Is tolerance to summer flooding correlated with distribution patterns in river floodplains? A comparative study of 20 terrestrial grassland species

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It is generally assumed that floods during the growing season have a strong impact on the distribution of grassland plant species in river floodplains but this proposition has never been tested. We examined the survival and growth responses of twenty species, originating from mid- and high-level floodplain grasslands along the River Rhine in the Netherlands, to total submergence for a maximum of two months in an outdoor flooding experiment. Plant survival and biomass reduction with flooding duration was determined as well as biomass recovery after de-submergence.

Our results indicate that species survival is the most prominent factor correlated with species distribution in floodplain areas. Relatively flood tolerant species occurred mainly at low elevations along the floodplain while more flood sensitive species were restricted to high parts of the floodplain gradient. Biomass reduction rates during submergence were only marginally significantly correlated with species lower distribution boundaries along the flooding gradient. Biomass recovery rate was significantly correlated with species distribution patterns in the field only after 2 weeks of complete submergence, but not after 4 and 8 weeks. Our results suggest that the more flood tolerant species can have various ways to survive and recover from flooding, ranging from low rates of biomass loss and low recovery to relatively high rates of biomass loss and quick recovery.

Our results are consistent with the notion that disturbance by floods during the growing season is an important determinant of species lower distribution boundaries in river floodplains. They also suggest that high survival under flooding may be achieved by different physiological mechanisms. Such mechanisms are discussed in this paper.

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Grassland species of temperate river floodplains are regularly subjected to floods, predominantly during winter and spring. Such floods are natural phenomena caused by peaks in precipitation and snow melt in the catchment area (Vervuren et al. 2003). However, due to channelisation and drainage of wetlands combined with global warming, flooding has increased in frequency, also during the growing season (Blom et al. 1990). This may have important consequences for plant distribution because flooding is one of the major abiotic factors determining the distribution of plant species in river areas (Keddy 1984, Sykora et al. 1988, Crawford 1992, Vartapetian and Jackson 1997, Pollock et al. 1998, Van de Steeg and Blom 1998, Silvertown et al. 1999, Vervuren et al. 2003). Moreover, flooding during the growing season may be particularly important in

affecting plant survival (Andrews and Pomeroy 1989, Klimesova 1994, Siebel 1998, Sparks et al. 1998, Johansson and Nilsson 2002).

It is generally assumed that the lower boundary of a species corresponds to a species' tolerance to flooding (Squires and Van der Valk 1992, Sand-Jensen and Frost-Christensen 1999, He et al. 1999). Depending on the range of occurrence along the flooding gradient a plant can be subjected to a certain flooding duration, depth and frequency (Vervuren et al. 2003). For *Rumex* species it has been shown that flood sensitive species are restricted to high parts of the flooding gradient, while more tolerant species persist at lower elevations (Laan and Blom 1990, Voesenek 1990, Nabben et al. 1999). Similar differences may apply to a wider range of species distributed along the flooding gradient. However, to our knowledge, the extent to which grassland species of other taxa differ in their tolerance for total submergence and the degree to which the relationship between species flooding tolerance and the distribution along the flooding gradient has general validity has not been tested.

The most important constraint that plants have to deal with during flooding is oxygen deficiency (Crawford and Brändle 1996, Vartapetian and Jackson 1997). As a consequence, growth rate and biomass production of terrestrial plants under flooded conditions are reduced. However, some grassland species are able to cope with such conditions by physiological and morphological adaptations (Blom and Voesenek 1996, He et al. 1999, Vervuren et al. 1999, Voesenek et al. 2003). Adverse effects during submergence can be avoided by aerenchyma formation (Visser et al. 1997, Jackson and Armstrong 1999) and under-water photosynthesis to restore oxygen and sugar supply to plant tissues (He et al. 1999, Vervuren et al. 1999). In such cases plant biomass can partly or entirely be maintained. If species lack such adaptations or change from aerobic to anaerobic metabolism, which is a very inefficient process with regard to carbohydrate consumption (Armstrong et al. 1994, Crawford and Brändle 1996), biomass will be strongly reduced during flooding. Re-aeration after de-submergence can lead to the formation of free oxygen radicals and the oxidation of metabolites that have accumulated during submergence. Both processes can lead to serious damage to plants known as post-anoxic injury (Crawford and Brändle 1996), and can strongly reduce the capacity to recover biomass after de-submergence. Species' flooding tolerance can thus be characterized by its survival and biomass reduction rate during complete submergence and by its rate of biomass recovery rate after de-submergence. To what extent grassland species of river areas withstand summer floods, and the importance of this tolerance for species distribution along flooding gradients, has been unknown and was examined in this study.

The aim of the present study was to examine survival and growth responses of grassland species of river floodplains to total submergence during summer. For this purpose an outdoor flooding experiment with 20 terrestrial grassland plant species, originating from low-, mid- and high-level floodplain grasslands along the River Rhine in the Netherlands, was performed in the summer of 1999. Total plant biomass reduction with increasing flooding duration was determined as well as plant biomass recovery after de-submergence. Furthermore, 123 vegetation surveys were investigated at different sites and elevations along river floodplains. Relationships between survival and growth responses of the selected plant species and their field distribution along the flooding gradient were analysed.

Material and methods

Field data

In the summer of 2000 we selected six sites along the River Rhine and Waal (Fig. 1) with pastures and meadows, gradual slopes that are regularly flooded, except a few highly situated sites that were rarely, if ever, flooded. Within each site we positioned one or two transects perpendicular to the waterline, while keeping a minimum distance of 100 m between adjacent transects. Along each transect we sampled vegetation surveys while taking care to encompass a broad range of elevations. The area of each survey was at least 5 m^2 but was larger in homogeneous vegetation (up to 16 m^2 to prevent large within-survey variability in elevation) in order to increase the probability of including experimental species. In each survey the abundance of all higher plant species was recorded and cover/abundance of each species was recorded using the scale of Van der Maarel (1979). Thereafter the elevation of the survey was determined by levelling. For each survey we averaged values of four measurements. For comparison, measured NAP-levels (Dutch Ordnance Level) were transformed to Lobith gauge levels (m) by correcting for the decline of the river water level. In total 123 vegetation surveys were made.

