Colonisation processes in riparian fen vegetation

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Colonisation processes in riparian fen vegetation

Kolonisatie processen in oeverstroken van laagveenwateren
(met een samenvatting in het Nederlands)

Proefschrift

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CHAPTER 1

General introduction

J.M. Sarneel

The fen system

Fens are valuable, peat accumulating wetlands, characterised by a strong influence of groundwater and/or surface water (Clymo 1983; Succow 1988; Van Wirdum *et al*. 1992; Hájek *et al*. 2006). This creates a pH-buffered, mesotrophic environment that, compared to other ecosystems in the temperate climatic zone, naturally harbours relatively species-rich plant communities and a large number of rare species (Figure 1.1; Verhoeven and Bobbink 2001; Bedford and Godwin 2003). For instance in the state of Iowa, USA, 18% of the total state flora occur in fens although these fens occupy only 0.01 % of the state land area (Nekola 1994). Besides, fens provide several valuable ecosystem services. They facilitate water purification, retain water and decrease the risk of floods and droughts (Mitsch and Gosselink 2000). Furthermore, the constant wet conditions induce peat accumulation, and therefore, fens can function as carbon sinks (Maltby and Immirzi 1993). Fens used to be a common ecosystem type in many parts of the world, but large areas have been lost. Especially in Europe, the strong increases of urban, industrial and agricultural activities have decreased the total fen area drastically (in some countries the area loss has been estimated at 95%; Vermeer and Joosten 1992; Bedford and Godwin 2003; Middleton *et al*. 2006a). To counteract the negative effects of these land use changes, numerous conservation and restoration efforts have been taken to protect and maintain the relatively high biodiversity and natural processes in fens (Bedford and Godwin 2003; Van Diggelen *et al*. 2006).

Vegetation dynamics in fens

Fens are naturally dynamic systems that develop typically in slow-flowing and stagnant (lentic) water bodies such as occur in river

Figure 1.1: Colonisation of the open water by species growing from the bank. (a) Colonisation by species that expand while floating at the water surface. (b) Colonisation of open water by species that root in the pond bottom. (c) No colonisation. Photos by M. Christianen.

floodplains and landscape depressions. Succession from open water to wet forests is an important process that structures fens. This serial development has been described in detail by Den Held *et al*. (1992), Van Wirdum *et al*. (1992), Schaminée *et al*. (1995), Westhoff *et al*. (1971) and Verhoeven and Bobbink (2001) and is often referred to as 'terrestrialisation'. Terrestrialisation starts by the colonisation of an open water body by numerous aquatic species (e.g. *Potomageton* species, *Chara* species, *Hottonia palustris*, *Urticularia* species). These species are successively followed by semi-aquatic (*Calla palustris*, *Cicuta virosa*, *Thelypteris palustris*) and semi-terrestrial communities (with helophytes such as *Phragmites australis*, *Cladium mariscus* and *Typha angustifolia*; Den Held *et al*. 1992; Figure 1.1). Under oligo- to mesotrophic conditions, succession will induce the formation of a typical phase: the phase of floating peat mats (characterised by the Cicuto-Calletum, Caricetum elatae and Caricetum paniculatae plant associations; Schaminée *et al*. 1995). These floating peat mats consist of peat held together by a meshwork of roots and rhizomes that floats on, or just below the water surface. These floating mats are species-rich (>30 species m^{-2}) and may contain several red list plant and animal species (Such as: *Eriophorum gracile*; *Menyanthes trifoliata*; *Liparis loeselii* and *Boloria selene*; Vermeer and Joosten 1992; Bal *et al*. 2001; Verhoeven and Bobbink 2001). Under more eutrophic conditions other, more productive species colonise the open water. Although those species are occasionally found to induce the formation of floating peat mats (Lambert 1946; Sasser and Gosselink 1984), it is more typical that the floating mat phase is lacking under very nutrient rich conditions.

Accumulation of litter and peat on the mat can eventually induce bog formation (with *Sphagnum subnitens, S. palustre* and *Carex* species) and eventually, terrestrialisation will lead to the development of carr vegetation (including *Alnus glutinosa*, *Betula* and *Salix* species; Wiegers 1992), under both mesotrophic and eutrophic conditions. On a landscape scale, small to larger scale disturbances (e.g. flooding, erosion, grazing) reset the terrestrialisation process every now and then. This creates a heterogeneous landscape in which the different succession phases and all their associated species occur. Succession and disturbances are therefore thought to be of major importance for maintaining both the high biodiversity and the natural functioning of fen landscapes (Pons 1992). However, due to increased anthropogenic influences and changes in land use, the natural dynamics have almost

Table 1.1: Target coloniser species. Bold species names indicate red list species.

Colonisers	Buoyancy		Longevity[3]	Habitat preference	Canopy height (m)	Growth form
	Veg. [1]	Seed[2]				
Calla palustris L.	++ *	++	Unknown	Mesotrophic	0.1-0.2	Leaved rhizome
Comarum palustre L.	-	++	Transient	Oligo- to mesotrophic, phosphorous poor	0.15-0.9	Woody rhizome
Equisetum fluviatile L.	+	Spore	Unknown	Mesotrophic	0.5-1.5	Without true leafs
Glyceria maxima (Hartm.) Holmb.	n.d.	+	Transient	Eutrophic	0.8-1.8	Grass with long rhizome
Menyanthes trifoliata L.	++	++	Transient	Mesotrophic, slightly acidic, P-poor	0.13-0.3	Leaved rhizome
Phragmites australis (Cav.) Steud.	+	++	Transient	Meso- to eutrophic	1.0-4.0	Grass with long rhizome
Ranunculus lingua L.	n.d.	-	Unknown	Mesotrophic	0.5-1.5	Herb with rhizome
Thelypteris palustris Schott	-	Spore	Unknown	Oligo- to mesotrophic, slightly acidic	0.3-0.8	Woody leaved rhizome
Typha angustifolia L.	n.d.	+	Short-Term persistent	Meso- to eutrophic	1.0-2.0	Grass with long rhizome
Typha latifolia L.	n.d.	+	Variable	Eutrophic	1.0-2.0	Grass with long rhizome
Stratiotes aloides L.	+ *	n.d.	Unknown	Mesotrophic	0.05-0.2	Floating, emergent rosettes

[1] Buoyancy of vegetative propagules was determined experimentally (Box 1). ++ = very long > 6 months, + = long, - = short < 1 month.

[2] Buoyancy was obtained from Kleyer *et al*. (2008), Van de Broek *et al*. (2005), M. Soons (unpublished). Due to small methodological differences between these researches, the species were classified: ++ = very long floating times, + = long floating times, - = short floating times.

[3] From Kleyer *et al*. (2008).

* Species has specialised vegetative propagules. n.d. = Not Determined or Unknown.

disappeared. Consequently, many areas have lost their high variation in succession stages and their characteristic high biodiversity (Lamers *et al*. 2001; Van Belle *et al*. 2006). In Europe, fens and especially floating mats in mesotrophic conditions are considered a highly threatened ecosystem and a set of regulations and aims have been defined to conserve them (The Natura2000 Habitat Directive). The majority of the Dutch fen systems that contain floating mats have also been incorporated in the European Natura2000 network (www.lnv.nl).

The colonisation of the open water by (semi-) terrestrial species from the bank is a crucial process in the whole succession from open water towards floating mats. The species capable of doing so (Table 1.1; hereafter called 'colonisers') can expand into the open water either with rhizomes that float on, or just below, the water surface (Figure 1.1; Weeda *et al*. 1999; Azza *et al*. 2006) or by rooting in the pond bottom.

Plants that colonise open water by floating on, or just below, the water surface create dense floating rhizome mats on which litter and peat accumulates. This creates a habitat for other species that, with their roots, consolidate the mat (Westhoff *et al*. 1971). The buoyancy of these litter, root and rhizome complexes is maintained by the aerenchym of the rhizomes and entrapment of gas bubbles produced by anoxic reduction and fermentation processes in the peat (Nitrogen 68%, Methane 28%, Carbon dioxide 4%; Hogg and Wein 1988a). The ability to retain those gas bubbles is determined by the quality and the structure of the peat (Strack *et al*. 2005) and usually increases with ongoing peat accumulation. Hence, the relative contribution of rhizomes to the overall buoyancy of the mats decreases with age (Hogg and Wein 1988a, 1988b). For old mats, it has been calculated that *Typha* rhizomes accounted for 10% of the buoyancy (Hogg and Wein 1988b), but for young mats no data are available. This 'floating-colonisation' is observed along sheltered regions of fen pond banks (Schaminée *et al*. 1995), on fringes of already existing floating mats and also on clumps of bare floating peat or aggregates of floating litter. *Calla palustris*, *Cicuta virosa, Comarum palustre*, *Menyanthes trifoliata,* and *Thelypteris palustris* (Schaminée *et al*. 1995) are the most important representatives of colonisation of open water by floating on the water surface. These species have adaptations (thick, air-filled or stiff, woody rhizomes) that enable them to maintain their rhizome at the water surface. The expansion of such floating rhizomes is thought to be facilitated by the aquatic macrophyte *Stratiotes aloides* L. (Schaminée *et al*. 1995; Van Buggenum and Valkenburg 2009). By the formation of dense vegetation beds, the stiff-leaved rosettes of this species can provide structural support for the rhizomes of species growing from the bank.

Stratiotes is therefore thought to act as a keystone species (Smolders *et al*. 2003).

Equisetum fluviatile, Glyceria maxima, Phragmites australis and *Typha angustifolia,* colonise the open water with rhizomes that root in the sediment bottom. This usually limits the lakeward expansion to a certain water depth and requires a set of physical adaptations to the anoxic and dark environment in the deeper water layers (Coops *et al*. 1996; Andersson 2001; Mäkelä *et al*. 2004; Jackson 2006). These adaptations involve the formation of porous tissue to improve gas transport (aerenchym), metabolic changes to cope with anoxia (Taiz and Zeiger 1998), radial oxygen loss from the root-tips to prevent damage from phytotoxines that are formed at low redox potentials (Koncalová 1990) and changes in stem and leaf morphology to ensure survival to mechanical disturbance from waves (Coops and Van der Velde 1996). Due to the high proportion of aerenchym in the rhizomes (up to 67%; Coops *et al*. 1996) and gas accumulation (methane) in the peat, complexes of peat and rhizomes can achieve a density that is lower than water and start to float (Lambert 1946; Westhoff *et al*. 1971). Typical fen vegetation can then develop on these floating rhizome-soil complexes afterwards (Papchenkov 2003; Somodi and Botta-Dukát 2004).

The speed by which open water is colonised has been quantified by interpretation of areal photographs and a few studies with permanent quadrats (Table 1.2). Those studies mainly show that terrestrialisation of narrow fen ponds may occur within several decades. The fastest rate was reported by Bakker *et al*. (1994) who found that 75% of the open water disappeared in a complex of narrow rectilinear fen ponds in a relatively short period of 20 years (Table 1.2).

However, despite the great concern for preservation and restoration of the terrestrialisation process, surprisingly few studies actually experimentally quantified the occurrence of the different colonisation strategies in the field or experimentally tested mechanisms behind them (but see Azza *et al*. 2006; Welch *et al*. 2006). As colonisation processes are considered crucial in the formation of species-rich floating mats, this thesis aims at investigating the mechanisms and conditions that determine the colonisation of open water by species growing from the bank.

Dutch fen systems - History

During the Holocene, large fen complexes developed in the low countries (i.e. the Netherlands and Flanders) that are situated in the deltas of the Rhine, Meuse and Scheldt. Eventually, about 2000 years BP, more than half of this coastal plain was covered with fen and bog systems (Pons 1992).

Table 1.2: Summary of all the reported terrestrialisation rates in fen ponds.

Time span	Wetland	Observed changes over this period
1937-1957	[a] Fen: Westbroekse Zodden	- 74% of the open water was converted to a next phase on aerial photos. - 9% of the water turned into forest. - Estimated turnover time from aquatic to forest 30.7 year.
1944-1993	[b] Fen: Het Hol	- 37% of the open water was converted to a next phase on aerial photographs - Woodland cover increased with 191%.
1984-2000	[c] Fen lake: Neuchâtel	- Aquatic species decreased and woody species increased in permanent plots. - "in some of the permanent quadrats the vegetation was even no longer classified as aquatic in 1998-2000".
1931-1981	[d] Fen lake: Naardermeer	- Permanent plot data suggest that the transition from open water to forest takes about 50 years
1864-1994	[d] Fen lake: Naardermeer	- 51% of the open water disappeared (366 ha to 187 ha) - "Woodland increased" (103 ha to 247 ha).
1956-1989	[e] Fen meadow: Bollemaat	- Under a summer-mowing regime rich fens developed into acid vegetation types. With a winter mowing regime, *Phragmites*-dominated vegetation types developed. - Only 2000 m^2 of the 4.75 ha with summer mowing remained unchanged. -"We found embryonic bog vegetation in 1989 in sites where there was open water in 1958".

[a] Bakker *et al*. (1994)
[b] Van Belle *et al*. (2006)
[c] Güsewell and Le Nédric (2004)
[d] Barendregt *et al*. (1995)
[e] Van Diggelen *et al*. (1996)

During the Middle Ages, large parts were reclaimed for agricultural practices such as grazing and crop growth (Pons 1992). The drainage that was necessary for these activities caused soil oxidation and hence the soil subsided. Eventually, the peat areas became too low and too wet for growing crops, but suitable for fen meadows that were grazed and harvested for hay (Pons 1992). In the meantime, the economic development increased since the 17th century. This increased the need for turf as fuel and people started to excavate the peat. In the western part of the Netherlands, the peat was even excavated from below the water table and this created landscapes with numerous rectangular ponds (turf ponds). These ponds were typically 1-4 m deep, 30 m wide and a few hundreds of meters long. They were rapidly colonised by aquatic, semi-aquatic and semi-terrestrial plants and species-rich floating mats became a rather common phenomenon. This excavation created dynamic disturbances, which maintained a mosaic landscape in which different succession stages occurred simultaneously. Despite the anthropogenic origin, the Dutch fen ponds contained a high biotope diversity and floristically resembled natural stands (Bootsma and Wassen 1996; Verhoeven and Bobbink 2001).

Dutch fen systems – Current status

Presently, peat is no longer extracted for commercial purposes. Most floating mat vegetation that had been formed after peat excavation in the early 1900s, have now gradually turned into alder forests (Bakker *et al*. 1994; Van Belle *et al*. 2006). Since hardly any new ponds were created in Dutch fen areas over the past 60 years, those mats and the species that play key roles in the colonization of open water and the formation of floating vegetation mats have become rare (Figure 1.2; Verhoeven and Bobbink 2001; Lamers *et al*. 2002; Beltman *et al*. 2008). However, intensive management (mainly mowing) preserved small remnant populations of those species (Van Diggelen *et al*. 1996; Verhoeven and Bobbink 2001). Besides, starting about 1985, new ponds have been created to restore opportunities in which these remnant populations can initiate the succession towards species-rich floating mats again. Now, about 20 years later, these restored ponds show a variable and often disappointing restoration success (Beltman *et al*. 2008). The characteristic pioneer species have remained absent and the formation of floating mats has hardly ever been observed (Figure 1.2; Lamers *et al*. 2001, 2002, Beltman *et al*. 2008). More frequently, ponds have either been colonised by species that root in the pond bottom or have remained open, dominated by aquatic species (*Elodea nutallii* or *Ceratophyllum demersum*).

These disappointing results might be due to changes in the landscape surrounding the fen ecosystems. Here, agricultural intensification, industrialisation and increased urbanization have negatively impacted water and soil quality and have increased habitat fragmentation. As a result of this, the typical mesotrophic species that are associated to the formation of floating mats might either 1) fail to reach the restored ponds, or 2) fail to become established and persist, or 3) fail to expand into open water and induce the formation of floating mats. These factors have rarely been studied simultaneously. Although studies that have investigated only one of these processes have provided valuable insights into fen processes and discovered several potential causes for restoration failure, they did not reveal which of those causes is most crucial for successful restoration (Leng *et al*. 2009; Beltman *et al*. 2010). For the Dutch fens, Lamers *et al*. (2001) made a detailed description of all the possible causes behind the loss of floating mat vegetation. Changes in habitat quality, dispersal limitations and the invasion by musk rats (*Ondatra zibethica* L.) were identified as the most important potential causes. Therefore, I summarise how these factors can effect the development of floating mats below.

Habitat quality

As floating mats develop at the interface of surface water and bank soil, the quality of both is important for species that grow from the bank into the water. Over the last decades, several causes have changed the quality of both soil and water. Fens are typically surface- and groundwater-fed, resulting in a buffered water chemistry with high concentrations of HCO_3^-, Ca^{2+} and $Fe^{2+/3+}$ but relatively low nutrient concentrations (Clymo 1983; Succow 1988; Van Wirdum *et al*. 1992). As a result of increasingly strong drainage in the surrounding agricultural land, the water tables at local and regional scales have dropped from several decimetres to over one meter below the soil surface (Lamers *et al*. 2002; Nienhuis *et al*. 2002). As a result of the increasing extraction of drinking water in many sandy hill areas nearby fen landscapes, upward seepage of groundwater has stopped in most of the Dutch fens (Lamers *et al*. 2002). This has caused desiccation and acidification of the soil (Van Wirdum *et al*. 1992). In addition, atmospheric deposition and runoff from fertilised agricultural fields have led to eutrophication of both soil and surface water (Barendregt *et al*. 1995; Bobbink and Lamers 2002). The surface water quality has changed even more, because at present, it is mixed with alkaline, nutrient-rich (river) water from outside the fen areas (Lamers *et al*. 2002). Such allochthonous water is frequently supplied during dry periods in summer, to maintain a stable water level throughout the year. This not only adds more nutrients, but also raises chloride and sulphate concentrations. Under reduced conditions, sulphate is reduced to sulphide that has strong toxic effects on plants (Lamers *et al*. 1998; Van der Welle *et al*. 2006; Geurts *et al*. 2009). Even low sulphide concentrations (<15-50 µM) can be lethal for several plant species (Van der Welle *et al*. 2006). Besides, as sulphide has higher affinity for iron than phosphate, it will release phosphate from Fe~P compounds, a process called ‘internal eutrophication’ (Smolders *et al*. 2006; Geurts *et al*. 2008).

These changes have drastically modified the water quality, often resulting in a catastrophic shift from a clear-water state with many macrophytes to a turbid state characterised by algal blooms and a low biodiversity (Scheffer *et al*. 2001). Under such eutrophic, turbid conditions, the more mesotrophic species that invade the water by floating at the water surface disappear. Due to the turbidity of the water the opportunity for bottom rooted species to colonise the open water from the bank will also be restricted, because light limitation will restrict the colonisation to shallower water depths. To break the positive feedbacks maintaining this turbid state, the nutrient concentrations (mainly P) need to be lowered drastically. A reduction of the benthivorous fish stock is usually necessary too. These fish feed by digging in the pond bottom and hence can maintain a turbid state, even when nutrient concentrations would allow a macrophyte dominated and clear water state (Scheffer 1998).

Fragmentation and isolation

Due to the land use changes in the surroundings of fens, fens have become small patches in a matrix of intensively used agricultural lands, roads and cities that form a hostile habitat for many fen species. This fragmentation of the landscape has increased the distance and the number of barriers between suitable habitat patches. As a result, dispersal between fen areas has become very difficult (Beltman *et al*. 2010). Consequently, species might have remained absent in a suitable habitat patch simply because they were unable to reach it. Fragmentation has also decreased the size of habitat patches, which makes populations that occupy them more susceptible to stochastic extinctions. This has resulted in biodiversity loss and especially rare species, such as *Carex lasiocarpa*, *Comarum palustris* and *Stratiotes aloides*, have disappeared locally.

Seeds and diaspores that persist in the seed bank can form an important additional mechanism for settlement and regeneration of species, particularly in fragmented landscapes with impeded dispersal. When persistent seed banks are present, a fast recovery of plant communities is often observed after restoration efforts, even in fragmented landscapes (Beltman *et al*. 1996; Bakker *et al*. 1996; Thompson *et al*. 1997). However, the majority of species associated to the formation of floating mats have transient seed banks (Table 1.1; Kleyer *et al*. 2008) and active dispersal from remnant populations is therefore likely to be an important factor for successful colonisation of restored ponds. Some species, like *Stratiotes aloides*, hardly produce any viable seeds (Westhoff *et al*. 1971; Smolders *et al*. 1995) and such species likely have an extremely low probability to colonise new habitats.

In riparian zones of fens, the main dispersal vector is water (hydrochory; Middleton *et al*. 2006b) and seeds of fen species generally have traits that enhance their ability to float and remain buoyant (e.g. low tissue density, impermeable seed coat). Even the species that disperse through spores can have special adaptations to water dispersal, although such species also depend on dispersal via wind (Mahabalé 1968). In general, hydrochory is driven by water currents (Andersson *et al*. 200; Nilsson *et al*. 2002; Boedeltje *et al*. 2003) and flooding events (Bornette *et al*.; Vogt *et al*. 2004), but at the moment we lack knowledge about the dispersal mechanisms in lentic water bodies such as fen ponds. The clonal growth strategy of most of the

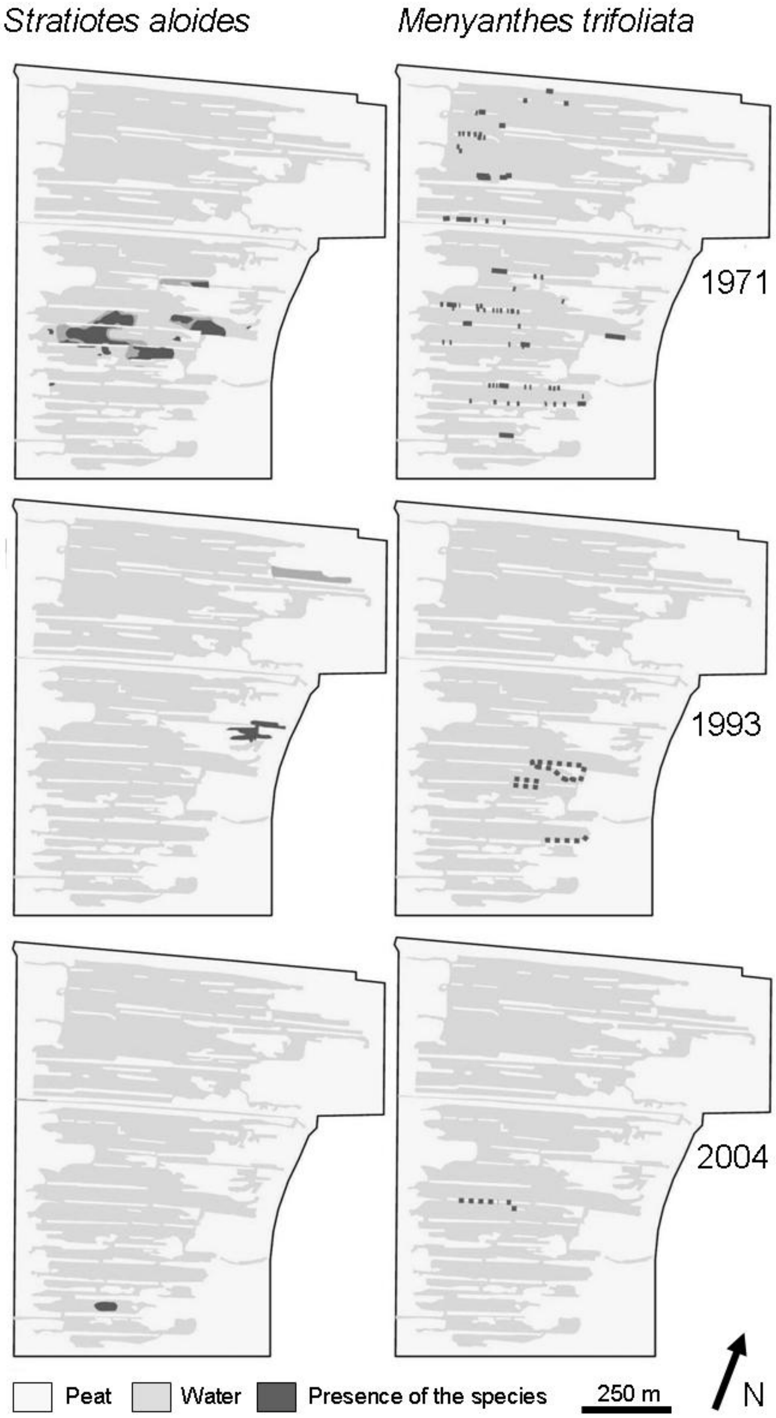

Figure 1.2: The decline of Stratiotes aloides and Menyanthes trifoliata between 1971 and 2004 in Tienhoven, a fen area in the Vecht-area (modified with permission from Beltman et al. 2008).

colonisers makes, vegetative propagules form an important alternative dispersal mode.

Biotic changes

Stratiotes aloides, the flagship species of Dutch fen conservation, is often thought to play a key role in the formation of floating mats. *Stratiotes* is an emergent macrophyte that forms stiff-leaved, floating rosettes, which by rapid clonal expansion can form dense beds that cover the entire pond surface (Figure 1.1). Such dense vegetation beds could provide structural support for the rhizomes of species growing from the bank. Besides, they will attenuate waves, which will decrease the probability of fracture of rhizomes in the water. *Stratiotes* is therefore thought to acts as a keystone species (sensu Paine 1969) in the formation of floating mats (Smolders *et al*. 2003). Unfortunately, *Stratiotes* has declined strongly over the past decades (Figure 1.2). Because of its hypothesised role in the colonisation of the open water this decline might have contributed to the disappearance of floating mats.

In 1941, the first muskrat was caught in the Netherlands (Bos *et al*. 2009). Since then, the density of this invasive North American species has increased considerably (in 2007, on average 0.91 muskrat was caught per hour of hunting in peat areas; Bos *et al*. 2009). As this species dwells in shorelines and its diet consists almost completely of herbaceous vegetation, including rhizomes (Doude van Troostwijk, 1976; Clark 1994; Connors *et al*. 2000), a high muskrat density could threaten the development of riparian zones and floating mats. Besides, by creating extensive holes and tracks in the banks, they undermine the stability of the banks which, therefore, may become more susceptible to erosion (Doude van Troostwijk, 1976).

Questions

Given the complexity and the number of possible factors that may have caused the decline in biodiversity and functioning of fens, there is a need for research on the role of the different possible mechanisms. Such knowledge is essential to identify major bottlenecks for restoration and will support decision making in nature management. As the formation of floating mats depends on the presence of a set of typical species (the ‘colonisers’) and their actual growth from the bank into water, this study focuses on the way these species colonise restored ponds and their expansion from the bank into open water. The central question of this thesis is:

> *Which mechanisms and conditions are important drivers for the colonisation of open water by species growing from the bank?*

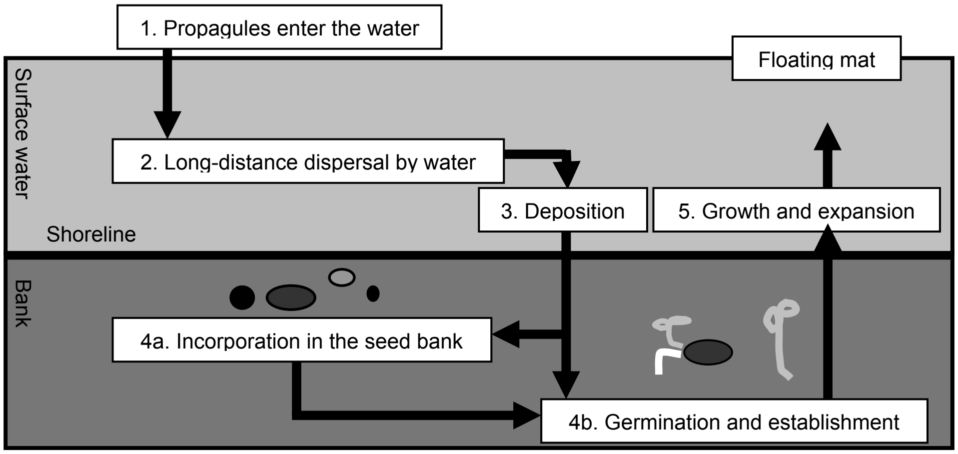

Figure 1.3: Schematic top view of a pond bank with the most important processes that are hypothesised to lead to the colonisation of open water and eventually to the formation of floating peat mats.

Research approach and questions

Figure 1.3 shows the processes that were hypothesised to play a role in the colonisation of open water by species growing from the bank. To start colonisation from the bank, an empty patch on the bank needs first to be colonised by propagules of species that are associated to the colonisation of the open water (Table 1.1; Figure 1.3, arrows 1-3). This requires active dispersal of seeds from remnant populations via water and, therefore, the mechanisms behind hydrochory were investigated with a series of field experiments on different spatial and temporal scales. These studies are described in Chapter 2. Apart from seeds, many of the typical colonisers also form (specialised) vegetative propagules (Eber 1983; Klosowski *et al*. 1995; Haraguchi 1996; Smolders *et al*. 1995). The potential of vegetative fragments as dispersal agent was assessed with a buoyancy and germination experiment described in ‘Intermezzo 1’. Once a seed or vegetative propagule is deposited on the bank, it can either germinate directly or be incorporated in the seed bank (Figure 1.3; arrow 4a & b). Therefore, the content of the seed bank and its relation to the dispersal process was also investigated in Chapter 2.

The next step towards successful colonisation of open water is that after a seed is deposited, it needs to germinate and become established (Figure 1.3; arrow 4b). Most species have specific requirements for germination and establishment. When these requirements are not met at the locations where their seeds are mainly deposited, this can form a serious bottleneck for the colonisation of open water. Therefore, the factors controlling the germination and establishment on sites where seeds are deposited were tested with a greenhouse and a field experiment in Chapter 3.

When a seedling has successfully become established on the bank, the plant needs to expand clonally and colonise the open water (Figure 1.3; arrow 5). This will only be possible under a specific set of circumstances. Given the frequent failure of restoration efforts, we were especially interested in those circumstances that currently impede the colonisation of open water. One major factor that is often held responsible for the decline in freshwater ecosystems is that land use changes in the areas surrounding the fens have drastically increased the nutrient inputs. In Chapter 4 we used a mesocosm experiment to investigate how nutrient availability in the surface water and bank soil influence the way species colonise the open water. Still, eutrophication is only one of the major changes that have occurred over the past decades, and other changes likely affect the colonisation of the open water too. Starting from our knowledge of the mechanisms gained in the previous chapters, we used a field survey to assess the importance of the major effects of land use changes on the colonisation of the open water by species growing from the bank in Chapter 5. The potential role of *Stratiotes* as keystone species in the colonisation process was assessed specifically in ‘Intermezzo 2’. More specifically, the following consecutive research questions were addressed:

1. What is the mechanism behind the dispersal of propagules in slow-flowing and stagnant water bodies such as fen ponds?
2. Which factors influence germination and establishment in fen shorelines and what are the probabilities for recruitment of colonisers on sites where their seeds are deposited?
3. How does N or P enrichment of the bank and surface water affect the growth of and colonisation by fen species?
4. Which factors determine the lack of colonisation of the open water in Dutch fen ponds?

Finally, based on the knowledge of basic mechanisms and the current situation in the field, Chapter 6 analyses the role of various factors interfering with restoration of succession towards floating mats in Dutch fens. There, I discuss the perspectives for restoration of species richness and ecosystem functioning in Dutch fens and provide a scheme to identify possible bottlenecks for restoration.

CHAPTER 2

The role of wind in the dispersal of floating seeds in shallow lakes and ponds

J.M. Sarneel, B. Beltman, A. Buijze, R. Groen, M.B. Soons

Abstract

For a rigorous assessment of the ecological role of wind in the dispersal of water-borne seeds in riparian zones of ponds and other stagnant or slow-flowing (lentic) water bodies, we investigated the relation between wind and dispersal at three temporal and spatial scales.

Firstly, we determined the direct effects of wind on hydrochorous dispersal speed and distance. Secondly, we related seed deposition over different seasons to the prevailing wind conditions. Thirdly, we evaluated the long-term (multiple years) effects of prevailing wind conditions on the pattern in and composition of seed banks.

Our results show that wind speed and direction strongly determine the dispersal process and the resulting deposition patterns of floating seeds in shallow lakes and ponds. Wind directly influenced dispersal speed and distance. Increasing wind velocity increased dispersal speed, but decreased dispersal distance. Over the seasons, wind-driven hydrochory resulted in directional transport following the prevailing wind direction. This directionality will have consequences for the colonisation of riparian zones in lentic systems, where more seeds are deposited at downwind banks. It also determines the effectiveness of dispersal through water connections at different geographical positions. Species composition of the seeds deposited was affected too, with proportionally more water-dispersed seeds at downwind shorelines. On the long term, however, seed banks in riparian zones reflected prevailing wind conditions poorly, showing that additional processes, such as differential germination and predation, play roles that are at least as important at this timescale.

Keywords: Colonisation, Dispersal mechanism, Hydrochory, Lentic water bodies, Wetlands

Introduction

As the local loss of species is a natural process in vegetation dynamics, colonisation events are essential to maintain local diversity and species richness (e.g. Palmer and Rusch 2001). Colonisation starts with the arrival of seeds, followed by germination and establishment. Hence, factors influencing dispersal exert a crucial influence on plant species distributions and (genetic) diversity (for an overview see Levin *et al*. 2003). It is therefore important to understand the mechanisms of dispersal in relation to the resulting seed deposition patterns and vegetation composition. This in turn has implications for nature management, restoration efforts and other human activities that affect connectivity between habitat patches (e.g. Bischoff 2002; Soons *et al*. 2005).

Riparian zones are disproportionately rich in plant species (Nilsson and Svedmark 2002; Renöfält *et al*. 2005). However, biodiversity of such habitats has decreased drastically over the past decades, particularly in lowland fens, which at the same time have become more and more fragmented (Tockner and Stanford 2002; Middleton *et al*. 2006b). Similarly, in riparian zones in the majority of Dutch fens, a group of characteristic pioneer species initiating succession and colonisation of open water (e.g. *Menyanthes trifoliata* L., *Comarum palustre* L.) have disappeared, and the subsequent succession phases, with numerous associated species (e.g. in floating rafts) have also almost disappeared (Verhoeven and Bobbink 2001). As habitat quality has improved considerably (e.g. nutrient loads and inputs of other chemical pollutants to the surface water and bank have been reduced), the sustained absence of these species is often attributed to a low dispersal capacity and a lack of (re)colonisation (Beltman *et al*. 2008). Previous research on the dispersal of seeds from and towards riparian zones has focussed on plant communities bordering streams, rivers and canals. In these systems, dispersal by water (hydrochory) is the dominant dispersal vector, transporting large numbers of seeds over long distances (up to 147 km in Nilsson *et al*. 1993; cited in Danvind and Nilsson 1997). Hydrochory is mainly driven by current velocity (Andersson *et al*. 2000; Nilsson *et al*. 2002; Boedeltje *et al*. 2003; Bang *et al*. 2007) and by flooding events (Bornette *et al*. 1998; Vogt *et al*. 2004; Jeffries 2008). However, water bodies in fens are often hydrologically isolated and therefore characterised by very low current velocity, a negligible discharge and the absence of large-scale flooding. Water movements are mostly driven by wind and consequently vary considerably more in direction and strength than currents in rivers. The resulting seed deposition patterns in fen ponds, therefore, are likely to differ from those in streams.

