Adaptation and dispersal
of native salt-marsh species
Elytrigia atherica

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Contents

Chapter *1*

General introduction

Salt marshes and *Elytrigia atherica*

Coastal salt marshes provide a unique habitat for plant and animal life. This habitat is restricted to a narrow zone between land and sea and is subjected to extreme environmental conditions due to periodic flooding by seawater and high salinity. As a consequence, the salt-marsh plant communities consist of a small number of highly adapted plant species and are considered rare and vulnerable to extinction (Doody, Johnston, & Smith 1993; Westhoff, Hobohm, & Schaminée 1993). Nevertheless, the salt-marsh vegetation provides an important habitat for a wide variety of animals, many of which are restricted to saline habitats (Pétillon *et al.* 2010; Schrama, Berg, & Olff 2012). Additionally, salt marshes are of great importance as staging and breeding area for migratory birds (Madsen, Cracknell, & Fox 1999; Koffijberg *et al.* 2006). For this reason an increasing number of the European salt marshes have been designated as nature reserves or as a part of National Parks in the past decades (Esselink, Fresco, & Dijkema 2002; Stock 2003; Townend *et al.* 2011). Currently, all salt marshes in the Wadden Sea area (40,000 ha) are under the protection of national nature legislation (Essink *et al.* 2005).
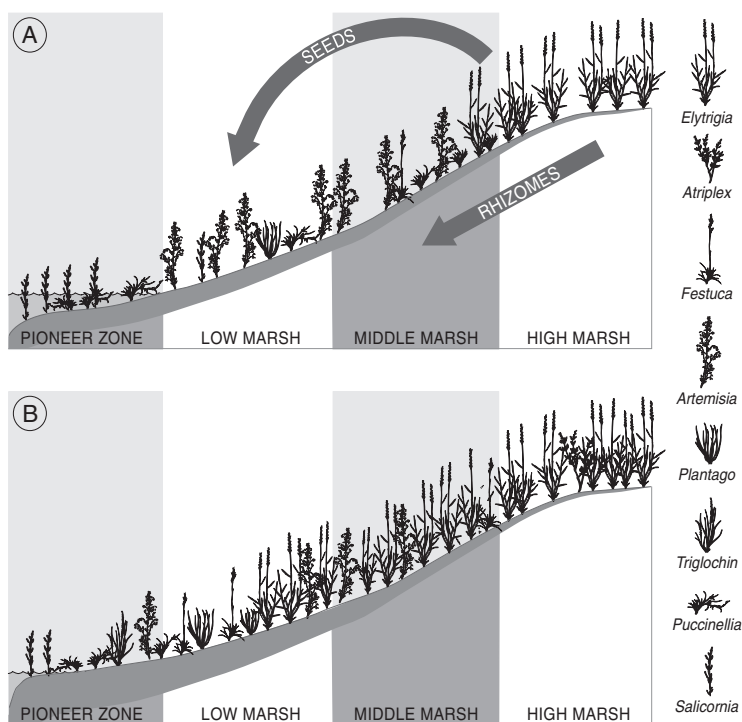

Figure 1.1 Changes in salt marsh succession on a salt marsh. In earlier succession (A) *E. atherica* occurs only at higher elevations. Over time, by means of seed dispersal and rhizomes *E. atherica* will expands to the lower marsh. At a late successional stage (B) *E. atherica* has invaded the lower marsh.
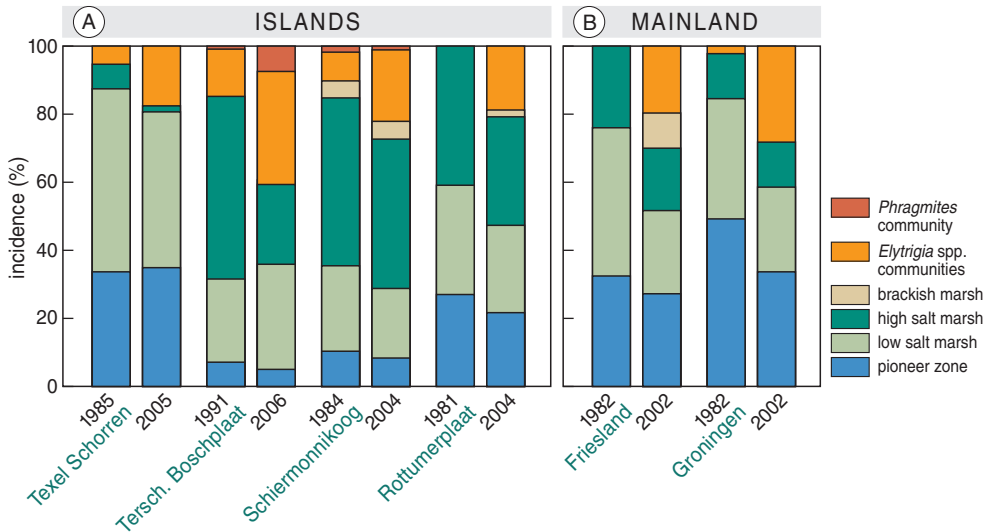
Figure 1.2 Vegetation change in four back-barrier salt marshes on islands and two mainland salt marshes in the Netherlands Wadden Sea over an approximately 25-year period. The graph represents the incidence of main vegetation zones and climax communities of *Elytrigia atherica* and *Phragmites australis* (Redraw from Esselink *et al.,* 2009).

Salt marshes show a strong zonation of the plant community depending on the elevation of the marsh and the distance to the sea (Adam 1990) (Fig. 1.1A). Differences in elevation are associated to differences in inundation frequency and thus sediment input, soil aeration and salinity. In time the zonation pattern changes as a result of succession. Increasing age of the salt marsh coincides with an increasing input of sediment and organic matter, resulting in increasing elevation and nutrient availability (Olff *et al*. 1997; Van Wijnen & Bakker 1997). The increasing nutrient availability allows taller growing plants to dominate the marsh (Fig. 1.1B). From a morphological point of view a distinction can be made between two salt marsh types; back-barrier marshes and mainland marshes. Back-barrier marshes are in principal natural salt marshes, developed under the lee of a barrier-beach or an artificial dune ridge. Mainland marshes are largely of anthropogenic origin, as their development is promoted by ditching and the construction of sedimentation fields.

The present total area of salt marshes along the Dutch coast is lower compared to that found in historical records around 1600. During the past centuries, extensive areas of salt marsh were embanked for coastal protection and agricultural exploitation (Dijkema 1987). Since the 1960s it was no longer economically feasible to embank salt marshes for agriculture. The remaining marshes have become older and higher, developing into suitable habitats for late successional plant species, such as the tall grass *Elytrigia atherica*. Around 1980, approximately 8% of the salt marsh along the Dutch coast was covered by *E. atherica* and this expanded to 17% coverage around 2000 (Dijkema *et al.* 2007) (Fig. 1.2).

Natural succession is an important player in the increase of *E. atherica* dominance. The spreading of *E. atherica* is a phenomenon due to natural succession on back-barrier island marshes without livestock grazing. During succession, the marshes become older and higher by accretion, developing into suitable habitat for late-successional species such as *E. atherica* (Olff *et al.* 1997). Also on mainland marshes the incidence and dominance of *E. atherica* is positively related to elevation and rate of increase of surface elevation (Heinze, Kiehl, & Neuhaus 1999). In later successional stages of salt marshes, more nitrogen is available as a result of silt accumulation (Olff *et al.* 1997). Van Wijnen & Bakker (2000) showed that atmospheric deposition was the dominant exogenous nitrogen source both in young and old marshes. Thus, anthropogenic eutrophication may have led to an increased nitrogen availability overall on the salt marsh, independent of the successional stage of the marsh. This would enable a late-successional species, such as *E. atherica*, to expand more rapidly and spread to lower and younger marshes (Bockelmann *et al.* 2002).

Additionally, the rapid increase of *E. atherica* occurred concurrently with a change in management regimes on many salt marshes. Although salt marshes tend to have a 'natural' appearance, the majority of salt marshes along the mainland coast of the Wadden Sea result from human interference (Dijkema 1983). Livestock grazing on European salt marshes can be traced back for couple of millennia (Davy, Bakker, & Figueroa 2009). Traditionally the mainland marshes were intensively grazed by sheep or cattle. As a result the majority of these marshes were covered by an extremely short homogeneous vegetation of the low-statured grasses *Puccinellia maritima* or *Festuca rubra* (Dijkema 1983; Kiehl *et al.* 1996). Starting in the 1980s, due to economic motivations of farmers or changes in nature management goals, grazing by livestock was discontinued in many marshes, resulting in up to 60% of back-barrier and up to 40% of mainland marshes in the Wadden Sea being ungrazed by 2008 (Esselink *et al.* 2009). Concurrently with this change in management regimes, namely cessation or reduction of livestock grazing in the past decades, the tall grass Elytrigia atherica has increased in abundance on several salt marshes along the northwest European coast; for example at Mont Saint Michel, France (Valéry *et al.* 2004), The Wash, UK (Norris, Blair, & Johnson 2007) and Schleswig- Holstein, Germany (Stock *et al.* 2005; Esselink *et al.* 2009).As numerous experiments have shown, large herbivores are able to prevent vegetation from being dominated by tall-growing species (Kiehl *et al.* 1996; Bos *et al.* 2002; Bakker & Olff 2003).

The mainland marshes consist of sedimentation fields with brushwood groynes. The fields are comprised of an extensive artificial system of a few large and many small ditches to allow inundation of the entire field. The intensive drainage system also facilitates fast discharge of the water, thus allowing oxygen to enter the soil and thus enhancing ripening development of the soil. Inundation frequency, as related to elevation and drainage conditions, was shown by Bockelmann *et al.* (2002) to be of great importance for the occurrence and dominance of *E. atherica*. Currently, artificial drainage by ditching is infrequently practiced: about 10% of back-barrier marshes and 30% of mainland marshes in the Wadden Sea were recently drained (i.e. <10 years ago) (Esselink *et al.* 2009).

Effect of invasion

Invasion by non-native species is considered as one of the most serious threats to the integrity of natural systems (Drake *et al.* 1989; Tyler, Lambrinos, & Grosholz 2007). Invaders can profit from the increased availability of nitrogen in disturbed systems, and may outcompete the species adapted to previously low-nutrient conditions (Vitousek *et al.* 1997). Invasion can result in extinction of native species, alteration of nutrient cycles, modification of food webs (Pétillon *et al.* 2005) and habitat loss. Wetlands are especially susceptible to invasions that lead to monocultures due to their positions in landscapes as sinks for many processes, resulting in the accumulation of the effects of disturbance, wetting, and eutrophication (Zedler & Kercher 2004). Expansion by native species can also lead to the same changes in communities and ecosystems as in the case of non-native species. Bakker (1989) showed that species richness decreased concurrently with the increase in the abundance of *E. atherica*.

Dispersal strategy

E. atherica is a clonal plant and as many clonal plant species, able to propagate both sexually (creating new genets from sexually produced seed) and vegetatively (producing genetically identical ramets, which are potentially independent plant units) (Cook 1983). It has been proposed that different modes of propagation are favourable under different environmental conditions encountered during the life of an individual or a population (Olivieri, Michalakis, & Gouyon 1995; Eriksson 1997). This advantage may be expressed at the genetic as well as the ecological level (Ellstrand & Roose 1987). For example, vegetative reproduction has the advantages of physiological aid from the mother plant to the daughter plant through vegetative connectors (D'Hertefeldt & Jonsdottir 1999) and absence of the costs of sexual reproduction (e.g. flowering, seed production) (Doust & Laporte 1991; Ronsheim & Bever 2000; Rydgren & Okland 2003). On the other hand, seed dispersal is a key component of a species capacity to establish populations in newly created patches of habitat (Piquot *et al.* 1998). Hence, long-distance seed dispersal is a means of escaping the competitive effect of clonal crowding and kin competition (Eriksson 1997; Howe & Miriti 2004). This suggests that the recent history of a population of plants capable of both clonal and sexual reproduction is reflected in its genetic structure as the consequence of their past life history and environmental conditions experience.

Grazing or mowing may have different effects on the reproductive strategies of plant species (Fernandez, Nunez, & Soriano 1992; Kerley, Tiver, & Whitford 1993). Some species respond to grazing by compensatory growth and producing more side shoots (ramets) (Mcnaughton 1983; Wallace, Mcnaughton, & Coughenour 1985), whereas other species may decrease or increase their seed production. As mentioned above, seedling recruitment may either be increased or limited by grazing. For many clonal species disturbances, such as grazing or fires, are needed for seedling establishment

(Eriksson 1989) as these reduce the competition from the established vegetation (Eriksson 1997). When seedling establishment is rarely observed in clonal plant populations, the general assumption is that the population is dominated by a few large clones. Only a few studies have dealt with the effect of disturbance by management on the clonal diversity of plant populations (Kleijn & Steinger 2002; Reisch & Scheitler 2009).

Thesis outline

In a previous project on the successful invasion of *E. atherica*, Bockelmann (2002) focused on the adaptive plasticity and genetic differentiation of the species, induced by environmental conditions. She showed that abiotic and biotic conditions differ between habitats, in particular inundation frequency and presence of herbivores represent different selection regimes, which resulted in restricted gene flow over small distances (Bockelmann *et al.* 2003). Additionally she showed that biotic factors had a much stronger effect on seedling growth and mortality than parental origin and were independent of inundation. However, parental origin strongly interacted with herbivory and competition, with seedlings performing better under the conditions that resembled their parental site (Bockelmann, Wels, & Bakker 2011).

The main objective of this project is to investigate how *E. atherica* has been able to spread so rapidly in the past decades on the salt marshes in the Wadden Sea. Firstly, we aim to understand the spatial and temporal dynamics of *E. atherica* on natural salt marshes. It is difficult to disentangle the relative influences of natural succession and human induced processes on the rapid spread of *E. atherica*. For this purpose we selected, in **chapter 2**, four naturally developed back-barrier salt marshes to address this problem. Naturally developed salt marshes feature a self-stimulated development and geomorphological condition and growth that are not affected by humans. Furthermore, the selected salt marshes have not been grazed by livestock or subject to other management regimes. Experimental studies of exclusion of cattle have shown a rapid increase of *E. atherica* on older marshes (Bakker, Bos, & De Vries 2003). However an increase of *E. atherica* has not been observed on all abandoned mainland marshes. In **chapter 3** we aim to reconcile the contradictory results from local studies demonstrating either strong dominance of *E. atherica* or hardly any increase at all after the cessation of livestock grazing. Thus, we evaluate which factors affect the dominance of *E. atherica* on mainland salt marshes after cessation of livestock grazing along the German and Dutch Wadden Sea coast (Fig. 1.3). We study the effect of factors, such as time since cessation of livestock grazing, elevation, surface elevation change rates and drainage conditions, on the increase of *E. atherica* on the mainland marshes.

In chapters 4, 5 and 6 we concentrate on the dispersal strategies of *E. atherica*. Only a few studies have dealt with the effect of disturbance by management on the clonal diversity of plant populations (Kleijn & Steinger 2002; Reisch & Scheitler 2009). A long-term grazing study on the back-barrier marsh of Schiermonnikoog and the
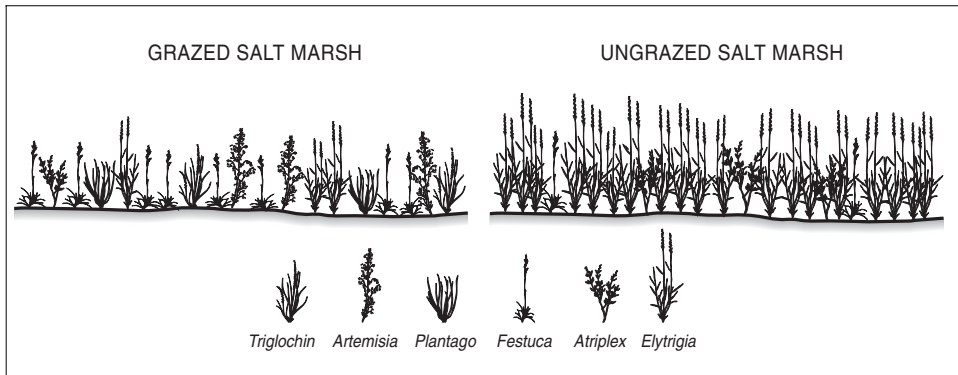
Figure 1.3 Changes in vegetation community on a middle marsh as after cessation of grazing.

availability of molecular markers provided a unique opportunity to study the effects of management regimes on the genetic structure of a clonal plant species. In **chapter 4** we address the question whether the clonal structure of *E. atherica* populations is affected by the different management regimes such as grazing, mowing and no interference. In **chapter 5** we assess what mode of propagation, sexual or vegetative, governs the successful spread of the species in new habitats (Fig. 1.1B). Additionally we aim to asses if there is a change in the balance of sexual versus vegetative reproduction as succession proceeds. In **chapter 6** we focus on the seed dispersal of *E. atherica*. We examine the processes that influence seed retention in intertidal systems by identifying simple functional relationships between dispersal vectors (tidal flow, wind-generated waves), trapping agents (vegetation), seed characteristics (buoyancy) and seed retention. We explore the effects of morphological seed traits, vegetation type and hydrodynamic variables on seed retention processes in both in the field and in a flume facility. In **chapter 7** we synthesize the findings of this thesis. Firstly, we discuss the rapid spread of *E. atherica* in the past decades on back-barrier and mainland marshes. Secondly, the dispersal strategies of *E. atherica* in relation to management and succession are be discussed. Finally, we will indicate the future challenges of *E. atherica* and the salt-marsh management.

Chapter 2

Spatio-temporal dynamics of the invasive plant species, *Elytrigia atherica,* on natural salt marshes

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Jan P. Bakker

Abstract   **Question**: In the past decades, the tall native invasive grass, *Elytrigia atherica*, has been increasing its frequency and dominance on salt marshes along the Wadden Sea coast. Is this rapid expansion an outcome of natural succession or is it driven by anthropogenic eutrophication resulting from atmospheric deposition?

Location: Salt marshes on four back-barrier islands in the Wadden Sea along the coast of the Netherlands and Germany.

Methods: We used a combination of time series of vegetation maps and chronosequence data of four naturally developed salt marshes to address our questions. These salt marshes have not been grazed by livestock or subject to other management regimes. By comparing the development of *E. atherica* within and between the four different salt marshes, we are able to study the spatial and temporal dynamics of the community dominated by Elytrigia atherica on natural salt marshes.

Results: The expansion rate of the *E. atherica* community is highest on young salt marshes (up to 30 years old) with vertical accretion rates of 0.35 cm/yr. The expansion rate decreases on older marshes and reverses direction, becoming negative, on the oldest marshes (around 90 years old), which have no vertical accretion and are under waterlogged conditions.

Conclusions: The expansion of *E. atherica* on natural, back-barrier islands along the Wadden Sea coast is more influenced by the age of the salt marsh and patterns in vertical accretion of soil than by uniformly spread atmospheric deposition.

Introduction

Eutrophication as a result of increased nitrogen input from watersheds to estuaries has become a major environmental concern in coastal systems. Invaders can profit from the increased availability of nitrogen in disturbed systems, and may outcompete the species adapted to previously low-nutrient conditions (Vitousek *et al.* 1997). Invasion by non-native species is considered as one of the most serious threats to the integrity of natural systems (Drake *et al.* 1989; Tyler *et al.* 2007). Invasion can result in extinction of native species, alteration of nutrient cycles, modification of food webs (Pétillon *et al.* 2005) and habitat loss. Wetlands are especially susceptible to invasions that lead to monocultures due to their positions in landscapes as sinks for many processes, resulting in the accumulation of the effects of disturbance, wetting, and eutrophication (Zedler & Kercher 2004).

Expansion by native species can also lead to the same changes in communities and ecosystems. The tall grass, *Elytrigia atherica*, is native to the salt marsh and occurs along the North Atlantic Coast from Northern Portugal to Southern Denmark. In the past decades *E. atherica* has been increasing in frequency and dominance on the salt marshes along the North Sea coast, for example at Mont Saint Michel (France (Pétillon *et al.* 2005)), The Wash (UK (Norris *et al.* 1997) and Schleswig- Holstein (Germany (Esselink *et al.* 2009)). Natural succession is an important player in the increase of *E. atherica* dominance. The present total area of salt marshes along the Dutch coast is lower compared to that found in historical records around 1600. Also the marshes have become older and higher, developing into suitable habitats for presumed climax species. Around 1980, approximately 8% of the salt marsh along the Dutch coast was covered by *E. atherica* and this expanded to 17% coverage around 2000 (Table 2.1) (Dijkema *et al.* 2005; Dijkema *et al.* 2007). This rapid increase has occurred concurrently with a change in management regimes on these salt marshes. Due to the economic reasons of farmers or changes in nature management ideals, grazing by livestock has been discontinued. As numerous experiments have shown, large herbivores are able to prevent vegetation from being dominated by tall-growing species (Kiehl *et al.* 1996; Bos *et al.* 2002; Bakker & Olff 2003).

Hence, from a management point of view for plant species richness, it is known that the spread of *E. atherica* can be counteracted by grazing (Van Wijnen *et al.* 1997) and mowing (Veeneklaas *et al.* 2011). We know how to reduce the dominance of *E. atherica*. It is however unknown whether *E. atherica* can continue to spread under natural conditions and remains to stay a climax species. The aim of this study is to detect if there is a world beyond *E. atherica* at later successional stages in naturally developed salt marshes.

In later successional stages of salt marshes, more nitrogen is available as a result of silt accumulation (Olff *et al.* 1997). Van Wijnen and Bakker (2000) showed that atmospheric deposition was the dominant exogenous nitrogen source both in young and old marshes. Hence, anthropogenic eutrophication may have led to an increased nitrogen availability overall on the salt marsh, independent of the successional stage of the marsh. This would enable a late-successional species, such as *E. atherica*, to expand

Table 2.1 An overview of change in abundance of *E. atherica* on Dutch salt marshes over time (Dijkema *et al.* 2009). The cover area (ha) of *E. atherica* and the total marsh area (ha) per region is shown. The intrinsic rate of natural increase (yr^{-1}) is calculated using Dijkema's data (Dijkema *et al.* 2005; Dijkema *et al.* 2009).

Study area	1980		1990		2000		2006		intrinsic rate of increase		
	E. atherica cover (%)	total area salt marsh (ha)	*E. atherica* cover (%)	total area salt marsh (ha)	*E. atherica* cover (%)	total area salt marsh (ha)	*E. atherica* cover (%)	total area salt marsh (ha)	1980–1990	1990–2000	2000–2006
Eems-Dollard	7.2	796	10.1	755	10.9	741	3.9	763	0.04	0.01	-0.14
Wadden Sea-Groningen mainland	2.0	1719	3.9	1367	14.4	1420	28.2	1355	0.04	0.34	0.10
Wadden Sea-Friesland mainland	0.0	1617	8.3	1684	9.5	1922	19.6	2277	0.49	0.07	0.15
Wadden Sea-North-Holland mainland	8.8	34	14.3	35	19.7	71	34.9	86	0.09	0.12	0.13
Wadden Sea-East barrier islands	11.1	2408	15.7	2489	17.5	2856	23.0	2911	0.04	0.04	0.04
Wadden Sea-West barrier islands	1.6	190	5.3	188	6.3	301	8.4	334	0.17	0.13	0.05
Wadden Sea-North-Holland barrier island	7.3	286	10.2	294	8.2	257	14.1	277	0.04	-0.07	0.10
Haringvliet-mouth	0.0	38	10.5	399	20.4	230			0.93	0.01	
Oosterschelde	0.2	1218	9.5	589	15.6	508			0.18	0.06	
Westerschelde-mouth	25.2	139	22.8	79	29.8	57			-0.07	-0.01	
Westerschelde	21.4	2381	37.2	2376	33.0	2395	29.1	2690	0.04	-0.03	0.00

more rapidly and spread to lower and younger marshes (Bockelmann 2002). As salt marshes expand, vegetation development is highly dependent on local drainage conditions and sediment input (Tessier *et al.* 2003). For example, on the Hamburger Hallig salt marsh (Germany), a distinct gradient was found with high sedimentation rates close to the intertidal flats and hardly any accretion about 1000m from the edge of the salt marsh (Schröder *et al.* 2002). Thus, areas further from the edge show less vertical accretion and subsequent soil waterlogging (Esselink *et al.* 2009).

It is difficult to disentangle the relative influences of natural succession and anthropogenically induced processes on the rapid spread of *E. atherica*. Therefore, we selected four naturally developed back-barrier salt marshes to address this problem in this study. Naturally developed salt marshes feature a self-stimulated development and geomorphological condition and growth that are not affected by humans. Furthermore, the selected salt marshes have not been grazed by livestock or subject to other management regimes. Sequential vegetation maps of the back-barrier islands of Terschelling (NL), Schiermonnikoog (NL), Norderney (D) and Spiekeroog (D) allow us to study the spatial and temporal dynamics of *E. atherica* on natural salt marshes. The differences in succession between nutrient-rich and nutrient-poor salt marshes will be discussed in the light of nutrient accumulation by sediment accretion.

The aim of our study is to distinguish between the effects of natural succession and anthropogenically induced processes on the rapid spread of *E. atherica* along the Wadden Sea coast. The first hypothesis is that anthropogenic eutrophication has enhanced the expansion rate of *E. atherica* over time. We expect the time series to reflect the increase in *E. atherica* but not the chronosequence data, as it is subject to spatially uniform deposition. The second hypothesis is that the expansion rate is affected by factors such as the age of the marsh, rate of vertical accretion and soil waterlogging. By studying the spread of the species on multiple islands, we expect to observe spatial differences in expansion rate. The third hypothesis is that the monoculture of the tall grass is not the climax stage of succession on the salt marsh, but that there is a world waiting beyond *E. atherica*.

