.81***	6.40*	43.32***	0.060

Table 5: F-values of root weight ratios from twelve different plant species grown in three different substrate types (S) at two water levels (W). Data of root weight ratio of *T. latifolia* based on arcsinsqrt-transformed data. Abbreviations: MCD= Marsh clonal dominants; MI= Marsh interstitials; TF= Tall forbs. *P<0.05, ***P<0.01, ***P<0.001. Degrees of freedom in parentheses.

Species	S(2)	W(1)	S*W(2)	MS (error) (30)
I. pseudacorus	25.15***	0.23	12.69***	0.001
P. australis	65.67***	7.81***	0.73	0.002
T. angustifolia	17.39***	9.39**	4.76*	0.005
T. latifolia	26.35***	2.70	18.75***	0.003
L. europaeus	15.33***	3.16	1.14	0.005
M. aquatica	35.17***	0.68	0.48	0.003
M. scorpioides	15.79***	0.26	0.89	0.005
R. amphibia	41.70***	4.83*	5.04*	0.001
S. latifolium	1.90	4.27*	1.40	0.003
C. arvense	46.12***	80.03***	0.25	0.002
E. cannabinum	6.45***	12.14***	13.20***	0.002
E. hirsutum	10.20***	15.94***	6.12***	0.002

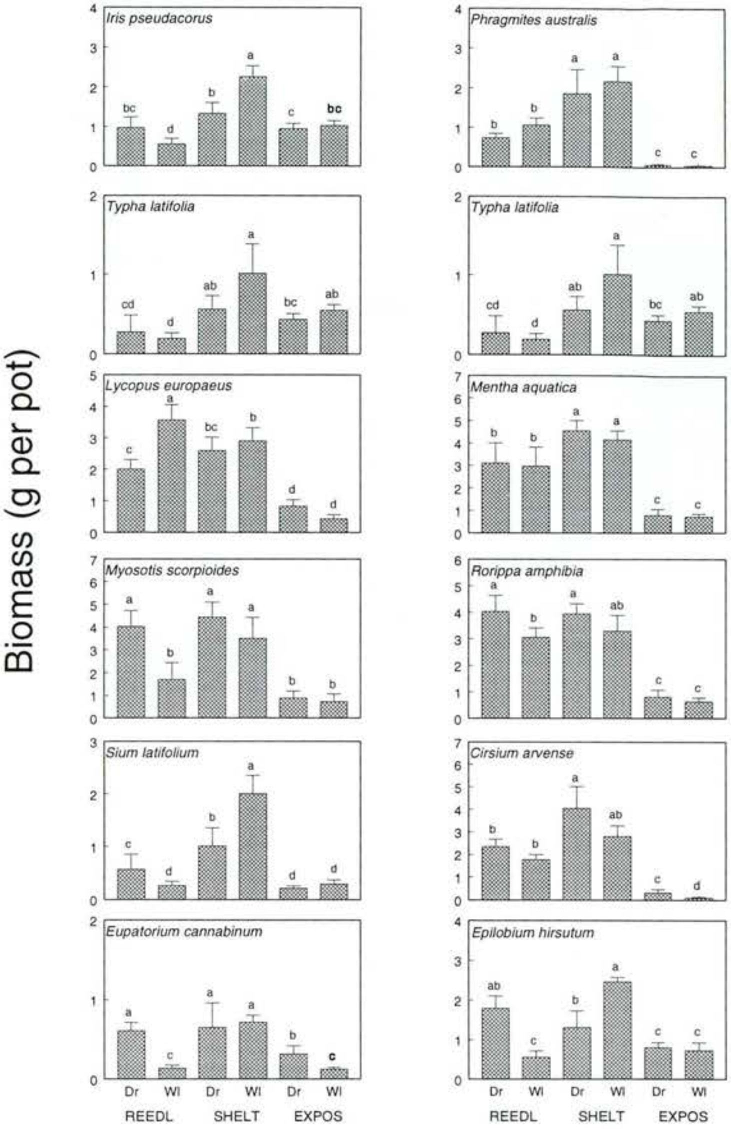

Figure 3: Total dry weight (means±SE, n=6) of plants grown in substrate with reed-litter (REEDL), substrate with organic matter originating from algae and submerged macrophytes (SHELT) and mineral substrate (EXPOS) under drained (Dr) and waterlogged (Wl) conditions. Significant differences of total biomass are indicated with different letters (Tukey, P<0.05). Note different scaling on y-axis

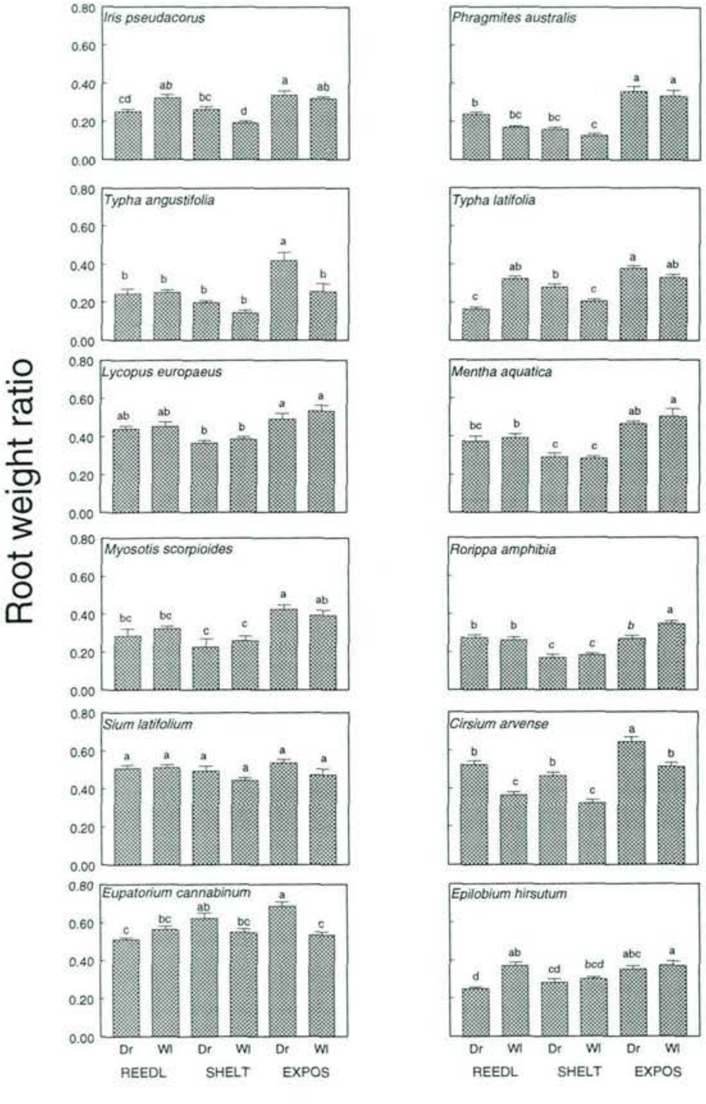

Figure 4: Root weight ratio (means±SE, n=6) of plants grown on substrate with reed-litter (REEDL), substrate with organic matter originating from algae and submerged macrophytes (SHELT) and mineral substrate (EXPOS) under drained (Dr) and waterlogged (Wl) conditions. Significant differences of total biomass are indicated with different letters (Tukey, P<0.05).

Interactions between water level and substrate type did not significantly affect RWR of *P. australis*, *M. aquatica*, *L. europaeus*, *S. latifolium*, *M. scorpioides* and *C. arvense* (Table 5). RWR in EXPOS-substrate was significantly higher than in other substrates for *P. australis* and *M. aquatica* and under drained conditions for *M. scorpioides* and *C. arvense*. *L. europaeus* and *S. latifolium* also had their highest RWR in EXPOS-substrate, but these RWR values were not significantly different from RWR in REEDL-substrate (Fig.4).

Root weight ratios of *T. angustifolia*, *T. latifolia*, *I. pseudacorus*, *E. cannabinum* and *E. hirsutum* were significantly affected by interactions between substrate type and water level (Table 5), but no clear effect of water level could be seen. All these species had the highest RWR in EXPOS-substrate (Fig.4).

Plant analyses

Shoot concentrations of N and P were used to calculate the N:P ratios, because these ratios best indicate which element might have been limiting growth. Under all circumstances N:P ratios were below 14 (Table 6), indicating that only N-availability could have been limiting biomass production (Koerselman and Meuleman 1996).

N-concentrations were always lowest in plants from the EXPOS-substrate, even for *T. latifolium* and *I. pseudacorus* (Table 7) which had relatively high biomass production in this substrate. Species with their maximum biomass production in both SHELT- and REEDL-substrate, i.e. *T. angustifolia* (waterlogged), *M. scorpioides*, *R. amphibia*, *E. hirsutum* and *E. cannabinum* (all drained) had either higher N-concentrations in REEDL-substrate (*M. scorpioides*, *E. hirsutum* and *E. cannabinum*) than in SHELT-substrate or equal concentrations (*R. amphibia*). Only *T. angustifolia* had a higher N-concentration when grown in SHELT-substrate. Species which had a lower biomass production in REEDL-substrate compared with the SHELT-substrate were *P. australis*, *T. latifolia*, *I. pseudacorus* (waterlogged only), *S. latifolium*, *M. scorpioides*, *E. hirsutum*, *E. cannabinum* (last three species only when waterlogged) and *C. arvense* (drained). These species also had obtained their maximum N-concentration in the REEDL-substrate. Only *P. australis* and *C. arvense* had more or less similar concentrations (Table 7). Shoot Fe-concentrations seemed to be unaffected by substrate type (Table 8).

Table 6: N:P-ratio in shoots of plants grown in three different substrates under drained and waterlogged conditions. Values represent means of duplicate samples (*Mentha aquatica* not determined). Abbreviations: MCD= Marsh clonal dominants; MI= Marsh interstitials; TF= Tall forbs; REEDL= organic substrate with reed-litter; SHELT= organic substrate without reed-litter; EXPOS; mineral substrate; Dr= drained; Wl= waterlogged .

Species	Group	REEDL		SHELT		EXPOS	
		Dr	Wl	Dr	Wl	Dr	Wl
I. pseudacorus	MCD	6.2	8.7	5.5	4.8	7.3	6.6
P. australis	MCD	3.7	6.7	4.8	6.3	n.d.	n.d.
T. angustifolia	MCD	7.2	8.3	3.8	3.6	12.8	10.9
T. latifolia	MCD	2.9	3.2	3.1	3.7	n.d.	n.d.
L. europaeus	MI	3.7	4.4	3.9	4.0	3.9	2.6
M. scorpioides	MI	2.2	4.4	1.7	2.7	2.5	2.4
R. amphibia	MI	3.3	3.8	3.0	3.2	2.9	3.6
S. latifolium	MI	5.4	5.3	4.3	4.3	4.3	3.2
C. arvense	TF	5.7	4.6	5.4	4.9	5.2	2.8
E. cannabinum	TF	7.3	10.8	8.1	9.0	9.1	9.7
E. hirsutum	TF	5.0	5.8	4.5	5.2	4.9	5.3

Table 7: N-concentration (mg.g^{-1} DW) in shoots of plants grown in drained and waterlogged organic and mineral substrates. Values represent means of duplicate samples (*Mentha aquatica* not determined). Abbreviations: MCD= Marsh clonal dominants; MI= Marsh interstitials; TF= Tall forbs; REEDL= organic substrate with reed-litter; SHELT= organic substrate without reed-litter; EXPOS; mineral substrate; Dr= drained; Wl= waterlogged.

		N-concentration					
Species	Group	REEDL		SHELT		EXPOS	
		Dr	Wl	Dr	Wl	Dr	Wl
I. pseudacorus	MCD	25.4	24.9	21.7	21.6	14.9	14.6
P. australis	MCD	12.6	22.0	17.9	21.0	n.d.	n.d.
T. angustifolia	MCD	15.8	16.8	19.4	28.5	n.d.	n.d.
T. latifolia	MCD	45.7	45.7	27.8	38.4	21.5	19.7
L. europaeus	MI	12.9	13.1	11.8	13.5	11.9	10.0
M. scorpioides	MI	8.1	12.6	7.0	9.0	8.4	10.3
R. amphibia	MI	7.4	8.1	7.4	8.4	6.4	9.5
S. latifolium	MI	30.9	32.4	19.6	19.6	23.6	21.6
C. arvense	TF	9.8	11.5	10.2	11.4	10.7	13.2
E. cannabinum	TF	23.3	25.0	18.1	18.5	14.7	16.7
E. hirsutum	TF	18.5	24.7	15.9	20.6	12.0	14.4

Table 8: Fe-concentrations (mg. g^{-1} DW) in shoots of plants grown in waterlogged substrates. Horizontal bars refer to cases with insufficient shoot dry weight to determine Fe-concentration. Abbreviations: REEDL= organic substrate with reed-litter; SHELT= organic substrate without reed-litter; EXPOS= mineral substrate.

Species	REEDL	SHELT	EXPOS
Phragmites australis	0.162	0.159	-
Typha angustifolia	0.122	0.182	-
Typha latifolia	-	-	-
Iris pseudacorus	-	0.073	0.087
Mentha aquatica	0.225	0.246	-
Lycopus europaeus	0.220	0.217	-
Sium latifolium	-	-	-
Myosotis palustris	0.222	0.177	0.162
Rorippa amphibia	0.127	0.112	-
Eupatorium cannabinum	-	-	-
Epilobium hirsutum	0.464	0.345	0.424
Cirsium arvense	0.714	0.544	-

Discussion

At both water levels, clear effects of substrate type were found. Water level however mainly affected growth in the REEDL-substrate. In the EXPOS-substrate waterlogging also inhibited biomass production, but only of the tall forb species *C. arvense* and *Eupatorium cannabinum.*

Ten out of twelve species, i.e. *I. pseudacorus* and *T. latifolia* excluded, produced significantly more biomass in the organic SHELT-substrate than in the mineral EXPOS-substrate. This increase of productivity with increasing organic matter content in both drained and waterlogged soils is in accordance with other studies (Wilson and Keddy 1985, Van den Brink *et al* 1995). Differences in productivity between the EXPOS- and SHELT-substrate are related to nutrient availability. Shoot nutrient concentrations indicated N-limitation and N-availability was indeed significantly lower in the mineral substrate as shown by the low NH_4-N in the substrate (Fig 1) and N-concentration in shoots (Table 7). In addition, mechanical impedence may have limited productivity in the mineral substrate, but none of the species showed increased productivity by waterlogging, although this usually mitigates the influence of soil compaction (Engelaar *et al* 1993).

Our results no longer confirm Wilson and Keddy (1985) and Van den Brink *et al* (1995) if plant performance in the REEDL-substrate is also considered. Although this substrate was highest in organic matter content biomass production was often decreased. Differences between both organic substrates could not be attributed to nutrient limitation. Van der Putten *et al* (1997), using the same reed-litter substrate, found no effect of fertilization on plant growth in this substrate. According to Koerselman and Meuleman (1996) N:P ratios in shoots indicated N-limitation. N-availability however did not differ significantly between both organic substrates (Fig 1). Shoot-N concentrations showed that luxury uptake of N had taken place in the REEDL-substrate, since their shoot-N concentrations were often higher than of conspecifics grown in the SHELT-substrate. Root weight ratios did not differ between plants grown in the SHELT- or REEDL-substrate. Nutrient limitation in one of both substrates would have resulted in strong differences in allocation to roots since species from eutrophic habitats, such as used in this study, quickly respond to nutrient limitation by enhanced allocation to roots (Chapin 1980).

Low density of the substrate will not have been a constraint either since this will only limit growth at soil bulk densities below 0.2 $kg.l^{-1}$ (Delaune *et al* 1979, Barko and Smart 1986, Spencer 1991). All three substrates exceeded this value. The detrimental effects of waterlogged reed-litter might be related to low redox potentials (DeLaune *et al* 1984, Pezeshki and DeLaune 1990), but these did not differ between both organic substrates. Redox potentials of both soils where indicative of iron reduction (Ponnamperuma 1984). Fe-concentrations however were always below values found to reduce growth in solution cultures and Fe-shoot concentrations were also below foliar concentrations found to be associated with yield reduction in solution cultures (Wheeler *et al* 1985, Snowden and Wheeler 1995).

Van der Putten *et al* (1997) and Clevering (1998) using seedlings and adults, respectively, of *P. australis* found growth-inhibiting effects of reed-litter derived organic matter at organic matter contents below the level of the SHELT-substrate in this study. Hence, growth differences between both organic substrates can also not be attributed to different amounts of organic matter.

Plant-induced increases of soil redox potentials were not related to performance in reed-litter substrate. Both *T. angustifolia* and *C. arvense* significantly increased soil redox potential in the reed-litter substrate (Fig. 2) but growth of the latter species was strongly decreased. Redox measurements however offer little information on the intensity of oxidation-reduction processes (Clevering and Van der Putten 1995, Brix and Sorrel 1996) and a higher oxygen demand may therefore have played a role in the reed-litter substrate. It is remarkable however that species such as *T. latifolia* and *P. australis*, with a strong capacity for rhizosphere oxidation (Conlin and Crowder 1989, Gries *et al* 1990, Armstrong *et al* 1992), were also inhibited in the decomposed reed-litter substrate. This indicates that in addition to the high oxygen demand of the reed-litter substrate, phytotoxic organic acids such as acetic and butyric acid may offer an important explanation for growth reduction in this substrate. These acids are released during anaerobic decomposition of structural carbohydrates, compounds that are abundant in reed-litter, but not in labile organic matter such as present in the SHELT-substrate (Mason and Bryant 1975, Godshalk and Wetzel 1978). Experiments with *Oryza sativa* showed a strong phytotoxic effect of acetic and butyric acid (Niranjan Rao and Mikkelsen 1977), *P. australis* was adversely affected by acetic acid in the medium (Armstrong *et al* 1996b).

Since differences in biomass production between the mineral substrate and the substrate with labile organic matter were related to nutrient-availability these differences are unlikely to play a role along eutrophic waterbodies. Species composition at sites with where labile organic matter has accumulated may however be different from mineral sites because of direct effects of wave exposure, which is often higher at mineral sites (Coops *et al* 1991).