This study was conducted to clarify the role of wind as a driving factor behind hydrochorous dispersal of plant seeds in slow-flowing or lentic water bodies, taking fen ponds as a model system. It is well-known that wind induces shear stress on the water surface, generating currents and waves in the windward direction (e.g. Shemdin 1972; Podsetchine and Schernewski 1999). This causes an accumulation of water at the downwind end of the lake or pond, which induces a reverse current in deeper layers. Litter or other floating material (such as seeds) would then be transported to downwind banks, where it is deposited at maximal wave height (Goodson *et al*. 2003; Stocker and Imberger 2003). For small lakes and ponds, local interactions of the wind profile with roughness of the landscape and lake properties (i.e. water depth, size and shape) can have strong effects on the actual size of currents and waves (Sarkkula 1991; Podsetchine and Schernewski 1999) and hence dispersal and deposition. We are aware of only one quantification of seed dispersal in slow-flowing water bodies, which reported maximal distances travelled of about 500 m (Beltman *et al*. 2005), but neither the mechanism, nor the ecological relevance of the dispersal process were assessed. Also the effect of wave disturbance on the species richness of the seed bank has been investigated, but the underlying mechanism has not been addressed (Gresson and Nilsson 1991).

As floating seeds are situated at the water surface and thus are directly subjected to the wind, we firstly hypothesise that there is a direct relation between wind speed and direction and seed dispersal speed and direction. Secondly, we hypothesise that over a longer time period, larger numbers of seeds are deposited on banks that are downwind of the prevailing direction. Thirdly, we expect that such greater numbers of seeds at downwind locations over the years result in more seeds stored in the seed bank of such locations. Finally, because pond banks downwind receive proportionally more seeds via water than via other vectors such as the air, we expect the species composition of drift material and seed bank to reflect this. We expect the proportion of seeds of species adapted to water dispersal to be higher at downwind banks. We investigated the above, in a thorough examination of the mechanisms and resulting patterns of wind-driven hydrochorous dispersal at different temporal and spatial scales, to evaluate the importance of wind-driven transport of floating seeds for the ecology of riparian zones along lentic water bodies.

Methods

To test our hypothesis that wind determines seed dispersal and deposition at different temporal and spatial scales, we (1) tracked dispersal trajectories of individual seed mimics while simultaneously measuring wind speed, (2) collected seeds deposited on banks of contrasting wind exposure and (3) performed a seed bank analysis.

Study system

The majority of lentic water bodies in species-rich fen systems in the Netherlands originate from peat excavations that lasted until the early 19th century. This created shallow (1.5 m deep) ponds, typically about 30 m wide and 100 - 900 m long, separated from each other by small strips (up to about 40 m wide) of fen peat with herbaceous or woody wetland vegetation. The ponds generally contain a high biodiversity of aquatic and riparian species, such as *Calla palustris* L., *Carex pseudocyperus* L., *Comarum palustre* L., *Menyanthes trifoliata* L. and *Stratiotes aloides* L. (Schaminée *et al*. 1995; Bal *et al*. 2001). The prevailing wind direction in the Netherlands is from the South West (SW), which means that the shorelines at the SW of ponds are predominantly upwind and those at the North East (NE) downwind (Figure 2.1). The simple, rectangular shape of many ponds provides an ideal situation to examine general principles behind the interaction of wind and hydrochory in slow flowing and stagnant water bodies. In two fen nature reserves, 'Westbroek' (52˚10N; 5˚07E) and 'De Weerribben' (52°46N; 5°55E), four to eight ponds with a SW-NE orientation were selected to investigate differences in seed deposition between the two ends of each pond.

Direct effects of wind speed

To determine how wind influences seed dispersal via water on short time scales, a seed tracking experiment was performed. A 45 m long transect with relatively homogeneous bank vegetation was established along the eastern bank of a SW-NE oriented pond in Westbroek. In March - June 2007, 46 small, brightly coloured seed mimics (polypropylene discs; Ø 4 mm; 2 mm high) were released one by one into the pond at 0.5 - 1 m distance from the riparian vegetation zone. Their movements were tracked till the end of the 45 m stretch or until they became fixed in the riparian vegetation for >10 minutes. Every minute, the distance travelled along the riparian zone (longitudinal distance) and the distance to the riparian vegetation zone (lateral distance) were noted. To measure wind speed simultaneously, a weather station (Eijkelkamp agrisearch equipment BV, Giesbeek) was installed on the bank at the centre of the transect. Wind speed was measured at 0.5, 2 and 5 m above the bank every 10 seconds, averaged and stored per minute. All dispersal trajectories were recorded on days with a wind direction parallel to the eastern pond bank (a deviation up to 25° was allowed), under a range of wind velocities (1.7 to 8.9 m s^{-1} at 5 m above the adjacent bank; bank height 0.5 m).

From each dispersal trajectory we calculated the total, the longitudinal, net lateral (difference between start and end position) and gross lateral (total distance travelled in lateral direction) dispersal distances (m; Figure 2.1). In addition, dispersal speeds (m s^{-1}; total, longitudinal and gross lateral), the range of lateral movement, the number of lateral directional changes and the number of contacts with the riparian vegetation (when the lateral distance was zero) were calculated per trajectory. These 10 variables were related to the mean wind speed measured during each trajectory using Pearson (when normally distributed) or Spearman (when not not-normally distributed) correlations. Because several seed mimics were lost for short periods of time (n = 8) or totally (n = 9), those trajectories were discarded from the analyses between wind speed and total dispersal distance. The current velocity of the water in a pond usually decreases laterally towards the bank (due to friction with the riparian vegetation) and consequently, dispersal speed would also decrease when seeds approach the bank. To test whether the dispersal speed was influenced by distance to the riparian vegetation within a trajectory, we carried out a stepwise multiple regression on total dispersal speed, with both wind speed and lateral distance to the riparian vegetation as potential explanatory variables. The regression line was fitted through the origin.

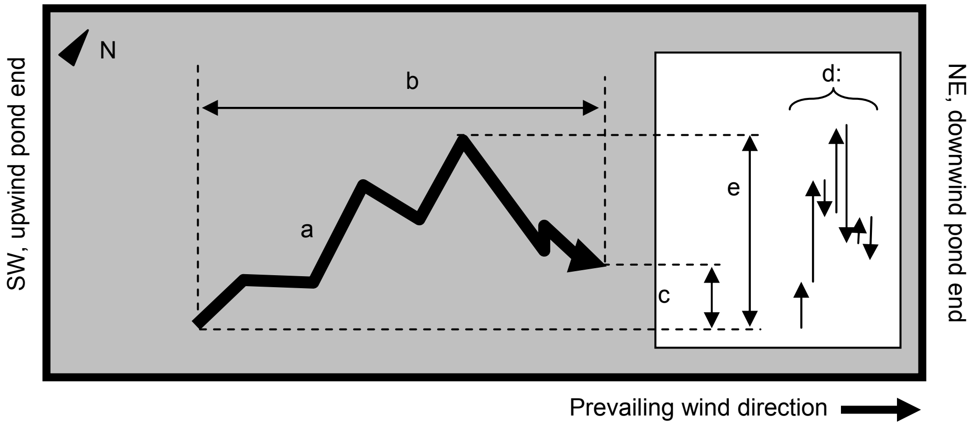

Figure 2.1: Schematic top view of a turf pond and a hypothetical dispersal trajectory (bold line). The letters indicate the different characteristics that were calculated for each trajectory. a) Total dispersal length, b) Longitudinal track length, c) Net lateral track length, d) Gross lateral track length and e) Lateral range.

Within-year variations in deposition

To test the effect of wind on hydrochory and the formation of deposition patterns over longer periods of time, four ponds with a SW-NE orientation were selected in Westbroek. During November 2006 and February, May and August 2007, 50 x 50 cm seed traps, in the form of artificial grass mats (cf. Wolters *et al*. 2004), were installed at the waterline in the centre of SW and NE banks of the four ponds. During November 2007, mats were placed in eight similar ponds, including the four ponds that had been sampled before. After 4 weeks, the mats were recollected and thoroughly rinsed. The seeds in the trapped material were counted and identified to species using Van der Meijden (2005) and Cappers *et al*. (2006). To relate the trapping data to the actual weather over these months, averaged daily wind speed and direction were obtained from the weather station in De Bilt, which is 9 km from Westbroek (Royal Netherlands Meteorological Institute; KNMI; www.knmi.nl/klimatologie/daggegevens).

The amount of seeds and species trapped at the SW and NE banks were compared using a repeated measures test, regarding both sides of each pond as the within-subject factor. The different months were analysed as between-subject factor, because we expected only a weak coupling between the months or none at all, due to large differences in vegetation phenology and composition. In addition, separate paired t-tests were conducted for each consecutive month sampled. A stepwise multiple regression was performed to relate differences in seeds trapped at SW versus NE banks to the mean wind speed and direction of the trapping periods.

Long-term variation in deposition patterns

To test if the long-term prevailing wind direction caused patterns in seed bank contents, seed banks were analysed using a seedling emergence test (Ter Heerdt *et al*. 1996; Thompson *et al*. 1997). In March 2007, seven isolated ponds with a SW-NE orientation were sampled in Westbroek (*n* = 4) and De Weerribben (*n* = 3). Soil cores (Ø 8 cm) were collected from the top 10 cm of SW and NE banks, just above the waterline, and brought to the laboratory. Assuming that natural stratification had taken place in the field, each core was homogenised and 300 g of each sample was spread thinly over a mixture of 50% sterilised sand and 50% potting soil in seed trays (395 x 430 x 75 mm) (Bakker *et al*. 1996; Ter Heerdt *et al*. 1996). These trays were placed in a greenhouse at a 15/9 h light/dark regime and mean temperature of 20 °C. Ten control trays, filled with the same substratum but without samples, were randomly placed between the other trays to check for airborne contamination. During 15 months all emerging seedlings were identified and removed regularly. To be able to calculate the number of viable seeds per litre of soil, 15 ml of fresh soil was taken from the sample and weighed. Differences between seeds and species per litre of soil from both sides of the ponds were tested using paired t-tests.

Variation in species composition

Buoyancy and terminal velocity are commonly used as proxies for the ability of a species to disperse via water or wind, respectively. Buoyancy is quantified as the percentage of seeds still floating after seven days (Kleyer *et al*. 2008) and terminal velocity is defined as the constant fall rate of a single propagule after a phase of acceleration, (m s^{-1}; Soons *et al*. 2004). As we expected that hydrochory would transport seeds predominantly towards downwind (NE) banks, we hypothesised that species better adapted to hydrochory would be more likely to reach the NE bank, whereas species better adapted to anemochory would be equally likely to reach SW or NE banks. To test this, we calculated a weighted mean buoyancy and terminal velocity for each sample collected in the trapping and seed bank experiments. This was done using data from the LEDA trait-base (Kleyer *et al*. 2008), appended with terminal velocity data from Soons (unpublished). Since the terminal velocities of Soons were consistently slightly lower ($P <$ 0.01) than those given in the LEDA trait-base, they were transformed using a regression equation derived from species present in both datasets ($n = 15$, $R^2 = 0.95$). Buoyancy data was available for 59% and 90% of the seeds trapped in the mats and found seed banks, respectively. Terminal velocity data was available for 88% and 71% of the seeds, respectively. The mean buoyancy and terminal velocity at SW and NE banks were compared statistically as described above for the number of species and seeds. All statistical analyses were carried out in SPSS 16.0.

Results

Direct effects of wind speed

Seed mimics were released at a mean wind speed of 5.6 m s^{-1} (± 0.25 S.E.; range 1.7 to 8.9 m s^{-1} measured at 5 m above the bank) and moved through the water with an overall mean velocity of 0.03 m s^{-1} (± 0.004 S.E.; range 0.002 to 0.10 m s^{-1}). Wind speed significantly influenced the hydrochorous dispersal trajectories (Table 2.1; Figure 2.2). With increasing wind speed, seed mimics travelled faster in total and in the longitudinal direction. In contrast, the gross lateral speed of the seed mimics was not affected significantly (Table 2.1). Surprisingly, the seed mimics dispersed over shorter distances when wind speed increased (total, longitudinal, net and gross

Table 2.1: Correlations between wind speed (m s^{-1}) and the 10 variables that describe the dispersal trajectories of floating propagules. [1] Pearson correlation. [2] Spearman correlation.

	Unit	N	Correlation coefficient	*P*-value
Speed				
Mean total dispersal speed[1]	m min^{-1}	46	0.46	<0.001
Mean longitudinal dispersal speed[1]	m min^{-1}	46	0.48	<0.001
Mean lateral dispersal speed (gross)[1]	m min^{-1}	46	-0.15	n.s.
Distance				
Total dispersal track length[1]	m	29	-0.40	0.03
Longitudinal track length[1]	m	37	-0.57	<0.001
Lateral track length (gross)[2]	m	29	-0.36	0.05
Lateral track length (net)[2]	m	37	-0.56	<0.001
Lateral range[2]	m	37	-0.62	<0.001
Change of lateral direction[2]	#	29	-0.23	n.s.
Touches with the riparian vegetation zone[2]	#	29	-0.23	n.s.

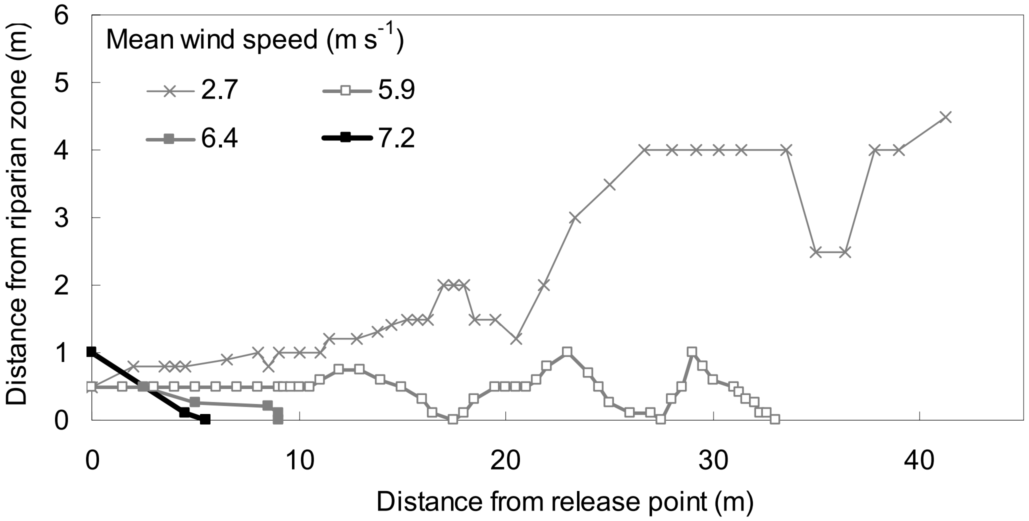

Figure 2.2: Four examples of dispersal trajectories (longitudinal and lateral movement) of seed mimics released along riparian vegetation at different wind speeds. Elapsed time between the data points is one minute.

lateral distance; Table 2.1). At low wind speeds, the seed mimics moved slower in the dominant dispersal direction, but were not easily trapped by the vegetation. In addition, at low wind speed, we observed that trapped seed mimics were often released again after a brief period of time, resulting in a 'bouncing' trajectory. However, correlations between wind speed and number of lateral directional changes and number of contacts with the vegetation were not significant. Overall, the dispersal speed was about half of the wind speed at 0.5 m height.

Dispersal speed of the seed mimics was also significantly positively related to lateral distance from the riparian zone: the mimics slowed down when approaching the vegetation. When this variable was added to a stepwise multiple regression model already containing wind speed, R^2 increased by almost 10% (Table 2.2). These processes are illustrated by the four dispersal trajectories shown in Figure 2.2.

Within-year variation in deposition patterns

In total, 155 different species were found in the 48 seed traps that had been placed at SW and NE banks of fen ponds throughout the year. The majority of the trapped seeds consisted of common riparian species like *Lycopus europaeus* L. (18%), *Carex paniculata* L. (17%), *Juncus effusus* L. (12%), *Alisma plantago aquatica* (7%) *Carex pseudocyperus* (6%) and *Rumex hydrolapathum* Huds. (5%). During all months sampled, the wind direction was predominantly W to SW, with a mean daily speed of approximately 3.3 m s^{-1} measured at 20 m height. Significantly more species were found in downwind NE mats (Repeated measures ANOVA; P = 0.01; Figure 2.3a). This was not simply the effect of vegetation differences, as a paired-t-test showed that the total number of species present in the vegetation surrounding the mats did not differ between SW and NE banks (Figure 2.3a). Between months no significant difference in species number in the mats was found.

A similar pattern existed for the numbers of seeds deposited on the mats (Figure 2.3b). The number of seeds was almost significantly higher in downwind NE mats (Repeated measures ANOVA; P = 0.06), but no significant differences were found between the months. The wind speed and directions were different during the five sampled months (Figure 2.3c) and this affected the dispersal and deposition. When the difference between the number of seeds trapped on SW and NE banks was correlated to these weather conditions, a positive correlation was found with the number of days per month with a SW wind (Multiple stepwise regression; r = 0.987; P = 0.002; Figure 2.3d), but not with mean wind speed per month.

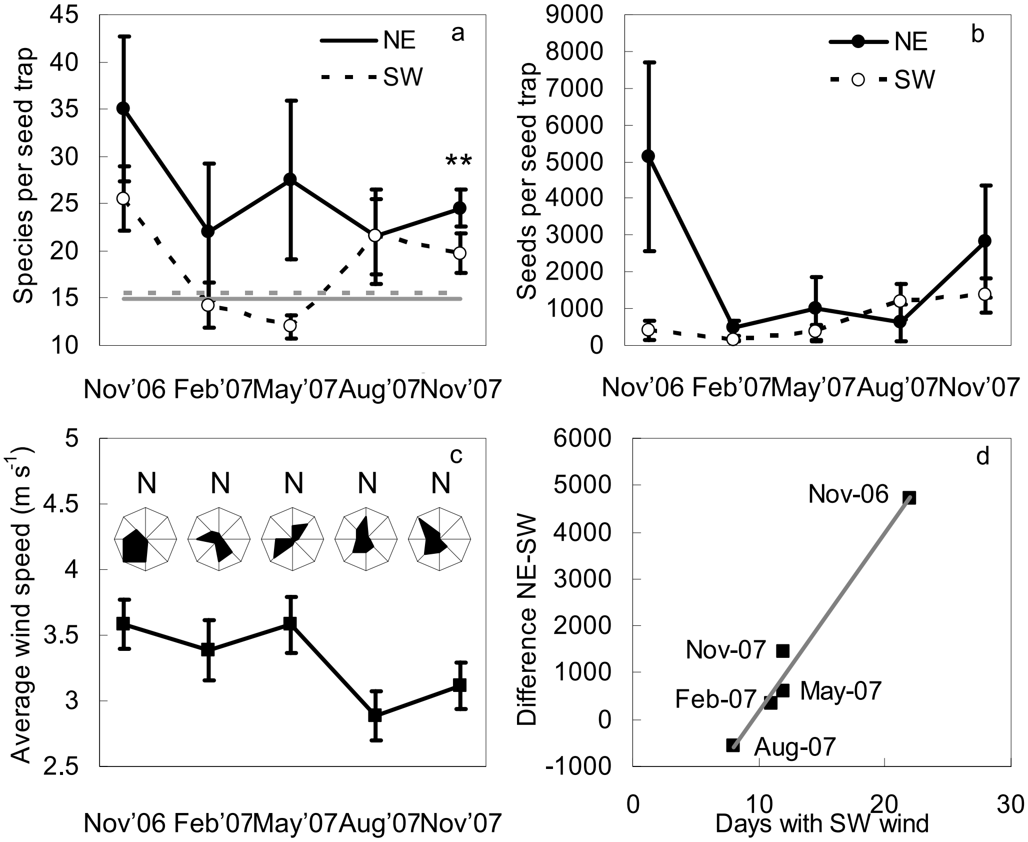

*Figure 2.3: a) Number of species (± SE) of seeds trapped in the mats (black lines) and number of species present in the local vegetation (grey lines; n = 8). b) Number of seeds (± SE) trapped in the mats. SW banks are indicated with open symbols and NE banks with filled symbols. Differences between pond banks are indicated by: * $P < 0.05$ and (*) $P < 0.10$. c) Mean wind speed (± SE) and frequency of the wind directions during the sampled months. d) Correlation between number of days with a SW wind (per month) and the difference between the numbers of seeds trapped in mats at SW and NE banks.*

Table 2.2: Unstandardised regression coefficients of stepwise multiple regression models relating environmental variables 'Wind speed at 0.5 m elevation (m s^{-1})' and 'Lateral distance to the riparian vegetation zone (m)' to 'Total dispersal speed (m min^{-1})' of 37 seed mimics. The increase in R^2 is significant ($P = 0.047$).

Regression coefficients	Model			
Variable included	*r* Wind	*r* Distance	*P*-value	R^2
Wind speed		0.45	<0.001	66%
Wind speed and Distance to riparian vegetation zone	0.40	0.47	<0.001	71%

Long-term variation in deposition patterns

Unlike the deposition pattern, the pattern in the seed bank did not reflect dispersal of seeds governed by the prevailing wind direction. Neither the number of species nor the amount of seeds per litre of soil differed between both sides of the pond (Figure 2.4).

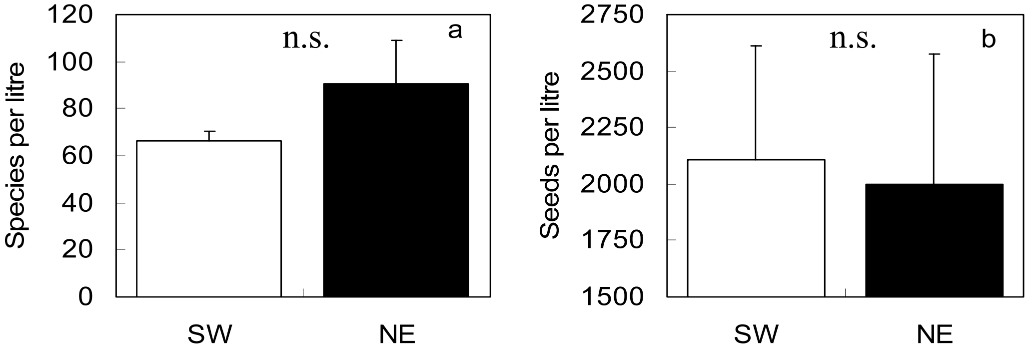

Figure 2.4: Seed bank composition. a) Number of species (± S.E.) and b) Number of seeds (± S.E.) per litre of soil from riparian zones at the SW and NE banks of seven fen ponds.

Variation in species composition

Weighted mean buoyancy, indicating the ability of the trapped seeds to disperse via water, was higher at NE banks than at SW banks (Repeated measures test; *P* = 0.01). The same analyses also indicated significant difference between the months, with a higher buoyancy in November 2007 compared to May and Augustus 2007 (Bonferoni post hoc test *P* = 0.07 and *P* = 0.02, respectively), because of a peak of relatively short floating species in August and a peak of long floating species in November. Weighted mean terminal velocity (m s^{-1}), used to quantify the ability of the trapped seeds to disperse by wind, was significantly higher at NE mats (*P* = 0.002), indicating that the proportion of seeds adapted to wind dispersal was lower there (Figure 2.5b). No differences between the months or interaction effects were found. Overall, those results suggest that relatively more seeds reached the NE mats via water.

The species composition of seed banks did not show a bias towards a certain dispersal vector. Both the mean buoyancy and mean terminal velocity were higher on NE banks, but these differences were not significant (Figure 2.5).

Discussion

Unlike dispersal via water in rivers and streams (lotic waters), dispersal via water in lakes and ponds (lentic waters) appears to be directly influenced by

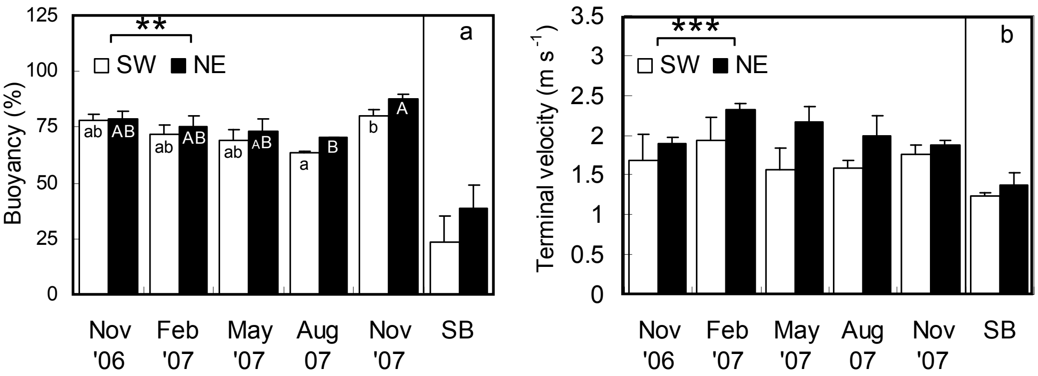

*Figure 2.5: a) Weighted means of the buoyancy (% of the seeds still floating after one week) of the seeds trapped in the mats at SW and NE pond banks and in the seed bank (SB). b) Weighted means of the terminal velocity (m s^{-1}) of the seeds. Differences between pond banks are indicated by: ***$P < 0.01$, ** $P < 0.05$, * $P < 0.10$. Differences between months are indicated with letters in the bars. Error bars indicate the standard error of the mean.*

wind speed and direction, and deposition patterns are consistent with dominating wind directions.Overall, our results show that wind-driven hydrochorous dispersal of floating seeds can be a relatively fast process, with speeds up to 0.10 m s^{-1}. Under favourable wind directions, long dispersal distances can be achieved at low wind speed, when seeds move slowly and erratically but do not become trapped in the vegetation. On longer time scales, and particularly over the season in which most seeds are dispersed (autumn), larger numbers of seeds and seeds of more species are transported to pond banks that are predominantly downwind. The species composition of deposited seeds also reflected an effect of wind ho hydrochory, with proportionally more water-dispersed seeds trapped at downwind banks. Over the years, no accumulation of seeds was found in the seed banks of predominantly downwind pond banks compared to upwind pond banks.

Direct effects

The results of our tracking experiment can be explained from the interaction between wind, water and pond morphology at different wind speeds. The simple morphology of our ponds and their location in an open landscape allowed for maximal influence of the wind on the water surface. Therefore, we assume that the relation between wind and dispersal speed approaches the upper limit for ponds in this size class. In general, increasing wind speed will increase the shear stress on the water and this will result in larger surface currents and higher waves (Shemdin 1972). Such faster currents result in faster transportation of seeds. This was also shown by Soomers *et* *al*. (in press) for seed transport in drainage ditches. However, wind direction is generally more variable at low wind speed (Wieringa and Rijkoort 1983) and water currents are more susceptible to follow these changes. As a result of this, we found longer, more variable dispersal trajectories at low wind speed. At high wind speed, the direction of the water mass changes less easily by small changes in wind direction, waves become larger and, due to shear with the riparian vegetation, water movement towards the bank increases (Stocker and Imberger 2003). Consequently, seed dispersal is faster but dispersal distances are shorter as seeds become trapped in the bank sooner.

Within-year variation in deposition patterns

Over longer periods of time, during which wind direction changes considerably, dispersal in stagnant or slow-flowing water bodies appears consistently directional. Hence, our results challenge the current opinion that in lentic water bodies hydrochorous dispersal distributes seeds evenly through the system (Vanormelingen *et al*. 2008; Soomers, in press). The exact deposition pattern will depend on the interaction between wind, water and pond morphology, but as all ponds have predominant downwind and upwind banks, wind-driven hydrochory will always result in more or less directional transport. Pond or lake size may play a role in this, however. In relatively small ponds such as ours, it is likely that many floating seeds reach the downwind side. In much larger lakes, seeds may sink before they reach the downwind end. In this case, the mechanisms remain the same, but fewer seeds are deposited at the downwind end of the lake (see also Grelsson and Nilsson 1991 and Nilsson *et al*. 2002, who suggest such a mechanism for lotic and lentic parts in river lakes).

The directionality of the transport found in our study has consequences for the ecological relevance of connections at different locations in a water body. Seeds are likely to leave a pond through connections located in the downwind banks. Consequently, such connections might function as a sink, removing seeds from a pond, whereas connections located predominantly upwind might form a source, supplying a pond with seeds coming from other locations.

Wind-driven hydrochory in our study resulted in somewhat larger to comparable numbers of trapped seeds and species per m^2 shoreline compared to studies on hydrochory in rivers (Nilsson and Grelsson 1990; Skoglund 1990; Vogt *et al*. 2004; Gurnell *et al*. 2007; Vogt *et al*. 2007) and salt mashes (Chang 2006). This implies that wind-driven hydrochorous transport in lentic water bodies is similar in transportation of numbers of seeds and species to hydrochory in lotic water bodies. Andersson *et al.* (2000) found a positive correlation between the number of deposited seeds and species richness in streams, leading to the expectation that in our fen ponds downwind riparian vegetation would have a higher biodiversity than upwind stands. However, this was not observed in our study, indicating that additional factors are at least equally important for the plant species richness of the bank. These are investigated in Chapter 3.

Long-term variation

A rough calculation estimates that it would take 2-10 years to deposit the number of seeds found in the top 10 cm of the soil, which is in the same order of magnitude as the mean age of the ponds (5 - 10 years). Two observations suggest that on longer timescales the seed bank composition is determined by additional factors. First, the number of seeds in the seed bank did not reflect the prevailing wind direction and second, the mean buoyancy of the seeds in the seed bank was much lower compared to the mean buoyancy of the seeds trapped in the mats. This may be the result of additional processes such as mixing activities of soil organisms (Willems and Huijsmans 1994), predation (Wurm 1998; Fraser and Madson 2008), differential germination success (Lenssen *et al.* 1998) and seed decay (Vogt *et al.* 2007). Hence, we may conclude that on time-scales from days to seasons, the ecological effect of wind-driven dispersal of floating seeds on seed transport and deposition in ponds and other lentic water bodies is large, but over longer periods (more than several years) it is reduced by additional processes playing at least equally important roles (see Chapter 3).

Species composition

The difference in mean terminal velocity indicates that not only the number of seeds, but also the species composition of the trapped seeds differs between upwind and downwind riparian zones. Both the presence and the absence of a relation between deposition of seeds and their buoyancy have been found previously (Nilsson *et al.* 1991; Andersson *et al.* 2000; Nilsson *et al.* 2002). Those differences can be attributed to the local hydrology and the mechanism behind hydrochory in those systems. Besides buoyancy, seed traits such as seed shape or the presence of wings and plumes might determine the hydrochorous dispersal as well. Those wings and plumes for instance, could act as sails and might be as important as buoyancy for transport via wind-driven hydrochory.

Wind-driven hydrochory in perspective

In this study we showed that dispersal of floating seeds in ponds or shallow lakes is determined by wind speed and direction and that the deposition pattern is consistent with the prevailing wind direction. In the long term (more than several years) the effects on vegetation and seed bank composition are obscured by additional processes playing roles that are at least equally important. Wind effects on hydrochorous dispersal have been shown to be important in other systems as well, be it more accidental, causing alterations to the major dispersal pattern that is determined by currents. In a free-flowing river, Andersson and Nilsson (2002) found remarkable differences between deposition patterns in different years. They attribute this to differences in wind conditions. Danvind and Nilsson (1997), Lacap *et al*. (2002), Chang (2006) and Reyns *et al*. (2006), reasoned likewise for their results in rivers and coastal marshes. Hence, wind is not only an important mechanism in wetlands for the transportation of airborne seeds (Soons 2006), it is also a common mechanism for the transportation of waterborne seeds.

In lentic water bodies, current velocity and wave size are relatively coupled as they are the direct result of wind speed. In rivers, however, current velocity and wave size can vary relatively independently because they are determined by separate mechanisms. This will have consequences for the dispersal in such water bodies, as the effect of waves is different from that of currents (Chang 2006). Waves tend to scatter and deposit seeds, whereas currents mainly transport. Disentangling the effects of waves from those of currents might further unite studies on hydrochory in different wetland types.

Conclusions

Our results clearly show that wind speed and direction are major determinants of the dispersal process and deposition patterns of floating seeds in ponds and other lentic water bodies. This results in a directional transport that follows the prevailing wind direction. This directionality is likely to have consequences for the colonisation of riparian zones at different locations in relation to wind directions. It is also likely to have consequences for the ecological relevance of connections between water bodies. Overall, we demonstrate that wind plays an important role in the dispersal of water-borne seeds, on scales from days to seasons, but that its role over longer periods of time (more than several years) is overruled by other ecological processes occurring at this scale. This emphasises once more the importance to consider the different scales at which ecological processes operate (cf. Sandal and Smith 2009).

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INTERMEZZO **1**

Dispersal of vegetative propagules in fen ponds

J.M. Sarneel

Introduction

It is generally acknowledged that besides dispersal via seeds, dispersal via a broad variety of vegetative plant parts provides an important alternative dispersal mode. This was established by numerous observations of vegetative propagules collected in seed traps in rivers (Eber 1983; Klosowski *et al*. 1995; Haraguchi 1996; Smolders *et al*. 1995; Boedeltje 2003; Riis and Sand-Jensen 2006; personal communication C. Vogt) and a few studies that investigated the dispersal patterns of these vegetative diaspores (Johansson and Nilsson 1993). In fen riparian zones, where the majority of species have clonal growth forms and a high proportion of aerenchyma, dispersal of vegetative propagules such as rhizomes, stolons, tubers, turions, simple stem fragments or even entire plants, might form an important dispersal mechanism. By hydrochory, vegetative diaspores have been reported to travel distances up to 5.4 km (Table B 1.1). Part of the variation in dispersal distances can be attributed to differences in flow and hydrology of the studied water bodies. However, variation in species traits may also play a role (Riis and Sand-Jensen 2006). For seeds, it has generally been assumed that the buoyancy of a seed is a good proxy for its ability for long distance dispersal by water (Kleyer *et al*. 2008). This may also apply to vegetative propagules. To assess the relative importance of vegetative diaspores in the dispersal of fen species we raised the following questions:

1. What proportion of the total number of propagules that is deposited on banks in fen ponds consists of vegetative propagules?
2. What is the range of buoyancies of vegetative propagules of fen species?

Table B 1.1: Reported (minimal and maximal) dispersal distances of vegetative diaspores.

Distance	*Species*	*Fragment length*
19 m min. 4605 m max	*Ranunculus peltatus* L.[a]	15-20 cm
21 m min. 658 m max	*Elodea Canadensis* Michx. [a]	15-20 cm
100 m min	*Ceratophyllum demersum* L.[b] *Elodea nuttalii* (Planch.) H. St. John[b] *Elytrigia repens* (L.) Nevski[b]	Unknown
102 m on average, 206.2 m max	*Dioscorea oppositifolia* L.[c]	Bulbils
300 m min	*Potamogeton pusillus* L.[b]	Unknown
600 m min	*Juncus bulbosus* L.[b]	Unknown
800 m min	*Potamogeton trichoides* Cham.&Schltdl.[b]	Unknown
1500 m for the majority, 3000 m max	*Ranunculus lingua* L.[d]	20-30 cm
3100 m min	*Myriophyllum spicatum* L.[b]	Unknown
5400 m min	*Ranunculus aquatilis* L.[b]	Unknown

[a] Riis and Sand-Jensen (2006)
[b] Boedeltje *et al.* (2003)
[c] Thomas *et al.* (2006)
[d] Johansson and Nilsson (1993)

Methods

Deposition measurements

During November 2006 and February, May, August and November 2007, 50 x 50 cm seed traps, in the form of artificial grass mats (cf. Wolters *et al.* 2004) were installed at the waterline in the centre of SW and NE banks ponds in Westbroek, a fen area in the Netherlands (for a more elaborate description see Chapter 2). After 4 weeks, the mats were collected from the field and thoroughly rinsed. The seeds and vegetative propagules in the trapped material were counted and identified using Van der Meijden (2005) and Cappers *et al.* (2006).