Methods

Species
E. atherica is a hexaploid, tall-growing, wind-pollinated species with the ability of reproducing clonally as well as sexually (Bockelmann *et al.* 2003). This species is also known as *Agropyron pungens*, *Elymus athericus*, *Elymus pycnanthus*, or *Elytrigia pungens*. It occurs on continental European salt marshes along the coast from northern Portugal to southern Denmark, and along the southern coasts of the British Isles.

Study sites
We studied natural salt marshes that had developed without the aid of sedimentation fields and also were not grazed by livestock, as the occurrence of *E. atherica* is negatively

Figure 2.1 Location of the study sites.

affected by cattle and sheep grazing (Bakker *et al.* 1985). Salt marshes on four barrier islands in the Wadden Sea were studied: the Boschplaat on Terschelling (53°26'N, 5°28'E) and the Oosterkwelder on Schiermonnikoog (53°30'N, 6°16'E) in the Netherlands; the Ostheller on Norderney (53°42'N, 7°17) and the Ostplate on Spiekeroog (53°46'N, 7°45'E) in Germany (Fig. 2.1 & 2.2). By using major creeks as boundaries, the salt marshes were subdivided into five or six sections (see Fig. 2.2).

The salt marsh of the Boschplaat on Terschelling developed between 1932 and 1936. A long artificial dune ridge was established north of sections T1-T6 (Fig. 2.2) on eastern sand banks (Roozen & Westhoff 1985). In the shelter of this dune ridge, the present salt marsh, the Boschplaat, developed and is currently 75 years old. To this day, the artificial dune ridge reduces the transport of sand and water from the North Sea, thus preventing the development of young marshes. Consequently, the entire salt marsh is of the same age and was established in 1934. The Boschplaat has never been grazed by livestock.
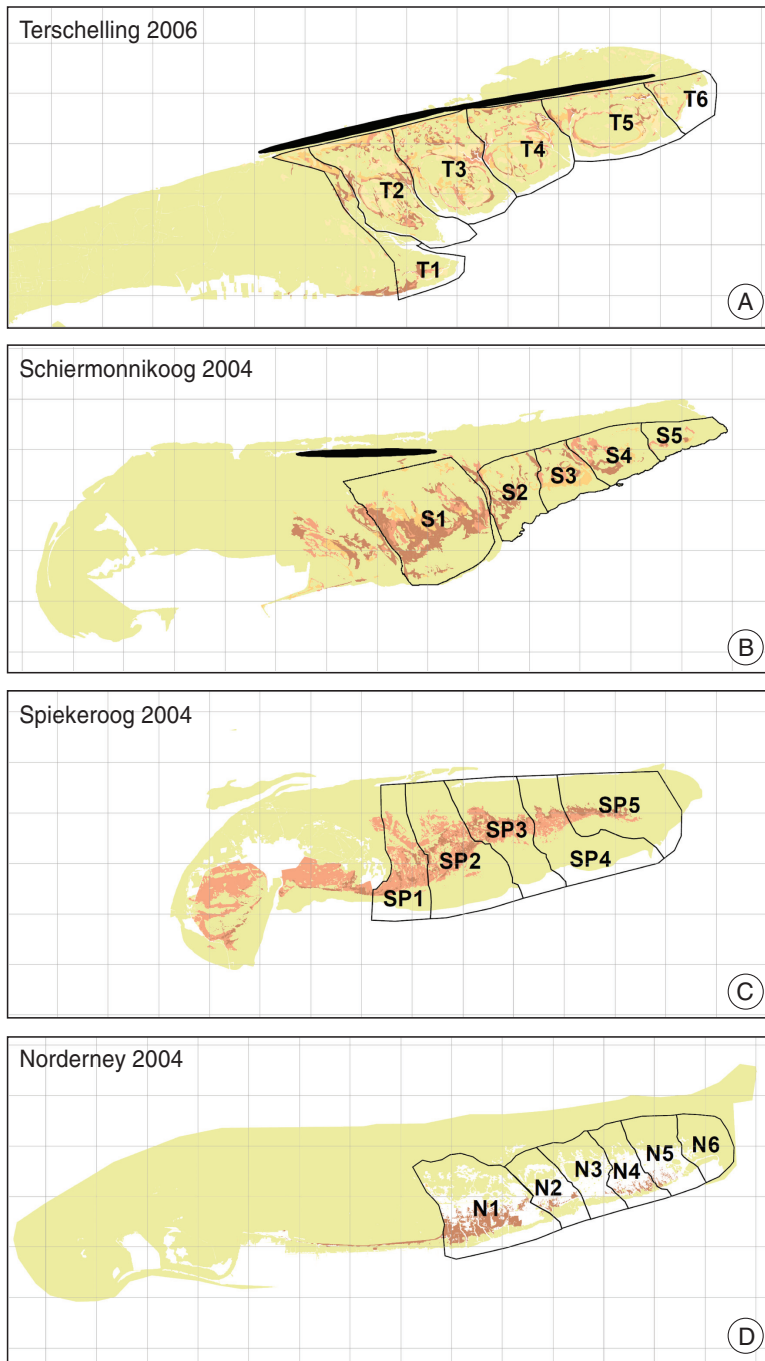
Figure 2.2 Maps of the four barrier islands with naturally developed salt marshes. The different sections indicated for each island are related to natural drainage creeks on the marsh. The position of artificial dune ridges is indicated as a black bar. The grid scale is 1km × 1km.
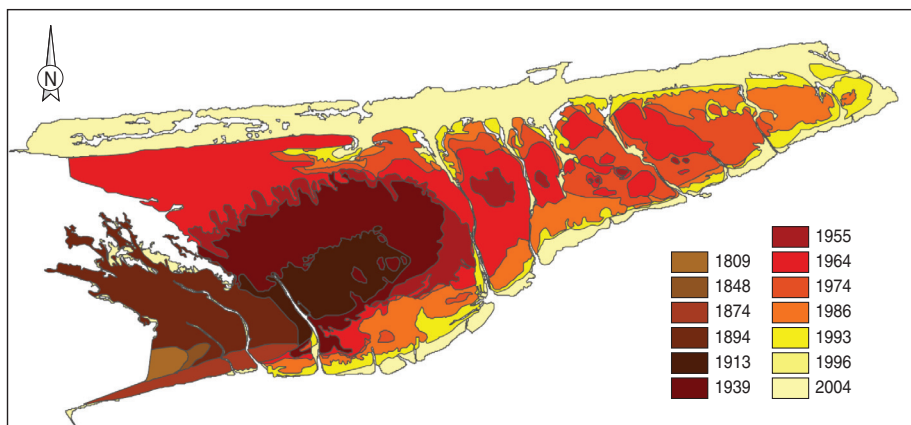
Figure 2.3 Salt-marsh development on Schiermonnikoog. Vegetated areas were mapped from aerial photographs and topographical maps (Jager 2006). The shading represents different ages of the salt marsh. The legend labels represent the estimated year of vegetation establishment.

In contrast, salt marshes have been developing sequentially on the Oosterkwelder of Schiermonnikoog since 1850. An artificial dune ridge was built in 1960 for coastal protection, but it was breached every winter (Fig. 2.2). From the mid-1960s onwards, the artificial dune ridge was only maintained in the area north of section S1. After this time, the eastern part of the island was not protected from the North Sea, thus giving the sea and winds a free hand in dune formation. Due to dominantly westward sea currents, there is a net transport of sediment along the shore from west to east. As a result of this tidal sand deposition, the island is gradually expanding eastward, allowing us to study the dynamic development of the eastern part of the island (Fig. 2.3) (Bakker 1989; Olff *et al.* 1997). Therefore, Schiermonnikoog displays a chronosequence of salt-marsh establishment from 1913 onwards in our study area. Until 1958, the western part of the Oosterkwelder (section S1) was extensively grazed by cattle. Sections S2-S5 have never been grazed by cattle.

The Ostplate of Spiekeroog developed naturally in the eighteenth century after embankment of the Harle Bay. On an aerial photograph from 1960, the high sand deposition of the Ostplate Spiekeroog with attendant little dunes can be observed. Between 1960 and 1980, the initial salt-marsh and dune development began on Ostplate, leading up to the present situation. Artificial constructions, such as drainage ditches or dune ridges, were not used to establish these salt marshes.

According to aerial photographs, the initial development of salt marshes on Norderney started before 1937. On an aerial photograph from 1937, the western part of the current salt marsh (section N1) and its artificial drainage system can be observed. Between 1938 and 1960 the drainage system was extend to section N2. The eastern salt marsh (section N3 till N6) developed naturally after 1938 in the absence of artificial constructions, such as dune ridges or drainage furrows.

Comparison of vegetation maps for short-term and long-term dynamics

The expansion of *E. atherica* was recorded by interpreting vegetation maps of different time periods (App. S2.1). All vegetation maps were developed by interpreting data collected from aerial photographs and field surveys. The criteria for classifying the plant communities varied between the maps because they were collected from different sources. To compare the sequential maps, we re-classified the plant communities according to the recently standardized typology for salt marshes in the Trilateral Monitoring and Assessment Programme of the Wadden Sea (TMAP2004) (Bakker *et al.* 2005) (Appendix S1), using the vegetation survey appendices of these maps.

The aforementioned subdivisions applied to each marsh (Fig. 2.2, Table 2.2) allowed us to study patterns along a gradient from west to east on the islands. Area changes between time periods were further analysed using a logarithmic growth equation. A growth formula normalizes area change, so that changes over time within small areas are more comparable to changes over time within large areas. Following Wilson & Bossert (1971) and Rice *et al.* (2000), the intrinsic rate of natural increase per year (*r*) was calculated using this logarithmic growth equation:

$$N = N_0 e^{rt}$$

N represents the total area at time 1, N_0 is the total area at time 0, *e* is the natural logarithm and *t*, the difference in years between time 1 and time 0. The intrinsic rate of increase of *E. atherica* community was calculated for each individual section on Schiermonnikoog, Terschelling, Norderney and Spiekeroog.

Results

Short-term temporal dynamics of *Elytrigia atherica* on natural marshes

The salt marshes of the four islands vary in age and local factors such as vertical accretion and waterlogging. By comparing the development of *E. atherica* within and between the four different salt marshes, we can monitor the spatial and short-term (1997/1999 – 2004/2006) temporal dynamics of the *E. atherica* community on natural salt marshes (Fig. 2.4, Table 2.2).

The cover of the *E. atherica* community on Terschelling increased from 15-20% of the vegetated salt marsh in 1999 to 20–30% in 2006. The intrinsic rate of increase of *E. atherica* did not differ between sections and was on average 0.049 (Fig. 2.4, Table 2.2). In contrast, the incidence of the *E. atherica* community on Schiermonnikoog was lower in the western than in the eastern salt marshes in both 1997 and 2004. In the oldest region (S1, 90 yrs), 25% of the vegetated salt marsh was covered by the *E. atherica* community, whereas in the youngest region (S5, 20 yrs), the community covered less than 10% of the area (Fig. 2.4, Table 2.2). A similar pattern was found on both German islands, where the highest incidences of the *E. atherica* community were found in older salt marshes to the west. On Norderney, the incidence of the *E. atherica* community
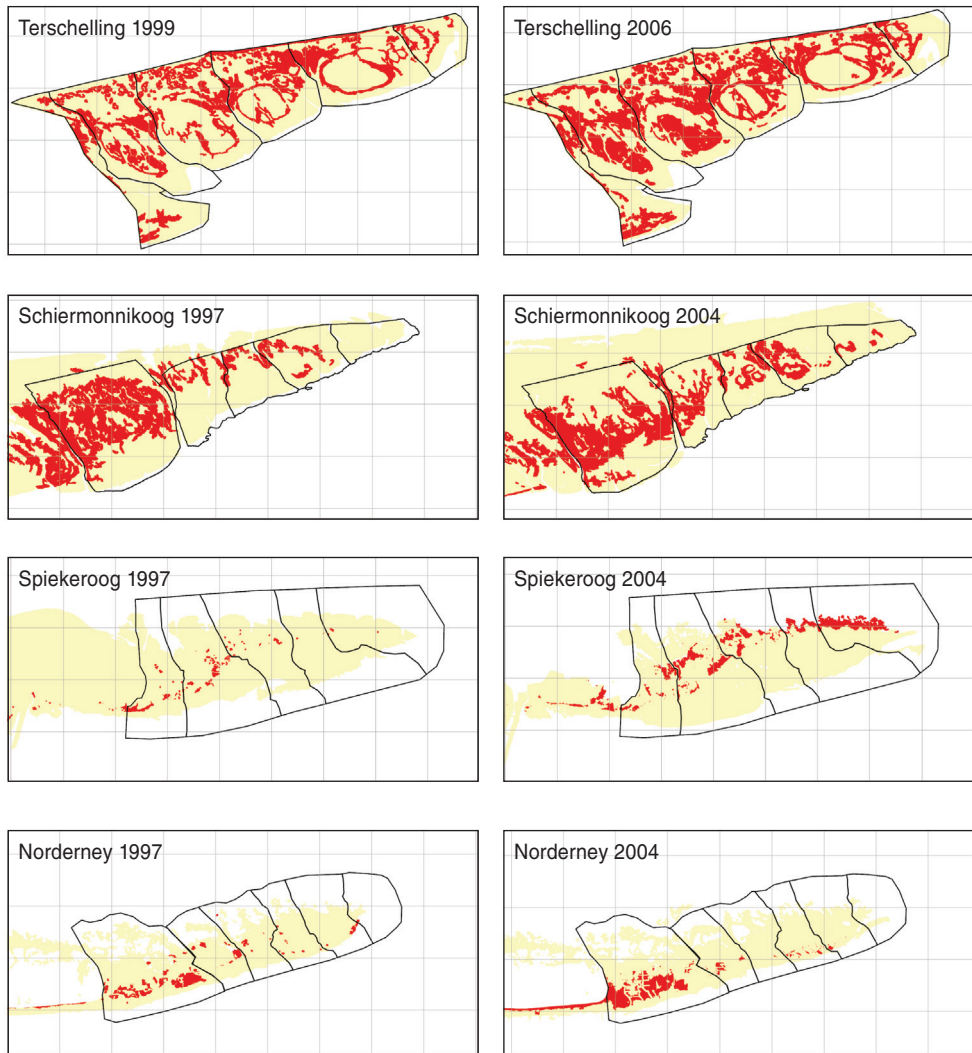
Figure 2.4 Distribution of the *E. atherica* community (TMAP type 3.7) during two subsequent mappings around the turn of the millennium in the back-barrier salt marshes of the islands of Terschelling, Schiermonnikoog, Spiekeroog and Norderney. The grid scale is 1km × 1km.

decreased from 25% in the west to 1% at the eastern-most section of the marsh. On Spiekeroog, a decreasing gradient of the incidence of *E. atherica* from west to east was observed in 1997. In 2004, this gradient had disappeared and an average incidence of 14% was observed over the entire salt marsh.

The highest mean rate of increase of the *E. atherica* community during the period from 1997 to 2004 was found at the 32- to 39-year-old marsh on Spiekeroog, followed by the 33- to 38-year-old marsh on Schiermonnikoog (Table 2.2). The expansion rate of

Table 2.2 Characteristics of the four barrier-island salt marshes divided into sections. The divisional boundaries between salt-marsh sections are based on the location of major natural creeks intersecting the marsh. The following characteristics are shown: year of initial vegetation establishment (VE); estimated annual vertical accretion rate (VA, cm yr^{-1})(unpublished sedimentation data); presence of an artificial dune ridge (*dune*) or artificial drainage system (*drain*); and vegetated surface area (ha) (estimated in 1997 and 2004 for Schiermonnikoog and Norderney, in 1999 and 2006 for Terschelling).

Island	section	VE	VA (cm yr^{-1})	Precence dune ridge or drain	Vegetated area (ha) 1997, 1999	2004, 2006	% cover E.atherica community 1997, 1999	2004, 2006	Intrinsic rate of increase of E. atherica community 1997 – 2004, 1999 – 2006
Terschelling	T1	1934	0.15	dune ridge	181	174	15.51	19.51	0.028
	T2	1934	0.15	dune ridge	276	282	19.57	23.78	0.031
	T3	1934	0.15	dune ridge	314	322	15.30	25.77	0.078
	T4	1934	0.15	dune ridge	246	262	21.16	29.06	0.054
	T5	1934	0.15	dune ridge	258	260	16.26	22.31	0.046
	T6	1934	0.15	dune ridge	90	89	13.28	20.17	0.058
Total area					1365	1389			mean ± s.e. 0.049 ± 0.007
Schiermonnikoog	S1	1913	0.0	dune ridge	456	472	23.90	25.62	0.015
	S2	1964	0.15	none	133	153	6.79	19.66	0.172
	S3	1974	0.15	none	97	114	11.38	14.03	0.054
	S4	1974	0.35	none	124	138	6.43	16.62	0.151
	S5	1986	0.35	none	60	85	1.67	8.23	0.278
Total area					870	962			mean ± s.e. 0.134 ± 0.046
Norderney	N1	<1937		drain	178	244	12.45	24.96	0.144
	N2	<1937		drain	65	166	5.51	3.20	0.056
	N3	<1937		none	58	155	1.87	0.29	-0.127
	N4	<1960		none	60	113	2.02	6.52	0.259
	N5	1960		none	54	91	2.23	4.25	0.166
	N6	1960		none	4	76	2.04	0.00	-0.291
Total area					419	845			mean ± s.e 0.035 ± 0.084
Spiekeroog	SP1	1960		none	77	117	9.09	11.97	0.108
	SP2	1960		none	142	211	4.22	10.90	0.194
	SP3	1966		none	108	114	1.22	15.79	0.381
	SP4	1966		none	96	100	0.04	6.00	0.690
	SP5	1966		none	42	66	0.48	24.24	0.625
Total area					465	608			mean ± s.e 0.399 ± 0.114

E. atherica on the older (65- to 72-year-old) marsh of Terschelling was much lower than on the younger marshes of Spiekeroog and Schiermonnikoog (Fig. 2.4). On Norderney, the rate of increase on the western salt-marsh section was similar to that found on Spiekeroog but a negative rate of increase was observed on the eastern sections.

Long-term dynamics of *Elytrigia atherica* community
The longest-running time series of detailed vegetation maps we were able to obtain were of Schiermonnikoog, taken from 1958 until 2004. In 1958, less than 1% of the vegetated salt marsh was dominated by the *E. atherica* community but this had increased to almost 25% of the ungrazed salt marsh in 2004 (section 1 in Table 2.3). In 1958, the vegetated salt marsh extended only until the eastern border of section 1. Eastward of section 1, only a few isolated dunes on sandbanks existed (Fig. 2.5). Van der Toorn (1959) identified 18 small stands of *E. atherica*, with areas ranging between 200 and 1200 m^2, on a map of the vegetated salt marsh. According to Neuteboom (1963), *E. atherica* occurred at high densities (35-50% cover) on the lower edges of old *Hippophae rhamnoides*-dominated dunes and higher regions of the *Festuca rubra* community on the salt marsh in section 1.

The surface area of *E. atherica* community, according to the SALT- typology, had doubled by 1984 (Fig. 2.5). This species had spread to the higher elevations of salt marshes

Table 2.3 Total cover area (ha) of the *E. atherica* community in sections with different periods of vegetation establishment (VE) and intrinsic rate of increase (yr^{-1}) on Schiermonnikoog and Terschelling, expressed in Hy (TMAP2004 typology). n/a = not applicable.

| | VE | 1958 | 1971 | 1992 | 1997 | 2004 | intrinsic rate of increase | | |
							1958-1971	1971-1992	1992-2004
Schiermonnikoog									
S1	1955	1	21	139	109	121	0.224	0.090	-0.012
S2	1964		0	21	9	30	n/a	n/a	0.030
S3	1974		0	10	11	16	n/a	n/a	0.039
S4	1986		0	3	8	23	n/a	n/a	0.170
S5	1996			0	1	7	n/a	n/a	n/a
Total		1	21	173	138	197	0.224	0.101	0.011
			1976		1999	2006		1976-1999	1999-2006
Terschelling									
T1	1934		5		28	34		0.079	0.028
T2	1934		40		54	67		0.013	0.031
T3	1934		40		48	83		0.008	0.078
T4	1934		10		52	76		0.072	0.054
T5	1934		9		42	58		0.069	0.046
T6	1934		1		12	18		0.134	0.058
Total			104		236	336		0.036	0.050
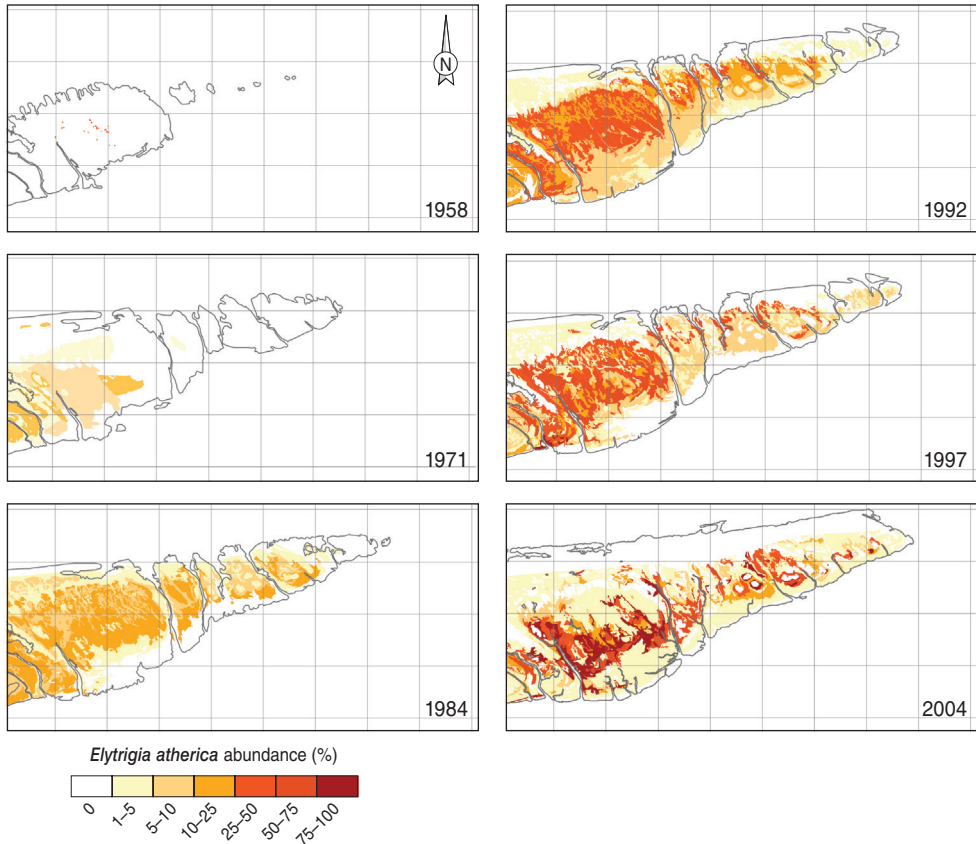
Figure 2.5 Distribution of the plant species, *E. atherica*, in the back-barrier salt marsh on the island of Schiermonnikoog during subsequent mappings during the period 1958 – 2004. Abundance of *E. atherica* is estimated by percentage plant cover, which in turn was calculated from the distribution of plant communities and their concurrent survey data. See Fig. 2.1 for the position of the artificial dune ridge. The grid scale is 1km × 1km.

which had been vegetated for at least ten years. On the older marshes (>50 yrs old), a strong increase in the dominance of *E. atherica* was observed. On the lower dunes and in the *Ammophila arenaria* community on seaward dune slopes, a high abundance of *E. atherica* (about 30%) was observed in the *Festuca rubra* community (Hf). However, *E. atherica* was not dominant enough for the vegetation to be classified as *E. atherica* community. The far eastern tip of the island (section 5) was classified as either dune, green beach vegetation or bare sand (Zonneveld *et al.* 1990).

By 1992, the *E. atherica* community covered almost 20% of the ungrazed salt marsh (von Asmuth & Tolman 1996). The first establishment of *E. atherica* stands on the younger salt marshes (those that had developed since 1986) were recorded. Along the levees of the local creek banks, however, the abundance was too low for the vegetation

to be assigned to the *E. atherica* community. While the expansion of *E. atherica* continued on the younger marshes (<50 yr), the spread stagnated at the oldest marsh of section 1 (Table 2.4). Concurrent with the expansion of the cover of the *E. atherica* community, there was an increase in abundance of *E. atherica* within communities. The highest intrinsic growth rate was found in the period between 1958 and 1971 (0.224 yr^{-1}) on the oldest marsh, which was 45 to 58 years old at that time. However, this section was released from grazing pressure by livestock in 1958. The growth rates recorded for the period from 1992 to 2004 generally decreased with the age of the marsh (Table 2.3). Also a high growth rate was found for the marsh between 1992 and 2004 in section 4, which was 23 to 30 years-old at the time.

