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Competition for light as a bottleneck for endangered fen species: an introduction experiment

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Abstract

Many endangered plant species remain absent in rewetted, previously drained fens.
We performed a 3-year introduction experiment with endangered fen species (9 *Carex-* and 6 bryophyte species) in 4 hydrologically restored fens to investigate which factors hamper establishment and survival. *Carex* species were introduced as adults and seedlings, mosses as gametophytes. Introductions were done on (initially) bare soil, which allowed us to exclude excessive competition for light during the first year.
First year survival of the transplants was high in all fens (mean survival = 96%), indicating that there were no direct abiotic constraints on establishment. However, survival analysis revealed that a decrease in relative light intensity (RLI) at the soil surface during consecutive years (indicating an increase in biotic competition for light) drove high mortality rates in most species. As a result, overall final survival was lowest in the two most productive (low light) fens (mean survival = 38%), while most transplants persisted in the two less productive (high light) fens (mean survival = 79%). Taller and faster-growing *Carex* species were able to outgrow light limitation near the soil surface, and thus had a higher overall survivability than smaller and slower-growing species. Light limitation also drove the loss of 5 out of 6 bryophyte species. We conclude that both dispersal limitation and asymmetric competition for light may explain the lack and loss of small and endangered plant species in rewetted fens. A minimum empirical threshold of c. 30% relative light intensity near the soil surface is required for successful introduction.

Keywords: biodiversity, competition, dispersal limitation, fen restoration, light availability

1. Introduction

Fens are groundwater-fed peatlands that are typically covered by plant communities of small sedges (*Carex* spp.) and bryophytes (typically *Sphagnum* at low-pH sites and dominance by species of the Amblystegiaceae and Calliergonaceae in more base-rich sites (Grootjans et al. 2006; Rydin and Jeglum 2013)). In the Northern hemisphere however, many fens have suffered from severe drainage, land use change and degradation (Lamers et al. 2015). Therefore, conservation and restoration of the remaining fens has become a priority in nature conservation (van Diggelen et al. 2006).

Fen restoration is usually focused on hydrological restoration (i.e. the restoration of high water levels and a continuous groundwater discharge with appropriate chemistry) (Mälson et al. 2008; Zak et al. 2010). However, many typical fen species often remain absent at restoration sites despite successful rewetting, and are replaced by more common wetland species (Aggenbach et al. 2013; Mälson et al. 2008; van Dijk et al. 2007). This trend is particularly clear for the smallest and most vulnerable fen species, e.g. *Carex dioica* (L.), *C. limosa* (L.), *C. lepidocarpa* (Tausch), *Tomentypnum nitens* ((Hedw.) Loeske), *Scorpidium scorpioides* ((Hedw.) Limpr.), or *Campylium stellatum* ((Hedw.) C.E.O. Jensen). The marked absence of typical fen species in rewetted fens may have at least three nonexclusive reasons.

First, rewetted fens have a "degradation legacy" in which long-term drainage has deteriorated diaspore banks and extirpated source populations of vulnerable species. Relict populations, if still present, are often heavily fragmented, small or genetically impoverished (de Vere *et al.* 2009; Lamers *et al.* 2015). Hence, spontaneous recolonization of rewetted fens by target species often appears to be hampered by dispersal limitation or limited propagule viability (Cobbaert et al. 2004; Middleton et al. 2006a).

Second, abiotic conditions in rewetted fens have usually been altered in comparison to pristine fens (Aggenbach et al. 2013; Zak et al. 2010), and conditions may have become hostile to some fen species. Fen species may for example be vulnerable to prolonged soil desiccation and concomitant base leaching and acidification (Cusell *et al.* 2013; van Diggelen *et al.* 2015), or they can be negatively affected by high concentrations of potential phytotoxins (e.g. iron (Fe^{2+}), hydrogen sulfide (H_2S), or ammonium (NH_4^+)) that often accumulate upon fen rewetting (Aggenbach et al. 2013; Geurts et al. 2009; Paulissen et al. 2004).

Finally, many rewetted fens are characterized by high macronutrient mobilization rates and nutrient levels (van de Riet et al. 2013; van Dijk et al. 2007; Zak et al. 2010). Although high nutrient availability is primarily also an abiotic filter, macronutrients are not often directly harmful to plants. In fact, it is the concomitant increase in plant primary production, which sets off strong biotic competition for light and growing space, that eventually determines vegetation assembly. Under eutrophic conditions, small and low-competitive species are easily outcompeted by taller and fast-growing competitors (DeMalach et al. 2017; Kotowski et al. 2006; Tilman 1988).

In this study, we performed a species introduction experiment in four rewetted fens to investigate fundamental mechanisms behind the loss of endangered fen species. Our setup allowed us to partially discriminate between direct abiotic constraints on species establishment and vigor, constraints imposed by nutrient-driven competition for light and growing space, and constraints on dispersal and (re-)colonization potential.