Because many species were not normally distributed along the elevation gradient, median plant elevation was used for analysis instead of mean values. For each median value 10th, 25th, 75th and 90th percentile values were calculated.

Experimental design

The species
Twenty terrestrial plant species were selected (Table 1) which are all characteristic of grasslands (pastures and hayfields) of low- and mid-level floodplains with frequent and prolonged flooding and high, occasionally

Fig. 1. Location of sampling sites for plant material and sites where vegetation surveys were made along the main branch of the River Rhine in the Netherlands.
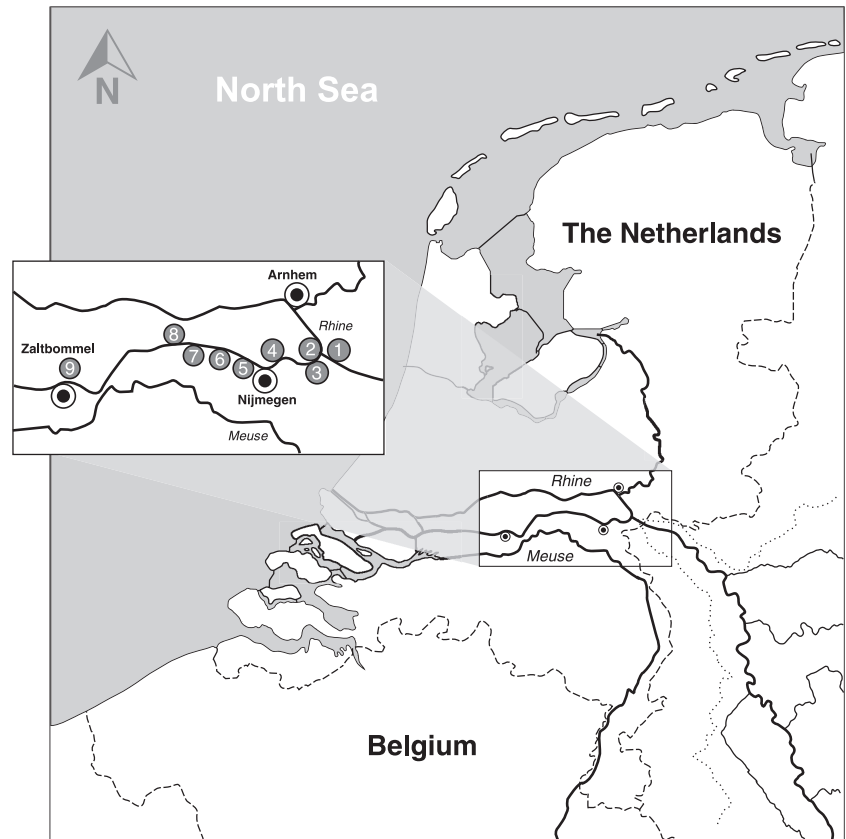
	Site	Coordinate	Surveys	Diaspores
1	Tolkamer	51°52′N 6°05′E	x	x
2	Doornenburg	51°53′N 6°02′E	x	x
3	Kekerdom	51°52′N 5°59′E	x	x
4	Bemmel	51°52′N 5°53′E		x
5	Beuningen	51°52′N 5°48′E	x	
6	Ewijk	51°53′N 5°45′E	x	x
7	Deest	51°54′N 5°40′E		x
8	Ochten	51°54′N 5°34′E		x
9	Neerijnen	51°50′N 5°17′E	x	x

flooded sites along the Rhine in the Netherlands. The species differ in their optimal elevation relative to the river. Seeds of each species were collected from 1995 to 1999 from single populations in floodplain grasslands along the main branch of the River Rhine in the Netherlands (Fig. 1). All selected species are perennials, except *Pastinaca sativa* and *Rumex crispus*, which are biennial or short-living perennials.

Plant material
The majority of the plants were grown from seeds. When vegetative material (young ramets or seedlings) was used, at least 20 genotypes per species were collected

to ensure ample genetic variation of the experimental material. Seeds were germinated on moist filter paper in petri dishes and incubated in a growth cabinet (12 h 25 μmol m^{-2}s^{-1} PPFR (Philips TL33), 25°C; 12 h dark, 10°C) for one week. Thereafter, germinated seeds were individually transferred into 800 ml pots on a mixture of sand and clay (1:1 v/v) and placed in a greenhouse.

Vegetative material was collected in the spring of 1999. This material was also transferred to the same pots and substrate mixture as mentioned for seedlings and placed in the greenhouse. The photoperiod in the greenhouse was kept at 16 h by 400 W high-pressure sodium lamps and supplemented normal daylight to a minimal light

Table 1. Selected species for the submergence experiment, species abbreviation, type of diaspore used for raising plants and floodplain sites where plant material was collected. For location of floodplain sites see Fig. 1. Nomenclature follows Van der Meijden (1996).

Species	Abbreviation	Diaspore	Origin
Achillea ptarmica L.	acp	Seed	Bemmel
Agrostis stolonifera L.	ags	Ramet	Ewijk
Alopecurus geniculatus L.	alg	Ramet	Ochten
Arrhenatherum elatius (L.) J. & C. Presl	are	Seed	Deest
Centaurea scabiosa L.	ces	Seed	Tolkamer
Elytrigia repens (L.) Nevski	elr	Seed	Ewijk
Festuca rubra L.	fer	Seed	Kekerdom
Inula britannica L.	inb	Ramet	Neerijnen
Medicago falcata L.	mef	Seed	Tolkamer
Pastinaca sativa L.	pas	Seedling	Kekerdom
Plantago lanceolata L.	pll	Seed	Tolkamer
Plantago major ssp. intermedia (Gilib.) Lange	plm	Seed	Doornenburg
Potentilla anserina L.	poa	Ramet	Kekerdom
Potentilla reptans L.	por	Seed	Bemmel
Ranunculus acris L.	raa	Seed	Neerijnen
Ranunculus repens L.	rar	Ramet	Neerijnen
Rumex acetosa L.	rua	Seed	Deest
Rumex crispus L.	ruc	Seed	Bemmel
Rumex thyrsiflorus Fingerh.	rut	Seed	Tolkamer
Thalictrum minus L.	thm	Seed	Kekerdom

intensity (PPFR) of 100 μmol m^{-2}s^{-1} at plant level. During the growth period the temperature in the greenhouse fluctuated between 19.0°C and 29.0°C. Plants were watered three times a week with tap water and fertilized twice during the growth period with half strength modified Hoagland nutrient solution (Johnson et al. 1957). Seedlings and seed grown plants were 10 weeks old at the start of the experiment. For species which were grown from ramets, it was impossible to determine exact plant age.