For Terschelling, we were able to obtain detailed vegetation maps from 1976, 1999 and 2006 (App. S2.2). In 1976, a distinct gradient in *E. atherica* incidence from west to east along the salt marsh could be observed. The intrinsic rate of increase between 1976 and 1999 showed a similar pattern. In later years, succession stagnated and a gradient from west to east could no longer be observed (App. S2.2, Table 2.3). In sections T2 and T3, the *E. atherica* community covered about 15% of the salt-marsh surface in 1976, approximately 40 years after completion of the artificial dune ridge. In other sections of the marsh, the *E. atherica* community formed less than 10% of the cover. In 1999, values of abundance between 13% and 21% were measured for the *E. atherica* community (Table 2.2). In both 1999 and 2006, the expansion rate and abundance of *E. atherica* were comparable between all sections of the marsh. We found the highest intrinsic rate of increase in section T6 during the period between 1976 and 1999 (0.134 yr^{-1}) (Table 2.3). The mean rate of increase over all six sections in the period 1976–1999 was 0.063 yr^{-1} (± 0.02 s.e.) when the marsh was 42 to 65 years old. When the marsh on Terschelling was 65 to 72 years old (1999–2006), a comparable mean rate of increase was observed over all sections (0.049 yr^{-1} ± 0.007 s.e.).

Climax community *Elytrigia atherica*
By overlaying sequential vegetation maps of Schiermonnikoog, we were able to observe which plant communities were the predecessors and/or successors of the *E. atherica* community (Hy, *E. atherica* dominant or cover >50%). In 35% of the cases, the Hy community was the climax community during succession on the salt marsh. *E. atherica* mainly started to reach significant levels of abundance in the *Juncus gerardii* community, *Festuca rubra* community (Hf) and *Artemisia maritima* community (Hz). However, the developmental sequence depended upon the age of the salt marsh. On the salt marsh older than 50 yrs, the *A. maritima* community was the primary predecessor of the *E. atherica* community, whereas it was the *F. rubra* community on the younger marsh. In addition on the salt marsh older than 50 years, fluctuations in late successional plant communities were observed between years. Dominance of the species *E. atherica*, *F. rubra* and *J. gerardii* varied between the years, resulting in different classifications of plant communities. For example, locations where *F. rubra* communities were observed in 1992 were dominated by the *E. atherica* community in 1997, and again recorded as the *F. rubra* community in 2004. In the oldest section (S1), the *E. atherica* communities, which

include tall plants, did not maintain their position as the climax communities, but developed into low-statured *F. rubra- Agrostis stolonifera* communities (Hg) with small patches of *Phragmites australis* in waterlogged areas high on the marsh.

On Terschelling, an approximately 75-year-old salt marsh, the dominance of *E. atherica* as a species increased in spite of the observed low intrinsic rate of increase of the *E. atherica* community (Fig. 2.4). On higher elevated and well drained areas, such as found along the foot of the dunes and on the creek bank levees on the salt marsh, the cover reached up to 80%. In 1976, dominance by *E. atherica* was mainly observed on the dunes in sections 1 and 2. Despite the overall increase of *E. atherica* on the marsh of Terschelling in subsequent years, a decrease in dominance was observed at these dune locations. Within these dunes, the *E. atherica* community was succeeded by brackish, waterlogged dune-slack vegetation dominated by the *P. australis* community or brackish grasslands with *A. stolonifera* and *J. gerardii*. This vegetation change is comparable to the successional development found on the older inner marsh of Schiermonnikoog.

As only vegetation maps of 1997 and 2004 were available for Spiekeroog and Norderney, long-term vegetation changes could not be analysed on the German islands.

Discussion

We found different rates of expansion of *E. atherica* on natural salt marshes. The spread occurred at higher rates on young marshes than on older marshes. Young marshes have relatively high levels of vertical accretion, which is linked to greater nitrogen input into the soil, whereas older marshes have low levels of nutrient input (Van Wijnen & Bakker 2001). Since the amount of atmospheric deposition of nitrogen is similar on young and older marshes (Van Wijnen & Bakker 2000), we conclude that the spread of *E. atherica* is more likely due to natural processes than caused by anthropogenic input of nutrients. We suggest that the spread of *E. atherica* can be counteracted by waterlogging at older salt marshes.

In contrast, increase of atmospheric deposition of nitrogen has led to grass encroachment in coastal dunes (Veer 1997; Kooijman *et al.* 1998; Remke *et al.* 2009; Provoost *et al.* 2011). Coastal dunes have mineral and nutrient-poor soils. Studies have shown that it is the early stages of succession, such as open and semi-fixed dunes, which are most sensitive to atmospheric deposition of nitrogen. In later stages, such as fixed dune grasslands, rates of N-mineralisation exceed that of N-depostion and nitrogen is no longer the limiting nutrient (Veer 1997; Kooijman *et al.* 1998; Jones *et al.* 2004).

Increase and rate of expansion of the *Elytrigia atherica* community over time
On Schiermonnnikoog, Norderney and Spiekeroog, the marshes extended over time from west to east. Concurrently with the development of these islands, a decrease in expansion rate with increasing salt-marsh age has been observed. The establishment of the artificial dune ridge on Terschelling in 1934 initiated vegetation development over the entire eastern part of the salt marsh at the same time. Corresponding with this

trajectory of vegetation development, little variation was found in the expansion rate between the different sections of the salt marsh on Terschelling (Table 2.3).

In the earlier years of salt-marsh development on Schiermonnikoog (as seen from the historical vegetation map (Blaauw 1971)) and on the young marshes of Schiermonnikoog and Spiekeroog, a high intrinsic rate of spread (>0.2 yr^{-1}) was found, which is consistent with the competitive and clonal nature of *E. atherica* and its potential to spread rapidly. A similar rapid expansion of *E. atherica* has been observed at other study sites along the Wadden Sea coast (Leendertse *et al.* 1993; Dijkema *et al.* 2005). Young stands of *P. australis* along the coast of Chesapeake Bay also showed a similar pattern (Rice *et al.* 2000).

The expansion rates were lowest on the oldest salt marshes in this study. This suggests that the rate of expansion stagnates or decreases in old stands of *E. atherica* as fewer suitable sites remain for *E. atherica* to colonise. In the oldest section of Schiermonnikoog, *E. atherica* occupied nearly 60% of salt marsh in 2004 (Fig. 2.5). A similar levelling off in expansion, as a result of reaching equilibrium or simply because of limited space, has been found in old and well-established stands of *P. australis* (Rice *et al.* 2000) and *Spartina alterniflora* (Rice *et al.* 2000; Civille *et al.* 2005). Although the expansion rate stagnated at the older sites, the abundance of *E. atherica* actually increased.

Climax community of *Elytrigia atherica*

In the 1960s, concerns regarding the decrease of species diversity due to domination by competitive grasses were already stated by (Neuteboom 1963). However, the main species of concern during that period was the low-statured *F. rubra*. The increase in domination by grasses on salt marshes has gradually been acknowledged by nature managers and conservationists. Today, the tall *E. atherica* is the commonly acknowledged candidate to take over grasslands and is assumed to be the climax stage of salt-marsh vegetation on mainland and back-barrier island salt marshes (Dijkema *et al.* 2005).

Although the *E. atherica* community has been described as the climax stage of succession, a decrease of the *E. atherica* community was observed in 2004 on the oldest part of Schiermonnikoog at a large distance from the intertidal flats. The decrease of the *E. atherica* community may be related to local environmental changes. Esselink *et al.* (1998) and Schröder *et al.* (2002) have shown that there is a decreasing gradient of annual vertical accretion in relation to distance from the intertidal flat. The input of sediments is related to the frequency and duration of tidal innundations and, along with the sediments, organic matter including nitrogen is imported (Olff *et al.* 1997; Van Wijnen & Bakker 1997). A decrease of sediment input on older salt-marsh site may lead to a decrease of available nitrogen. Moreover, a decrease of sediment input accompanied by soil autocompaction may also lead to the formation of depressions with stagnant water. This process may be further enhanced by sea-level rise, and coinciding with poor drainage, will lead to waterlogged conditions after tidal inundations and rainfalls. On Schiermonnikoog and Terschelling, former *E. atherica* stands have recently been replaced by stands of *P. australis* and *J. gerardii*. This process occurred high on these marshes under conditions of waterlogging and little vertical accretion.

A comparable process, leading to the decline of *Elytrigia repens*, was caused by water-logging on brackish mainland salt marshes (Esselink *et al*. 2000). Laboratory experiments revealed that the root system of *E. atherica* is negatively affected by inundation (Bouma *et al*. 2001). This suggests reduced competitive abilities under saturated conditions. In order to unravel the effects of reduced sediment input, further field measurements and experiments are needed as the effects can be due to reduced elevational increase or reduced nutrient input.

Expansion rates on natural, back-barrier and man-made, mainland marshes
On salt marshes of the barrier islands, we found a high intrinsic rate of spread per year for *E. atherica* in young marshes up to the age of 30 years with a relatively high vertical accretion of 0.35 cm/yr and this decreased to low rates at older marshes with a very low vertical accretion of 0.15 cm yr^{-1}. These were marshes that had never been grazed by livestock. However, at old (>100 years) back-barrier salt marshes with no vertical accretion but a history of livestock grazing, an expansion of *E. atherica* stands to dominant levels, with a high intrinsic rate of spread, was recorded. This is in accordance with results collected from permanent plots 30 years after cessation of livestock grazing, whereby *E. atherica* showed a rapid expansion (from 1% cover to 60% cover) within the three years following the first 10 years of livestock abandonment (Veeneklaas *et al*. 2011). Also on man-made mainland marshes along the Dutch Wadden Sea coast, aged up to 100 years and with very high vertical accretion rates of about 1 cm yr^{-1}, high expansion rates of 0.10 yr^{-1} or greater were recorded (Dijkema *et al*. 2007) (Table 2.1). The high expansion rates at these salt marshes were all found at sites which had been released from livestock grazing. In a further study, we are aiming to disentangle the relationships between the spread of *E. atherica*, cessation of livestock grazing and vertical accretion in salt marshes.

Studies have shown that *E. atherica* is expanding on salt marshes in the past decade (Fig. 2.1) (Esselink *et al*. 2000; Dijkema *et al*. 2009). However, all these study sites showing this expansion at a landscape scale were on previously cattle-grazed salt marshes with sedimentation fields and drainage ditches. The comparison of the four back-barrier islands allowed us to study the spatial and temporal dynamics of the *E. atherica* community on natural salt marshes, which had never been grazed by livestock.

Conclusion

The decrease in annual expansion rate over time of the *E. atherica* community (shown by the sequential maps) is very comparable to the decreasing trend in annual expansion rate along the chronosequence of Schiermonnikoog and Spiekeroog. Contradictory to our first hypothesis, our findings show that the expansion of *E. atherica* is strongly related to the age of the salt marsh. Thus, we conclude that the rapid expansion of *E. atherica* along the Wadden Sea coast is not likely to be caused by increased anthropogenic nutrients but more likely a result of successional processes on young and/or

natural marshes, and changes in vertical accretion and/or cessation of livestock grazing in older natural marshes and marshes with sedimentation fields. Decrease of *E. atherica* community at the oldest marshes suggest that this community is not the climax stage and that there is a world beyond *E. atherica* at later successional stages in naturally developed salt marshes.

**Appendix S2.1** A list of the vegetation maps and descriptive reports used to study the increase of *E. atherica*. Depending on the available information, the data is formatted as TMAP, Ea >25%, Ea >50% (Salt97)1.

Island	Survey year	Scale	Type source	Data
Schiermonnikoog	1958	unknown	Map (van de Toorn 1959)	TMAP
	1963	unknown	Report (Neuteboom 1963)	
	1969	1:10000	Map + report (Blaauw 1971)	TMAP
	1973	1:1600	Report (Bosch & Huisman 1976)	
	1976	1:25000	Report (de Graaf 1978)	
	1984	1:10000	Map + report (Zonneveld *et al.* 1991)	TMAP, Ea>25%, Ea>50%
	1992	1:10000	Report (Prins 1992)	
	1992	1:12500	Map + report (von Asmuth and Tolman 1996)	TMAP, Ea>25%, Ea>50%
	1997	1:10000	Map + report (Gennip et al. 2000)	TMAP, Ea>25%, Ea>50%
	2004	1:10000	Map + report (Jager 2006)	TMAP, Ea>25%, Ea>50%
Terschelling	1976	1:5000	Map + report (Leeuw & Vuijk 1977)	TMAP, Ea>25%, Ea>50%
	1999	1:10000	Map + report (Gennip & Severijn 2002)	TMAP
	2006	1:10000	Map + report (Reitsma J.M. *et al.* 2008)	TMAP
Norderney	1997	1:10000	GIS (Ringot 1997)	TMAP
	2004	Pixel 1024 cm^2	GIS (Petersen 2006)	TMAP
Spiekeroog	1997	1:10000	GIS (Ringot 1997)	TMAP
	2004	Pixel 1024 cm^2	GIS (Petersen 2006)	TMAP

All relevant vegetation maps were provided in ArcGis format except for Blaauw (1971) and Leeuw & Vuijk (1977). The vegetation maps derived from the last two sources were digitized using a colour photograph and appended features retrieved from Blaauw (1971) and Prins (1992) for Schiermonnikoog, and Leeuw & Vuijk (1977) for Terschelling. Each vegetation map table contained per polygon the following features: i) original community (as described by the author of each map); ii) TMAP community-typology (Bakker *et al.* 2005); iii) cover percentage *E. atherica* >25% and co-dominant community per polygon (according to SALT97 (de Jong et al. 1998)); iv) cover percentage *E. atherica* >50% community per polygon (according to SALT97, (de Jong et al. 1998)); and v) total cover percentage of E. atherica, as species, per polygon (estimated in the field by Jager (2006)). Vegetation and area changes between years were analysed and calculated in ArcGis 9.3, using overlay, union and join commands. The area for plant communities dominated by *E. atherica* per polygon was calculated by multiplying the community cover percentage with the area of the polygon.

Appendix S2.1 Distribution of the plant species, *E. atherica*, in the back-barrier salt marsh on the island of Terschelling during subsequent mappings in 1976, 1999 and 2006. Abundance of *E. atherica* is given as percentages plant cover. The abundance has been estimated from the distribution of plant communities and their concurrent survey data. The grid scale is 1km × 1km.

Elytrigia atherica abundance (%)

0–10 11–25 26–50 51–75 76–100
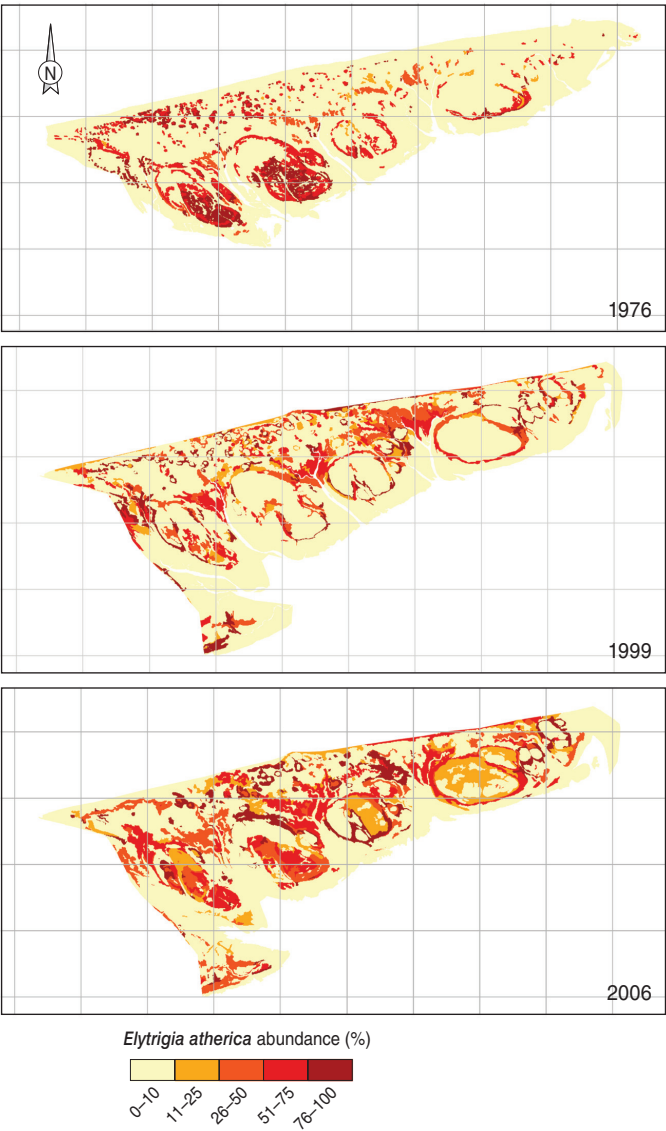
Chapter 3

Elytrigia atherica invasion of mainland marshes depends on abiotics and management

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Abstract In the past decades, due to economic motivations of farmers or changes in nature management goals, grazing with livestock has ceased on a great number of mainland salt marshes along the Wadden Sea coast. Most of these marshes had been established from sedimentation fields with intensive ditching. Concurrent with this management change, the tall native grass, *Elytrigia atherica*, has been increasing in both incidence and dominance. An increase of *E. atherica*, however, has not been observed on all abandoned mainland marshes. Our aim was to evaluate under which circumstances and for what reasons *E. atherica* will come to dominate a mainland salt marsh after cessation of livestock grazing. Long-term measurements on vegetation, elevation, sedimentation and drainage conditions allowed us to determine which factors apart from grazing management may affect the rate of increase of *E. atherica* on mainland salt marshes. Contrary to expectation, a negative relation was found between the dominance of *E. atherica* and the number of years since cessation of grazing. The incidence and dominance of *E. atherica* on mainland marshes was related to elevation and drainage conditions. This study shows that when abiotic conditions, namely inundation frequency and drainage, are optimal for *E. atherica*, this species can dominate the marsh within a few years. However, if drainage is neglected, this dominance does not occur.

Introduction

Coastal salt marshes provide a unique habitat for plant and animal life. For this reason, an increasing number of the European salt marshes have been designated as nature reserves or as a part of National Parks in the past decades (Esselink, Fresco, & Dijkema 2002; Stock 2003; Townend *et al.* 2011). Currently, all salt marshes in the Wadden Sea area (40,000 ha) are under the protection of national nature legislation (Essink *et al.* 2005). Although many salt marshes tend to have a 'natural' appearance, the majority of salt marshes along the mainland coast of the Wadden Sea result from human intervention (Dijkema 1983). Livestock grazing on European salt marshes can be traced back for couple of millennia (Davy, Bakker, & Figueroa 2009). During the past centuries, extensive areas of salt marsh were embanked for coastal protection and agricultural exploitation (Dijkema 1987). Since the 1960s, it has no longer been economically feasible to embank salt marshes for agriculture (Wolff 1992). However, the marshes are maintained for reasons of coastal and nature protection in front of the seawall, and partly continued to be grazed by livestock.

Traditionally the mainland marshes were intensively grazed by sheep or cattle. As a result the majority of these marshes were covered by an extremely short homogeneous vegetation of *Puccinellia maritima* or *Festuca rubra* (Dijkema 1983; Kiehl *et al.* 1996). Starting in the 1980s, due to economic motivations of farmers or changes in nature management goals, grazing by livestock was discontinued in many marshes, resulting in up to 60% of islands and up to 40% of mainland marshes in the Wadden Sea being ungrazed by 2008 (Esselink *et al.* 2009). Concurrently with this change in management regimes, namely cessation or reduction of livestock grazing in the past decades, *Elytrigia atherica* has increased in abundance on several salt marshes along the northwest European coast; for example at Mont Saint Michel, France (Valéry *et al.* 2004), The Wash, UK (Norris, Blair, & Johnson 2007) and Schleswig-Holstein, Germany (Stock *et al.* 2005; Esselink *et al.* 2009). By around 1980, approximately 8% of the salt marshes along the Dutch mainland coast were covered by *E. atherica* and this expanded to 17% coverage around 2000 (Dijkema *et al.* 2009; Esselink *et al.* 2009).

The spreading of *E. atherica* is a phenomenon due to natural succession on back-barrier island marshes without livestock grazing. During succession, the marshes become older and higher by accretion, developing into suitable habitat for late-successional species such as *E. atherica* (Olff *et al.* 1997; Veeneklaas *et al.* 2013). Also on mainland marshes, the incidence and dominance of *E. atherica* is positively related to elevation and rate of increase of surface elevation (Heinze, Kiehl, & Neuhaus 1999). Older island marshes are grazed by livestock as are mainland marshes and experimental exclusion of livestock resulted in the establishment and spreading of *E. atherica* after a decade (Bakker, Bos, & Vries 2003). An increase of *E. atherica*, however, has not been observed on all abandoned mainland marshes. For example, , *E. atherica* did not become dominant over whole the marsh in Hamburger Hallig (Germany) even after decades of cessation of grazing (Schröder, Kiehl, & Stock 2002).

Remaining salt marshes along the mainland coast of the Wadden Sea feature

sedimentation fields. These are surrounded by brushwood groynes and many still have a dense drainage system (Esselink *et al.* 2009). These sedimentation fields with brushwood groynes reduce wave energy and thus enhance trapping of suspended sediment by vegetation. The fields are comprised of an extensive system of a few large and many small ditches to allow inundation of the entire field. The intensive drainage system also facilitates fast discharge of the water, thus allowing oxygen to enter the soil and thus enhances development of the soil. Once the soil is firm and covered by dense salt-marsh vegetation, livestock grazing can be practiced. When grazing is ceased, the intensive drainage system is often no longer maintained. For example, after establishment of the National Park 'Schleswig-Holsteinisches Wattenmeer', the formerly intensive sheep grazing was strongly reduced or abandoned and also the maintenance of the artificial drainage system was neglected in the abandoned areas (Kiehl *et al.* 1996; Stock 1997). Furthermore, grazing was deemed no longer feasible and was stopped as a consequence of the cessation of the maintenance of the drainage system in 2000 on some marshes along the Dutch coast (personal observation Dijkema). Inundation frequency, as related to elevation and drainage conditions, was shown by Bockelmann *et al.* (2002) to be of great importance for the occurrence and dominance of *E. atherica*. Currently, artificial drainage by ditching is infrequently practiced: about 10% of island marshes and 30% of mainland marshes in the Wadden Sea were recently drained (i.e. < 10 years ago) (Esselink *et al.* 2009).

The main aim of this study is to reconcile the contradictory results from local studies demonstrating either strong dominance of *E. atherica* or hardly any increase at all after the cessation of livestock grazing. Thus, we will evaluate which factors affect the dominance of *E. atherica* on mainland salt marshes after cessation of livestock grazing in 33 transects spanning from the seawall to the intertidal flats along the German and Dutch Wadden Sea coast. We hypothesise that (i) the incidence and dominance of *E. atherica* increase over time after cessation of livestock grazing and that (ii) the probability of dominance of *E. atherica* after cessation of grazing depends on elevation, surface elevation change rates and drainage conditions.

Methods

Species

E. atherica is a hexaploid, wind-pollinated tall grass species with the ability of reproducing clonally as well as sexually (Bockelmann *et al.* 2003). The species is also known as *Agropyron pungens*, *Elymus athericus*, *Elymus pycnanthus*, or *Elytrigia pungens*. It occurs on continental European salt marshes along the coast from northern Portugal to southern Denmark, and along the southern coasts of the British Isles.

Study sites

We studied mainland salt marshes that had been developed with the aid of sedimentation fields along the Wadden Sea coast. The salt marshes are located along the Frisian

Figure 3.1 Location of the study sites.

and Groningen coast in the Netherlands and in National Park 'Schleswig-Holsteinisches Wattenmeer' in Germany (Fig. 3.1). The topography of the study sites is characterised by sedimentation fields with a dense artificial drainage system (Fig. 3.2) (Kiehl *et al.* 1996; Dijkema *et al.* 2011). For this study, we selected sites where the grazing management had been ceased for at least two or more consecutive years.

Data collection
On the German mainland marshes, 79 permanent plots (4 to 90 m2) were established along 19 transects from the seawall to the intertidal flats since 1991 for monitoring changes in plant communities. Eleven of the 19 transects were (partially) located on ungrazed marshes. The percentage cover of all plant species was recorded annually (Schröder, Kiehl, & Stock 2002; Stock 2012a). Adjacent to the permanent plots, surface elevation change was annually recorded using a sedimentation-erosion bar (Schröder & Lüning 2000; Stock 2012b). To correct for year-to-year differences, for example due to extreme storms, the mean annual surface elevation change was calculated over the period 2002-2008. In each permanent plot, elevation (relative to Mean High Tide) was measured in 2001, 2006 and 2012. For years when elevation was not measured, elevation was estimated using the mean annual surface elevation change.
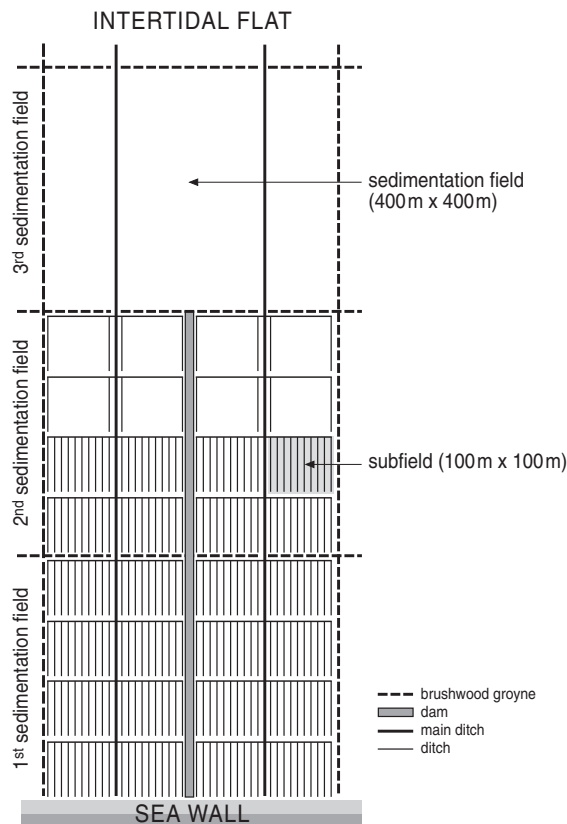
Figure 3.2 Scematic lay out of a mainland salt marsh, showing the intens drainaige system en brushwood groynes.