Inhibitory effects of litter-derived organic matter also play a role along eutrophic waterbodies since these effects were not related to nutrient-availability. Due to the specific impact of reed-litter substrate on growth of shoreline plant species presence of litter in the substrate will affect the outcome of species interactions. Therefore species composition in mineral sites will be different from sites where litter accumulation takes place. Litter accumulation will not enhance species richness by favouring interstitials above clonal dominants. Some clonal dominant species, such as *T. angustifolia* (this study) and *Glyceria maxima* (Van der Putten *et al* 1997) grow well in reed-litter substrates whereas this study has shown that many interstitials are inhibited in these substrates. At a large scale, however, variability in litter accumulation may enhance species diversity because species composition at organic sites will differ from mineral sites. The litter sensitive *T. latifolia* is usually restricted to the open water fringe of the emergent vegetation (Segal 1982) where waves and currents prevent the accumulation of organic matter. Although the sensitive *P. australis* may remain dominant at litter-rich sites its vigour is strongly reduced (Buttery *et al* 1965, Schröder 1987, Van der Putten 1997). In flooded parts of such highly organic *P. australis* stands increased abundance of *T. angustifolia* (Granéli 1989, Coops 1995) and *L. europaeus* (Klosowski 1988) has been noticed. In our experiment, the latter two species also performed best when grown in waterlogged reed-litter.

The inhibitory effect of waterlogging in reed-litter on many marsh species and tall forbs indicates that lower limits on the water-depth gradient of many shoreline species may be constrained by litter acccumulation. A similar influence of reed-litter on depth penetration has previously been shown for *P. australis* (Weisner 1987, 1991). The susceptibility of marsh species to waterlogged reed litter implies that these species require a littoral zone where water level fluctuates, since the regular drawdowns will ameliorate substrate conditions.

6. ARE WEAK COMPETITORS STRONG TOLERATORS? RESPONSE OF SMALL AND TALL MARSH PLANT SPECIES TO COMBINED AND SEPARATE EFFECTS OF CANOPY SHADE AND WATERLOGGING

Abstract

Since competitive ability of plant species is strongly related to morphological traits tall plants are usually strong competitors. Small species on the other hand are assumed to be better tolerators of adverse environmental conditions. It has however rarely been tested whether these weak competitors are also more tolerant for two environmental factors acting simultaneously. In this study, it was investigated if small marsh species are better able to tolerate separate and combined effects of canopy shade and waterlogging than tall marsh species. Two small perennial species, *Mentha aquatica* L. and *Myosotis scorpioides* L., and two tall perennial species, *Epilobium hirsutum* L. and *Eupatorium cannabinum* L., were grown outdoors for three months both shaded and unshaded in either moist or waterlogged soils.

All species produced maximum biomass when grown in moist soil and unshaded. Strongest reduction occurred when plants were waterlogged and shaded. Interactions between shade and waterlogging significantly affected the biomass production but there were no specific responses. When only shaded the root weight ratio (RWR) of *Myosotis scorpioides* was reduced to a larger extent than of the other three species, whereas the increase of specific leaf area (SLA) was significantly smaller for *Myosotis scorpioides* than for the other three species. These differences in morphological adaptations indicate that *Myosotis scorpioides* was better able to sustain prolonged periods of shading. However, this feature did not occur in the other small perennial *Mentha aquatica*. Waterlogging decreased biomass production of all species except of the tall forb *Epilobium hirsutum*. The tall forb *Eupatorium cannabinum* was reduced to a greater extent than the two small marsh species.

The results have shown that the distinction between weak and strong competitors on the basis of plant size may not be valid when two or more adverse environmental conditions operate simultaneously. It is argued that for a succesful prediction of species composition in habitats with several interacting adverse conditions life-history characteristics of species have to be considered as well.

Introduction

Among plant species tradeoffs exist between competitive ability and the ability to tolerate extreme environmental conditions (Smith and Huston 1989, Grace 1990, Grime *et al* 1997). Competitive ability is strongly determined by size-related morphological traits (Shipley *et al* 1989, Boutin and Keddy 1993, Hills *et al* 1994). Experiments in a wide range of ecosystems, including calcareous grasslands (McLellan *et al* 1997), old-fields (Miller and Werner 1987, Gurevitch *et al* 1990) and wetlands (Gaudet and Keddy 1988, Stockey and Hunt 1994) have confirmed that large plants are generally less affected by competition than smaller plants. Strong competitors therefore dominate the fertile, undisturbed sites, whereas weak competitors are restricted to habitats where either resource or nonresource conditions limit plant growth (Grace and Wetzel 1981, Wilson and Keddy 1986*b*, Keddy 1989, Austin 1990, Campbell and Grime 1992).

In wetland vegetation, where flooding is an important factor limiting plant growth, a zonation of strong and weak competitors may be recognized. Tolerant species inhabit the frequently flooded places, whereas tall competitive herbs often dominate vegetation at higher elevations (Menges and Waller 1983, Hills et al 1994). Although flooding is an important factor in wetlands it may not be the only factor determining plant distribution. In floodplain forests for example both flooding and light availability (as determined by the tree canopy) are related to species distribution (Menges and Waller 1983, Siebel and Bouwma 1998).

A similar combination of stress factors, i.e. canopy shade and flooding, may be experienced by species inhabiting reed marshes. At the highest, least frequently flooded positions vegetation is dominated by tall competitive herbs such as *Epilobium hirsutum* L. and *Eupatorium cannabinum* L. At lower elevations, where frequent shallow floods occur, the flood tolerant competitor common reed, *Phragmites australis* (Cav.) ex. Steudel, dominates and is accompanied by small marsh species such as *Mentha aquatica* L. and *Myosotis scorpioides* L. (Gryseels 1989, Fojt and Harding 1995, Van de Rijt *et al* 1996). Because of their small stature the latter two species may be considered as weak competitors which may become subject to shade of the reed canopy and, due to their position on the shoreline, to waterlogging.

Light interception by a canopy reduces both light quantity and red:farred ratio in the understorey (Smith 1982). A common response for species lacking physiological adaptations to shade (Murchie and Horton 1997) is an increased elongation of stems in order to reach the top of the canopy (Morgan and Smith 1979, Schmitt and Wulff 1993, Van Tienderen and Van Hinsberg 1996). Otherwise, plants may be able to persist by increasing the size of the photosynthetic apparatus and by a conservative use of carbohydrates (Grime 1966). This can be achieved by allocating more biomass to stems instead of roots since roots have relatively high maintenance and respiration costs (Corré 1983*a*, Lambers *et al* 1983, Amthor 1984).

Waterlogging may influence the ability of plants to grow in the shade because it can lead to a decrease of chlorophyll concentration and impairment of leaf expansion (Talbot *et al* 1987, Dale and Causton 1992*b*). In addition, waterlogging enhances respiration of carbohydrates in roots (Drew 1990) thereby providing a respiratory burden which may be too heavy when another stress factor, such as canopy shade, is also present. Waterlogging may also inhibit root extension and nutrient uptake (Laan *et al* 1989*a*), thereby limiting possibilities for increased photosynthesis (Seemann *et al* 1987).

In the experiment reported in this paper we compared the separate and combined effects of waterlogging and canopy shade on two weak and two strong competitive species from wetland vegetation. The hypothesis to be tested was that the weak competitors *Mentha aquatica* and *Myosotis scorpioides* are better able to tolerate both separate and combined effects of shade and waterlogging than the strong competitors *Epilobium hirsutum* and *Eupatorium cannabinum*.

Under field conditions other marsh species accompany *P. australis* in zones that are waterlogged or shallowly flooded during the growing season. Higher water levels are restricted to winter and early spring (Breen *et al* 1988, Blom *et al* 1996). Hence, during the growing season, when canopy shade plays a role, our studied species will not experience higher levels of flooding than waterlogging. We also compared our experimental results with field data collected in reed marshes. These data were used to investigate relationships between the occurrence of the studied species and the performance of the dominant *P.australis* as the factor determining light availability.

Material and methods

Plant material

Plants used for this experiment were raised from seeds, collected from single populations along the River Nieuwe Merwede in the Netherlands (51°45'N, 4°45'E). Seeds were stored dry and dark at room temperature until further use. Thirtysix days before the start of the experiment seeds were germinated on 1 mm glass beads watered by tap water in a chamber with a day/night of 16h/8h at 25°C/10°C. Seedlings with two cotyledons and two true leaves were transplanted to plastic trays with a homogenuous 1:1 mixture of sand and clay. From June 8-28 1995, seedlings were grown in a greenhouse with a light period of 16 h and a minimum temperature of 23 °C in alternation with a dark period of 8h and a minimum temperature of 15 °C. Before the start of the experiment plants were removed from trays, soil was carefully rinsed from the roots under gently flowing tap water and fresh weight was determined for each individual plant. Ten plants of each species were separated into roots and shoots and dry weights were determined to calculate the fresh/dry weight ratio.

After determining the fresh weight each plant was placed in a PVC cylinder (15 cm diameter, 50 cm height) filled with the same substrate as used in the plastic trays. To avoid nutrient limitation a 7.5 g tablet of Osmocote (7.5%NH_4-N, 7.5% NO_3-N, 7.2% P_2O_5 and 12% K_2O) was placed in every cylinder 5 cm below the soil surface.

Light and water level treatments

Shade was produced by frames (90 x 90 x 100 cm, l x w x h) covered with a moss green filter (Lee no. 122) to reduce the red:farred ratio. Neutral density net was placed on top of the moss green filter to reduce light intensity to 7% of full daylight. Plants receiving full daylight treatment were placed under frames covered by a colourless plastic film to obtain micro-climatic conditions comparable to shaded cages. The top of the cylinders was 10 cm below the bottom of the frames, so that the space in between allowed air circulation while preventing light transmission as was confirmed by light quantity measurements (data not shown).

In order to control for photo-degradation of the moss green filter red:farred ratio and light quantity were measured in three shade cages and three daylight cages at the start of the experiment (July 11), halfway and at the end (November 1) by MACAM Spectro radiometer SR9910-PC (MACAM Photometrics Ltd.). At the beginning the red:farred ratio was 0.20±0.03 and at the end 0.24±0.005 (both means±SE with three replicates).

In the field light interception by reed-shoots lasts from the beginning of May until the end of October (Dykyjova *et al* 1970, Geisslhofer and Burian 1970). Measurements of light quantity made by Ondok (1973) in monospecific reed stands showed a constant level of light extinction from July until the end of September of more than 95%. In a reed stand with interstitial marsh species Lenssen *et al.* (chapter 3) measured light extinction of 90% in June and 98% in August. Thus, compared with the field situation maximal light extinction under experimental conditions was somewhat lower, whereas the gradual decrease in light availability caused by growing reed shoots was not taken into account and the period of shading in field conditions lasts longer than the experimental period.

Water treatments were realised in four adjacent outdoor ponds at the University of Nijmegen. At the start of the experiment, 1 July 1995, plants were placed in the ponds under the cages, water levels in all ponds were maintained at 10 cm below the top of the PVC cylinders to guarantee an adequate water supply for the roots of the transplanted seedlings. After one week, the water level in two ponds was gradually raised to the top of the cylinders ('waterlogged treatment'). In the two other ponds the water level was lowered to 20 cm below the top of the cylinder ('moist treatment').

Experimental design

Treatment and harvest date were assigned to each plant. At the start of the experiment plants were placed under the frames with one replicate of the combination of species and harvest date under each frame. Per pond three shaded and three daylight frames were randomly placed in two parallel rows. There were six replicates for each combination of species, harvest date and treatment (i.e. 'shade moist', 'shade waterlogged', 'daylight moist' and 'daylight waterlogged').

Determination of mortality and harvesting of plants

Plants were harvested 27, 63 and 92 days after the start of the experiment. At each harvest date the total number of dead plants (including the ones of the subsequent harvests) were counted. Harvested plants were separated into roots, rhizomes, stolons, main stem, branches, leaves and inflorescences. Leaf area was determined using a digital image processor (Imaging Technology Inc., Woburn, MA). Data of leaf area were used to calculate leaf area ratio (LAR, the quotient of total leaf area to total plant dry weight which represents an index of plant leafiness) and the specific leaf area (SLA, the quotient of total leaf area to total leaf dry weight). Petioles were pooled with branches to allow comparison of stem dry weight between species with petioles (*Mentha aquatica* and *Eupatorium cannabinum*) and without petioles (*Myosotis scorpioides* and *Epilobium hirsutum*). Dry weights were determined after drying at 70 °C for at least 48 hours. Dry weight of roots, stems and leaves were divided by total dry weight of the plant and will be referred to as weight ratios.

Field distribution

Whereas the distribution of the four studied species along flooding gradients is already well known, little information is available on the distribution of these species in relation to the light climate in the *P. australis* dominated zone. Since the light availability is directly determined by the performance of the species which dominates the top of the canopy, i.c. *P. australis* (Ondok 1973), relationships between presence/absence and performance of *P. australis* were determined.

Relevés were sampled in *P. australis* dominated vegetation along the River Nieuwe Merwede and Haringvliet (51°42'N,4°25'E;51°49'N,5°1'E; most western and most eastern sampling site respectively) between 2 July 1996 and 3 September 1996. In each relevé all higher plant species were recorded and performance of *P. australis* was assessed between 21 August and 3 September, when *P. australis* is at peak aboveground biomass (Van der Toorn and Mook 1982). Within each relevé the number of *P. australis* stems were counted in a randomly chosen subplot of 0.4 m x 0.4 m, clipped off at ground level and used for estimating the total aboveground dry weight after drying at 70°C for at least 48 hours. The mean stem weight of *P. australis* within the relevé was estimated by taking the quotient of total aboveground dry weight of *P. australis* and the number of stems. From this data set all relevés bordering the open water were excluded because here direct effects of wave exposure may have influenced species composition; 45 relevés were used for further analysis.

Statistical analysis

The influence of light and water level on the monthly survival of the four species was tested with a log linear analysis for goodness of fit against a binomial distribution (Sokal and Rohlf 1995) using type 3 analysis in SAS procedure GENMOD (SAS Institute 1993).

Dead plants were treated as missing values and because of this all analyses of variance were performed with Type III Sum of Squares (Sokal and Rohlf 1995) in SAS procedure GLM (SAS Institute Inc. 1989). In the ANOVA design used each frame was treated as a block factor in which the effect of pond was confounded.

Analyses were performed with ln-transformed values of total dry weight and arcsin-transformed values of weight ratios to reduce heterogeneity of variances. Within significant species x light interactions differences between species were split up in six pair-wise, non-orthogonal contrasts keeping the overall experimental error rate at 5%. Differences in overall species means were investigated with the Tukey-Kramer procedure (Sokal and Rohlf 1995). Logistic regression was used to test the relationship between presence of species (only if adult) and performance of *P. australis*. Significance was assessed with the likelihood ratio chi-square test (Trexler and Travis 1993)

Results

Survival

In waterlogged soils less plants survived than in moist soils (Table 1). The effect of water level was significant at month one and three (Table 2). In the second month the effect of water level depended on plant species. This significant interaction was probably due to the low survival of waterlogged *Eupatorium cannabinum* (Table 1).

Significantly fewer plants had survived under daylight conditions in the second month of the experiment (Table 1+2) but survival under influence of light was not species dependent (Table 2). During the third month, *Myosotis scorpioides* seemed to have survived better in daylight than in shade (Table 1), although statistical analysis did not show a significant effect of species, light or of interaction between both factors. The low total number of plants, which has lead to a low power of the test, may have caused this lack of significance.

Table 1: Survival (%) for successive months of tall forbs (*Epilobium hirsutum* and *Eupatorium cannabinum*) and reed marsh interstitials (*Mentha aquatica* and *Myosotis scorpioides*) grown in the daylight and in the shade in moist and waterlogged soils.

	Shade		Daylight	
	Moist	Waterlogged	Moist	Waterlogged
Month 1				
Epilobium hirsutum	100	100	100	100
Eupatorium cannabinum	100	100	100	94
Mentha aquatica	100	100	94	94
Myosotis scorpioides	100	94	100	100
Month 2				
Epilobium hirsutum	100	100	83	100
Eupatorium cannabinum	92	92	100	64
Mentha aquatica	100	100	92	92
Myosotis scorpioides	100	92	92	100
Month 3				
Epilobium hirsutum	83	67	83	83
Eupatorium cannabinum	50	67	67	75
Mentha aquatica	100	67	100	67
Myosotis scorpioides	67	33	100	83

Table 2: G-values for survival (each month separately) for four different species (S) grown under two light conditions (L) at two water tables (W). Degrees of freedom are listed in parentheses; * P<0.05, ** P<0.01, ***P<0.001; - interactions which could not be calculated with log-linear analysis.

	L (1)	W (1)	S (3)	L*S(3)	W*S(3)	W*L(1)	W*L*S(3)
Month 1	1.22	11.61***	3.29	-	-	-	-
Month 2	12.80***	1.03	11.35**	7.43	15.20**	-	-
Month 3	2.97	6.60*	7.48	3.29	3.25	0.56	-

Table 3: F-values of Model III ANOVA of total dry weight (DWtot), leaf area ratio (LAR), leaf weight ratio (LWR), specific leaf area (SLA), stem weight ratio (SWR) and root weight ratio (RWR) for four different species (S) under influence of water level (W) and light (L) at three different harvests (H). Results of DWtot are based on ln-transformed data. Data of LAR, LWR, SLA, SWR and RWR were arcsinsqrt-transformed prior to analysis. Df= degrees of freedom; * P<0.05; ** P<0.01; *** P<0.001.

Factor	Df	DWtot	LAR	LWR	SLA	SWR	RWR
H	2	388.9***	134.3***	61.3***	47.4***	15.5***	26.3***
H*W	2	9.5***	1.7	3.9*	2.1	1.1	5.0*
H*L	2	62.0***	1.8	6.2**	5.2**	12.6***	1.7
H*W*L	2	4.8*	0.6	2.0	2.7	3.5*	0.5
H*C(within W*L) (= Error 3) (MS)	40	0.4	0.0	0.0	0.0	0.1	0.1
S	3	39.4***	0.5	0.2	0.1	60.7***	36.3***
S*W	3	7.3***	0.8	2.4	3.0*	7.2***	5.7**
S*L	3	15.0***	1.7	2.5	5.4**	18.2***	2.8*
S*W*L	3	1.5	0.0	1.1	0.6	3.2*	2.4
S*C(within W*L) (= Error 2) (MS)	60	0.4	0.1	0.1	0.0	0.0	0.0
W	1	113.4***	20.2***	25.5***	15.5***	19.5***	2.2
L	1	368.7***	230.7***	128.9***	161.3***	116.2***	62.0***
W*L	1	13.8**	0.5	4.8*	5.1*	0.3	0.7
C(within W*L) (= Error 1) (MS)	20	0.7	0.0	0.1	0.0	0.1	0.0
S*H	6	11.6***	4.2	4.0**	4.0**	18.4***	5.5***
S*H*W	6	7.1***	1.4	2.6*	1.5	3.0*	3.6**
S*H*L	6	1.8	1.6	1.3	0.3	6.5***	0.4
S*H*W*L	6	1.6	1.7	1.0	2.2	4.7***	1.3
S*H*C(within W*L) =residual(MS)	80	0.3	0.0	0.1	0.1	0.0	0.1

Biomass production

The interaction between water level and light significantly influenced biomass production (Table 3). Reed marsh species did not differ from the response of tall forb species since the interaction between species, water level and light treatment was not significant (Table 3). The biomass production of all species was reduced when shaded. Except *Epilobium hirsutum* all species had lower biomass when waterlogged. All species had their lowest biomass production when both shaded and waterlogged (Fig. 1a).