Maximum flooding duration

The flooding characteristics of the different elevations at which the studied species grow were analysed for the period 1960–1999 by comparing the relative elevation (normalized to the elevation at Lobith) of the floodplain with the water levels of the river and calculating the timing and duration of the flooding events. Water level data were provided by the RIZA Water Data Desk. Since we focus on floods during the growing season only flooding characteristics in the period May until September were considered (Vervuren et al. 2003). From these data it followed that a flood peak at high elevations (>12.5 m) lasted for a maximum of 25 days. At mid levels (between 11.0 and 12.5 m) this flood endured 80 days. Based on this analysis we decided to submerge the studied species for a maximum period of two months. Because long lasting summer floods are very rare, it was expected that differences between species with regard to survival and growth responses upon flooding, would arise within an artificial flooding period of this length.

Flooding treatment

Before the start of the submergence treatment, roots and shoots of 10 randomly selected plants per species were gently washed with tap water and total dry weight (dried for 24 h at 105°C) was determined. At the same time, all other plants were distributed over five outdoor basins (width: 8 ×12.5 m; depth 1 m) at the Botanical Garden complex of the Nijmegen University and gradually filled with tap water. Plants were placed in a randomised block design with basin as a block. Each basin contained 32 plants per species. All plants were completely submerged for one week up to a maximum period of two months (July–August 1999). Water level was kept 70 cm above the soil surface in the pots to avoid restoration of leaf–air contact of some species due to shoot elongation or leaf floating. During submergence plants were not fertilised. To prevent growth of plank tonic algae, *Daphnia* spec. was added to the basins. During flooding, light quantities at plant level were measured twice in each basin using a LI-COR (Lincoln, Nebraska) photometer (model LI-18513) with an underwater quantum sensor (LI-192SB). Average light attenuation in the water at plant level (±50 cm from the water surface) was 20–22%. Minimum and maximum water temperatures were measured daily in each basin using standard thermometers. Temperatures fluctuated between 18°C and 26°C.

Harvesting

Every week 20 plants per species were taken out of the water (four from each basin) and moved to the greenhouse. This group was split into two groups of 10 plants. The first group was harvested after 3 days recovery to assess survival. A three day delay was necessary because immediately after de-submergence it was often not clear whether or not a plant was alive. Some plants looked vital after de-submergence but appeared to be dead after a few days. In such cases, turgor was maintained in the

water while the root–shoot junction was already disconnected by decay. Roots and shoots were washed gently with tap water, dead roots (black) were removed and survival and dry weight of roots and shoots (dried for 24 h at 105°C) was determined. Survival was based on physical appearance: plants with green, turgid leaves and green buds were designated as to be alive (Nabben et al. 1999). From the second group of 10 plants, survival and total dry weight was determined after a three-week regeneration period in the greenhouse under drained conditions. These plants were watered when necessary but not fertilized. Biomass data of a non-submerged control group were lacking for this group. The light conditions in the greenhouse during the recovery period were similar to the conditions at which plants were grown initially. Day and night temperatures fluctuated between 18.5°C and 26.5°C.

To determine the maximal potential relative growth rate (RGR) of the species, total dry weight after four weeks growth under drained conditions was determined for another group of ten plants per species.

Data analysis

Survival

To determine plant species survival, the flooding duration at which 50% of the plant individuals from a particular species had died, the so-called median lethal time (LT_{50}), was determined. For each flooding duration all harvested plants of one species were grouped (total of 20 plants) and the number of plants that survived was counted. With the SAS LIFEREG procedure (SAS/STAT 6.0) Weibull survival curves were fitted through the survival data and values for LT_{50} were derived from these curves. If 100% (or almost 100%) of the plants of a species survived eight weeks of submergence, we did not fit a curve through the data, because reliable LT_{50} values could not be derived. Survival was then set at 60 days, as a conservative estimate, because the largest reliable estimate for LT_{50} was 59.4 days (*Inula britannica*). This was done for *Elytrigia repens*, *Potentilla anserina*, *Potentilla reptans* and *Rumex crispus*.

All plants of *Festuca rubra*, *Centaurea scabiosa* and *Pastinaca sativa* died within a period of 14 days, resulting in too few observations to fit survival curves. In such cases LT_{50} was estimated by taking the midpoint between 100% survival and 0% survival.

Rate of biomass reduction and recovery capacity rate

Biomass reduction rate during submergence (expressed as $g\,g^{-1}\,day_{flooding}^{-1}$) was determined by regression analysis of plant biomass of surviving plants versus flooding duration (using SPSS 10.0.7). Such an analysis was performed for three days after de-submergence to determine the biomass response to flooding and a similar analysis was performed for plants that had grown drained for three weeks after de-submergence. This latter analysis was run to determine species ability to recover from flooding. For some species all plants died within two or three weeks after onset of the flooding treatment. As a consequence, only one or two data points were obtained, making it impossible to perform a reliable regression analysis.

Plant biomass recovery three days and three weeks after de-submergence versus flooding duration may differ due to reduced recovery with increasing flooding duration. To explicitly compare both recovery measurements we run an ANCOVA to analyse whether the capacity to recover plant biomass after de-submergence decreased significantly with increasing flooding duration (STATISTICA 5.5). In addition, biomass recovery rate (expressed as $g\,g^{-1}\,day_{recovery}^{-1}$) after de-submergence was determined by regression for three flooding durations, i.e. two, four and eight weeks flooding, based on differences between average biomass (ln dry weight) after a three days and three weeks re-growth period.

The possible effect of phylogeny on the outcome of the species comparisons was judged to be small because differences in flooding responses within a family and/or genus and also within monocots and dicots were large. Therefore, we considered it unnecessary to carry out phylogenetic corrections of the data.