The mainland marshes along the Dutch coast have been monitored since 1960. Along the Frisian and Groningen coast, 25 transects were established. Of the 25 transects, 22 were (partially) located on ungrazed marshes. Each transect consists of a series of three to ten sedimentation fields (400m × 400m) from the seawall to the intertidal flats (Fig. 3.1). Each sedimentation field was further divided in 16 subfields of 1 ha, to distinguish whether the measured data applied for the entire sedimentation field or just part of it. For each subfield, the percentage cover of all plant species in the transects was recorded annually. Every four years, the elevation was measured along permanent lines within the subfields (Dijkema *et al.* 2011). For each subfield, the annual surface elevation change was estimated using the elevation data. The degree of ditch maintenance was annually estimated per subfield according to the following index: (1) ditches present but plot poorly drained, (2) ditches present, plot moderately drained, (3) ditches present, plot well drained. This classification allowed us to study the effect of drainage, elevation and sedimentation rate and the interaction between these factors on the incidence and abundance of *E. atherica*.

Data analysis

A database containing time series per plot was established, in which each plot represented a permanent sampling plot along the German coast or a subfield along the Dutch coast. In the analysis, only one year of the time series per plot was selected. The selected year was either the year when *E. atherica* cover had reached >50% or the final year of the time series if *E. atherica* did not come to dominate the specific plot. Years of abandonment were calculated until the selected year of the time series. Moreover, only plots within the elevation range of 0 – 100 cm (+MHT) were included in the analysis. This is because below 0 cm (+MHT), the plots can be assigned to the pioneer zone of the salt marsh, where the late successional species *E. atherica* will not occur. At higher elevations (above 100cm (+MHT)), the salt marsh develops into a brackish plant community and is less affected by tidal flooding. In total, 45 plots within eleven transects were analysed in the German marshes, and 65 plots within 22 transects in the Dutch marshes. When the vegetation surveys indicated that the cover of *E. atherica* was >25%, the plot was assigned to the vegetation type, *E. atherica*. A distinction between >25% and >50% cover was made within the vegetation type, *E. atherica*. The occurrence of *E. atherica* (cover >50%) in relation to duration of abandonment and local abiotic conditions was analysed using stepwise enter logistic regression models in SPSS 17.0 (SPSS Inc.).

Results

Site characteristics

On average, the selected sites had been abandoned for a period of at least ten years before *E. atherica* came to dominate the marsh. Along the German coast, salt marshes were abandoned for a longer period before *E. atherica* covered more than 50% of the marsh (Table 3.1). The mean elevation of the selected salt marshes was 42 cm above Mean High Tide. The elevation was lowest on the sedimentation fields along the coast of Groningen (Table 3.1). The mean annual sedimentation rate was highest along the Frisian coast.

Table 3.1 Characteristics of selected plots (4-90 m^2 in Germany, 1 ha in the Netherlands) between 0 cm and 100 cm above MHT after the cessation of grazing, along the mainland Wadden Sea coast during the year of selection. The selected year was either the year when *E. atherica* cover had reached >50% or the final year of the time series if *E. atherica* did come to dominate the specific plot.

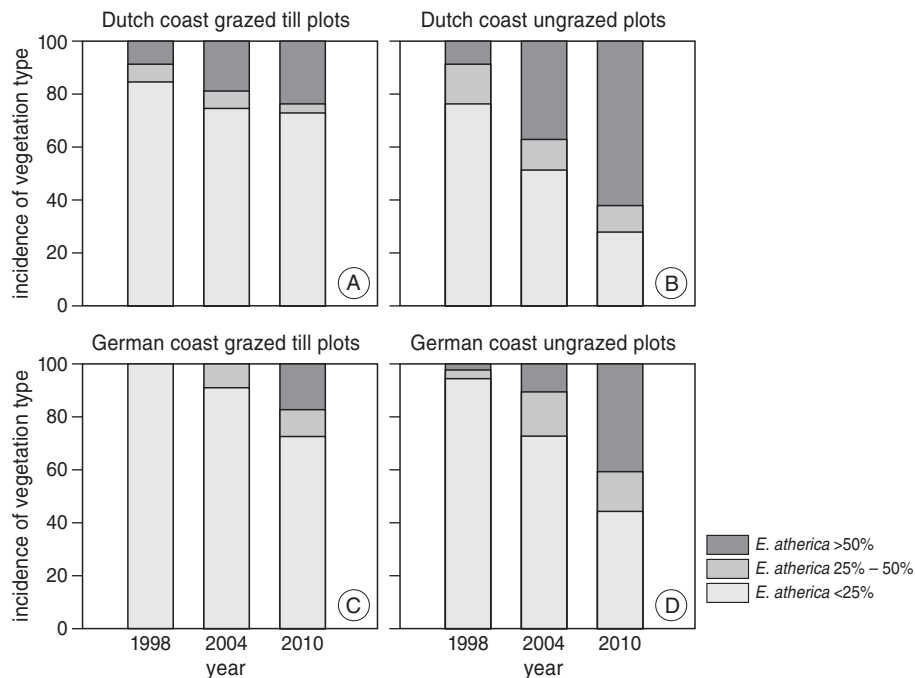|	Years after cessation of grazing mean ± s.e.	Max. years ungrazed	Elevation (cm + MHT) mean ± s.e.	Surface elevation change (mm/yr) mean ± s.e.	Drainage score mean ± s.e.	n
Friesland	7.0 ± 0.7	20	45.2 ± 3.2	17.8 ± 2.2	1.2 ± 0.17	32
Groningen	8.7 ± 0.8	20	36.1 ± 1.5	12.2 ± 2.1	2.1 ± 0.09	33
Germany	14.3 ± 0.9	21	44.7 ± 2.0	12.0 ± 1.2	n/a	45

Figure 3.3 Increase of *E. atherica* vegetation in monitored plots (located 0 – 100cm + MHT) along the Dutch and German mainland coast: A) plots along Dutch coast with grazing regime (n = 175); B) ungrazed plots along Dutch coast (n = 65); C) plots along German coast which grazing regime (n = 34); D) ungrazed plots along German coast (n = 45). The vegetation was categorised in three types.

Concurrent with the cessation of grazing, the maintenance of the drainage systems was often neglected. At all sites along the German coast, all maintenance of the drainage systems has been ceased and drainage conditions were not recorded during monitoring. Anecdotal observations of German salt marshes (personal communication Stock) indicate that on plots with low elevation, the artificial drainage system has partly filled up with sediment and drainage is blocked whereas on higher elevated locations, the drainage system is maintaining itself and functioning well. Along the Dutch coast, the maintenance of the drainage system differed per site and owner. On some Dutch sites, maintenance of the drainage system was stopped concurrently with cessation of the grazing regime. Drainage conditions were best maintained on the salt marshes of Groningen; on average these sedimentation fields remained moderately drained. After stopping maintenance of the drainage system, the drainage conditions also remained moderately good in Groningen. Some creeks even increased in depth and became a danger for live stock (personal observation Dijkema, www.kwelderherstelgroningen.nl). In contrast, the Frisian marshes were poorly drained (Table 3.1), and after cessation of maintenance, the remaining drainage systems gradually filled up with sediment.
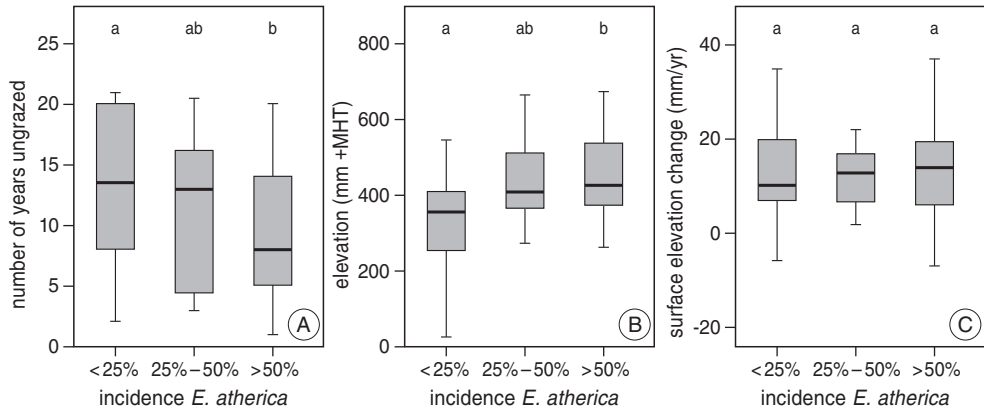
Figure 3.4 Incidence and cover of *E. atherica* (*E. atherica* cover <25%, *E. atherica* cover 25 – 50%, *E. atherica* cover >50%) in relation to A) number of years ungrazed, B) elevation and C) surface elevation change rate. Different letters show significant differences (p < 0.05) between means based on Tukey-test.

Dominance of *Elytriga atherica*

During a monitoring period of 12 yrs, an increase of *E. atherica* vegetation type was observed along all the mainland marshes. In 2010, approximately 50% of the ungrazed and 20% of the grazed mainland salt marshes were dominated by *E. atherica* (with cover >50%). The increase in incidence and dominance of *E. atherica* on the ungrazed marshes was up to 60% in the Dutch marshes (Fig. 3.3A,B), and up to 40% on the German marshes (Fig. 3.3C,D). In the German marshes, the spread of *E. atherica* has only become apparent during the past decade.

E. atherica in relation to grazing, elevation, surface elevation change

The data show a significant negative relation between dominance of *E. atherica* and years of abandonment (Wald 6.545, p < 0.01, n = 110) (Fig. 3.4A). The dominance of *E. atherica* was strongly positively related to the elevation of the salt marsh (Fig. 3.4B, Wald 10.739, p < 0.001, n = 110). The three different regions in the Wadden Sea showed this same relation. However, on the Frisian and German salt marshes, the average elevation of plots where *E. atherica* dominated the vegetation (>50%) is around 50 cm + MHT, whereas on the Groningen salt marshes, *E. atherica* was able to dominate the salt marsh at a lower elevation of around 40 cm + MHT. There was no significant relation between *E. atherica* and the rate of surface elevation change, or an interaction between surface elevation change and elevation.

E. atherica in relation to drainage conditions

As the drainage conditions were not scored on the German plots, the analysis on the effect of drainage was only conducted for the Dutch salt marshes. Neglect of the artificial drainage system clearly resulted in lower dominance of *E. atherica*. Better drainage
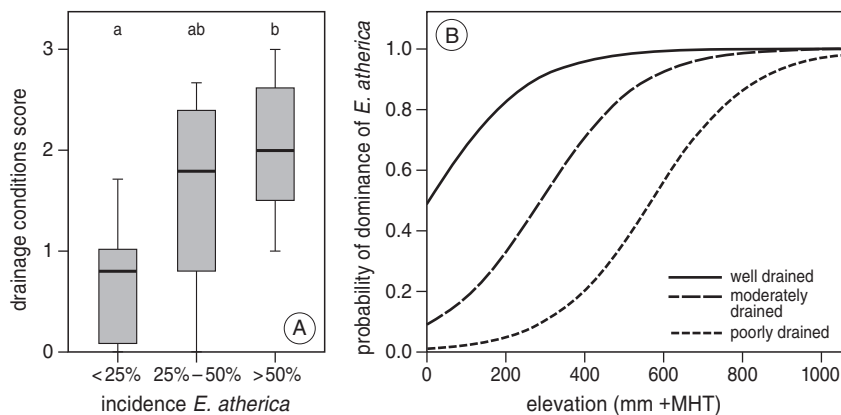
Figure 3.5 A) Incidence and cover of *E. atherica* (*E. atherica* cover <25%, *E. atherica* cover 25 – 50%, *E. atherica* cover >50%) in relation to drainage conditions (1 = poorly drained, 2 = moderately drained, 3 = well drained). Different letters show significant differences (p < 0.05) between means based on Tukey-test; b) Probability of *E. atherica* dominance per drainage category as a function of elevation.

conditions had a significant positive effect on *E. atherica* (Fig. 3.5A, Wald 15.144, p < 0.01, n = 65). Secondly, a trend (not significant) was observed that *E. atherica* was more dominant on plots with higher elevation. The data show that when sites are well drained, *E. atherica* can expand to dominate at lower elevations on the marsh (Fig. 3.5B). There was no significant relation between dominance of *E. atherica* and number of years abandoned or rate of surface elevation change when drainage conditions were taken into account.

Discussion

We hypothesised that the incidence and dominance of *E. atherica* increased with the number of years after cessation of grazing. Contradictory to our first hypothesis, our results revealed a significant negative relation between dominance of *E. atherica* and years of cessation of grazing on mainland salt marshes. With respect to our second hypothesis, the incidence and occurrence of *E. atherica* was slightly positively related to elevation, but not affected by the rate of surface elevation change. Better drainage conditions did have a positive effect on the incidence and dominance of *E. atherica*.

Period of cessation of grazing
The negative relation between the dominance of *E. atherica* and the number of years of cessation of grazing found in this study could be the result of the initial selection by farmers over which sedimentation fields would be abandoned along the coast. Economic and logistic reasons may affect the preference for abandoning certain sedimentation

fields. The first fields to be abandoned are most likely the sedimentations fields with the lowest production rate of palatable species or which are least suitable for livestock grazing due to poor drainage conditions. The abiotic conditions at these fields are also less optimal for *E. atherica*. Additionally, it is quite likely that the high-marsh sedimentation fields, more conveniently located adjacent to the sea wall, are maintained and grazed for a longer period. As a result of intensive grazing, the spread of *E. atherica* was controlled at these fields, and *E. atherica* remains present in low densities.

E. atherica is a successful clonal plant, able to spread extensive distances underground through rhizomes, and rapidly increases in dominance after abandonment (Veeneklaas *et al.* 2011). The observed negative relationship suggests that the increase of *E. atherica* is not affected by the duration of abandonment, but more likely by the abiotic conditions of the abandoned sites. If the abiotic conditions, i.e. inundation frequency depending on elevation and drainage, are optimal for the species, it is merely a matter of a couple of years for *E. atherica* to dominate the marsh, but if elevation is low and the artificial drainage of the marsh is diminishing, *E. atherica* will not be able to dominate the vegetation.

Surface elevation change and elevation
Surface elevation change encompasses two processes: it can increase the amount of nutrients in the soil, as suspended sediment transported by inundations (Olff *et al.* 1997); it can also increase elevation when sediment input is higher than auto compaction (Nolte *et al.* 2013). The results presented indicate that the incidence and dominance of *E. atherica* is not correlated with the rate of surface elevation change. A high number of studies have shown that salt-marsh succession is induced by sedimentation, since increased sediment deposition leads to increased nitrogen (N) accumulation (Roozen & Westhoff 1985; Leendertse, Rozema, & Andrea 1997; Olff *et al.* 1997). However, these studies were conducted on back-barrier marshes with a relative thin clay layer on top of a sandy sub-soil. Van Wijnen & Bakker (1997) showed that on back-barrier marshes, clay thickness is highly correlated with N-pool, and N-mineralisation (Bakker, Bouma, & Van Wijnen 2005), but overestimated at sites with thicker clay layers. Studies on mainland marshes did not find such a response of *E. atherica* to fertiliser application (Kiehl *et al.* 1997; Bockelmann & Neuhaus 1999, personal communications Kai Jensen 2010). It is likely that biomass production on mainland marshes cannot increase due to fertilisation as a result of self-shading (Kiehl, Esselink, & Bakker 1997). In accordance with the latter studies, our results suggest that nitrogen limitation does not play a role on mainland marshes and sedimentation as a nitrogen source is not a determinant of the incidence or dominance of *E. atherica*.

Even though sediment input may not directly affect the occurrence *E. atherica*, as mainland marshes are not nitrogen limited (Kiehl *et al.* 2001), the rate of surface elevation change does affect the elevation of the mainland marshes and therefore indirectly the occurrence of *E. atherica*. Stock (2011) measured positive surface elevation change rates of 20 mm/year close to the intertidal flats and hardly any change in surface elevation at a distance of 1 km from the intertidal flats. Concurrent to this, *E. atherica* did

spread close to the intertidal flats, but not at sites with no surface elevation change on ungrazed parts of the salt marsh (Schröder, Kiehl, & Stock 2002). The present study has shown that the dominance *E. atherica* is strongly correlated to elevation. The rate of surface elevation change is thus one predictor to estimate when a salt marsh will reach the elevation suitable for *E. atherica*.

Drainage

The present study has shown that the occurrence and dominance of *E. atherica* is strongly related to the drainage conditions of a salt marsh. The main purpose of a drainage system is to divert flooding seawater in order to promote soil aeration, which is one of the major factors controlling plant growth in salt marshes (Armstrong *et al.* 1985; Adam 1990; Bakker *et al.* 2002; Marani *et al.* 2006). Esselink *et al.* (2000) has shown that the neglect of artificial drainage systems clearly resulted in a lower drainage index of the permanent plots in later years. On a back-barrier marsh at great distance from the intertidal flats, a decrease of *E. atherica* was recorded. The species was succeeded by *Phragmites australis*, indicating waterlogged conditions (Veeneklaas *et al.* 2013). The low incidence of *E. atherica* on sites with waterlogged conditions, as observed by Schröder *et al.* (2002) and Veeneklaas *et al.* (2013), suggests that *E. atherica* does not like 'wet feet'. Bockelmann *et al.* (2002) also suggest that besides inundation frequency, inundation duration may be of great importance for plants. Personal observations in German and Dutch salt marshes have shown that on plots with low elevation, the artificial drainage system has partly filled up with sediment and drainage is blocked whereas on higher elevated locations, the drainage system is maintaining itself and functioning well. Thus, the cessation of ditch maintenance on the German salt marshes is one of the important factors explaining the slow invasion of *E. atherica* on those salt marshes compared to the Dutch marshes.

Conclusion

This study has shown that the incidence and dominance of *E. atherica* on mainland marshes is mainly determined by elevation and drainage conditions. *E. atherica* can occur at lower elevations when soil aeration is sufficient as a result of good drainage. As mainland marshes are not nitrogen limited, sedimentation is not of importance for nutrient supply. However, sedimentation rates should be incorporated in models in order to predict when the elevation increase of a salt marsh will result in a suitable habitat for *E. atherica*.

Chapter **4**

Effect of grazing and mowing on the clonal structure of *Elytrigia atherica*: a long-term study of abandoned and managed sites

Roos Veeneklaas
Anna Bockelmann
Thorsten Reusch
Jan Bakker

Abstract Physical disturbance by large herbivores can affect species diversity at the community level and concurrently genetic diversity at the species level. As seedling establishment is rarely observed in clonal plants, short-term experiments and demographic studies are unlikely to reveal the response of clonal plants to disturbances. A long-term (30-year) field experiment and the availability of molecular markers allowed us to investigate the clonal structure of populations of *Elytrigia atherica* subjected to different management regimes. The long-term field study provided us with five replicated blocks that had been subjected to three different management regimes, grazing by cattle, mowing and abandonment. In this study we examined the effects of herbivore grazing and mowing on clonal richness and genetic diversity of populations in salt marshes using multilocus microsatellite genotypes. In addition,phenotypic traits and spatial positions of *E. atherica* ramets were determined for 20 samples in a 5 × 10 m plot in each of the blocks. Abundance and phenotypic traits were affected by the management regimes, resulting in a higher abundance in abandoned fields and plants having shorter and narrower leaves in managed fields. Biomass removal did affect the clonal structure of populations and increased the genetic diversity compared to that in abandoned fields. However, no distinct difference was found between the two management regimes, mowing and grazing. Although seedling recruitment has rarely been observed, the present study shows that such rare events have occurred within the populations studied. Thus, molecular tools can greatly increase our understanding of vegetation dynamics and processes within populations growing under different conditions.

Introduction

Plant community structure, composition and dynamics can be greatly affected by land-use practices such as mowing or grazing by large herbivores (Kleijn & Steinger 2002, Ehrlén *et al.* 2005). Studies have shown that grazing, trampling and/or mowing can alter succession,plant species diversity, structural heterogeneity and productivity (Ritchie & Olff 1999). Large herbivore-exclusion experiments have shown that herbivores can promote or reduce species diversity (Bakker 1989, Crawley 1997, Olff & Ritchie 1998). Through biomass removal and trampling gaps are created in the existing vegetation. This mechanism reduces competition, enhances seedling recruitment and colonization and hence local plant species diversity (Collins & Barber 1986). Other studies have shown that herbivores can have a devastating effect on seedling survival (Bakker & De Vries 1992, Milchunas *et al.* 1992, Crawley 1997). There are a few detailed studies of the effect of grazing and mowing on population structure (Kölliker *et al.* 1998, Kotanen & Bergelson 2000, Bühler & Schmid 2001, Bockelmann 2002). These studies focus on monitoring the fate of individual plants and describing differences in phenology and morphology (Watkinson & Powell 1993). However, much more can be learned about the effect of disturbance on population structure.

Most clonal plant species combine vegetative and sexual reproduction and as a consequence have complex population structures. Secondly, grazing or mowing may have different affects on the reproductive strategies of plant species (Fernandez *et al.* 1992, Kerley *et al.* 1993). Some plant species respond to grazing by compensatory growth and producing more side shoots (ramets) (Mcnaughton 1983, Wallace *et al.* 1985), whereas other species may decrease or increase their seed production. As mentioned above, seedling recruitment may either be increased or limited by grazing. For many clonal species disturbances, such as grazing or fires, are needed for seedling establishment (Eriksson 1989) as these disturbances reduce the competition from the established vegetation (Eriksson 1997). When seedling establishment is rarely observed in clonal plant populations, the general assumption is that the population is dominated by a few large clones. However, genetic studies on clonal diversity have shown the contrary (Richards *et al.* 2004, Scheepens *et al.* 2007). Thus, analysis of spatial and genetic population structure is an important tool for inferring the most likely processes involved in any observed effect of disturbance (Kleijn & Steinger 2002).

Only a few studies have dealt with the effect of disturbance by management on the clonal diversity of plant populations (Kleijn & Steinger 2002, Reisch & Scheitler 2009). Both studied the effect of disturbance by comparing sites with different land use. Due to large distances between and the different environments at the sites, the treatments could not be treated as a pair. Experimental studies on the processes determining clonal structure and diversity are lacking, in particular at small spatial scales of several m^2, and at a large temporal scale of decades.

Salt marshes along the European Wadden Sea have been grazed since their establishment, around 1000 years A.C. Over the past few decades the management regimes of the salt marshes of the Wadden Sea have changed. The cessation of grazing of the salt

marsh has lead to the dominance of a few species, and hence a decrease in the number of plant communities and plant species (Van Wijnen & Bakker 1997). The native wheat grass *Elytrigia atherica* is one of the species that has been increasing in abundance and dominating as a result of this change in the management regime. The palatable, tall grass *E. atherica* is capable of both vegetative and sexual propagation. In 1972 a long-term field experiment was initiated to study the effects of management on species composition and diversity (Bakker 1989). Three different management regimes, i.e. grazing by cattle, mowing and abandonment were established and monitored for nearly 30 years in replicated blocks. While in the mown fields only biomass was removed, grazing by cattle additionally introduced trampling effects. The study by Bakker (1989) showed that abandonment of the fields resulted in a decrease in species diversity and increase in the abundance of *E. atherica* (Fig. 4.1).

This long-term field experiment and the availability of molecular markers provide a unique opportunity to study the effects of management regimes on the genetic structure of a clonal plant species. We used the polymorphism displayed by microsatellite markers to identify genets and determine the clonal structure and genetic diversity. The aim of the study is to address whether the clonal structure of *E. atherica* populations is affected by the different management regimes. As biomass removal may reduce competition and enhance seedling recruitment, a higher clonal diversity is expected in the grazing and mowing treatments. Mowing was carried out only once a year, whereas cattle remove biomass throughout the season by repeated grazing and trampling, hence leading to small-scale heterogeneity and more diversity in the grazed plots. This brings about the following questions: (i) How do the different management regimes (abandoned, grazing and mowing) affect the clonal diversity in a clonal palatable grass species? (ii) Do different management regimes affect species diversity in a way similar to their affect on the clonal structure and genetic diversity of *E. atherica*?

Methods

Study species
Elytrigia atherica (Link) Kerguélen ex Carreras Mart (*Triticeae*: *Poaceae*) is a tall-growing grass that reproduces clonally via rhizomes and sexually (Bockelmann *et al.* 2003). The species is native to salt marshes and occurs along the North Atlantic Coast from Northern Portugal to Southern Denmark. It is wind-pollinated, as are most *Poaceae*. The main dispersal units are spikelets, which bear up to five seeds (A. C. Bockelmann, personal observation.). Spikelets have no obvious dispersal mechanism. The species is hexaploid (6x = 2n = 42; Stace 1995, Hess *et al.* 1998). *Elytrigia atherica* is usually out-crossing (Dewey 1983) but is also self-compatible (Bockelmann 2002).