Buoyancy measurements

Vegetative propagules of ten species *Acorus calamus* L., *Calla palustris* L., *Comarum palustre* L., *Equisetum fluviatile* L., *Hydrocharis morsus-ranae* L., *Mentha aquatica* L., *Menyanthes trifoliata* L., *Phragmites australis* (Cav.) Steud., *Stratiotes aloides* L. and *Thelypteris palustris* Schott. were collected from the field at the end of September 2006. Only free-floating rhizome and plant fragments were collected. As the banks had been mown or dredged a few days before, it can be assumed that the collected fragments were fresh and had not been floating for a long time. *Calla*, *Hydrocharis* and *Stratiotes* produce specially adapted vegetative propagules and these were collected directly from the plant, from at least 3 populations. On the 11th of October 2006, 20 individual propagules of each species were put afloat in containers (50 x 50 cm), filled with rainwater to a depth of 60 cm. The rhizomes of three randomly selected species were placed together in one container. During the first two weeks, the depth of the propagules to the water surface was measured at two day intervals. After this period, the depth of each propagule was measured on a weekly basis. When a propagule had reached a position deeper than 50 cm, it was registered as being ‘sunk’. Each week, the water was stirred by making 3 circles with a long wooden stake to mimic natural turbulence. The specialised propagules of *Calla*, *Hydrocharis* and *Stratiotes* were too small for the large containers and were placed in smaller pots (19 cm x 12.5 cm) filled to a water depth of 8 cm. The same procedure was followed for these pots. After 6 months all propagules (except for the aquatic species *Stratiotes* and *Hydrocharis*) were taken from the containers and planted in trays (395 x 430x 75 mm) in the greenhouse. The trays were filled with potting soil and watered regularly.

Table B 1.2: Mean number of seeds and vegetative propagules found in the seed traps (50 x 50 cm) that were placed on NE and SW pond ends.

Sampled month	Pond side	Seeds		Vegetative propagules		% Vegetative
		Average	S.E.	Average	S.E.	
Nov-06	SW	405	*263*	24	*13*	6
	NE	5132	*2564*	4576	*2283*	47
Feb-07	SW	135	*31*	13	*10*	9
	NE	465	*218*	3633	*3269*	89
May-07	SW	369	*207*	92	*86*	20
	NE	986	*869*	141	*108*	13
Aug-07	SW	1202	*487*	9	*5*	1
	NE	629	*501*	112	*54*	15
Nov-07	SW	1364	*463*	377	*197*	22
	NE	2827	*1511*	1907	*877*	40

Results

Deposition

On average, between 1 and 89 % of the total number of propagules trapped in the mats consisted of vegetative diaspores (Table B 1.2). The total number of vegetative propagules varied enormously between the sampled ponds and the sampling periods (Table B 1.2). Most vegetative propagules were deposited at downwind pond ends (repeated measures, within-subjects effect; *P* = 0.013) and in winter (between-subjects effect; n.s.). A large number of propagules trapped were from the submerged and floating aquatic species *Elodea* spec. and *Lemna minor* L. (up to 13372 and 6222 propagules and on average 66% and 14% of the vegetative propagules, respectively). The most frequent emergent riparian species found were *Sparganium* species, *Mentha aquatica* and *Glyceria maxima* (Hartm.) Holmb.

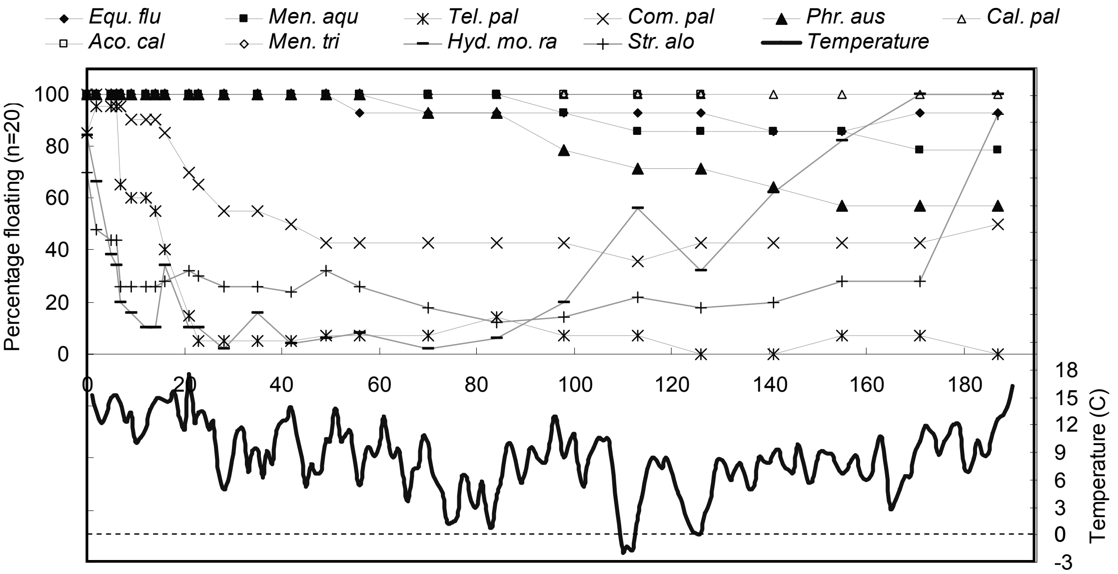


Figure B 1.1: Percentage of the propagules of the different species that remained floating between 11 October 2006 and 13 April 2007 (left axis) and temperature changes over time (right axis). Species names are abbreviated as the first three letters of the generic and species name.

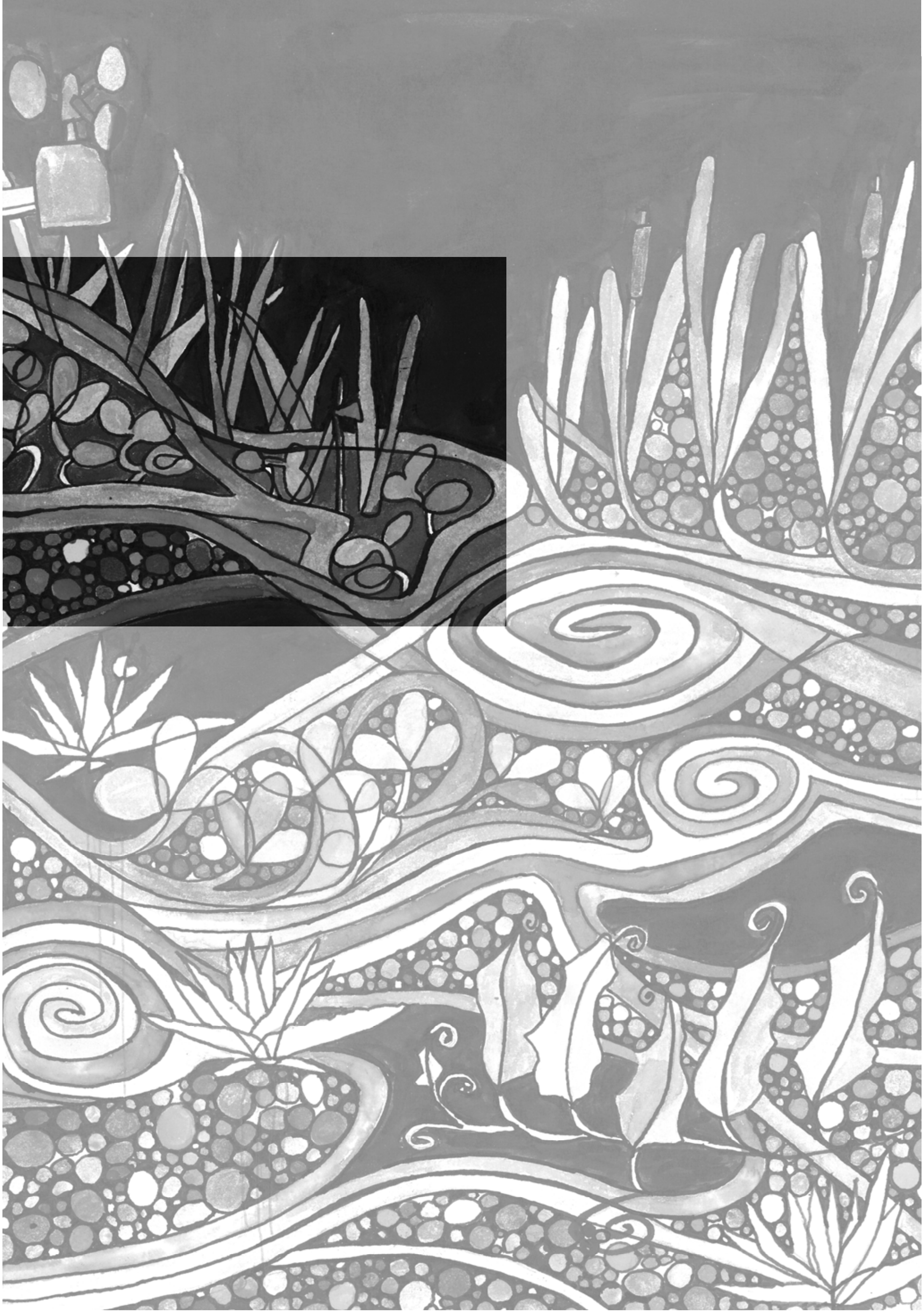
Buoyancy

The selected species differed widely in their ability to float (Figure B 1.1). Propagules of *Hydrocharis* and *Stratiotes* were the first to sink, followed by the woody stem fragments of *Comarum* and *Thelypteris*. Sinking was a relatively gradual process in these last two species. Respectively 5 and 11 propagules took several days or even a week to reach the bottom of the container. After about 100 days, the first samples of *Phragmites* started to rot and sank, followed by the slender-rhizome species *Mentha* and *Equisetum* that also disintegrated. The thick rhizomes of *Acorus* and *Menyanthes* and the propagules of *Calla* remained buoyant over the entire six months. Not a single propagule of these species sank. These floating times are high compared to the floating times of seeds. In the Leda trait-base reliable buoyancy data are available for 778 species (Kleyer *et al*. 2008). For 313 species, over 50% of the seeds have already sunk after just one week.

Surprisingly, the propagules of *Stratiotes* and *Hydrocharis* increased buoyancy when spring started and almost all propagules were floating again when the experiment ended (Figure B 1.1). This fluctuation is known for mature *Stratiotes* rosettes, but apparently juvenile *Stratiotes* and *Hydrocharis* plants show the same behaviour. Leaves were formed when they started to float again. While floating, 72% of the propagules of *Calla* started to form tiny leaflets. The viability of the propagules of the other species was generally high but was not related to the buoyancy. *Mentha* had the lowest budding percentage (36%), followed by *Comarum*, *Acorus*, *Equisetum* and *Menyanthes* (57, 58, 67 and 69%, respectively). *Thelypteris* and *Phragmites* had the highest percentage of budding rhizomes (73 and 98%, respectively).

Conclusions

This study clearly demonstrates that vegetative propagules may form an important dispersal mode in fen ponds. First, high numbers of vegetative diaspores are dispersed by hydrochory, especially during the winter months. Second, they often form a large proportion of the total amount of floating diaspores. Third, the vegetative diaspores of many fen species have high buoyancies and thus are well adapted to (long distance) dispersal via water. In the field, the combination of properties ‘buoyancy’, ‘size’ and ‘shape’ would determine the actual dispersal distances that can be achieved by vegetative diaspores (Johansson and Nilsson 1993). It will be interesting to compare the dispersal behaviour of vegetative diaspores to the dispersal of seeds (described in Chapter 2). As some species (*Hydrocharis* and *Stratiotes*) regained buoyancy in spring, after sinking over the winter season, their dispersal capacity may easily be underestimated in short term experiments. Therefore, buoyancy experiments with vegetative propagules should last for a very long time (several months). Overall, dispersal of floating vegetative propagules is likely to be highly effective for long distance dispersal and the colonisation of fen banks, as a high proportion of the propagules was still able to bud after six months.



CHAPTER 3

Post dispersal probability of germination and establishment on the shoreline of shallow lakes and ponds

J.M. Sarneel and M.B. Soons

Abstract

Many riparian pioneer species occur predominantly on shorelines sheltered from the wind. However, water-borne dispersal of floating seeds (which in slow-flowing or stagnant water bodies is driven by wind), deposits the majority of seeds at downwind, exposed shorelines. We hypothesised that differences in germination and establishment between sheltered and exposed shorelines caused this difference, and tested this for a set of five typical fen species.

Seeds of *Berula erecta*, *Calla palustris*, *Comarum palustre*, *Glyceria maxima* and *Mentha aquatica* were sown at sheltered and wind exposed shorelines in eight fen ponds. Seed germination and establishment, temperature, light availability, water level, wave impact, litter and seed deposition and vegetation height were recorded during 16 weeks in late spring and summer. In an additional greenhouse experiment we examined the effects of each separate environmental factor.

The results show that wind-induced water currents that drive hydrochory also cause differences in recruitment conditions. In the greenhouse experiment litter addition decreased germination, but in the field, higher solar radiation at exposed banks overruled this effect. In the field the number of seedlings decreased strongly over time, and eventual recruitment was determined by the degree to which seeds and seedlings were washed away by wave action. The probability to be washed from the shore was highest at exposed shorelines (where waves were larger) and this resulted in a higher recruitment at upwind, sheltered shorelines. Thus, wind exposure affects seed deposition as well as subsequent probability of establishment, with waves and water levels playing key roles.

Keywords: Colonisation, Hydrodynamics, Restoration, Riparian vegetation, Wetlands

Introduction

In Dutch fens, pioneer species that colonise open water and induce the formation of floating peat mats, such as *Comarum palustre* L. and *Calla palustris* L., have become rare (Lamers *et al.* 2001; Verhoeven and Bobbink 2001; Beltman *et al.* 2008). Consequently, the floating peat mats and the large number of species associated to them (up to 30 species m^{-1}), have become rare too. This has raised great concern for their restoration but unfortunately, establishment of new populations of these pioneer species has seldom been observed over the past 20-30 years. The exact causes for this remain largely unclear as the habitat quality has considerably increased (in most cases by substantially reducing nutrient inputs; Beltman *et al*. 2008) and remnant populations are often still found in most fen areas. As dispersal is often acknowledged as a crucial process (Poschlod and Bonn 1998; Tockner and Stanford 2002; Soons *et al.* 2005; Ozinga 2008), the lack of new colonisation events of pioneer species in fens has been attributed to a limited dispersal from these remnant populations to restored habitat patches (Beltman *et al.* 2005; Beltman *et al*. 2008).

The main dispersal vectors in fens are water and wind (Soons 2006), and in riparian zones dispersal via water (hydrochory) plays a crucial role (Sarneel *et al*. 2010). Hydrochory in slow-flowing or stagnant (lentic) water bodies such as fen ponds is wind-driven and follows the prevailing wind direction (Sarneel *et al*. 2010). The majority of floating seeds will therefore be deposited in on downwind, wave exposed shorelines (Sarneel *et al*. 2010). However, the desired coloniser species grow mainly in habitats sheltered from the wind (Schaminée *et al.* 1995; Azza *et al*. 2002, 2007). Thus, it is likely that not only dispersal itself, but also the subsequent recruitment (germination and establishment), limits new colonisation events. Therefore, we investigated the post-dispersal probability of germination and establishment along the wind-driven seed deposition gradient in lentic water bodies. For a broad range of wetland species, the effects of many environmental factors on recruitment have been studied (e.g. Lenssen *et al.* 1998; Xiong *et al*. 2003), but the relation between seed deposition patterns and conditions for recruitment remains largely unclear.

The wind-driven surface currents and waves that transport seeds will inherently result in higher waves and greater turbulence at the downwind side (Shemdin 1972; De Wandre 2005), which may also affect germination and establishment (Moggridge and Gurnell 2009). This influence of waves could either be direct, by depositing and removing seeds and seedlings from the bank, or indirect, by influencing the recruitment conditions (light, moisture and temperature) experienced by the seeds and seedlings. Four habitat characteristics were hypothesised to influence these recruitment conditions. First, higher waves at downwind shorelines will deposit seeds higher on the bank. This would result in a lower moisture content of the site where seeds are deposited. Moisture is known to affect seed germination and establishment, albeit in a species-specific way (Lenssen *et al*. 1998). Second and third, waves will deposit greater amounts of litter and seeds on downwind banks, which could reduce recruitment (Xiong *et al*. 2003) through reducing light and seedling competition (Cheplick 1991, 1993). A fourth, complicating factor is that differences in geographical orientation of the shoreline, which determine seed input and hydrodynamic forcing, also affect the input of sunlight. This, in turn, could result in differences in temperature and moisture between upwind and downwind shorelines. The prevailing light climate at the soil surface will however also depend on the height and density of the standing vegetation. Overall, light availability should also be considered while disentangling the effect of wave action and turbulence from other factors. Sedimentation processes are also related to turbulence and could result in differences in chemical composition of the soil (Grelsson and Nilsson 1991). However, for our fens, unpublished data (n = 17 banks) showed that nutrient availability was not related to wave exposure. Therefore, we did not investigate this in the present study, although nutrient availability can effect germination as well.

Our main objective was to evaluate the probability of post-dispersal recruitment for seeds deposited on shorelines in fens. Specifically, we 1) investigated the differences in germination and establishment between exposed and sheltered shorelines and 2) determined the factor(s) responsible for the differences. We focused on a group of important pioneer colonisers that mainly grow on sheltered banks, although wind-driven currents are likely to transport the majority of their seeds to downwind, exposed banks. We hypothesised that this could be explained by differences in germination and establishment and hypothesised that those differences are the result of contrasting recruitment conditions at sheltered, upwind and exposed, downwind sites (as described above).

Methods

A greenhouse experiment was designed to test the effects of different habitat characteristics prevailing at exposed and sheltered banks under controlled conditions. We performed a field experiment to test which factors determine germination and establishment at exposed and sheltered banks under natural conditions.

Study system

We investigated the germination, establishment and overall recruitment of five typical pioneers in species-rich fen systems in the Netherlands. Fen ponds in these systems originate mainly from peat excavation that lasted until the early 1900s. This created shallow (1.5 m) ponds, typically about 30 m wide, about 100 to 900 m long and separated from each other by strips of intact fen peat (± 40 m wide) with species-rich herbaceous or woody wetland vegetation. Thus, these fen areas predominantly consist of riparian habitats, inhabited by for instance *Phragmites australis* (Cav.) Steud., large *Carex* species, *Mentha aquatica* L. and *Rumex hydrolapathum* Huds. The prevailing wind direction in the Netherlands (South West; SW) creates water currents that transport seeds towards North East (NE) banks. The upwind SW banks thereby receive lower seed inputs (Chapter 2). In the fen reserve '*Westbroek*' (52˚10N; 5˚07E) all ponds have the same SW-NE orientation, which makes them ideal for testing differences in recruitment between sites with contrasting wave action and seed inputs. We selected eight ponds of varying lengths (130 - 900 m).

The coloniser species selected are all characteristic of riparian fen plant communities inducing the formation of floating mats (Den Held *et al*. 1992). In the past, they commonly colonised open water from the shoreline, but they are now scarce in the riparian vegetation or do not expand into the water (Beltman *et al*. 2008). They play a comparable ecological role in our study system. Hence, we regard them as one functional group, characteristic of riparian fen vegetation that colonises the open water from the bank (Lamers *et al*. 2001). *Berula erecta* (Huds.) Coville, *Calla palustris* L., *Comarum palustre* L., *Glyceria maxima* (Hartm.) Holmb. and *Mentha aquatica* L. all expand clonally from the bank into the water, either by forming dense rhizome networks on the water surface or by rooting in the pond bottom. All species were present in at least one of the selected ponds. Seeds of these species were obtained from cultivated plants (*Calla* and *Glyceria*; Jellitto Staudensamen B.V. Schwarmstedt, Germany; 2007), or were collected in the field (Biodivers B.V., Reewijk, The Netherlands; 2007) and stratified under cold (5 ˚C), wet and dark conditions for three weeks prior to the experiments.

Greenhouse experiment

Starting on 29 April 2008, a greenhouse germination experiment was carried out to test the effect of different habitat characteristics prevailing at downwind (windward) and upwind (leeward) shorelines. Five different treatments were applied to disentangle effects. Each treatment was replicated four times.

(1) *Control*: For each species, 25 seeds were sown in 9.5 x 9.5 cm pots on sterilised potting soil. In each of the four replicates, the five species were put together in one large tray, which was filled with water to maintain a constant water level at the soil surface (waterlogged soil, representing the shoreline). Temperature was measured using temperature loggers (Thermochron G-type, KN laboratories, Inc., Ibaraki) in an extra pot filled with soil, added to each replicate. (2) *Drier deposition site*: To examine the effect of waves that deposit seeds at higher, and therefore drier, locations at downwind banks, we added a treatment in which the seeds were sown as in the control treatment, with the exception that the water level in the large tray was lowered to field capacity, 4 cm below soil level. (3) *Seed density*: To evaluate the effect of increased seedling competition caused by a higher seed input at downwind banks, we added a treatment where 25 *Rumex hydrolapathum* seeds were added to each pot. This species was chosen because it germinates fast and was very abundant in seed traps in the field (on average 5% and up to 48% of the total number of seeds; unpublished data). (4) *Litter*: To test for an effect of the great quantities of litter deposited on downwind banks, we added 17 g of litter on top of each pot in this treatment. This resembled the natural deposition on downwind banks measured in a seed trapping study (0.19 g litter per cm^{-2}; Nov 2007; unpublished data). Litter (mainly from *Sparganium* species and *Carex paniculata*) was collected in the field (April 2008), rinsed to remove all seeds and cut into small pieces to fit the pots. (5) *Shade*: In the field experiment, banks with a different input of floating seeds also differed in exposure to the sun. To examine the effect of the reduced light availability at upwind banks (at the SW pond side, hence facing NE), neutral density shading cloth was put over the pots in one treatment (reducing the total photon flux by 25%, as measured in the field on upwind banks). For all treatments, newly germinated seedlings were marked on a weekly basis over a period of 16 weeks using small iron pins. Every four weeks, the numbers of pins and seedlings were counted to determine the total germination and survival percentages.

Field experiment

To test the combined effect of habitat differences between upwind and downwind banks on germination and establishment, a field experiment was conducted in eight ponds in '*Westbroek*'. On 25 April 2008, we selected four bare patches of 10 x 15 cm at each upwind and each downwind pond bank. Those patches were located around the centre of each bank and had at least

75 cm between them. In each patch we applied a different sowing treatment. In the first treatment, 50 seeds of each of the five species were sown in lines of 10 cm long, perpendicular to and starting directly above the waterline (*'Sowing'* treatment). The lines of the five species were sown in random order. In this treatment seeds were able to float away due to wave action, which would result in biased germination scores if not accounted for. Hence, to determine the proportion of seeds that float away, 50 brightly coloured polypropylene granules (*'Seed mimics'* ; Ø 4mm; Hampe 2004) were placed next to the seeds sown. In a second and third treatment seeds were prevented from floating away. 50 Seeds per species were put in nylon mesh bags (10 x 15 cm, mesh-size 0.3 mm), which were divided in 5 parallel compartments (10 x 3 cm). Two bags were placed on each bank. The first bag was collected after four (*'Bag T4'*) and the second after eight weeks (*'Bag T8'*). A possible effect of the bag itself was tested with an extra treatment in the greenhouse experiment. Here, 25 seeds of each species were put in bags (5 x 3 cm, n = 4) and treated as a *Control* treatment. After eight weeks, germination percentages did not differ from the *Control* (independent samples t-test, SPSS 14.0). Therefore, we assumed that germination in the field was also similar inside and outside the bags. In the fourth and last field treatment we quantified the number of seedlings that originated from seeds present naturally. Therefore, we counted the number of seedlings of the experimental species in a bare patch in which no seeds were sown (*'No sowing'*). After 4, 8, 12 and 16 weeks (T4, T8, T12 and T16, respectively) we counted the number of visible seed mimics (without disturbing the soil) and the total numbers of seedlings for all species. At T16, soil and litter collected from 10 x 15 cm around the *Seed mimics* was carefully examined for remaining seed mimics.

To quantify the factors that may have influenced germination and establishment, we also measured habitat characteristics and recruitment conditions. Each 4 weeks, light availability at NE and SW banks was determined using a line quantum sensor (LI-191SA, Li-Cor, Lincoln, NE, USA) placed above the vegetation and a quantum sensor (LI-190, Li-Cor, Lincoln, NE, USA) on the soil surface in each plot. To measure temperature, one temperature logger (see above) was placed at the waterline in the deposition zone, between the treatments. In each pond, water level was measured every six hours using pressure loggers (Schlumberger, The Hague) and corrected for air pressure. For each plot, we used these data to calculate the number of six-hour time intervals that at least 50% of the plot area was submerged. Further, wave action at both sides of the pond was quantified using a PTX 1830 pressure sensor (Druck Inc., New Fairfield), which measured the water level every 20 s for five minutes. This was measured in open water (*Far*; 2 m from the emergent riparian vegetation) and close to the bank (*Near*; 0.75 m from the bank) on 4 and 12 March 2009 (wind speed 5.5 m s^{-1} and 4.3 m s^{-1} at 20 m height, respectively; www.knmi.nl). From these measurements, the mean absolute deviation from the mean water pressure was calculated, as a simple estimate of average wave height (Appendix 3.1). This variable was called wave impact. The density of seeds arriving at each bank was quantified in a previous study using seed traps (Chapter 2). Further, after 4, 8, 12 and 16 weeks, vegetation height and cover and litter layer depth and cover were quantified for each of the four plots on a bank. At T16, the litter and the vegetation (30 x 30 cm surrounding each plot) were harvested, dried (48 hours, 70 °C) and weighed.

Data analyses

As the five selected species represent one functional group, the numbers of seeds and seedlings of the individual species were summed and mean germination and survival percentages were calculated. This approach also proved the best way of dealing with the relatively large number of zero values for the individual species. Differences between treatments in the greenhouse experiment were tested using One-way ANOVA with a Dunnett's *post hoc* test (SPSS 14.0), after arcsine transformation of the germination and establishment percentages.

In the field experiment, germination percentages ($G_{\%}$) were calculated by dividing the total number of seedlings in the '*Bag T4*' treatments (4 weeks after the start) by 250 (five species x 50 seeds). The '*Bag T4*' treatment was selected because germination was highest at that time. Establishment success was calculated for each sample as the total number of seedlings found in a plot, divided by the number that could have potentially germinated in that plot, taking into account that a given fraction floated away before germination. For example, after eight weeks (T8):

$$Est_8 = \frac{S_8 - N_8}{(250 - F_4) \cdot G_{\%}} \tag{1}$$

where S_8 and N_8 are the total number of seedlings of the experimental species in the '*Sowing*' and '*No sowing*' plots, respectively, both at T8 and from the same bank. F_4 is the number of seeds that floated away before germination, based on the remaining number of seed mimics in that specific plot at T4. In the same way Est_{12} and Est_{16} were calculated, leaving the denominator unchanged.

Whereas our calculated establishment success (*Est*) represents survival of seedlings from the seeds that were not washed away, we also calculated a recruitment success (*Recr*) that indicates the probability that any seed sown would germinate and survive over 8, 12 or 16 weeks. This was done by removing F_4 from equation 1.

First, we tested whether the habitat characteristics of the four different patches selected on each bank were comparable at T0 (Block test, SPSS 14.0). Then, environmental differences between SW and NE banks (n = 8) were compared with a paired t-test for each time step. Lastly, separate mixed-effects ANOVA's were performed to test the relationships between $G_{\%}$, Est_8, Est_{12}, Est_{16} and *Recr* and each measured environmental variable one by one in *R* (version 2.8.0; 2008). Fixed effects were included for each individual environmental variable and a random effect was included for pond, thereby accounting for the relationship between the two sides (upwind and downwind) of each pond. Due to our relatively low sample size (n = 8), it was not possible to perform a regression analysis with all the variables in one model. Finally, a stepwise linear regression was performed to test the relation between *Recr* and the percentage of seed mimics that remained at a bank. Week was also included as an independent variable. However, week number was not included as a significant factor in the regression model and results did not change when week was forced into the model. Therefore, we show only the results of the stepwise regression model without week as a variable.

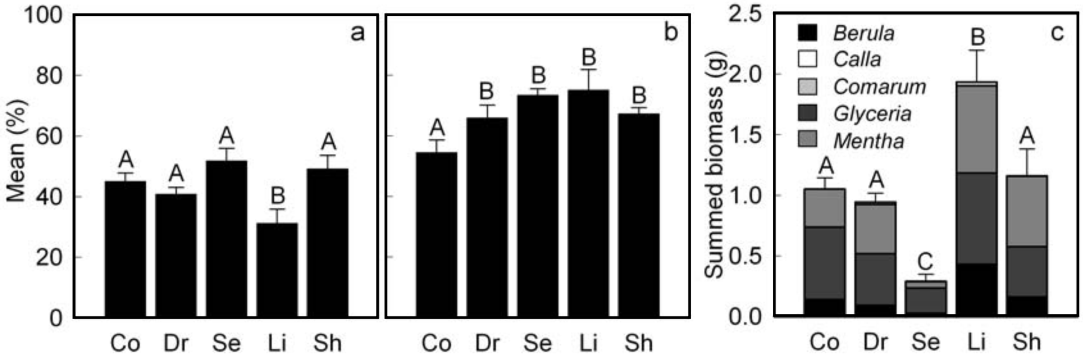


Figure 3.1: a) Mean germination percentages (± SE), b) survival percentages (± SE) and c) total dry biomass produced by the five species in the different greenhouse treatments, at the end of the experiment (week 16). Co = Control, Dr = drier deposition site, Se = seed addition, Li = litter addition, Sh = shade. Significant differences are indicated with letters above the error bars.

Results

Greenhouse experiment

The majority of the seeds germinated within 4 weeks. Mean temperature in the pots was 21˚C, with no significant differences between treatments. Overall, only litter addition significantly decreased the germination percentage (One-way ANOVA; P = 0.013; Figure 3.1a). This pattern was consistent among the different species. Survival of seedlings was higher than the control in all treatments (Figure 3.1b). This was mainly because the survival of *Comarum* in the control was very low, while the other species did not show differences in seedling survival. Although there were only small differences in germination and survival between the treatments, the development of the seedlings in the treatments differed strongly. In the seed addition treatment, the added *Rumex* seeds germinated very fast and the seedlings of all experimental species remained smaller (Dunnett's *post hoc* test; P = 0.029; Figure 3.1c). In contrast, litter addition affected the growth of the seedlings positively (Dunnett's *post hoc* test; P = 0.01; Figure 3.1c). These findings were again very consistent among species. The differences in growth did not affect the mean survival (Figure 3.1b), which was high (>75%) in all species except *Calla* (only one out of 94 seedlings survived).

Field experiment: Germination and establishment

The majority of seeds also germinated within 4 weeks in the field, as shown by the '*Bag*' treatments (Figure 3.2a). Germination percentages at downwind (NE) banks were higher than that at upwind (SW) banks (trend in Mixed-effects ANOVA; Figure 3.2a, P = 0.055). Germination and establishment of the experimental species of natural origin in the '*No sowing*' plot was negligible (Figure 3.2b). The number of seedlings of the experimental species in the 'S*owing*' plots was much lower than in the '*Bag*' treatments (Figure 3.2a, c), and declined strongly over time (Figure 3.2c). At T4, the number of seedlings in the '*Sowing*' treatment was about 35% of that in the '*Bag T4*' treatment. This is consistent with the finding that, on average, only 40% of the seed mimics were still present in the '*Sowing*' plots at T4, and indicates that many seeds or young seedlings may have been washed away. Correcting the establishment percentages in the *'Sowing'* plot for the number of seeds that floated away (Eq. 1) enabled separation of the direct and indirect effects of waves on establishment.

At upwind (SW) banks, establishment success was still 100% at T8 (One-sample t-test; P > 0.05; Figure 3.2d), indicating that all seedlings that had germinated from sown seeds and were not washed away before germination were still present. At the same time, at downwind (NE) banks, establishment success was already much less than 100% (One-sample t-test; *P* = 0.042), meaning that seedlings had either died or disappeared from downwind plots. During our study period, establishment declined drastically, but remained consistently higher at upwind (SW) than at downwind (NE) banks. Not only did establishment decline over time, but careful examination of the soil and litter of the plots after 16 weeks showed that almost all seed mimics had been washed away (Figure 3.2e). In 13 plots (out of 16), less than two seed mimics were found. However, significantly more seed mimics were retained at upwind banks than at downwind banks (paired t-test T16; *P* = 0.05).

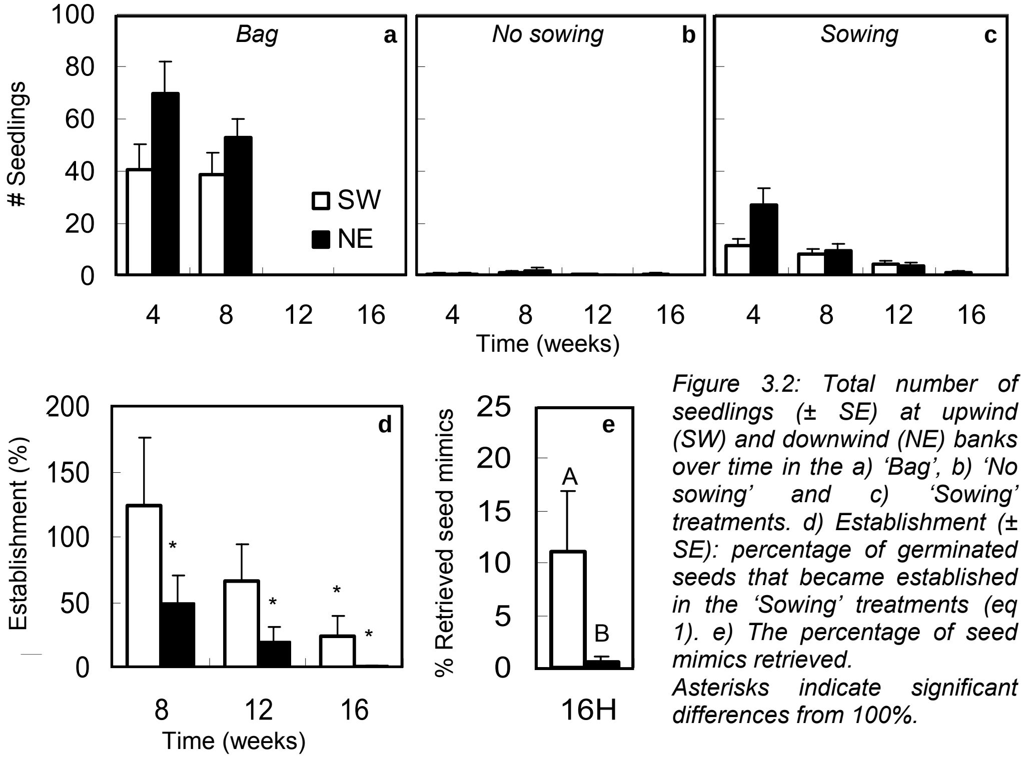


Figure 3.2: Total number of seedlings (± SE) at upwind (SW) and downwind (NE) banks over time in the a) 'Bag', b) 'No sowing' and c) 'Sowing' treatments. d) Establishment (± SE): percentage of germinated seeds that became established in the 'Sowing' treatments (eq 1). e) The percentage of seed mimics retrieved.
Asterisks indicate significant differences from 100%.