Responses to flooding versus field distribution

Relationships between the distribution of the studied grassland species on river floodplains and species responses to complete submergence (survival, biomass reduction rate and biomass recovery rate), as well as relationships between those responses were investigated by Spearman rank correlation analysis (using SPSS
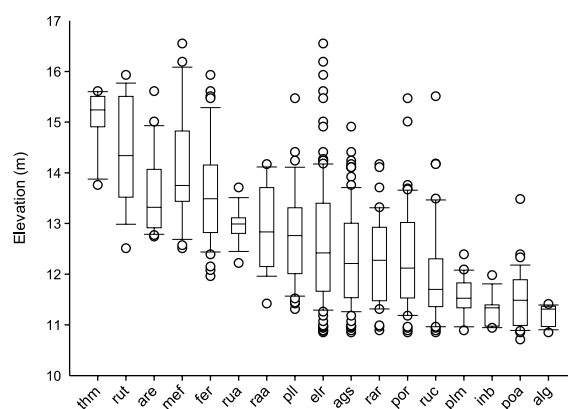

Fig. 2. Species occurrence along the elevation gradient based on 123 vegetation surveys made at 6 floodplain sites along the River Rhine in the Netherlands (Fig. 1). The end of the boxes defines the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles. Circles define data points beyond the 10th and 90th percentiles. For species abbreviations see Table 1.
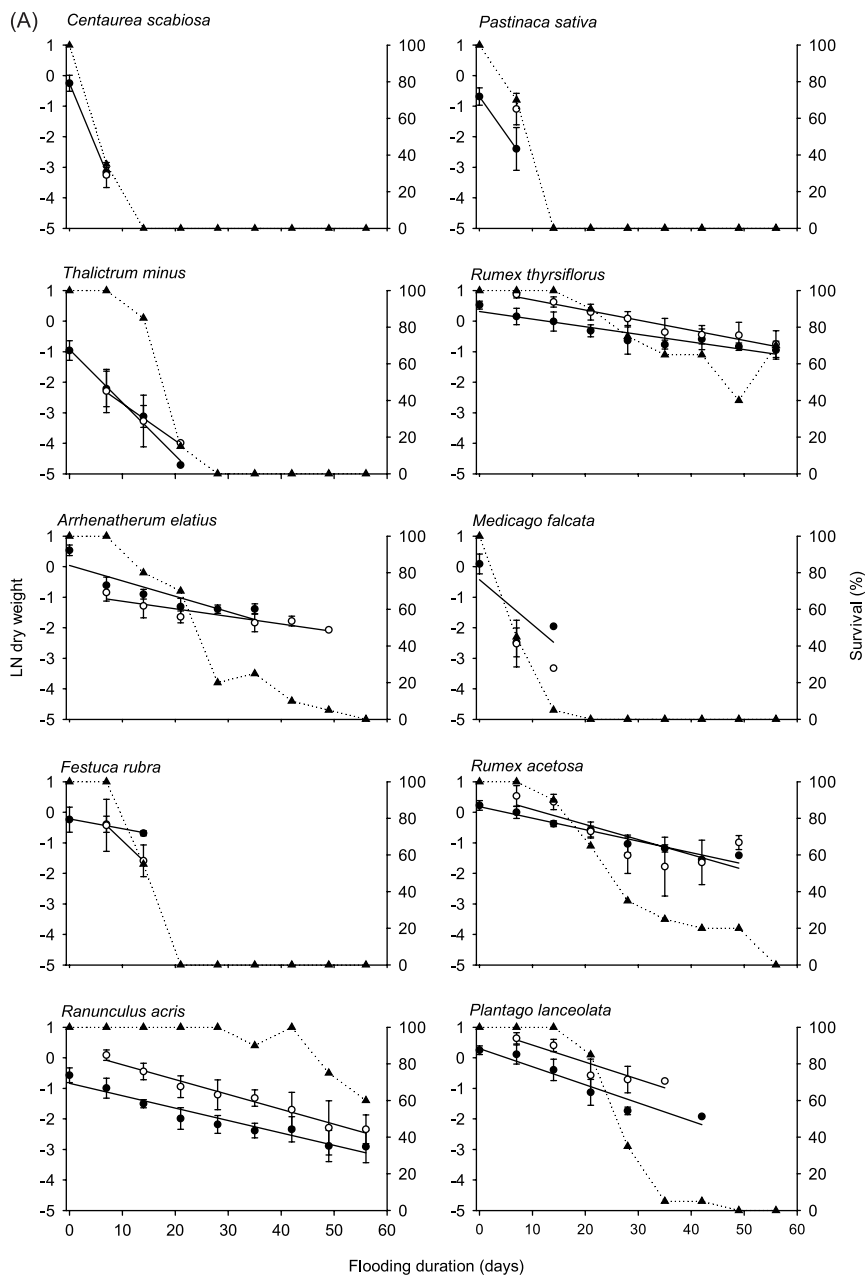
Fig. 3. Relationships for high-elevational (A) and low-elevational (B) species between flooding duration and survival (▲, right-hand axes) (n =20) and between flooding duration and biomass reduction (based on ln-transformed data, ±SE), three days after sampling (●, left-hand axes) and after a three weeks recovery period (○, left-hand axes) (n =10). For *Medicago falcata*, *Pastinaca sativa* and *Centaurea scabiosa* the minimum required number of recovery data points to perform a reliable regression analysis was not available.

10.0.7). To determine which of these responses that best explained species' lower distribution limits, a stepwise regression analysis was performed (SPSS 10.0.7). Elevation was expressed as median, 10th (species 'lower boundary') percentile or 90th percentile ('upper boundary') values based on presence/absence data of the species in the surveys. In these analyses all species were included except *Centaurea scabiosa*, *Pastinaca sativa* and *Achillea ptarmica* because for those species were less than the minimum required number of data points to compute a reliable set of percentiles.