Both the significant species*light and species*water level interactions were not caused by a difference between the reed marsh species on the one hand and tall forb species on the other hand. Contrast analysis of the species*light interaction (results not shown) revealed that *Eupatorium cannabinum* was significantly different from the other three species. This effect of shade on *Eupatorium cannabinum* was probably an artefact, caused by the relatively low biomass production of unshaded, waterlogged *Eupatorium cannabinum* (Fig. 1a).

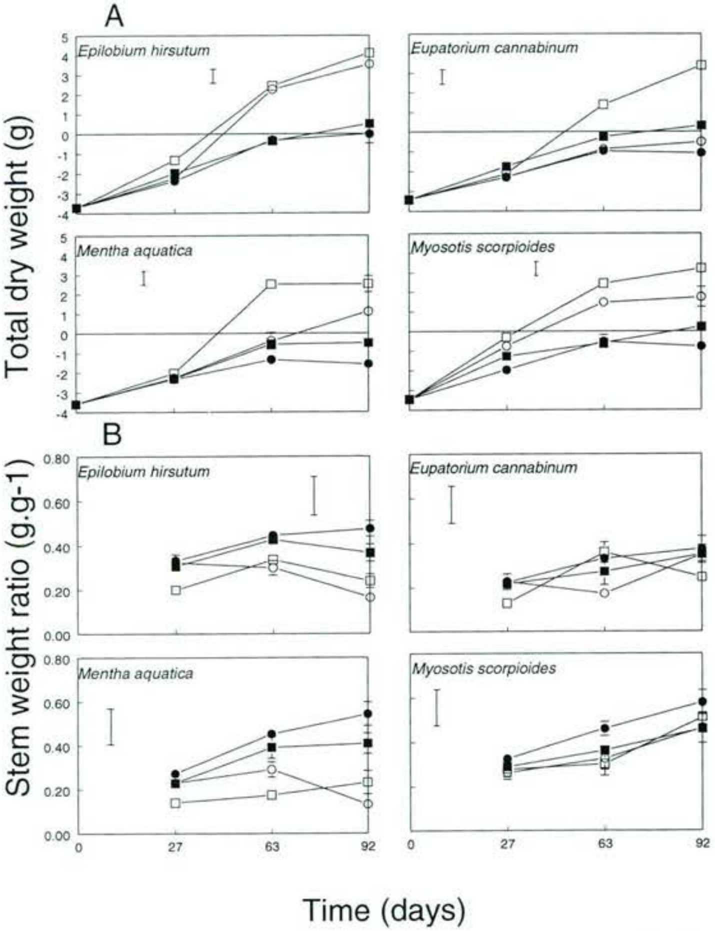

Figure 1: Changes in total dry weight (A; ln-transformed values) and stem weight ratios (B) of *Epilobium hirsutum*, *Eupatorium cannabinum*, *Mentha aquatica* and *Myosotis scorpioides* grown for 3 months under daylight-moist (□), daylight-waterlogged (○), shaded-moist (■) and shaded-waterlogged (●) conditions (mean ± SE; n= 2-6). If error bars are not visible they fall within the range of the symbol. Vertical bars represent least significant range ($P<0.05$).

Waterlogging alone did not reduce biomass production of *Epilobium hirsutum*. Both *Mentha aquatica* and *Myosotis scorpioides* were reduced by waterlogging but not as strongly as *Eupatorium cannabinum*. Contrast analysis of the interaction between species and water level revealed that *Epilobium hirsutum and Myosotis scorpioides* were similar, *Mentha aquatica* was significantly different from *Epilobium hirsutum* and *Eupatorium cannabinum* was significantly different from all other studied species.

Stem weight ratio

The stem weight ratio (SWR) was significantly influenced by the interaction between species, harvest, water level and light (Table 3). All possible interactions between species, water level and light were probably caused by rhizome formation by *Epilobium hirsutum* and stolon formation of *Mentha aquatica,* which played an important role in biomass allocation in the daylight. In the daylight SWR of these species were less than in the shade and this difference was especially visible from 63 days onwards when the first rhizomes and stolons had been formed. *Eupatorium cannabinum* and *Myosotis scorpioides* had very little difference in SWR between plants grown in shade and daylight (Fig. 1b). When ANOVA was performed on SWR data with biomass of rhizomes and stolons included in SWR, no interactions were found between species and water level or species and light (results not shown).

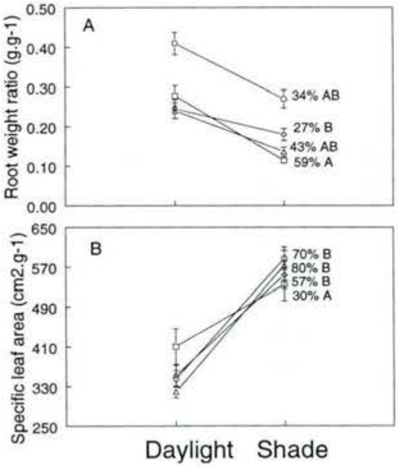

Figure 2: Difference in root weight ratio (a) and specific leaf area (b) between plants grown in daylight and shade for *Epilobium hirsutum* (△), *Eupatorium cannabinum* (○), *Mentha aquatica* (◇) and *Myosotis scorpioides* (□). Points for each species and light level represent means averaged across water levels and harvests. Significant differences (based upon contrast analysis of species*light interaction, experiment-wise error rate $P<0.05$) between species are indicated by different letters. Percentage reduction in RWR and percentage increase in SLA under shade is indicated at the right of each species line.

Root weight ratio

No significant effects of the interaction between species, light and water level on RWR (root weight ratio) was found although interactions between species and light and species and water level significantly influenced the RWR (Table 3).

All species invested less dry weight in roots when shaded. The magnitude of this reduction differed between species, but there was no distinction between reed marsh species and tall forbs. Shaded *Myosotis scorpioides* had the strongest reduction of RWR. This reduction was significantly higher than of *Mentha aquatica*, the species with the smallest reduction of RWR in shade (Fig. 2a).

Leaf indices

LAR is the product of LWR (leaf weight ratio) and SLA. LAR, LWR and SLA were significantly increased by shading (Table 3, Fig. 2b and 3). When shaded the LAR of all species was increased by enhanced allocation of biomass to leaves, thereby realising a higher LWR in the shade than in daylight, and by formation of thinner leaves, leading to increased SLA of shaded plants (Table 3, Fig. 2b+3b). LAR and LWR were not significantly affected by species or interactions between species and light, but species differed in the degree in which they increased their SLA (Table 3). Contrast analysis of the interaction between species and light showed that the SLA of *Myosotis scorpioides* was significantly less increased than the SLA of the other three species, which did not differ among each other (Fig.2b).

Field distribution

Presence of *Epilobium hirsutum* and *Eupatorium cannabinum* in the *P. australis* dominated zone was too low to allow statistical testing of relationships with performance of *P. australis*. The distribution of both weak competitive species was differently related to *P. australis* performance (Fig. 4). As indicated by the significant relationship of *Mentha aquatica* with mean stem weight of *P. australis*, *Mentha aquatica* was restricted to sites where performance of *P. australis* was strongly decreased. *Myosotis scorpioides* however occurred over a wider range of *P. australis* performance and its presence was not significantly related to performance of the dominant (Fig. 4).

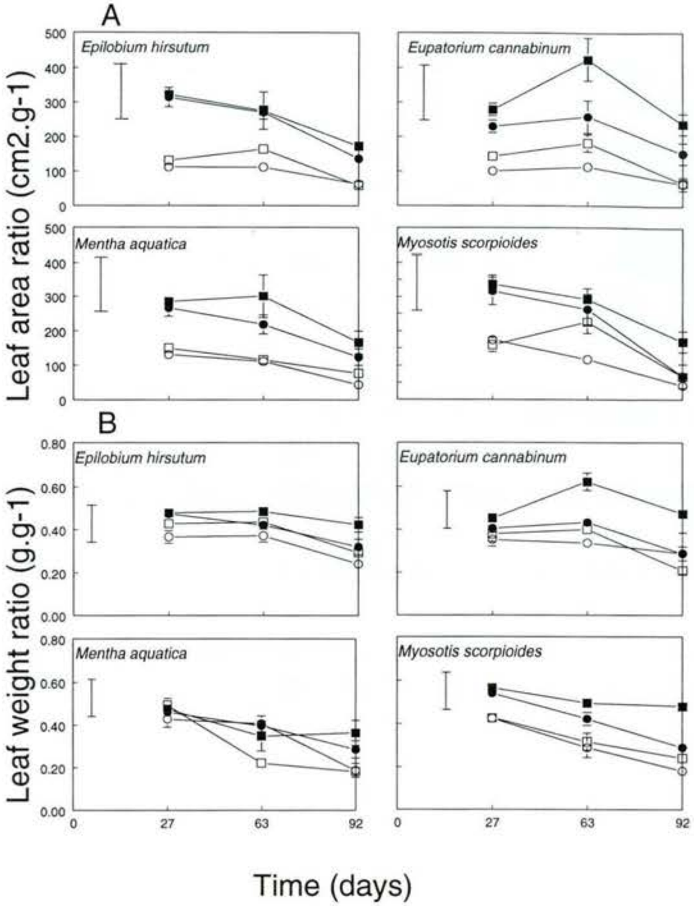

Figure 3: Changes in leaf area ratio (A) and leaf weight ratio (B) mean ± SE; n= 2-6) of *Epilobium hirsutum*, *Eupatorium cannabinum*, *Mentha aquatica* and *Myosotis scorpioides* grown for 3 months under daylight-moist, daylight-waterlogged, shaded-moist and shaded-waterlogged conditions (mean ± SE; n= 2-6). Meaning of symbols according to legend in Figure 2. Error bars which are not visible fall within the range of the symbol. Vertical bars represent least significant range (P<0.05).

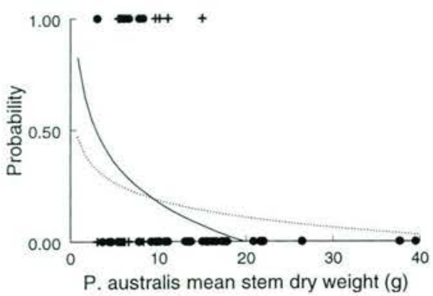

Figure 4: Fitted logistic regression curves of *M. aquatica* (●; solid line) and *M. scorpioides* (+; dashed line) versus *P. australis* mean stem dry weight. *M. aquatica* significantly decreased with increased stem weight of *P. australis* ($P<0.001$; 8 ocurrences in 45 samples), occurrence of *M. scorpioides* was not significantly related to *P. australis* stem weight (8 occurrences in 45 samples).

Discussion

Interactions between light and water level significantly affected biomass production: biomass of plants grown in both shaded and waterlogged conditions was generally lower than of plants grown only waterlogged or shaded. Interactions between shade, waterlogging and species were however insignificant (Table 3) and the hypothesis that the weak competitors *Mentha aquatica* and *Myosotis scorpioides* are better able to tolerate combined effects of shade and waterlogging than the strong competitors *Eupatorium cannabinum* and *Epilobium hirsutum* must therefore be rejected. Significant differences among species were found in responses to separate effects of shade and waterlogging, but this significance was not based on a difference of the small marsh species *Mentha aquatica* and *Myosotis scorpioides* from the tall *Eupatorium cannabinum* and *Epilobium hirsutum*.

When shaded, biomass production of *Eupatorium cannabinum* appeared to be less reduced than of the other three species, but this was caused by the strong reduction in waterlogged daylight conditions. Reduction in biomass production of *Epilobium hirsutum* by shade did not differ from both reed marsh species. However, reduced growth in shade is common for species of both open and closed habitats (Grime 1966, Smith 1982, Corré 1983*b*, Dale and Causton 1992*c*) and may be of adaptive significance because it reduces respiration (Grime 1966, Corré 1983*b*). A reduced respiration can be achieved by increased allocation to stems at the expense of leaves and roots (Corré 1983*b*). In this study there were no indications that the four species differed in their allocation to stems and leaves, but allocation to roots revealed some clear differences between species. In the shade all species decreased their RWR, but *Myosotis scorpioides* did so to a much greater extent than the other three species. When shaded *Myosotis scorpioides* is therefore more economic in its carbohydrate utilization because root tissue has high respiratory and maintenance costs (Corré 1983*b*, Lambers *et al* 1983, Amthor 1984).

All species maintained biomass production by increasing the size of their photosynthetic system under shaded conditions (expressed by LAR) which is in accordance with observations from other studies (Lambers and Poorter 1992, Dale and Causton 1992*a*). It indicates that none of the studied species is a real 'shade' species since these will mainly respond to changed light conditions at the chloroplast level, whereas 'sun' species or species from habitats intermediate to 'sun' and 'shade' habitats will show adaptations at the leaf level (Murchie and Horton 1997). When shaded all species increased LAR by forming larger, thinner leaves, i.e. via an increase of SLA, instead of increasing the leaf weight ratio. *Eupatorium cannabinum* and *Myosotis scorpioides* realised the higher LAR by an increase of both LWR and SLA, although increase in SLA of *Myosotis scorpioides* was more moderate than for other species. A high SLA enables plants to achieve high growth rates but decreases resistance to fungal attack or possibilities for persistence under adverse conditions (Grime 1966, Lambers and Poorter 1992).

From the moderate increase in SLA and the high reduction in RWR it can be concluded that *Myosotis scorpioides* is better able to survive long term shading than the other three species. In addition, *Myosotis scorpioides* was the only species which produced inflorescences when shaded, whereas all species did so when grown in daylight (results not shown).

Field distribution of the four studied species confirms that *Myosotis scorpioides* can endure higher degrees of canopy shade than the other three species. *Epilobium hirsutum* and *Eupatorium cannabinum* are also not subject to canopy shade under field conditions because their leaves are always positioned in the top of the canopy and never occur in the understorey. *Myosotis scorpioides* was found under a wider range of *P. australis* performance than *Mentha aquatica* (Figure 4), which indicates that it can endure higher degrees of shading than *Mentha aquatica*. This finding however does not indicate that *Myosotis scorpioides* is better able to tolerate combined effects of canopy shade and waterlogging under field conditions. In our study area the *P. australis* dominated vegetation has its lower limit around the mean high water level (Coops *et al* 1996*b*, Van de Rijt *et al* 1996), which implies that water levels will regularly drop below soil surface during the growing season. Hence, with regard to the separate effects of shade our experimental study has measured responses, which are ecologically relevant.

Biomass allocation patterns offer little information about the tolerance to waterlogging and will therefore not be discussed. Although waterlogging did not affect biomass production of the tall forb *Epilobium hirsutum*, the other tall forb *Eupatorium cannabinum* was more affected by waterlogging than the small species *Mentha aquatica* and *Myosotis scorpioides*. So except for *Epilobium hirsutum* the experimental responses to waterlogging were in accordance with distribution patterns in the field where *Eupatorium cannabinum* inhabits higher elevations of the flooding gradient than *Mentha aquatica* and *Myosotis scorpioides* (Brock *et al* 1987, Gryseels 1989, Fojt and Harding 1995).

Our approach, i.e the use of morphological traits, plant size in particular, to distinguish weak and strong competitive species, has been justified in a number of studies (Gaudet and Keddy 1988, Boutin and Keddy 1993, Hills *et al* 1994, Grime *et al* 1997). Except *Mentha aquatica* with regard to shade and *Epilobium hirsutum* with regard to water level, our results confirm that weak competitors are more tolerant than strong competitors to a single environmental factor. The distinction between weak and strong competitors on the basis of plant size was no longer valid when two adverse environmental conditions were operating simultaneously. In order to succesfully predict the species composition of habitats where several adverse conditions interact a distinction of species based upon life-history characteristics may be more successful. Menges and Waller (1983) for example found a relatively high proportion of spring-active perennials and spring ephemerals in the most frequently flooded parts of the floodplain forest. Species inhabiting vigorous *P. australis* stands are also either spring-ephemerals (Blom *et al* 1996) or plants with a different phenology from the dominant (Zonneveld 1960, Haraguchi 1993).

7. IMPACT OF WATER LEVEL ON VEGETATIVE REPRODUCTION CONTRIBUTES TO ZONATION OF PLANT SPECIES AT SHALLOW FLOODED SITES

Abstract

Along land-water gradients tall forbs are usually restricted to places above the mean water level, whereas reed marsh interstitials inhabit the shallow flooded zone. We experimentally investigated whether the effect of water level on vegetative reproduction is the cause of this zonation. *Epilobium hirsutum* was used as a representative of tall forbs and *Mentha aquatica* represented reed marsh interstitials. In an outdoor experiment under drained, waterlogged and flooded conditions growth, biomass allocation and vegetative reproduction were measured.

When flooded, stem elongation of *M. aquatica* was enhanced, whereas stem length of *E. hirsutum* was decreased. These results corresponded well with their position on the shoreline. Biomass production however did not reflect the zonation since *M. aquatica* was more decreased in biomass production by waterlogging than *E. hirsutum*. When waterlogged and flooded *E. hirsutum* invested a high proportion of biomass in adventitious roots. *M. aquatica* also formed adventitious roots but maintained an equal proportion of below ground roots at all water levels and this species seemed therefore better able to supply its below ground roots with oxygen. Waterlogging and flooding had a strong, negative impact on vegetative reproduction of *E. hirsutum*, whereas *M. aquatica* was not affected. Most rhizomes of *E. hirsutum* emerged from aboveground parts of the stem base and were oriented in an upward direction when waterlogged and submerged. At these water levels total dry weight allocated to rhizomes and mean rhizome weights were significantly reduced.

The results are discussed in relation to the impact of oxygen-deficiency in roots and rhizomes on hormonal balance and carbohydrate use. It was concluded that adaptations to soil flooding as displayed by *E. hirsutum*, although successful in the short term, are inadequate for long-term maintenance in flooded soils.

Introduction

Lower limits of emergent macrophytes at water depth gradients are often determined by the influence of water level on vegetative reproduction (Grace and Wetzel 1982, Grace 1989, Coops *et al* 1996*b*). In deep water vegetative reproduction may be limited if a higher allocation of biomass to stems and leaves is required to avoid flooding of the photosynthetic apparatus (Grace 1989, Clevering 1998*a*). At higher parts of the shoreline, where only roots and stem base may become flooded, the amount of biomass available for vegetative reproduction will be less limited. Here, zonation of species is often related to the resistance of plants against adverse soil conditions due to waterlogging (Justin and Armstrong 1987) or to stem elongation upon short-term flooding (Ridge 1987, Blom and Voesenek 1996).