Field experiment: Environmental differences

In April, at the start of the experiment, the four patches selected on each of the 16 studied banks did not differ significantly from each other for any of the environmental variables. However, several of the hypothesised differences between upwind and downwind pond banks were found to exist. The wind-driven currents that transport seeds resulted in a larger wave impact at downwind banks (Figure 3.3a), both in the open water (paired t-test; *Far*: *P* = 0.01) and within 0.75 m from the bank (paired t-test; *Near*: *P* = 0.03). Wave exposure of the plots varied over time as the mean water level of the ponds first dropped and then increased again (after 14 weeks) due to evaporation and rainfall (Figure 3.3b). As upwind banks were a little less steep (paired t-test; P = 0.029), the plots on upwind banks were submerged during a slightly (but not significantly) longer time period. Other habitat characteristics, which are more indirect effects of waves, also differed between upwind and downwind banks. More litter (on average SW: 0.95 g versus NE: 2.2 g dry weight at T16; paired t-test; P = 0.009) was deposited on plots at downwind (NE) banks. In another study, conducted in the same ponds, we showed that the number of deposited seeds was also higher at downwind banks (on average in November 2007 SW: 5455 seeds m^{-1} versus NE: 11306 seeds m^{-1}; Chapter 2). Consequently, there was a trend that seedling density (the number of seedlings per plot) of non-experimental species was higher in downwind plots at time steps 4 and 8 (paired t-tests; P = 0.09 and P = 0.06, respectively).

As the differences in geographical orientation that determine differences in wind and wave action also determine light input from the sun, light availability at downwind banks (facing south) was higher than at upwind banks (facing north) at the start of the experiment (paired t-test T0; P = 0.007; Figure 3.3c). During our study however, light availability at downwind banks decreased to values comparable to upwind banks. Probably due to a greater exposure to the sun, maximum temperatures were significantly higher during the first two months at downwind banks (paired t-tests; P = 0.01 and P = 0.06, respectively; Figure 3.3b) and mean temperature showed a weak trend to be higher over the first four weeks (paired t-test; P = 0.10; Figure 3.3b). Although the vegetation was 10-15 cm higher at downwind banks at T16, standing biomass of a 30 x 30 cm area surrounding the ‘*Sowing*’ plots was not significantly different between upwind and downwind banks (paired t-test).

Field experiment: Germination, establishment and recruitment in relation to environmental factors

Out of all the variables tested, mean germination percentage in the ‘*Bag T4*’ treatment only correlated significantly to light availability (positive correlation, mixed-effects ANOVA; P = 0.05). This accounts for the higher germination percentages at the downwind banks where light availability was significantly higher. The percentage of seed mimics that had floated away at T4 did not correlate to any of the measured environmental variables, not even to wave impact, which we had assumed to be the major factor explaining the number of remaining seed mimics at NE banks. The number of seedlings from experimental species that emerged in the ‘*No sowing*’ plots also did not

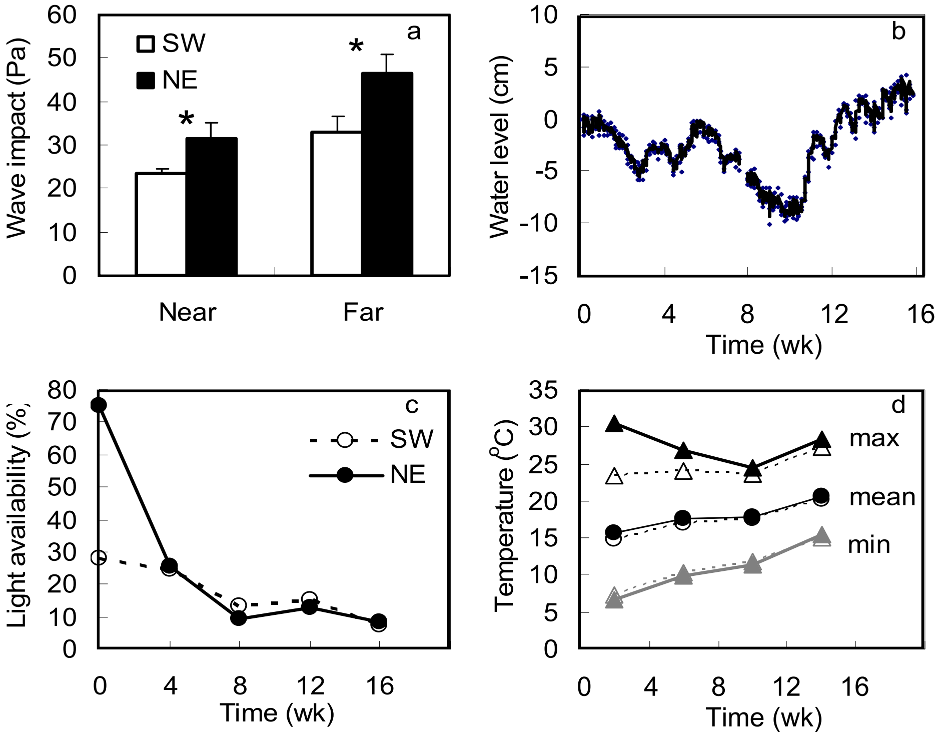


Figure 3.3: a) Wave impact (the mean of all absolute deviations from the mean water pressure; See Appendix 3.1) (± SE). Significant differences between the two ends of the pond are indicated by asterisks. b) Mean water level fluctuations during the field experiment. c) Mean light availability at soil level as a percentage of the total flux above the canopy, for the different banks during the field experiment. d) Mean temperature of the different banks during the field experiment. Open symbols, upwind (SW) banks. Closed symbols, downwind (NE) banks.

relate to any of the environmental variables. Hence we were unable to detect responses of natural recruitment to environmental differences, but as shown in Figure 3.3b, natural recruitment was generally very low. Establishment success (Est_8, Est_{12} and Est_{16}) in the 'S*owing*' plots (Eq. 1) was also not affected by any of the measured environmental variables. This is not surprising considering that the plots at both sides of the pond became more similar after T4 for several of the environmental variables (light, temperature; Figure 3.3). The addition of litter and extra seeds on downwind banks did not significantly affect establishment.

The overall recruitment percentage (*Recr*) combines the direct and indirect effects of waves on establishment of all seeds sown. None of the environmental variables affected *Recr* (mixed-effects ANOVA's). Recruitment decreased strongly over time (see symbols for different weeks in Figure 3.4a, b), indicating a low probability for survival over one growing season. However, there was a strong correlation between the recruitment percentages and the percentage of remaining seed mimics over time (Stepwise linear regression; $P < 0.001$; Figure 3.4). This pattern was also observed within T4, as banks that retained high numbers of seed mimics, also had high recruitment percentages (Pearson correlation; $P = 0.059$; Figure 3.4a, b; black dots). As the slope of this relationship for the plots at downwind banks is very close to 1 ($\beta = 1.03$; $R^2 = 0.618$), the number of seed mimics washed away almost equalled the amounts of seeds and seedlings that disappeared over time. On upwind banks, the slope of the curve was less steep ($\beta = 0.51$; $R^2 = 0.336$). Besides, the confidence interval did not include a slope of 1 and the intercept was significantly different from 0 ($P = 0.017$). This indicates that overall, recruitment was higher at these upwind banks as seeds and seedlings appeared to have a lower probability to be washed away from those banks.

Discussion

The aim of this study was to test which conditions determine germination and establishment of fen pioneer species at sites that receive contrasting seed inputs by wind-driven hydrochory. In general in fen ponds, wind-driven waves and currents affected the habitat characteristics and recruitment conditions at upwind and downwind pond banks. The higher light availability in downwind, south facing banks correlated positively to germination. Other, wave-imposed differences between upwind and downwind banks, i.e. seed and litter deposition, appeared to affect germination and seedling growth when tested experimentally as individual factors in the greenhouse.

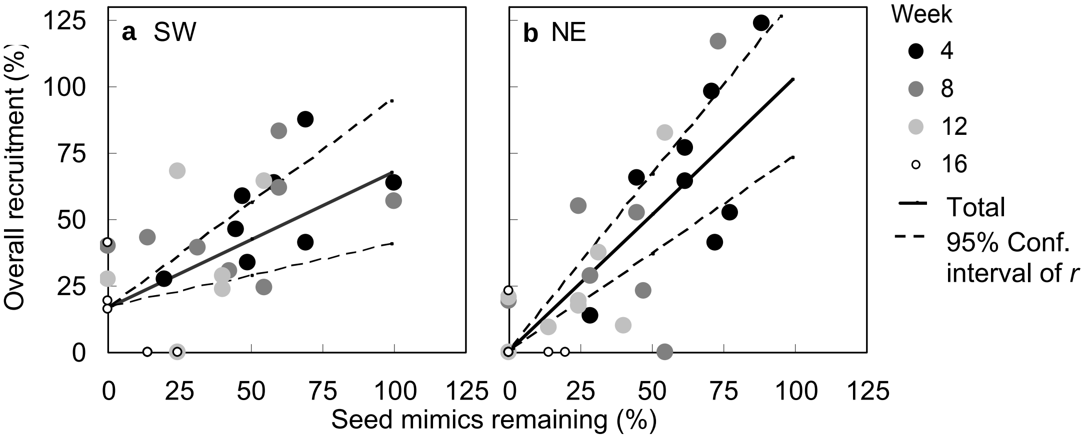


Figure 3.4: a) Relation between the (square root transformed) percentage remaining seed mimics and the (square root transformed) recruitment percentages at a) upwind (SW) banks and b) downwind (NE) banks.

In the field however, they did not affect germination and establishment, as germination and establishment were above all strongly related to the probability of seeds and seedlings of becoming washed away. The probability to become washed away was highest at downwind banks, which retained a smaller number of seed mimics and had a very strong (almost 1:1) correlation between recruitment percentages and remaining seed mimics. This indicates that in the field, recruitment of the pioneer species is strongly limited by the probability of seeds and seedlings to be washed away into the open water. This eventually can lead to more successful recruitment of fen pioneer species on upwind or lee-side shorelines, even though seed input is lower there.

Germination

The negative effect of litter on germination found in the greenhouse experiment was not detected in the field, where the larger amounts of litter deposited on windward banks did not have consequences for germination. In fact, germination without the effect of seeds being washed away (in the '*Bag*' treatment) was only significantly related to light, with higher rates on the relatively more illuminated downwind, south facing banks, which also received most litter. This suggests that in the field the effect of additional litter was overruled by differences in light availability. Other studies also report effects of both litter and light on germination (Xiong and Nilsson 1999), but especially the effect of litter has been shown to be susceptible to interaction effects with other environmental characteristics (Xiong *et al*. 2003). We assume that the complex interactions with factors such as temperature, soil or water type caused the differences between effects measured in the greenhouse and in the field. However, in a natural situation (like in the '*Sowing*' plot) any effect of separate variables will be diminished by wave action, washing seeds and seedlings away from the shore.

Other studies in wetlands (Lenssen *et al*. 1998; Blomqvist *et al*. 2005; Van den Broek and Beltman 2006) report germination percentages comparable to those in the bag treatment, but higher percentages compared to the *'Sowing'* treatment. This seems to support our main findings that wave impact is highly important, as these studies focussed not specifically on shorelines, but on wet grasslands, fen meadows and marshes where, like in the bag treatment, the probability to become washed away is low. Secondary seed movement after initial deposition is also found in other systems where strong water movements occur, such as floodplains and tidal marshes (Lacap *et al*. 2002; Hampe 2004; Chang 2006; Gurnell *et al*. 2007). In such systems, secondary dispersal may play an even more important role than previously thought.

Establishment

In relation to the general decline of riparian fen pioneer species, we found indications of strong seed limitation in the field. In the treatments where we tested for the presence of seeds from natural origin, only a few seedlings were found (on average 39 seedlings m^{-2}). In addition to that, survival of these seedlings over the first growing season was extremely low (0 - 17% after 16 weeks), especially at downwind banks (max 5.3% survival after 16 weeks). Therefore, we conclude that new colonisation events by coloniser species in fen riparian zones are restricted by strong seed and establishment limitations. Establishment was especially low in *Comarum palustre* and *Calla palustris*. Out of 800 seeds sown for each species, only 3 and 16, respectively, germinated in the field and even fewer individuals (1 and 0, respectively) became established. Establishment is frequently found to be a crucial step in colonisation events in other wetland types as well (e.g. Lenssen *et al*. 1998; Clark *et al*. 2007; Vogt *et al*. 2007). For instance, Andersson *et al*. (2000) found that recruitment in a 50 m river stretch correlated to the availability of suitable habitat (bare ground) and not to seed input.

Other studies on wetlands report establishment percentages of 10% - 50%, which are roughly comparable to what we found (12% after 16 weeks) if we corrected the germination percentages for the number of seeds that floated away. Overall recruitment percentages were lower, only 2.1%, which again shows that the removal of seeds and seedlings has a strong effect. These seeds and seedlings may have been redistributed via water flows, for example to less exposed sites (personal observations), or they may have been lost to the population. On larger spatial scales, effective colonisation might therefore also depend on the probability of secondary seed dispersal and the establishment of floating seedlings in a habitat that is more suitable (personal observations; see also Prentis *et al*. 2006). Other studies also suggest that seeds are able to move over considerable distances after initial deposition, (2 – 250 m; Lacap *et al*. 2002; Hampe 2004) and become established at new sites. However, this additional dispersal would increase the residence time in the water as well which increases the probability to sink (and subsequently die). This would enlarge the probability that a seed is lost to the population. Much less is known about water-borne dispersal of seedlings and only accidental observations are reported, but the general impression is that seedlings of many species may float for some time at least (Pers. Obs.; Boedeltje 2005; Prentis *et al.* 2006; Ozinga 2008).

Water level fluctuations in lentic water bodies

Given the strong positive relationship between remaining seedlings and remaining seed mimics (as a proxy of the probability to become washed away), it can be concluded that wave impact on shorelines is of great importance for local seedling survival and recruitment. Wave impact is determined by the orientation of the bank to the wind direction and by the water level. This implies that water levels can have a strong effect on germination and establishment, as they determine the range of shoreline that is reached by the water and waves. Nowadays, water tables in the Netherlands and in many agricultural landscapes in other countries are maintained at relatively high constant levels over summer and low levels over winter, minimising or even reversing the natural water table fluctuations (Lamers *et al*. 2002). Consequently, seeds are deposited at relatively low locations on the bank in late autumn/early winter, which is the main dispersal season. Because the water level does not change or even rises in summer, seedlings that emerge in spring are very susceptible to becoming washed away. Under a natural regime, the water level drops in spring and summer and seeds and seedlings would remain safe from being washed away during germination and early establishment. This effect might form an additional explanation for the observed vegetation changes following intensified regulation of the water table in agricultural landscapes (Leyer 2005).

Conclusions

Our results show that the wind-induced water currents in lentic water bodies not only strongly affect hydrochorous dispersal, but also cause differences in recruitment conditions along the seed deposition gradient. Wind-driven hydrochorous dispersal leads to deposition of seeds in greater numbers at downwind shorelines, where their establishment is strongly reduced. This is due to a high probability of seeds and even seedlings to become washed away from downwind banks. Consequently, within one growing season, eventual recruitment is highest at upwind, more sheltered shorelines and new colonisation events of coloniser species depend on local establishment limitations as well as seed limitations. The effect of seeds and even seedlings being washed away from the shore overruled effects of other environmental factors such as the amount of litter deposited. The current water regime in the fen areas studied, with relatively constant water levels that are equally high (or higher) in summer than in winter, could therefore be an important bottleneck for the occurrence of new colonisation events by riparian species.

Acknowledgements

We thank Jos Verhoeven and Boudewijn Beltman for their suggestions and comments, colleagues and students for assisting with the practical work and the State Forestry Service for access to ‘*Westbroek*’. The NIOO-CEME is acknowledged for providing the PTX 1830 pressure sensor and Matthijs Benit (TU Delft) for advice on wave calculations. This study was conducted within the National Research Programme ‘Ontwikkeling + Beheer Natuurkwaliteit’, funded by the Dutch Ministry of Agriculture, Nature and Food Quality.

Total

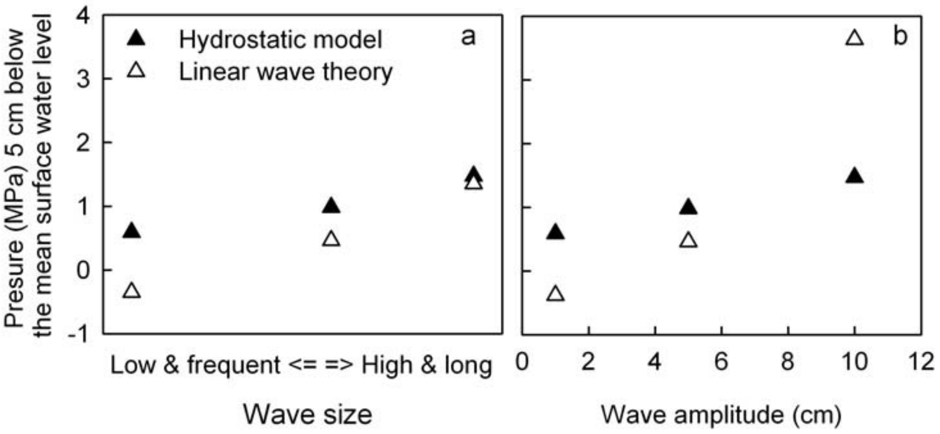


Figure A 1: a) Relation between water pressure at 5 cm below the average water level and overall wave size. Along the x-as, wave amplitude increased from 1 to 5 to 10 cm and simultaneously frequency decreased from 3 to 2 to 1.5 Hz. b) Relation between water pressure and wave amplitude of a middle long wave (2 Hz) for the different hydrodynamic calculation models

Appendix 1: Relation between ‘wave impact’ and wave height

We calculated ‘wave impact’ as the mean absolute deviation from the mean pressure measured and assumed a hydrostatic pressure distribution in our data. In this appendix we explore the differences to more complex but also more correct calculations following linear wave theory. In the case of short, small waves (about 40 cm long and up to 10, 20 cm high) in deep water (>2 times wave height), such as in our study, the following relation between wave amplitude and measured pressure exists (Crapper 1984):

$$p - \overline{p} = \rho \cdot g \cdot a \cdot \cosh(k \cdot a) \qquad (1)$$

where *p* is the measured pressure (Pa) and $\overline{p}$ is the average pressure of that sample, *ρ* is the density of the water (1000 kg m^{-3}), *a* is the wave amplitude (e.g., the crest of a wave; m) and *k* is written as (Crapper 1984):

$$k = \frac{2 \cdot \pi}{L} \qquad (2)$$

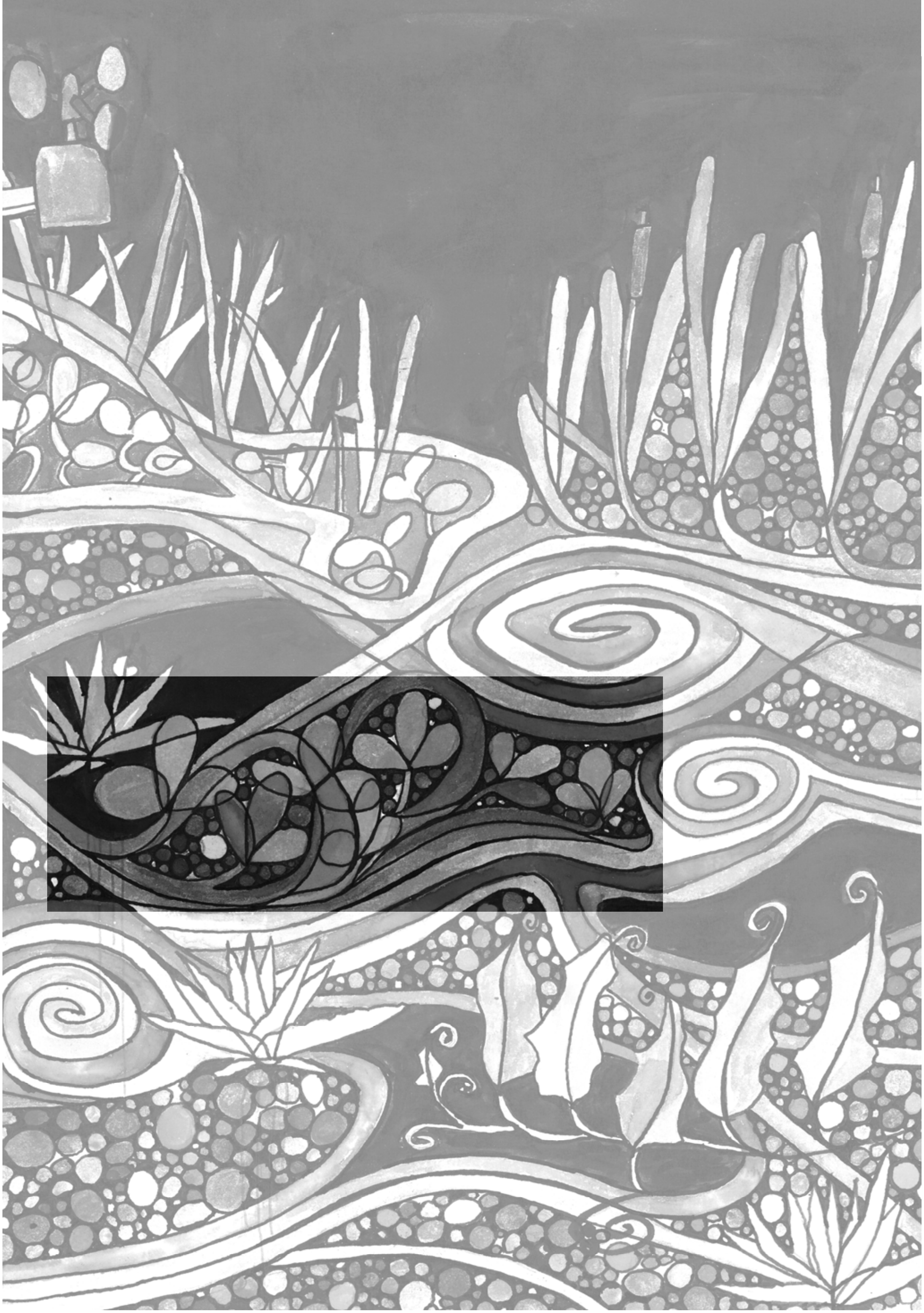
where wave length (*L*; m) is defined as (Crapper 1984):

$$L = 1.56 \cdot T^2 \qquad (3)$$

and *T* is the wave period (s) under the assumption of deep water. For long, high waves it is not necessary to know the wave period (Figure A 1a) as the relation between pressure and wave amplitude follows (Crapper 1984):

$$p = \rho \cdot g \cdot a \qquad (4)$$

With these models we calculated pressures for waves with different frequencies and amplitudes (Figure A 1). This shows that the assumption of a hydrostatic pressure distribution overestimates the pressure of a certain wave (Figure A 1a). Further, assuming a hydrostatic pressure distribution (Eq. 4) when dealing with small waves (with a small amplitude) results in a relative overestimation of the smaller, and an underestimation of larger waves (Figure A 1b). This means that our calculations of wave impact at upwind (SW) and downwind (NE) will underestimate the differences between the banks; they were probably larger in reality.



CHAPTER 4

The response of shoreline vegetation in fens to nutrient enrichment of either the bank or the surface water

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M.M. Nijzink, M.B. Soons, and J.T.A. Verhoeven

Abstract

Riparian ecosystems harbour great floral and faunal diversity and fulfil several important ecological functions. Although the effect of riparian vegetation on water quality is well-studied, the impact of eutrophication on the riparian ecosystem itself and, in fens, on the succession from open water towards floating peat mats remains unclear. We used a large controlled mesocosm experiment to study the complex interactions of eutrophication of bank soil and/or surface water with vegetation development and the colonisation of open water. Because litter accumulation in fens is assumed to control succession towards floating mats, this process was also studied. The mesocosms consisted of a bank and a water compartment planted with typical fen species. We applied different combinations of nutrients (N and/or P) to either the bank or the surface water.

Nutrient addition (mainly P) resulted in increased growth and larger colonisation distances on the bank as well as in the water, with clear cross-over effects. Only eutrophic species increased shoot density after fertilisation. Nutrient addition further resulted in higher litter production and stimulated decomposition. Litter accumulation was calculated to be highest on fertilised banks. These processes imply that eutrophication accelerates succession. Although this could be seen as positive, because it could induce faster colonisation of the open water, it is not likely to result in the formation of species-rich floating mats aimed for by nature managers and policy makers. Rather, eutrophication tends to lower biodiversity and the eutrophic species that increase in dominance are less capable of the formation of such mats.

Keywords: Colonisation, Decomposition, Eutrophication, Mesocosm experiment, Nitrogen, Phosphorus, Succession, Wetland.

Introduction

On the interface between aquatic and terrestrial ecosystems, riparian zones and shorelines provide important biological and physical transition zones (Ward *et al*. 2002). They function as a separate ecosystem that is characterised by a relatively high floral and faunal diversity and plays a crucial role in the nutrient cycle of aquatic systems (Nilsson and Svedmark 2002; Renöfält *et al* 2005). Over the past decades, increased nitrogen (N) and phosphorus (P) inputs to terrestrial and aquatic ecosystems have caused drastic changes in their quality and functioning. Yet, surprisingly little is known about the effects of nutrient enrichment of the surface water and the bank soil on vegetation structure and development of riparian and shoreline communities themselves (Saunders and Kalff 2001). As an illustration, when the search key (Nitrogen OR Phosphorus) AND (riparian AND vegetation) is entered in Scopus, less than ten out of over 300 retrieved articles focus on the effects of eutrophication on plant growth and vegetation development, while most papers focus on mineralisation and water quality.

Lowland fen areas, including those in the Netherlands, have a relatively high proportion of riparian and shoreline ecosystems as they consist of a complex network of streams, ponds and marshland, combined with numerous man-made canals and ditches. However, in the Netherlands, characteristic species that play key roles in the colonisation of open water and the formation of floating vegetation mats, have become very rare over the past 25 years (Verhoeven and Bobbink 2001; Lamers *et al*. 2002; Beltman *et al*. 2008). Moreover, as many of the existing floating mats have gradually turned into alder forests by succession, a large number of species associated with those mats have also become very rare (Bakker *et al*. 1994; Van Belle *et al*. 2006). As these fen complexes are embedded in an agricultural landscape, it is likely that this degradation is related to eutrophication due to agricultural activities or supply of nutrient-rich river water. Although nature conservation agencies are much concerned about the deterioration of the shoreline communities, the exact cause-effect relations behind this development are not clear (Van Belle *et al*. 2006). Moreover, in spite of numerous measures to reduce nutrient levels in the bank and the surface water, many typical fen species remain absent in the majority of the Dutch fens (Lamers *et al*. 2002; Beltman *et al*. 2008). Therefore, a better understanding of the interaction between enrichment of the bank and the surface water and these species is required for the development of more effective restoration measures.

There is a growing body of evidence that nutrients dissolved in surface water are available for plants in the riparian zone (Schade *et al*. 2002; Grimm *et al*. 2003; Hultine *et al*. 2008). Plants colonising the surface water are therefore likely to be directly affected by nutrient availability in both bank and surface water. However, the relative importance of dissolved nutrients in surface water might be small, especially when compared to the nutrient availability in mesotrophic or eutrophic peat bank soils. The colonisation of the open water towards floating mats might also be influenced indirectly by the nutrient availability in water and soil through the effects on litter production and decomposition. Eutrophication will probably affect litter accumulation, which is generally assumed to control the succession towards floating mats e.g. by providing new substratum for the roots, affecting germination and by changing the hydrology (Van Wirdum *et al*. 1992; Van der Putten *et al*. 1996; Bakker *et al*. 1997). Litter accumulation is the net result of litter production and decomposition and both processes are affected by eutrophication. First, plants in an eutrophic environment produce more litter with higher nutrient concentrations (Shaver and Mellillo 1984). Second, eutrophication will enhance decomposition because both enrichment of the litter and the surrounding substrate have been shown to increase decomposition rates (Knops *et al*. 2002; Rejmánková and Houdková 2006; Graf and Rochefort 2009). Hence, in a eutrophicated situation more litter is produced, but it may also be broken down more rapidly. The net effect of eutrophication on litter accumulation has seldom been assessed for freshwater wetlands (but see: Rejmánková and Houdková 2006). Therefore, besides studying the way in which nutrient enrichment of the bank and surface water directly affects typical shoreline fen species and their colonisation, we also aimed at understanding the effect of nutrient enrichment on litter quality and breakdown.

We investigated this in a controlled mesocosm experiment with a given set of characteristic plant species and addressed the following three questions: 1) How do different types of nutrient addition (N or P) to the bank and surface water affect macrophyte growth in the surface water and on the bank? 2) How do these nutrient additions affect the colonisation strategy of different plant species? and 3) How do they affect tissue quality and litter breakdown? Regarding colonisation, we hypothesise two possible ways in which the availability of nutrients would influence plant growth. First, if nutrient availability increases, plants produce more biomass and hence would colonise over larger distances. Second, plants in short supply of nutrients may start to explore for better sites and would colonise over relatively larger distances per unit of biomass when compared to nutrient rich situations (Grime 1977). In this case we expect that the shoot density per colonised distance would increase by nutrient addition. As a null-hypothesis, we assume that eutrophication stimulates both litter production and decomposition and that these processes could balance out by which net litter accumulation would not be affected (Rejmánková and Houdková 2006).

Methods

The experimental set-up

To establish representative fen banks, 24 polyethylene mesocosms (1.0 x 1.0 m, 30 cm deep) were placed at the Radboud University Nijmegen Botanical Gardens in March 2005. Each mesocosm was divided into a 'terrestrial' compartment (40% of the surface area; hereafter referred to as bank) and a 'water' compartment, separated from each other by a plywood board covered with anti-rooting cloth (Figure 4.1a). Plants were still able to grow over or under this board into the water and they frequently did. The terrestrial compartment (40% of the surface area) was filled with unfertilised sphagnum peat (Holland Potgrond, Poeldijk, The Netherlands), creating a very gradually sloping bank. The water compartment was filled with demineralised water and a thin sediment layer (5 cm) of peat. During the experiment, water depth was maintained at 25 cm (± 5 cm). Because the initial pH of the peat was low (between 4 and 5), all banks were limed with 175 g "Dolokal" (75% $CaCO_3$, 10% $MgCO_3$, and 5% MgO) to achieve a pH between 5 and 6 before the start of the experiment. To increase and maintain the alkalinity to 1.5 meq L^{-1} (a common value in Dutch fens; Lamers *et al*. 2006), 20 g $NaHCO_3$ was added to the surface water in April, May and August 2005. All treatments received once a background dose of $CaPO_4$ in May 2006 (6.7 kg P ha^{-1}), to avoid P-deficiency of the oligotrophic sphagnum peat after 1 year.

In April 2005, seven emergent macrophyte fen species: *Calla palustri* b ., *Equisetum fluviatile* L., *Glyceria maxima* (Hartm.) Holmb., *Juncus effusus* L., *Menyanthes trifoliata* L., *Ranunculus lingua* L. and *Thelypteris palustris* Schott, were planted on the bank of each mesocosm. *Glyceria* and *Juncus* are typical for eutrophic shoreline vegetation (hereafter named: eutrophic macrophytes) whereas the other species are associated to mesotrophic situations and the formation of floating mats (mesotrophic macrophytes). Four aquatic species were placed in the water compartment. These were *Ceratophyllum demersum* L. and *Elodea nuttallii* (Planch.) H. St. John, typical for more eutrophic situations, and *Potamogeton compressus* L. and

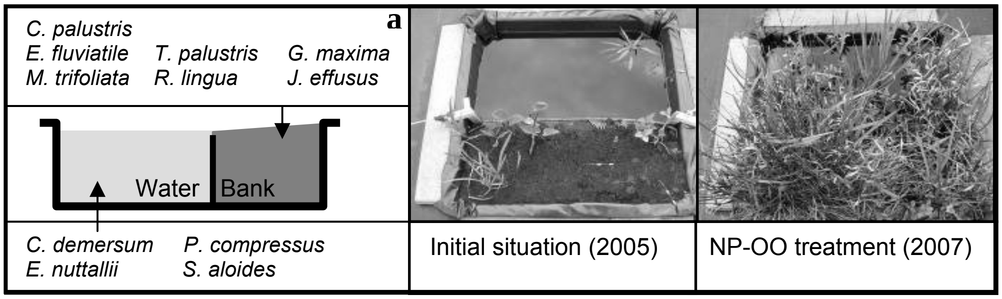


Figure 4.1: Experimental design of the mesocosms. A) A schematic cross-section of a mesocosm in which the planted species are indicated. B) Top view of the initial situation and the situation after the third growing season, showing the treatment where N and P were added only to the bank. Full species names can be found in Table 4.1.

Table 4.1: Yearly measured, non-destructive growth and colonisation parameters per species.

Bank species	Number of plants	Initial FW (g)	Growth parameter per compartment	Clonal growth
Calla palustris L.	3	50.6 ± 8.4	Number of leaves	Distance between the two most remote plants
Equisetum fluviatile L.	3	11.1 ± 2.1	Number of shoots	Distance between the two most remote plants
Glyceria maxima (Hartm.) Holmb.	2	14.0 ± 4.1	Number of shoots	Distance between the two most remote plants
Juncus effusus L.	4	15.8 ± 1.6	Number of shoots	Tussock diameter
Menyanthes trifoliata L.	3	28.0 ± 5.3	Number of leaves	Distance between the two most remote plants
Ranunculus lingua L.	2	23.0 ± 11.7	Number of leaflets	Distance between the two most remote plants
Thelypteris palustris Schott	2	15.7 ± 4.1	Number of leaves	Distance between the two most remote plants
Aquatic species				
Ceratophyllum demersum L	10	21.9 ± 1.1	Number of shoots	Total stem length
Elodea nuttallii (Planch.) H. St. John	10	4.0 ± 0.2	Number of shoots	Total stem length
Potamogeton compressus L.	2	2.7 ± 0.9	Number of shoots	Total shoot length
Stratiotes aloides L.	3	55.1 ± 9.9	Number of rosettes	Summed rosette diameter

Stratiotes aloides L. from mesotrophic situations. All species were collected in Dutch fens.

The 24 mesocosms were randomly allocated to six fertilisation treatments, each with four replicates. The treatments were: (1) NP-NP; N & P added to both the bank and the water compartment, (2) OO-NP; N & P added only to the bank, (3) NP-OO; N & P added only to the water,

(4) NO-OO; only N added only to the water, (5) OP-OO; only P added only to the water and (6) OO-OO; no fertilisation. The first two letters of the codes indicate the nutrients added to the water and the latter two the nutrients added to the bank.

The bank was fertilised each year in May with $CaPO_4$ (70 kg P ha^{-1} y^{-1}) and NH_4NO_3, (600 kg N ha^{-1} y^{-1}; half in the form of slow release Osmocote granules). These applications reflect very heavily fertilised agricultural areas (Schils and Snijders 2004). N (NH_4NO_3) and P (NaH_2PO_4) addition to the water occurred in three-weekly doses, resulting in a yearly dose of 6 kg P ha^{-1} and 25 kg N ha^{-1}, leading to concentrations that occur in polluted surface waters in fens (Lamers *et al*. 2006). Surface water was sampled regularly with iodated polyethylene bottles (100 ml), while bank pore water (10, 20 and 30 cm distance from the waterline) was sampled anaerobically with 10 cm long Rhizon soil moisture samplers (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) and vacuum glass infusion bottles (30 ml).

At the end of each growing season, plant cover of the emergent macrophytes was estimated for both bank and water compartments separately and the fresh weight of the submerged aquatic species was weighed. Besides, several non-destructive parameters of growth and colonisation were determined (Table 4.1). In September 2007, at the end of the third growing season, in addition to the non-destructive measurements listed in Table 4.1, all aboveground biomass of the emergent macrophytes was harvested and sorted into dead (brown) and living material (green) per species and per compartment. The samples were dried for 24 h at 70 ºC, weighed and subsequently ground to determine N and P concentrations (see below).