2. Material and methods

2.1. Study sites

We selected four fens in the Netherlands: Langstraat (LS: 51°41'1.06"N; 4°58'27.53"E), Holmers (HO: 52°54'9.85"N; 6°37'47.32"E), Drentsche Aa (DA: 53°1'13.88"N; 6°40'10.28"E), and Peizermade (PE: 53°10'5.26"N; 6°30'18.43"E). All fens have been drained in the past and have a history of decades of agricultural use (mainly haymaking). Nowadays, the sites are owned by nature conservation agencies and high groundwater levels were restored > 10 years ago by closing drainage ditches (sites DA, HO and PE) and/or by removing several decimeters of degraded top soil (sites LS and HO). In 2014-2016, groundwater levels in the fens fluctuated between (min) 29.8 cm below and (max) 20.3 cm above soil surface (Appendix Fig. A1). The sites differed in duration of inundations (water level was at or above surface 8% of the time in LS, 34 % in PE, 35 % in DA, and 60 % in HO; Appendix Fig. A1). Deep inundation (> 15 cm) as well as deep water table drawdown (< -20 cm) was uncommon and mainly restricted to winter (max = 3 % of time at HO) and summer (max = 6% of time at HO) respectively. The soils of sites DA and PE were predominantly peat soils (> 40 cm peat), site HO had a shallow (±10 cm) peat layer on top of a silty mineral soil, whereas site LS was covered by peaty sand. Pore water pH ranged between an average of 6.3 (site LS) and 6.7 (site HO), while concentrations of dissolved Ca and HCO_3^- were relatively high at all sites (means > 1.3 and > 2.5 mmol L^{-1} respectively, Table 1). Sites differed substantially in concentrations of dissolved Fe, which were very high in PE (2.21 mmol L^{-1}) and DA (2.26 mmol L^{-1}) and an order of magnitude lower in HO (0.18 mmol L^{-1}) and LS (0.09 mmol L^{-1}). All fens were fully vegetated and followed a gradient in herb peak standing crop (in metric tons ha^{-1}): PE = 5.6 ± 1.1, DA = 4.3 ± 0.7, LS = 2.0 ± 0.9, HO = 1.6 ± 0.6 (means ± SD)). Natural vegetation composition of the herb layer also varied among fens: site DA was dominated by species such as *Carex rostrata* (Stokes) and *Equisetum fluviatile* (L.)*;* site PE by *Carex disticha* (Huds.), *Menyanthes trifoliata* (L.) and *Calamagrostis canescens* ((Weber) Roth), site LS by *Carex lasiocarpa* (Ehrh.)*, Carex oederi* subsp. *oedocarpa* ((Andersson) Lange)*, Pedicularis palustris* (L.)*, Agrostis canina* (L.) and *Hydrocotyle vulgaris* (L.)*,* and site HO was covered by a diverse mixture of wetland species including *Lotus pedunculatus* (Cav.), *Juncus* spp., *Carex nigra* ((L.) Reichard), *Mentha aquatica* (L.), *Galium palustre* (L.) and saplings of *Salix* sp.. Cover of the natural bryophyte layer on average ranged between 35-95 % and was primarily composed of relatively common true moss species such as *Calliergonella cuspidata* ((Hedw.) Loeske) (all sites), *Climacium dendroides* ((Hedw.) F. Weber & D. Mohr) (sites PE and DA), *Brachythecium* sp. (sites HO and DA), *Calliergon cordifolium* ((Hedw.) Kindb.) (site PE), *Philonotis fontana* ((Hedw.) Brid.) (site HO) and *Rhytidiadelphus squarrosus* ((Hedw.) Warnst.) (site DA). Permits for field experiments were granted by Staatsbosbeheer (LS, HO, DA) and Natuurmonumenten (PE).

Table 1: Pore water and soil chemistry at the four study sites (Means ± standard deviations). Pore water values are based on three sampling rounds (growing seasons of 2014, 2015 and 2016) in each of the eight plots per site (n = 24). Soil chemistry values are based on five soil samples per site collected in summer 2016.

	Unit	Site DA		HO		LS		PE	
Pore water		mean	SD	mean	SD	mean	SD	mean	SD
pH	/	6.6	0.1	6.7	0.1	6.3	0.2	6.5	0.2
NO_3^-	$\mu mol\ L^{-1}$	1.1	0.7	0.5	0.6	0.5	0.4	6.0	11.2
NH_4^+	$\mu mol\ L^{-1}$	23.5	32.1	11.0	18.2	35.9	50.6	36.8	47.1
Na	$\mu mol\ L^{-1}$	570.5	56.7	473.3	94.7	873.6	231.3	340.5	72.8
Al	$\mu mol\ L^{-1}$	3.7	1.8	0.6	0.4	4.9	1.7	8.1	4.1
Ca	$\mu mol\ L^{-1}$	1482	335	2435	1567	1359	714	2402	703
Fe	$\mu mol\ L^{-1}$	2257	633	186	177	88	76	2218	1072
K	$\mu mol\ L^{-1}$	45.8	58.4	58.1	73.2	135.9	152.6	50.2	71.4
Mg	$\mu mol\ L^{-1}$	262.9	69.6	256.2	179.0	303.4	184.7	269.6	80.4
Mn	$\mu mol\ L^{-1}$	20.8	11.3	10.7	10.7	9.7	8.5	37.7	20.5
P	$\mu mol\ L^{-1}$	3.4	2.2	2.6	3.3	1.5	0.6	26.9	24.3
S	$\mu mol\ L^{-1}$	19.8	7.2	16.0	7.0	76.6	87.9	76.2	29.6
HCO_3^-	$\mu mol\ L^{-1}$	4405	1417	4263	1543	2500	565	5290	1565
Soil		mean	SD	mean	SD	mean	SD	mean	SD
Bulk density	$kg\ L^{-1}$	0.12	0.01	0.43	0.11	0.33	0.21	0.23	0.09
P	$mmol\ kg^{-1}$	81.2	11.1	80.9	73.1	12.4	6.4	32.8	12.5
P-olsen	$mmol\ kg^{-1}$	4.98	2.58	2.52	0.88	0.65	0.41	1.94	0.26
Ca	$mmol\ kg^{-1}$	146	11	290	91	223	112	151	55
Mg	$mmol\ kg^{-1}$	16.9	1.3	29.2	4.2	25.3	15.1	16.4	2.3
K	$mmol\ kg^{-1}$	22.8	5.1	16.1	1.9	9.5	8.5	12.5	1.9
Fe	$mmol\ kg^{-1}$	1657.6	304.5	721.2	535.2	89.9	44.6	385.7	147.5

2.2. Study species and propagule collection

In 2012 and 2013 we collected ripe seeds of 9 species of typical small- to medium-sized fen sedge (*Carex pulicaris, C. davalliana, C. dioica, C. limosa, C. appropinquata, C. diandra, C. lepidocarpa, C. flava* and *C. chordorrhiza*) and gametophytes of 6 species of bryophyte (*Scorpidium scorpioides*, *S. revolvens*, *Campylium stellatum*, *Tomentypnum nitens*, *Calliergon giganteum*, and *Paludella squarrosa*) (Appendix Table A1). The bryophytes belong to the ecological group of "brown mosses" (representing the Amblystegiaceae, Meesiaceae and Calliergonaceae). All species are typical for well-developed fens in large parts of the Northern hemisphere, and are endangered (red list "vulnerable" or worse) or have disappeared in the Netherlands.

Due to past drainage and deterioration of most fens in the Netherlands (Lamers et al. 2015), many fen species have either gone regionally extinct, or, are only left in small relict populations with limited viability. Therefore, we also collected propagules in other European countries with larger populations (Appendix Table A1). We aimed to minimize the impact of propagule collection: for sedges we collected small amounts of ripe seeds, and for mosses we collected one or two living gametophyte mats (15 cm x 15 cm) per species. The use of gametophyte fragments is a well-established method for bryophyte introductions (Borkenhagen and Cooper 2016; Graf and Rochefort 2010; Mälson and Rydin 2007).