Results

Species distribution

Most species show a limited range of occurrence along the flooding gradient (Fig. 2). Although some species, such as *Elytrigia repens*, *Potentilla reptans* and *Rumex crispus*, occur along the whole elevation gradient, their distribution range between 25th and 75th percentiles is situated at low elevations. The range in height between the 25th and 75th percentile differs between species
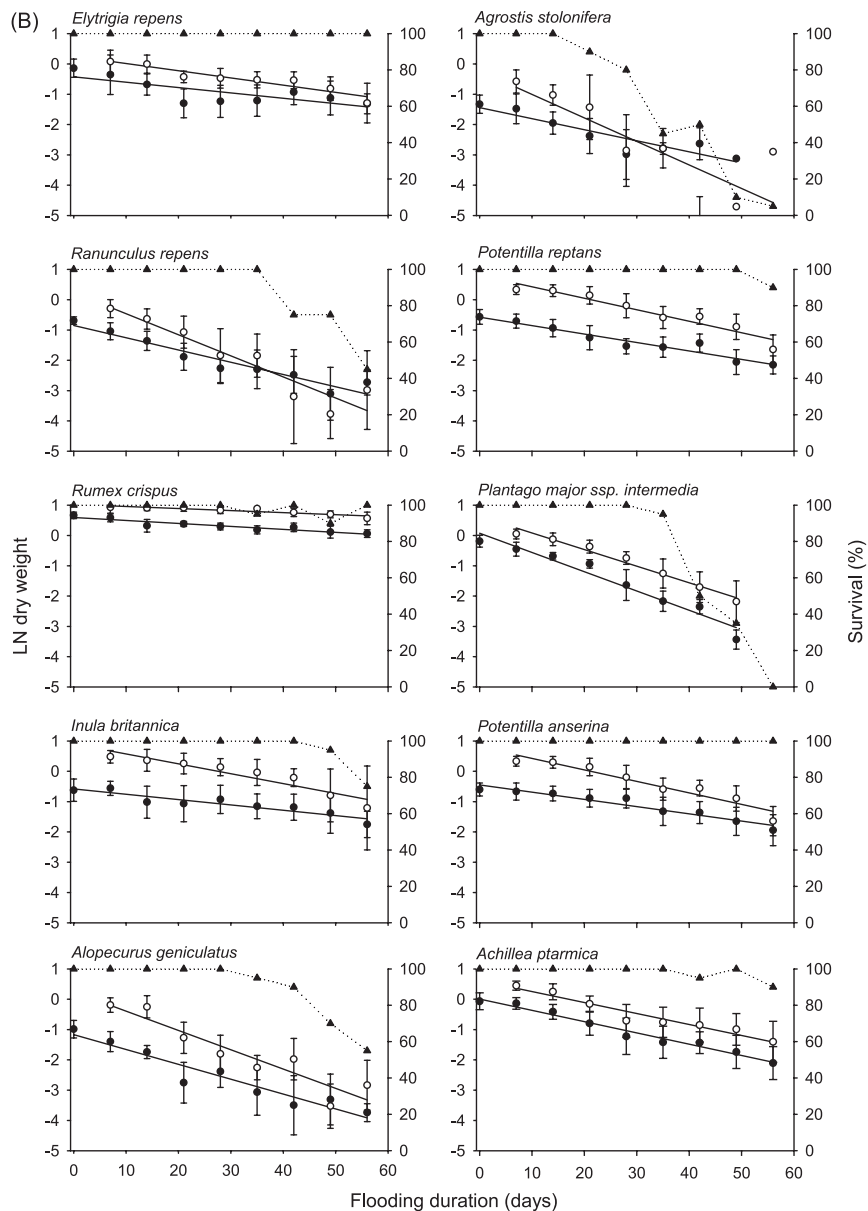
Fig. 3 (*Continued*)

(Fig. 2). Low elevated species had relatively similar lower limits, which indicates that these limits are also shaped by geomorphological processes (e.g. erosion due to wave action or sand deposition by the river).

Survival

Species differed markedly in flooding tolerance. Most species survived flooding up to one week (Fig. 3). Only *Centaurea scabiosa*, *Medicago falcata* and *Pastinaca sativa* were affected by submergence already in the first week. When flooding lasted for more than one week, the survival of most species was reduced. LT_{50} values for the different species ranged from 6.8 up to 59.4 days. All plants of *Rumex crispus*, *Elytrigia repens* and *Potentilla anserina* survived the entire flooding period of 56 days, and 90% of the plants of *Achillea ptarmica* and *Potentilla reptans* survived this flooding period.

The location of species along the elevation gradient was significantly correlated with LT_{50}. This relation applied to median as well as, 10th and 90th percentile elevation levels (Fig. 4A). Flooding tolerant species occured at low elevations while more sensitive species were restricted to high elevations.
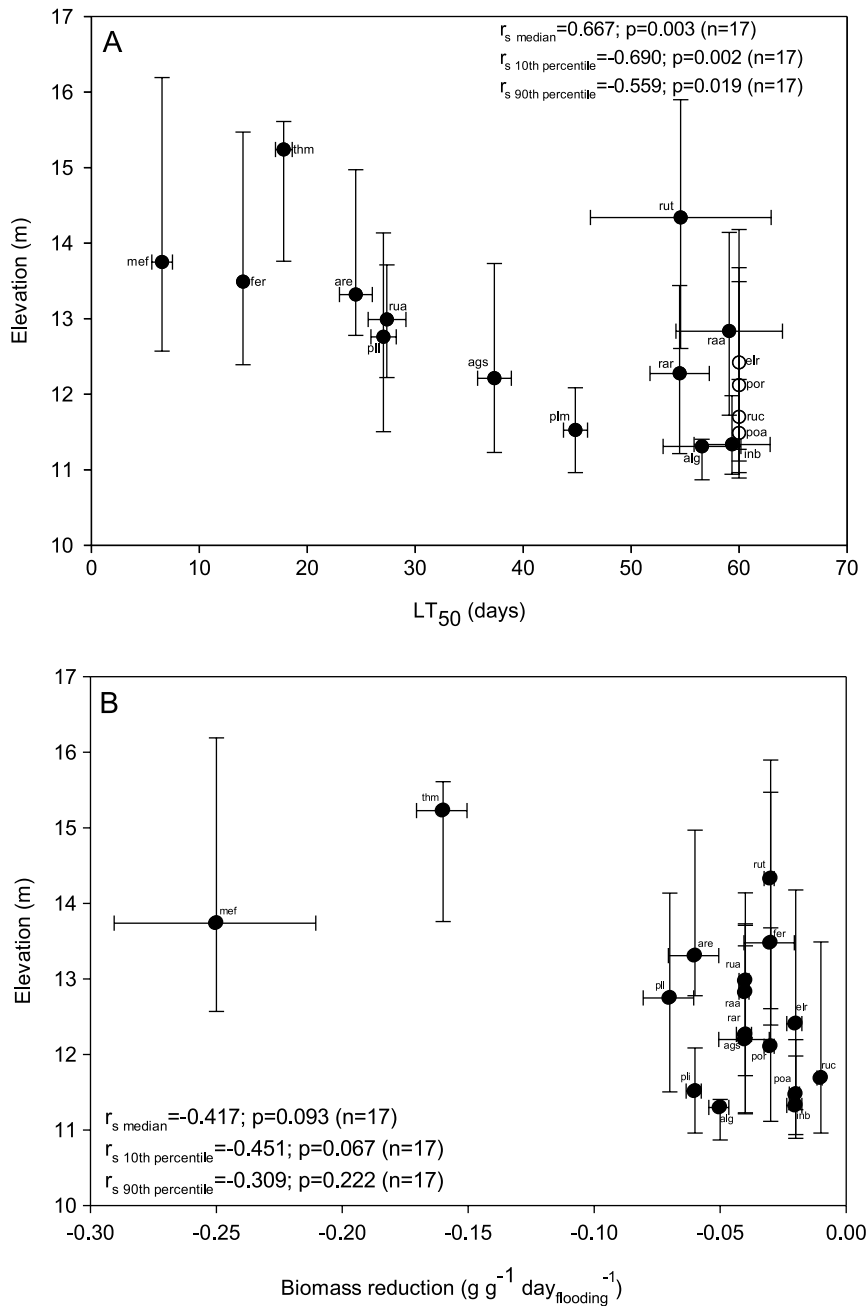
Fig. 4. Relationship between median, 10th and 90th percentile elevation levels of species at six sites along the main branch of the River Rhine floodplains in the Netherlands and (A) survival (expressed as $LT_{50} \pm SE$ as derived from Fig. 3) and (B) plant biomass reduction during flooding (expressed as $g\,g^{-1}\,day_{flooding}^{-1} \pm SE$). LT_{50} values for species which survived 100% or almost 100% after 56 days of submergence were set conservatively at 60 days (indicated by open circles). Plant biomass reduction was based on total plant biomass three days after de-submergence from flooding. For species abbreviations see Table 1.

