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

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ARTICLE INFO

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.

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 and 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).

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 × 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, 12/12 h 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 ± 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 ± 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 × 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