2.3. Preparation of plant material

Carex seeds received a cold-moist stratification treatment in full darkness (4°C) for a minimum of 3 months to break seed dormancy. Next, seeds were germinated on moist filter paper in an incubator under a fluctuating day-night regime (24/15°C, 12/12h photoperiod). Half of the plants were germinated in spring 2013 and transported to a greenhouse nursery: these plants were considered "adult" at the time of introduction into the field in spring 2014 (height 18 ± 7 cm; leaf count 56 ± 30 (mean $\pm$ SD)). The other half of the plants were germinated in spring 2014 and transplanted as "seedlings" in the experiment three weeks later (height 10 ± 4 cm, leaf count 11 ± 6 (mean $\pm$ SD)). We aimed to introduce a total of 32 seedlings and 32 adult plants per *Carex* species, but low seed collection success and low germination rates resulted in a smaller sample size for *C. flava* and *C. chordorrhiza* respectively (Appendix Table A1).

The bryophytes were cultivated in plastic containers (39 cm x 28 cm, height 14 cm) prior to introduction. The containers were filled with a layer of limed clean white sand, and demineralized water was regularly added to keep the sand waterlogged. All species gradually expanded within the containers, with the exception of *Paludella squarrosa* which survived but did not expand. Before introduction into the field, the bryophyte mats were separated into 32 smaller mats per species (20.3 ± 9.3 cm^2; mean $\pm$ SD). The limited growth of *Paludella squarrosa* resulted in only 12 mats for this species.

2.4. Field experiment

Mid-April 2014, all individual sedges (268 seedlings and 235 adults) and 172 bryophyte mats were equally divided and introduced at the four study sites. At each site we selected an area of 4.5 m x 9.5 m in which 8 experimental (main) plots of 1 m x 1 m were established (Fig. 1). The experimental area was unmown and not grazed by livestock for the duration of the experiment.

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Preparation of the experimental plots was as follows: first, we cut the existing vegetation. To prevent rapid resprouting from the clipped vegetation, we manually removed the top 10 cm of the soil (in sods), tilted the sods upside down, and then pressed the sods back into the hole. The outer 25 cm edge of each plot was covered with root canvas. By doing so, we created a 0.5 m x 0.5 m patch of bare light-saturated soil without leaving a depression. The reasoning for starting from a patch of bare soil rather than introducing plants into existing (closed) vegetation was because we wanted to separate potential direct negative effects of the abiotic environment (abiotic filtering) from indirect negative effects of competition for light and growing space with an (existing) herb layer. By the initial removal of biotic competition, we were thus able to temporarily delay competition-induced mortality.

Each experimental plot was further subdivided in 25 (10 cm x 10 cm) sub-plots (Fig. 1). As the primary aim of our study was to investigate fundamental mechanisms behind the lack and loss of endangered fen species on the plant-level (individual-based approach), we introduced and monitored individual plants rather than clumps of several individuals. Individual plants were therefore assigned to each sub-plot so that each main plot always contained 2 individuals (1 seedling and 1 adult) of each sedge species and 1 gametophyte mat of each bryophyte species. Sedges were planted directly into the soil, and bryophyte mats were simply pressed on top of the soil. A small wooden label was pushed into the soil at 1 cm from each individual plant to facilitate future recovery.
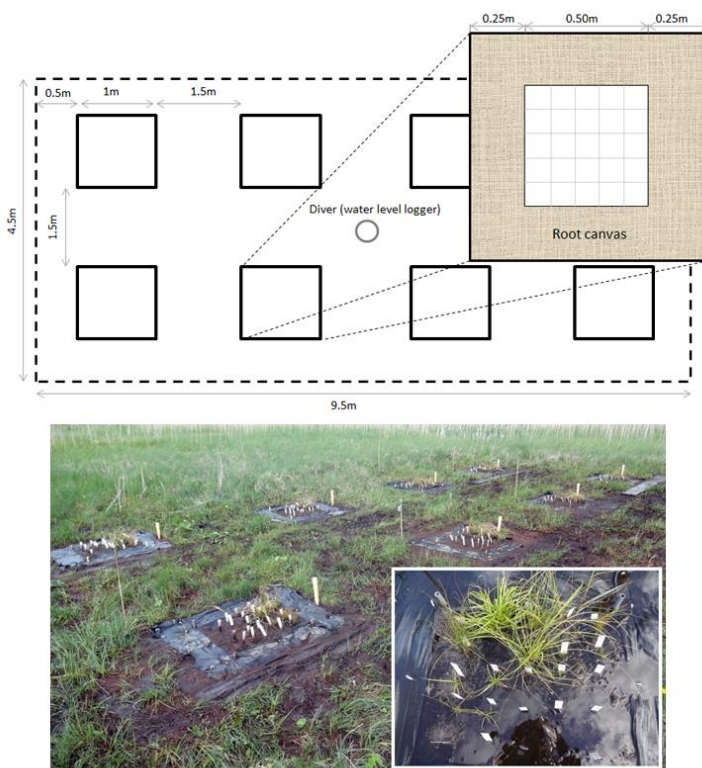
Figure 1: Experimental design of the field experiment. A total of eight (1 m x 1 m) experimental (main) plots was established at each of the four study sites. Each experimental plot was further subdivided in 25 (10 cm x 10 cm) sub-plots in which one individual plant (sedge or moss) was introduced. A wooden label was placed next to each individual plant.

2.4.1. Plant performance and species richness

The field experiment was monitored six times in total divided over three growing seasons: April 2014 (week 0), May 2014 (week 3), August 2014 (week 16), May 2015 (week 54), August 2015 (week 68), and July 2016 (week 111). We checked survival of each individual sedge or bryophyte mat: the criterion for plant survival was the presence of photosynthetically active (green) tissue. For surviving sedges, we additionally measured the height (cm) of each individual plant (from the base of the stem to the highest living leaf, excluding inflorescences). At each visit we also recorded total species richness per main (1 m x1 m) plot, including both introduced target species as well as naturally (re-)appearing non-target species.

2.4.2. Abiotic conditions

We collected pore water samples in all main plots once per growing season (2014, 2015 and 2016) using MacroRhizon samplers (filter size = 0.15 µm, Rhizosphere Research Products, The Netherlands). pH of the pore water was measured directly in the field using portable equipment (WTW Multi 340i, WTW, Weilheim, Germany). Total inorganic carbon (TIC) was determined on an infrared gas analyzer (ABB Advance Optima): HCO_3^- concentrations were calculated based on pH. Concentrations of NH_4^+ and NO_3^- were determined on an Auto Analyzer 3 System (Bran + Luebbe). Pore water subsamples were acidified with 0.7 mL 65% suprapure HNO_3 per 100 mL sample and analyzed on ICP (IRIS Intrepid II) for total dissolved concentrations of Ca, Mg, K, Na, Fe, Mn, P, and S, and Al. In July 2016, we collected five soil samples (10 cm x 10 cm x 10 cm) of the top soil per site. Samples were dried (72 h at 70 °C) and weighed. 200 mg of soil was digested with 4 mL HNO_3 (65%) and 1 mL H_2O_2 (30%) using a microwave labstation (Milestone srl) to measure total Ca, Mg, K, P, and Fe with ICP. Plant-available P was extracted according to Olsen et al. (1954). Soil and pore water data are summarized per site in Table 1.