Almost 45% of the variation in median species distribution was explained by LT_{50}. The coefficient of determination was even slightly higher if 10th percentile elevation values were used instead of median elevation ($R^2 = 0.48$). 10th percentile values may be considered as an estimate of the lower distribution limit of a species.

Biomass reduction rate and recovery capacity

In all species we found a significant reduction of total plant biomass with increasing flooding duration ($p < 0.05$, Fig. 3). Biomass reduction rates differed considerably between species and species lost approximately 1 to 25% of total plant biomass per day of

flooding. The lower distribution boundary (10th percentile) was only marginally significantly correlated with the biomass reduction rate (Fig. 4B). The weak correlation is possibly due to the lack of estimates for field distribution data of some extremely flood intolerant species from high elevations, such as *C. scabiosa* and *P. sativa*. The rate of biomass reduction was significantly correlated with species survival (Table 3). Species with high survival rates had relative low biomass reduction rates compared to species with lower values for LT_{50}, which reduce biomass much faster.

Total plant biomass after a three weeks recovery period, also decreased significantly with increasing flooding duration for all species (Fig. 3). Slopes of the regression lines for plant biomass recovery three days and three weeks after de-submergence depending on flooding duration, differed for six species (as indicated by significant interactions between recovery and flooding duration, Fig. 3, Table 2). In such cases the recovery capacity decreased with increasing flooding duration. These six species are flood tolerant (e.g. *Potentilla anserina*) as well as flood intolerant species (e.g. *Arrhenatherum elatius*). The other species showed recovery rates proportional to the degree of biomass reduction, not differing depending on flood durations.

Biomass recovery capacity was significantly correlated with species distribution along the flooding gradient after two weeks of flooding, but not after four and eight weeks flooding (Fig. 5). The reason for the lack of correlation after four and eight weeks flooding might be that the more flood sensitive species had died. Apparently, amongst the more tolerant species, there were no systematic differences with respect to recovery capacity.

Biomass recovery capacity after two weeks flooding was also significantly and positively correlated with plant species survival (Table 3). However, there were no significant correlations between biomass reduction rate and recovery capacity for any of the flooding duration's (two, four and eight weeks). Most species which, at least partly, survived the entire flooding period were either largely able to maintain their biomass during flooding (e.g. *Rumex crispus*) or maintained the capacity to recover biomass (e.g. *Achillea ptarmica*). However, some tolerant species significantly decreased their capacity to recover (e.g. *Potentilla anserina*).

Stepwise regression analysis showed that the most important factor with regard to species' lower boundary (10th percentile) along the flooding gradient was the survival rate ($R^2 = 0.49$; $p = 0.001$; $n = 17$). The correlation coefficients of the other parameters (reduction rate and recovery capacity) were not significant ($p > 0.5$). Therefore, only the LT_{50} was significant correlated with species lower distribution boundaries along the flooding gradient.

Discussion

Species survival and distribution

Floods may negatively affect plant performance and survival and consequently play a major role in determining species distribution and diversity along flooding gradients (Sykora et al. 1988, Squires and Van der Valk 1992, Carter and Grace 1990, He et al. 1999, Silvertown et al. 1999). Here we showed that the flooding tolerance of a number of floodplain grassland species was significantly correlated ($R^2 = 0.48$) with the lower distribution boundaries along flooding gradients. The 20 species studied differed strongly in their tolerance to complete submergence. The number of days of flooding which killed 50% of the plants (LT_{50}), ranged from 5.6 up to

Table 2. Results of ANCOVA for biomass reduction rate of 20 terrestrial grassland species from low-, mid- and high-level river floodplains in response to block, biomass recovery period after 0 or 3 weeks (R) and flooding duration (T). F values and significance levels are presented as well as MS values for the error (Block $\times$ R $\times$ T). For *Centaurea scabiosa*, *Festuca rubra*, *Medicago falcata* and *Pastinaca sativa* were not enough data available to perform an ANCOVA analysis.