Shallow flooded and dry parts of many West-European eutrophic shorelines are characterised by a zonation of reed marshes and tall forb stands. Reed marshes are dominated by *Phragmites australis* Cav. Trin ex Steudel, which is accompanied by interstitial marsh species such as *Mentha aquatica* L. Above the mean high water table the vegetation is dominated by tall forbs of which *Epilobium hirsutum* L. is an important representative (Brock *et al* 1987, Gryseels 1989, Ter Heerdt and Drost 1994, Fojt and Harding 1995, Van de Rijt *et al* 1996).

The zonation of tall forb and reed marsh vegetation is only partly related to different waterlogging tolerances of constituent species or different abilities for stem-elongation. Under experimental conditions waterlogged *E. hirsutum* produces an equal amount of biomass as when drained (Etherington 1984), whereas biomass production of *M. aquatica* was inhibited by waterlogging (chapter 6). According to qualitative observations by Ridge (1987) both species are able to enhance stem elongation upon flooding. Hence, there is a discrepancy between the ecological distribution of the tall forb *E. hirsutum* and the reed marsh interstitial *M. aquatica* and the performance under experimental conditions such as have been studied thus far. It has not been examined to which extent the zonation of tall forbs and interstitial reed marsh species may, just as in deep water, be caused by the impact of water level on vegetative reproduction.

Although *E. hirsutum* and *M. aquatica* represent different clonal growth forms both species rely on vegetative reproduction for expansion and persistence. *E. hirsutum* is a pseudoannual (cf. Suzuki and Hutchings 1997) forming rhizomes at below ground parts of the shoot, which become disconnected once the main shoot decays at the end of the growing season. At the apex of each rhizome a winter rosette is formed which produces one new shoot in the next spring (Shamsi and Whitehead 1973). *M. aquatica* forms long, horizontal runners at above ground (and sometimes below ground) parts of the stem. Connections between main shoot and runners are also lost at the end of the growing season. However, contrary to *E. hirsutum*, each runner of *M. aquatica* may produce several daughter shoots. These daughter ramets usually remain underdeveloped throughout the year in which the stolon was formed (personal observations).

In the present study the impact of water level on the growth and reproduction of *E. hirsutum* and *M. aquatica* was investigated. We hypothesised that the species occurring at the drier zone of the shoreline, i.e. *E. hirsutum*, will be less able to allocate biomass to rhizomes when waterlogged and flooded than a marsh species such as *M. aquatica*, naturally occurring at waterlogged and shallow flooded sites.

Material and methods

Plant material

For the sake of simplicity we will hereafter refer to the stolons of *M. aquatica* as rhizomes also. At 25 April 1996, rhizomes of *E. hirsutum* and *M. aquatica* were collected from a shoreline of the River Nieuwe Merwede (51°45'N, 4°45'E). We dug out winter rosettes of *E. hirsutum* which consisted of roots, a rhizome and the apical rosette. From each rhizome of *M. aquatica* we separated one node from which both roots and a shoot with leaf-buds emerged. At each side of the node a 3.5 cm piece of rhizome was left attached. We maintained a minimum distance of 1 m between each collected rhizome of *M. aquatica* and *E. hirsutum* and used only one ramet from each rhizome of *M. aquatica* to incorporate some degree of genetic diversity.

The vegetative material was planted in moist sand and stored at 5°C with a day/ night regime of 11 h/13 h until further use.

Experimental procedure

At the start of the experiment, 8 May 1996, we selected 48 medium-sized ramets of each species and measured fresh weight of each ramet. A subset of ramets was weighed before and after drying for at least 48 h at 70°C to determine dry/fresh weight ratios. That same day each ramet was planted in a separate plastic container (diameter at bottom 55 cm and at top 65 cm, height 55 cm) filled with 10 L of a 1:1 (v/v) mixture of sand and river clay and 7.5 g Osmocote slow-release fertiliser (7.5% NH_4-N, 7.5% NO_3-N, 7.2% P_2O_5 and 12% K_2O). Containers were placed outdoors in the experimental garden of the Netherlands Institute of Ecology (Heteren). At random positions within each of eight blocks we placed two replicates of each combination of species and water level (48 containers per species, 96 containers in total). For one week, ramets were grown in moist soil, after which three water level treatments were imposed: drainage, waterlogging and flooding. Holes were made in the bottom of the containers of drained treatments, at 1 cm above the soil surface in case of waterlogging and at five cm above the top of the shoot in case of flooding. Water level treatments were maintained manually by adding ground water and water in the containers was replenished at least twice a week. In order to restrict algal blooms Daphnids were added to the containers with flooded plants and filamentous algae were regularly removed manually from both waterlogged and flooded treatments. Redox measurements at 5 cm depth in drained and waterlogged containers (one reading per container) at 22 August 1996 indicated that flooding of the soil had caused a considerable decrease in soil-redox potential (drained 303 ± 8 mV; waterlogged: -97 ± 29 mV; both means ± SE, n=8).

Plant measurements

At the same day as treatments were imposed the shoot base of each ramet was tagged. At the first day and three, six, nine and twenty days thereafter shoot length was measured as the distance between shoot base and top.

After ten weeks, eight plants of each combination of species and water level were harvested. In the next eight weeks, stems of some plants had been broken by wind whereas others had suffered from insect attacks. These plants were excluded at the final harvest (week eighteen) so that only five replicates could be used for further analysis. At harvest shoots were cut off at ground level and separated into leaves, stems (including main stem, branches and petioles), inflorescence, adventitious roots and rhizomes. Roots attached to the shoot above soil surface were regarded as adventitious roots. Below ground parts were separated into roots and rhizomes after the soil was carefully washed away. Rhizomes were counted and dry weights of all plant parts were determined after drying at 70°C for at least 48 hours. Proportion of each plant organ to total biomass was calculated and will hereafter be referred to as weight ratio. Mean rhizome weight of each plant was calculated as the ratio between total rhizome weight and number of rhizomes.

We noticed that many rhizomes of waterlogged and flooded *E. hirsutum* were oriented upwards and originated from aboveground parts of the stem base. At the first harvest these phenomena were quantified by measuring the angle between the stem base and the top of the rhizome. We also counted number of rhizomes attached at above- and below ground parts of the main stem.

Data analysis

The influence of water level on total shoot length was tested with ANOVA in SAS procedure GLM with the time of measurement as a within plant repeated factor. The GLM-option Polynomial was used to compensate for differences in time intervals between length measurements (SAS Institute Inc. 1989). Influences of water level and harvest on total dry weight and weight ratios were tested with ANOVA according to a randomised block design. Prior to analysis values of total dry weight were ln-transformed and weight ratios were arcsinsqrt transformed. Total dry weights were analysed using initial dry weights as co-variable, both were ln-transformed.

The influence of water level and harvest on biomass invested in inflorescence and rhizomes and on mean rhizome weight and number of rhizomes was examined by analysis of covariance with vegetative dry weight (= leaves + stems + roots + adventitious roots) as the covariable. Direct effects of water level and harvest could thus be distinguished from effects of plant size. Linear regression analysis was used to test for relationships with plant size (Samson and Werk 1986, Hartnett 1990, De Kroon and Schieving 1991). Ln-transformed data were used for analysis of covariance and linear regressions because these better met statistical assumptions. Since ln-transformed data may influence the value and significance of the intercept (Samson and Werk 1986) significance of slope was used to detect size-dependent reproduction. Due to unequal sample sizes, caused by a different number of replicates between harvest one and two, all F-values were calculated with type III Sums of Squares (Sokal and Rohlf 1995). Means were compared by least significant difference.

Results

Shoot lengths of both species were significantly influenced by interaction between time and water level (Table 1). Water level did not affect the shoot length of *E. hirsutum* during the first nine days and flooded plants did not elongate faster than drained and waterlogged plants. After twenty days however, drained and waterlogged plants were significantly taller than flooded plants (Fig. 1). Contrary to *E. hirsutum*, *M. aquatica* elongated faster when flooded and after six days, when most shoots had protruded the water layer, they were significantly taller than those of drained plants (Fig. 1). Waterlogged plants also elongated faster than drained plants but differences were significant after twenty days only (Fig. 1).

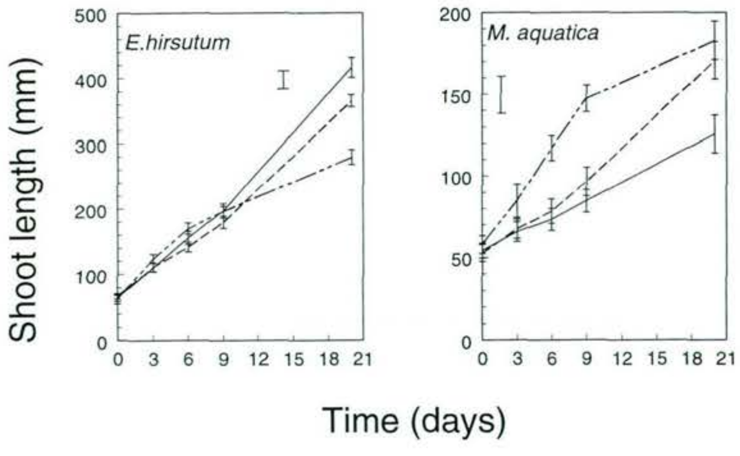

Figure 1: Shoot length of plants (means ± SE, n=16) during the first twenty days of the experiment for plants grown under drained (——), waterlogged (------) and flooded (—· ·—) conditions. Vertical bar represents least significant difference (P<0.05). Note different scaling on y-axis.

Table 1: F-values of total shoot length under influence of block, water level (W) and time (T). Lengths were measured at the same plant at the onset of the experiment and three, six, nine and twenty days thereafter and time was therefore analysed as a within plant repeated measurement. Degrees of freedom are listed in parentheses. * P<0.05; **P<0.01; *** P<0.001.

	Block (7)	W (2)	T (4)	W*T (8)
E. hirsutum	0.86	2.27	290.93^{***}	11.32^{***}
M. aquatica	3.74^{*}	13.78^{***}	659.22^{***}	25.26^{***}

Table 2: F-values of total dry weight, biomass allocation parameters under influence of block, water level (W) and harvest (H). Abbreviations/symbols: Df= degrees of freedom; DWtot= total dry weight; RWR= Root (below ground) weight ratio; ARWR= Adventitious root weight ratio; SWR= Stem weight ratio; LWR= leaf weight ratio; * P<0.05; **P<0.01; *** P<0.001. DWtot was analysed with initial dry weight as co-variable. ARWR was only determined for plants grown under waterlogged and submerged water levels (corresponding df-values are given in parentheses).

	Df	DWtot	RWR	ARWR	SWR	LWR
E. hirsutum						
Block	7 (7)	0.50	0.75	0.62	1.25	0.25
(W)	2 (1)	30.54^{***}	25.8^{***}	71.08^{***}	2.62	2.70
(H)	1 (1)	273.88^{***}	4.18	0.03	51.36^{***}	72.89^{***}
W*H	2 (1)	25.56^{***}	4.47	0.54	0.54	2.01
error (MS)	25 (15)	0.02	0.00	0.00	0.00	0.00
M. aquatica						
Block	7 (7)	0.50	0.44	1.08	1.66	2.34
W	2 (1)	29.54^{***}	0.29	5.33^{*}	1.42	15.88^{***}
H	1 (1)	243.18^{***}	42.22^{***}	1.00	47.82^{***}	59.14^{***}
W*H	2 (1)	6.82^{*}	0.07	3.84	0.9	5.69^{**}
error (MS)	25 (15)	0.05	0.00	0.01	0.00	0.0

Total dry weights of both species were significantly affected by water level (Table 2). After ten weeks total dry weights were reduced by waterlogging and more reduced by flooding. At the final harvest, differences between drained and waterlogged *E. hirsutum* were not significant and total dry weights were significantly reduced by flooding only (Fig. 2). *M. aquatica* also had the highest dry weight when drained, but dry weight was already significantly less when waterlogged (Fig. 2).

The allocation of biomass to below ground roots and adventitious roots of *E. hirsutum* was significantly affected by water level (Table 2). Flooded plants invested a significantly higher proportion of their biomass in adventitious roots than waterlogged plants, whereas less biomass was allocated to below ground roots (Fig. 2). Allocation to stems and leaves was not affected by water level (Table 2) but at the second harvest a significantly lower proportion of biomass was present in stems and leaves whereas a higher proportion was allocated to inflorescences and rhizomes (Fig. 2).

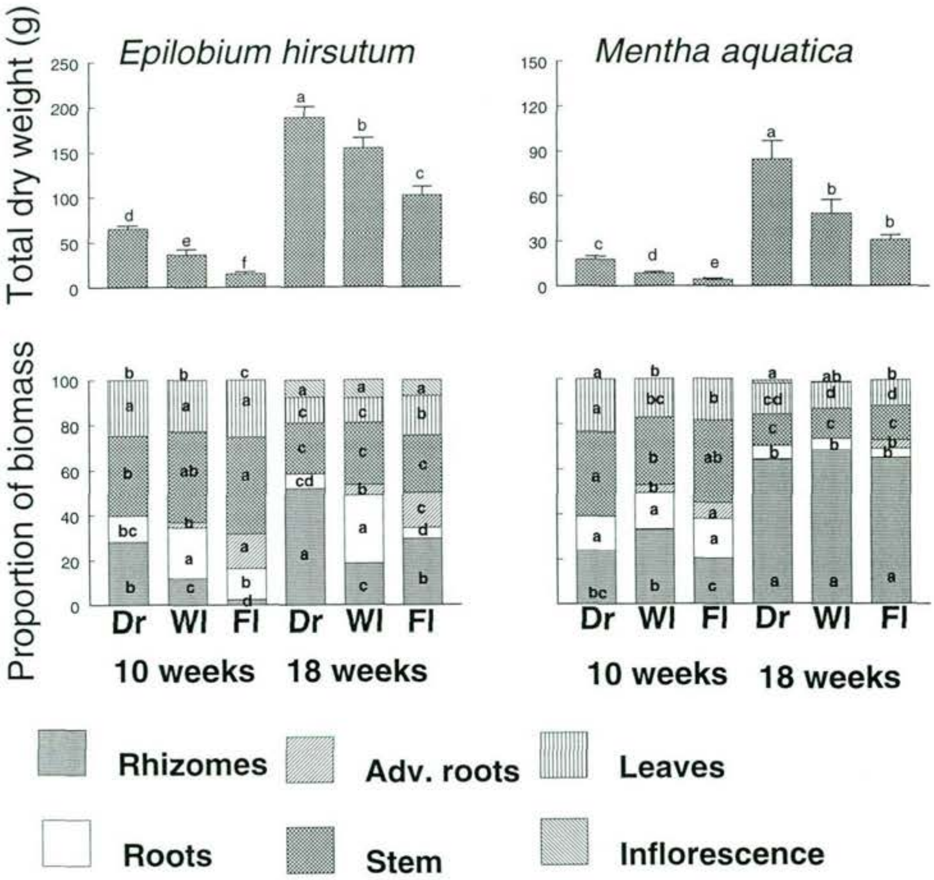

Figure 2: Total dry weights (mean ± SE, n=5-8; upper half) and proportions of weight allocated to different plant organs (mean, n=5-8; lower half) of plants grown for 10 and 18 weeks under drained (Dr), waterlogged (Wl) and flooded (Fl) conditions. For each species and plant organ significant differences between means are indicated with different letters. Letters for inflorescence dry weight were placed on top of the bar if inflorescence weight ratio was too low to visualise. Note different scaling on y-axis of total dry weights.

M. aquatica also formed adventitious roots when flooded, but contrary to *E. hirsutum*, it maintained an equal proportion of below ground roots at all water levels (Table 2, Fig. 2). Allocation to stems was not affected by water level, but the proportion of biomass invested into leaves was significantly affected by the interaction between water level and harvest time (Table 2). This interaction was due to the relatively low leaf biomass of waterlogged and flooded plants (Fig. 2).

Table 3: Morphology of rhizome-stem base connections of *E. hirsutum* after ten weeks of growth in drained, waterlogged or flooded conditions. Proportion of rhizomes for each of three categories of angle between top of rhizomes and stem base and proportion of rhizomes attached to above- and below ground parts of the stem base (both measures: %, means±SE, n=8) .

	Angle			Position at stem base	
	90°	90°-45°	0°-45°	Above ground	Below ground
Drained	88±5	8±3	4±3	16±4	84±4
Waterlogged	4±4	60±17	36±17	78±11	22±11
Flooded	17±7	67±12	15±12	96±4	4±4

Table 4: F-values of ANCOVA for inflorescence dry weight, total rhizome dry weight, mean rhizome dry weight and number of rhizomes under influence of block, water level (W) and harvest (H) with vegetative dry weight as the covariable. Degrees of freedom are listed in parentheses. * P<0.05; **P<0.01; *** P<0.001, n.d.= not determined (insufficient data to allow statistical testing).

	Block (7)	W (2)	H (1)	W*H (2)
E. hirsutum				
- Inflorescence	0.39	0.68	0.90	0.15
- Total rhizome dry weight	0.43	111.77^{***}	153.54^{***}	101.7^{***}
- Mean rhizome dry weight	0.20	65.82^{***}	146.65^{***}	55.32^{***}
- Number of rhizomes	0.20	52.33^{***}	0.63	6.94^{*}
M. aquatica				
- Inflorescence	n.d.	n.d.	n.d.	n.d.
- Total rhizome dry weight	0.95	1.83	17.00^{**}	1.01
- Mean rhizome dry weight	3.17	1.89	5.67	0.35
- Number of rhizomes	1.06	0.25	1.07	0.89

The impact of water level on vegetative reproduction strongly differed between both species. Waterlogging and flooding did not alter the orientation and position of *M. aquatica* rhizomes (results not shown). However, when waterlogged and flooded the majority of *E. hirsutum* rhizomes had a sharp angle with the stem base, indicating that its rhizomes were growing upwards. Rhizomes also emerged from aboveground parts of the stem instead of from below ground parts at higher water levels (Table 3).

The interaction between water level and harvest significantly affected *E. hirsutum*'s allocation to rhizomes (Table 4). After eighteen weeks, the relationship between dry weight of rhizomes and vegetative dry weight was significant for drained plants only. At all water levels more biomass was allocated to rhizomes at the end of the growing season (Table 5; harvest two). However at both harvests mean and total rhizome weights of waterlogged and flooded *E. hirsutum* did not depend on their size (Table 5) and were always lower than those of drained plants (Table 6).