We determined relative light intensity (RLI) near the soil surface of each plot at each visit using an Accupar LP-80 ceptometer (Decagon Devices Inc., Pullman, WA, USA), which measures photosynthetically active radiation (PAR, in µmol m^{-2} s^{-1}). In short, a horizontal bar fitted with multiple PAR sensors was horizontally inserted just above the soil or moss surface below the herb layer, while a reference light sensor was simultaneously logging the amount of incoming PAR at approximately 1 m above the vegetation. Relative light intensities (in % PAR) at soil surface level can then be calculated. This we repeated in three positions per main plot, and we averaged the values to attain one representative RLI value per plot. RLI below the herb layer is a good proxy for the amount of biotic competition for light: low RLIs indicate that most incoming PAR is captured and utilized by the vegetation, and are typical below a dense and highly productive herb layer (Kotowski and van Diggelen 2004).

2.5. Data analyses

All analyses were performed in R 3.2.3 (R Development Core Team 2017). Significance was accepted at $p < 0.05$.

We analyzed plant survival in two ways using the R package *survival* (Therneau and Lumley 2016). First, we investigated differences in plant survival between the three plant functional groups (adults and seedlings of *Carex*, bryophytes) among the four study sites (LS,

HO, DA and PE), regardless of species. Second, we analyzed survival of the plants grouped per species versus RLI, regardless of plant functional group or study site. We chose this approach as one survival model that includes all factors (Survival versus site, functional group, species and RLI) was statistically not feasible. Differences in survival distributions of the three plant groups among study sites were tested with log-rank tests: results were visualized with Kaplan-Meier curves. Cox Proportional Hazard models (CoxPH) were used to test for the effect of (final) RLI on survival probabilities of the 15 different species. We report the hazard ratio as a parameter that quantifies survival: hazard ratios < 1 indicate decreasing death risk with increasing RLI whereas hazard ratios > 1 indicate increasing death risk with increasing RLI. In addition to CoxPH, we used logistic regression (logit-link) to estimate the survival probability of each species at the end of the experiment versus RLI, in which survival (value = 1) or death (value = 0) of an individual plant was plotted against the corresponding RLI-value of the experimental plot. We did this to extract inflection points for all species, i.e. the RLI below which survival probability of a species was < 50 %.

Finally, we calculated two proxies that define *Carex*-species morphology and growth strategy, which we compared with survival fractions of the species at the end of the experiment: (1) maximum adult height (cm) and (2) seedling relative height increment rate (cm week^{-1}). Maximum height was defined as the mean height of the 25% tallest (living) individuals of the species at the end of the experiment, height increment rate is the growth of (living) sedges during week 3-16, excluding the first three weeks (week 0-3) to overcome potential negative effects of transplantation. As survival fractions are bounded between 0 and 1, we used Beta-regression (with logit-link) (Cribari-Neto and Zeileis 2010).

3. Results

### 3.1. RLI, species richness and survival probabilities

RLI averaged about 87 % at the start of the experiment and gradually decreased over time (Fig. 2a). At the end of the experiment, RLI was still relatively high for sites LS and HO (> 30 %) and much lower for sites DA and PE (< 15 %). The gradual decrease in RLI corresponded with an initial increase in total species richness (including the introduced species, Fig. 2b). The initial increase in species richness was followed by a strong decrease at sites DA and PE (the sites with the lowest final RLI), so that final species richness was highest at sites LS and HO (the sites with the highest final RLI).

Survival distributions of the introduced plants across sites followed similar patterns for all three functional groups: survival was very high during the first growing season (week 0-16, mean survival = 96%), but then decreased with time from the second growing season onwards (Fig. 3). Overall survival of adult plants, seedlings and bryophytes was highest in sites LS and HO (mean overall survival = 79 %) and lowest in sites PE and DA (mean overall survival = 38%) (Fig. 3abc, Appendix Table A2).

Survival distributions also differed among the three functional groups: overall, adult plants had a higher survival than seedlings and bryophytes (log-rank χ^2 = 16.8, df = 1, p < 0.001 and χ^2 = 20.8, df = 1, p < 0.001 respectively), but seedlings and bryophytes had equal survival probabilities (log-rank χ^2 = 0.1, df = 1, p = 0.72). Final survival percentages per species per site can be found in Appendix Table A3.

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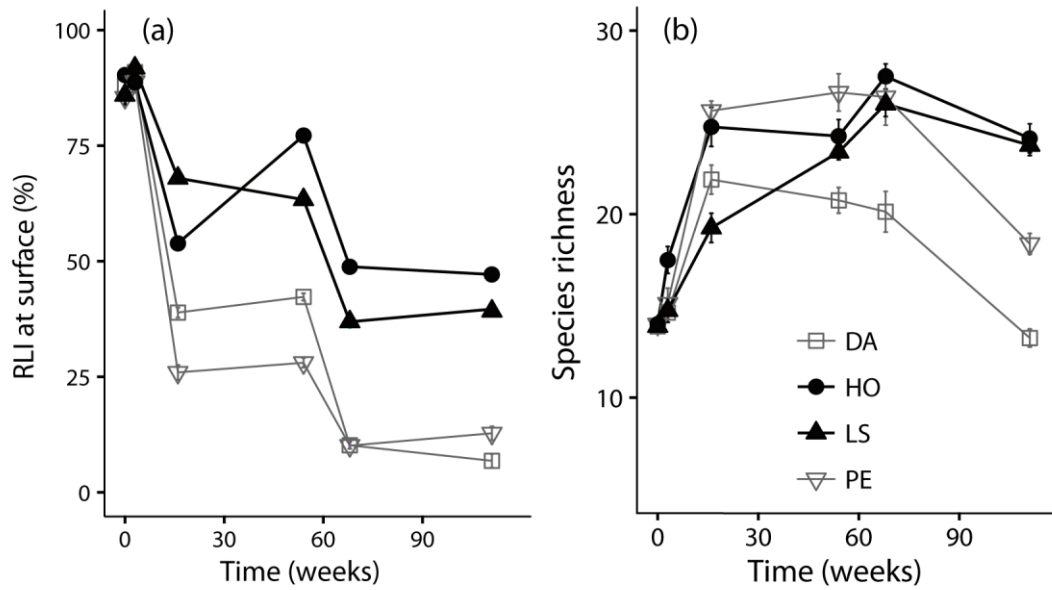
Figure 2: Evolution of (a) relative light intensity (RLI in %) and (b) total species richness in the main experimental plots. Data are grouped per study site (HO, LS, PE and DA), vertical bars represent standard errors (n=8). Total species richness includes both the introduced species as well as naturally re-appearing species.
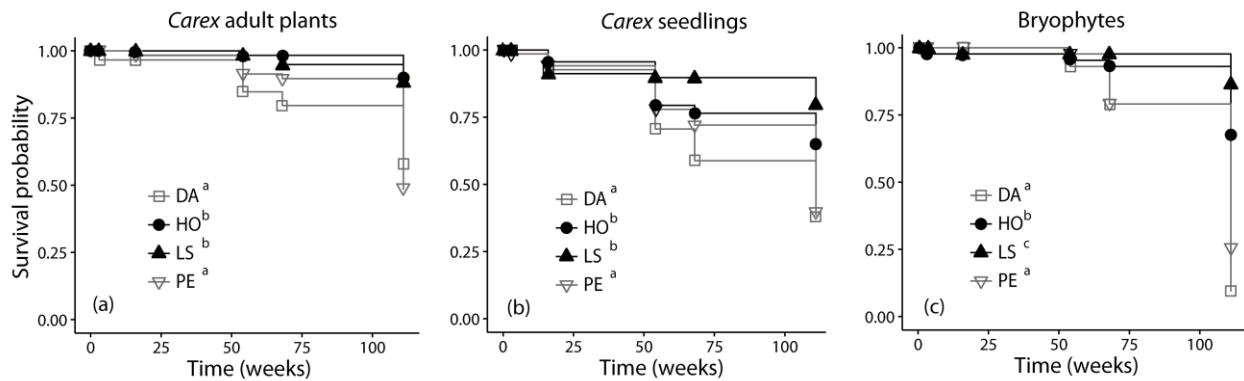