| Species	Block	R	T	R $\times$ T	Error
Df	4	1	1	1	8
Achillea ptarmica	0.392	58.00***	312.9***	0.056	0.210
Agrostis stolonifera	0.347	9.128*	168.5***	31.28***	0.408
Alopecurus geniculatus	0.572	24.75**	143.6***	2.251	0.903
Arrhenatherum elatius	3.217	66.96***	138.7***	16.32**	0.131
Elytrigia repens	2.770	48.79***	132.5***	3.009	0.154
Inula britannica	0.596	50.60***	32.74***	2.995	0.758
Plantago lanceolata	7.828**	55.60***	306.9***	0.013	0.083
Plantago major ssp. *Intermedia*	0.958	252.6***	2009***	10.17*	0.038
Potentilla anserina	2.764	142.9***	278.6***	15.14**	0.158
Potentilla reptans	1.652	198.3***	262.0***	6.135*	0.191
Ranunculus acris	1.185	65.57***	239.7***	1.487	0.343
Ranunculus repens	0.115	1.772	73.90***	5.998*	1.500
Rumex acetosa	2.240	0.009	106.1***	3.460	0.275
Rumex crispus	0.622	297.7***	106.8***	3.163	0.034
Rumex thyrsiflorus	9.433*	37.06***	270.6***	4.953	0,109
Thalictrum minus	0.879	19.84**	99.49***	0.753	0.351

* P <0.05; ** P <0.01; *** P <0.001.
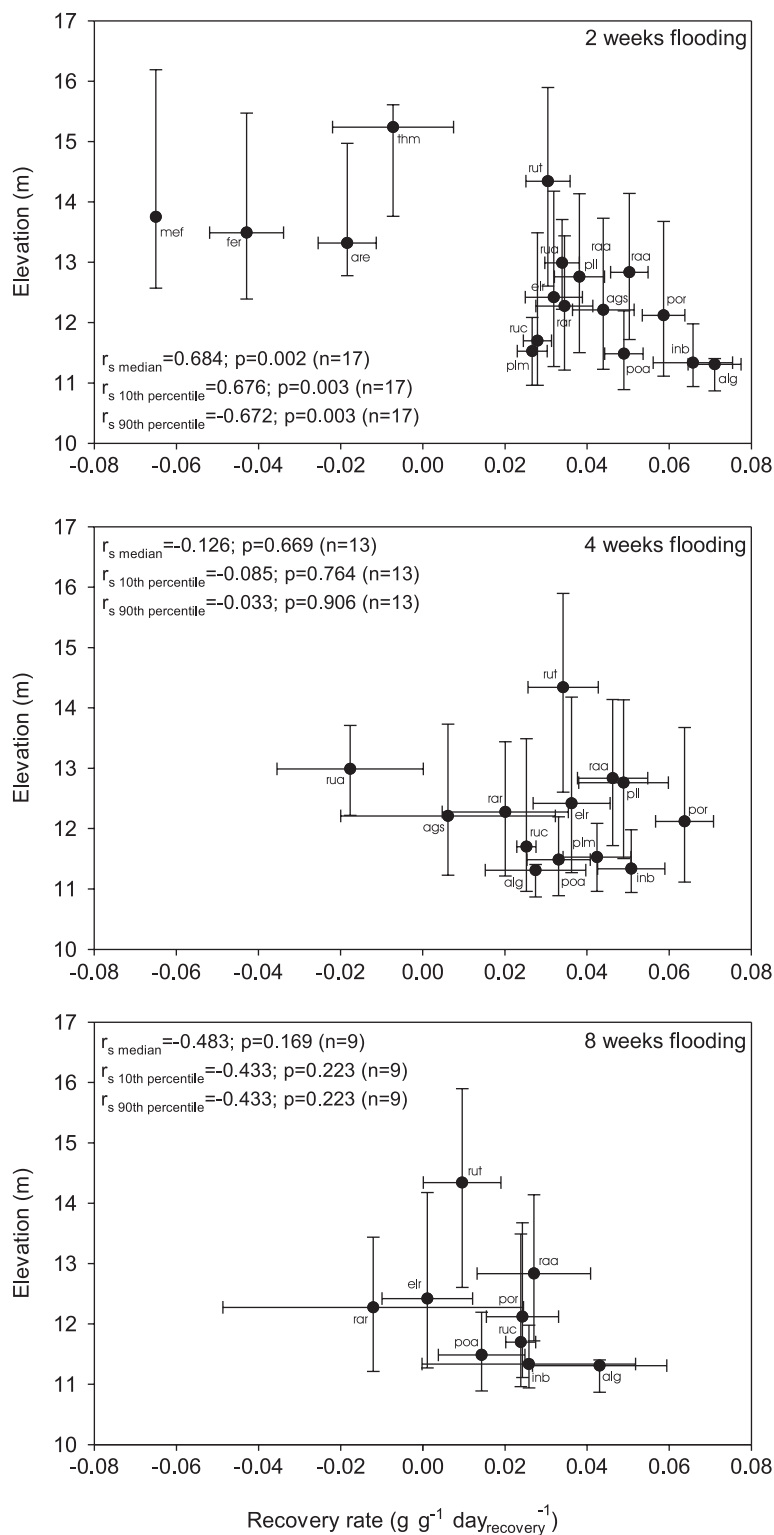
Fig. 5. Biomass recovery rates after 2, 4 and 8 weeks of flooding, based on differences between average biomass (ln dry weight) after a three-day and three-week re-growth period (expressed as $g\,g^{-1}\,day_{recovery}^{-1} \pm SE$). For species abbreviations see Table 1.

Table 3. Correlation matrix concerning relations between flooding characteristics. Distribution along the flooding gradient (median, 10th and 90th percentile values), survival (LT_{50}), biomass reduction rate during submergence (Reduction rate) and biomass recovery after 2 weeks submergence (Recovery cap.$_{2\ wks}$). Correlations for biomass recovery after 4 and 8 weeks flooding are omitted because no significant relations were found. All correlations were based on 17 replicates. * P <0.05; ** P <0.01; *** P <0.001; § 0.05 <P < 0.1.