Decomposition

To estimate the net litter accumulation, a litter bag decomposition experiment was conducted with litter from *Glyceria* that was harvested in September 2006. *Glyceria* was chosen because this species produced a substantial amount of litter in the majority of the mesocosms. Litter from fertilised banks (enriched litter) and non-fertilised banks (poor litter) was kept separate. The litter was air dried, cut to smaller pieces (5 cm), and put in 10 x 10 cm mesh bags (0.3 mm). In early November 2006, these were inserted vertically to a depth of 5-7 cm in bank compartment of the NP-NP and OO-OO treatments. Bags were retrieved after 3, 6, 9 and 10 months. For the poor litter, harvesting took place after 6 and 10 months due to limited availability of this litter type. Retrieved litter was carefully cleaned and subsequently dry weight, N and P concentrations were determined.

Chemical analysis

The pH of the water samples was measured directly using a combined pH electrode with an Ag/AgCl internal reference (Orion Research, Beverly, CA), and a TIM800 pH meter. Subsequently, surface water samples were filtered through glass microfibre filters (type GF/C, Whatman, Brentford, UK). Citric acid (0.6 mmol L^{-1}) was added to prevent metal precipitation. The samples were stored in iodated polyethylene bottles at -20 ºC until further analysis. Concentrations of NO_3^- and NH_4^+ in the water samples were measured colourimetrically with an Auto Analyzer-3 system (Bran and Luebbe, Norderstedt, Germany), using hydrazine sulphate (Kamphake *et al*. 1967) and salicylate (Grasshoff and Johannsen 1972), respectively. Total P was measured using an ICP Spectrometer (IRIS Intrepid II, Thermo Electron Corporation, Franklin, MA). A homogenized portion of 150 mg dried plant material was used to determine N and P concentrations in *Glyceria* using a salicylic acid thiosulphate modification of the Kjeldahl digestion (Bremner and Mulvaney 1982). N and P concentrations were determined colourimetrically on a continuous flow analyzer (SA-40, Skalar Analytical, Breda, the Netherlands).

Statistical analysis

Pore water concentrations from different positions on the bank were averaged, as they were significantly indifferent. To test differences between nutrient concentrations between the treatments, a one-way ANOVA was performed using the overall mean of the different sampling dates per mesocosm (Log (+1) transformed to improve homogeneity of variance). Treatment effects on biomass production, colonisation estimates and nutrient concentrations in the plant were tested for the bank and water separately. The four aquatic species were considered as one functional group and hence their biomasses were summed per mesocosm. N and P use efficiency (NUE and PUE, respectively) were calculated under the assumption of steady state conditions as the yearly aboveground litter production divided by the total nutrient loss (Vitousek 1982). Testing of all these variables was done with a one-way ANOVA and a Dunnett's *post hoc* test, comparing all treatments to the control (OO-OO) treatment. All statistical analyses were carried out in SPSS 16.0.

Results

Nutrients

The nutrient additions to the bank compartment resulted in significantly higher NO_3^-, NH_4^+ and Total-P concentrations in the pore water of the bank

(Figure 4.2a-c). Probably due to runoff after rain events and diffusion, the P concentration in the water compartment was increased by fertilisation of the bank also (Figure 4.2f). N concentrations in the water did not show this effect. The other way around, nutrient addition to the water compartment did not significantly increase the concentration in the bank pore water. In all treatments where N was added to the water, the concentrations of NO_3^- were higher (Dunnett's; $P = 0.07$, $P = 0.02$ and $p<0.001$ for NP-NP, NP-OO and NO-OO, respectively). Besides, in the NO-OO treatment the NH_4^+ concentration in the water compartment was higher than in the control treatment (Dunnett's; $P < 0.001$). P addition to the water did not increase total P concentrations in the water significantly (Figure 4.2f). Overall, the nutrient additions were quite well reflected in the nutrient concentrations in the bank pore water and surface water. However, they varied considerably over time and decreased steeply towards the end of each growing season. The mean pH of the bank ranged from 5.4 to 5.8 and did not differ between the treatments. The pH of the water compartment ranged from 6.7 in the treatments where the bank was fertilised to 7.3 in the other treatments.

Vegetation

Not all species survived in all mesocosms, nor did all bank species invade the water. Over the first year, predominantly *Calla* and *Menyanthes* grew from the land into the water in all treatments. *Glyceria*, *Equisetum* and *Ranunculus* followed over the next growing seasons. However, the latter two only reached very low cover percentages in the water compartments. *Thelypteris* disappeared in most mesocosms. After three growing seasons, both the bank and the water compartments of the NP-NP and OO-NP treatments were completely covered with emergent macrophytes (Figure 4.1b). The eutrophic species (*Glyceria* and *Juncus*) dominated on the banks of these treatments (together covering 30 - 70%), whereas *Menyanthes* prevailed in the water compartments (20 - 60%). In the other treatments, total cover percentages were lower, ranging between 20 and 30% on the bank and between 5 and 20% on the water. Besides, the water compartments of the NP-OO, OP-OO and NO-OO treatments were (partly) covered with floating filamentous algae. The differences in cover percentages were reflected in differences in the total aboveground biomass (Figure 4.2). Both eutrophic and mesotrophic macrophytes produced significantly more biomass on the bank and in the water compartment after NP addition to the bank (Figure 4.3; Table 4.2). The aquatic species almost disappeared in those treatments. As the water compartments were totally filled by emergent macrophytes growing from the bank, the aquatic species were probably

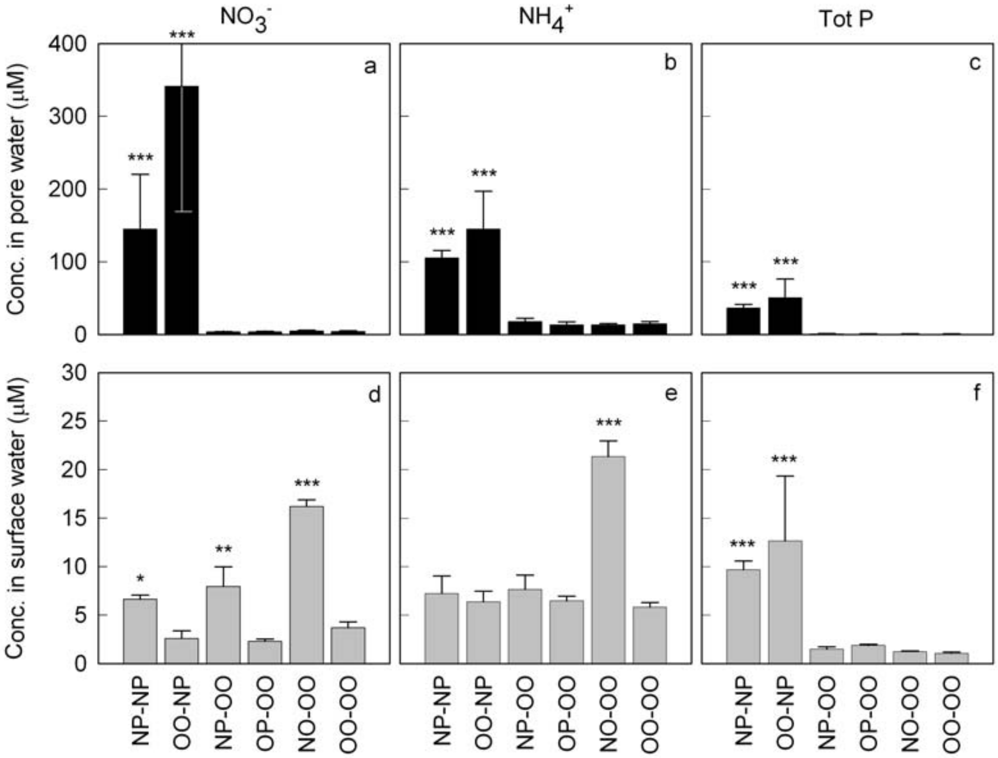


*Figure 4.2: Mean NO_3^-, NH_4^+ and total P concentrations (µM) in bank pore water (a-c) and surface water (e-f) in the different treatments (±S.E.). The first two letters in the treatment codes indicate the nutrients added to the water compartment and the following two letters indicate nutrient addition to the bank. An asterisk above the bar indicates that that treatment significantly differs from the control treatment (OO-OO). *** $P < 0.01$, ** $P < 0.05$, * $P < 0.10$.*

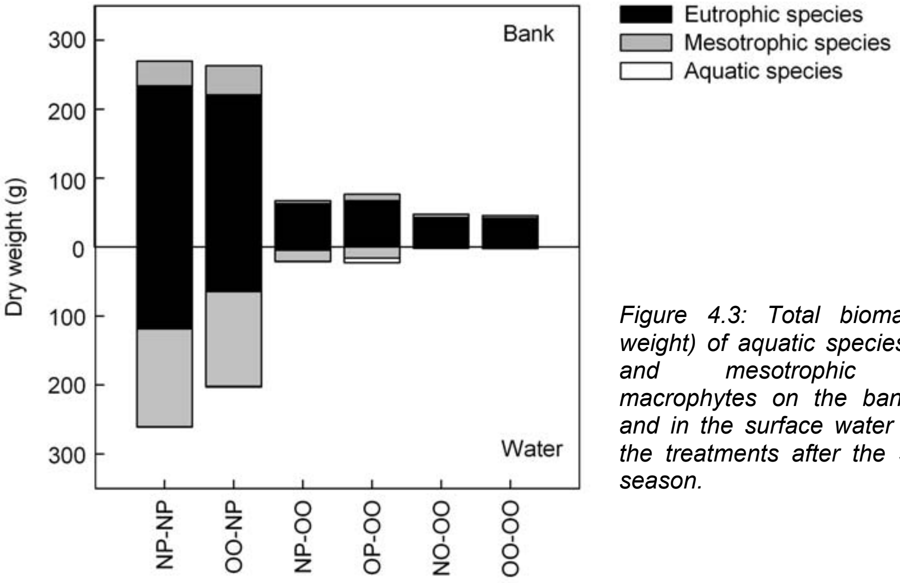


Figure 4.3: Total biomass (g dry weight) of aquatic species, eutrophic and mesotrophic emergent macrophytes on the bank (0.4 m^2) and in the surface water (0.6 m^2) in the treatments after the 3rd growing season.

outcompeted. In the treatments where P was added only to the surface water, total biomass increased both on the bank and in the water. Surprisingly, on the bank, only eutrophic species (mainly *Juncus*) increased in biomass, whereas in the water compartment, the mesotrophic emergent macrophytes increased (Figure 4.3; Table 4.2). Aquatic species (i.e. *Elodea* and *Ceratophyllum*) had the highest biomass in the OP-OO treatment (Figure 4.3; Table 4.2). In all treatments, most biomass was produced on the bank. Whenever P was available in the water compartment, ± 20 - 50% of the total biomass of the bank species was produced in the water compartment. As this biomass on the bank and in the water compartment was formed by the same species, it suggests that the availability of P in the surface water has triggered plants to grow into the water.

Generally, the parameters that were measured as indicators of colonisation distance were lower in the water compartment than on the bank. In the treatments where the bank was fertilised, the colonisation distances both on the bank and in the water were larger, except for *Ranunculus* and *Thelypteris* (Figure 4.4a) that were indifferent. Nutrient additions to only the water did not affect the colonisation parameters, either on the bank or in the water itself, except for *Menyanthes* which colonised over a significantly longer distance in the water compartment in the OP-OO treatment ($P = 0.05$; Figure 4.4). Only the eutrophic macrophytes (*Juncus* and *Glyceria*) significantly increased their shoot density per colonised distance when nutrients were added to the bank (respectively; NP-NP: 0.006 and 0.08 and OO-NP: 0.02 and 0.02). Some of the mesotrophic species (*Equisetum* and *Menyanthes*) also had this tendency, but this was not significant (Figure 4.4b). As vegetation density is often linked to competitive strength, this could provide a mechanism by which *Juncus* and *Glyceria* eventually could outcompete the mesotrophic macrophytes on banks with NP addition. Nutrient enrichment of the water did not result in differences in growth structure on the bank (Figure 4.4b).

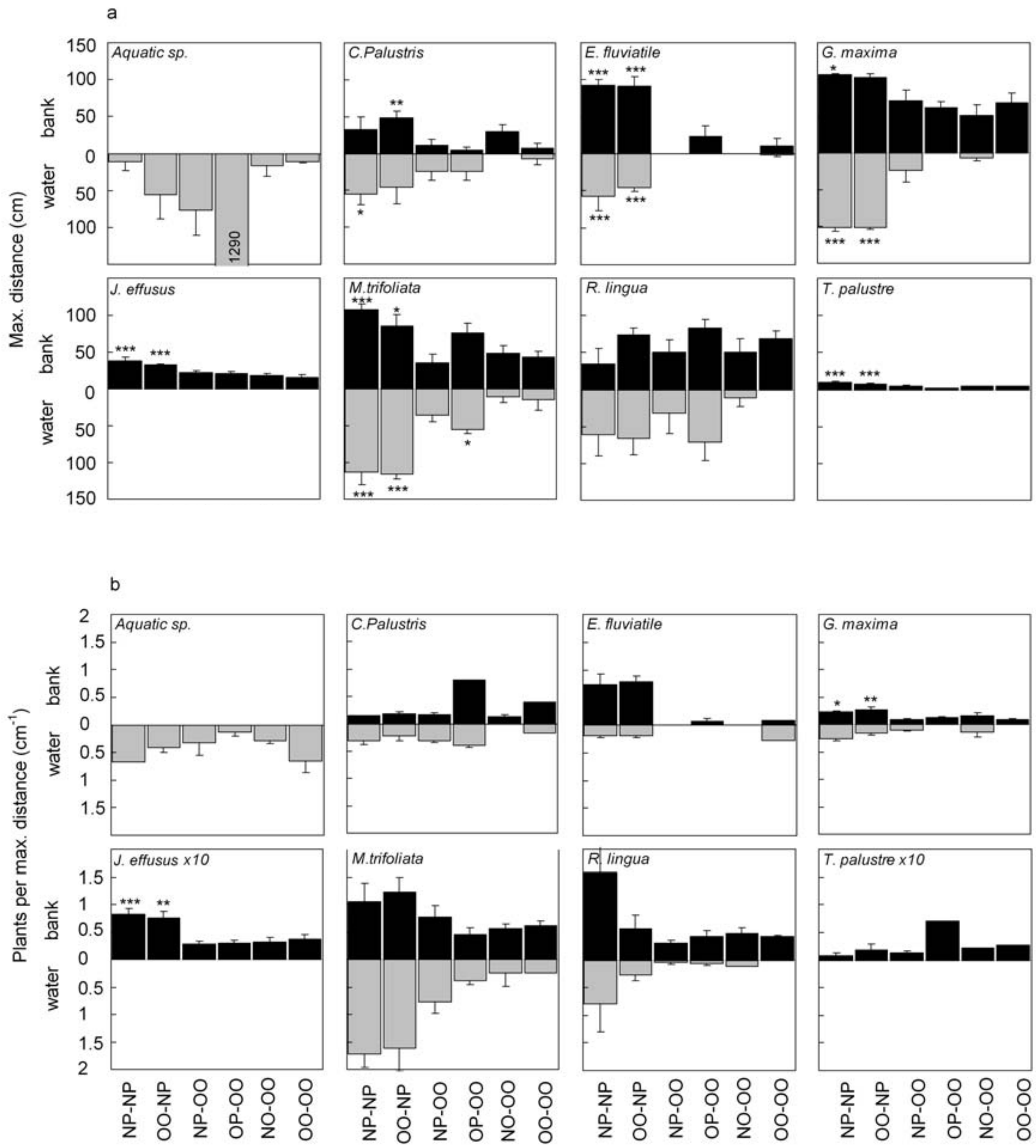
Summarizing, upon NP addition to the bank, more biomass was produced, resulting in longer colonisation distances and a denser vegetation structure. This was mostly due to the eutrophic macrophytes, which increased shoot density per unit of colonised distance. P availability in the water compartment stimulated the colonisation of the water, mainly by aquatic and emergent mesotrophic species.

Table 4.2: P- and F-values of the one-way ANOVA's on the harvested biomass on the bank and in the water compartment, in the different treatments. The first two letters of the treatment codes indicate the nutrients added to the water compartment and the last two the type of fertilisation of the bank.

		NP-NP	OO-NP	NP-OO	OP-OO	NO-OO	*F*-value	*P*-value
Bank	Eutrophic species	<0.01	<0.01	n.s.	0.08	n.s.	34.7	<0.01
	Mesotrophic species	<0.01	<0.01	n.s.	n.s.	n.s.	12.1	<0.01
	Total	<0.01	<0.01	0.12	0.03	n.s.	45.8	<0.01
Water	Eutrophic species	<0.01	<0.01	n.s.	n.s.	n.s.	26.2	<0.01
	Mesotrophic species	<0.01	<0.01	<0.01	<0.01	n.s.	56.3	<0.01
	Aquatic species	n.s.	n.s.	n.s.	<0.01	n.s.	11.4	<0.01
	Total	<0.01	<0.01	<0.01	<0.01	n.s.	61.7	<0.01

Table 4.3: Biomass, nutrient concentrations, NUE and PUE of Glyceria on the banks and in the water compartment of the different treatments and F- and P-values of One-way ANOVA's.
Bold values indicate significant differences to OO-OO (P < 0.05). Post hoc tests were not performed on nutrient concentrations of plants that grew in the water compartment, due to the low number of replicates.

Bank	NP-NP	OO-NP	NP-OO	OP-OO	NO-OO	OO-OO	*F*-value	*P*-value
Biomass living (g)	20.4	18.7	1.50	0.88	0.9	0.87	2.3	0.089
Biomass dead (g)	**44.6**	**49.1**	2.93	2.8	2.6	2.2	7.0	<0.01
N in living tissue (mg g^{-1})	23.2	22.4	14.7	15.4	17.3	17.6	4.1	0.012
N in dead tissue (mg g^{-1})	**18.9**	**17.2**	8.0	9.9	10.3	9.9	16.1	<0.01
NUE (g^{-1})	**54.7**	**58.5**	126	104	98.8	104	12.0	<0.01
P in living tissue (mg g^{-1})	**2.6**	**2.02**	0.21	0.27	0.27	0.26	20.4	<0.01
P in dead tissue (mg g^{-1})	**2.0**	**1.56**	0.08	0.13	0.10	0.12	22.3	<0.01
PUE (g^{-1})	**544**	**670**	12471	8255	10738	10117	14.1	<0.01
Water								
Biomass living (g)	**26.9**	16.2	3.03	0.0	0.17	0.0	3.72	0.017
Biomass dead (g)	**91.9**	**60.5****	1.71	0.0	0.29	0.0	9.88	<0.01
N in living tissue (mg g^{-1})	14.2	12.4	12.21		16.9		0.36	n.s.
N in dead tissue (mg g^{-1})	13.3	10.4	7.02		9.2		3.80	0.066
NUE (g^{-1})	77.9	96.7	142.53		108		11.01	<0.01
P in living tissue (mg g^{-1})	1.6	1.4	0.15		0.14		4.37	0.05
P in dead tissue (mg g^{-1})	1.1	1.0	0.08		0.13		6.14	0.023
PUE (g^{-1})	987	1121	11932		7661		655.0	<0.01



Λ *Figure 4.4: a) Mean colonisation distance (Table 4.1) in the water compartment and on the bank of each species separately (±S.E.). b) Number of plants or shoots per cm colonised distance (±S.E.). *** $P < 0.01$, ** $P < 0.05$, * $P < 0.10$. Full species names can be found in Table 4.1. The number of replicates was too low to perform post hoc tests for the water compartments.*

Litter accumulation

As biomass production was stimulated by enrichment of the bank (Figure 4.3), litter production by *Glyceria* was much higher in the enriched treatments, both in the water and on the bank (Table 4.3). Nutrient concentration in litter increased significantly in the NP-NP and OO-NP treatments (Table 4.3). This was the result of increased nutrient concentrations in living tissue (significant for P; Table 4.3) and much lower NUE and PUE in those treatments. NUE and PUE were about half of that calculated for the banks without nutrient addition (Table 4.3). Nutrient addition to the surface water did not affect the litter quality significantly.

Unexpectedly however, litter quality did not affect decomposition rates (Figure 4.5). After ten months, the percentage weight loss did not differ significantly between litter from banks with and without nutrient addition (Figure 4.5). However, both litter types decomposed significantly faster in the NP-NP treatment, indicating that there was a strong influence of site conditions on decomposition (Two-way ANOVA; $P = 0.001$). The net effect of both the enhanced litter production and increased decomposition was calculated under the assumption that all standing biomass would die before the next growing season. This showed that after ten months 17.6 g per bank (44.0 g m^{-2}) *Glyceria* litter would remain in the NP-NP treatment while only 1.30 g (3.25 g m^{-2}) would remain in the OO-OO treatment (Table 4.4). During decomposition, both N and P concentrations decreased in the litter in the NP-NP treatment while concentrations increased in the OO-OO treatment. As a result, large amounts of nutrients could be mobilized during decomposition in the NP-NP treatment while in the OO-OO treatment a P immobilization could be expected (Table 4.4).

Table 4.4: The calculated litter accumulation (mg yr^{-1}) and N and P released (mg) per year per bank (0.4 m^2). T (time) is indicated in months.

Treatm.	*Glyceria* per bank* (g yr^{-1})	Weight loss t=10 (%)	Biomass left t=10 (g)
NP-NP	64.95	72.95	17.57
OO-OO	3.02	57.04	1.30

Cont.	[N] litter t=0 (mg g^{-1})	[N] litter t=10 (mg g^{-1})	N left (mg yr^{-1})	N release (mg yr^{-1})	[P] litter t=0 (mg g^{-1})	[P] litter t=10 (mg g^{-1})	P left (mg yr^{-1})	P release (mg yr^{-1})
NP-NP	18.85	16.53	290.28	933.88	2.03	0.96	16.87	115.06
OO-OO	9.86	12.08	15.66	14.10	0.12	0.30	0.39	-0.04

* The sum of living and dead biomasses harvested in 2007, assuming that all plants would die before the next growing season.

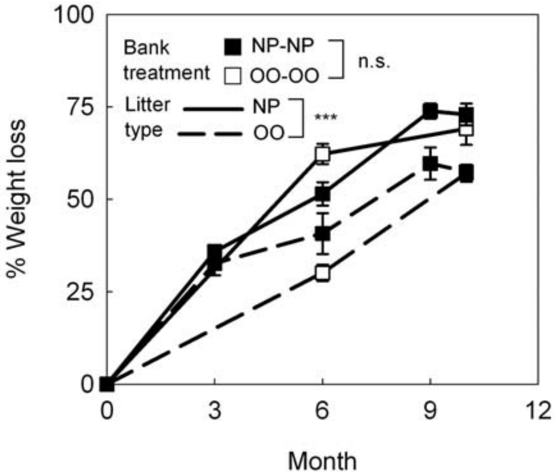


*Figure 4.5: Weight loss through decomposition for the different types of Glyceria litter in the different treatments (±S.E.). Litter harvested from banks without fertilisation (open symbols) and litter harvested from banks with three years NP addition (closed symbols) were placed in the NP-NP (solid lines) and OO-OO (dashed line) treatments. *** P < 0.01, ** P < 0.05, * P < 0.10.*

Discussion

In the present study we evaluated the importance of nutrient enrichment of water and bank for (1) the growth of shoreline species, (2) their colonisation strategy and (3) the litter quality and accumulation in shorelines of fens. We will discuss each question separately and evaluate the implications for the functioning of shoreline ecosystems, in particular the succession towards floating peat mats.

Effects on plant growth

The results clearly show that nutrient levels in the surface water and in the bank are both important for the development of riparian vegetation as they both did affect the growth of shoreline species and the colonisation of the surface water. Interestingly, the growth of plants was not only enhanced in the compartment where the nutrients were added to, but productivity also increased in the other compartment. This effect was very strong with enrichment of the banks, which stimulated the growth into the water (be it with a large contribution of eutrophic species) and smaller for enrichment of the water, which had a minor but significant effect on plant growth on the bank. Of course the difference in nutrient dose applied on bank and water will have affected the strength of this effect, but this shows that at least for nutrient poor banks, surface water quality can influence biomass production on the bank. Overall, the productivity in the mesocosms with enriched banks was quite similar to the productivity of shorelines in the field (3 sites; results not shown) and to data reported for *Glyceria* vegetation in Wassen *et al*. (1995, 2003) and Güsewell *et al*. (2007). Nutrient enrichment commonly increases biomass of plants in terrestrial as well in aquatic systems (Grimm *et al*. 2003), generally accompanied by a shift from nutrient competition towards light competition (Grime 1977), favouring taller, competitive species, often grasses (Van de Riet *et al*. 2009). This was also observed in our study, where the taller *Glyceria* and *Juncus* dominated the fertilised banks. The observation that *Menyanthes,* which is much shorter in height, invaded the water compartment rapidly and had a relatively high biomass there can be interpreted as an escape from the unfavourable light conditions on the bank. However, after the first year, *Glyceria* also fiercely invaded the water compartment, suggesting that sooner or later *Menyanthes* would be outcompeted there too, eventually resulting in a species-poor situation.

We only found effects of the surface water quality on the bank vegetation when the banks were nutrient-poor. This is in line with the few other studies that reported effects of polluted surface water. Those were also conducted on unfertilised banks (Schade *et al*. 2005; Hultine *et al*. 2008; Banach *et al*. 2009; Geurts *et al*. 2009). Eutrophication of banks, on the other hand, can obscure the effects of surface water pollution (also with SO_4^{3-}; Geurts *et al*. 2009). Nutrients in the surface water are hypothesised to increase biomass of the plants on the bank via the two following mechanisms (Schade *et al*. 2005; Hultine *et al*. 2008). First, by plastically adjusting their root architecture to the bank zone where surface water infiltrates (Robinson 1994; Hodge 2004), plants can directly take up nutrients originating from the enriched surface water (Schade *et al*. 2005; Hultine *et al*. 2008). Second, Schade *et al*. (2002) found indications that enrichment of the surface water stimulates mineralisation of organic compounds in the whole infiltration zone, thus increasing nutrient availability for plant growth in a wider zone compared to the previous mechanism. We found no evidence that excludes or favours either one of these mechanisms.

Effects on colonisation

With nutrient addition, more biomass was produced and in accordance to our first hypothesis, plants colonised over larger distances. The second way in which we assumed that nutrients might influence the colonisation strategy, was that plants in eutrophic situations would invest relatively less in rhizome exploration but instead increase shoot density. This was only observed for the eutrophic species. As shoot density is positively related to competitive ability (Grime 1977), these species may eventually outcompete the mesotrophic species, resulting in a species shift. This is often observed in enriched ecosystems (Smith and Smith 1998). Compared to pioneer species in mesotrophic fens, eutrophic shoreline species such as *Glyceria* and

Juncus invade the water less frequently, or not at all, by floating on the water surface (Weeda *et al*. 1999). Instead, these eutrophic species more often colonise surface water with rhizomes rooting in the bottom. This process will usually stop at a certain water depth due to wave action (Riis and Hawes 2003; Azza *et al*. 2006, 2007), light limitation (Boedeltje *et al*. 2001) or herbivory (Perrow *et al*. 1997; E. S. Bakker unpublished). Hence, following nutrient enrichment the formation of species-rich floating mats becomes very unlikely, especially because eutrophication also tend to result in vegetation with a lower biodiversity (Grime 1977).

Effects on litter accumulation

Decomposition rates in *Glyceria* were comparable to those in other studies on *Glyceria* (B. P. Van de Riet unpublished). Our finding that decomposition was mainly determined by site conditions is in line with several other reciprocal litter experiments (Rejmánková and Houdková 2006; Moore *et al*. 2007). On the community level, decomposition and litter accumulation would also depend on species composition, as species differ in litter production and decomposition rates (Graf and Rochefort 2009; B. P. Van de Riet unpublished). The mesotrophic species produced only a small amount of litter (on average 4 g on the fertilised banks), compared to *Glyceria* that produced on average 20 g per bank. Given that *Glyceria* became dominant after fertilisation, overall litter accumulation in fen shorelines might even be more contrasting when assessed for a whole plant community. In the succession sere from open water to carr or brown-moss fen (Van Wirdum *et al*. 1992) accumulation rates of organic material are highest in the phases of expanding floating mats (1 kg m^{-2} yr^{-1}; Bakker *et al*. 1997). Results from the same study suggested that the litter accumulation rates of the different phases control their ‘turnover time’ (i.e. time needed for transition from one phase to the next; Bakker *et al*. 1997). Therefore, the expanding floating mats represent the shortest phase in the complete succession from open water to alder forest. Huotari *et al*. (2007) found litter accumulation to play a comparable role in the succession of cut-over peat bogs. This implies that eutrophication, by increasing litter accumulation, would accelerate succession, most likely however, towards undesired end stages (Van Wirdum *et al*. 1992; Verhoeven and Bobbink 2001). In fact, Bakker *et al*. (1994) calculated that the life span of the semi-aquatic phase decreased between 1937 and 1989, and nutrient inputs into the fen have increased drastically during this period. In wetlands, litter quantity and quality have been shown to strongly affect other processes like germination (Wilby *et al*. 2001; Xiong *et al*. 2003), establishment (Van der Putten *et al*. 1997) and hydrology (Van Wirdum *et al*. 1992), which might induce the vegetation changes toward later succession phases (Van der Putten *et al*. 1997).

Implications

With this research we showed that nutrient additions will change the functioning of riparian ecosystems in fens. Nutrient addition (mainly P) resulted in increased growth and larger colonisation distances on the bank as well as in the water, but only eutrophic species increased shoot density after nutrient addition. This may have caused their increased dominance on enriched banks. Nutrient addition further resulted in a higher litter production and stimulated decomposition. Litter accumulation was calculated to be highest on banks with NP addition. These processes imply that the succession would accelerate. Although this could be seen as positive, because it could lead to faster formation of floating mats, it is not likely that succession under very eutrophic conditions would result in the species-rich vegetation type aimed for by nature managers and policy makers (Van Wirdum *et al*. 1992; Verhoeven and Bobbink 2001). First, eutrophication often results in vegetation with a lower biodiversity (Grime 1977). Second, the eutrophic species that increase dominance are less capable of the formation of such mats (Weeda *et al*. 1999). As a consequence, restoration and management activities, such as mowing or reduction of the nutrient concentrations in both water and bank will be necessary to create more resilient opportunities for the formation of species-rich floating mats. Although field studies would be required to test these assumptions, this mesocosm study provided valuable insights in the functioning of fen riparian ecosystems in reaction to eutrophication of both surface water and bank soil.

Acknowledgements

This study was conducted within the National Research Programme ‘Ontwikkeling + Beheer Natuurkwaliteit’, funded by the Dutch Ministry of Agriculture, Nature and Food Quality. We especially thank B. Willers for his daily care of the mesocosms and thank many other colleagues and students for assisting with the practical work and analyses.

INTERMEZZO **2**

The keystone function of *Stratiotes aloides L.*

J.M. Sarneel and M.M. Nijzink

Introduction

Stratiotes aloides L., the flagship species of Dutch fen conservation, is often thought to play a key role in the formation of floating mats in shallow fen ponds. *Stratiotes* is an emergent macrophyte that forms stiff leaved, floating rosettes that by rapid clonal expansion can form dense beds (up to 35 rosettes per m^2) that can cover the entire pond surface (Figure B 2.1). By the formation of such dense vegetative beds, this species is assumed to provide structural support for the rhizomes of other colonising species growing from the bank. *Stratiotes* would thereby act as a keystone species (*sensu* Paine 1969) in the formation of floating mats (Smolders *et al.* 2003). Especially species with rhizomes that have a density higher than water (such as *Comarum palustre* L. and *Thelypteris palustris* Schott), and hence have to invest in support tissue to maintain their position at the water surface, might benefit from the presence of *Stratiotes*. In addition, the dense mats will attenuate the waves which will decrease the probability of fracture for the rhizomes in the water.

Stratiotes is, however, relatively intolerant to the effects of the land-use changes that have occurred in the Dutch fen landscapes in the 20th century (Chapter 1). It has been observed to die back in waters with high ammonium and sulphide concentrations (Smolders and Roelofs 1996; Smolders *et al*. 2003). Besides, it hardly ever forms seeds and hence might be especially vulnerable to the increased fragmentation of the landscape. As a consequence, the *Stratiotes* population has declined considerably in the Dutch fen areas over the last decades (Beltman *et al.* 2008; see also Chapter 1; Figure 2). Because of its hypothesised keystone function in the succession towards floating mats this decline might have contributed to the disappearance of those vegetation types.

Here, we investigated the keystone function of *Stratiotes* and hypothesised that if species growing from the bank benefit either from the structural support of *Stratiotes* rosettes or from their ability to attenuate waves, the rhizomes of these species would be less rigid in the presence of dense *Stratiotes* vegetation than at sites without *Stratiotes*. This is because we assume that the presence of *Stratiotes* decreases the necessity of support tissue to maintain a position at the water surface. We tested this for the woody rhizomes of the fern species *Thelypteris palustris*.

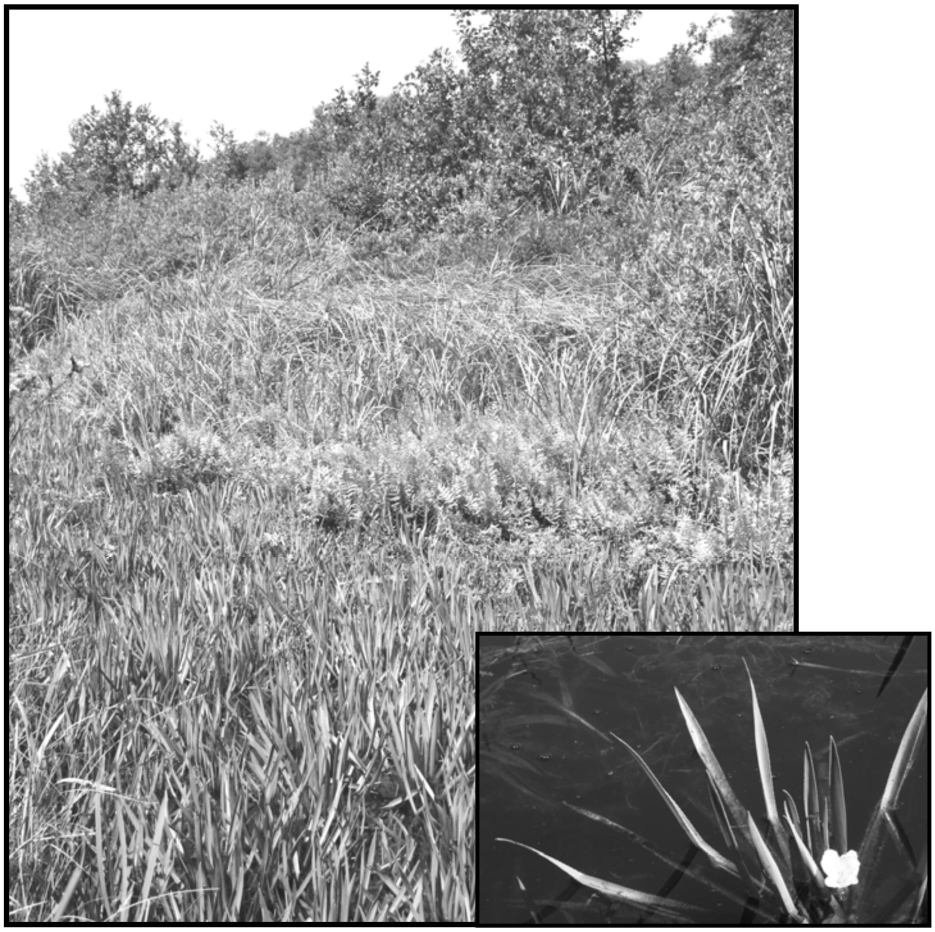


Figure B 2.1: Dense Stratiotes aloides vegetation that is invaded by Thelypteris palustris and sedge vegetation in Het Hol.