Figure 3: Kaplan-Meier survival curves of individual (a) adult *Carex* plants (9 species), (b) *Carex* seedlings (9 species), and (c) bryophytes (6 species) through time. Data are grouped per study site (HO, LS, PE and DA), superscripts (abc) indicate significant differences between groups ($P < 0.05$).

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3.2. Species survival versus RLI and species morphology

CoxPH revealed that 11 out of 15 of the introduced species (6 *Carex* and 5 bryophyte species) had a higher survival probability with increasing RLI (Hazard ratios < 1, Table 2). Of the remaining three *Carex* species, two species (*C. appropinquata* and *C. diandra*) had a lower (albeit non-significant) survival probability with increasing RLI (Hazard ratio > 1, Table 2), and one species (*C. flava*) had a higher but non-significant survival probability with increasing RLI. For the bryophytes, we found no significant effect of RLI on *Paludella squarrosa*: 10 out of 12 individual mats had eventually disappeared regardless of RLI.

For the species that were significantly affected by RLI, logistic regression models further revealed the RLI-inflection points per species (i.e. RLI at which the species' survival probability equals 50 %), which varied between 0 and 50.5 % (Table 2, Appendix Fig. A2).

Overall final survival of the *Carex* species (cumulative of the four study sites) was higher for tall (Fig. 4a, Appendix Table A4) and fast-growing species (Fig. 4b, Appendix Table A4) than for small, slow-growing species. In accordance, the two *Carex* species that were not negatively affected by low RLI (*C. appropinquata* and *C. diandra*, Table 2) were also the tallest species with highest relative height increment rates (Fig. 4). Tall *Carex* species tended to have higher relative height increment rates, but the correlation between species increment rate and maximum height was non-significant ($r_s = 0.60$, df = 7, p = 0.09).
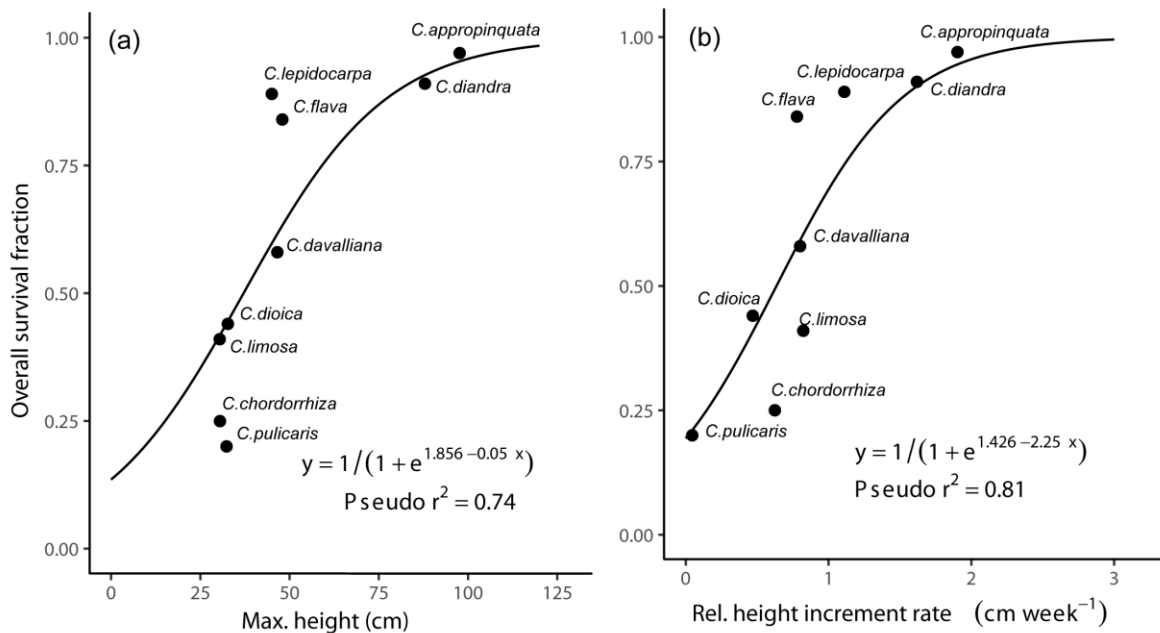
Figure 4: Overall survival fraction per *Carex* species at the end of the experiment (four study sites combined) in relation to (a) maximum height (cm) and (b) relative height increment rate (cm week-1) of the corresponding species. Maximum height is the mean height of the 25% tallest (living) individuals per species at the end of the experiment, relative height increment is the net height increment of seedlings during the first growing season after introduction (13 weeks). Models were fitted using Beta-regression (logit-link).