	Median	10th perc	90th perc	LT_{50}	Reduction rate
median	–	–	–	–	–
10th perc	0.980***	–	–	–	–
90th perc	0.939***	0.921***		–	–
LT_{50}	−0.667**	−0.690**	−0.559*	–	–
Reduction rate	−0.417§	−0.451§	−0.309	0.762***	–
Recovery cap.$_{2\ wks}$	−0.684**	−0.676**	−0.672**	0.639**	0.313

more than 60 days. For four species LT_{50} was conservatively set at 60 days. However, for *Rumex crispus* it is known that LT_{50} far exceeds 60 days (Vervuren et al. 2003). Therefore, if actual values for LT_{50} were known, correlation coefficients and coefficients of determination would probably be higher as all four species occur at low elevations. Relatively flood-tolerant species occurred mainly at low floodplain elevations while more sensitive species were restricted to high parts of the floodplain gradient. The LT_{50} appeared to be the most important factor explaining species distribution along flooding gradients. Adding species biomass reduction rate during flooding or the recovery capacity after de-submergence to a step-wise regression model did not increase the regression coefficient.

In particular, species with low tolerances to flooding determine the zonation patterns in the field. Some broad ranged species, such as *Elytrigia repens*, can be found along the whole gradient. Not only the lower but also the upper distribution limits (90th percentile values) were significantly correlated to the species' flooding tolerance in terms of LT_{50} (Table 3). This relationship suggests the occurrence of a trade-off between a species' flooding tolerance and the performance under non-flooded conditions (Keddy 1984, Crawford 1992) with competitive ability or drought tolerance determining the upper distribution limits (Blom et al. 1994). Species such as *E. repens*, which occurs along the whole flooding gradient, is probably adapted to conditions at low as well as high elevations of the flooding gradient.

Our results suggest that disturbance by floods during the growing season may be an important determinant of the lower distribution boundaries of plant species in river floodplains. However, it should be noted that we only measured the response of adult plants and may therefore not completely account for the distribution patterns observed in the field. A complete investigation of life-history patterns should be conducted to fully understand the mechanisms behind zonation patterns along flooding gradients.

The last major summer flood in our study area took place in 1987, about 12 years before the species distributions reported in this study were recorded. Before this event, long lasting summer inundation's occurred in 1980 and in 1983 (Sykora et al. 1988, Vervuren et al. 2003). It is striking, that in spite of the relatively long period without prominent summer floods, species flooding tolerance (LT_{50}) is still significantly correlated with the lower distribution boundaries (10th percentile) along the flooding gradient, suggesting that lower vegetation boundaries remain relatively stable, even during longer periods without severe summer floods.

This observation may be explained by two possible mechanisms. Firstly, in spite of more severe effects of summer floods on plant survival, the more common winter floods may also contribute to the maintenance of relatively stable zonation patterns by preventing flood intolerant species to migrate to lower regions of the flooding gradient. However, in a subsequent comparative study we have shown that tolerance to winter floods is not correlated with distribution patterns in the field (Van Eck et al., unpubl.). Although other studies have shown that summer flood sensitive species are less affected by winter floods (Klimesova 1994, Siebel 1998, Van Eck et al., unpubl.), which is due to lower respiration rates at lower water temperatures in the winter season (Andrews and Pomeroy 1989, Van Eck et al., unpubl.), their higher frequency may maintain the distribution as shaped by more dramatic summer floods. Secondly, dispersal limitation of high-elevated species may also restrict or slow down recruitment at low parts of the floodplain gradient. Recruitment limitation may thus be an important mechanism behind the delayed migration of high-elevated species to low floodplain sites during periods without summer floods (Bischoff 2000, Van Eck et al., unpubl.).

Biomass reduction and recovery

As expected, flood tolerant, low elevated species, had high recovery rates after de-submergence following two weeks of flooding, while the more flood sensitive, high elevated species, recovered to a lesser extent or lacked the capacity to recover at all. Flood sensitive species lost 3–25% of their biomass per day during submergence. Such species probably suffer from oxygen stress and

apparently do not possess sufficient adaptations, like under-water photosynthesis (He et al. 1999, Vervuren et al. 1999) or aerenchyma formation (Visser et al. 1997, Jackson and Armstrong 1999) to ensure oxygen and carbohydrate supply to their tissues. Some flood sensitive species did not recover at all after de-submergence but further lost biomass, e.g. *Festuca rubra*, *Medicago falacata* and *Thalictrum minus*. Re-exposure to oxygen after a period of oxygen deprivation may lead to post anoxic injury in such species. Post-anoxic stress is known for a wide variety of plant species and can lead to serious damage to plants (Crawford and Brändle 1996, Nabben 2001).

For none of the flooding durations the recovery capacity was significantly correlated with the biomass reduction rate. The recovery rate after two weeks of flooding was significantly and positively correlated with the relative growth rate (RGR) under drained conditions, as determined in this experiment ($r_s = 0.57$, $p < 0.05$, $n = 16$). With the rate of biomass loss linked to flooding adaptations and the biomass recovery rate connected with growth rate, these two traits seem to have a different physiological basis, which may explain their independence.

For the majority of flood tolerant species the recovery rate after de-submergence remained the same for all flooding durations, indicating that the relative ability to recover from flooding was not compromised by increasing flooding duration. The large variation in reduction rate and recovery rate was large among species. This suggests that the more tolerant species had various ways to survive and recover from flooding. Some tolerant species can lose relatively large amounts of their biomass during flooding, probably as a consequence of the change from aerobic to anaerobic metabolism, which is an energetically inefficient process that strongly reduces carbohydrates (Crawford and Brändle, 1996, Vartapetian and Jackson, 1997). Other species, however, may maintain most of their biomass during submergence. For example, a species like *Rumex crispus* is extremely tolerant and may survive complete submergence for two years with little biomass loss (Vervuren et al. 2003). This species is able to perform under-water photosynthesis and therefore able to restore sugar and oxygen supply to all tissues (Vervuren et al. 1999). Surprisingly, *Rumex crispus* had one of the lowest recovery rates, indicating that maintenance of biomass does not necessarily allow a plant to quickly regain growth upon de-submergence.

We conclude that, for the more tolerant species either rapid biomass loss with fast recovery or little biomass loss with slow recovery leads to high survival rates. From an ecological point of view the variable speed with which the species recover after flooding may be less important, because after floods of relatively long duration competition may be strongly decreased (Lenssen et al. 2004).

This underscores the primary role of survival in response to flooding rather than the way in which survival is achieved.

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