Method

We collected 8 rhizomes of *Thelypteris* that colonised the surface water in the presence of *Stratiotes* and 9 rhizomes from places where *Stratiotes* was absent. All samples were taken in the Molenpolder (52˚09N; 5˚05E). In the field we measured the depth at which *Thelypteris* and *Stratiotes* grew in the water column. We also measured the distance over which the *Thelypteris* rhizome had colonised the water. A *Thelypteris* rhizome consists of a main stem with several (branched) side branches which form dense and thoroughly connected rhizome mats. We only harvested branches at the free, pondward end of the rhizome mats and did not include (parts of the) rhizome that were within the dense rhizome network. We carefully wrapped the harvested rhizomes in wet tissue to prevent rigidity changes due to the loss of water. The rhizomes were transported in a large cool box to the lab, where the rigidity of the rhizome was determined at the base, middle and distal end of the main rhizome.

The rigidity of a rhizome depends on its diameter, shape and elasticity, a property of the rhizome material. The material elasticity was measured with a universal testing machine (Instron Model 5542, Canton, USA) which applies a small, downward force (F; N) on the middle of a rhizome fragment that is placed horizontally over two supports. It measures this force and the resulting deflection (δ; m). Material elasticity (E; MPa) is then calculated using the theoretical relation given by Gere and Timoshenko (1999):

$$E = \frac{F \cdot L_{sp}^{3}}{I \cdot 48 \cdot \delta} \tag{1}$$

where L_{sp} is the distance between the supports (m) and I, the second moment of area (m^4), represents the contribution of stem thickness and shape to rigidity. For the rhizomes of *Thelypteris*, which have a circular diameter, I is given by:

$$I = 0.25 \cdot \pi \cdot r^4 \tag{2}$$

The diameter (r, m) was measured at the middle of the sample. The overall rhizome rigidity, which combines the effect of shape, size and material rigidity, is then defined as the flexural stiffness (N m^2):

$$\text{Flexural stiffness} = E \cdot I \tag{3}$$

Besides, the total rhizome length, the length of the side branches, their fresh weight and the weight of the leaves were measured. To calculate the tissue density of the main rhizome we determined its volume by submerging the rhizome and measuring the water displacement.

For the flexural stiffness, differences between the different sections of the rhizome and differences between rhizomes from places with and without *Stratiotes* were tested with a repeated measures test in SPSS 16.0. The different rhizome sections were considered as the within-subject factor and the different sample sites as the between-subject factor. All other variables were tested with independent samples t-tests.

Results

The mean density of *Thelypteris* rhizomes was slightly higher than water (1.27 g ml^{-1}) and did not differ between rhizomes from places with and without *Stratiotes*. A repeated measures test showed that the flexural stiffness of *Thelypteris* rhizomes increased towards the top (Figure B2.2a; *P* = 0.03). The rhizomes were more flexible (i.e. they had a lower flexural stiffness) in the presence of dense *Stratiotes* vegetation, but this effect was not significant. The length of the rhizomes did not differ between both treatments. Although the traits of the rhizomes did not show significant differences between the *Stratiotes* treatments, their placement within the water column did. In the presence of *Stratiotes*, the rhizomes of *Thelypteris* were somewhat closer to the waterline and the leaves protruded significantly higher above the water (*P* = 0.008; Figure B 2.2b). Figure B 2.2 further shows that *Thelypteris* is indeed carried by *Stratiotes. Thelypteris* could benefit from this higher position as it implies that its leaves are less frequently submerged. Whether those leaves also receive more light remains open for further research. The presence of *Stratiotes*, with leaves also protruding from the water, increases the competition for light, especially below the water surface.

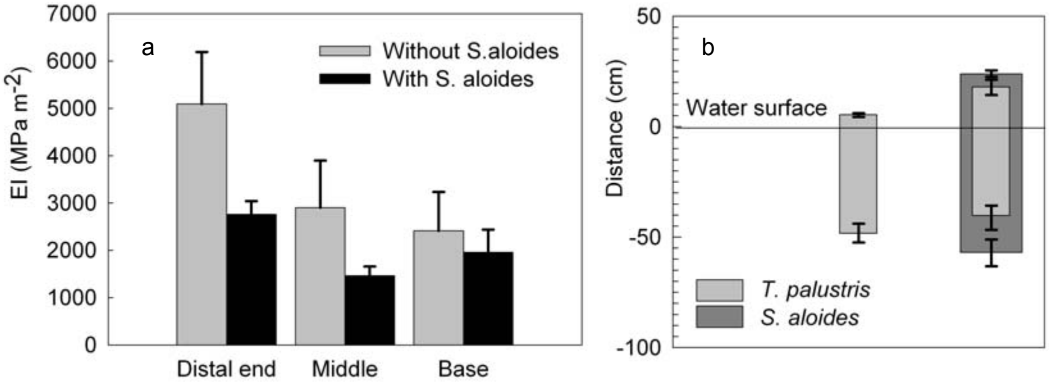
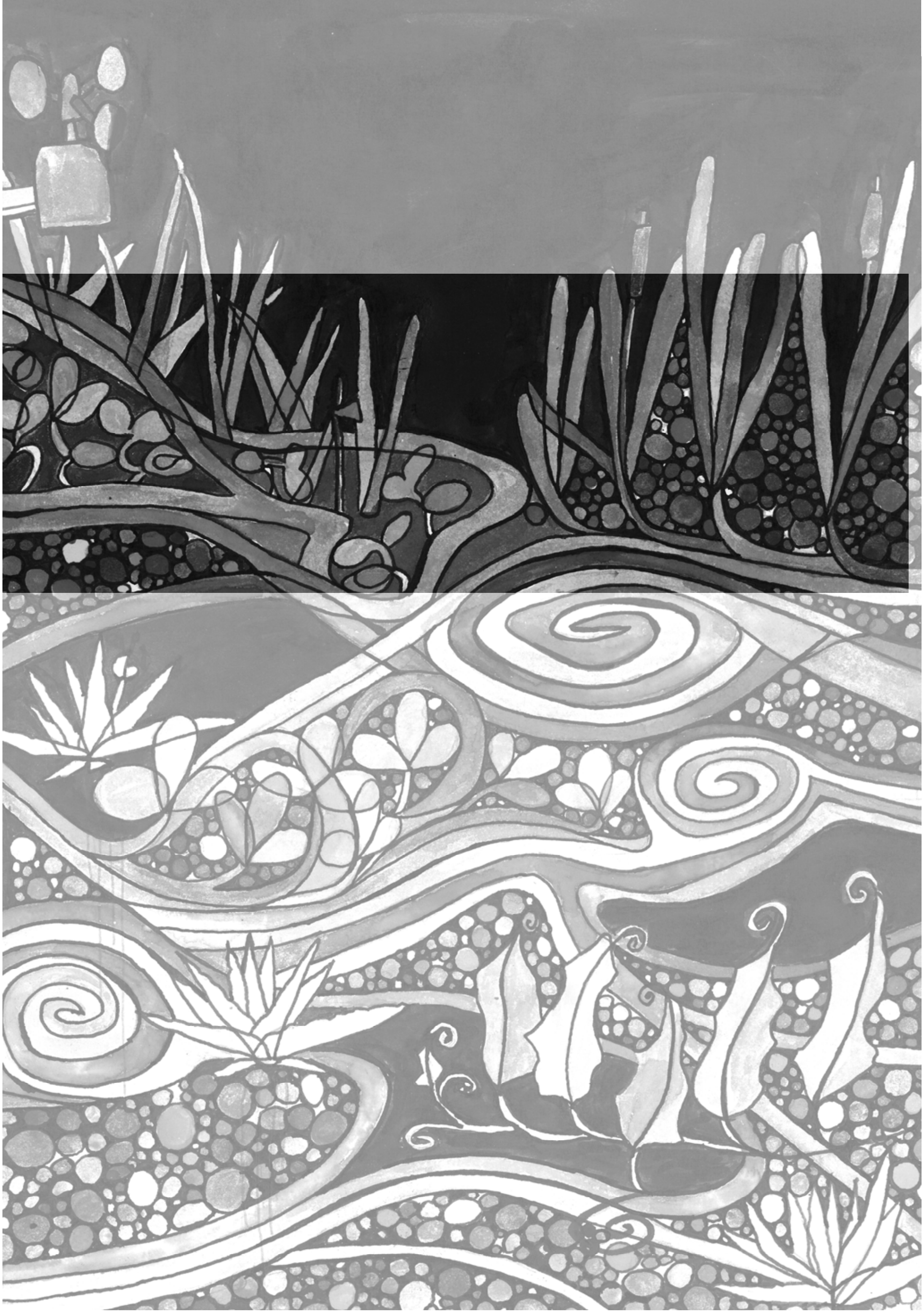


Figure B 2.2: A) Stiffness (MPa m^{-2}) of the different sections of the Thelypteris rhizome. B) The distribution of Thelypteris palustris plants above and below the water table in the absence and in the presence of Stratiotes aloides. Error bars indicate the Standard Error.

Conclusions

Overall, this pilot study shows that rhizomes of *Thelypteris* are carried by dense *Stratiotes* vegetation. As hypothesised, this influences the way in which *Thelypteris* grows into the open water. In the presence of *Stratiotes* the rhizomes grow closer to the water surface and protrude higher from the water surface. Contrary to our hypothesis, other rhizome traits (density, flexural stiffness, length) are not affected significantly. Whether these adaptations of *Thelypteris* indeed result in a better colonisation when *Stratiotes* is present remains open for further research.



CHAPTER **5**

Multiple effects of land use changes impede the colonisation of open water in fen ponds

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Abstract

Dutch fen areas have become embedded in intensively used landscapes, resulting in a loss of characteristic fen species and the succession process in which they play a role. Especially species that colonise open water by growing from the bank hence inducing the formation of species-rich floating peat mats, have disappeared. Despite many restoration efforts they have not returned. The factors hypothesised to be responsible for this are associated to decreased habitat quality, hampered dispersal and altered biotic interactions (in particular, loss of the keystone species *Stratiotes aloides* and invasion of muskrats). We investigated which factors are most likely responsible.

We compiled a large dataset on the occurrence of colonisers in 62 fen ponds. In each pond we determined species richness and the expansion into open water. We related these to habitat quality (chemical composition of soil and surface water, pond morphology), dispersal potential (distance to remnant populations, likelihood of dispersal) and biotic interactions (presence of *Stratiotes* and muskrats).

A factor analysis showed that regional differences influence both the expansion into open water and the species richness of the bank. Plants expanded further into open water and banks were more species-rich in areas with older ponds and lower muskrat densities. Colonisation was also related to local factors, most importantly water turbidity and depth. A high turbidity hampered colonisation. Whenever the water was clear, colonisation was higher in shallow ponds and in deep ponds if *Stratiotes* was present. Species richness was negatively correlated to nutrient availability in the soil and positively to hydrological isolation, which decreased sulphate concentrations. We also found that species richness was higher at sheltered banks. Overall, we conclude that multiple habitat characteristics (turbidity, water depth, nutrient and sulphate concentrations) and the interaction with muskrats and *Stratiotes* play a role in the lack of restoration success in Dutch fen ponds. Dispersal limitations seem to be overruled by habitat limitations as colonisation often fails even when sufficient propagule sources are present, or when connectivity is high. Restoration and conservation should therefore aim at increasing habitat quality and *Stratiotes* cover and decreasing regional populations of muskrats.

Keywords: Fragmentation, Muskrat invasion, Restoration, *Stratiotes aloides* L., Succession, Wetlands.

Introduction

Over the past decades, many fen areas have become embedded in intensively used landscapes, often resulting in a decreased biodiversity and a changed ecosystem functioning. Fens are groundwater- or surface water-fed, peat accumulating wetlands that may contain a relatively high proportion of open water. These water bodies are first colonised by a wealth of aquatic species, successively followed by semi-aquatic and semi-terrestrial species (Table 5.1; hereafter called colonisers). The colonisers invade the water either by floating on or just below the water surface or by rooting in the pond bottom. Under oligo- to mesotrophic conditions, such colonisers can induce the formation of floating peat mats (Van Wirdum *et al*. 1992; Schaminée *et al*. 1995; Somodi and Botta-Dukát 2004), a species-rich succession phase (> 30 species m^{-2}) that contains several red list plant and animal species (Vermeer and Joosten 1992; Bal *et al*. 2001; Verhoeven and Bobbink 2001). This process is thought to be facilitated by *Stratiotes aloides* L. as its stiff-leaved, floating rosettes could provide structural support for the rhizomes of other species that invade the water (Schaminée *et al*. 1995, Smolders *et al*. 2003). Under very eutrophic conditions, the floating mat phase is frequently omitted and species that root in the pond bottom take over (Van Wirdum *et al*. 1992). In either case, succession will eventually lead to the formation of (forested) peatland. Local disturbances (e.g. flooding, grazing or erosion) will halt or even reset succession, thereby creating a landscape in which different succession phases and all their associated species occur. Succession, and more specifically, the colonisation of the open water therefore plays a crucial role in the maintenance of the high biodiversity of fens (Bedford and Godwin 2003; Lamers *et al*. 2002).

However, due to the drastic land use changes of the 20th century, the habitat quality for many fen species has deteriorated and dispersal between the remaining suitable habitat fragments has become very difficult (Beltman *et al*. 2010). Consequently, many fen species have failed to persist in, or have failed to reach, remaining fen fragments. This has led to the loss of characteristic fen species (including the colonisers and *Stratiotes*) and impedes the succession process, including the colonisation of open water, in which those species play a role.

This deterioration is also observed in the Netherlands, where species-rich fens with floating mats used to be a rather common phenomenon (Bakker *et al*. 1994; Verhoeven and Bobbink 2001; Lamers *et al*. 2002). Since about 1700, peat excavations, dredging the peat even from below the water table (Pons 1992; Vermeer and Joosten 1992), created a landscape with numerous rectilinear ponds. Despite their anthropogenic origin, these ponds contained a wealth of succession phases and a large number of rare plant and animal species (Bootsma 2000; Verhoeven and Bobbink 2001). Around 1940 the excavation of turf ponds stopped because the demand for turf ceased. After that, succession has gradually turned the majority of remaining ponds into alder forest (Bakker *et al*. 1994; Van Belle *et al*. 2006). Because this occurred simultaneously with the drastic changes in the landscape surrounding the fens, many species, especially those associated to the formation of floating mats, have become rare (Verhoeven and Bobbink 2001; Lamers *et al*. 2002; Beltman *et al*. 2008). Despite many attempts to restore the habitat quality and the (semi-) natural dynamics by the creation of new open water, the formation of new floating mats has almost never been observed over the last 20-30 years (Lamers *et al*. 2001; Beltman *et al*. 2008). These disappointing results of restoration efforts are often regarded as the consequences of the main land use changes, which can be categorised into 1) decreased habitat quality, 2) restricted dispersal and 3) altered biotic interactions. We summarise these changes below.

1. After the 1950s, multiple antropogenic activities have changed the habitat quality of fen areas drastically. First, drainage has increased, which has changed the hydrology of almost all fen areas (Lamers *et al*. 2002) resulting in desiccation and acidification of soils (Van Wirdum *et al*. 1992; Barendregt 1993; Lamers *et al*. 2002). Second, nutrient concentrations of both soil and surface water have been enhanced by the supply of nutrients via increased atmospheric deposition (to 30-45 kg N ha^{-1} y^{-1} in the late 1980s), agricultural run-off (Barendregt *et al*. 1995; Bobbink and Lamers 2002) and via (allochthonous) nutrient and sulphate-rich water that has been supplied to fen areas during dry periods in summer to maintain stable water tables (Lamers *et al*. 2002).

Table 5.1: Target coloniser species. Bold species names indicate red list species.

Colonisers	Buoyancy		Longevity[3]	Habitat preference	Canopy height (m)	Growth form
	Veg. [1]	Seed[2]				
Calla palustris L.	++ *	++	Unknown	Mesotrophic	0.1-0.2	Leaved rhizome
Comarum palustre L.	-	++	Transient	Oligo- to mesotrophic, phosphorous poor	0.15-0.9	Woody rhizome
Equisetum fluviatile L.	+	Spore	Unknown	Mesotrophic	0.5-1.5	Without true leafs
Glyceria maxima (Hartm.) Holmb.	n.d.	+	Transient	Eutrophic	0.8-1.8	Grass with long rhizome
Menyanthes trifoliata L.	++	++	Transient	Mesotrophic, slightly acidic, P-poor	0.13-0.3	Leaved rhizome
Phragmites australis (Cav.) Steud.	+	++	Transient	Meso- to eutrophic	1.0-4.0	Grass with long rhizome
Ranunculus lingua L.	n.d.	-	Unknown	Mesotrophic	0.5-1.5	Herb with rhizome
Thelypteris palustris Schott	-	Spore	Unknown	Oligo- to mesotrophic, slightly acidic	0.3-0.8	Woody leaved rhizome
Typha angustifolia L.	n.d.	+	Short-Term persistent	Meso- to eutrophic	1.0-2.0	Grass with long rhizome
Typha latifolia L.	n.d.	+	Variable	Eutrophic	1.0-2.0	Grass with long rhizome
Stratiotes aloides L.	+ *	n.d.	Unknown	Mesotrophic	0.05-0.2	Floating, emergent rosettes

[1] Buoyancy of vegetative propagules was determined experimentally (Box 1). ++ = very long > 6 months, + = long, - = short < 1 month.

[2] Buoyancy was obtained from Kleyer *et al*. (2008), Van de Broek *et al*. (2005), M. Soons (unpublished). Due to small methodological differences between these researches, the species were classified: ++ = very long floating times, + = long floating times, - = short floating times.

[3] From Kleyer *et al*. (2008).

* Species has specialised vegetative propagules. n.d. = Not Determined or Unknown.

The supply of sulphate-rich water has also induced the release of extra phosphate from Fe-P compounds (internal eutrophication; Smolders *et al*. 2006) and the formation of sulphide, which is toxic to plants even in low concentrations (10-50 μM; Smolders and Roelofs 1995; Van der Welle *et al*. 2006; Lamers *et al*. 1998; Geurts *et al*. 2009).

2. As human land use intensity has increased, the fen areas have become smaller and less connected to each other. This increased the distance and the number of barriers between suitable habitat patches. Hence, dispersal between the remaining fragments has become very difficult (Soons *et al*. 2005). Consequently, species might be absent in suitable patches or restored fen ponds simply because they have been unable to reach them. The seed bank can form an important buffer against this effect of fragmentation, but most colonisers form only short-lived seed banks (Chapter 2) and hence, active dispersal from remnant populations is more important (Van den Broek and Beltman 2006; Beltman *et al*. 2010). Dispersal in fens is driven by wind-generated currents, and deposition patterns reflect prevailing wind directions (Chapter 2).

3. The third set of changes assumed to negatively affect the formation of floating mats are the invasion of muskrats (*Ondatra zibethicus* L.) and the loss of *Stratiotes aloides* L. The first muskrat was caught in 1941, but currently, almost one muskrat is caught per hour of hunting in peat areas (Bos *et al*. 2009). As this invasive North American species dwells in shorelines and its diet consists almost completely of herbaceous vegetation, including rhizomes (Doude van Troostwijk, 1976; Connors *et al*. 2000; Clark 1994), a high muskrat density would be a possible threat to the colonisation of open water. The decline of *Stratiotes* may also impede the colonisation of open water as this species could act as a keystone species, providing structural support and shelter to waves to floating rhizomes that invade the water.

These three factors (habitat quality, dispersal limitations and biotic changes) have rarely been studied simultaneously (Leng *et al*. 2009; Beltman *et al*. 2010). Instead, the majority of studies tended to focus on the relation between habitat characteristics and the conservation and restoration of biodiversity. Only recently the importance of dispersal limitations and the need to restore ecosystem processes rather than biodiversity alone have been acknowledged (Barendregt *et al*. 1995; Lamers *et al*. 2002; Leng *et al*. 2009; Verhoeven *et al*. 2008). In this study, we consider all three problems and investigate whether the loss of characteristic species and lack of succession towards species-rich floating fen is best explained by site conditions, dispersal limitations or changed biotic interactions.

Methods

In 2006 and 2008, we performed an extensive field survey in six fen nature-areas in the Netherlands (Figure 5.1). In total, we sampled 61 fen ponds (4 - 12 per area) of different ages. For each pond, we determined three response parameters that indicate the current state of the development towards species-rich floating mats. Using multivariate statistics we related these to a large number of parameters indicative of the habitat quality of soil and water, the likelihood of dispersal and the presence of biotic interactions with muskrats and *Stratiotes*.

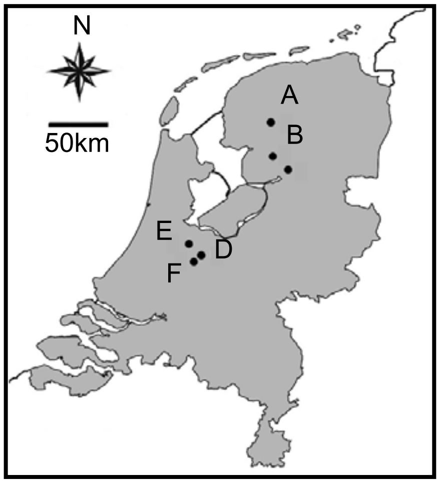

Figure 5.1: Locations of the sampled fen areas in the Netherlands. A: De Deelen (450 ha), B: De Weerribben (3500 ha) C: De Wieden (5676 ha), D: Westbroek (200 ha), F: Molenpolder (190 ha), E: Terra Nova (85 ha).

Study sites

All areas in which ponds were sampled are protected by nature agencies, but some commercial use is allowed (reed cutting, fishing, grazing and recreational boating). Each area consists of a network of lakes, ponds, canals and ditches, alternating with reedland, alder forest and some grass and sedge meadows. Management of the banks and shorelines in the areas consists of mowing and sheep grazing. To decrease turbidity, the Molenpolder has been dredged in 1992-93 and this measure was successful (Verberk *et al*. 2007). In the more eutrophic fen system Terra Nova, the fish stock has been reduced to provoke a shift to a clear water state, but this has shown only short term effects on turbidity (Bontes *et al*. 2006). A strictly stable water table is maintained in all areas, but in De Deelen some level fluctuations (± 25 cm around the mean) have been allowed since 2003. To maintain stable water tables, water is let out in wet periods and water from outside the area (allochthonous water) is supplied during drier (summer) periods. Usually this nutrient-rich allochthonous water enters an area from one point, preferably situated in one of the larger lakes. Water quality then improves towards more remote and hydrologically isolated parts of the area, where most of the sampled ponds were located (Geurts, unpublished data).

Characterisation of colonisation

Succession toward floating peat mats depends upon the presence of coloniser species (Table 5.1) in the pond. Hence, to test under what circumstances these species were absent or present, we determined the presence of ten typical colonising species in each pond (Table 5.1). If these species are present, they have to grow from the bank into the water. As a straightforward measure of the colonisation of open water, we measured the distance between the bank and the farthest (semi-)terrestrial plant in the water on a representative stretch of the bank. We incorporated distances achieved by any emergent species, but submerged and floating-leaved aquatic species were left out. Next to the colonisation process, biodiversity is an important aim of restoration and conservation. Therefore, we also characterized the species richness of the sampled pond banks with a Braun Blanquet relevé of 2 m^2 parallel to the waterline (Barkman *et al*. 1964). Relations between these three response variables and independent variables indicative of 1) the habitat quality of soil and water, 2) the connectivity (the probability of dispersal of the set of typical colonisers into the pond) and 3) biotic interactions (with the keystone species *Stratiotes* and muskrats) were analysed with multivariate statistics.

Habitat quality

The most important habitat characteristics for shoreline species are determined by the chemical composition of the soil and surface water and by the morphology of the pond (Table 5.2). For each sampled bank, these were determined as follows: A water sample from the surface water was taken with a 100 ml glass flask. The bank pore water was sampled anaerobically with 10 cm-long soil moisture samplers (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) and vacuum glass infusion bottles (100 ml), within 20 cm from the margin with the open water. All samples were first stored in a cool box and later at -20 ºC until further analysis. In the lab, pH was measured on the day of sampling (WTW Measurements Systems, Ft. Myerss, FL). The $Fe^{2+/3+}$ Al^{3+}, Ca^{2+}, Mg^{2+}, HCO_3^-, Cl^-, SO_4^{2-}, NO_3^-, NH_4^+, PO_4^{3-}, Na^+ and K^+ concentrations of the pore water and the Ca^{2+}, SO_4^{2-}, NO_3^-, NH_4^+, PO_4^{3-}, Na^+ and K^+ concentrations of the surface water were measured using a continuous flow analyser and flame emission spectroscopy (SKALAR SA-40, Breda, The Netherlands).

Bank and pond morphology (Table 5.2) are also important features of the shoreline habitat as they determine, among other things, the probability of flooding (Bank height, Bank steepness), light availability (Orientation, Secchi depth) and disturbance by waves (Orientation, Pond depth, Effective fetch).

These were quantified as follows: Bank height above the water table and water depth close to the bank (within 30 - 50 cm) and in the middle of the pond were measured with a ruler. Secchi depth was measured as the depth at which the Secchi disk was not visible anymore. Bank steepness was measured as the angle between a stick that was placed on the bank (perpendicular to the waterline) and another stick that was kept horizontal with use of a spirit level. The orientation of the bank was estimated as the angle between the bank-to-water transition and the North with use of a compass. Here, low values (< 90) indicate an East to South facing bank, while North to North-East facing banks have high values (> 270). As a measure for wave impact, the effective fetch (i.e. the distance of open water in the direction of prevailing wind direction; m) was measured from aerial photographs. It was calculated as the weighted mean of the distances of open water in front of the sample point in a range of -42° to +42° from the prevailing wind direction (in 6° increments).

Dispersal and connectivity

Next, we determined a set of variables that represent the probability that propagules will enter the pond via water (which is the main dispersal vector) (Table 5.2). To receive propagules via water, connections with other water bodies are needed and therefore, we counted these in each pond. Further, as dispersal follows the prevailing wind direction (Chapter 2; Soomers *et al*. in press), the geographical orientation of these connections in relation to the prevailing wind direction (SW) is of importance. For this, we classified the ponds into four categories: 0 - isolated (ponds with no connections); 1 - low probability of seed supply (ponds with connections, but not in the SW side of the pond); 2 - some probability of seed supply (ponds with connections in the SW side of the pond, but connected to a ditch perpendicular to the prevailing wind direction, limiting transport through that ditch); 3 - high probability of seed supply (ponds with connections in the SW side of the pond and connected to another SW oriented water body).

The probability that species enter a (restored) pond will also depend on the presence of source populations in the direct or more remote vicinity. The likelihood of dispersal increases when there are more ponds in the surroundings, as a result of a greater availability of suitable habitat (Mäkelä *et al*. 2004; Beltman *et al*. 2010). We therefore determined the number of ponds within the km^2 surrounding the sampled pond from aerial photographs (Google earth 2009). Further we obtained the presence of colonisers (Table 5.1) per km^2 from the Floron database. The Floron database divides the Netherlands into grid cells of one km^2 and contains the vegetation composition of each cell in two time spans: species present before 1950 and present after 1975. For each time span, we calculated the proportion of the ten colonisers that was present in the km^2-cell containing the sampled pond. To quantify the presence of these species on a larger regional scale, we calculated the proportion of the ten colonisers present for each km^2-cell in the $5 \times 5\ km^2$ area around the pond. These 25 proportions were averaged to obtain a measure for the regional availability of colonisers (Table 5.2).

Biotic interactions

As muskrats are a mobile species, we quantified the disturbance by muskrats per area and obtained the number of muskrats caught per hour of hunting from water boards. The possible role of *Stratiotes* as keystone species in the succession is assessed explicitly in Intermezzo 2. In this field study we incorporated this factor by recording presence of *Stratiotes* for each pond.

Statistical analyses

To derive the most important relations from such a large dataset, the following strategy was used. First, a factor analysis with Varimax rotation was conducted including all 37 variables (SPSS 16.0). Missing values were replaced by the mean. Percentage data were arc-sine transformed. A factor analysis groups the large set of variables into clusters (hereafter called: components) of variables that are highly correlated. These components can be interpreted based on the underlying variables (e.g. when NO_3^-, NH_4^+ and PO_4^{3-} group together, this component reflects nutrient richness). Via stepwise multiple regression the component scores (a new variable for each component, calculated by SPSS 16.0) of the extracted components were related to the three dependent colonisation characteristics (Table 5.2). Next, the individual variables that were highly correlated ($r > 0.4$ after rotation) to the component(s) selected by the stepwise regression, were included in a second stepwise multiple regression analysis. This allowed us to assess which of these variables could, by themselves, form an explanation for the relation found between restoration success and the components composed by SPSS.

Results

In our dataset the measured variables were grouped in twelve components which explained almost 80 % of the total variance (Table 5.3). The first component grouped most of the salt concentrations in the soil pore water (Mg^{2+} S, Ca^{2+} S, Na^+ S, Na^+ W, SO_4^{2-} S; S = soil pore water; W = surface water) and hence the score on this component can be interpreted as an

Table 5.2: Variables measured for each sampled pond.

Independent variables: Colonisation characteristics	
Col. Dist.	The distance between the bank and the farthest plant in the water (m)
Richness	Total number of species per relevé ($2m^2$)
Col. in pond	Percentage of the set of colonisers present in the pond
Chemical composition	
Element + W	pH and Ca^{2+}, SO_4^{2-}, NO_3^-, NH_4^+, PO_4^{3-}, Na^+ and K^+ concentrations in the surface water (µM)
Element + S	pH and $Fe^{2+/3+}$ Al^{3+}, Ca^{2+}, Mg^{2+}, HCO_3^-, Cl^-, SO_4^{2-}, NO_3^-, NH_4^+, PO_4^{3-}, Na+ and K+ concentrations in the bank pore water (µM)
Morphological variables	
Depth at bank	Water depth measured close to the bank (cm)
Depth Pond	Water depth measured in the middle of the pond (cm)
Secchi	Secchi depth, measure of turbidity (cm), in the middle of the pond
Angle	Bank angle of the sampled bank
Height	Vertical height above the water of the sampled bank (cm)
Orientation	Orientation of the sampled bank towards the north
Dispersal related variables	
# Conn.	Number of connections to other ponds or ditches
SW Conn.	Probability of seed input during SW winds*
Eff. fetch	Effective fetch (weighted distance of open water in front of the sample point) (m)
Surface	Pond surface (m^2)
Ponds-km	Number of ponds within the surrounding km^2
Km 1975	% of the colonisers present in the surrounding km^2 after 1975
Km 1950	% of the colonisers present in the surrounding km^2 before 1950
Reg. 1975	% of the colonisers present in the surrounding 5 x 5 km^2 after 1975
Reg. 1950	% of the colonisers present in the surrounding 5 x 5 km^2 before 1950
Other variables	
Age	Age of the pond (years)
Stratiotes	Presence of Stratiotes aloides (0/1)
Rat	Muskrat density (# caught $hour^{-1}$)

* This probability was indicated in classes: 0 = Isolated pond, no connections; 1 = Connection(s) but not in SW end of the pond; 2 = Connection(s) in the SW end of the pond but with a ditch that is not SW oriented; 3 = Connection(s) in the SW end of the pond connected with a SW oriented ditch.

Table 5.3: The correlations (>0.4) between the constructed components and the original variables, the cumulative variance explained by these components and the results of the stepwise regression of the components with the three dependent variables.

	1	2	3	4	5	6	7	8	9	10	11	12
Mg^{2+} S	0.9											
Ca^{2+} S	0.8											
SO_4^{2-} S	0.8											
Na^{+} W	0.7											
Na^{+} S	0.7											
Cl^{-} S	0.5	- 0.4										
Km 1975		0.9										
Reg 1975		0.9										
Age		- 0.7										
Rat		0.7					- 0.5					
pH S			-0.8									
Surface			0.8									
Al^{3+} S			0.6									
$Fe^{2+/3+}$ S			0.6			0.5						
Ponds_km			0.5					0.4				
Ca^{2+} W												
NH_4^{+} W				0.9								
K^{+} W				0.8								
NO_3^{-} W				0.8								
PO_4^{3-} W				0.6		0.4						
Angle					0.8							
NO_3^{-} S					0.7							
HCO_3^{-} S					- 0.6							
Height					0.6				-0.5			
NH_4^{+} S						0.8						
PO_4^{3-} S						0.8						
Reg 1950							0.8					
pH W							- 0.7					
Km 1950							0.5				0.5	
Secchi								0.8				
Depth pond								0.7				
K^{+} S								0.7				
Stratiotes									0.6			
Depth at bank			- 0.5						0.6			
# Conn.										0.9		
SO_4^{2-} W										0.6		
SW Conn.										0.5		
Eff. Fetch											0.8	
Orientation												0.8
Cumm. var.	*10.4*	*20.7*	*29.2*	*36.3*	*43.0*	*49.5*	*55.3*	*60.8*	*66.0*	*71.3*	*75.6*	*79.6*

Correlations	1	2	3	4	5	6	7	8	9	10	11	12
Distance		-0.27						-0.28				
Richness		-0.25				-0.28				-0.26		0.28
Col. in pond		-0.29	-0.42									

indication of the overall conductivity of the bank soil pore water. The second component contained variables that can be interpreted as regional influences (Presence of species after 1975, Age, Rat density, Cl^- S), as those variables varied more on a regional scale compared to others that varied more locally. Age was probably included in this component because the areas with a high muskrat density, predominantly contained young ponds. The age of the ponds was more variable in the other areas. The third component consisted of variables that related to the acidity (pH S, Al^{3+} S, $Fe^{2+/3+}$ S, Ca^{2+} W) and the quantity of open surface water (Ponds per Km^{-2}, Pond surface). Apparently, ponds in larger complexes are also larger, have more acidic banks and contain higher concentrations of Al^{3+} and $Fe^{2+/3+}$ in the pore water of the bank. These ponds are also shallower close to the bank. Very clearly, the fourth component grouped variables concerning the nutrient status of the surface water (NH_4^+, K^+, NO_3^- and PO_4^{3-}). Remarkably, the nutrient concentrations in the bank pore water were grouped in other components, indicating that the nutrient concentrations of the water and the bank varied relatively independently from each other in the field. The fifth component contained bank height and angle, and banks with a steep and high morphology contained higher NO_3^- but lower HCO_3^- concentrations in the pore water of the bank. The sixth component indicated the nutrient availability in the bank pore water. Not surprisingly, iron in the pore water, which is highly associated to internal eutrophication, and hence to phosphate, was included in this component as well. The PO_4^{3-} concentration in the surface water also correlated to this component, but the correlation coefficient was rather low. The seventh component contained another set of regional variables (Rat density, Presence of species before 1950) and the pH of the surface water was also negatively correlated to this component. Water depth and Secchi depth correlated positively to the eighth component. In fact, Secchi depth often equalled water depth, showing that the turbidity was generally low, allowing light to penetrate to the pond bottom. As the number of ponds per km^2 also correlated to this component, this component also shows that ponds within larger complexes were somewhat deeper. Component nine showed that ponds in which *Stratiotes* was present, generally exhibited steep banks below the water table (i.e. they were relatively deep close to the bank), a low bank height above the water and higher K^+ concentrations in the soil pore water. The 10th component was related to the degree of isolation, and showed that ponds with more connections have a higher sulphate concentration in the surface water, probably due to larger mixing with sulphate rich water from outside the area.