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Table 2: Results of Cox Proportional-Hazard models (hazard ratios) and Logistic Regression models (odds ratios) of survival probability of the introduced plants (grouped per species) versus relative light intensity (RLI, in %). For CoxPH: Hazard ratios <1 indicate decreased hazard (longer survival) with increasing RLI, hazard ratios > 1 indicate increased hazard (shorter survival) with increasing RLI. Inflection points (50%-survival thresholds) are also indicated (in % RLI). Asterisks indicate significance with *p < 0.05, **p < 0.01, *** p < 0.001.

	CoxPH Survival	Logistic regression	
	Hazard ratio	Odds ratio	Inflection point (RLI (%))
Carex pulicaris	0.98**	1.08**	50.5
Carex davalliana	0.97**	1.05**	19.0
Carex dioica	0.94***	1.12***	30.7
Carex limosa	0.94***	1.11***	33.2
Carex appropinquata	1.06	0.94	NA
Carex diandra	1.00	1.00	NA
Carex lepidocarpa	0.93*	1.09*	0.0
Carex flava	0.85	1.24	NA
Carex chordorrhiza	0.96**	1.12*	44.5
Scorpidium scorpioides	0.94***	1.10***	37.8
Scorpidium revolvens	0.91***	1.14***	23.7
Campylium stellatum	0.95***	1.08**	40.9
Tomentypnum nitens	0.93**	1.11**	19.4
Calliergon giganteum	0.95*	1.07*	10.8
Paludella squarrosa	0.98	NA	NA

4. Discussion

We delayed (excessive) biotic competition in four rewetted post-agricultural fens by initiating species introduction on patches of bare light-saturated soil. Hence, we were able to evaluate target species survival while partially discriminating between direct short-term effects of the abiotic environment and indirect biotic effects: the underlying assumption is that rapid (first-year) mortality would be related to an unfavorable or hostile abiotic environment, whereas delayed (second- and third-year) mortality would primarily be related to increasing biotic competition for light and growing space.

4.1. Direct abiotic constraints

We conducted our experiment in four different fens, each characterized by a unique set of abiotic conditions (Table 1). At least two direct abiotic constraints may fundamentally hamper the development of small sedge and brown moss communities in rewetted fens. First, water levels have a large effect on vegetation assembly in fens (Vitt et al. 2016), and there are differences in optimal hydrological niches among fen species (Borkenhagen and Cooper 2016). Nonetheless, relatively high and stable water levels are a general prerequisite, and prolonged deep desiccation may directly cause death or limited fen species performance (Manukjanova et al. 2014). Also, prolonged water table drawdown can indirectly limit survival of fen species: concomitant oxidation reactions lead to H^+ production, acidification and base cation leaching (Lamers et al. 1998; Van Haesebroeck et al. 1997), and this often induces a shift from rich fen species towards fen meadow species or *Sphagnum* dominance (Kooijman et al. 2016; Soudzilovskaia et al. 2010). However, since our study sites had been successfully rewetted in the past, groundwater levels were within the range for small sedge and brown moss communities (Goebel 1996), and pore water pH (> 6), HCO_3^- (> 2 mmol L^{-1}) and Ca (> 1 mmol L^{-1}) concentrations were sufficiently high (Table 1). Water levels, pH and base availability are therefore unlikely to have had a negative impact on survival in our experiment.

      Second, rewetted fens can be rich in potential phytotoxins that can hamper survival. For instance, high concentrations of H_2S and NH_4^+ as well as reduced forms of metals such as Fe^{2+} and Mn^{2+} can cause necrosis and death within weeks (Geurts et al. 2009; Paulissen et al. 2004; Snowden and Wheeler 1993). Two study sites (DA and PE) were characterized by pore water concentrations of dissolved iron that were far above potentially phytotoxic thresholds (> 2 mmol Fe L^{-1}) (Aggenbach et al. 2013; Snowden and Wheeler 1993). However, a very high first year survival of the introduced sedges and mosses at all sites (mean survival = 96 %) rules out phytotoxicity as a relevant factor in our experiment. Overall, these results suggest that abiotic conditions were, at least in the short term, largely within the target species' fundamental niches at all sites.

4.2. Biotic constraints

Species can be absent simply because they are unable to naturally (re-)colonize a fen after hydrological restoration (Cobbaert et al. 2004; Middleton et al. 2006a). The role of dispersal limitation in an area can easily be verified: if manual species introductions are successful in the long term (i.e. species can establish, propagate and eventually form a healthy population), then

this is a solid indicator for dispersal limitation for those particular species in that area. The high survival rates that we observed at two sites (LS and HO) support the hypothesis of dispersal limitation as a bottleneck, as least for those two sites.

As time progressed, the introduced plants grew taller while the pre-existing natural vegetation gradually recolonized the (initially) bare soil experimental plots. As a result, biotic competition for light and growing space increasingly affected performance of the introduced plants, eventually constraining their survival. Survival analysis showed that 11 out of the 15 introduced fen species were negatively affected by low relative light intensities at soil surface level (RLI). For the *Carex* species, the only three species that were not significantly affected by RLI were also the tallest species with more rapid growth and high overall survival (*Carex flava, C. diandra,* and *C. appropinquata*). It is likely that taller and faster-growing species were simply able to outgrow light limitation that is typically found near the soil surface of productive sites, thereby producing even more shade for the smaller species which seemed to lack the competitive ability to cope with light stress. This pattern confirms the theory that interspecific competition is dependent on species-specific traits and is therefore highly asymmetrical (DeMalach et al. 2017; Gaudet and Keddy 1988). The RLI-inflection points of the *Carex* species that were negatively affected by light limitation, i.e. the RLI value at which the species' survival probability drops below 50 %, varied between 0 % (*C. lepidocarpa*) and 50.5 % (*C. pulicaris*) with an average of 29.7 %.

For the bryophytes, 5 out of 6 species were negatively affected by low RLI values. Bryophytes generally cannot compete for light with a herb layer due to obvious morphological constraints, and therefore a too dense herb layer is assumed to be a bottleneck for many bryophyte species (van der Wal et al. 2005). The RLI-inflection points of the bryophyte species varied between 10.8 % (*Calliergon giganteum*) and 40.9 % (*Campylium stellatum*), with an average of 26.5 %. These results imply that endangered bryophytes and small sedges generally disappear below values of 25-30% RLI at soil surface level, which corresponds with an estimated herb standing crop that exceeds 4 metric ton ha^{-1} (Emsens et al. 2015).