Study site
The present study was conducted on a salt marsh on the Dutch Frisian island of Schiermonnikoog (53°30'N, 6°10'E). The study site is situated in the western part of the salt

marsh and has an area of approximately 32 ha. This part of the salt marsh was grazed by young cattle until 1958, after which the salt marsh was abandoned. In 1972, the grazing regime (from beginning of May until end of October) was resumed in a fenced area with a stocking rate of 1.3 to 1.7 head of cattle/ha. Five distinctive plant communities, initially dominated by *Juncus maritimus, Festuca rubra/Armeria maritima, Elytrigia atherica, Festuca rubra/Limonium vulgare* and *Artemisia maritima*, respectively, were selected to study the changes in vegetation (Bakker & De Vries 1992). Within each plant community, concurrently with the introduction of the cattle, a mowing experiment was initiated in the un-grazed salt marsh adjacent to the fence (Bakker 1978, Bakker 1989). In each plant community there were three plots (5 × 10 m), adjacent to each other, assigned to cattle grazing, mowing in June and abandonment. The distance between the five blocks, each with three treatment plots, varied between 100 m and 400 m. Changes in the vegetation were recorded annually by scoring the cover of all species occurring in permanent quadrats of 2 × 2 m in each plot from 1972 onwards. In total 15 permanent quadrats, three treatments per plant community, and five distinct plant community vegetation relevés, based on the decimal scale (Londo 1976) were annually recorded. Plant species nomenclature follows Van der Meijden (2005).

The sampling in the present study was conducted in November 2000. In each plot the tiller density was sampled three times by randomly placing a 10 × 10 cm frame within the plot and counting the tillers within this frame. Within each plot phenotypic traits, such as shoot length, width and length of upper leaf were measured for ten randomly selected tillers. Secondly, in each plot 20 tillers of *E. atherica* were randomly selected for genetic analysis. The location (xy-coordinates) of these tillers was measured and drawn on a map. Young leaf tissue of these mapped tillers was collected and preserved for DNA extraction by drying with silica-gel. In total 300 tillers were selected for genetic analysis.

Genetic analysis

The distinction between genets and ramets within the sampled tillers of *Elytrigia atherica* was based on the microsatellite polymorphism displayed at four marker loci. For this study, four microsatellite markers originally designed for two other species of *Poaceae, Elymus caninus* (Sun *et al*. 1998) and *Triticum aestivum* (Röder *et al*. 1998), were used (Table 4.1). Detailed information on cross-species modification of these microsatellite primers is described by Bockelmann (2002) and Bockelmann *et al*. (2003). DNA was extracted following the CTAB method (Doyle & Doyle 1987). The extracts were purified and subjected to PCR with fluorescence-labelled primers following (Bockelmann *et al*. 2003). DNA samples were stored at –20°C until further analysis. The PCR products of primer pairs WMS6 and WMS44, and primer pairs of WMS2 and ECGA89, respectively, were pooled. The products were visualized on an ABI-377 automated sequencer (Applied Biosystems), together with an internal size standard (Gene Scan^{TM} –350 ROX ^{TM}, Applied Biostystem). To check the repeatability of the results positive and negative controls were always used. When errors were detected or weak DNA signal and/or banding pattern observed, amplifications were repeated. Samples with weak banding patterns for one of the four primers were not included in subsequent analyses.

Data analysis

As *E. atherica* is a hexaploid species a codominant analysis for microsatellite markers could not be applied (Bockelmann *et al.* 2003, Scheepens *et al.* 2007). Therefore, the banding patterns of the microsatellite markers for each individual sample were interpreted as multilocus fingerprints. To ensure repeatable and reliable genotype assignment, the scoring of polymorphic fragments was automated (Bonin *et al.* 2004). Polymorphic fragments were scored as present or absent, resulting in a multilocus genotype per ramet, using the software packages GENESCAN and GENOTYPER (Applied Bioystems). Secondly, the scoring data of identical multilocus genotypes, multilocus genotype pairs with a genetic distance of only two bands and a random subset of genotypes were manually checked. In the manual check not only the presence of fragments was scored, but also the amplification intensity of each fragment. As *E. atherica* is a polyploid species, the amplified fragments show different intensities. The chance of overestimating the number of clones was reduced by incorporating amplification when assigning identical multilocus genotypes.

The probability of a particular multilocus genotype is given by the product of the single locus genotype probabilities in random mating conditions $P_{dgen} = \Pi p_i$, in which p_i is the frequency of fragment presence at each locus in the observed multilocus genotype (Sydes & Peakall 1998). The probability of obtaining $n-1$ more copies of that genotype by chance is given by $(P_{dgen})^{n-1}$, where n is the number of times the genotype was observed (Mandel 2010).

Clonal richness for each plot was determined as the number of genets detected (G) divided by the sample size (N) (Kleijn & Steinger 2002). Simpson's index for diversity corrected for finite samples was used as a measure of genotypic diversity per plot, $D_G = 1 - \sum n_i (n_i - 1) / N (N - 1)$, where n_i is the number of individuals in the *i*-th genotype, N is sample size (Ellstrand & Roose 1987, Kleijn & Steinger 2002, Arnaud-Haond *et al.* 2007). Distance between identical genet pairs was measured using geographical information software (ArcGis 9.3).

For each phenotypic trait, the mean and standard error of the randomly selected tillers per plot were calculated. We applied analysis of variance to test for effects of treatment (abandoned, grazed and mown) on phenotypic traits and clonal structure using the software program SPSS 16 (2007) for statistical analyses. Transformations

Table 4.1 Microsatelites primer pairs of *Triticum aestivum* (WMS) and *Elymus caninus* (ECGA) used to asses multilocus genotypes of *Elytrigia atherica*.

Primer name	Repeats in orginal species	Repeats in *E. atherica*	Fragment size range (bp)	No. of fragments
WMS 2	$(CA)_{18}$	$(CA)_{10}$	185-295	5
WMS 6	$(GA)_{40}$	$(GA)_{15}$	138-164	12
WMS 44	$(GA)_{28}$	no data	115-147	9
ECGA 22	$(GA)_{17}$	$(GA)_{12}G(GA)_4$	190-208	7

were used in the analysis in order to adhere to assumptions of normality and homogeneity of variance, but the measured values were used to produce the figures. The response data was first tested in a general linear model, in which the different plots were fixed and the blocks (initial plant community) random factors. When the block effect was not significant, this random factor was emitted from the analysis.

Results

Effect of management on plant community

Although the experiment was initially (1972) set up in different plant communities, there were no significant differences between the communities in species diversity (one-way ANOVA, F = 0.41, df = 25, P = 0.8) or standing biomass in 2000 (one-way ANOVA, F = 1.901, df = 25, P = 0.148). However, the different management regimes resulted in changes in the initial communities. In 1972 *E. atherica* occurred only in one block, the *E. atherica* plant community. After 15 years *E. atherica* rapidly started to dominate the abandoned plots. This expansion resulted in the establishment of an *E. atherica* plant community, independent of the initial plant community, in all the abandoned plots (one-way ANOVA, F = 15.151, df = 13, P = 0,001, post hoc Tukey) (Fig. 4.1A). Over the years the species diversity (number of species in 4 m^2) diverted between the treatments. In 2000 the abandoned had the lowest species number and the grazed plots the highest number of species (one-way ANOVA, F = 14.258, df = 13, P = 0.001, post-hoc Tukey) (Fig. 4.1B). The initial plant community, as a random factor, had no significant effect on species diversity

Effect of management on plant morphology

In all management regimes *E. atherica* was present at widely varying densities. In the abandoned plots *E. atherica* formed monocultures, whereas in the grazed and mown plots its abundance (as percentage cover) was often <1% (Fig. 4.1A). The ramet density of *E. atherica* was at least twice as high in the abandoned plots than the managed plots, however not significantly so (Table 4.2). There were significant differences in the phenotypes recorded in the abandoned versus grazed and mown treatments. The mown and grazed plants had significantly shorter shoots and narrower leaves then the ramets on unmanaged plots (Table 4.2). Except for the length of the primary leaf, the initial plant community, as a random factor, had no significant effect on plant morphology.

Effect of management on clonal structure

A total of 38 different sized fragments were recorded among the four primer pairs that could be reliably scored when comparing duplicates and all were polymorphic. The highest number of polymorphic fragments was found at locus WMS 6, including 15 fragments, locus WMS44 showed nine fragments. The primer pairs WMS2 and ECGA89 amplified seven fragments each. The number of fragments scored per sampled ranged between 6 and 18. On average 11 amplified fragments were scored per sample.
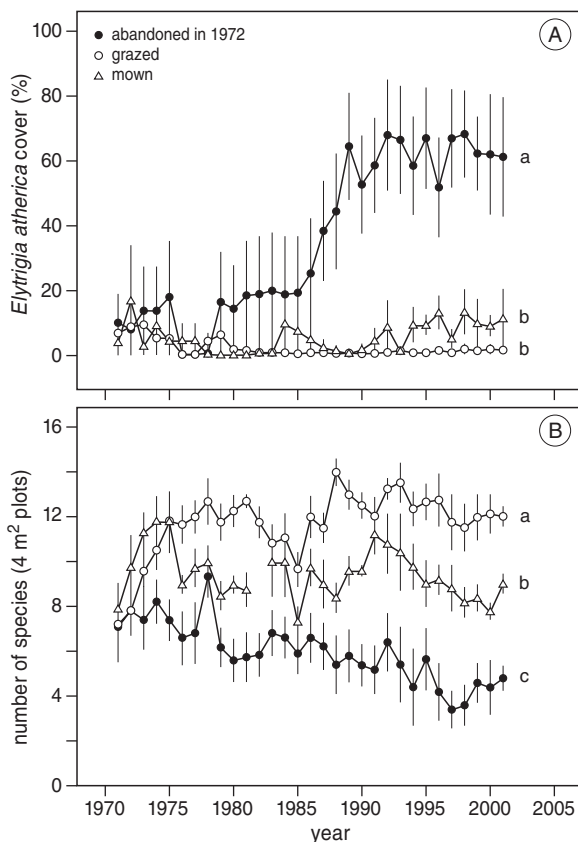
Figure 4.1 Effect of management on (A) the cover of *Elytrigia atherica* and (B) number of species in plots that were abandoned in 1972 (closed circles) and permanent quadrats (2 × 2 m) that were grazed by cattle (open circles) or mown (triangles). Different letters indicate significant (P < 0.05) differences in 2000.

Table 4.2 Phenotypic traits of *Elytrigia atherica* (n = 10) and tiller density (n = 3) in each management regime (N = 5). Data were analyzed using one-way ANOVA, density was log-transformed and leaf width square-root transformed. Differetne letters indicate significant differences (P < 0.01) between management regimes based on post hoc Tukey HSD test.

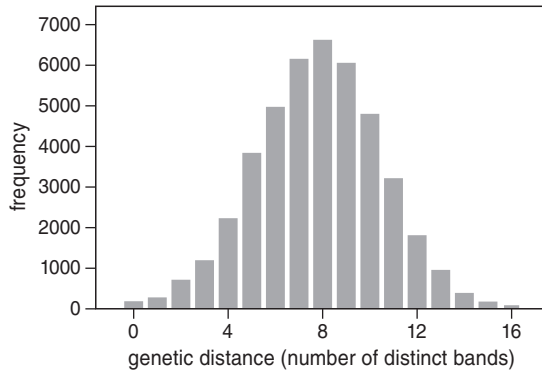|	Abandoned	Grazed	Mown	F-value	df
Density (#ramets/100 cm^2)	52.1 ± 9.6[a]	17.7 ± 8.2[a]	13.1 ± 2.7[a]	3.534	14
Leaf number per tiller	5.12 ± 0.25[a]	4.28 ± 0.24[a]	4.64 ± 0.19[a]	3.372	14
Shoot length (cm)	53.4 ± 2.4[a]	23.6 ± 3.1[b]	23.8 ± 1.9[b]	50.767	14
Leaf width (cm)	4.45 ± 0.14[a]	3.33 ± 0.15[b]	3.13 ± 0.13[b]	25.330	14

Figure 4.2 Frequency distribution of band differences among 296 different *Elytrigia atherica* multi-locus genotypes based on four microsatellite loci.
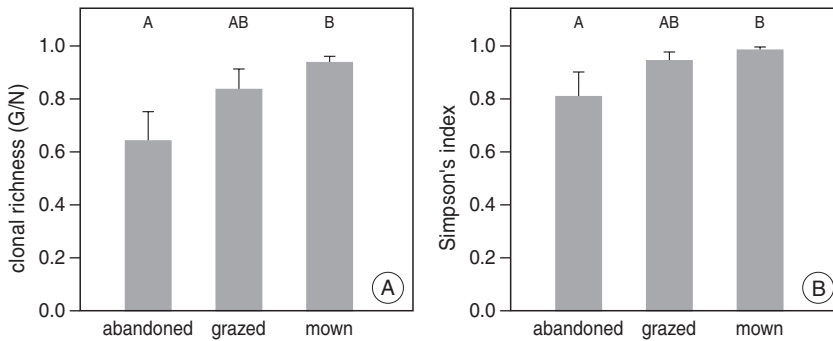

Figure 4.3 Effect of management regime, i.e. abandoned, grazed or mown, on the clonal diversity of *Elytrigia atherica*, measured in terms of (A) clonal richness and (B) the Simpson diversity index. The bars represent the means for 20 tillers and standard errors for the results for the different management regimes, and those with different letters differ significantly ($P < 0.05$), based on Mann-Whitney U tests.

Among 296 samples a total of 236 different multilocus genotypes were found, resulting in a grand clonal diversity of 0.77. A histogram of genetic distances among multilocus genotypes shows band differences between multilocus genotypes are large (Fig. 4.2). At least 95% of the multilocus genotype pair combinations differed in more than two fragments. This 95% interval was also used as the threshold genetic distance. Multilocus genotype pairs that differed in only two fragments were checked and assigned manually.

The probability P_{gen} values ranged from 0.03 to 1.3×10^{-6}. The values of $P_{dgen}n^{-1}$, the probability of drawing more than one copy of a certain multilocus genotype from a randomly mating population, were always lower than 0.05 (range from 0.03 to $2.3 \times$
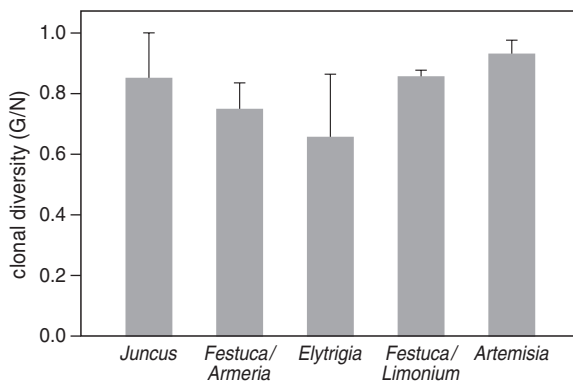
Figure 4.4 Comparison of the clonal richness of *Elytrigia atherica* in different plant communities. The bars represent mean clonal richness and standard error recorded in the three management regimes within each plant community. There are no significant differences in clonal richness in the different plant communities.

10^{-20}). This implies that multiple occurring multilocus genotypes were always considered to originate from clonal reproduction. The clonal richness, represented by G/N, was significantly higher in the managed than the abandoned plots (Kruskal-Wallis, χ^2 = 6.54, df = 2, P =0.04, Fig. 4.3A). In the abandoned plots more than 40% of the shoots were of clonal origin, whereas in the managed plots less than 20% originated from vegetative propagation. In comparison with the other plant communities, the G/N was relatively low in the *E. atherica* plant community, which had a G/N ranging from 0.75 to 0.93. However, the difference between the plant communities was not significant. In the abandoned *E. atherica* plot there were only five genets in the 19 ramets (G/N = 0.66, Fig. 4.4).
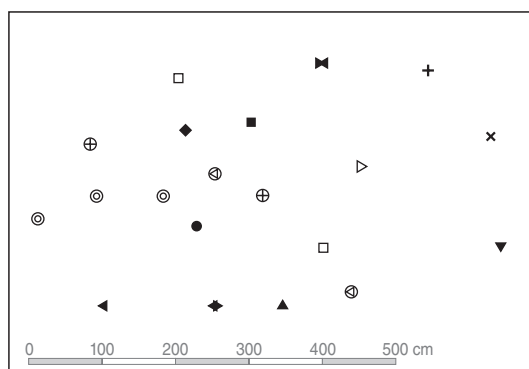

Figure 4.5 Distribution of clones in the abandoned plot in the *Festuca/Armeria* community, an example of intermingled plots. Open circles with identical inner symbols represent ramets with identical genets. Closed symbols represent unique ramets.
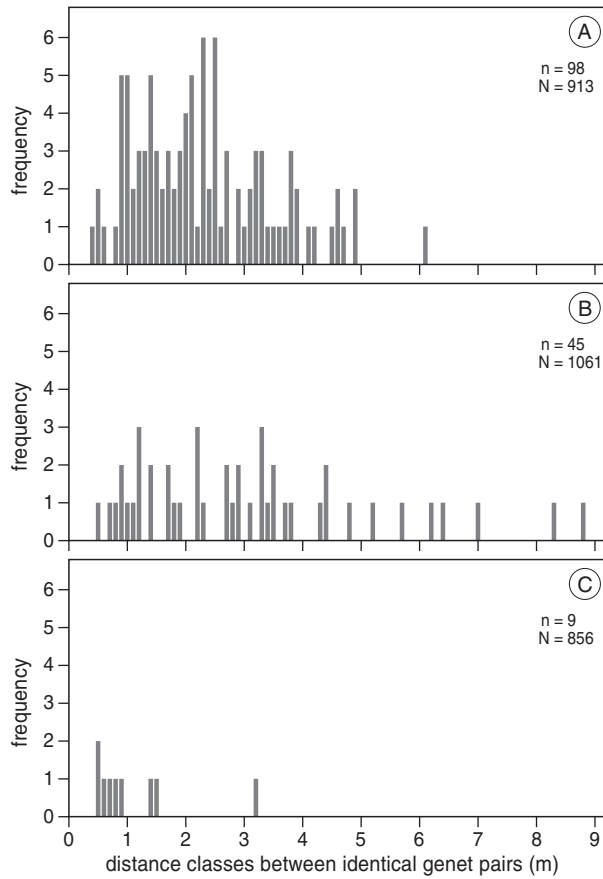
Figure 4.6 Frequency distribution of distances between ramets of the same genet in (A) abandoned, (B) grazed and (C) mown plots, with the number of identical genet-pairs indicated by n and total sample-pair combinations possible by N.

Table 4.3 Effect of management regime on the clonal structure of *E. atherica* based on 20 tillers per management regime. Clone size is the mean number of ramets per genotype (± S.E.). The mean distance between identical genotypes is expressed in terms of clone distance (m), and the maximum distance between two identical genotypes in each treatment. Data were analysed by Kruskal-Wallis test, for which the χ^2 and df are given: different letters row-wise indicate significant (P < 0.05) differences.

	Abandoned	Grazed	Mown	χ^2	df	P
Clone size	1.87 ± 0.47[a]	1.23 ± 0.12[ab]	1.07 ± 0.02[b]	6.54	2	0.04
Mean clone distance (m)	2.19 ± 0.21	3.90 ± 0.88	1.25 ± 0.41	5.23	2	0.07
Maximum distance between identical genets (m)	3.50	8.80	3.15	-	-	-

The Simpson's diversity index was very high in all management regimes. It was significantly higher in the mown than the abandoned plots (Kruskal-Wallis, $\chi^2 = 6.06$, df = 2, P = 0.05). Grazing resulted in an intermediate Simpson's diversity (Fig. 4.3B). For both clonal richness and Simpson's diversity, the grazed plots were intermediate to the abandoned and mown plots (Fig. 4.3A, B). The initial plant community, as a random factor, had no significant effect on genetic diversity or richness parameters. Sometimes clones grew intermingled within plots and between plots (Fig. 4.5). Twice, a pair of identical genets was found to occur in two fields with different management regimes. The distance between one of these pairs was 41m, which occurred in the abandoned and grazed *E. atherica* community plot. This pair had a genotype with low likelihood of occurring by chance ($P_{gen} = 0.0028$). Most pairs of identical genets (n) were found in the abandoned plots, $\approx$ 11% of all possible sample-pair combinations (N), with an average clone size of 1.87 ramets per genet (Table 4.2, Fig. 4.6). In the grazed and mown, 4% and 1%, respectively, of the sample-pairs had an identical genotype (Fig. 4.6). The lowest average distance between identical genet pairs was found in the mown plots (Table 4.3, Fig. 4.6).The observed minimum distance between identical genet pairs was 36 cm.

Discussion

Consistent with the initial hypothesis, we found higher clonal richness (as G/N) in mown plots compared to abandoned plots. Although the expectation was that the abandoned and managed plots would differ, the grazed plots were intermediate between abandoned and mown plots for both clonal richness and in terms of Simpson's diversity index. Apparently, the clonal structure is not only affected by biomass removal by herbivores, but possibly also by other mechanisms.

Clonal diversity
Elytrigia atherica dominated the abandoned plots, while this species was rare in grazed and mown plots. As *E. atherica* is a tall grass, its high abundance corresponds to a high standing biomass throughout the year with concomitant competitive suppression of seedling recruitment. The apparent differences in canopy height between the managed and abandoned plots could have resulted in the difference in clonal structure between the mown and abandoned plots. Two possible processes could explain the low clonal diversity in the tall and dense canopy of the *E. atherica* community in the unmanaged plots. First,the high standing biomass could limit seedling recruitment, as competition for light will be high (Wilson & Tilman 1991, Bakker & De Vries 1992). In many clonal plant species seedling recruitment is highly dependent on disturbance, e.g. removal of above-ground biomass (Watkinson & Powell 1993, Eriksson & Jakobsson 1998). Secondly, disturbance, like mowing and grazing, may enhance the transport of seeds and bulbils (Mouissie *et al.* 2005, Reisch & Scheitler 2009). Thirdly, with the increase in the number of individuals there is an increase in intraspecific competition among adult

plants (Bengtson 2003), which may result in a loss of genets and decrease in clonal diversity (Gray *et al.* 1979, Watkinson & Powell 1993). Low genetic diversities resulting from intraspecific competition are recorded for various species at different timescales: *Lolium perenne* (Mcneilly & Roose 1984), *Calamagrostis epigejos* (Lehmann 1997), *Spartina alterniflora* (Travis & Hester 2005) and *Elytrigia atherica* (Scheepens *et al.* 2007).

Contrasting effects of grazing and mowing on clonal diversity

A higher clonal diversity was expected in the grazed than in the mown areas. Mowing took place once a year, whereas cattle may remove biomass throughout the season by repeated grazing and trampling leading to small scale heterogeneity and hence a greater species diversity in the grazed plots. However, in the present study there was no difference in species diversity in the different management regimes. Kleijn & Steinger (2002) found different effects of mowing and grazing on the clonal structure of *Veratum album*. The low clonal diversity in grazed populations of *V. album* is most likely due to large herbivores avoiding this toxic species and as a consequence enhancing its vegetative reproduction. *Elytrigia atherica* is a palatable species for cattle therefore selective removal did not play a role in this study.

Large herbivores often have positive effects on seedling recruitment by opening up the vegetation through grazing and creating gaps through trampling (Bakker 1987, Silvertown & Smith 1988, Bullock *et al.* 1994). The percentage of bare soil was highest in the grazed parts of the salt marsh site and seed germination of lower marsh species is higher in the grazed plots (Bakker & De Vries 1992). Gap size can affect seedling establishment (Silvertown & Smith 1988, Watt & Gibson 1988). The amount of bare soil ranged between 20% and 40% in both grazed and mown treatments at this salt-marsh study site (Bakker & De Vries 1992). Therefore, it is unlikely that gap size affected the clonal structure in the present study.

Despite the higher germination recorded in the grazed plots, Bakker & De Vries (1992) found a higher seedling survival in mown than in grazed plots at the present study site. In grasslands, small-scale disturbances may enhance germination but not necessarily seedling survival (Eriksson 1997). In *Trifolium repens,* disturbance enhances germination but the survival of seedlings was higher in undisturbed vegetation (Barrett & Silander 1992). Kuijper et al (2004) show that when neighbouring plants are removed the survival of seedlings of *E. atherica* was lower than when subject to competition from neighbouring plants. The mown regimes are low disturbance areas, as the vegetation was cut and removed only once annually, whereas cattle grazed the site from early May till end October. The destruction of seedlings by trampling might explain the higher survival in the mown than the grazed plots in several instances, especially in the heavily grazed *Festuca-Limonium* community (Bakker & De Vries 1992).

Effect of management on clonal structure

Watkinson & Powell (1993) predict using a simulation model, that genet density of *Ranunculus repens* is higher in areas of high seedling recruitment whereas inequality of clone size tends to be higher in areas of low seedling recruitment. Kerley *et al.* (1993),

Kleijn & Steinger (2002) and Liston *et al.* (2003) record a larger clone size in the presence of grazers. A greater clone size in undisturbed populations is also recorded for *Calamagrostis epigejos* (Lehmann 1997) and *Lolium perenne* (Mcneilly & Roose 1984). Reusch (2006) records enhanced seedling recruitment in response to physical disturbance in *Zostera marina*. Conversely, in populations of *Puccinellia maritima* the mean clone size is larger in grazed than undisturbed habitats and seedling establishment more frequent in un-grazed marsh, unlike that recorded in our study (Gray *et al.* 1979). In the present study we observed an inequality of clone size when there was little disturbance and intraspecific competition as on the abandoned marsh. The single mowing event reduces competition and creates a window of opportunity for seedling recruitment. The presence of large numbers of small sized genets and the highest total number of genets in the mown plots support this. Although disturbance by cattle create windows of opportunity for seedling recruitment the continuous nature of the disturbance reduces the chance of successful recruitment. This is supported by the few but large clones in the grazed areas, where the greatest distance between identical clones was observed (Fig. 4.4). In conclusion, this study indicates that management does have an effect on clonal structure, but there is a threshold. Disturbance can enhance seedling recruitment but too much disturbance will enhance clonal dispersal.