The number of *E. hirsutum* was significantly affected by interactions between water level and harvest (Table 4). Whereas the number of rhizomes remained constant for drained and waterlogged plants, numbers were size-dependent when flooded (Table 5). Rhizome number of flooded plants at the end of the growing season did not differ from waterlogged plants (Table 6).

Table 5: Significance of linear relationships between vegetative dry weight of plants (independent variable) and sexual (inflorescence dry weight) and vegetative reproductive characteristics of *E. hirsutum* (all based upon ln-transformed data). For each harvest linear relations were tested for plants grown under drained (Dr), waterlogged (Wl) and flooded (Fl) conditions. For sexual reproduction data of water levels and harvests were pooled. *P<0.05, **P<0.01, ***P<0.001.

	Harvest 1 (10 weeks)			Harvest 2 (18 weeks)		
	Dr	Wl	Fl	Dr	Wl	Fl
y= total inflorescence dry weight						
- Slope		0.896^{**}				
- R^2		0.80				
y= total rhizome dry weight						
- Slope	0.49	0.553	0.385	0.98^{**}	0.27	0.41
- R^2	0.12	0.30	0.15	0.97	0.07	0.16
y= mean rhizome dry weight						
- Slope	2.29	0.68	0.274	-0.03	0.56	0.057
- R^2	0.08	0.36	0.07	$0.9.10^{-3}$	0.31	0.32
y= number of rhizomes						
- Slope	0.10	0.01	0.1	0.01	-0.01	0.26^{*}
- R^2	0.15	0.002	0.31	0.003	0.09	0.84

Table 6: Total rhizome dry weight (Tot Rh DW), mean rhizome dry weight (M Rh DW) and number of rhizomes (N rhizomes; mean±SE, n=8-harvest 1, n=5-harvest 2) of plants grown under drained (Dr), waterlogged (Wl) or flooded (Fl) conditions. Degrees of freedom are listed in parentheses. * P<0.05; **P<0.01; *** P<0.001. Significant differences between means within the same row are indicated with different letters. Significance test for Tot RH DW based upon ln-transformed data.

	Harvest 1 (10 weeks)			Harvest 2 (18 weeks)		
	Dr	Wl	Fl	Dr	Wl	Fl
E. hirsutum						
- Tot Rh DW (g)	18.5±1.3 c	2.9±0.5 d	0.3±0.06 e	95.9±5.8 a	25.7±2.3 bc	27.7±2.3 b
- M Rh DW (g)	1.12±0.1 c	0.5±0.09d	0.06±0.01 e	7.70±1.36 a	3.16±0.27 b	2.36±0.05 b
- N Rhizomes	16.8±0.6 a	7.3±0.9 c	5.2±0.4 c	16.2±1.3 a	8.2±0.6 bc	11.8±1.1 b
M. aquatica						
- Tot Rh DW (g)	5.4±1.1 c	2.9±0.5 c	1.0±0.3 d	55.0±9.1 a	33.9±7.5 ab	20.5±2.2 b
- M Rh DW (g)	0.60±0.14 c	0.40±0.07 c	0.15±0.05 d	4.98±1.19 a	4.65±1.41 ab	1.91±0.22 b
- N Rhizomes	9.8±1.0 abc	8.1±1.0 bc	7.1±0.9 c	12.4±1.8 a	7.8±0.8 bc	10.8±0.7 ab

Table 7: Significance of linear relationships between vegetative dry weight of plants (independent variable) and sexual (inflorescence dry weight) and vegetative reproductive characteristics of *M. aquatica* (all based upon ln-transformed data). For each harvest plants grown under different water levels were pooled and for mean rhizome dry weight and number of rhizomes all data were pooled. *P<0.05, **P<0.01, ***P<0.001.

	Harvest 1 (10 weeks)		Harvest 2 (18 weeks)
y= total rhizome dry weight			
- Slope	0.815***		0.942***
- R^2	0.64		0.87
y= mean rhizome dry weight			
- Slope		1.59	
- R^2		0.68***	
y= number of rhizomes			
- Slope			0.572***
- R^2			0.30

Contrary to *E. hirsutum*, water level did not affect the vegetative reproduction of *M. aquatica*. Differences in total rhizome dry weight (Table 6) were positively related to harvest time and plant size (Table 7). As indicated by the steeper slope, more biomass was allocated to rhizomes at the end of the growing season (Table 7). Mean rhizome weight and rhizome number were not influenced by harvest time and depended on plant size only (Table 7). Regardless of harvest time, larger plants produced more rhizomes but this relationship explained only 30 % of the variance (Table 7). The equal number of rhizomes of drained and flooded plants (Table 6), which were strongly different in size (Fig. 2), should therefore be attributed to other factors than plant size. The amount of biomass of *E. hirsutum* allocated to inflorescences, was not influenced by water level or harvest time (Table 4) but only by plant size (Table 5).

Discussion

Stem elongation response upon submergence of both *E. hirsutum* and *M. aquatica* species was in accordance with their position on flooding gradients. Compared to *E. hirsutum*, which is most abundant above the mean water table, the 'shallow water' species *M. aquatica* has a higher change of getting fully submerged after sudden rises of the water level. *M. aquatica*'s ability for enhanced shoot elongation will increase its survival of flooding (Ridge 1987, Voesenek *et al* 1993*b*, Setter and Laureles 1996). The inability of *E. hirsutum* to respond to submergence by enhanced shoot elongation may partly explain why it inhabits higher elevations than *M. aquatica*. These results with adult plants deviate from a previous study with seedlings in which *E. hirsutum* enhanced shoot lengths when submerged, whereas *M. aquatica* did not (chapter 4). This different behaviour of seedlings and adults confirms findings of Van der Sman *et al.* (1993) that the ability to respond to flooding by stem-elongation may vary with developmental stages of plants.

High water levels did not lead to enhanced biomass allocation to stems and leaves. This response to flooding of the photosynthetic apparatus is common for emergent macrophytes inhabiting deep water parts of the shoreline (Grace 1989, Coops *et al* 1996*b*, Clevering 1998*b*). In the present study, shoots were above the water table during the main part of the study period. Higher water levels even decreased leaf weight ratio of *M. aquatica*. This was not necessarily a direct effect of water level and may reflect differences in ontogeny (Coleman *et al* 1994), caused by growth retarding effects of high water levels.

The root apparatus was most affected by high water tables. When flooded, *E. hirsutum*'s below ground roots were to a large extent replaced by adventitious roots, which are essential for its growth in flooded soils (Etherington 1984). Adventitious roots are formed when the functioning of below ground roots is constrained by anaerobic conditions caused by soil flooding and inadequate internal ventilation of the plant (Koncalova 1990, Visser *et al* 1996*a*, Clevering 1998*a*). *M. aquatica* is able to supply its below ground roots with oxygen (Pedersen and Sand-

Jensen 1997). This may explain the maintenance of an equal proportion of below ground roots at all water tables as well as the relatively small proportion of adventitious roots.

The formation of inflorescence by *E. hirsutum* was not affected by water level. Although many authors have reported changes of investment in sexual structures under influence of environmental conditions (Loehle 1987, Hartnett 1990, Schmid and Weiner 1993) we found no evidence that water level has a similar impact. Vegetative reproduction of *E. hirsutum* was however strongly affected by water level.

The changes in rhizome position and orientation of waterlogged and flooded *E. hirsutum* may be best explained by the changed hormonal status of soil flooded plants. Gibberelins are responsible for stimulation of lateral bud growth and induction of erect instead of horizontal rhizome orientation (Hutchings and Mogie 1990). The responsiveness to gibberelins is enhanced by increased levels of auxins and ethylene (Blom *et al* 1994). Compared to *M. aquatica*, *E. hirsutum* had formed a high proportion of adventitious roots, which indicates that large amounts of auxin and ethylene had accumulated in the stem base (Visser *et al* 1995, 1996*b*). This accumulation will have increased the sensitivity to gibberelins, resulting in vertically oriented shoots originating from the stem base. *M. aquatica* will have accumulated less auxin and ethylene at the stem base due to its adequate internal ventilation system (Pedersen and Sand-Jensen 1997) which allows unlimited transport of auxin to the roots (Visser *et al* 1995) and release of ethylene to the atmosphere.

For drained *E. hirsutum* the amount of biomass allocated to rhizomes was size-dependent, which is a common phenomenon for pseudo-annual species (Worley and Harder 1996, Verburg *et al* 1996, Dong *et al* 1997). However, for waterlogged and flooded plants the biomass of rhizomes was no longer related to the size of the whole plant. This relationship also seemed to be lost for *M. aquatica* growing at the highest water level. Anoxic conditions in the rhizomes may have resulted in high carbohydrate use in the rhizomes, thus decreasing dry weights of these organs (Braendle and Crawford 1987).

In a wide range of clonal growth forms weight of perennating organs determines the size of shoots in the next year (Mook and Van der Toorn 1982, Jerling 1988, Cain 1990, Clevering *et al* 1995). Especially in tall and dense vegetation large perennating organs are essential for survival of offspring (Jerling 1988). The present study has shown that *E. hirsutum* can dominate only above the mean high water table because the resources allocated to perennating organs strongly decrease as soon as soils are waterlogged. *M. aquatica* experienced adverse conditions for rhizome formation at higher water levels, which explains why it can persist at shallow flooded sites. The different impact of water level on vegetative reproduction must be considered as more important for the zonation of reed marshes and tall forb stands than differences in stem-elongation responses upon flooding. Wetlands dominated by *P. australis* are characterised by small fluctuations of the water table (Breen *et al* 1988, Blom *et al* 1996) and adult plants of species inhabiting this habitat, such as tall forbs and reed marsh interstitials, will therefore only occasionally be submerged.

Our results confirm earlier studies in which zonation of species on flooding gradients correlated with the impact of water level on vegetative reproduction (Grace and Wetzel 1982, Grace 1989, Coops *et al* 1996*b*, Clevering 1998*b*). In these studies, decreased rhizome production was explained by increased allocation to stems and leaves, aimed at avoiding shoot submergence. We have shown that even waterlogging may impose serious constraints on vegetative reproduction and that adaptations to soil flooding as displayed by *E. hirsutum*, although successful in the short term, are inadequate for long term maintenance at soil-flooded sites.

8. COMPETITION AT A COMMUNITY BOUNDARY BETWEEN PLANTS WITH DIFFERENT CLONAL GROWTH STRATEGIES

Abstract

Phragmites australis, a clonal plant with highly integrated ramets, is often intermingled with the pseudoannual *Epilobium hirsutum* at dry ends of flooding gradients. We investigated whether this co-occurrence represents a transitional phase in the replacement of *P. australis* by *E. hirsutum* or whether both species coexist. Coexistence may occur if competition between both species is symmetric or if the effects of intraspecific competition exceed those of interspecific competition. Therefore we investigated both modes of competition in a two-year field experiment by measuring shoot numbers, total aboveground biomass, shoot size and shoot lengths in plots where *P. australis* or *E. hirsutum* was removed and in plots where both species remained.

Neither shoot lengths nor shoot weight of *P. australis* was affected by intra- or interspecific competition, but shoot numbers and total aboveground biomass significantly increased when *E. hirsutum* was removed. This indicates that *E. hirsutum* inhibited *P. australis* by limiting the formation of new shoots. *E. hirsutum* was not negatively affected by *P. australis.* It performed even better in mixed plots than in monocultures. Shoot weight of *E. hirsutum* was negatively related to density of its own shoots and shoots were significantly smaller in monoculture. In contrast to what has been reported for other pseudoannuals shoot mortality of *E. hirsutum* was density dependent.

Our results have shown that *P. australis* is negatively influenced by competition of *E. hirsutum*, but that the latter is not affected by *P. australis*. Despite this asymmetric competition coexistence is possible because *E. hirsutum* is more inhibited by intraspecific than interspecific competition.

Introduction

Along flooding gradients zones of plant communities occur which are usually dominated by single clonal plant species (Spence 1982). Although boundaries between different communities are usually sharp (Bertness 1991*a,b*, Studer-Ehrensberger *et al* 1993) dominant species of the adjacent communities may also co-occur in a transitional zone (Buttery and Lambert 1965, Yamasaki 1990).

This co-occurrence may be a temporary phenomenon if one species is gradually replacing the other, but it may also be based upon stable coexistence. Coexistence is possible if both species compete with each other symmetrically, i.e. their equivalence in competitive abilities prevents each others' exclusion (Goldberg and Barton 1992, De Kroon *et al* 1992, Hara 1993, Herben *et al* 1997). This may be the case if competition is for nutrients (Schwinning and Weiner 1998) but under eutrophic conditions, competition is mainly for light. For annuals this results in asymmetric competition between shoots, leading to increased shoot-size variability during the growing season, resulting in density-dependent mortality and consequently a decreased shoot size variability (Weiner 1990, Schwinning and Weiner 1998). Shoot competition is different for most clonal plants because the growth rate of larger plants is decreased by storage of resources. Shoot self-thinning is therefore often avoided, although shoot-size variability may increase strongly during the growing season (Suzuki and Hutchings 1997). In higly integrated clonal plants asymmetric competition between adjacent shoots may be prevented by effective control of shoot density (De Kroon and Kwant 1991) or resource translocation between shoots (Hara *et al* 1993).

In addition to resource competition, clonal plants may also have to compete for the limited amount of belowground space that can be occupied by rhizomes (Herben and Hara 1997). Due to this lateral component of clonal growth shoot interactions are often not the only factor determining the outcome of competitive interactions. Once the available space is filled even asymmetric shoot interactions may lead to symmetric competition between clones (De Kroon *et al* 1992) or species (Herben *et al* 1997). Coexistence may also be the result if intraspecific competition is stronger than interspecific competition. This may be the case if species are limited by different resources (Tilman 1982). For clonal plants however, the degree of intra- and interspecific competition may also depend on the clonal growth form. Clonal plants with a high degree of ramet integration, such as *Phragmites australis* (Cav.) Trin. ex Steudel, often exhibit low degrees of intraspecific competition (Hara *et al* 1993, Ekstam 1995). A high degree of integration allows clonal plants to occupy patches for a long period, but these species may be disadvantaged if they grow in species mixtures instead of monospecific stands. On the other hand, species with a low degree of physiological integration between ramets often perform better in mixed stands (Schmid and Harper 1985, Schmid and Bazzaz 1987).

In eutrophic, ungrazed shorelines, *P. australis* dominates the lower, frequently flooded parts, but decreases in vigour at dry ends of flooding gradients. Under these suboptimal conditions *P. australis* intermingles with tall competitive herbs such as the pseudoannual *Epilobium hirsutum* L. (Shamsi and Whitehead 1973), which may dominate the parts above the mean high water table (De Boois 1982, Gryseels 1989, Yamasaki 1990, Fojt and Harding 1995, Van de Rijt *et al* 1996). *E. hirsutum* is a pseudoannual (sensu Suzuki and Hutchings 1997); each shoot emerges from a single piece of rhizome and because there are no physical connections with other shoots during growth (Shamsi and Whitehead 1973).

We investigated whether there is coexistence between *P. australis* and *E. hirsutum* by measuring the effects of intra- and interspecific competition in a two-year removal experiment. Special attention was paid to intraspecific competition of *E. hirsutum*, since intraspecific shoot competition of *P. australis* has already been studied in detail by Van der Toorn and Mook (1982), Hara *et al* (1993) and Ekstam (1995).

Material and Methods

Experimental procedure

Competition is usually investigated along experimentally created density gradients (Goldberg *et al* 1995). However, creating such gradients by planting ramets at different densities would result in poor representations of natural *P. australis* stands, which have a deeply buried, extensive rhizome network. An artificial increase of density by planting in existing stands was considered too much of a disturbance (Aarssen and Epp 1990, Goldberg *et al* 1995). Therefore, we restricted our study to natural densities.

On a shoreline of the River Nieuwe Merwede (51°45'N, 4°45'N) in a mixed stand of *P. australis* and *E. hirsutum* thirty plots (2x2m each) were laid out in October 1994. In the plots shoots of both species were counted to determine initial density. Elevation of plots was measured with leveling equipment and related to data from neighbouring gauge stations to determine the daily minimum and maximum water level of the study site.

In April 1995, three treatments were installed: removal of *P. australis*, removal of *E. hirsutum* and removal of neither species (mixture). Ten replicates of each treatment were obtained by randomly assigning each plot to a treatment. Due to severe damage in the winter of 1995/1996 only six replicates of each treatment could be used for the second season.

P. australis and *E. hirsutum* were removed by clipping off their shoots at ground level. This was repeated every fortnight throughout two successive growing seasons. Seedlings and vegetative shoots of other species were removed from all plots. At the beginning of each growing season underground rhizome connections with plants outside the plots were severed with a spade up to a depth of 1 m.

Plant measurements

In 1995 (May-September) and 1996 (May-August) numbers of shoots per species were counted in each plot every month. In August 1995 and 1996, aboveground biomass was measured non-destructively by determining relationships between dry weight of shoots and length and diameter of shoots. These relationships were determined using regression analysis on shoots harvested at the same time from the adjacent, undisturbed vegetation. The regression equations with the best fit for each species and year are listed in Table 1. Plots were subdivided into four equal-sized parts and within each subplot maximally ten shoots were randomly selected for measurement.

In 1996, shoot-lengths of *P. australis* and *E. hirsutum* and diameters of *E. hirsutum* (at ground level) were measured each month (May-August). Stem diameters of *P. australis* were not measured because they do not change during the growing season (Hara *et al* 1993).

Table 1: Regression equations used in the removal experiment for estimating aboveground dry weight. Ln-transformed data of shoot-dry weight (LnDW) were fitted on ln-transformed and untransformed data of stem-diameter or stem-length. Equations with best fit are given. For all slopes and intercepts P<0.001.

	Equation	R2adj	dF
P. australis			
1995	LnDW=-7.39+1.88*ln(length)	0.69	40
1996	LnDW=-9.32+2.30*ln(length)	0.88	30
E. hirsutum			
1995	LnDW=-1.96+2.29*ln(stem diameter)	0.95	39
1996	LnDW=-2.26+2.52*ln(stem diameter)	0.87	25

Data analysis

The total aboveground biomass per species and plot was calculated by summation of estimated dry weights of measured shoots and subsequently multiplying this sum with the ratio between the total number of shoots and the number of measured shoots. Effect of treatment on total aboveground dry weight was analyzed with ANOVA repeated measures using time (year) as a within-plot repeated factor. Because analysis was carried out using data of both years only the six replicates could be used that remained undisturbed until the end of the experiment.