The loss of one moss species (*Paludella squarrosa*) could not be explained by light limitation, as it also largely disappeared in the low-productivity fens. Although the lack of statistical significance in this species may be related to a limited sample size (n = 12) or poor vitality at the moment of introduction, the decline of this species may also be linked to other factors. *Paludella squarrosa* is at its southern distribution limit in the Netherlands, so the failure may have macro-climatic reasons. We did not investigate which exact plant-functional mechanisms explain the negative impact of low RLI on plants' performance, but it is most likely that direct photosynthetic constraints imposed by the limited availability of photosynthetically active radiation in the lower herb and moss layer of productive fens explains the low survival.

4.3. Study limitations

Some limitations of our experiment need to be addressed. First, we did not study germination and establishment success from seeds or sporophytes. Moreover, plants were deliberately introduced on (initially) bare soil but not within dense existing vegetation, meaning that we created priority effects that may have favored early survival. Hence, we can draw no conclusions with respect to germination, nor to establishment success within existing vegetation. Second, we monitored survival over a relatively short time span. This implies that an equilibrium in vegetation structure and composition within the experimental plots has probably not yet been reached, and the final

outcome of the experiment may still change with time. Also, long-term survival can be affected by other factors as well. For example, irregular climatic extremes such as extreme drought can also cause local extinctions of vulnerable species. Such extremes have for example been shown to impact species distributions in other ecosystem types (Stampfli and Zeiter 2004; Vervuren et al. 2003). Third, we did not study genotypic variation among donor populations although genetic within-species differences could affect reintroduction outcomes (Zedler 2000). This may have blurred outcomes of this experiment as we used donor material from a variety of climatologically distinct regions. Finally, our study took place in post-agricultural, largely vegetated and rewetted fens. Therefore, our results may not be applicable to fens that are in a different stage of degradation, for example mined and cut-over peatlands in which physical abiotic constraints such as frost heave may play an additional role (Cooper and MacDonald 2000).

4.4. Recommendations for fen restoration and conservation

We identified dispersal limitation and excessive competition for light as two potentially important driving mechanisms behind the lack of endangered species in rewetted fens with a history of agricultural use. Although dispersal limitation can be lifted by targeted (re-)introductions, as also shown by various other studies (Cooper and MacDonald 2000; Mälson and Rydin 2007; Middleton et al. 2006a; Vitt et al. 2011), light limitation due to a too productive herb layer is more difficult to tackle. A solution to increase light availability in degraded post-agricultural fens may be to completely remove the eutrophied top soil prior to introduction (Emsens et al. 2015), or to suppress the productivity of the herb layer and gradually deplete nutrient pools by regular mowing (Hajkova et al. 2009; Middleton et al. 2006b).

Overall, we emphasize that reintroductions of low-competitive fen species are only promising if certain conditions are met. First, abiotic conditions should be directly suitable for target species, i.e. relatively stable groundwater levels near the soil surface and a sufficiently high base cation availability and pH ($pH_{pore\ water} > 6$). Second, nutrient availability and concomitant competition for light should be sufficiently low to allow long-term survival of vulnerable fen species. As a rule of thumb for small sedge and true moss communities we propose a minimum empirical threshold of 30 % RLI at soil surface level, which corresponds with an estimated herb standing crop < 4 metric ton dry weight ha^{-1}.

Data accessibility

Data have been archived in the Mendeley Data repository

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Appendix A

Table A1: *Carex* and bryophyte species used in the introduction experiment with country of propagule collection (Sw = Sweden, Sl = Slovakia, Pl = Poland, Nl = The Netherlands, Sp = Spain, Pyrenees). Numbers (N) represent the total number of introduced individuals (all sites combined).

Carex species	N adult plants	N seedlings	Donor country
Carex pulicaris (L.)	32	32	Sw
Carex davalliana (Sm.)	32	32	Sl, Pl
Carex dioica (L.)	31	32	Sw
Carex limosa (L.)	32	32	Sw
Carex appropinquata (Schumach.)	32	32	Nl, Sw
Carex diandra (Schrank)	32	32	Sp
Carex lepidocarpa (Tausch)	32	32	Pl, Sp
Carex flava (L.)	0	32	Sl, Pl
Carex chordorrhiza (Ehrh. ex L.f.)	12	12	Sw
Total	235	268	
Bryophyte species	N bryophyte mats		
Scorpidium scorpioides ((Hedw.) Limpr.)	32		Nl
Scorpidium revolvens ((Sw.) Rubers)	32		Sw
Campylium stellatum ((Hedw.) C.E.O. Jensen)	32		Nl
Tomentypnum nitens ((Hedw.) Loeske)	32		Sl, Sw
Calliergon giganteum ((Schimp.) Kindb.)	32		Sl, Sw
Paludella squarrosa ((Hedw.) Brid.)	12		Sw
Total	172		

Table A2: Comparison of survival distributions between different study sites (DA, HO, LS and PE) within functional groups (adult plants, seedlings, bryophytes). Values indicate χ^2 values from a long-rank test (df = 1). Asterisks indicate significant differences with *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$.

	Adult (*Carex*)			Seedlings (*Carex*)			Bryophytes		
	Survival			Survival			Survival		
Study site	HO	LS	PE	HO	LS	PE	HO	LS	PE
DA	16.5***	13.9***	0.20	8.9**	23.2***	0.50	27***	47.5***	2.0
HO		0.10	23.4***		3.50	6.4*		4.2*	14.4***
LS			19.1***			20.2***			31.0***

Table A3: Percentage final survival of introduced sedges (*Carex* spp.) and bryophyte mats at each of the four study sites (DA, HO, LS and PE), grouped per species.

|	Site			
Species	**LS**	**HO**	**PE**	**DA**
Carex pulicaris	25.0	50.0	0.0	6.3
Carex davalliana	100.0	62.5	25.0	43.8
Carex dioica	93.8	68.8	0.0	12.5
Carex limosa	87.5	68.8	6.3	0.0
Carex appropinquata	100.0	87.5	100.0	100.0
Carex diandra	75.0	100.0	93.8	93.8
Carex lepidocarpa	100.0	100.0	75.0	81.3
Carex flava	100.0	100.0	75.0	62.5
Carex chordorrhiza	66.7	33.3	0.0	0.0
Scorpidium scorpioides	100.0	37.5	0.0	0.0
Scorpidium revolvens	100.0	87.5	12.5	12.5
Campylium stellatum	100.0	25.0	0.0	0.0
Tomentypnum nitens	75.0	100.0	37.5	25.0
Calliergon giganteum	87.5	87.5	87.5	12.5
Paludella squarrosa	0.0	66.7	0.0	0.0
Total	84.0	74.0	38.7	37.3

Table A4: Coefficients (± SE) of the two beta-regression models (logit-link) for maximum height of the species (model 1) and relative height increment rate of the seedlings (model 2). Asterisks indicate statistical significance with **p < 0.01, *** p < 0.001.