Conclusions

Few experimental studies have focused on the effect of disturbance on genet dynamics, recruitment or genotypic diversity, and none that we are aware of deal with medium temporal (i.e. 30 years) and spatial (100 m^2) scales as in the present study. The studies discussed above mention an effect of disturbance on genet dynamics and recruitment. Only in some of these studies was a significant effect of management on the genetic diversity and differentiation between populations recorded. Lehman (1997), Liston *et al.* (2003) and Reusch (2006) did find any differences in clonal structure of populations subject to disturbance. However, this effect was also weak, which will be reflected in clonal diversity. Despite, the large demographic differences recorded in populations of *Brachypodium pinnatum* (Schlapfer & Fischer 1998), *Piptochaetium napostaense* (Tomas *et al.* 2000) and *Festuca idahoensis* (Matlaga & Karoly 2004) caused by different grassland managements, the clonal diversity or genetic variation was unaffected by the treatments. The timescale of these manipulative studies, ranging from three years (Reusch 2006) to 64 years (Matlaga & Karoly 2004), could be for some populations or species too short to observe such changes in clonal diversity or genetic differentiation. Other studies suggest that the morphological differentiation between plants with different grazing histories seems to be the outcome of a phenotypically plastic response of adapted genotypes.

In the *E. atherica* populations studied there was a clear difference in clonal structure and clonal diversity in response to different management over a period of nearly 30 years. Using molecular tools to study the clonal structure of populations can help us

understand and unravel the dynamics and processes in populations subject to different conditions. As hypothesized biomass removal does affect the clonal structure of populations and increases the genetic diversity. However, no distinct differences were recorded for the two management regimes, mowing and grazing.

Chapter 5

Clonal structure of *Elytrigia atherica* along different successional stages of a salt marsh

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L. van de Zande
J. P. Bakker

Abstract   *Elytrigia atherica* is a tall clonal grass species typical of higher salt marshes, but is gradually invading to the lower marshes. At young successional stages of a salt marsh, *E. atherica* is found sparsely dispersed in small groups of ramets. These patches increase in size and ramet density over time, eventually forming extensive swards as succession proceeds. This study investigates the change in the clonal diversity of *E. atherica* stands during colonization as a result of its reproductive strategy. Clonal diversities of differently sized patches of *E. atherica* were investigated on two lower salt-marsh sites of different age, 25 and 35 yrs respectively. Microsatellite fingerprint patterns were used to determine genet identities and to estimate relatedness and genetic differentiation between the sites, between patches within sites and within patches. The majority of the patches on both sites contained more than one genet. On the older site the clonal diversity was higher than on the younger site. However, the clonal diversity tended to decrease with increasing patch size. Low genetic differentiation was found between the two sites, indicating habitat differentiation, whereas differentiation between patches within sites was high. It is reasoned that different environmental conditions could have resulted in different clonal structures: On an older marsh, the increase of successful seedling recruitment, due to more suitable environmental conditions, leads to an increase in clonal diversity. Over time, with increasing ramet density, intra-specific competition is likely to increase, resulting in a decrease of clonal diversity.

Introduction

Many clonal plant species are able to propagate both sexually (creating new genets from sexually produced seed) and vegetatively (producing genetically identical ramets, which are potentially independent plant units) (Cook 1983). It has been proposed that different modes of propagation are favourable under different environmental conditions encountered during the life of an individual or a population (Olivieri *et al.* 1995, Eriksson 1997). This advantage may be expressed at the genetic as well as the ecological level (Ellstrand & Roose 1987). For example, vegetative reproduction has the advantages of physiological aid from the mother plant to the daughter plant through vegetative connectors (D'Hertefeldt & Jonsdottir 1999) and absence of the costs of sexual reproduction (e.g. flowering, seed production) (Doust & Laporte 1991, Ronsheim & Bever 2000, Rydgren & Okland 2003). On the other hand, seed dispersal is a key component of a species capacity to establish populations in newly created patches of habitat (Piquot *et al.* 1998). Hence, long distance seed dispersal is a means of escaping the competitive effect of clonal crowding and kin competition (Eriksson 1997, Howe & Miriti 2004). This suggests that the recent history of a population of plants capable of both clonal and sexual reproduction is written in its genetic structure as the consequence of past environmetal conditions..

The balance between sexual and vegetative reproduction, i.e. the relative production of sexually and vegetatively produced offspring by individual plants, will determine the clonal diversity of the population (Watkinson & Powell 1993, Eriksson 1997). First, life-history traits of the species determine the allocation to both types of propagation. Second, external factors such as seedling and stochastic mortality, competition and herbivory may have effects on clonal diversity. Biotic and abiotic factors such as inter-specific competition or facilitation and nutrient availability may affect this balance as well. Thus, the balance between sexual and vegetative reproduction is dynamic and may show spatio-temporal dependence on the prevailing environmental conditions (Mandujano *et al.* 1998, Nabe-Nielsen & Hall 2002).

The wheatgrass species, *Elytrigia atherica*, is capable of both vegetative and sexual reproduction. In the past, the occurrence of *E. atherica* was limited to the higher elevations of salt marshes (Bakker 1989, Van Wijnen *et al.* 1997). However, during the past three decades, the species has been expanding exponentially at higher elevations in many European salt marshes (Bakker *et al.* 1993). This increase in *E. atherica* has led to a concurrent decrease in species diversity (Leendertse *et al.* 1997, Bockelmann *et al.* 2003). In addition, the species has recently been expanding its range rapidly by invading lower elevations of the marshes (Bockelmann 2002, R.M. Veeneklaas unpublished data),.

At young successional stages with low elevations, *E. atherica* can be found sparsely dispersed in groups of ramets that we will refer to as patches. Patches may establish from a single, or a few seedlings, followed by vegetative spread. These patches increase in size and ramet density over time (R.M. Veeneklaas unpublished data). Such densely packed patches may trap dispersed seeds during tidal inundations. Eventually the

patches merge and form extensive swards in late successional stages, dominating most other salt-marsh species (Olff *et al.* 1997, Van Wijnen *et al.* 1997). Based on the observation that both biotic (e.g. herbivory, Kuijper *et al.* 2004) and abiotic (e.g. nutrient availability, Van Wijnen & Bakker 1999) conditions acting on *E. atherica* are very dynamic over time, it is likely that the clonal diversity of *E. atherica* stands during colonisation will change as a result of a changing reproductive strategy (Kik *et al.* 1990). In addition, autonomous patch processes, such as the increase of intra-specific competition in denser patches (Tilman 1988), may influence the clonal diversity. It is, however, difficult to predict the direction of change in clonal diversity during succession, as some environmental factors could have opposing effects or have different influences on vegetative or sexual reproduction (Kik *et al.* 1990).

Previously, studies on the genetic diversity of *E. atherica* in European salt marshes were conducted on macrogeographical scales (Bockelmann *et al.* 2003, Refoufi & Esnault 2006). However, a specific study aimed at clonal diversity on a microgeographical scale is necessary in order to understand the clonal structure of invasive, clonal plants such as *E. atherica*. The aim of this study is to assess 1) what mode of propagation, sexual or vegetative, governs the successful spread of the species in new habitats, and 2) whether there is a change in the balance of sexual versus vegetative reproduction as succession proceeds? To this end the clonal diversity of *E. atherica* on two adjacent sites, differing in successional stage and consequently in environmental conditions but within one salt-marsh system will be measured. Genets will be distinguished by using microsatellite markers as DNA fingerprints (Bockelmann *et al.* 2003). Relatedness and genetic differentiation between genets and groups of genets will be estimated to assess if differences in environmental conditions between the two sites pose different selection pressures on the individuals (Stanton *et al.* 1997).

Materials and Methods

Species, sites and sampling design

Elytrigia atherica (Triticeae: Poaceae) (Link) Kerguélen ex Carreras Mart. (sensu (Van der Meijden 2005) is a hexaploid, tall-growing, wind-pollinator with the ability of reproducing clonally as well as sexually (Bockelmann *et al.* 2003). It occurs on salt marshes along the coast from northern Portugal to southern Denmark and along the southern coasts of the British Isles.

To study the clonal structure at a microgeographical scale of *E. atherica* along a successional gradient, we selected two adjacent salt-marsh sites, deferring in successional stages, but within one salt-marsh system. The study sites are situated on the Oosterkwelder of the Dutch back-barrier island of Schiermonnikoog (53°30'N, 6°10'E). The island of Schiermonnikoog gives us the unique possibility to investigate the long-term succession of *E. atherica* by space for time substitution within one salt-marsh system. The island grows eastwards due to tidal sand deposition on the eastside of the island as a consequence of the dominant sea currents. This has resulted in a chronosequence of

salt-marsh sites in the shelter of the island (Bakker 1989, Olff *et al.* 1997). Another advantage of this study site is that the eastern part of the island has not been affected by human impact such as artificial sand walls or livestock grazing.

In the present study, two ungrazed salt-marsh sites of 25 and 35 years old, respectively, were chosen. In 1999 the first seedlings of *E. atherica* were observed at the lower marsh of the young site. Unfortunately it is unknown when the first seedlings established at the lower marsh of the older site. For this reason, the salt marsh age is indicated as the time since initial colonisation by terrestrial plants (information based on aerial photographs) (Olff *et al.* 1997). The two sites were situated approximately 1500 metres apart. Three blocks per site were established with approximately 20 metres distance between the blocks, each covering 2500 m^2. The occurrence of *E. atherica* was mapped in each block and ramet densities (m^{-2}) in the centre of each patch were determined. A patch was defined when neighbouring ramets were standing within 50 cm of each other. On the younger site, *E. atherica* occurs in many, predominantly small patches (<5 m^2), whereas on the older site, patch sizes are more evenly distributed over size classes (Table 5.1). In addition, *E. atherica* is more abundant on the older salt marsh (coverage 21.1%) compared to the younger salt marsh (coverage 1.2%). Furthermore, clay thickness and surface elevation were determined in the centre of each square meter in every block. Clay thickness was measured with an auger, the length of the clay column being easily distinguishable from the sandy substrate upon which the clay layer has formed. Surface elevation was measured with a theodolite calibrated to a fixed point of which the elevation with respect to regional Mean High Tide (MHT) was known.

For the genetic analysis, a small (<5 m^2), an intermediate (5–20 m^2) and a large (>20 m^2) patch were randomly selected in every block if possible. Leaves were sampled on crossings of a 25 cm × 25 cm grid within a 1m × 1m plot located in the centre of each patch, whereas outside this 'core' area, sampling took place at increasing grid sizes of up to 2m × 2m (Fig. 5.1) (design modified from Van der Velde *et al.* 2001). The sample size of small, intermediate and large patches ranged from 5–20, 19–34 and 30–59 respectively and for their core areas 5–17, 13–25 and 11–24 respectively. In addition, several samples were taken from the edges of adjacent patches. In overall 125 samples were collected at the younger site, and 283 samples at the older site. The coordinates of leaf samples were recorded and young leaf tissue (8–10 cm) was put in 2-mL air-tight tubes containing dried silica-gel.

Table 5.1 Patch size class distribution as the average number (S.E.) of small (S), intermediate (M) and large (L) patches for the three blocks (2500 m^2 each) on the 25 and 35 year old salt-marsh site.

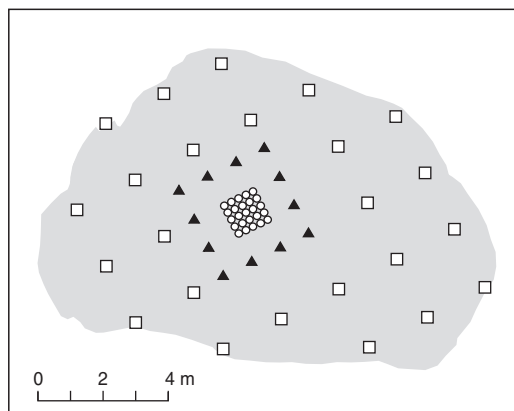| Site	Patch size class		
	Small	Intermediate	Large
25 yrs	18.67 (2.60)	1.67 (0.33)	0 (0)
35 yrs	5.67 (2.73)	4.67 (1.20)	6.67 (1.20)

Figure 5.1 Sampling scheme at patch level for genetic analysis. The open circles represent the core of the patch, sampling level at 25cm × 25cm. The black triangles represent the sampling level at 1m x 1m, and the open squares the 2m × 2m sampling level.

Microsatellite analysis

DNA was extracted by grinding 0.5 cm2 leaf tissue with a metal pestle in a 1.5 mL eppendorf tube after the sample had been frozen in liquid nitrogen. The genomic DNA was purified using a CTAB (hexadecyltrimethylammoniumbromide)-based method (Doyle & Doyle 1987) and was stored in 0.1x TE [1 mM Tris-HCl (pH 8.0), 0.1 mM EDTA (pH 8.0)] at –20°C.

For this study 5 microsatellite markers were used: ECGA22, ECGA89, WMS2, WMS6 and WMS44, originally designed for *Elymus caninus* (Sun *et al.* 1998) and *Triticum aestivum* (Röder *et al.* 1998) (see Bockelmann *et al.* 2003 for detailed information). Each 10 μL PCR reaction consisted of 1× PCR-buffer (Roche Diagnostics, Almere, NL), 0.2 mM of each dNTP (Roche Diagnostics, Almere, NL), 0.5 μM of labeled forward and 0.5 μM of reverse primer (Biolegio, Malden, NL; Applied Biosystems, Warrington, UK), 0.1% (wt/vol) bovine serum albumine (BSA; Roche Diagnostics, Almere, NL), 0.25 units *Taq*-polymerase (Roche Diagnostics, Almere, NL) and 5–10 ng of genomic DNA. Amplifications were performed with a PE 9700 PCR thermocycler (Perkin-Elmer). After an initial denaturation at 94°C for 3 min, 10 cycles of 1 min at 94°C, 1 min at annealing temperature (50–65°C), and 1 min at 72°C were performed, followed by 16–28 cycles of 30 s at 94°C, 1 min at annealing temperature (50–65°C), and 1 min at 72°C. Finally an extension period of 20 min at 72°C was applied. A subset of the samples was amplified twice or thrice to validate the results. PCR products were visualized by electrophoresis on an ABI PRISM™ 377 automated fragment analyser (Perkin-Elmer). The size of the fragments was scored against an internal lane standard (ROX-350).

As *E. atherica* is a hexaploid species, a codominant analysis for microsatellite markers is highly complicated. Scoring of allelic configurations in polyploids has been achieved in other studies (Esselink *et al.* 2004). However, owing to the alloploid nature of the

species the number of alleles detected per individual varies according to locus (Refoufi and Esnault 2006), it proved to be impossible to quantitatively analyse peak sizes (allele dosage). Therefore, the banding patterns of the microsatellite markers for each individual sample were interpreted as DNA-fingerprints. Both absence and presence of fragments (alleles) was interpreted as characters, yielding individual genotypes that enable genet identification (Jarne & Lagoda 1996).

Following Sydes and Peakall (1998), the probability of a particular multilocus genotype is given by the product of the single locus genotype probabilities in random mating conditions:

$$P_{\text{dgen}} = \Pi p_i$$

in which p_i is the frequency of band presence at each locus in the observed multilocus genotype.

After establishing multilocus genotypes of the samples from the obtained fingerprints, these data were linked to the sample coordinates. Clonal diversity was estimated as G/N (Ellstrand and Roose 1987) in which G is the number of different genotypes found and N is the number of samples analysed. Samples from the core area of patches were used to calculate the clonal diversities. This has the advantage that patches are comparable irrespective of their surface area. The overall Simpson's diversity index was calculated per block per site using only the samples from the core areas of patches.

Genetic Relatedness

Analysis of Molecular Variance (AMOVA) was used to estimate variance components for fingerprint genotypes, partitioning the variation among individuals/within patches and among patches/within sites and among sites (Excoffier *et al.* 1992). An AMOVA was also used to calculate pairwise differences as Φ_{ST}, an analogue of F_{ST}, between the younger and older site and between patches within sites which were tested for significance with a permutation procedure. The AMOVA analysis was only exercised on the samples of the core, and identical genotypes were omitted.

To study the genetic relatedness by geographical distance within sites, the similarity coefficient of band-sharing between samples was calculated as $S_{xy} = 2n_{xy} / (n_x + n_y)$, where n_x and n_y are the numbers of bands present in samples x and y, respectively, and n_{xy} is the number of bands shared by x and y (Lynch 1990, Kappe *et al.* 1995). For the comparison between patches within sites, 9 random samples in the core of a patch were selected and compared pair-wise with 9 samples of the other patches, to avoid multiple sampling. The sample size was limited to the maximum number of samples of the small patches. The geographical distance between patches is expressed as the mean of the distance between pairs. The similarity coefficient within patches was calculated by selecting 8 samples randomly within a patch for the old site, and 4 random samples in the young site and compared pair-wise. For the S between and within patches, a comparison was done between S_{genet} and S_{ramet}. For S_{genet}, every genotype could only occur once in the analysis, where as for S_{ramet} all samples were likely to be drawn.

By using spatial statistics, spatial autocorrelation of clones was determined giving the clonal probability that at a certain distance interval two ramets belong to the same genet. The spatial autocorrelation of clones within patches for the two sites was estimated by values of Moran's I statistic for different distance classes. Using SPAGeDi software, Moran's I was calculated according to Hardy and Vekemans (2002).

Results

Surface elevation and clay thickness

The difference in surface elevation between both sites was quite small, but statistically significant (25 yrs site = 40 ± 0.4 cm + MHT; 35 yrs site = 38 ± 0.3 cm +MHT; $t_{756.3}$ = 4.39; P < 0.001). A significantly thicker clay layer was observed on the older site (16.8 ± 0.3 cm) in comparison to the younger site (6.3 ± 0.1 cm) ($t_{915.5}$ = −58.86; P < 0.001). In addition, *E. atherica* occurred at plots with a relatively high clay thickness. A larger difference in mean clay thickness between plots with and without *E. atherica* was found at the younger site compared to the older site (Table 5.2). Regarding the mean difference in surface elevation between plots with and without *E. atherica*, *E. atherica* is more strongly confined to the higher areas at the younger site, whereas at the older site this difference is less distinct.

Clonal diversity and environmental effects

A total of 42 differently sized bands were found that were reproducible, out of which 35 were polymorphic. Among 410 samples, a total of 186 different multilocus genotypes was found, making a grand clonal diversity of 0.45. The Simpson's diversity index (*D*) was 0.64. Values of P_{dgen}, the probability of drawing more than one copy of a certain multilocus genotype within a randomly mating population, were always lower than 0.002 (range 0.002 – $6.2*10^{-12}$.) This implies that multiple copies of a given multilocus genotype can be considered to have been clonally reproduced.

Table 5.2 Comparison of surface elevation and clay thickness between plots with and without *E. atherica* on the 25 and 35 year old sites. Means and standard error are shown below. The t, d.f. and P values (*** <0.001, ** <0.01) of the accompanied two-tailed t-test (unequal variances assumed).

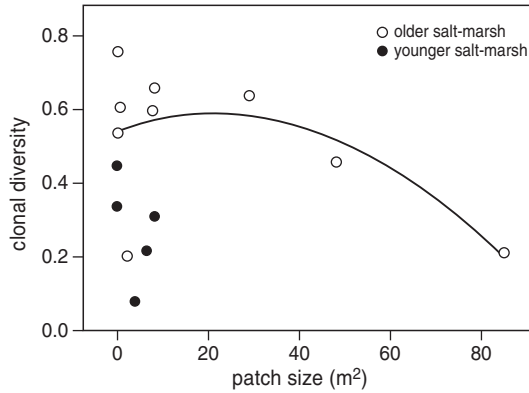| Site	absent	present	t	df	P
Clay thickness (cm)					
25 yrs	5.87± 0.09	8.41± 0.26	-9.09	98.25	***
35 yrs	16.44± 0.23	17.23± 0.18	-2.68	529.04	**
Surface elevation (cm+MHT)					
25 yrs	37.6 ± 0.4	50.7± 0.5	-20.93	232.01	***
35 yrs	35.6 ± 0.4	40.0± 0.3	-8.83	516.44	***

Figure 5.2 Relationship between clonal diversity within the patch centre and total patch size in m^2 for the older salt-marsh site and younger salt-marsh site. A quadratic polynomial curve is fit for the older site ($R^2 = 0.386$).
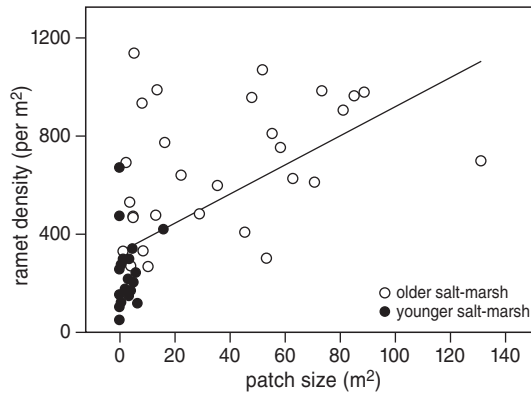
Figure 5.3 Relationship between ramet density in centre of patches and patch size. A linear regression is fit through both the older salt-marsh site and younger salt-marsh site (Spearman's rank coefficient, $r_s = 0.765$, $P < 0.01$, $N = 47$).

In general, the patches were found to be multiclonal (13 out of 14 examined patches) and all genets (i.e. an individual consisting of one or more genetically identical ramets) were restricted to their patch. The different genets grew intermingled within patches. The mean clonal diversity on the older site (mean $G/N = 0.51$, $N = 9$) was significantly higher than on the younger (mean $G/N = 0.28$, $N = 5$) site (2-tailed t-test, equal variances assumed; $t_{12} = 2.41$; $P = 0.033$) (Fig. 5.2). This implies that the older site harbours relatively more genets per unit area than the younger site. The difference in clonal diversity remained significant ($t_9 = 2.74$; $P = 0.023$), when the three largest patches from the older site were omitted from the calculation to compare only approximately even-sized patches.

At the older site, a high variance in clonal diversity was found for patches smaller than 10m^2. Although there were only three relatively large patches, clonal diversity tended to decrease with patch size (Fig. 5.2). Thus, larger patches contain less genets than smaller patches, but no significant relationship could be detected here. Ramet densities, measured within all patches at a site, showed a similar pattern: large variance but predominantly low densities in small patches and significantly increasing with increasing patch size (Spearman's rank coefficient, r_s = 0.765, P < 0.01, N = 47) (Fig. 5.3). However, no significant relation was found between ramet density and clonal diversity.

Genetic Relatedness

Analysis of Molecular Variance (AMOVA) showed that genetic differentiation between the two sites was relatively low whereas differentiation between patches within sites was very high at both sites (Table 5.3), though somewhat higher on the older site than on the younger site.

The mean similarity coefficient (S) between patches was relatively high in both sites (Table 5.4). At the younger site, a significant lower similarity was found for S calculated at genet level than at ramet level. However, at the older site, taking identical genets into the similarity coefficient (S_{ramet}) did not result in an increase of similarity. At both sites,

Table 5.3 Summary of analyses of molecular variance (AMOVA) performed on character data from genets belonging to patches grouped by site or by patches from the younger and the older site. The significance test was based on 16 000 permutations.

Source of variation	d.f.	Sum of squares	Variance components	% of variation	Φ_{ST}	P
Between sites	1	21.849	0.391	9.73	0.09728	<0.001
Within sites	132	479.233	363.056	90.27		
Between patches, young	4	36.218	130.898	37.73	0.37727	<0.001
Within patches, young	25	54.015	216.061	62.27		
Between patches, old	8	178.374	178.906	44.66	0.44657	<0.001
Within patches, old	95	210.626	221.712	55.34		

Table 5.4 Band similarity coefficient (S) on genet and ramet level. A comparison between patches within sites (two-tailed t-test, equal variances assumed).