For each year the effect of removal on number of shoots per species was tested with ANOVA, using time (month) as a within-plot repeated factor and using the shoot number in the last month of the previous year (October 1994 and September 1995) as covariable. In each plot with *E. hirsutum*, mortality of this species was determined by calculating the difference in shoot number between subsequent counts. Because *E. hirsutum* can only form new shoots at the end of the growing season (Shamsi and Whitehead 1973) this difference is not affected by shoot natality. Density dependent mortality of *E. hirsutum* was tested by investigating the significance of the Pearson correlation between mortality and the initial density at the start of the period over which mortality was calculated. In removal plots number of conspecific shoots represented the initial density whereas in mixed plots the number of *P. australis* shoots was used as initial density. Prior to correlation analysis normality of distribution was tested with Kolmogorov-Smirnov statistic (Sokal and Rohlf 1995). Treatment effects on shoot numbers and aboveground dry weight (as repeated factors) were also tested with elevation of each plot as a covariable to detect possible influences of elevation. However, these tests did not reveal a significant impact of differences in elevation between plots and are therefore not presented in this paper.

Effect of treatment and month on length and diameter of shoots was tested with ANOVA using plot as a factor nested within treatment. Variability in shoot lengths and diameters were expressed as the coefficient of variation (CV). Effects of treatments on CV in 1996 were tested with ANOVA using the factor time (month) as a within-plot repeated factor.

Effects of intra- and interspecific competition on dry weight of individual shoots were analysed with regression analysis using shoot density in May as predictor variable. For tests of intraspecific competition, density of plots with removal of other species was used. Density of shoots of the other species in mixed plots was used for testing interspecific competition.

Initial density (i.e. in May) was used because this is a better indicator of potential interactions than final density, which may also reflect effects of competition, such as shoot mortality (Goldberg *et al* 1995). Since a maximum of forty shoots was measured per plot there was more than one y-value for each x-value (initial density). Therefore the residual sums of squares was partitioned into a 'pure error' term (sums of squares around the density means) and a 'lack of fit' term (the residual sums of squares minus the 'pure error' sums of squares). The significance of the regression was tested by the F-ratio of the regression mean sums of squares and the lack of fit mean sums of squares. Significance of the lack of fit was tested by the F-ratio of the lack of fit mean sums of squares and the 'pure error' sums of squares. If the lack of fit was significant, the within-plot variation was considered to be too large to accept a significant relationship between shoot dry weight and initial shoot density, even if the regression slope was significantly different from zero (Sokal and Rohlf 1995).

Results

Water levels in the river during the experimental period differed between years. Throughout 1995 and especially during spring, water levels were higher than in 1996 (Fig. 1).

The total aboveground dry weight of *P. australis* was significantly affected by the interaction between year and treatment (Table 2). In 1995 its aboveground biomass was not affected by removal of *E. hirsutum*, whereas it was significantly greater following *E. hirsutum* removal in 1996 (Fig. 2). Aboveground biomass of *E. hirsutum* was significantly different between years due to higher productivity in 1996 than in 1995. However, removal of *P. australis* did not influence aboveground biomass of *E. hirsutum* (Fig. 2, Table 2).

Table 2: ANOVA of total aboveground biomass in August 1995 and 1996 of *P. australis* and *E. hirsutum* with or without removal (treatment) of the other species. The yearly determined biomass (Year) was analysed as a within plot repeated factor. * P<0.05; ** P<0.01; *** P<0.001.

	df	F-value	
Factor		*P. australis*	*E. hirsutum*
Treatment	1/2	2.52	0.18
Year	1/6	9.93^{**}	28.73^{***}
Treatment*Year	1/6	5.83^{*}	0.07

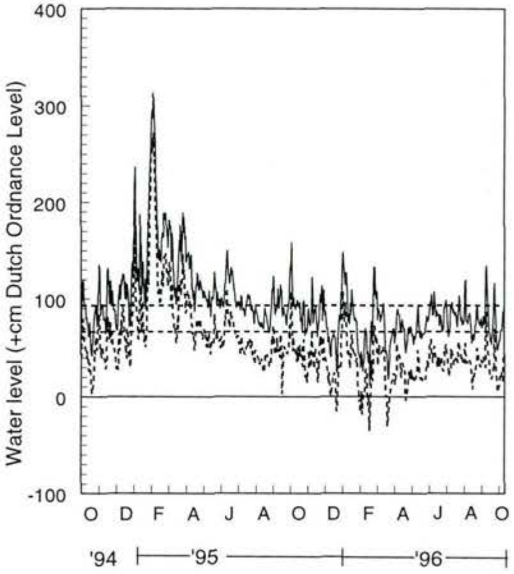

Figure 1: Minimum (broken line) and maximum (solid line) water level (cm above Dutch Ordnance Level) in the main river channel during the experimental period. Elevations of highest and lowest plot are indicated with horizontal broken lines.

During both years shoot numbers of *P. australis* were significantly influenced by interactions between treatment and month (Table 3). For both treatments the number of *P. australis* shoots increased until August, but most shoots were formed when *E. hirsutum* was removed (Fig. 3). In 1995, shoot-numbers of *E. hirsutum* were not significantly affected by removal of *P. australis* (Table 3). For both treatments however, there was a small but significant decrease of shoot numbers during the growing season (Fig. 3, significance of 'month' in Table 3). In 1996, shoot numbers seemed to increase until June, after which they decreased (Fig. 3). Although shoot-numbers appeared to be higher in monoculture plots (Fig. 3), there was no significant effect of treatment. However, interaction between treatment and month was close to significance (Table 3). Correlation between mortality and density of conspecific shoots was close to significance in 1995 (i.e. between July and August) and significant in 1996. Mortality of *E. hirsutum* was never significantly related to density of *P. australis* (Table 4).

Table 3: ANOVA of number of shoots during the growing season of 1995 and 1996 of *P. australis* and *E. hirsutum* with or without removal of the other species. The factor month was treated as a within-plot repeated factor. Number of shoots at the end of the previous year (October 1994 and September 1995) was used as covariable. Df- degrees of freedom (effect, error). * P<0.05; ** P<0.01; *** P<0.001.

Factor	Df	F-value *P. australis*	 *E. hirsutum*
1995			
Treatment	1/17	6.01^{*}	2.50
Month	4/72	21.70^{***}	5.91^{***}
Treatment * Month	4/72	7.57^{***}	0.05
1996			
Treatment	1/10	14.58^{**}	2.46
Month	3/33	31.30^{***}	9.28^{***}
Treatment * Month	3/33	3.62^{*}	2.68 (P=0.06)

Table 4: Pearson correlation coefficients (r) for mortality of *E. hirsutum* and density at the start of the period over which mortality was calculated. Effects of initial density of conspecifics (in plots with removal of *P. australis*) and initial density of *P. australis* (in mixed plots) were tested. n= number of observations; * P<0.05.

Period	N	r (vs. *E. hirsutum*)	r (vs. *P. australis*)
1995			
May-June	10	no mortality	no mortality
June-July	10	0.04	0.14
July-August	10	0.60 (P=0.06)	0.49
August-September	10	0.15	0.34
1996			
May-June	6	no mortality	no mortality
June-July	6	0.85^{*}	0.65
July-August	6	0.76 (P=0.07)	0.26

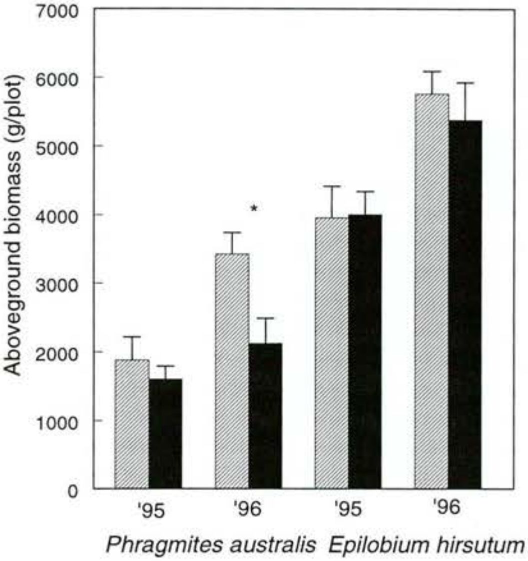

Figure 2: Total aboveground dry weight of *P. australis* and *E. hirsutum* in August 1995 and 1996 (Means ± SE, 1995: n=10; 1996:n=6) inplots with (closed bars) or without the other species (hatched bars). Asterisk indicates significant difference between treatments within one year (P<0.05).

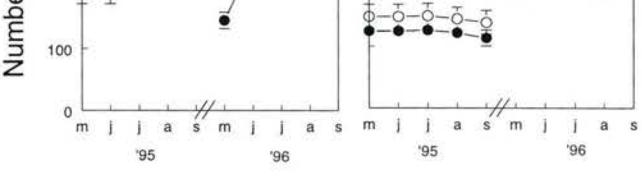

Figure 3: Number of stems at each month of growing season in 1995 and 1996 in plots with other species (closed symbols) or without other species (open symbols) (Means ± SE; 1995: n=10; 1996: n=6).

Mean shoot length of *P. australis* was significantly different between plots, but not affected by removal of *E. hirsutum*. *P. australis* mean shoot length increased from June onwards for both treatments (Table 5, Fig. 4). In monocultures the delay in shoot length increment until June was accompanied by an increased shoot-size variability (CV stem length in Fig. 4), explaining the significant interaction between treatment and month. At the end of the growing season variability did not differ from variability in May (Fig. 4).

Shoot lengths of *E. hirsutum* were significantly influenced by removal of *P. australis* (Table 5). *E. hirsutum* shoots remained tallest in mixed plots throughout the growing season (Fig. 4). Variability in shoot length changed significantly during the growing season (Fig. 4) and was much lower in August than in May. Shoot diameters also increased significantly during the growing season but these were not affected by removal of *P. australis* (Table 5, Fig. 4). Variability in shoot diameters also changed significantly, but no general decrease or increase was observed in the course of the growing season. Removal of *P. australis* did not influence the variability in length and diameter of *E. hirsutum* shoots (Table 6, Fig. 4).

Table 5: F-values of stem length of *P. australis* as well as stem length and stem diameter of *E. hirsutum* in plots with or without removal (Treatment) of the other species. In each plot a maximum of forty shoots was measured once a month from May 1996 until August 1996. Plot was analysed as a factor that was nested within treatment. Df= degrees of freedom (effect, error), * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

		P. australis	*E. hirsutum*	
	df	length	length	Diameter
Plot (P)	10/1751	14.81^{***}	25.91^{***}	15.87^{***}
Treatment (T)	1/10	0.23	19.10^{**}	3.35
Month (M)	3/30	359.77^{***}	1135.22^{***}	51.42^{***}
P*M	30/1751	1.65^{*}	1.27	1.19
T*M	3/30	0.59	10.61^{***}	0.73

Table 6: F-values of coefficient of variation (CV) of shoot length of *P. australis* and CV length and -diameter of *E. hirsutum* shoots in plots with or without removal (Treatment) of the other species. The monthly (M) determined size variability was treated as a within-plot repeated factor. df= degrees of freedom (effect/error), * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

		P. australis	*E. hirsutum*	
	df	CV length	CV length	CV diameter
Treatment (T)	1/10	0.27	0.26	1.08
Month (M)	3/30	10.25^{***}	45.92^{***}	3.60^{*}
T*M	3/30	5.32^{**}	1.10	0.15

In the removal plots the individual biomass of *P. australis* stems was either uncorrelated (1995), or positively correlated with the initial stem density (in 1996 and across both years; Table 7). For each year, a significantly negative relationship between biomass of *E. hirsutum* shoots and initial density was found in monocultures. Shoot biomasses of both species were not related to the shoot density of the other species (Table 7).

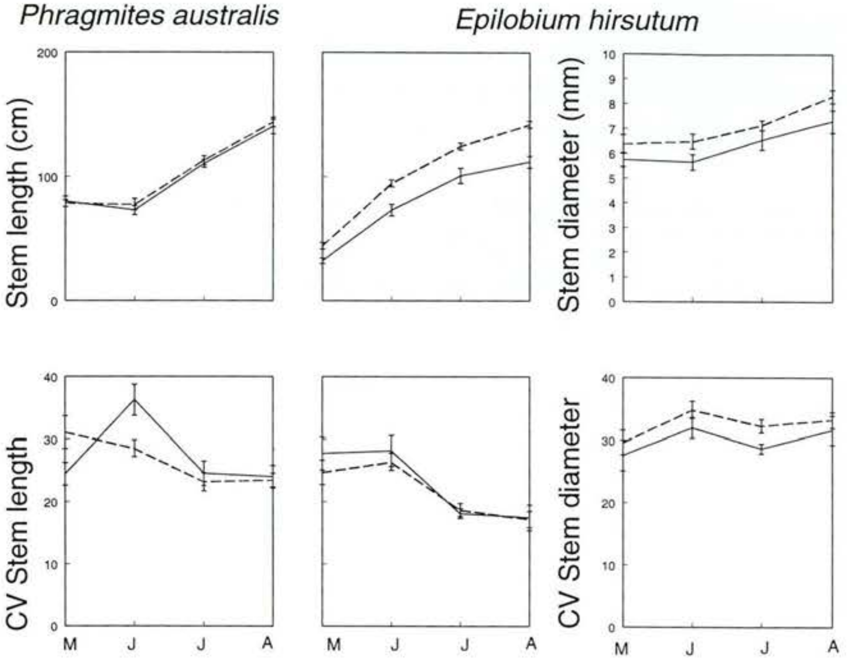

Figure 4: Shoot length and variability (CV) of shoot length of *P. australis* and *E. hirsutum* and mean and variability of stem diameter of *E. hirsutum* (all: means ± SE; n=6) in May, June, July and August 1996 in plots without other species (solid line) and with other species (broken lines).

Table 7: Regression analysis of biomass of individual shoots in August versus initial shoot density in May of the same year or May of the previous year (1995-1996). Regression coefficients are only given if lack of fit was insignificant and regression significant (see 'Materials and Methods' section for further details). Tests of intraspecific competition are based on plots with removal of other species whereas control plots were used for tests of interspecific competition. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

	F-lack of fit	F-regression	Regression coefficient	R^2(adj)
Intraspecific competition				
Phragmites australis				
1995	-	-	-	
1996	0.74	47.70^{**}	$3.31.10^{-3}$	0.12
Epilobium hirsutum				
1995	1.59	5.44^{*}	$-2.15.10^{-3}$	0.02
1996	0.48	65.67^{**}	$-2.44.10^{-3}$	0.11
Interspecific competition				
Phragmites australis				
1995	-	-	-	-
1996	3.69^{*}	0.36	-	-
Epilobium hirsutum				
1995	1.76	1.78		
1996	0.95	0.04	-	-

Discussion

Plant interactions have been assumed to decrease the vigour of *P. australis* at its upper limit along flooding gradients (Buttery and Lambert 1965, Yamasaki 1990). To our knowledge this study provides the first experimental evidence confirming this assumption.

Interspecific competition affected both shoot number and total aboveground dry weight of *P. australis*. Biomasses of individual *P. australis* shoots were however not density dependent and neither shoot lengths nor variability in shoot lengths were affected by removal of *E. hirsutum*. For both treatments shoot-size dynamics of *P. australis* followed a pattern conforming to earlier findings of Hara *et al* (1993).

At the community level, *P. australis* was inhibited by *E. hirsutum* as was shown by the significantly higher number of shoots in monoculture plots for both years and the significantly higher aboveground biomass in the monoculture at the end of 1996. The spring floods in 1995 may have inhibited the increase of aboveground dry weight in monocultures in 1995. Submergence of emerging shoots is known to be deleterious to *P. australis* (Weisner 1988, Ostendorp 1991). An alternative explanation is that standing crop of *P. australis* may be determined by standing crop in the previous year (Van der Toorn and Mook 1982), which causes a time-lag (of one year) before removal of competitors is noticeable in aboveground dry weight.

Inhibition of *P. australis* by *E. hirsutum* was not related to shoot interactions (competition for light) but to the suppression of new shoot formation. This finding indicates that competition between both species is for space, but small-scale investigations are required to confirm this (Silvertown *et al* 1994, Herben *et al* 1997). In addition, the canopy of *E. hirsutum* may suppress the young emerging reed shoots. These are formed later in the growing season in response to damage by the rhizome feeding larvae of the moth *Rhizedra lutosa* (Van der Toorn and Mook 1982) and could have caused the increased shoot size variability in June. Effective suppression of these extra shoots may explain why an increase in shoot size variability did not occur in mixed plots (Fig. 4).

Loss of competitive ability of *P. australis* at higher elevations has been explained by the inability of *P. australis* to form a dense canopy early in the growing season because its shoots have to emerge from deeply buried rhizomes (Buttery and Lambert 1965, Yamasaki 1990, Crawford 1992). This was clearly not the case in the present study. At the start of the growing season shoot lengths of *E. hirsutum* were much less than those of *P. australis* (Fig. 4).

In contrast to *P. australis*, *E. hirsutum* experienced intraspecific competition as shown by the significantly negative relationship between shoot weight and initial density in monoculture plots (Table 7). Intraspecific competition for light usually results in strong increases in shoot size variability after which the variability declines due to density dependent mortality (Weiner 1990, Schwinning and Weiner 1998). Although variability in shoot sizes did not increase, the decreased size-variability coincided with the period of density-dependent mortality (Fig. 4, Table 4). Density dependent shoot mortality has been reported for a number of clonal species with interconnected shoots (De Kroon and Kwant 1991, Hara and Srutek 1995), but never for pseudoannuals (Suzuki and Hutchings 1997). An additional explanation for the decreased shoot-size variability is that the smallest shoots of *E. hirsutum* increased in shoot length without increasing their diameter (Schwinning and Weiner 1998). This would explain why a similar pattern of shoot-size variability was observed in stands with *P. australis*, where no density dependent mortality occurred.

With regard to shoot mortality (Table 4) and shoot weight (Table 7) we found no evidence for suppression of *E. hirsutum* by *P. australis*. *P. australis* seemed to limit the amount of space available for vegetative expansion of *E. hirsutum*, since there was an almost significant increase in shoot number after removal of *P. australis* in 1996. Because of the growth form of *E. hirsutum* (see Shamsi and Whitehead 1973) this increase in shoot numbers did not occur in

1995 because all shoots had been formed in 1994, i.e. before the treatments were installed. There were no significant differences in total aboveground biomass of *E. hirsutum* between plots with and without *P. australis* and biomasses were only different between years. This may have been due to many other factors, such as the high water levels in spring 1995.

Our finding that *P. australis* performed better in monocultures than in species mixtures with regard to standing crop and shoot number confirms studies on other clonal species with highly integrated ramets (Schmid and Harper 1985, Schmid and Bazzaz 1987). *E. hirsutum* performed better in mixed cultures than in monocultures, which indicates that the intra- and interspecific interactions of pseudoannuals are similar to clonal species with a low degree of ramet integration (Schmid and Harper 1985, Schmid and Bazzaz 1987).