	Model coefficient (logit link)	SE
Model 1		
Intercept	-1.856**	0.622
Maximum height (cm)	0.050***	0.013
Model 2		
Intercept	-1.426**	0.472
Relative height increment rate (cm week^{-1})	2.245***	0.524
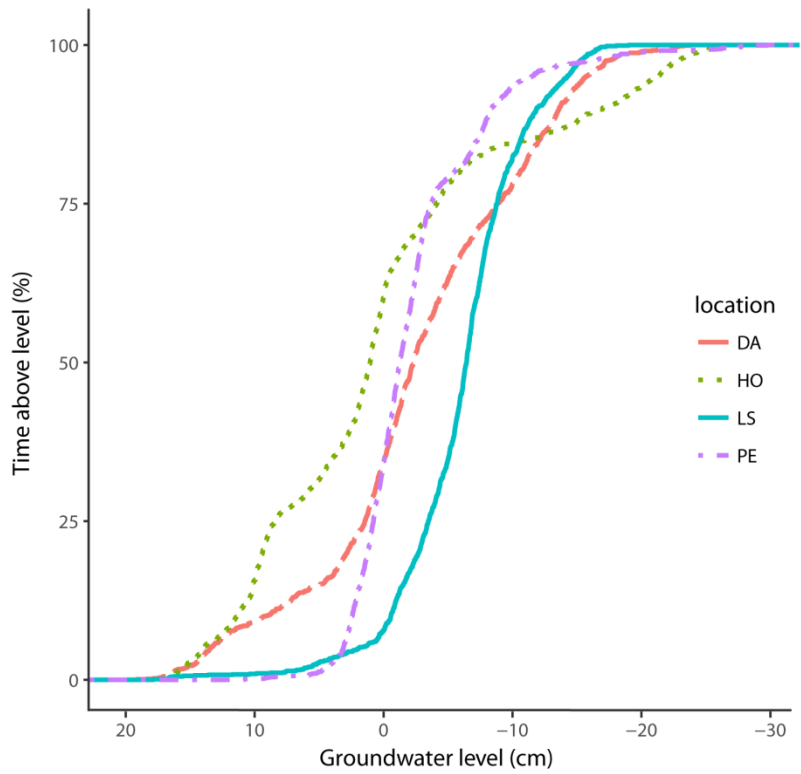
Figure A1: Cumulative relative frequency curves of groundwater levels at the four study sites (DA, HO, LS and PE). Soil surface is at zero cm. Data were collected over three consecutive years (2014-2016).
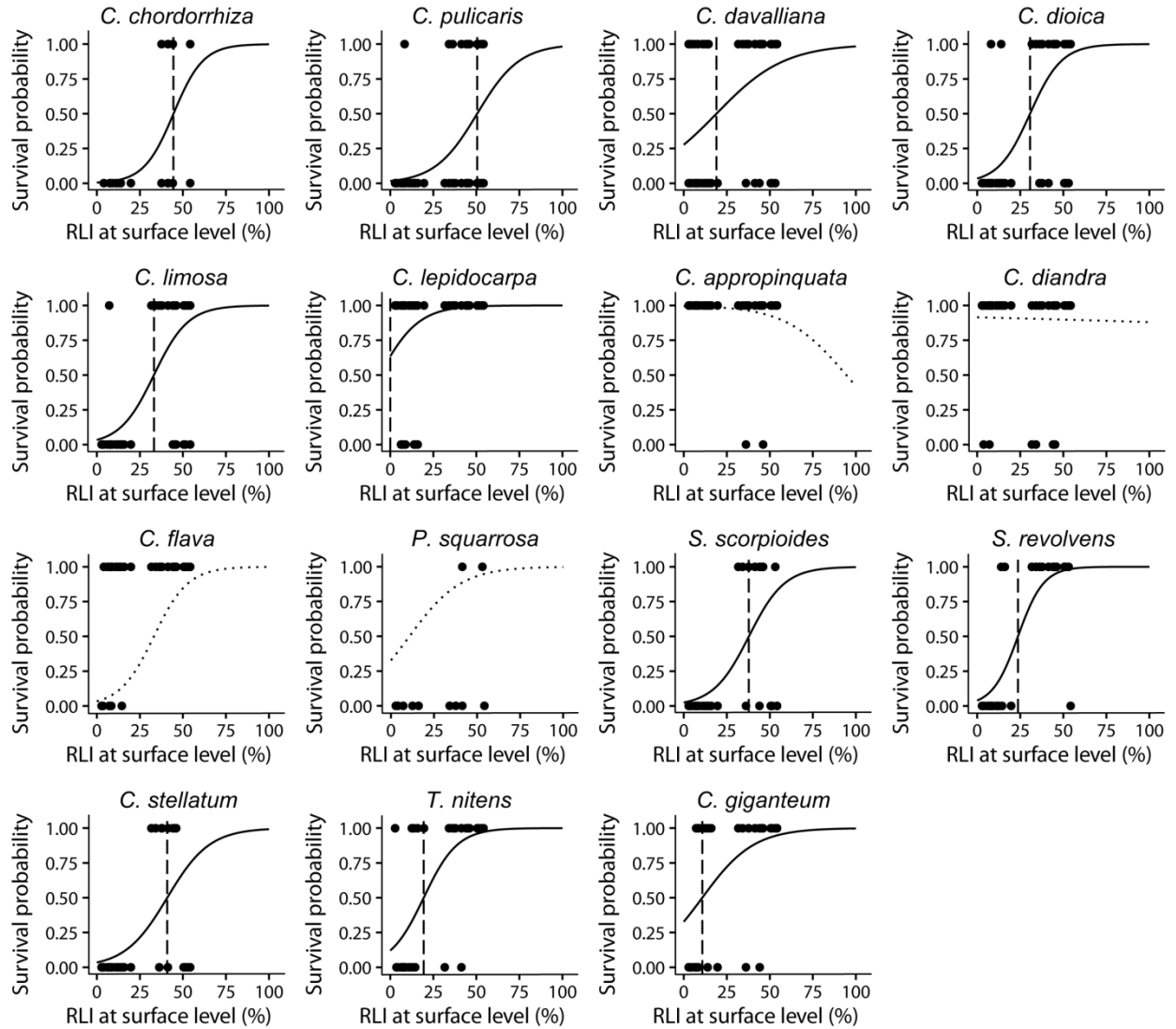
Figure A2: Logistic regression curves of survival probabilities of introduced fen species versus relative light intensity (RLI) at the soil surface. Solid curves indicate significant regression models ($p < 0.05$), dotted curves indicate non-significant regression models. Vertical dashed lines indicate the inflection points (i.e. RLI at which survival probability equals 50 %).