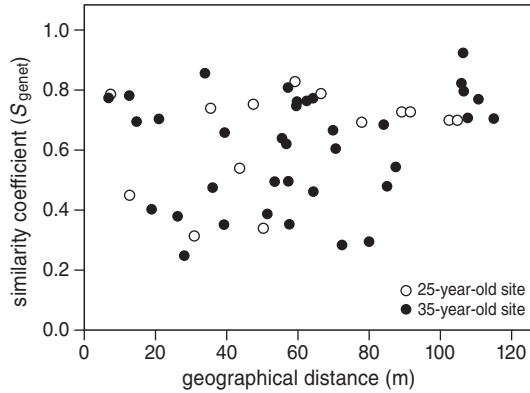| site	Similarity coefficient (S)		t	df	P
	genet	ramet			
25 yrs	0.88 ± 0.028	0.95 ± 0.017	2.26	30	0.03
35 yrs	0.89 ± 0.019	0.90 ± 0.023	0.35	61	0.73

Figure 5.4 Band similarity coefficient for genets between patches related to the geographical distance between the patches. Pairs from the 25 yrs old site are indicated with open circles and from the 35 yrs site are indicated with closed circles.
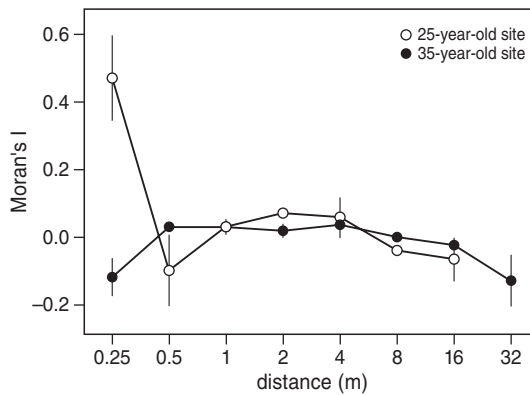
Figure 5.5 Correlogram of clonal probability as Moran's I statistics. The average Moran's I of all patches with corresponding standard error are shown per distance class. The 25 yrs old site is indicated with open circles and the 35 yrs site is indicated with closed circles.

no significant correlation was found between *S* and geographic distance (Spearman rank, $r_s = 0.219$, $P = 0.12$, $N = 51$; Fig. 5.4). The similarity coefficient within patches did not significantly decrease when identical genets were omitted in the analysis (25 yrs site, $S_{genet} = 0.95$, $S_{ramet} = 0.88$, $t_{equal\ variances} = 1.85$, df = 7, $P = 0.11$; 35 yrs site, $S_{genet} = 0.90$, $S_{ramet} = 0.89$, $t_{equal\ variances} = 0.25$, df = 16, $P = 0.81$).

The clonal probability (i.e. the chance to pick two ramets of the same genet) is relatively high, with a probability of 0.5, at the younger site at distances <25 cm. However, the clonal probability drops abruptly at distances >25 cm (Fig. 5.5). At the older site no autocorrelation is found at any of the distance classes.

Discussion

Clonal diversity and environmental effects

Although seedlings in clonal plant populations have rarely been observed in empirical field studies (Eriksson 1997), most populations of such species studied exhibited a considerable amount of genetic variation (Ellstrand and Roose 1987, Widen *et al.* 1994, Jonsson *et al.* 1996). This can be explained by the fact that even at a low rate of sexual recruitment within a population a comparable level of clonal diversity can be maintained as in populations of non-clonal species (Watkinson and Powell 1993, Bengtson 2003). The grand clonal diversity of 0.45 found in the present study is high compared to the mean of 0.17 (±0.05 S.E.; N =26) and 0.27 (±0.04 S.e.; N = 45) calculated from two reviews on isozyme based studies of clonal plant populations by Ellstrand & Roose (1987) and Widén (1994) respectively. However, Ellstrand and Roose (1987) found a positive correlation between the clonal diversity and the number of markers used, and which is usually higher in DNA-based studies compared with isozyme-based studies. As isozyme-based studies often do not capture all genetic variation, they may not be comparable with DNA based studies. In the present study, the combinations of four of the five primers already distinguished 73-94% of the genotypes, implying that most genotypes present have also been sampled.

In DNA-based studies, a high variability in values of clonal diversity is observed. For instance, Szmidt *et al.* (2002) found a clonal diversity of 0.72 in *Empetrum hermaphroditum*, whereas Persson and Gustavsson (2001) found a clonal diversity of 0.22 in their study on *Vaccinium vitis-idaea*. In populations of the clonal salt-marsh grass, *Spartina alterniflora*, higher clonal diversities ranging from 0.62 to 0.92 were observed (Travis & Hester 2005). Bockelmann *et al.* (2003) found their study on populations of *E. atherica* that the number of identical genotypes ranged from 0 to 17%, indicating that the clonal diversity can reach 1.0 in the studied species. However, as the clonal structure is usually unknown a priori, the chosen sampling scale often yields highly variable estimates of clonal diversity (Ellstrand and Roose 1987, Widen *et al.* 1994, Hangelbroek *et al.* 2002), which therefore may not be comparable among different studies. In addition, levels of clonal diversity may also be variable among populations within a species (Aspinwall & Christian 1992, Jonsson *et al.* 1996, Lehmann 1997, Hangelbroek *et al.* 2002), making the mean clonal diversity less informative. The overall Simpson's diversity index of 0.64 indicates that there is a substantial clonal diversity and that variation in clone size is small. In other words, there is no skewed distribution in clone size towards some large clones among many smaller ones.

In 13 out of 14 patches, more than one genet was found, indicating that patches are usually not the result of only one vegetatively propagating seedling. Multiclonality in patches has also been found in other species, e.g. in *Pteridium aquilinum* (Parks & Werth 1993), *Ilex leucoclada* (Torimaru *et al.* 2003) and *Vaccinium stamineum* (Kreher *et al.* 2000).

Interestingly, the older site was found to have a significantly higher clonal diversity than the younger site (Fig. 5.2). On the contrary, studies on *Puccinellia maritima* (Gray *et al.* 1979) and *Agrostis stolonifera* (Kik *et al.* 1990), found a lower number of clones in the

mature populations. Gray *et al.* (1979) suggested two possible processes affecting the population clonal structure. On the one hand, during development of a marsh a decrease in the number of genotypes is expected, as individuals may be eliminated by competition. On the other hand individuals in the pioneer marsh may be continuously replaced in the mature marsh by new individuals, in which case the number of individuals per unit area between pioneer and mature sites may be similar. However, there is a third possibility; the number of clones could increase over time due to increasingly favourable conditions on the marsh. The data indicate that both the first and third option occur in the studied populations of *E. atherica*, as explained below.

Two important environmental variables affecting clonal structure can explain the differences in clonal diversity between the young and old sites. First, herbivory can have a profound effect on seedling establishment (e.g. (Hangelbroek *et al.* 2002). Colonisation of new sites by *E. atherica* happens mainly through seedling recruitment (Bockelmann *et al.* 2002). Seeds of *E. atherica* are able to establish successfully on 10-year-old salt marsh when hares and geese are excluded, whereas under natural conditions, grazing by hares and geese prevent or at least slow down invasion of *E. atherica* on young successional stages. On older successional stages, however, herbivory on seedlings is less severe (Kuijper & Bakker 2003, Kuijper *et al.* 2004, Kuijper *et al.* 2005). Hence, the probability for successful establishment will be higher at the older site in comparison to the younger site.

The second variable potentially affecting the clonal structure of *E. atherica* is clay thickness. Clay thickness is positively correlated with soil nitrogen content and increases over time due to tidal sediment deposition (Olff *et al.* 1997, Van Wijnen & Bakker 1997). Nitrogen is the limiting factor to salt-marsh succession and succession of *E. atherica* in particular (Kiehl *et al.* 1997, Leendertse *et al.* 1997) and is also likely to be involved in salt tolerance of salt-marsh plants (Stewart & Lee 1974, Mansour 2000). With an increasing age of the salt marsh, increasingly favourable conditions for seedling recruitment of *E. atherica* are present along the chronosequence. Whereas extensive clones can redistribute nutrients (D'Hertefeldt and Jonsdottir 1999) and thus are less affected by locally thin clay layers, seedlings cannot cope with a nitrogen deficit. Seedlings are therefore highly dependent on the site conditions where they germinate. The data on surface elevation and clay thickness indicate that the older site is more favourable for seedling recruitment compared to the younger site due to its thicker clay layer. Seedlings on the older site are less likely to face a nutrient constraint and probably have a higher salt tolerance. On the younger site *E. atherica* is more strongly confined to areas with a relatively higher elevation (Table 5.2), possibly to reduce inundation and salt stress.

Clonal diversity and intra-specific competition
The lower herbivory and the higher nutritive status on the older site could affect the seedling recruitment and clonal growth positively, explaining the different clonal diversities between the sites. However, for dense patches, intra-specific competition is likely to play a counteracting role (Bengtson 2003), resulting in a loss of genets and therefore a decrease in clonal diversity (Gray *et al.* 1979, Watkinson and Powell 1993). Empirical

evidence for intra-specific competition decreasing clonal diversity has been found for various species at different time-scales (e.g. (Mcneilly & Roose 1984, Hartnett & Bazzaz 1985, Lehmann 1997, Travis and Hester 2005).

In *E. atherica*, intra-specific competition acts at the patch level rather than the site level, because the age of a patch is not necessarily correlated with the age of the salt marsh. Thus, intra-specific competition is assumed to become more important as patches increase in ramet density with increasing patch age. Vegetation density could facilitate for an input of new individuals as vegetation density is positively correlated with seed entrapment (Buitenwerf & Veeneklaas pers. comm.). Though vegetation density or, more importantly, canopy height can also be expected to be negatively correlated with seedling recruitment due to light competition ((Tilman 1988, Aspinwall and Christian 1992, Piquot *et al.* 1998, Kanno & Seiwa 2004). Although the sample size is too small, the three largest sampled patches hint a negative trend in clonal diversity with increasing patch size.

Travis *et al.* (2002) found a high genetic diversity within young populations of *Spartina alterniflora*. Apparently, the seedling recruitment in populations of *S. alterniflora* is limited to the first 30 years after initial colonisation, whereafter clonal diversity will decrease very slowly (Travis and Hester 2005). As *E. atherica* resembles the clonal salt marsh species, *S. alterniflora*, the same structural patterns could be found for *E. atherica* when populations get older, as in the three large patches in our study. Thus, aging of the population will possibly lead to a decrease of seedling recruitment and a severe clonal competition may determine the clonal diversity to a large extent (Gray *et al.* 1979, Kik *et al.* 1990).

Genetic Relatedness
The present study revealed low differentiation between sites (Φ_{ST} = 0.097), indicating habitat differentiation between these lower-marsh sites. This is consistent with the study by Bockelmann *et al.* (2003) that showed low but significant differentiation between sites at comparable distances on the same island of Schiermonnikoog. In addition, Refoufi & Esnault (2006) found a comparable average genetic differentiation among populations (0.067) at the same spatial scale in Mont Saint-Michel Bay. In both studies, this genetic differentiation between sites with distances <60 km could not be attributed to isolation-by-distance. Bockelmann *et al.* (2003) therefore attributed this differentiation to measured differences in biotic or abiotic conditions between sites (lower marsh vs. higher marsh).

The band-sharing coefficient (*S*) between patches was relatively high for both sites, but no correlation was found between *S* and geographic distance (Fig. 5.4). Thus, the patches established most likely independently from one another, and the differentiation occurs on patch level. At the younger site, S_{ramet} was higher than S_{genet}, indicating that identical genets increased the relatedness within the patch. At the older site, including the identical genets in the analysis did not increase the relatedness. The high similarity within patches would suggest that seedlings establishing in the patch mainly originate from within the patch.

An alternative explanation for the high relatedness between genets within patches is that, if minor differences in band patterns are present, somatic mutations are the cause for these differences. Mutations would also increase the clonal diversity falsely ((Hsiao & Rieseberg 1994, Torimaru *et al.* 2003, Nagamitsu *et al.* 2004). One band difference was found in 13.9% of of pairs from the random subset of samples used for calculating the band-sharing coefficient and often different primers were involved, suggesting that it is unlikely that these differences were the result of somatic mutations within a genet. In addition, the level of genetic differentiation between the sites (Table 5.3) was comparable with the results from Bockelmann *et al.* (2003). Finally, a bias in genet identification due to somatic mutations would probably arise equally in all samples. Therefore, conclusions drawn on comparisons between sites would still be meaningful if somatic mutations indeed account for some of the clonal diversity found.

Within patches a spatial relatedness was found only at short distances (<25 cm) at the 25 yr old site, though no spatial pattern was found at the older site. In studies on spatial autocorrelation of clones, a high positive autocorrelation has often been found as a result of the spatial limit of clonal dispersal (Chung & Epperson 2000, Namroud *et al.* 2005). In the present study, such spatial clonal structure is not found. Clones have no sharp boundaries within the patch, possibly due to intermingled growth of the genets.

As this study had been conducted within a single salt-marsh system, cautions have to be taken on the generality of our conclusion. However, other studies have shown that our study site is a good representative for other salt-marsh systems within the Wadden Sea with respect to the spreading of *E. atherica* towards the lower salt marsh (Van Wijnen and Bakker 1997, Bos *et al.* 2002)

In summary, sexual and vegetative propagation in *E. atherica* are both important in colonizing new sites. Seedling establishment increases with succession of the salt marsh as environmental conditions improve. Seedling establishment, i.e. sexual reproduction, therefore, becomes increasingly dominant, thereby affecting genetic and demographic structure of *E. atherica* on the older salt marshes. However, severe clonal competition may decrease the clonal diversity in the long run, tipping the balance to vegetative reproduction.

Chapter 6

To move or not to move: determinants of seed retention in a tidal marsh

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Abstract The effects of moisture conditions, seed morphology, vegetation structure and hydrodynamic variables on seed retention were examined in a system where the dominant dispersal agent is water. Experiments were conducted in a tidal salt marsh and in a flume facility where hydrodynamic variables could be controlled.

Moisture condition of seeds greatly influenced which factors were most important in determining seed retention. Seed type (buoyancy) was the most important factor when seeds were dry with seeds possessing very low floating capacity (*Plantago maritima*) being retained in greater numbers than seeds with intermediate floating capacities (*Suaeda maritima* and *Elytrigia atherica*).

In contrast, hydrodynamic variables dominated retention processes when seeds were waterlogged. The application of waves in addition to flow velocity dislodged more seeds than flow velocity alone.

Vegetation structure influenced retention in both dry and wet conditions but less so than other factors. Denser, less rigid vegetation types retained greater numbers of seeds than more open, more rigid vegetation types.

Results suggest that buoyancy traits appear to determine whether seeds move in the drier summer and autumn months, after initial detachment from parent plants but the intensity of wave action will determine whether waterlogged seeds stay in a microsite during the wetter months of late autumn to early spring.

Keywords: community organisation, flume tank, hydrochory, hydrodynamics, salt marsh

Introduction

Seed dispersal, as the initial constraining factor affecting species richness, can influence the structure of plant populations (Kudoh *et al.* 2006) and communities in systems dominated by water dispersal (Honnay, Verhaeghe & Hermy 2001; Nilsson *et al.* 2002; Jansson *et al.* 2005). A recent review, however, suggested that spatial variation in seed deposition in most empirical studies is more easily related to landscape elements that trap seeds than to the theoretical probability of dispersal distance travelled from the seed source (i.e. dispersal kernel) (Levine & Murrell 2003). Predicting where seeds stop is a less appreciated component of the dispersal kernel than how seeds move (Levine & Murrell 2003). In this study, we examine the processes that influence seed retention in tidal systems by identifying simple functional relationships between dispersal vectors (tidal flow, wind-generated waves), trapping agents (vegetation), seed characteristics (buoyancy) and seed retention. Seed retention refers to retention on the ground rather than retention in the gut, as defined in studies on the internal dispersal of seeds by birds and mammals. These relationships are needed to build and test quantitative models in water-driven systems as has been done in wind-driven systems (Johnson & Fryer 1992; Schurr *et al.* 2005).

Morphological traits of seeds, such as size, shape, presence of appendages and seed-coat properties, and compound traits such as floating capacity can influence patterns of seed distribution not only by influencing dispersal processes (Nilsson *et al.* 2002) but also as a result of interactions with trapping agents (Chambers, Macmahon & Haefner 1991; Peart 1984). In systems where water largely mediates seed dispersal (hydrochory), seed traits such as low mass, large surface area to volume ratio, buoyant appendages, air traps, waxy seed coat and a tenacious attachment to adult plant material (Praeger 1913; Schulthorpe 1967; Hutchinson 1975) may enhance dispersal by increasing the floating capacity. In contrast, traits such as mucilaginous seed coats, hygroscopic awns and collapsing pappi may enhance retention (Chambers & MacMahon 1994).

Both the probability of dispersal and dispersal distance have been shown to decrease with increasing vegetation density (Redbo-Torstensson & Telenius 1995; Watkinson 1978). In tidal marshes, vegetation forms important elements of landscape structure providing resistance to tidal currents (Green 2005) and wind-generated waves (Möller *et al.* 1999). Tidal currents result in net movement of water particles but their velocity can be considerably dampened by vegetation (Shi, Pethick & Pye 1995; Neumeier & Ciavola 2004) and dense vegetation can decrease the generation of turbulence (Nepf 1999). Orbital water movements linked to wave action can more effectively dislodge particles (such as sediments or seeds) vertically but cause no net movement of water particles. Orbital water movements refer to the oscillatory path of water particles as waves pass through water. The magnitudes of these effects are strongly dependent on vegetation characteristics such as shoot density and stiffness (Bouma *et al.* 2005).

In this study, we explore the effects of morphological seed traits, vegetation type and hydrodynamic variables on seed retention processes in a tidal marsh dominated by

hydrochory by examining these processes both in the field and in a flume facility where hydrodynamic variables can be controlled. We hypothesize that seed retention will be higher in: 1) species with low floating capacity than species with higher floating capacity; 2) denser, less rigid vegetation types compared to a more open, more rigid vegetation types and in 3) a flow treatment compared to a combined treatment (flow and waves).

Methods

Field study

We conducted the field study in a back-barrier salt marsh on the Dutch island of Schiermonnikoog (53° 30' N, 6° 10' E). Five sites approximately 50 m apart were selected for the presence of three contrasting community types in close proximity (details below). Within each site, we sowed in each community type four patches (10 cm × 10 cm) with 30 dry seeds each of three contrasting species (details below). We sprayed a little water on the seeds to prevent them from being blown away by the wind. Seeds were left on the marsh surface for one tidal inundation, after which turfs encompassing the patches were cut from the vegetation and examined for seeds under a magnifying glass. We analyzed one control patch for each set of four vegetation patches in order to detect presence of naturally dispersed seeds. The experiment was performed during a spring tide (7 to 8 April, 2004) when plots were inundated with 0.10 to 0.25 m of sea water. Wind conditions were moderately high with a mean speed of 37.4 km/h and maximum sustained speed of 50.4 km/h.

To test hypothesis 1, we selected 'seeds' from three salt-marsh species for their contrasting physical traits. Seeds of *Plantago maritima* have a relatively short floating capacity due to a seed coat that becomes mucilaginous when wetted (Table 6.1). Achenes of *Suaeda maritima* and grains of *Elytrigia atherica* have similar, relatively long floating capacities for this system (Table 6.1). The term 'seed' will be used to describe both true seeds and small, indehiscent fruits.

We compared three contrasting vegetation types that are common in low-elevation marshes (hypothesis 2). *Spartina anglica* is a loosely tufted, tall grass with stout, inflexible stems. *Limonium vulgare* forms low rosettes with leathery, basal leaves and taller flowering stalks. *Puccinellia maritima* is a creeping grass with soft, half-prostrate stems. Plant nomenclature follows van der Meijden (1996).

Flume study

A race-track flume tank was used to establish the effect of hydrodynamic conditions (flow vs. combination environments, hypothesis 3) on retention of three contrasting seed types (same species as used in the field experiment, hypothesis 1) in two contrasting stand types (*Festuca rubra, L. vulgare*, hypothesis 2). We used a *F. rubra* stand instead of *Puccinellia maritima* because tillers of *F. rubra* are similar in morphology to those of *P. maritima* but are present in much higher densities and we wished to conduct experi-

Table 6.1 Seed traits of species commonly found in salt marshes on Schiermonnikoog. Mass measurements were taken from the LEDA database (Knevel *et al.* 2003). Measurements for length and width were taken from the Digital Seed Atlas of the Netherlands (Cappers, Bekker & Jans 2006). Residence time at 50% and at 100% describe the time passing before 50% and 100% of initially floating seeds sink in laboratory experiments. Two sources were used for the floating capacity Species given in bold type were used in the seed retention experiments. *dimorphic seeds

Species	Unit	Special traits	Mass (mg)	Length (mm)	Width (mm)	Residence time in hours (h) or days (d)			
						Koutstaal *et al.* (1987)		Geertsema (2000)	
						50 %	100 %	50 %	100 %
Seriphidium maritimum	Seed	Mucilaginous seed coat	0.75	2.45	1.203			≤1 d	8 d
Salicornia sp.	Seed		0.18	1.316	0.769	1.5 – 2 h	≤1 d	≤1 d	26 d
Plantago maritima	**Seed**	**Mucilaginous seed coat**	**0.95**	**2.162**	**0.964**	**0.5 – 1 h**	**7 h**	**≤1 d**	**40 d**
	Fruit			3.55	1.49	5 – 10 d	14 – 21 d		
*Atriplex prostrata**	Achene		1.28	3.08	2.84	1 h	7 h	≤1 d	40 d
			3.31	1.83	1.70				
Juncus gerardi	Seed		0.03	0.713	0.348			≤1 d	43 d
*Spergularia media**	Seed	Wings on some seeds	0.10	1.57	1.46	1 – 1.5 h	5 – 7 h	10 d	43 d
				0.825	0.693				
Limonium vulgare	Nut		0.84	4.16	0.84	2.5 h	5.5 h		
Puccinellia maritima	Grain		0.18	2.08	0.76	2 – 20 h	2 – 4 d		
Triglochin maritima	Seed	Wings	0.48	3.23	0.69			≤1 d	>90 d
Festuca rubra	Grain		0.79	3.64	0.87	9 – 38 h	4 – 5 d	≤1 d	>90 d
Suaeda maritima	**Achene**		**0.89**	**1.52**	**1.35**	**2 – 30 h**	**7 – 14 d**	**≤1 d**	**>90 d**
Elytrigia atherica	**Grain**		**3.86**	**5.18**	**1.21**	**1 – 2 d**	**5 – 12 d**	**≤1 d**	**>90 d**
Aster tripolium	Achene	Pappus	0.90	3.82	1.16	2 – 4 d	7 – 15 d	4 d	70 d
Spartina anglica	Grain			12.62	1.2	1.5 – 5 d	4 – ≥ 60 d		
Glaux maritima	Seed		0.36	1.749	1.294			10 d	61 d
Atriplex Portulacoides	Fruit	Persistent bracteole	3.43			45 d	≥ 120 d		

ments with highly contrasting vegetation types in the flume. Stand of *Festuca rubra* and Limonium vulgare were grown in monoculture in containers under greenhouse conditions. *F. rubra* was sown to grow at a mean field density of 5600 tillers/m². Adult plants of *L. vulgare* were transplanted from the field and replanted at a mean field density of 25 plants/m². Different saturation conditions of seeds (dry, waterlogged) were used because we expected this to have a significant effect on seed buoyancy and both conditions occur in the field. We used a flume facility to be able to repeat experiments, by having a controlled and reproducible hydrodynamic environment.

Two vegetation containers (1.0 m × 0.25 m) were placed side by side for each run in the test section of the flume where the soil could be made level with the bottom of the flume. Behind the containers, we placed a net, with an opening exactly fitted to the dimensions of the flume (mesh size ~0.5 mm), in order to catch dislodged seeds.

For the dry-seed experiment, seeds were initially dry and exposed to water for a maximum of two hours during the experimental procedure, which is not long enough for most of the seeds to completely lose their floating capacity (Table 6.1). We applied three hydrodynamic treatments sequentially to the same seed lot: i) simulating flooding by filling the flume, ii) applying a strong unidirectional flow (0.40 m/s) and iii) applying wave action (amplitude = 0.39 m, period = 1.7 s) combined with a low unidirectional flow (0.20 m/s). The hydrodynamic treatments were designed to reflect the natural order of hydrodynamic factors that vegetation is subjected to in tidal salt marshes (i.e. tidal current always before waves). For the third hydrodynamic treatment, we used a combination of waves and low-velocity flow because pilot studies showed that applying only wave action resulted in temporary vertical displacement but no net movement of seeds, as predicted by the orbital movement of water during wave action. Fifty seeds each of all three species were sown approximately 20 cm downstream of the leading edge of the vegetation container. After sowing, treatment i was applied by filling the flume to a height of 0.30 m with a partition placed across the flume channel so that seawater would approach the vegetation from one direction. The salinity of the seawater used in the flume experiments was 31 ‰. Very low flow velocity was generated in order to make sure that floating seeds were caught in the net. Strong unidirectional flow and wave action with low unidirectional flow were applied for 30 minutes each. Between treatments, we collected seeds from the net. This protocol was repeated five times for each stand type, each time with a new vegetation container.

For the waterlogged-seed experiment, 50 seeds of each of the three species were sown as in the dry-seed experiment but with the flume already filled with water to a depth of 0.30 m. The seeds had been soaked in water for a minimum of 24 hours prior to the experiment and only seeds which had lost all floating capacity were used. When all seeds had sunk and settled in the vegetation, we applied a strong-flow velocity (0.40 m/s) for 30 minutes. After emptying the net of seeds, we added 50 more seeds of each of the three species and then applied wave action (amplitude = 0.39 m, period = 1.7 s) combined with a slow-flow velocity (0.20 m/s) for 30 minutes. The net was emptied and dislodged seeds were collected. This protocol was repeated five times for each stand type, each time with a new vegetation container.

In the race-track flume (circumference of 17.55 m, width of 0.6 m), unidirectional flow velocity was generated through the use of a drive belt. Sinusoidal waves were generated by moving a board that moved back and forth lengthwise along the flume by means of a fly-wheel construction. A more detailed description and diagram of the flume facility at the Netherlands Institute of Ecology in Yerseke is given in Bouma *et al.* (2005) and at http://www.nioo.knaw.nl/SCIENCE/FACILITIES/FLUME/Flume.htm.