The last two components were related to the effective fetch of the sampled bank and the orientation of this bank towards the north, respectively.

Overall, the relations found in the field reflect the major gradients such as electrical conductivity, nutrient availability and isolation (Table 5.3). Those relationships and those that were less predicable (e.g. between NO_3^- S and Bank height) should be kept in mind when correlations between the dependent variables and single environmental variables are interpreted.

Presence of coloniser species

On average, a pond contained 3.5 coloniser species. This was on average less than half (42%) of the regional coloniser pool, as on average 8.5 coloniser species were found after 1975 within the km^2 - cell that contained a pond. *Phragmites australis* was present in most of the km^2 - cells surrounding the ponds, and when it was present there, it almost always occurred in the pond (Figure 5.2). Other species were less frequently found. *Thelypteris palustris* and *Typha angustifolia* were found in 63% and 59% of the ponds when they were present in the surrounding km^2 - cell of that pond, respectively. *Menyanthes trifoliata*, *Calla palustris*, *Ranunculus lingua* and *Comarum palustre* were less frequently found, even though they occurred within a km^2 for 50 - 90% of the sampled ponds (Figure 5.2). *Calla* and *Ranunculus* were found in only 10% of the ponds where those species were present within the km^2 - cells surrounding that pond.

A stepwise multiple regression model showed that the number of colonisers in the pond related negatively to both component 6 (nutrient availability in the soil) and 8 (water depth) and this model explained 26% of the variance (Table 5.3). Thus, more colonisers were found in shallow ponds with a lower nutrient availability in the bank pore water. Further investigation by a second stepwise regression with the variables included in those components showed that the number of colonisers in a pond was negatively related to water depth (standardised $r = -0.314$) and the PO_4^{3-} concentration in the surface water (standardised $r = -0.289$). This model explained 20% of the variance ($P = 0.004$; Figure 5.3a).

Colonisation distance

In the 62 sampled ponds, a variety of plant species colonised the open water over, on average, 2.4 m. The majority of the longest distances were achieved by species that rooted in the bottom sediment of the pond (mainly *Phragmites*), especially in the ponds younger than 25 years. The stepwise multiple regression analysis showed that the colonisation distance was negatively related to both component 2 (regional factors) and 8 (water depth)

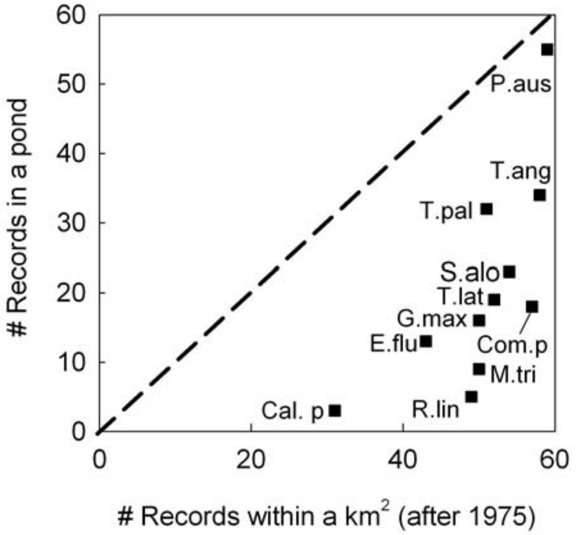

Figure 5.2: Number of km^2 - cells in which the different colonisers were found and number of ponds in which they were also found. Complete species names in Table 5.1.

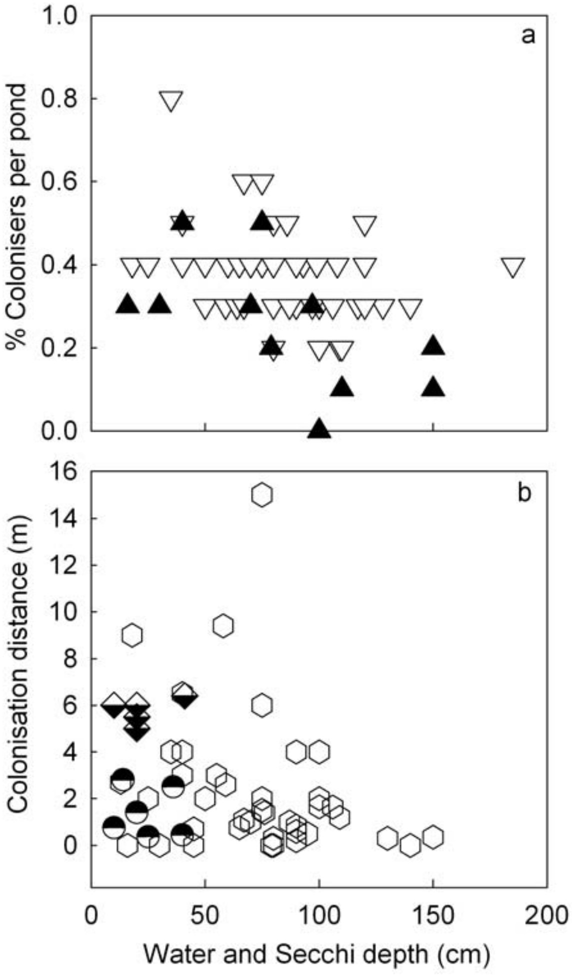

Figure 5.3: a) Relation between water depth in the middle of the pond and number of colonisers in the pond. Ponds with an above average amount of PO$_4^{3-}$ in the surface water (0.5 µM) are indicated by closed triangles. Ponds with lower concentrations are indicated by open triangles. b) Relation between Secchi depth (cm) and colonisation distance (m). Ponds where the Secchi depth equalled the pond depth are indicated with open hexagons. Deep ponds where the Secchi depth is low (< 40 cm) because of dense Stratiotes beds are indicated with diamonds. Deep turbid ponds with a low Secchi depth are indicated with circles.

colonisers in the pond correlated in turn to colonisation distance (P = 0.006; r = 0.37). This relation implies that more colonisers in a pond will increase the probability that the 'best' (*Typha* or *Phragmites*) coloniser is present. When richness was correlated to the components extracted by the factor analysis, component 2 (regional factors), 6 (nutrients in the bank), 10 (connectivity) and 12 (bank orientation) were included in a stepwise multiple regression model (R^2 = 0.28; Table 5.3). This showed that richness is higher in older ponds with a lower musk rat density and lower percentage colonisers in the area, in ponds with a lower nutrient availability, in ponds more isolated from allochthonous surface water and on sheltered (SW) banks. However, when the variables that correlated strongly to those components were put in a second stepwise multiple regression analysis, no significant model could be extracted. This demonstrates that the combination of variables within the components, rather than one single variable, determines the species richness of the bank.

Discussion

This study aimed at determining which of the consequences of land use changes have caused the decreased species richness and the lack of succession towards floating mats in Dutch fen areas. Our results show that species richness of fen pond banks and successful colonisation of open water are not directly related to each other and dependent on different environmental factors. Colonisation of open water is related to a set of variables that act on a regional scale (most importantly muskrat density and pond age) and to local pond depth and turbidity of the water. Whenever the water is clear, colonisation is enhanced in shallow ponds and in deep ponds if *Stratiotes* is present. A high turbidity impedes colonisation of open water. Also, more coloniser species are present in shallow ponds with clear water and low nutrient concentrations in pond and bank, especially low PO_4^{3-} in the surface water. Species richness on the bank is negatively correlated to nutrient availability (both N & P) in the soil. It is also higher at more hydrologically isolated sites, where water sulphate concentrations are lower, and it is higher at sheltered (SW) banks. Also, like colonisation distance, it is related to regional factors of which muskrat density and pond age are most clear. We will proceed with discussing the mechanisms that most likely cause these relations and their implications for restoration and conservation.

Habitat quality

Regional variables appeared to be very important for both the colonisation of open water and the species richness of the bank. This means that the six areas sampled differed considerably. Other studies on fens have reported large differences between areas due to a strong influence of regional factors such as water level fluctuations, human population density, road density and predation (Barendregt *et al*. 1995; Bootsma *et al* 1996; Vermaat *et al*. 2007). In our study, the most important regional differences between areas were muskrat density, pond age, number of colonisers in the area and Cl^- concentration in the bank soil. Although we were unable to distinguish which of them is most important, muskrat density and pond age are more likely to have direct causal relationships with colonisation and species richness than the Cl^- concentration and the presence of coloniser species. We will discuss these relationships later.

In our study, nutrient availability was an important local factor. The NO_3^-, NH_4^+ and PO_4^{3-} concentrations in soil and surface water did not differ significantly between the areas, with the exception of a higher PO_4^{3-} concentration in the surface water in De Deelen. Decreasing nutrient availability of the bank soil increased the species richness of the bank and the number of colonisers in the pond. Furthermore, the number of colonisers in the pond was negatively related to the PO_4^{3-} concentration in the surface water. This is in agreement with De Lyon and Roelofs (1986), who found that many colonisers (*Menyanthes*, *T. latifolia* and *Comarum*) prefer a low P concentration in the surface water (< 0.5, < 2.0 and < 2.2 μM, respectively). Fens have most often been reported as P-limited (Lamers *et al*. 2002; Wassen *et al*. 2005; Van Belle *et al*. 2006; Chapter 4), but N has also been found to affect the vegetation composition in floating fens (Smith and Wallstein 1986; Somodi and Botta-Ducát 2004). On average, however, the nutrient concentrations were in the lower range of those reported in literature on fens (De Lyon and Roelofs 1986; Beltman *et al*. 1996; Thorman and Bayley 1997; Hájek *et al*. 2002; Van Belle *et al*. 2006; Beltman *et al*. 2008). As especially older studies report higher values, this indicates that the water quality of fens may be improving (Beltman *et al*. 2008).

The colonisation distance appeared relatively unaffected by the chemical composition of the soil and surface water, but water depth and turbidity are important. A high turbidity hampers the colonisation, whereas in clear water colonisation is enhanced by a dense cover of *Stratiotes* or a shallow water depth. A high turbidity of the water is usually correlated to a high nutrient availability, which leads to algal blooms and increases the amount of small suspended organic particles by enhancing decomposition (Scheffer 1998; Geurts 2010). This decreases the light and oxygen availability in the pond bottom and thereby limits the lakeward expansion of (bottom rooted) colonisers. Larger colonisation distances were found in clear and shallow water, where coloniser species characterised by the bottom rooted strategy prevailed. In clear but deep water, larger distances were achieved if *Stratiotes* was present, in which case the colonisation by floating rhizomes is enhanced. *Stratiotes* itself also requires a low turbidity (Smolders *et al*. 2003) – and may even decrease turbidity as it excretes allelopathic substances that decrease the algal concentration (Mulderij *et al*. 2006).

The expansion of helophytes that root in the bottom is frequently found to be limited to shallow water (< 1 - 1.5 m), either by light limitation, disturbance from waves, as a consequence of the anoxic environment, or a through combination of these factors (Coops and Van der Velde 1996; Andersson 2001; Mäkelä *et al*. 2004; Jackson 2006; Azza *et al*. 2006, 2007). Based on our study, we cannot specify which of these factors cause the lack of colonisation at greater water depths. We did not find any clear evidence for wave damage, as effective fetch was small (< 100 m) and did not relate to colonisation success in our factor analysis. However, species richness was highest at sheltered sites, a finding in accordance with a previous study that shows that germination and establishment of colonisers are highest at sheltered shorelines (Chapter 3).

Dispersal and connectivity

According to Island theory (MacArthur and Wilson 1967), species richness in a pond would decrease with increasing isolation and decreasing species richness of the supplying population. These factors are frequently observed to play a crucial role in the colonisation of banks and water bodies (Morris *et al*. 2002; Mäkelä *et al*. 2004; Milsom *et al*. 2004; Leng *et al*. 2009; Beltman *et al*. 2010). However, our results do not indicate such a relation. In fact, they show the opposite: higher species richness was found in more isolated ponds and when the regional occurrence of colonisers was lower. Several studies have also reported this kind of counter-intuitive relations between isolation of a pond and its species richness (Cottenie and De Meester 2004; Scheffer *et al*. 2006). There are several explanations for this phenomenon. The first, most straightforward explanation is that dispersal does not play a key role, or is at least overruled by effects of some other factor. According to the combination of variables in the factor analysis, ponds that were better connected had a higher sulphate concentration, probably due to more mixing with allochthonous sulphate-rich water. Besides, ponds with higher regional abundances of colonisers were younger and had higher rat densities. These conditions are likely to have decreased the species richness and colonisation of the open water. Therefore, these factors have likely prevailed over the effects of dispersal. A second explanation for the counter-intuitive relation between isolation and species richness is that isolated ponds frequently lack benthivorous fish (Scheffer *et al*. 2006). The feeding behaviour of such fish increases turbidity and induces a shift to a species-poor situation when nutrient availability increases. Isolated ponds that lack those fish have a higher species richness and turn into a turbid state less easily when the habitat conditions deteriorate (Scheffer *et al*. 2006).

Overall, all of these relations suggest that a lack of source populations and a low probability of dispersal from those populations are not the major reasons behind the low species richness and lack of colonisation in the field. Instead, habitat conditions and biotic interactions appear more important.

Biotic interactions

We found indications that both the interactions with muskrats and with *Stratiotes* affect the species richness of the bank and the colonisation of open water. In one pond we found direct evidence of muskrat herbivory in the form of rhizome parts that were eaten by musk rats. Observations of local rat catchers confirmed that plants expanded rapidly into the open water when a pond had been freed from rats (personal communications). However, in our factor analysis we could not separate the effect of muskrats from the other regional factors.

Evidence of the importance of *Stratiotes* was more indirect. Long colonisation distances were achieved in deep water bodies only if *Stratiotes* was present. In these ponds, colonisers expanded into the open water mainly by floating at the water surface. This is sensitive to disturbances and is therefore restricted to sheltered places (Westhoff *et al* 1972; Azza *et al*. 2006, 2007). A pilot study on the keystone function of *Stratiotes* shows that in the presence of dense rosette beds of this species, rhizomes of *Thelypteris palustris* remained closer to the water surface and protruded their leaves higher above the water (Intermezzo 1). However, the rigidity that was necessary to maintain at the water surface was not affected significantly. Overall, these results indicate that *Stratiotes* could play a role in the colonisation of deeper water bodies.

In general

This study confirms that, in general, colonisation of the open water in fen areas is very limited. The characteristic high biodiversity reported for the early succession phases in fens (Verhoeven and Bobbink 2001) has seldom been found in recent years. Our data set shows that, overall, quite a number of the multiple effects of land use changes have strong negative effects on the species richness of fen banks and the colonisation of the open water.

The major negative changes in habitat quality (increased turbidity, nutrient and sulphate concentrations) and changes in species interactions (with muskrats and *Stratiotes*) are the clearest factors. Dispersal limitations seem to be overruled by habitat limitations as colonisation often fails even when sufficient propagules sources are present. Not surprisingly, time is also an important factor. The succession rates reported in other studies range from 26 to > 50 years for the total transition from open water to floating herbaceous fen or floating carr. However, in our study, even in the 17 ponds that were older than 45 years, colonisation of open water was mostly very limited (less than 1 m within 7 of these ponds). It is a hopeful sign, however, that nutrient availability is now rather low compared to the previous decades and that typical mesotrophic colonisers (*Calla*, *Menyanthes* and *Comarum*) are still present and could make a start to colonise the open water. Given that the ponds in which this occurs are relatively young, careful management of those ponds may result in the formation of the so valued species-rich floating mats.

Implications for restoration and conservation

Our results indicate that restoration efforts aiming to improve the biodiversity of fen pond banks and the colonisation of open water should focus especially on measures that decrease water turbidity and phosphate and sulphate concentrations, and the nutrient concentrations (N and P) of pond banks. Also, the availability of sheltered shorelines should be increased. Moreover, our results show that it is important to restore dense floating *Stratiotes* beds and decrease muskrat density. Because several regional variables appeared of major influence, restoration should be considered from a landscape perspective (see e.g. Verhoeven *et al* 2008 for an integrated landscape-based approach).

A pilot study on 24 banks in three fen areas showed that soil layers deeper than 35 cm contain significant lower amounts of P (Sarneel, unpublished). Therefore, sod cutting could be an effective measure to reduce the nutrient concentrations in the bank. The risk of removing a valuable seed bank together with this top soil is low, because many of the colonisers have transient seed banks (Chapter 2). However, populations of coloniser species on the banks that could function as a source of propagules are, for this same reason, of great importance and should therefore be left intact. To improve water quality, measures that decrease the nutrient concentrations in agricultural runoff, decrease the supply of river water and, more locally, increase hydrological isolation have already been shown to be effective. These measures will also decrease the total nutrient load and sulphate concentrations in the system, thereby also reducing the risk of mobilisation of PO_4^{3-} from Fe-P complexes in the soil and the formation of toxic sulphide concentrations (> 50 μM; Van der Welle *et al* 2006). It should be noted however, that hydrological isolation of individual ponds decreases the probability of dispersal, a factor that should not be ignored totally (Chapter 2). Whenever it is not possible to reduce the external loads, or hydrological isolation of ponds within an area is undesirable, Fe, Al or Ca compounds could be added to bind P, but these additions have serious drawbacks (Geurts 2010). A detailed analysis of all the measures that potentially increase the water quality, and the relation between water quality and aquatic diversity is given by Geurts (2010) and Lamers *et al*. (2010).

The reduction of nutrient loads is also fundamentally important in order to decrease the turbidity of the water. To break the positive feedbacks that maintain a turbid state, sludge removal (Verberk *et al* 2009) and fish stock reduction (Scheffer 1998) have been shown to be successful. Our results show that, whenever the water is clear, colonisation is enhanced in shallow ponds and in deep ponds only when *Stratiotes* is present. Shallow ponds will enhance the colonisation by species that root in the pond bottom, whereas deep ponds with *Stratiotes* are more often colonised by rhizomes that float at the water surface (Azza *et al*. 2006, 2007). It is therefore very important to restore the habitat conditions of *Stratiotes*. This sensitive species is, however, soon out-competed when nutrient concentrations increase, and dies from low iron and high sulphide and ammonium concentrations in the root zone (Smolders and Roelofs 1996; Smolders *et al*. 2003).

Although we can not completely separate the effect of muskrats from other differences between areas, our results, in combination with observations of rat catchers, indicate that it is likely that herbivory by muskrats forms a constraint for colonisers to expand into the water (Doude van Troostwijk 1976). This necessitates a firm approach (in the form of year round hunting) to decrease the population density of this species or, preferably, eradicate it in fen areas (Bos *et al*. 2009).

Overall, successful restoration of species-rich plant communities that colonise the water and induce the formation of floating peat mats requires a multifaceted approach to address the various negative effects of land use changes.

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CHAPTER 6

Summary and perspectives for restoration

J.M. Sarneel

Objectives

Fen systems are valuable wetland types with a relatively high biodiversity, not only because they have a relatively high species richness per area, but also because they often consist of a patchwork of natural species-rich succession stages (Bedford and Godwin 2001; Verhoeven and Bobbink 2001). The natural succession in fens, from open water to wet (alder) forest, is called terrestrialisation. Under oligo- to mesotrophic conditions a succession phase with especially high species richness can be formed: the phase of floating peat mats. The formation of these mats starts with the colonisation of open water by semi-aquatic and semi-terrestrial species that grow from the bank into the water with rhizomes that either root in the pond bottom or float at the water surface (hereafter called ‘colonisers’). However, as many fen areas have become isolated patches in intensively used landscapes, the habitat quality for many fen species has deteriorated and dispersal between the remaining suitable habitat fragments has become difficult. Consequently, many fen species (including the colonisers) have failed to persist in the remaining fen fragments, or have failed to reach them. This has led to the loss of characteristic fen species and the impediment of the formation of floating mats. In the Netherlands, where floating peat mats used to be a rather common phenomenon, this decline has raised great concern for the restoration and conservation of the species diversity and natural ecosystem functioning of fens. However, despite numerous restoration measures, such as the creation of new ponds where succession could start again, the formation of new floating mats has hardly ever been observed in the last 20-30 years.

To understand why, and establish which factors impede restoration of fen ponds, this thesis studied the mechanisms and conditions that drive the colonisation of open water by species growing from the bank.

Summary of the most important mechanisms and conditions for colonisation

Figure 6.1 shows the processes that were hypothesised to play a role in the colonisation of the open water by species growing from the bank. Following this scheme, I will point out the most important findings of this thesis. To start colonisation from the bank, an empty patch on the bank first needs to be colonised by propagules of coloniser species (Figure 6.1 arrow 1-3). In most fen areas remnant populations of the typical colonisers are still present (Chapter 5), and active dispersal of propagules from those remnant populations via water (hydrochory) could therefore be a key mechanism in the colonisation of open water.

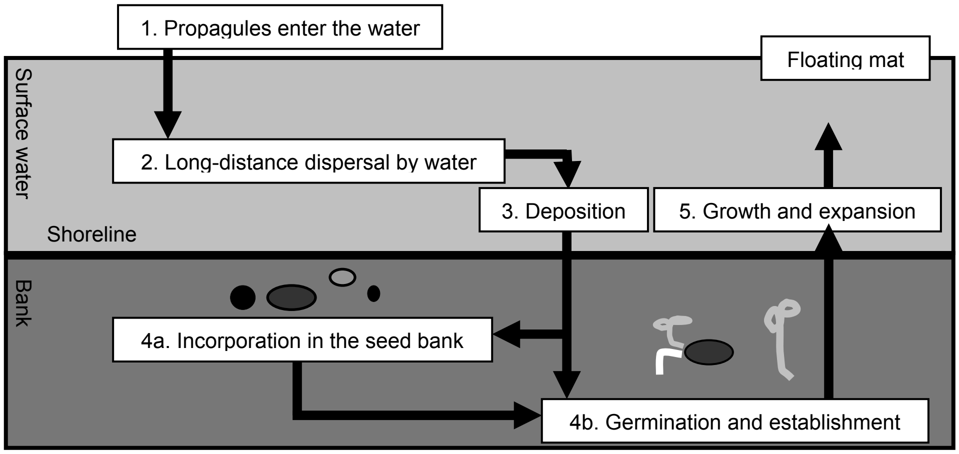


Figure 6.1: Schematic top view of a pond bank with the most important processes that are hypothesised to lead to the colonisation of open water and eventually to the formation of floating peat mats.

In **Chapter 2** several field studies were combined to investigate the mechanisms behind water dispersal (hydrochory) in fen ponds at different spatial and temporal scales. The results clearly show that wind speed and direction are major determinants of the hydrochorous dispersal process and determine the movement and deposition patterns of floating seeds in fen ponds. Wind-driven hydrochorous dispersal in fen ponds and other stagnant and slow-flowing (lentic) water bodies can be a relatively fast process, with dispersal speeds up to 0.10 m s^{-1}. Under favourable wind directions, the longest dispersal distances were reached at low wind speeds, when seeds moved slowly and erratically but did not become trapped in the shoreline. At higher wind speeds, seeds moved faster but dispersal distances were shorter. On longer time scales (months), during which wind direction and speed changed considerably, dispersal of floating seeds appeared consistently directional and followed the prevailing wind direction. Over the dispersal season, more seeds of a larger number of species were transported to pond banks that were predominantly downwind. This directionality is likely to have consequences for the colonisation of banks with different orientations to the prevailing wind direction, and will determine the effectiveness of connections (e.g. ditches) between water bodies. Over the years, this directional transport did, however, not result in an accumulation of seeds in the seed banks of predominantly downwind pond banks. At such long time scales, other processes, such as differential germination, seed mortality or predation, likely reduce the effects of dispersal. Our seed bank analysis also shows that the colonisers were frequently absent in the seed bank of fen ponds and constituted on average only 5% of all the seeds found. This suggests that for the establishment of coloniser species, the role of the seed bank may be small. The return of species in a restored pond will therefore depend on the transport via water. This in turn, is determined by the spatial configuration of the water bodies and their connections, given the frequent and (potentially) long-distance dispersal of diaspores by water and the directionality of this transport mechanism.

Intermezzo 1 shows that apart from seeds, numerous vegetative fragments were transported by the water and deposited in the shorelines of fen ponds. Especially during winter, large numbers of vegetative propagules were found. These were mainly fragments from aquatic species but also included vegetative fragments from semi-terrestrial species and colonisers like *Glyceria* and *Calla*. The vegetative fragments of the tested colonisers generally had high buoyancies and remained viable after long periods of floating in the water. Observations in the field also confirm budding of floating vegetative propagules during early spring and summer. Therefore, the dispersal of floating vegetative propagules may be just as important as the dispersal of seeds.

Once the seeds or vegetative propagules have arrived at a bank, the next important step is the germination and establishment of the diaspores that have been deposited (Figure 6.1; arrow 4). Chapter 2 showed that seeds were not deposited randomly, but were directionally transported by wind-driven currents. Therefore, the germination and establishment and the factors controlling them at upwind and downwind banks were investigated in **Chapter 3**. In a greenhouse and a field experiment, seeds of *Berula erecta* (Huds.) Coville*, Calla palustris* L., *Comarum palustre* L., *Glyceria maxima* (Hartm.) Holmb. and *Mentha aquatica* L. were sown. The results of these experiments show that the wind-induced water currents that drive the dispersal of seeds also cause differences in recruitment conditions between more exposed (downwind) and more sheltered (upwind) banks. At banks with contrasting orientations (upwind, sheltered banks vs. downwind, exposed banks), differences were found in wave impact, light availability, temperature, litter and seed deposition. The greenhouse experiment suggested that the larger amount of seeds and litter deposited on downwind, exposed banks affect the germination and growth of colonisers, but these effects were less clear in the field. There, after initial rapid germination in the first four weeks, the number of seedlings decreased drastically over the 16 weeks that the experiment lasted. This decrease was observed both on upwind, sheltered banks and on downwind, exposed banks, but it was much stronger on the exposed banks. At these exposed banks, the decrease in number of seedlings correlated almost 1:1 with the probability of being washed from the bank by wave action. This was quantitatively investigated with small, brightly coloured plastic granules that were placed next to the sown seeds. At the end of the field experiment, establishment was highest on sheltered banks, where more plastic granules had remained and waves were smaller. The high probability to become washed away overruled effects of other environmental variables on germination and establishment. This shows that new colonisation events of pioneer species in riparian zones in fen ponds are strongly determined by local establishment limitations that most likely depend on disturbance by waves. Moreover, this implies that the common practice to level off natural water table fluctuations could impede the establishment of colonisers. With natural water level fluctuations, seeds would be deposited high on the bank in winter. In spring, when the water tables are lower, seeds and seedlings are unlikely to become washed away. When water tables are not lower in spring, or are even higher during spring and summer, as is common practise in the water level management of the investigated fen polders, seeds that are deposited during winter are much more likely to become washed away.

When a plant has become established successfully on the bank, the next step in the colonisation of the water body is clonal expansion into the open water (Figure 6.1; arrow 5). This will only be possible under specific circumstances. Given the frequent failure of restoration efforts, we were especially interested to identify those circumstances that currently impede the colonisation of the open water. One major factor that is often held responsible for the decline in freshwater ecosystems is the nutrient input to the surface water and bank. These inputs have increased drastically due to several land use changes in the areas surrounding water bodies. Therefore, we specifically investigated whether nutrient inputs indeed play an important role in the colonisation process.

In **Chapter 4** we studied the complex interactions between nutrient enrichment of bank soil or surface water and vegetation development and colonisation of the open water, using a large controlled mesocosm experiment. Because litter accumulation in fens is thought to control succession towards floating mats, the effects of eutrophication on this process were also studied. 24 Mesocosms were divided in a surface water and a bank compartment on which two eutrophic and four mesotrophic coloniser species were planted. Nutrient levels (N and P) were manipulated experimentally in both the surface water and the bank compartment. Both nutrient levels (N and P) in the surface water and in the bank soil affected the growth of the colonisers and their expansion into the water. Interestingly, the growth of plants was not only enhanced in the compartment (either bank or surface water) where the nutrients were added, but productivity also increased in the other, unfertilised compartment. This effect was very strong for enrichment of the banks, which stimulated the growth into the water (be it with a large contribution of eutrophic species) and smaller for enrichment of the water, which had a minor but significant effect on plant growth on the bank. In reaction to nutrient addition, only the species that are associated to eutrophic conditions (*Glyceria maxima* and *Juncus effusus*) increased their shoot density. This might explain their increased dominance on enriched banks.

Nutrient addition also resulted in a higher litter production and in higher nutrient concentrations (N and P) in the litter. A reciprocal decomposition experiment showed that decomposition was enhanced in mesocosms with nutrient addition but was not affected by litter quality. The net litter accumulation was calculated to be higher after fertilisation than in non-fertilised conditions. As litter accumulation is generally assumed to control the succession rate of early succession stages, these processes imply that nutrient addition could accelerate succession. Although this could be seen as positive, because it might induce faster colonisation of open water, it is not likely that eutrophic conditions will result in the formation of species-rich floating mats aimed for by nature managers and policy makers. First because eutrophication often results in vegetation types with a lower biodiversity and second, because the eutrophic species that increase in dominance are less capable of the formation of such mats.

Still, eutrophication is only one of the major changes that have occurred over the past decades. Other important changes in fen areas are increased drainage, supply of nutrient and sulphate rich water during dry periods (to maintain a stable water table in the fen area), invasion by herbivore muskrats and the decline of the keystone species *Stratiotes aloides* (Lamers *et al*. 2001, 2002). The latter species is thought to provide structural support and shelter to waves for rhizomes of other colonising species that invade the water.

The keystone function of *Stratiotes* was further assessed in **Intermezzo 2**, where potential beneficial effects on *Thelypteris palustris* Schott rhizomes were investigated. I compared rhizomes that had expanded into open water in the presence and absence of dense *Stratiotes* vegetation. In the presence of dense *Stratiotes* vegetation, *Thelypteris* invested less in rigidity of its rhizomes to maintain them at the water surface, but this was not significant. Rhizome length and tissue density did not differ. However, in the presence of *Stratiotes*, the rhizomes of *Thelypteris* grew closer to the water surface and the leaves protruded significantly higher above the water. This shows that *Stratiotes* at least to some extent alters the way *Thelypteris* invades the water.

Starting from the knowledge of the mechanisms gained in the previous chapters and from literature, the importance of the three major effects of land use changes: decreased habitat quality of soil and water, dispersal limitations and changes in biotic interactions (i.e. the increase of muskrat herbivory and the decline of *Stratiotes*) was evaluated in **Chapter 5**. The effects of these changes were considered regarding the major aims of fen restoration: biodiversity and the colonisation of open water. The results show that species richness and colonisation are two different attributes of fen systems that do not correlate and, moreover, are related to different environmental variables. A factor analysis on a large field-dataset (collected in 62 fen ponds of different ages with and without natural colonisation of the open water) shows that more colonisation of the open water was found in pond complexes with a lower muskrat density, with less colonisers in the surrounding area, with ponds of a higher age which had a higher Cl^- concentration. These variables all varied considerably more on a regional than on a local scale, indicating that regional differences between the areas are important. Colonisation was further related to local pond morphology, mainly water depth, and to turbidity. Whenever the water had a low turbidity, colonisation was enhanced in shallow ponds and in deep ponds if *Stratiotes* was present. Shallow ponds were most likely colonised by species that rooted in the bottom, while the presence of *Stratiotes* could enhance the colonisation by species that float at the water surface. A high turbidity impeded colonisation of open water.

Further, the factor analysis in Chapter 5 showed that the species richness of banks, the other important aim of fen restoration, was, like the colonisation of the open water, negatively correlated to muskrat density and other regional differences. Besides these regional influences, species richness was negatively correlated to nutrient availability (N & P) in the soil and positively to hydrological isolation. The latter is explained by the decreased mixing with nutrient- and sulphate-rich water in more isolated water bodies. Further, corresponding to the results in Chapter 3, species richness was higher at sheltered (SW) banks. Overall, the results of this field survey suggest that the major changes in habitat quality (nutrient and sulphate concentrations and turbidity) and changes in species interactions (with muskrats and *Stratiotes*) do all play a role in the lack of restoration success in Dutch fens. The effects of these habitat characteristics seem to overrule the effects of dispersal limitations, as colonisation often fails even when sufficient propagules sources are present. If no propagule sources are present (e.g. in case species are absent in the entire area or for species that hardly produce seeds, such as *Stratiotes*) dispersal may form a more important bottleneck. This means that for restoration, it is very important to consider the whole range of possibly important factors (Nutrient availability, sulphate concentrations, turbidity, water depth, propagule sources, muskrat density, the presence of *Stratiotes*) instead of focussing on only one or a few.

Restoration perspectives

The research in this thesis confirms the general slow pace of colonisation of open water in Dutch fen ponds in the past 20-30 years. The mean distance over which the water was colonised from the bank was scarcely 2.5 m, although *Typha* species and *Phragmites australis* (Cov.) Steud., in general colonised over longer distances (on average 5.0 and 4.1 m, respectively; $n \geq 10$). These species frequently colonised the water by rooting in the pond bottom. Colonisation of open water by rhizomes that floated on or just below the water surface was seldom observed. None of the other typical coloniser species colonised the water over more than about 2 m. In fact, in 6 of 16 ponds that were older than 45 year, plants had colonised the open water over distances smaller than 1 m. Besides, the total cover by semi-terrestrial plants was estimated to be, on average, only 7% (range 0 - 60%) of the pond surface. Also the characteristic high biodiversity reported in Verhoeven and Bobbink (2001) for the initial succession phases, was seldom found in our field survey. On average, 12 species were found per relevée (2 m^2) and in only 6 of the 62 ponds more than 20 species were recorded per relevée.

Given the complexity and the large number of variables that were related to the colonisation of the open water and the species richness of the bank, it is difficult to provide restoration measures that are applicable to all Dutch fen areas. This means that each situation needs to be critically evaluated and restoration aims should be defined carefully. The research in this thesis provides important background knowledge for such a critical evaluation. The major findings are summarised in Figure 6.2 and this scheme can be used as tool to evaluate the requirements for restoration of species-rich ponds where colonisers expand successfully into the surface water. Because the restoration of habitat quality does not automatically result in the return of species into a restored pond, nor do invading species colonise successfully in a non-suitable habitat, the scheme has two different tracks, one considering the presence of species and the other considering the habitat characteristics that determine whether those species will be able to persist and expand into the surface water or not.

The track that will lead to the successful return of species into a pond starts with the localisation of potential propagule sources. In theory, propagules can be provided by the seed bank and by other populations in the surrounding area. However, the coloniser species are frequently absent in the seed banks of Dutch fen pond shorelines (Beltman *et al*. 2010; Chapter 2) and restoration should therefore not rely on the seed bank for the return of those species. Fortunately, source populations of most colonisers are still present within most Dutch fen areas (Chapter 5). As almost all coloniser species have propagules (seeds, spores and/or vegetative fragments) with high buoyancies, dispersal via water (hydrochory) will be very important. Given that the hydrochorous transport of seeds from those source populations predominantly follows the prevailing wind direction (Chapter 2), upwind populations have a higher potential to function as source populations than those located downwind of restoration sites. However, to facilitate the transport of propagules, connections between water bodies are needed. The second step in the track towards the successful return of species is therefore to consider the probability of dispersal through such connections. This will depend on their orientation and location within a pond in relation to the prevailing wind direction. Connections located in downwind pond banks might function as a sink, removing seeds from a pond, whereas upwind connections might form a source, supplying a pond with seeds coming from other locations. This transport from one pond to another can occur rather fast and a high proportion of floating seeds can be transported via such connections.