The co-occurrence of *P. australis* and *E. hirsutum* in the field can not be explained by symmetric, interspecific competition. Although *P. australis* was inhibited by *E. hirsutum*, no evidence was found for the reverse, so that the competition between these species is asymmetric. Asymmetric competition has also been shown between shrubs and herbs on shorelines (Keddy 1989) and salt marsh perennials (Bertness 1991*a,b*), but in these studies the asymmetric competition led to competitive exclusion and hence to very sharp boundaries between communities. In our system asymmetric competition will still allow coexistence, because *E. hirsutum* is more strongly inhibited by intraspecific competition than by interspecific competition. In addition, the unpredictability of the environment in our study area may also contribute to preventing competitive exclusion. The major environmental factor is water level, which fluctuates strongly due to variable river discharges. In dry years, unfavourable for *P. australis, E. hirsutum* will expand at the expense of *P. australis*, whereas during wet years with high water tables growth of *E. hirsutum* will be inhibited, allowing *P. australis* to expand.

9. GENERAL DISCUSSION

The aim of this study was to elucidate the ecosystem mechanism (*sensu* Van Andel *et al* 1993) underlying the zonation of monospecific reed, species-rich reed and tall forb dominated vegetation. In this chapter, the results of the separate studies will be discussed and combined to a qualitative model, which demonstrates the possible impact of management measures on reed marsh vegetation development. Finally, the possibilities for development and conservation of a species-rich *Phragmites australis* zone will be presented. In addition, possibilities for restoration of former reed marshes, which are now dominated by tall forbs will be discussed.

Species richness of *Phragmites australis* dominated vegetation

Until now, knowledge of factors which adversely affect *Phragmites australis* has mainly been obtained by experimental studies or incidental field observations. The systematically collected field data presented in chapter 2 have shown that adverse effects of soil organic matter (Van der Putten *et al* 1997, Clevering 1998*a*), litter layers (Jordan *et al* 1990, Campbell *et al* 1992) and dry soil conditions (Van der Toorn and Mook 1982) may also play a role in the field. Nevertheless, a relatively small fraction of the variability in *Phragmites australis* stem density and standing crop was explained by these factors. This indicates that in the field, many factors influence the performance of *Phragmites australis* simultaneously. In addition to the factors measured in the field study, performance in the previous year (Mook and Van der Toorn 1982), insect herbivory (Van der Toorn and Mook 1982, Mook and Van der Toorn 1982, 1985, Tscharntke 1989), competition (Buttery and Lambert 1965, Yamasaki 1990) and standing litter (Graneli 1989) may also have played an important role. Decreased performance of *Phragmites australis* seems to be an important prerequisite for increased species richness, since *Phragmites australis* standing crop, together with litter cover, was significantly related to species richness (Chapter 2-fig. 3). In addition to reed performance, exposure and flooding may also determine species richness (Keddy 1984, Nilsson 1987, Nilsson and Grelsson 1990, Pollock *et al* 1998). Chapter 3 provides evidence that flooding influences the species richness, especially at the species-rich reed marsh zone. At the rarely flooded tall forb zone, on the other hand, the number of species was enhanced by canopy removal. Hence, where flooding frequency is low, plant interactions are the main factor determining species richness.

Thus, flooding and exposure determine the potential number of species, whereas the amount of litter and the performance of *Phragmites australis* determine how many species are actually present. The next step in the research project was to asses when the invasion of monospecific reed leads to increased species richness of the *Phragmites australis* dominated vegetation and when it leads to dominance of tall forbs.

Zonation of species-rich reed marshes and tall forbs

Seed bank

The relative abundance of species in the seed bank did not determine the balance between interstitial marsh species and tall forbs (chapter 3). The limited correspondence between composition of seed bank and vegetation is common for non-tidal freshwater wetlands (Keddy and Reznicek 1982, Schneider and Sharitz 1986, Welling *et al* 1988). Provided that seeds are present, the environmental factors determine which species establish succesfully (chapter 3, Stockey and Hunt 1994, Weiher and Keddy 1995).

Germination and seedling establishment

Both marsh interstitials and tall forb species had their maximum emergence in cleared plots at the reed marsh zone. However, most tall forbs were unable to persist for more than one growing season (chapter 3). Therefore, germination requirements were similar, but seedlings of tall forb species were less tolerant to flooding than marsh interstitials. The experimental study in chapter 4 confirmed these results. Both species groups required moist soils for germination and were unable to germinate in waterlogged and flooded soils or in soils drier than field capacity.

Experiments also showed that seedlings of the tall forb species *Cirsium arvense* and *Eupatorium cannabinum* are more susceptible to total inundation than marsh interstitials. Other important tall forb species, *Urtica dioica* and *Rubus caesius*, are also strongly affected by total inundation in their seedling stage (Klimesova 1994, Siebel 1998). I found circumstantial evidence that seedlings of *Epilobium hirsutum* are more susceptible to total submergence than marsh interstitials. Survival of total submergence of *Epilobium hirsutum* strongly depended on the developmental stage of the seedlings and hence on the carbohydrate status of the plants (see chapter 4). In the field experiment (chapter 3) none of the *Epilobium hirsutum* seedlings managed to persist at the frequently flooded reed marsh zone.

Persistence and vegetative reproduction

During later stages of the life-cycle, waterlogging and flooding may also discriminate between tall forbs and reed marsh interstitials. Due to their low stem- and root porosity, *Eupatorium cannabinum* and *C. arvense* will be less able to supply roots and rhizosphere with sufficient oxygen in flooded soils (chapter 4). Another important tall forb species, *Urtica dioica*, is also unable to sustain growth when partially flooded (Siebel 1998) and its growth is strongly decreased at a high ground water table (Srutek 1997).

Epilobium hirsutum seems to be the most flood-tolerant tall forb species since its stem- and root porosity were similar to marsh interstitials *Lycopus europaeus*, *Mentha aquatica* and *Myosotis* *scorpioides* (chapter 4). Nevertheless, the apparently tolerant *Epilobium hirsutum* will have difficulties in dominating waterlogged and flooded parts of the shoreline because here its vegetative reproduction is strongly inhibited (chapter 7). In flooded soils, total dry weight allocated to rhizomes and mean rhizome weight of *Epilobium hirsutum* are strongly reduced. This will lead to smaller ramets the following year (Van der Toorn and Mook 1982, Jerling 1988, Cain 1990, Clevering *et al* 1995) and, especially in tall and dense vegetation, to a decreased chance of survival for offspring (Jerling 1988). *Epilobium hirsutum* will be able to persist in flooded soils but always as a subordinate component of the vegetation (personal observations). Due to the fact that tall forbs lack effective adaptations for growth (*U. dioica*, *Eupatorium cannabinum*, *C. arvense*) or vegetative reproduction (*Epilobium hirsutum*) in flooded soils, tall forb vegetation reaches its lower limits in zones which are waterlogged during summer (Van Donselaar-ten Bokkel Huinink 1961, Coops 1996). In tidal areas, tall forbs have their lower limits at flooding frequencies of 100 times per year, i.e. ± 20 cm above mean heigh water level (Wolff 1988, Van de Rijt *et al* 1996).

Reed marsh interstitials occur below the tall forbs, i.e. from waterlogged zones, to a maximum of 60 cm of water depth (Van Donselaar-ten Bokkel Huinink 1961, Klosowski 1988, Coops 1996). Although experiments have shown that growth of marsh interstitials such as *Mentha aquatica* and *Myosotis scorpioides* is suboptimal in flooded soils (chapter 6) their adequate internal ventilation system (chapter 4) and their ability to sustain vegetative reproduction (chapter 7) will allow them to persist at lower positions than tall forbs.

Replacement of reed by tall forbs

As argued above, hydrological conditions determine whether marsh interstitials or tall forbs can persist after performance of *Phragmites australis* is decreased. Optimal conditions for tall forbs will eventually lead to replacement of reed by tall forb vegetation. Although the clonal growth form of *Epilobium hirsutum* does not allow this species to outcompete *Phragmites australis* completely (chapter 8), tall forb species without strong intraspecific competition, such as *U. dioica*, may be more succesful (Hara and Srutek 1995). Indeed, a number of authors have reported the replacement of *Phragmites australis* by *U. dioica* (De Boois 1982, Gryseels 1989, Van de Rijt *et al* 1996) in reed marshes where hydrological measures have led to lowered water tables.

Species distribution within the reed marsh zone

It was shown above that water level is the main factor determining variation in species composition perpendicular to the shoreline. However, species composition may also vary parallel to the water level, and within the elevational range inhabited by reed marsh interstitials, organic matter and light availability seem to be important factors for species distribution.

Within reed marshes *Phragmites australis* is the major source of soil organic matter (Pieczynska 1993, Boschker *et al* 1995). In chapter 5 it was shown that this type of soil organic matter may lead to variation in species composition because interstitials differ from each other in their ability to resist adverse substrate conditions. Since these adverse substrate conditions were not caused by nutrient limitation, the inhibitory effects of litter-derived organic matter will also play a role in eutrophic waterbodies. Before these effects can be fully understood, it should be clear whether this organic substrate is lethal, i.e. triggering similar responses as on *Phragmites australis* (cf. Armstrong *et al* 1996*a,b,c*) or whether growth in waterlogged organic soils is just suboptimal. Based on present knowledge, I conclude that permanently flooded conditions restrict the opportunities for persistence of many marsh interstitial species in highly organic soils. Regular drawdowns will be necessary because they improve oxygen availability in the rhizosphere and because they accelerate decomposition of organic matter (chapter 5, Clevering 1998*a*).

Light availability for reed marsh interstitials is mainly determined by performance of the dominant *Phragmites australis*. Since marsh interstitials have different abilities to sustain prolonged periods of canopy shade, performance of *Phragmites australis* may also determine the distribution of species within the reed marsh zone (chapter 2). Species such as *Caltha palustris* and *Cardamine amara* for example, are able to avoid severe canopy shading because their phenology is different from *Phragmites australis* (Zonneveld 1960). Others, such as *Myosotis scorpioides,* can persist under relatively shaded conditions by means of morphological adaptations (chapter 6). Marsh interstitials such as *Mentha aquatica* however, are unable to adapt to canopy shade (chapter 6) and these species will be restricted to sites where productivity of *Phragmites australis* is strongly decreased.

Mechanism of zonation

The insight into the ecosystem mechanism underlying the zonation of monospecific reed, species-rich reed and tall forb vegetation as obtained by this study, is summarized in a qualitative model (Fig. 1). As argued in chapters 5 and 6, litter-derived soil organic matter and canopy shade do not discriminate between marsh interstitials and tall forb species. These factors are therefore not incorporated in the model. The model starts from a monospecific stand of *Phragmites australis* and explains the vegetation development that may take place after e.g.

lowering of the water table or cessation of commercial use. The model is also applicable to succession on bare soils after exceptionally high floods or intensive grazing has removed existing vegetation (Van der Valk and Davis 1978). However, on dry parts of bare soils, tall forb vegetation will develop immediately without the interactions between tall forbs and *Phragmites australis* (see for example Ter Heerdt and Drost 1994).

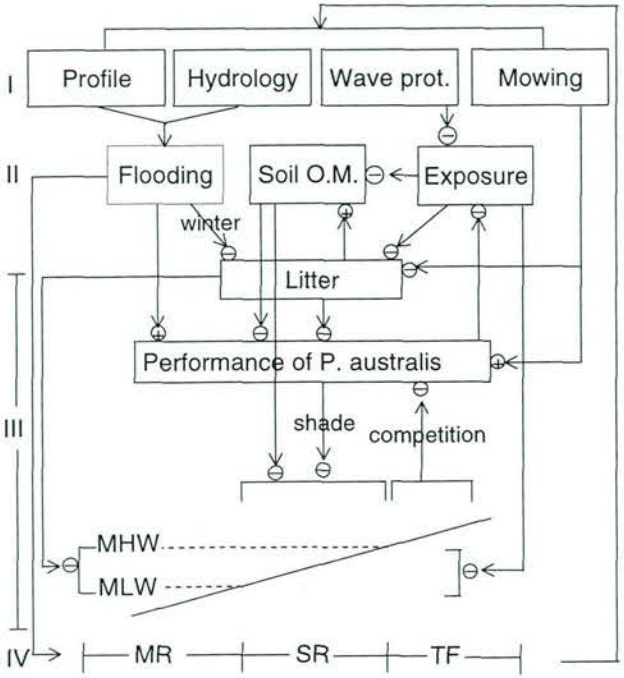

Figure 1: Summarizing model describing relationships between management tools, environmental factors and species interactions on zonation of monospecific reed (MR), species-rich reed (SR) and tall forb vegetation (TF). For detailed explanation of management tools (**I**), environmental factors (**II**) and vegetation processes (**III**) see chapter 1, figure 2. **Performance of Phragmites australis** is best interpreted as aboveground peak standing crop, i.e. in August (chapter 2). Flooding is the only factor determining the zonation of monospecific reed (**MR**), species-rich reed (**SR,** dominant *Phragmites australis* accompanied by interstitial marsh species) and tall forb vegetation (**TF**, vegetation dominated by tall forbs). Other environmental factors either favour (+) or restrict (-) species richness of the elevational zone suitable for a species-rich reed marsh, i.e. between the mean high water level (**MHW**) and the mean low water level (**MLW**). No influence of environmental factors is indicated for the tall forb zone as species richness here is mainly determined by plant interactions.

A species-rich *Phragmites australis* dominated vegetation can only develop at (i) sheltered sites where (ii) growth conditions of *Phragmites australis* are suboptimal and strong litter accumulation is prevented and (iii) water levels fluctuate between a few centimeters below the soil surface and 60 cm above.

The first requirement is a sheltered shoreline since both marsh interstitials and tall forbs are dicotyledonous species and therefore ill-adapted to waves and fast flowing water (Haslam 1978). In well-developed shoreline vegetation, monospecific stands of *Phragmites australis* at the water fringe will provide sheltered conditions because they reduce much of the wave energy (Coops *et al* 1996*a*). If space is limited, wave-breaking constructions before the actual shoreline may be necessary.

Secondly, adverse conditions for *Phragmites australis* will improve light availability for seeds and seedlings of other species. Particularly important for increased species richness are factors which inhibit *Phragmites australis* more than the interstitial species or leave the interstitials unaffected (Palmer 1994). Examples of the latter are herbivory by specialistic insects (Van der Toorn and Mook 1982, Mook and Van der Toorn 1982, Tscharntke 1989), standing litter (Graneli 1989) and, possibly, winter frost (Van der Toorn and Mook 1982). Litter and soil organic matter are factors which affect both *Phragmites australis* and other species and both will inevitably accumulate at sheltered sites provided they are not mown every winter. Small amounts of litter may already be used as a hibernating place for reed-damaging insects (Van der Toorn and Mook 1982) and hence indirectly affect *Phragmites australis*. Large amounts of litter will decrease species richness (chapter 2) by inhibiting germination, seedling establishment (Van der Valk 1986, Cowie *et al* 1992) and adults of small and creeping species (Gryseels 1989, Nilsson and Grelsson 1990). Accumulation of litter-derived organic matter may also increase species richness, because it inhibits *Phragmites australis* (chapter 2). Since it also provides adverse conditions for other marsh species (chapter 5), species richness in organic soils will be limited but will be higher than in monospecific reed. Flooding during winter will remove litter from the *Phragmites australis* dominated zone (Day *et al* 1988) and hence prevent strong formation of litter layers and soil organic matter. If litter is not removed naturally, it is necessary to cut the vegetation and remove the litter every third winter (Gryseels 1989).

The third condition for marsh interstitials is fluctuating water levels because they can only pass all phases of their life cycle in the zone of the shoreline where drained and flooded conditions alternate during the growing season or between successional growing seasons. Drained conditions are necessary for germination (chapter 4) and regular aeration of the soil accelerates decomposition of organic matter and improves growth conditions for plants rooting in the organic substrate (Chapter 5, Clevering 1998*a*). Flooding during the growing season prevents the tall forbs from gaining dominance because their seedlings are less tolerant to total inundation than marsh interstitials (chapter 4) and because flooding hampers their vegetative expansion (chapter 7).

The second and third requirement for species-rich reed marshes are best met by allowing water levels to fluctuate according to a natural pattern. This pattern is mainly determined by precipitation and evaporation and for most waterbodies in Northwestern Europe this implies that levels will be highest in winter and early spring and gradually drop during the growing season. Due to the climatic variability, alternations of floods and drawdowns may occur within a single growing season or between years.

The present mode of water level management is the biggest constraint in development of species-rich reed marshes. In order to meet demands of agriculture and ship-traffic, water levels are often kept at the same level throughout the year or are artificially lowered during winter and increased during summer, but strictly controlled within each season. By preventing water level fluctuations, the zone that is suitable for marsh interstitials disappears. This occurs because the shoreline is either continuously flooded or dry during the growing season, and the absence of winter floods leads to high accumulation of litter. Natural water level fluctuations are essential for marsh interstitials as well as for the development of helophyte stands of *Scirpus* spec., *Typha* spec. and *Phragmites australis* (Clevering 1995*a*, Coops 1996). Restoring or maintaining the natural water level dynamics should therefore be the basis of ecological wetland management.

The profile of the shoreline may be adapted to increase the area that is suitable for the species-rich zone or any desired vegetation type. In general, slopes should be as gentle as possible because such slopes extinct more wave energy; have a larger area where dry and flooded conditions alternate; and, within this area, have a higher variability in flooding frequency allowing more species to coexist (Keddy 1984, Pollock *et al* 1988).

Conservation and restoration of reed marshes

In many European wetlands, water levels have been artificially lowered which has often lead to replacement of reed marshes by tall forb vegetation (De Boois 1982, Gigon and Bocherens 1985, Gryseels 1989, Fojt and Harding 1995). In order to restore these marshes, it has been proposed to raise water levels. At the Rhine-Meuse Delta in the Netherlands, different forms of water level management have been evaluated with a computer model. This model was based upon correlative data, i.e. the present relationships of reed marshes and tall forb stands with flooding frequency as a basis for future vegetation development (Van de Rijt *et al* 1996). My thesis has provided experimental evidence for causal relationships between flooding frequency and zonation of reed marshes and tall forb stands and therefore justifies measures aimed at restoring natural hydrology.

Whether raised water levels are sufficient to turn deteriorated *Phragmites australis* into healthy stands is an important question yet to be answered. Raised water levels will remove tall forbs and thereby release the remaining *Phragmites australis* from competition. As suggested by the performance of *Phragmites australis* measured in the field competition experiment (chapter 8) competitive release from tall forbs does not necessarily lead to improved conditions for *Phragmites australis*. After two years removal of all other species in a transition zone from reed marsh to tall forb vegetation, *Phragmites australis* had obtained a standing crop of 855±192 g m^{-2} (August 1996; means ± SD; n=6). In the same area, i.e. shores along the River Nieuwe Merwede, standing crops of *Phragmites australis* measured in August 1996 in natural monospecific stands at the open water fringes (data collected as part of the field investigation-chapter 2) were still significantly higher, i.e. 1758±931 g m^{-2} (means ± SD; n=15).

Another important effect of increased water levels is the inhibition of further herbivory by the moth *Rhizedra lutosa*, which as a larvae inflicts considerable damage upon the rhizomes (Van der Toorn and Mook 1982). Once water levels are raised, excessive callus formation will take place in the damaged rhizomes. This callus impedes gas transport within the rhizomes (Armstrong *et al* 1996*a*). As a consequence, parts of the rhizome apparatus will die-off and during their decomposition phytotoxins are released which initiate a chain of events eventually resulting in dieback of *Phragmites australis* (Armstrong *et al* 1996*b*). This decline of *Phragmites australis* after reflooding may lead to belts of *Typha angustifolia*, *Glyceria maxima*, *Phalaris arundinacea* or *Carex acutiformis* instead of *Phragmites australis*. In that case, an important objective of nature management, i.e. restoring species-rich *Phragmites australis* stands as an important food source and habitat for wildfowl and other avifauna, will not be achieved. Hence, the reversibility of deteriorated *Phragmites australis* is a research topic that deserves top priority.

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Summary

Restoring and conserving natural values of reed marshes has become an important objective of water management in the last two decades. Much effort has therefore been put into the construction of wave protection devices and creation of more gentle slopes. Despite these measures, the actual vegetation on anthropogenically influenced shorelines barely resembles the natural situation: the vegetation below the waterline mainly consists of monospecific stands of common reed, *Phragmites australis*. At dry parts of the land-water gradient, this vegetation is often immediately succeeded by species-poor tall forb stands dominated by, among others, *Epilobium hirsutum* or *Urtica dioica*. The naturally occurring transition zone between monospecific reed and tall forbs, in which *P. australis* is accompanied by marsh interstitials such as *Mentha aquatica* and *Myosotis scorpioides*, is usually absent. In other areas, especially where traditional management has ceased and/or where water tables have dropped, many reed marshes have changed into tall forb stands.

In order to assess the appropriate management measures for increasing species richness of reed marshes and inhibiting the expansion of tall forbs, it was investigated which processes underly the zonation of species-rich reed marshes and tall forb stands along eutrophic waterbodies. This mechanism was unravelled by first identifying the conditions which allow establishment and persistence of other species in monospecific reed. Consequently it was assessed which conditions favour reed marsh interstitials above tall forbs. Finally, the role of competition in the replacement of *Phragmites australis* by the tall forb *Epilobium hirsutum* was investigated.

Descriptive and experimental field studies revealed that the flooding regime and wave exposure limit the potential number of species. The actual number of species within *Phragmites australis* dominated vegetation was negatively related to litter cover and peak standing crop of the dominant. Peak standing crop of *Phragmites australis* was negatively related to litter cover, elevation and soil organic matter content.

The factors that determine the development of the vegetation into a species-rich reed marsh or into a tall forb stand were consequently elucidated. Since species richness is achieved by favouring weak competitive, interstitial species above strong competitors, I selected representatives of reed marsh interstitials and tall forb dominants for detailed experiments. The factors which discriminate between the marsh interstitials and tall forb dominants were identified by comparing responses of representatives of both species groups to relevant environmental conditions, i.e. soil organic matter, canopy shade and hydrology.

Hydrology emerged as the main factor determining the balance between marsh interstitials and tall forb species. Water level does not discriminate between both species groups during germination since both groups require moist soil for germination and are unable to germinate

when the soil is flooded. Despite the similar germination requirements seedlings of tall forb species were less tolerant to total inundation than reed marsh interstitials. The tall forbs *Cirsium arvense* and *Eupatorium cannabinum* had a limited amount of aerenchyma in stems and roots and these species will therefore be strongly inhibited by soil waterlogging or partial flooding. Aerenchyma content of another important tall forb species, *Epilobium hirsutum*, was however not different from marsh interstitials and in waterlogged soils its biomass production was even higher than that of the marsh interstitials *Myosotis scorpioides* and *Mentha aquatica*. Nevertheless, the apparently tolerant *Epilobium hirsutum* will not be able to dominate waterlogged and flooded parts of shoreline vegetation because its vegetative reproduction is strongly inhibited at these positions. In an outdoor experiment, waterlogging and higher water levels strongly reduced the biomass allocation to rhizomes and mean rhizome weights of *Epilobium hirsutum*, whereas vegetative reproduction of the marsh interstitial *Mentha aquatica* was not affected by water level.

A two-year field competition experiment has shown that, if *Epilobium hirsutum* is able to reproduce vegetatively, *Phragmites australis* will be (further) decreased in vigour by competition with *Epilobium hirsutum*. There was a clear assymmetry in competitive abilities since *Phragmites australis* was not able to suppress *Epilobium hirsutum*. Thus, *Phragmites australis* will eventually be outcompeted by tall forbs at higher elevations of the shoreline.

Only at positions where the water table is usually at or above soil surface will *Phragmites australis* be able to maintain dominance and will reed marsh interstitials be able to persist. Although reed marsh interstitials performed best in moist, well-aerated soils under experimental conditions they will be excluded by the competitive tall forbs from these positions. Reed marsh interstitials find a refugium at lower positions because they are better adapted to partial and total inundation than tall forbs. At these positions however a number of interstitials may have difficulties to persist if the substrate contains organic matter derived from reed litter. This was concluded from an experiment in which specific growth responses were measured on plants growing in waterlogged reed litter substrate. In soils with organic matter of aquatic plant origin all species performed equally well when waterlogged. Canopy shade was also shown to decrease growth of some marsh interstitials and in combination with waterlogging interstitials were reduced to the same extent as tall forbs.

It was concluded that a species-rich reed marsh can only develop at sheltered sites where growth conditions of *Phragmites australis* are suboptimal, strong litter accumulation is prevented and water levels fluctuate in such a way that drawdowns and floods alternate. Drawdowns are necessary because marsh interstitials cannot germinate in flooded soils. In addition, regular drawdowns may prevent strong accumulation of organic matter because soil aeration accelerates decomposition and directly improves growth conditions for plants in reed litter substrate or under a *Phragmites australis* canopy. Regular floods will prevent seedling establishment and vegetative expansion of tall forbs. Floods therefore prevent competitive exclusion of marsh interstitials and *Phragmites australis* and will also remove litter from the reed marsh zone.

Conservation and restoration of a species-rich reed marsh zone is best guaranteed when water levels fluctuate according to a natural pattern. Due to variability in precipitation and evaporation, occasional floods may occur during the growing season and years with relatively high water levels will alternate with relatively low levels. The present mode of water level management is a big constraint for the development of species-rich reed marshes because water levels are often kept at the same level throughout the year or are artificially lowered during winter, increased during summer and strictly controlled within each season.

SOORTEN RIJKDOM IN RIET MOERASSEN

Samenvatting

Op natuurlijke oevers langs voedselrijke wateren bestaat, gaande van water naar land, een zonering van vegetatietypen. In de diepste delen is riet (*Phragmites australis*) vaak de enige soort, maar hogerop is de vegetatie soortenrijker omdat concurrentie-zwakke moerassoorten als watermunt (*Mentha aquatica*), moerasvergeetmijnietje (*Myosotis scorpioides*) en wolfspoot (*Lycopus europaeus*) hier het riet begeleiden. Aan de landzijde van de oever domineren ruigtesoorten als harig wilgeroosje (*Epilobium hirsutum*), grote brandnetel (*Urtica dioica*), koninginnekruid (*Eupatorium cannabinum*) en akkerdistel (*Cirsium arvense*) en is riet slechts sporadisch aanwezig. Door tal van menselijke invloeden is deze natuurlijke zonering bijna nergens meer terug te vinden. Uitgestrekte rietlanden zijn veranderd in soortenarme ruigtes. Zelfs op natuurvriendelijk ingerichte oevers overheersen ruigtesoorten en ontbreken riet en andere moerasplanten.

Oeverbeheerders willen soortenrijk riet graag behouden of herstellen, maar hun maatregelen zullen alleen succes hebben wanneer ze zijn gebaseerd op gedegen kennis van de processen die leiden tot de natuurlijke zonering van riet, soortenrijk riet en ruigte. Dit proefschrift beschrijft het onderzoek naar deze processen. Eerst werd vastgesteld wanneer andere soorten de mogelijkheid hebben zich in een rietbegroeiing te vestigen. Vervolgens kwamen de oorzaken van een verschillende standplaats van moeras- en ruigtesoorten aan bod. Soortenrijk riet zal zich namelijk alleen handhaven op plaatsen die geschikt zijn voor moerassoorten. Waar ruigtesoorten zich kunnen handhaven en uitbreiden zullen ze de moerassoorten verdringen en op den duur tevens de dominantie van riet overnemen. De rol van concurrentie bij deze verschuivende dominantie was het derde en laatste aandachtspunt van dit onderzoek. Tot slot zullen de onderzoeksresultaten vertaald worden naar adviezen voor beheer van riet moerassen.

Mogelijkheden voor andere soorten in door riet gedomineerde begroeiingen bleken sterk samen te hangen met strooisel- bedekking en vitaliteit van de dominante soort. Zowel op plaatsen zonder strooisel- duidend op sterke golfslag- als op plaatsen met volledige bedekking door strooisel kwamen weinig soorten voor. Dit gold ook voor plaatsen met vitaal riet omdat de hoge stengels veel licht onderscheppen en zodoende laaggroeiende moerassoorten belemmeren. Alleen waar riet was aangetast door, onder andere, insekten, vorst of ophoping van strooisel in en op de bodem was het aantal soorten relatief hoog. Alhoewel de vitaliteit van riet dus het aantal soorten lijkt te bepalen leidde, in een veldproef, verwijdering van riet in een soortenrijke rietzone niet tot meer soorten. Overstromingen maakten de vestiging van veel soorten namelijk

weer ongedaan. In rietmoerassen bepalen dus niet alleen strooisel bedekking en vitaliteit van riet het aantal soorten, maar ook fysische factoren zoals golfslag en overstromingen. In de hoger gelegen ruigte nam het aantal soorten wel toe na verwijdering van de hier dominerende soorten. Deze vegetatie overstroomt namelijk alleen 's winters waardoor golfslag en overstromingen, in tegenstelling tot lichtonderschepping, geen rol van betekenis spelen.

Om te achterhalen welke factoren de balans tussen moeras- en ruigtesoorten bepalen zijn vertegenwoordigers van beide groepen in een reeks experimenten blootgesteld aan omstandigheden die zich kunnen voordoen in een rietbegroeiing. Het verloop van de waterstand gedurende het jaar kwam naar voren als de bepalende factor. Beide groepen kunnen niet kiemen bij waterstanden ter hoogte van het maaiveld of daarboven. Zaailingen en volwassen planten van alle soorten groeiden optimaal wanneer het water beneden het maaiveld stond en minder goed bij hogere waterstanden. Kiemplanten van moerassoorten bleken echter veel beter aangepast tegen overstroming dan ruigtesoorten. Akkerdistel (*Cirsium arvense*) en koninginnekruid (*Eupatorium cannabinum*) bijvoorbeeld vormden in natte bodems nauwelijks luchtweefsel in scheut en wortels. Dit maakt het onmogelijk om door zuurstoftransport via stengels en wortels het zuurstofgebrek in de bodem (een belangrijk gevolg van overstroming) het hoofd te bieden. Bovendien bleken de meeste planten niet in staat om volledige onderdompeling langer dan zes weken te overleven. De moerassoorten watermunt, moerasvergeetmijnietje en wolfspoot bleken wel te beschikken over veel luchtweefsel. Bovendien overleefden alle planten een volledige overstroming van acht weken.

Harig wilgeroosje was in de meeste proeven veel toleranter dan de andere ruigtesoorten en week qua hoeveelheid luchtweefsel en overleving van zaailingen niet af van moerassoorten. Zaailingen in overstroomde bodems groeiden zelfs beter dan jonge planten van watermunt en moerasvergeetmijnietje. De schijnbaar tolerante harig wilgeroosje zal op den duur echter niet in staat zijn de vegetatie op regelmatig overstroomde delen van de oever te domineren. In tegenstelling tot watermunt was bij harig wilgeroosje de investering van biomassa in wortelstokken namelijk veel minder in overstroomde bodems. Bovendien wezen de wortelstokken omhoog en ontsprongen ze uit bovengrondse delen van de stengel. Deze afwijkende vorm duidt op een hormonale verstoring in de plant, die waarschijnlijk het gevolg is van een slechte zuurstofvoorziening in de wortels.

Zowel in het zaailing– als in het volwassen stadium zijn moerassoorten dus beter bestand tegen overstroming dan ruigtesoorten. In combinatie met andere omstandigheden kunnen hogere waterstanden echter ook problemen opleveren voor moerassoorten. In bodems met organisch materiaal afkomstig van rietstrooisel remden waterstanden ter hoogte van het maaiveld de groei van moerassoorten sterk af. Onder een (nagebootst) bladerdek van riet verdroegen moerassoorten overstroomde bodems niet beter dan ruigtesoorten.

De laatste fase van het onderzoek richtte zich op de rol van concurrentie bij de vervanging van riet door ruigtesoorten. In een twee jaar durende veldproef bleek riet zich sterk te kunnen uitbreiden na verwijdering van harig wilgeroosje. Concurrentie speelt dus duidelijk een rol bij de verruiging van riet. Maar zelfs na twee jaar verwijdering van alle concurrenten had het riet in de proefvlakken ter hoogte van de ruigtezone niet dezelfde produktiviteit als het riet op optimale standplaatsen lager op de oever. Behalve concurrentie zijn er dus andere factoren die de achteruitgang van riet op droge oeverdelen veroorzaken.
Verwijdering van riet leidde niet tot meer of grotere planten van harig wilgeroosje. In gemengde vakken waren stengels van deze ruigtesoort zelfs groter dan in vakken met alleen harig wilgeroosje. Hieruit bleek dat alleen intraspecifieke concurrentie (tussen exemplaren van de eigen soort) en hoge waterstanden in het voorjaar de groei en dichtheid van harig wilgeroosje beïnvloeden.

Het onderzoek in dit proefschrift heeft aangetoond dat de overgang van riet naar soortenrijk riet of ruigte alleen mogelijk is op droge en ondiepe plaatsen die beschermd zijn tegen extreme golfslag en waar het riet aan vitaliteit heeft ingeboet. Een verschil in tolerantie tegen overstromingen als zaailing en volwassen plant bleek de belangrijkste oorzaak voor de verschillende standplaats van moeras- en ruigtesoorten. Bovendien is duidelijk geworden dat moerassoorten zich alleen kunnen handhaven op plaatsen die regelmatig overstromen en droogvallen. De lage waterstanden maken kieming van moerassoorten mogelijk en zorgen voor een tijdelijke verbetering van groeimogelijkheden voor planten in bodems met veel rietstrooisel of onder een dicht bladerdek van riet. Regelmatige overstromingen voorkomen de vestiging en uitbreiding van ruigtesoorten en verwijderen bovendien veel strooisel.
Een natuurlijk verloop van het waterpeil biedt daarom de beste garanties voor behoud en herstel van soortenrijke rietoevers. Door variatie in neerslag en verdamping kunnen binnen een groeiseizoen peilschommelingen optreden en zullen jaren met natte en droge zomers elkaar afwisselen. Daarnaast zal het water in najaar en winter meestal hoger staan dan in het groeiseizoen waardoor strooisel regelmatig verwijderd wordt uit de oeverzone met riet en moerassoorten. Het huidige peilbeheer, met een constant waterpeil gedurende het gehele jaar of met een kunstmatig verlaging in de winter en een kunstmatig verhoging in de zomer, is verre van natuurlijk en vormt daarom een grote belemmering voor ontwikkeling van soortenrijk riet.

NAWOORD

Dit is weliswaar mijn proefschrift, maar zeker niet alleen mijn werk. Met name mijn co-promotor Wim van der Putten heeft een onmisbare rol gespeeld in alle fasen van het onderzoek. Ook zonder mijn promotor, Kees Blom, had dit boekje er niet gelegen. Samen wisten ze de duimschroeven soms stevig aan te draaien, maar hun begeleiding was altijd een mooie balans tussen inhoudelijke kritiek en stimulerende opmerkingen. Precies zoals je mag verwachten van begeleiders! Veel heb ik bovendien te danken aan de inhoudelijke en praktische assistentie van Frank Menting. Onze samenwerking was vanaf het begin al vanzelfsprekend en daarom juist heel bijzonder.

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(DWW) en J.W.B. Nienhuis (waterschap de Brielse Dijkring) een belangrijke bijdrage aan het project.

Rest mij nog te zeggen dat het uiten van mijn dankbaarheid jegens Maya een boekwerk van ten minste de omvang van dit proefschrift vergt.

CURRICULUM VITAE

John Lenssen werd geboren op 7 oktober 1967 te Nijmegen. In 1986 behaalde hij het eindexamen VWO aan het St. Thomascollege te Venlo. Daarna is hij begonnen aan de studie biologie aan de Katholieke Universiteit Nijmegen. Zijn hoofdvak aquatische oecologie bestond uit een onderzoek naar de invloed van macrofauna op de afbraak van bladmateriaal in verzuurde en neutrale aquatische systemen. Tevens werd een bijvak aquatische oecologie gevolgd aan de University College in Cork (Ierland). In 1992 studeerde hij af na het doorlopen van bijvakken op het gebied van milieurecht, popularisering van natuurwetenschappen (stage bij Stichting Natuur en Milieu) en plantenoecologie via een stage bij het Riza te Lelystad.

Van november 1992 tot en met april 1993 werkte hij aan het Nederlands Instituut voor Oecologisch Onderzoek te Heteren aan een beheershandleiding voor water- en oevervegetaties van rijkswateren. Dit project werd uitgevoerd in opdracht van Rijkswaterstaat directie Zuid-Holland en Dienst Weg- en Waterbouwkunde en Waterschap de Brielse Dijkring. In juli 1993 begon hij voor dezelfde opdrachtgevers aan het project 'Oecologische beperkingen bij het herstel van oeverbegroeiingen'. De resultaten hiervan zijn weergegeven in de brochure 'Soortenrijke oevers: sturen tussen riet en ruigte' en in dit proefschrift.