However, one should bear in mind that the number of connections will also determine the mixing with nutrient and sulphate-rich water that is supplied during drier periods to maintain a stable water level (Lamers *et al*. 2002). Under anoxic conditions, sulphate is reduced to sulphide which is toxic to plants and can induce the release of phosphorus from Fe-P complexes. Chapter 5 showed that in ponds with more connections, the effects of increased mixing with nutrient and sulphate enrichment indeed prevailed over the effects of increased dispersal. It is therefore very important to consider the source and the quality of the surface water before connections are made.

If there are no remnant populations within a fen area, or the probability of dispersal from such populations is very low (because they are located in isolated or in downwind locations) or the creation of connections is undesirable because this would have a strong negative effect on water quality, coloniser species may need to be re-introduced by sowing or planting. An investigation of germination and establishment of coloniser species from natural (background) seed deposition shows that recruitment (at least in one fen area) strongly limited by seed availability (Chapter 3).

If propagules are able to enter a pond, the next requirement for successful return of species after restoration is that deposited seeds have to germinate and become established. Chapter 3 shows that new colonisation events are mainly determined by establishment success. Establishment is enhanced on shores with a low wave impact. The wave impact on a shore can be estimated from its orientation to the prevailing wind direction and the length of the open water in this direction (effective fetch). Shelter from wind by trees, shelter from waves by constructions that function as wave breakers and morphological adaptations that decrease the effective fetch could therefore enhance establishment. The intensity of the wave impact on the patches where seeds are deposited is also related to water table fluctuations. Nowadays, water tables in the Netherlands, and in many agricultural landscapes in other countries, are maintained relatively high over summer and low over winter, levelling off or even reversing the natural fluctuations (Lamers *et al*. 2002). The consequence of this is that water tables in summer are quite constant and comparable or even higher than water levels in winter and seeds deposited in winter are very likely to become washed away before, or just after germination. Under a natural regime, the summer water table will not reach the winter level at which the majority of the seeds were deposited and seedlings may be safer from being washed away during germination and early establishment. More natural water level fluctuations could therefore form an effective measure to enhance the establishment in fen shorelines.

If the targeted species are already present within the pond or the dispersal of propagules is not hampered, the habitat quality will determine the eventual vegetation composition and the expansion into open water. The land use changes that could currently impede the dispersal of species also have resulted in drastic changes of the habitat quality of fens. This strongly affects both species richness and the colonisation of open water in fen ponds. Because the colonisers grow in the interface between bank and water, both the characteristics of the water and the bank soil are important (Chapter 4). In the field, soil and water quality varied relatively independently and therefore they should be considered separately (Chapter 5). Not all habitat characteristics are equally important and the most important parameters that should be considered for restoration are the phosphate and sulphate concentration and turbidity of the water, the nutrient richness (N and P) of the bank and the pond depth. In line with other studies (Lamers *et al*. 2002; Wassen 2005; Van Belle *et al*. 2006), both the mesocosm study (Geurts *et al*. 2009; Chapter 4) and the field survey (Chapter 5) indicate that phosphate is the major limiting nutrient in fen ponds. To decrease the phosphate concentrations in the surface water, several measures can be taken (Geurts 2010; Lamers *et al*. 2010). First, the supply of river water and agricultural runoff should be reduced. This will also decrease the sulphate concentrations in the surface water, which is important too, as high sulphate concentrations correlated to a low biodiversity of the banks (Chapter 5;

Geurts *et al*. 2009). This is probably related to the toxicity of sulphide that is formed under anoxic conditions and its affinity for iron, by which phosphate is released from Fe-P complexes. Whenever it is not possible to reduce the external input sufficiently, $Fe^{2+/3+}$, Al^{3+} or Ca^{2+} can be added to the fen water, but these additions have serious drawbacks (Geurts 2010).

To enhance colonisation, the turbidity of the water should be low. Turbidity is usually correlated to nutrient availability too. High nutrient concentrations lead to algal blooms and increase the amount of small suspended organic particles by enhancing decomposition. To break the positive feedbacks maintaining a turbid state and return to a clear water state, the nutrient concentrations (mainly P) need to be lowered drastically. Further, sludge removal (Verberk *et al*. 2007) and fish stock reduction (Scheffer 1998) have been shown to be effective additional measures to improve water clarity. When those measures fail, or are not possible, covering the sludge with a layer of sand can form a last option to lower the turbidity. A detailed analysis of all the measures that potentially increase the water quality and the relation between water quality and aquatic diversity is given by Geurts (2010) and Lamers *et al*. (2010).

To reduce the nutrient concentrations in the bank, sod cutting could be an effective measure. Unpublished data shows that deeper layers (< 35 cm) of the bank have considerably lower P and somewhat lower N concentrations than the top 10 cm. Besides, the risk of removing a valuable seed bank together with the removed top soil is low, as the seed bank of shorelines did not contain a high number of coloniser species or red list species. However, populations of coloniser species on the banks that could function as a source of propagules should, for the same reason as mentioned before, be left intact.

Pond morphology, especially water depth, also affected the colonisation of open water. Whenever the water was clear, the colonisation was enhanced in shallow ponds and in deep ponds if *Stratiotes* was present. Other studies in shallow lakes have shown that the expansion of helophytes that root in the bottom is frequently found to be limited to shallow water (<1 m), either by light limitation, as a consequence of the anoxic environments, by disturbance by waves or a by combination of those factors (Coops and Van der Velde 1996; Andersson 2001; Mäkelä *et al*. 2004; Azza *et al*. 2006, 2007; Jackson 2006). Deeper water was found to be more associated to the colonisation by rhizomes that float at the water surface (Azza *et al*. 2006, 2007). In such ponds, the interaction with *Stratiotes* will be important.

Therefore, in addition to a critical evaluation of the dispersal of colonisers and the habitat quality, it is important to consider the interactions with other organisms (*Stratiotes* and muskrats). As said before, in deep ponds (<90 cm) the interaction with *Stratiotes* could facilitate the expansion of rhizomes into open water (Chapter 5; Box 2). Further, as their emergent rosettes attenuate waves, the presence of *Stratiotes* may enhance the establishment on the bank (Chapter 3). *Stratiotes* is a sensitive species that is soon out-competed when nutrient concentrations increase, and dies from low iron and high sulphide and ammonium concentrations in the root zone (Smolders and Roelofs 1996; Smolders *et al*. 2003). In Dutch fens, *Stratiotes* occurred preferably in ponds with a high K^+ concentration in the bank soil, a low bank height and a steep bank below the water surface. This pond morphology will enable the *Stratiotes* rosettes to reach the bank and provide its structural support to colonisers that expand from that bank into the open water. So, if the return of *Stratiotes* is very likely in a newly created pond, a morphology with low banks that are steep beneath the water surface could facilitate the colonisation. (Re)-Introduction of *Stratiotes* may also be considered, but given its sensitivity to iron, sulphide and ammonium, additional measures may be necessary. So far, the re-introduction of *Stratiotes* has shown limited success in several pilot studies (Lamers *et al*. 2006, 2010; personal observations).

Muskrats and other herbivores could form another potential threat to the colonisation of open water. Areas where the muskrat density was lower in general had higher colonisation distances and a higher species richness of the banks. Although we can not completely separate the effect of muskrats from the effect of other factors, our results are in line with observations of rat catchers, that herbivory by muskrats might form a real constraint for colonisers to expand into the water. In its native range, mortality from cold during winter and the effects of extreme water level fluctuations (Clark 1994) regulate this species, but because such situations hardly occur in the Netherlands, year round hunting is necessarily to keep populations small (Doude van Troostwijk 1976; Bos *et al*. 2009).

In fen ponds where all conditions described above are met, with the result that propagules of the colonisers have been able to invade the pond, germinate, become established and expand clonally into the open water (Figure 6.1), it is very likely that in due course of time floating pet mats will form. Studies on aerial photographs and permanent quadrats show that terrestrialisation generally occurs within several decades (Table 6.1). However, most of these studies studied succession in systems where anthropogenic activities increased during the study. Therefore, studies that combine (permanent-plot) information from disturbed and undisturbed situations could provide insight into the interaction of these changes with time. Careful monitoring and evaluation of the developments in restored ponds over time could also provide valuable information. Unfortunately, this is frequently neglected or implemented erratically. The scheme in Figure 6.2 provides a useful tool to determine which parameters should be monitored. Overall, a firm approach to the multiple factors that impede colonisation of open water in fens is necessary to successfully restore a situation in which species rich plant communities colonise the water and induce the formation of floating peat mats.

Table 6.1: Summary of all the reported terrestrialisation rates in fen ponds.

Time span	Wetland	Observed changes over this period
1937-1957	[a] Fen: Westbroekse Zodden	- 74% of the open water was converted to a next phase on aerial photos. - 9% of the water turned into forest. - Estimated turnover time from aquatic to forest 30.7 year.
1944-1993	[b] Fen: Het Hol	- 37% of the open water was converted to a next phase on aerial photographs - Woodland cover increased with 191%.
1984-2000	[c] Fen lake: Neuchâtel	- Aquatic species decreased and woody species increased in permanent plots. - “in some of the permanent quadrats the vegetation was even no longer classified as aquatic in 1998-2000”.
1931-1981	[d] Fen lake: Naardermeer	- Permanent plot data suggest that the transition from open water to forest takes about 50 years
1864-1994	[d] Fen lake: Naardermeer	- 51% of the open water disappeared (366 ha to 187 ha) - “Woodland increased” (103 ha to 247 ha).
1956-1989	[e] Fen meadow: Bollemaat	- Under a summer-mowing regime rich fens developed into acid vegetation types. With a winter mowing regime, *Phragmites*-dominated vegetation types developed. - Only 2000 m^2 of the 4.75 ha with summer mowing remained unchanged. -“We found embryonic bog vegetation in 1989 in sites where there was open water in 1958”.

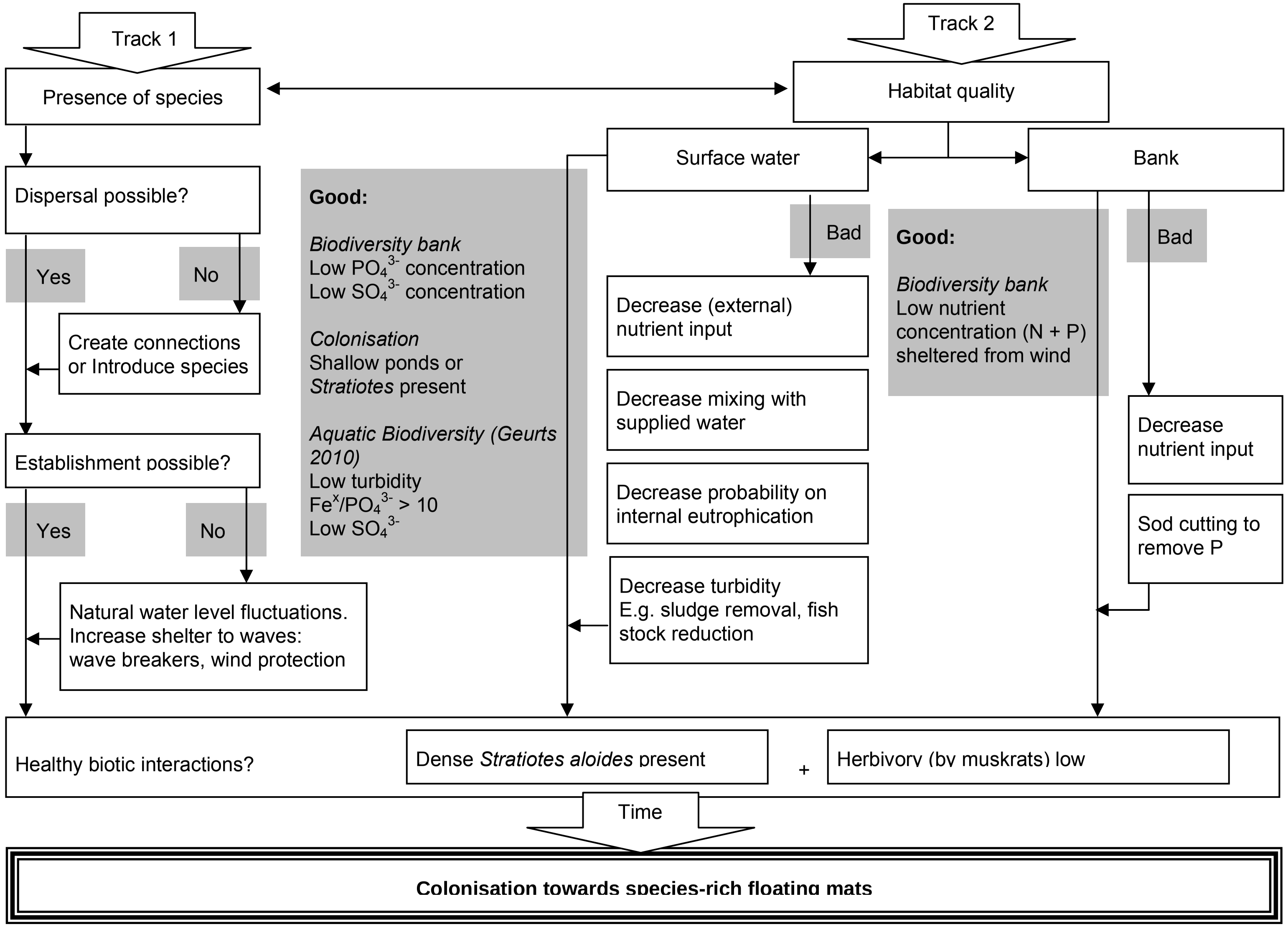
[a] Bakker *et al*. (1994)
[b] Van Belle *et al*. (2006)
[c] Güsewell and Le Nédric (2004)
[d] Barendregt *et al*. (1995)
[e] Van Diggelen *et al*. (1996)

Natural water level fluctuations.
Increase shelter to waves;

Bank

Decrease nutrient input

> Next page: Figure 6.2: Steps that need to be considered for a critical evaluation of the bottlenecks for the restoration of succession towards floating mats.


Track 1
Presence of species
Track 2
Habitat quality
Dispersal possible?
Yes
No
Create connections or Introduce species
Establishment possible?
Yes
No
Natural water level fluctuations. Increase shelter to waves: wave breakers, wind protection
Good:
Biodiversity bank
Low PO_4^{3-} concentration
Low SO_4^{3-} concentration
Colonisation
Shallow ponds or Stratiotes present
Aquatic Biodiversity (Geurts 2010)
Low turbidity
$Fe^x/PO_4^{3-} > 10$
Low SO_4^{3-}
Surface water
Bad
Decrease (external) nutrient input
Decrease mixing with supplied water
Decrease probability on internal eutrophication
Decrease turbidity E.g. sludge removal, fish stock reduction
Bank
Good:
Biodiversity bank
Low nutrient concentration (N + P) sheltered from wind
Bad
Decrease nutrient input
Sod cutting to remove P
Healthy biotic interactions?
Dense Stratiotes aloides present
+
Herbivory (by muskrats) low
Time
Colonisation towards species-rich floating mats

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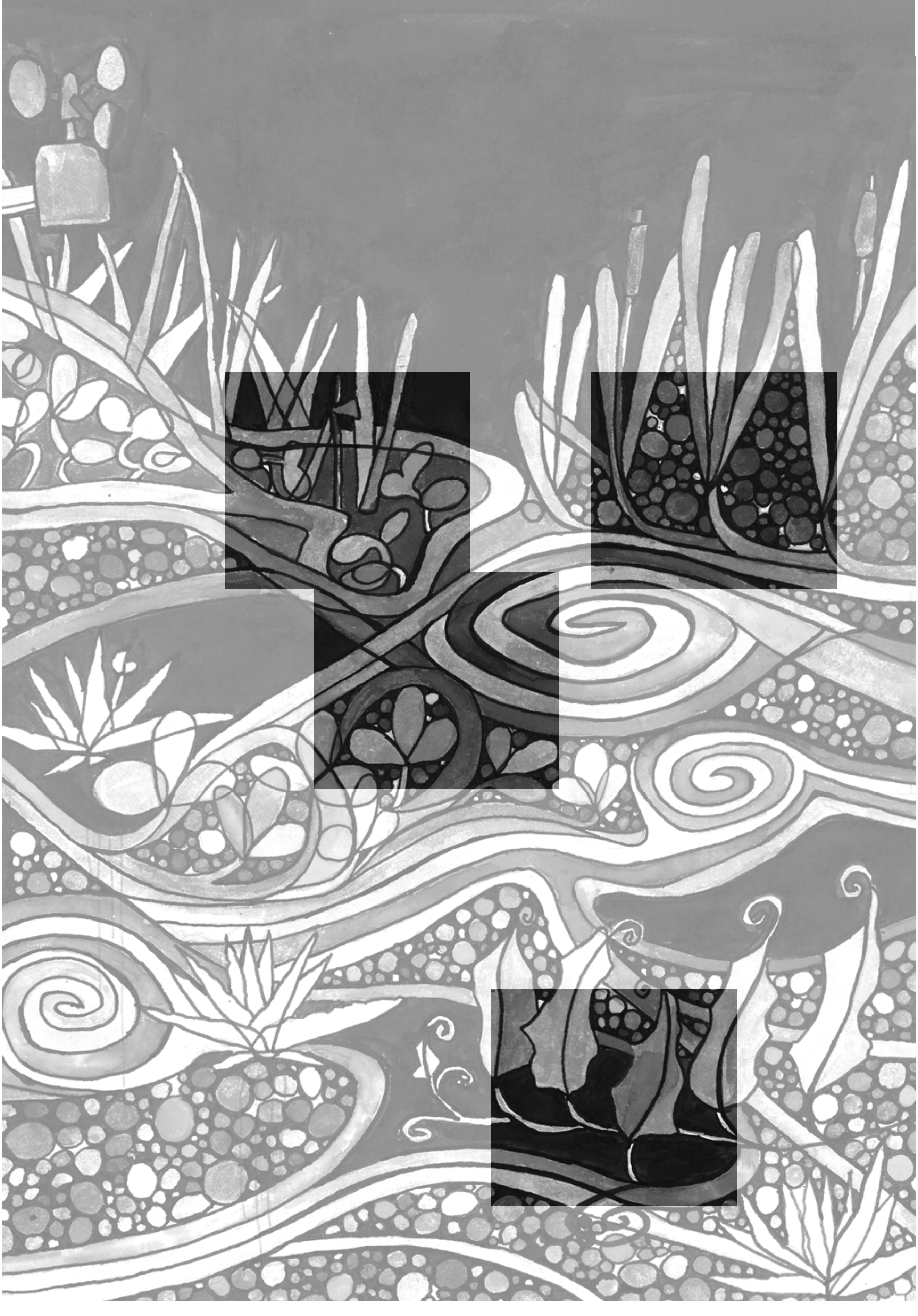


Samenvatting

Biodiversiteit in Nederlandse laagvenen

Verlanding is een typerend proces voor laagveenwateren en start vaak doordat planten vanaf de oever het water ingroeien. Doordat er allerlei plantenmateriaal ophoopt tussen deze koloniserende planten, wordt het water langzaam weer land. Vaak wortelen deze planten in de bodem van het meertje of plas, maar soms koloniseren ze het water met behulp van wortelstokken die op het wateroppervlak blijven drijven. Als dit gebeurd ontstaat er een stuk drijvend (veen) land, een kragge. Zo'n kragge biedt een geschikte vestigingsplaats voor veel bijzondere planten soorten en daardoor heeft dit verlandingsstadium een hele hoge biodiversiteit. Uiteindelijk zullen boomsoorten zoals zwarte els zich in de kragge gaan vestigen en zal de kragge plaats maken voor het laatste stadium van de verlanding: (broek)bos.

Ondanks dat Nederlandse laagvenen al sinds de middeleeuwen sterk door de mens beïnvloed zijn, waren ze even divers als natuurlijke veengebieden. De vervening (het winnen van turf) heeft hier een belangrijke rol in gespeeld. Om aan de groeiende vraag om brandstof te voldoen werd het veen zelfs onder het grondwater niveau gestoken. In de plassen (petgaten) die daardoor ontstonden vormden zich in de loop van tijd kragges en bleef het waardevolle karakter van veel veengebieden behouden. Veel natuurbeschrijvingen uit het begin van de vorige eeuw roemen dan ook de verscheidenheid en de diversiteit van Nederlandse laagveenwateren.

Achteruitgang van Nederlandse laagvenen

Echter, toen de vervening rond de tweede wereld oorlog stopte omdat andere energiebronnen belangrijker werden, zorgde verlanding ervoor dat veel plassen en kragges in bos veranderden. Als gevolg hiervan nam de diversiteit van Nederlandse laagvenen sterk af. Daarnaast zorgde de intensivering van de industrie en de landbouw voor nog meer achteruitgang. Door bemesting en ontwatering van het land om de resterende veengebieden heen werden in het veen, het water en de oever voedselrijker en de veenbodem droger en zuurder. De intensivering van het landgebruik heeft er ook voor gezorgd dat de huidige laagveengebieden kleine, geïsoleerde stukjes zijn geworden. Hierdoor wordt de verspreiding van (planten) soorten tussen deze geïsoleerde stukken erg moeilijk. Door al deze veranderingen hebben veel laagveengebieden karakteristieke plantensoorten zoals waterdrieblad, krabbenscheer en waterscheerling verloren. Tot overmaat van ramp doemde er nog een extra bedreiging op in de vorm van de muskusrat, die een voorliefde bleek te hebben voor laagveenoevers en wortelstokken van diverse koloniserende planten.

Pogingen tot herstel

Zo rond 1980 werd deze achteruitgang onderkend en werden er maatregelen getroffen die de waterkwaliteit sterk verbeterde en de toevoer van grote hoeveelheden voedingstoffen via de landbouw aan banden legde. Daarnaast werden op een heel aantal plekken nieuwe petgaten gegraven, zodat verlanding en daarmee kraggenvorming opnieuw zou kunnen plaatsvinden.

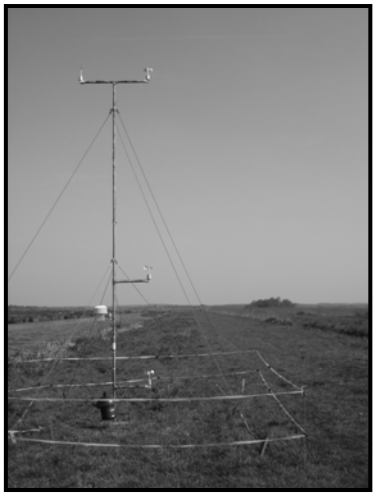
Nu zo’n 25 jaar later, blijkt het resultaat van veel van deze activiteiten teleurstellend. Nieuwe kragges worden niet gevormd, en ook blijven veel karakteristieke laagveensoorten (te) zeldzaam. Omdat eigenlijk nog niet precies bekend is welke processen de verlanding sturen, is het ook heel moeilijk te achterhalen wat het teleurstellende resultaat van al deze beheersmaatregelen veroorzaakt heeft.

Doel

Daarom heb ik mij in dit onderzoek gericht op het in kaart brengen van de processen die aan de kolonisatie van het water ten grondslag liggen. De centrale vraag hierbij was:

- *Welke (a)biotische processen sturen het verloop van de kolonisatie van het water door verlandingssoorten?*

Verspreiding en kieming in laagveenwateren



Wil verlanding ook op nieuwe plekken kunnen optreden, dan moeten de planten die dit proces in gang kunnen zetten, wel op de herstelde oever aanwezig zijn of er kunnen komen. Veel zaden van oeverplanten blijven vrij lang op het water drijven. Daarom zijn waterstromingen erg belangrijk voor de verspreiding van oeverplanten. Het water in laagvenen stroomt echter nauwelijks. Alleen door windwerking op het wateroppervlak ontstaat er een stroming in de richting van de wind.

De verspreiding van planten zaden via het water wordt dan ook vooral gestuurd door de windrichting en -snelheid. Opvallend genoeg werden de grootste verspreidingsafstanden gevonden bij lage windsnelheden (en een gunstige windrichting = parallel aan de oever). Zaden bewogen zich dan weliswaar langzaam door het water en veranderden vaak van richting, maar ze spoelden niet zo snel aan. Bij hogere windsnelheden bewogen zaden zich sneller voort (tot wel 10 cm per seconde) maar ze spoelden wel eerder aan. Ook over langere periodes (maanden) was het effect van wind duidelijk zichtbaar. Ondanks dat de windrichting gedurende de bemonsterde maanden veelvuldig veranderde, volgde de verspreiding toch voornamelijk de overheersende windrichting. Meer soorten en grotere aantallen zaden spoelden aan op de benedenwindse oever (in het noordoosten).

Bij bemonstering van de zaden die opgeslagen liggen in de bodem van de oever (de zaadbank) bleek echter dat dit directionele transport van zaden niet resulteerde in een accumulatie van zaden in de zaadbank van voornamelijk benedenwindse oevers. Waarschijnlijk bepalen op dit soort langere tijdschalen (jaren) andere processen (kieming, zaadsterfte of predatie) waar veel of weinig zaden aanwezig zijn. De zaadbank analyse liet ook zien dat veel typische koloniseerders zoals waterdrieblad, slangenwortel, maar ook riet nauwelijks in de zaadbank aanwezig waren. Slechts 5% van het totaal aantal zaden in de

zaadbank was van deze soorten. Dit suggereert dat de vestiging van typische koloniseerders na herstelmaatregelen vooral zal afhangen van directe verspreiding van zaden vanaf restpopulaties, en niet van kieming van zaden uit de zaadbank. Die directe verspreiding zal dus vooral afhangen van de ruimtelijke oriëntatie van het petgat en de aanwezigheid en plaatsing van verbindingen via water.

Niet alleen zaden, ook vegetatieve diasporen kunnen significant bijdragen aan het verspreiden van veel oeversoorten. Deze bleken namelijk veelvuldig voor te komen in het water, ze bleven erg lang (> 6 maanden) drijven, en waren daarna nog kiemkrachtig!

Vervolgens zal een zaadje of vegetatieve diaspore wel moeten kunnen kiemen en vestigen op de plek waar het is aangespoeld. We zagen al dat de verspreiding de zaden niet op willekeurige plekken op de oever afzet, maar dat windaangedreven stromingen de verspreiding sturen.

Deze stromingen hebben echter ook gevolgen voor de kieming en vestiging van aangespoelde zaden. Zo zullen zaden aanspoelen op oevers aan de noordoostelijke kant van een petgat, waar ook veel golfslag is, veel strooisel is afgezet en de instraling door de zon groot. Na de kieming, die vooral afhing van de beschikbaarheid van licht, nam het aantal kiemplanten op de oever sterk af. Golfslag bleek de belangrijkste oorzaak van deze afname. Veel zaden en zelfs ook kiemplanten spoelden van de oevers af. Deze afname was het sterkst op de noordoostelijke oever, met als gevolg dat de uiteindelijke vestiging het hoogst was op de luwere oevers aan de zuidwestelijke kant van het petgat.

Dit heeft een belangrijke implicatie. Namelijk, doordat tegenwoordig fluctuaties in de waterstand sterk geminimaliseerd en gecontroleerd worden, zullen zaden, die zich vooral in de herfst en winter verspreiden, in de lente kiemen in een omgeving met veel golfslag. Het zou best kunnen dat een natuurlijk waterpeil, waarbij de waterstand zomers veel lager is dan in de winter, gunstig is voor de vestiging van veel soorten omdat tijdens de kieming het waterpeil lager is dan de plek waar de zaden aanspoelden.

Voedselrijkheid en de kolonisatie van het water

Maar niet alleen is het belangrijk dat de koloniserende soorten weer terugkomen in de laagveenoevers, de planten moeten ook van de oever af het water in gaan groeien. Uit het mesocosm experiment bleek dat zowel de waterkwaliteit als de kwaliteit van de oever hier invloed op hadden. Wanneer we voedingsstoffen toevoegden aan danwel de oever danwel het water deel van de mesocosm bakken (mini laagveentjes) zagen we een sterke toename van de groei van planten het water in. Er was zelfs te zien dat toevoegen van extra voedingstoffen aan het water (vooral fosfaat) een (klein) effect had op de groei van planten op de oever. Wat verder opviel was dat soorten die karakteristiek zijn voor voedselrijke plekken (pitrus en liesgras), na toevoegen van voedingstoffen aan het mesocosm, dichtere vegetaties gingen maken. Omdat vegetatiedichtheid bepalend is voor de concurrentie om licht, is dit waarschijnlijk de reden waarom deze soorten dominant werden op bemeste oevers. Deze soorten zijn helaas minder goed in staat een drijvende veenmat te vormen, zij groeien vooral via de petgatbodem het water in. Daarom kan zware bemesting een belemmering vormen voor het ontstaan van kragges met een hoge biodiversiteit.

Vaak wordt verondersteld dat krabbenscheer de kolonisatie van planten met drijvende wortelstokken stimuleert. Krabbenscheer is een stekelige, stugge, drijvende waterplant die dichte matten vormt. Het bleek dat de groei van moerasvaren (een typische koloniserende en veenmatten vormende soort) enigszins door krabbenscheer beïnvloed werd. Wanneer deze soort ondersteund werd door krabbenscheermatten groeide hij dichter bij het wateroppervlak, en investeerde hij iets minder in de stijfheid van zijn wortelstok om bij het wateroppervlak te blijven.

De vorming van een drijvende veenmat heeft ook sterk te maken met de ophoping van (dood) organisch materiaal (strooisel). Uit het mesocosm experiment bleek het volgende: Doordat de toevoeging van voedingstoffen de groei van planten stimuleerde, werd er ook meer strooisel gevormd. De toevoeging van voedingstoffen versnelde echter ook de afbraak van dit strooisel door micro-organismen. Netto bleek echter dat deze versnelling niet in staat was al het extra geproduceerde strooisel af te breken. Er bleef daardoor meer strooisel over in de voedselrijke bakken. In het experiment zorgde de toevoeging van voedingstoffen (stikstof en fosfaat) al met al voor een versnelling van de verlanding. Of extra voedingstoffen buiten in de natuur ook zo'n gunstig effect zullen hebben is nog maar de vraag. Tijdens de relatief korte tijd dat het mesocosm experiment duurde (3 jaar) namen de soorten van voedselrijke situaties al sterk toe in dominantie. In de natuur is niet alleen de uitgangssituatie al veel voedselrijker, de soorten van voedselrijke situaties hebben ook veel meer kansen (tijd) om dominant te worden. Daarnaast zijn deze soorten minder goed in staat tot de vorming van een kragge. Daarom wordt de vorming van een kragge minder waarschijnlijk in erg voedselrijke situaties.

Kolonisatie in natuurgebieden

De natuur buiten is altijd veel complexer dan losse experimenten kunnen aantonen. Daarom is het belangrijk om naast begrip van de mechanismen, ook zicht te krijgen op de factoren die in de natuur zelf het meest bepalend zijn. Uit een grote veldstudie waarin 62 petgaten bemonsterd werden bleek dat er veel factoren zijn die in het veld de kolonisatie van het open water beïnvloeden. Hoge sulfaatconcentraties in het water, een grote muskusratten dichtheid en troebel water bleken de belangrijkste belemmeringen voor planten om ver het water in te groeien. Daarnaast groeiden planten verder het water in in ondiepe petgaten. Echter, de ondiepe petgaten werden vooral gekoloniseerd door planten die in de onderwaterbodem wortelden. Diepe petgaten waar krabbenscheer aanwezig was werden ook goed gekoloniseerd, en deze petgaten werden meestal gekoloniseerd door planten met drijvende wortelstokken.

De verspreiding van soorten die goed vanaf de oever af het water kunnen koloniseren bleek in het veld minder belangrijk. Namelijk, het aantal koloniserende soorten in een petgat was hoog, ondanks dat een petgat erg geïsoleerd lag of de aanwezigheid van die soorten in de omgeving van dat petgat laag. Voor het herstel van petgaten met verlanding en kraggevorming is het dus belangrijk een set van randvoorwaarden te scheppen. Het schema in figuur 6.2 is hiervoor een bruikbaar hulpmiddel.

Tot slot nog een hoopvolle waarneming: ondanks dat de koloniserende soorten vaak ontbraken in de bemonsterde petgaten waren er een heel aantal petgaten waarin deze soorten nog wel aanwezig waren en ook daadwerkelijk van de oever af het water in groeiden. Deze petgaten waren allemaal vrij jong. Zorgvuldig monitoren van deze petgaten zal moeten uitwijzen of hier na verloop van tijd weer kragges zullen worden gevormd.

Dankwoord

Er zijn veel verschillende manieren om dankjewel te zeggen, en evenveel mensen om het tegen te zeggen. De een zegt het met bloemen, de ander met een lied of een gedicht, met een zoen, een omhelzing. Weer een ander schrijft een lieve lange brief.

Ik kies ervoor om het gewoon te zeggen, uit de grond van mijn hart en met alles wat in me is:

BEDANKT

Zonder jullie allemaal was het echt niet gelukt. Dank je wel voor alle hulp, alle steun, het meedenken, het meepuzzelen, het meedromen en meeleven. Ook bedankt voor de lekkere maaltijden, rare ideeën, spontane plannen, het voederen van visjes en de hulp bij lab- en kas werk. En zeker ben ik jullie ook dankbaar voor alle medewerking aan promotiestukjes, alles wat ik van je geleerd hebt, het meeworstelen, jullie pogingen om het te begrijpen, het voorbeeld dat je bent geweest, het bestaan van stengelstijfheid, de aanmoediging, en de manieren waarop je mij hielp aan andere dingen te denken en los te komen. Ook bedankt voor het zwemmen, de bootjes, het verduren van mijn frustraties, gezang, gestuiter en geklaag, en tot slot: Bedankt voor het meefeesten om het resultaat.

Ik heb me heel erg gedragen en gesteund gevoeld door jullie allemaal. Ik ben God dankbaar want ik voel me een gezegend mens.

Judith

Curriculum vitae

Judith Maria Sarneel werd geboren op 3 april 1981 te Leiderdorp. Ze haalde haar VWO diploma bij het Andreas College te Katwijk met een vakkenpakket dat bestond uit Biologie, Scheikunde, Wiskunde B, Engels, Duits, Nederlands, Geschiedenis en als extra vak Tekenen (Havo-niveau).

Daarna begon zij eerst aan een studie creatieve therapie bij de Hogeschool van Utrecht, maar na het behalen van haar propadeuse begon zij in 2000 met de opleiding biologie in Utrecht. Ze haalde haar propadeuse biologie Cum Laude. Tijdens haar studie liep ze stage bij de vakgroep Landschapsecologie waar ze onderzoek deed aan zaden die opgeslagen liggen in bodems van laagveenplassen. Hierna deed zij acht maanden onderzoek bij het Nederlands Instituut voor Oecologisch Onderzoek (NIOO) in Yerseke. Daar onderzocht zij de biomechanische eigenschappen van planten in het getijdegebied. Daarnaast assisteerde ze tijdens haar studie bij diverse master cursusen.

In september 2005 behaalde zij haar doctoraal examen, waarna zij een half jaar als junior onderzoeker aan de slag ging binnen het O+BN Laagveenwateren project. Binnen hetzelfde project werkte zij vervolgens ruim vier jaar aan haar promotieonderzoek, onder leiding van Jos Verhoeven, Merel Soons en Boudewijn Beltman. Gedurende de laatste anderhalf jaar van haar promotietraject vervulde zij de taak van secretaris binnen het Centrum voor Wetland Ecologie en organiseerde daarvoor twee symposia. Na het afronden van haar proefschrift werkte zij een aantal maanden als docent aan de Hogeschool van Utrecht.

Sinds augustus 2010 is zij werkzaam bij het NIOO te Nieuwersluis, waar zij als post-doc onderzoek doet naar de effecten van een flexibel peilbeheer op de ontwikkeling van oevervegetaties.

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