Flow velocity measurements were taken by an Acoustic Doppler Velocimeter (ADV) (Nortek AS, Oslo, Norway) during current generation by the drive belt. The ADV measured the flow velocity in a grid of 69 cm × 22 cm at three height levels (5 cm, 11 cm and 17 cm above the soil level). The distance between grid points for the x (direction of current generation), *y* and *z* velocities were 3 cm, 5.5 cm and 6 cm respectively. To give a general description of current velocities, the flow velocities along the x, y and z directions were averaged over time for each point along each axis, and then over space for each measuring height.

In order to relate changes in hydrodynamics to vegetation properties, we quantified the structure of the stands. We measured percentage cover values using random point quadrat measurements in all the vegetation containers. A hundred points each were recorded at soil level, 5 cm above the soil and at canopy height, and averaged at each measuring height. We also clipped all vegetation in three areas of 10 cm × 10 cm per container for estimating biomass (g dry plant material/m^2) after the experiments were conducted.

Data analysis

We applied analysis of variance (ANOVA) to test for effects of seed type (taxonomy), vegetation type and hydrodynamic treatments on seed retention using the software program, SPSS (2003). The response variable used was percentage retention: % retention = seeds found in turfs after treatment / seeds sown in vegetation before treatment × 100% (field experiment); % retention = 100% - seeds caught in net / seeds present before treatment × 100% (flume experiment). Transformations were used in analyzing both the field (arcsine-square root) and flume (dry: arcsine-square root; waterlogged: arcsine) experiments in order to adhere to assumptions of normality and homogeneity of variance but percentage values of seed retention were used to generate figures.

For the field experiments, a general linear model ANOVA with two fixed factors (seed type, vegetation type), one random factor (site), three two-way interactions (seed type × vegetation type, seed type × site, vegetation type × site) and one three-way interaction (seed type × vegetation type × site) was used. We used a repeated-measures ANOVA model with one within-subjects factor (hydrodynamic treatment) and two between-subjects factors (seed type and vegetation type) for the flume experiments. When the assumption of sphericity was violated, we used the conservative Greenhouse-Geisser correction for degree of freedom and Bonferroni's method for multiple comparisons as advised by Field (2000). For analysis of the waterlogged-seed experiment, the sum of seeds not dislodged by the flow treatment and the 50 seeds of each species added to the vegetation before applying the combined treatment was used in calculating

percentage retention. Therefore, the combined treatment was potentially applied to more than 150 seeds.

Results

Seed retention in the field study

Seed type was the most important factor in explaining seed retention in the field experiment ($F_{2,8}$ = 49.3, P < 0.001) (App. S6.1). Significantly higher numbers of *P. maritima* seeds were retained followed by seeds of *S. maritima* and then *E. atherica* (Tukey's multiple comparisons), differing slightly from predictions by hypothesis 1 (Fig. 6.1). There was a highly significant but small interaction effect between seed type and vegetation type ($F_{4,16}$ = 9.6, P < 0.001). Seed retention was significantly lower for seeds of *P. maritima* in stands of *S. anglica* than in other vegetation types, whereas vegetation type did not have an effect on the retention of *E. atherica* and *S. maritima* seeds. Site was not a significant factor. The control patches captured very few seeds, with the exception of *S. maritima* seeds which were found in site 5 (App. S.62). We corrected the percentage retention by adding the mean number of seeds found in control patches of each vegetation type for each site to the total of seeds added in the beginning for each seed type (30).

Seed retention in the flume study

As in the field study, seed type was an important factor in explaining patterns in seed retention for the experiment using initially dry seeds ($F_{2,24}$ = 27.8, P < 0.001) (App. S6.3). The greatest numbers of seeds of *P. maritima* were retained, followed by seeds of *S. maritima* and *E. atherica* (Bonferroni's multiple comparisons), as predicted by hypothesis 1 (Fig. 6.2). There was no difference in percentage of seeds retained of the latter two species. Vegetation type also had a significant effect on seed retention but explained much less of the variability than seed type ($F_{1,24}$ = 12.9, P = 0.001). Stands of *F. rubra* retained greater numbers of seeds than those of *L. vulgare*, as predicted by hypothesis 2 (Figs 6.2A, B). The effect of the hydrodynamic treatment was also significant ($F_{1.363, 0.120}$ = 4.403, P = 0.033 using Greenhouse-Geisser correction for degrees of freedom) although it was less important than either seed type or vegetation type in influencing seed retention.

When seeds were waterlogged, the hydrodynamic treatment was the most important factor in explaining patterns of seed retention ($F_{1,24}$ = 335.9, P < 0.001) (App. S6.4). The combined treatment dislodged more seeds than the flow treatment, as predicted by hypothesis 3 (Fig. 6.3). There was also a significant effect of vegetation type with stands of *F. rubra* generally retaining more seeds than that of *L. vulgare* ($F_{1.24}$ = 65.0, P < 0.001). A very small but significant three-way interaction effect between the hydrodynamic treatment, seed type and vegetation type ($F_{2,24}$ = 3.675, P = 0.041) showed that there are no consistent effects of seed type on seed retention processes once the seeds are waterlogged.
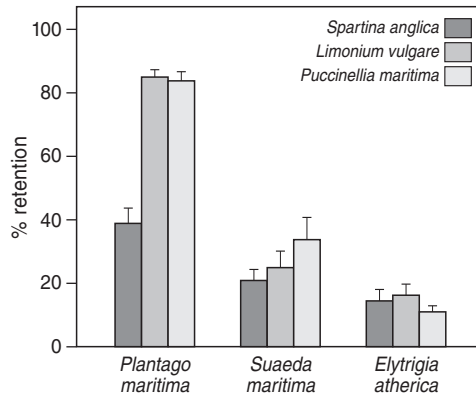
Figure 6.1 Percentage retention of *Plantago maritima*, *Suaeda maritima* and *Elytrigia atherica* seeds in the field study for *Spartina anglica*, *Limonium vulgare* and *Puccinellia maritima* community types. 30 seeds of each species were sown in each patch after a spring tide on 8 April 2004 when plots were inundated with 10–25 cm of sea water. Error bars represent 1 standard error. (n = 5).
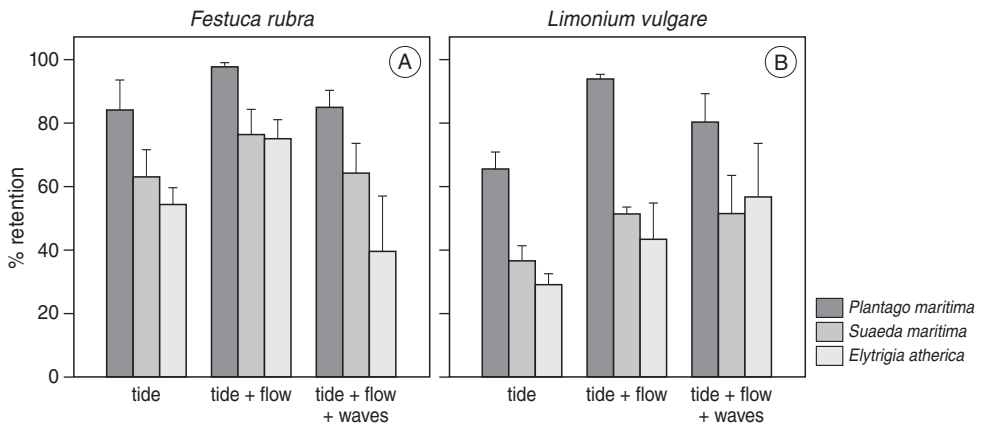

Figure 6.2 Percentage seed retention found in two stand types (*Festuca rubra*, *Limonium vulgare*) for initially dry seeds. Three hydrodynamic treatments (upcoming tide, tide + flow, tide + flow + waves) were applied to dislodge seeds in a flume facility. Results for seeds of *Plantago maritima*, *Suaeda maritima* and *Elytrigia atherica* are shown. 50 seeds of each species were sown at the beginning of each replicate run. The 'waves' treatment was a combined treatment of flow current and waves. Error bars represent 1 standard error. (n = 5).

Effect of vegetation type on hydrodynamics in the flume study

There were key structural differences between *F. rubra* and *L. vulgare* stands. The vertical structure of the stands was more evenly spread for *F. rubra*, whereas much of the plant material was concentrated at ground level for *L. vulgare* (Fig. 6.4A). The canopy cover of *F. rubra* (84%) was significantly higher than that for *L. vulgare* (66%) (t-test: $t_8 = 3.09$, P = 0.02). The canopy height was also generally higher for *F. rubra* with a peak at 6 cm
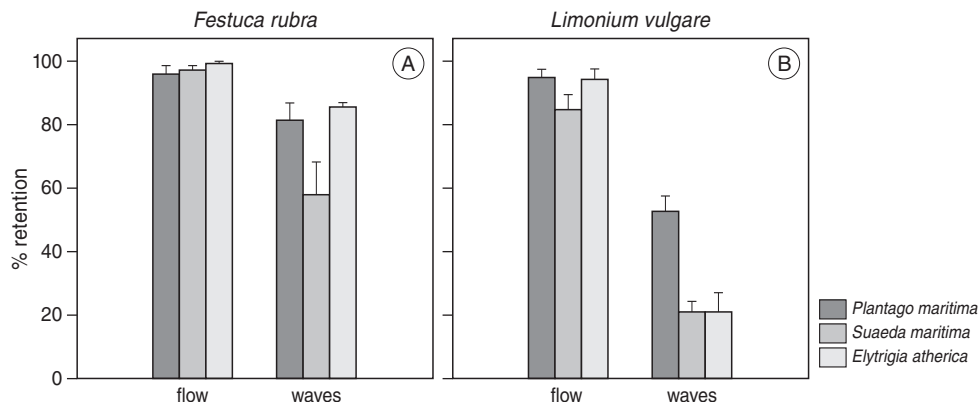
Figure 6.3 Percentage seed retention found in two stand types (*Festuca rubra*, *Limonium vulgare*) for waterlogged seeds. Two hydrodynamic treatments (flow, waves) were applied to dislodge seeds in a flume facility. Results for seeds of *Plantago maritima*, *Suaeda maritima* and *Elytrigia atherica* are shown. 50 seeds of each species were sown at the beginning of each of the two hydrodynamic treatments. The 'waves' treatment was a combined treatment of flow current and waves. Error bars represent 1 standard error. (n = 5).

compared to 4 cm for *L. vulgare* (Fig. 6.4B). However, biomass was significantly higher for *L. vulgare* (312 gm^{-2}) than *F. rubra* (106 gm^{-2}) (t-test: t_6 = -2.81, P = 0.03).

When only flow velocity was generated in the flume, the mean stream-wise flow velocity at all levels above the soil was greater in *F. rubra* than in *L. vulgare* stands (Fig. 6.4C). The flow velocity measured at 5 cm (located in the vegetation) was lower than at the other two higher heights (located mostly above the canopy of the vegetation) (Fig. 6.4C). However, both lateral and vertical velocities at 5 cm were more variable for *L. vulgare* stands (lateral velocity σ (standard deviation) = 3.11, vertical velocity σ = 1.33) than for *F. rubra* stands (lateral velocity σ = 0.73, vertical velocity σ = 0.88). The combination of water depth (0.3 m), wave amplitude (0.039 m), vegetation heights (<0.18 m; Fig. 6.4B) and length of vegetated surface (1.0 m) used in the experiments diminished the level of wave attenuation by the vegetation to a non-detectable level.

Discussion

Our study indicates that different factors may influence the probability of seed movement or retention in tidal systems during the processes of primary and secondary dispersal. Once seeds are waterlogged, movement or retention may depend greatly upon hydrodynamics because seeds lose their floating capacity. This is important because final distributions of seeds will be highly influenced by interactions between waves, tidal currents and trapping agents (vegetation, microtopography).

When seeds are initially dry, differences in floating ability and traits for retention become more important in explaining patterns of seed retention. In the field experi-
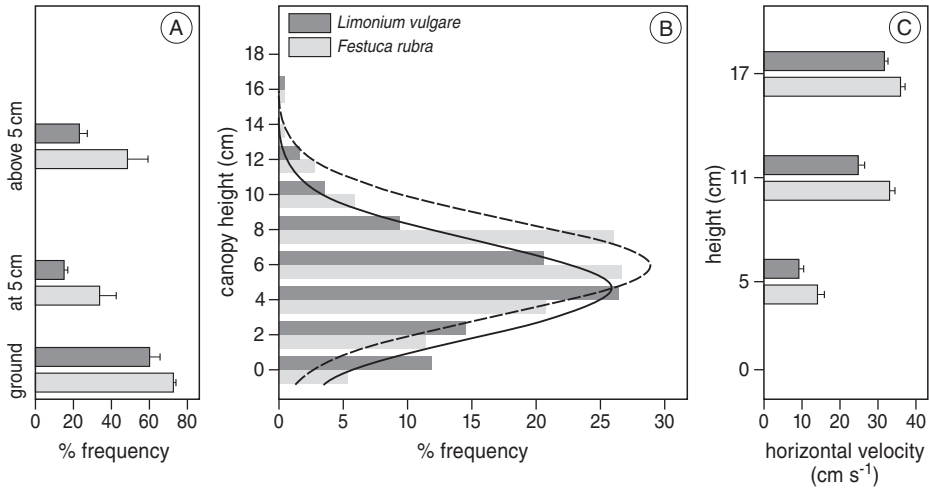
Figure 6.4 Parameters of vegetation type and hydrodynamics at different heights: A) percentage frequency of hits for vegetation recorded with point quadrat method at ground, 5 cm above ground and above 5 cm, B) percentage frequency of canopy heights and C) measured horizontal flow velocity at 5 cm, 11cm and 17 cm above ground. Measurements for *Limonium vulgare* are in dark grey and those for *Festuca rubra* in light grey. The dotted line represents the smoothed distribution in canopy height for *F. rubra*, whereas the solid line represents that for *L. vulgare*. Error bars represent 1 stand error.

ment, seed type was more important than vegetation type in explaining patterns of seed retention. The field experiment was not replicated over time under different hydrodynamic conditions but was completed once under moderately rough weather conditions. However, results were similar to the dry-seed experiment in the flume study, where differences in structure were greater between stand types, the current flow was higher and the wave amplitude was lower than in the field study. Seed type was still the most important factor determining the outcome of the dry-seed experiment.

Due to the design of the flume experiment, there are some carry-over effects of the hydrodynamic treatments as they were always applied in the same sequence (e.g. flow-only treatment before combined flow-and-wave treatment). These effects are stronger in the "dry" than in the "waterlogged" experiment because seeds were replenished between treatments in the latter study. However, seeds are subjected to these "carry-over" effects also in the field in that they always experience tidal inundation, tidal currents and waves in this order.

In contrast to our study, some minor differences were found between the distribution of small cypress seeds and more buoyant, larger tupelo fruits in a swamp but most of the patterns in distribution were explained by trapping by emergent vegetation types (Schneider & Sharitz 1988). However, the differences in floating ability between seeds of bald cypress and tupelo (2×) were much less than those between *P. maritima* and the other two species in our study (~ 48×) (Table 6.1). Retention of *P. maritima* seeds (with mucilaginous seed coats) was much higher, whereas seeds of *S. maritima* and *E. atherica* behaved more or less similarly.

Hydrodynamic variables are more important determinants of retention under wet conditions than seed type in our study. A mechanistic model on secondary dispersal by wind predicts that seed traits could have an effect on the probability of seed movement by influencing the "wind interception parameter" and threshold lift-off velocity (Schurr *et al.* 2005). The wind interception parameter refers to the dimentionless ratio between wind velocity experience by the seed and wind velocity at reference height. If the thickness of the seed, as it lays on the ground, falls below a threshold aerodynamic roughness length, this parameter will become 0 resulting in no movement of the seed. The threshold lift-off velocity is determined by balance between wind drag and friction between the seed and the ground surface. As such, the mass-to-area ratio of the seed will influence threshold lift-off velocity. In our study, seeds of *E. atherica* are one order of magnitude heavier than seeds of *S. maritima* (Table 6.1) yet differed very slightly in retention ability (and only in the field experiment). Either we did not include enough variability in seed traits (although we included both intermediate and large seeds for our system) or the inclusion of turbulence in the form of waves swamps the effect of these two parameters. Turbulence was not included in model of Schurr *et al.* (2005).

The third important parameter in Schurr *et al.* (2005) is the obstacle encounter rate by the seeds. However, these "obstacles" can also change the properties of the dispersal vector itself, as acknowledged by a mechanistic analytical model for wind dispersal (Katul *et al.* 2005). As water currents encounter vegetation structures, current velocity decreases due to energy losses through hydrodynamic drag and generation of turbulence. Horizontal velocity at 5 cm above the soil was less than half that at 11 cm and 17 cm in the flume study. In other flume studies (Shi *et al.* 1995; Neumeier & Ciavola 2004), the flow velocity in the lower part of the vegetation was always very slow compared to that higher up in the canopy and above the canopy. Current velocity also decreased with increasing distance from the leading edge of the bed in another flume study on Zostera marina beds, whereas mean turbulence intensity increased (Gambi, Nowell & Jumars 1990). This transference of kinetic energy into turbulent energy was also demonstrated in our study, although this process was more marked for the more open stands of *L. vulgare* than for denser *F. rubra* stands. As water flow passes through vegetation, turbulence intensity increases with the introduction of sparse vegetation but then decreases as stem population increases further (Nepf 1999), possibly through interactions between wakes produced behind individual stems (Green 2005). If turbulence intensity, resulting from interactions between flow velocity and vegetation resistance, is augmented by turbulence generated through wave action, it is logical that significantly more seeds would be dislodged and exported as in our waterlogged-seed experiment.

Studies on hydrochory in riparian systems have stressed the importance of hydrodynamic factors in transporting seeds with limited floating ability. For example, a greater proportion of species with short-term floating seeds were found along turbulent reaches than tranquil reaches of rivers in a Swedish riparian system (Nilsson *et al.* 2002). Andersson *et al.* (2000) speculated that the floating capacity of diaspores was not a crucial factor in structuring riparian communities in their study because flooding events

during spring result in such strong and turbulent currents that even seeds with short floating capacities are dispersed effectively. In flume experiments, Merritt & Wohl (2002) found that saturated seeds sink and get trapped in pool bottoms, thus requiring hydraulic remobilization to move downstream. However, very few other studies on hydrochory have specifically addressed the role of wave action on seed movement (but see Nilsson *et al.* 2002). In addition, the majority of hydrodynamic studies in vegetation have focussed on unidirectional flow rather than waves (see references in Bouma *et al.* 2005) but the present study clearly demonstrates that it is necessary to include waves in order to understand transport processes, especially for particles that have a limited floating capacity. The incorporation of updrafts caused by wind turbulence and thermals have resulted in mechanistic models that simulate more realistic dispersal kernels for wind dispersal (Soons 2006; Katul *et al.* 2005).

Seeds face two different challenges with respect to movement (or lack of movement). Firstly, they must disperse in order to colonize new areas, escape from higher mortality near con-specifics and/or find microsites that enhance establishment probability (Howe & Smallwood 1982). Secondly, once reaching a suitable microsite, they must stay long enough to imbibe water, germinate and establish. Current results provide us with an insight into how the balance of dispersal and retention affect seed distribution over time in our open, temperate, hydrochorous systems. Seeds are likely driest in summer and early autumn during the period of initial detachment from parent plants. Both dispersal probability and distance would be enhanced by higher buoyancy of initially dry seeds and strong tidal action is not necessary to dislodge seeds as they float up even with a gentle upcoming tide (Fig. 6.2). Redistribution of seeds, when they are able to dry out between tidal inundations and rain fall, will also be greatly influenced by buoyancy traits of seeds during this period. Seeds are more likely to be waterlogged from late autumn to early spring when further secondary dispersal by tidal water will continue to redistribute seeds. Tides are generally higher, storms also occur more frequently and there is more standing water present on the marsh surface during this period. Once waterlogged, many seeds will tend to remain at a microsite unless they are dislodged by wave action, as occurs during storms. On the salt marsh of Schiermonnikoog, the highest density and diversity of seeds captured by traps during secondary dispersal were found during a stormy period (Chang, Veeneklaas & Bakker 2007). After the last storm of the season, there may be some localized dispersal but many seeds will be retained at microsites, especially if located in dense vegetation, thus setting the template for seedling establishment or incorporation into the soil seed bank.

In conclusion, the same seed can have traits that facilitate dispersal and retention, depending upon the moisture conditions of the environment. Thus the level of moisture can exert strong control in this system, determining whether buoyancy or hydrodynamics, influenced by vegetation type, will most influence patterns of seed retention. Studies considering buoyancy traits alone, separated from their ecological context, were not able to greatly explain patterns of community structure in some cases (Andersson, Nilsson & Johansson 2000; Danvind & Nilsson 1997), whereas studies considering buoyancy traits in relation to hydrodynamic variables, landscape features and other

biotic influences were more successful (Nilsson *et al.* 2002). Further studies are needed to build empirical or mechanistic relationships between seed retention and predictor variables such as flow velocity, wave action, vegetation resistance and buoyancy. However, the present study provides insights in how to integrate the opposing processes of dispersal and of retention into a single conceptual model.

Appendix S6.1 ANOVA results for field experiment. Three seed types (*Suaeda maritima, Elytrigia atherica, Plantago maritima*), three vegetation types (*Spartina anglica, Limonium vulgare, Puccinellia maritima*)- and five sites were used. For the degrees of freedom, the df for the numerator is given followed by the denominator.

Source	df	MS	F	Significance
Seed type	2, 8	7.971	49.258	$P < 0.001$
Vegetation type	2, 8	0.700	25.713	$P < 0.001$
Seed × Vegetation	4, 16	0.664	9.602	$P < 0.001$
Site	4, 3.92	0.066	0.551	$P = 0.711$
Site × Seed	8, 16	0.162	2.341	$P = 0.070$
Site × Vegetation	8, 16	0.027	0.394	$P = 0.908$
Site × Seed × Vegetation	16, 135	0.069	1.843	$P = 0.031$
Error	135	0.038		

Appendix S6.2 Number of seeds found in control patches during the field experiment. There was one control patch per vegetation type (*Spartina anglica, Limonium vulgare, Puccinellia maritima*) per site (5). Seeds of *Suaeda maritima* and *Elytrigia atherica,* but not of *Plantago maritima,* were found. As very few seeds were found, only data for patches with captured seeds are shown.

Site	Vegetation type	Seed type	Number of seeds
1	*L. vulgare*	S. maritima	1
2	*L. vulgare*	S. maritima	3
2	*S. anglica*	E. atherica	1
4	*S. anglica*	E. atherica	1
4	*S. anglica*	S. maritima	2
5	*Puccinellia maritima*	S. maritima	3
5	*S. anglica*	S. maritima	8

Appendix S6.3 Repeated-measures ANOVA results for flume experiment using dry seeds. We used three seed types (*Suaeda maritima, Elytrigia atherica, Plantago maritima*), two vegetation types (*Limonium vulgare, Festuca rubra*) and three hydrodynamic treatments (upcoming tide, tide + flow, tide + flow + waves). The 'waves' treatment was a combined treatment of flow current and waves. n = 5

Source	df	MS	F	Significance
Within-subjects factors				
Hydrodynamics	1.363	0.530	4.403	0.033
Hydrodynamics × seed	2.726	0.047	0.389	0.743
Hydrodynamics × vegetation	1.363	0.235	1.955	0.168
Hydrodynamics × seed × vegetation	2.726	0.52	0.430	0.715
Error	3.935	32.710	0.120	
Between-subjects factors				
Seed	2	1.498	27.800	<0.001
Vegetation	1	0.694	12.874	0.001
Seed × vegetation	2	0.018	0.336	0.718
Error	24	0.054		

Appendix S6.4 Repeated-measures ANOVA results for flume experiment using waterlogged seeds. We used three seed types (*Suaeda maritima, Elytrigia atherica, Plantago maritima*), two vegetation types (*Limonium vulgare, Festuca rubra*) and two hydrodynamic treatments (flow, waves). The 'waves' treatment was a combined treatment of flow current and waves. n = 5

Source	df	MS	F	Significance
Within-subjects factors				
Hydrodynamics	1	7.740	335.915	<0.001
Hydrodynamics × seed	2	0.089	3.869	0.035
Hydrodynamics × vegetation	1	0.510	22.120	<0.001
Hydrodynamics × seed × vegetation	2	0.085	3.675	0.041
Error	24	0.023		
Between-subjects factors				
Seed	2	0.160	10.060	0.001
Vegetation	1	1.032	64.891	<0.001
Seed × vegetation	2	0.056	3.501	0.046
Error	24	0.016		

Chapter 7

General dicussion

In the past decades *Elytrigia atherica* has been increasing in frequency and dominance on the salt marshes along the North Sea coast. The rapid spread and dominance of *E. atherica* has led to monospecific stands of the tall grass, and hence a low species and structural diversity (Bakker 1989; Vàlery, Bouchard, & Lefeuvre 2004). This rapid expansion does not only affect the vegetation community but also the invertebrate fauna and bird populations. All salt marshes in the Wadden Sea are protected under national nature legislation and within EU Habitats Directives. The unified management aim of the Netherlands, Germany and Denmark (Trilateral Wadden Sea Plan) is to maintain and where possible to extend the area of salt marshes and gain a higher biodiversity (Esselink *et al.* 2009). The observed increase of late-successional salt-marsh communities at the expense of young successional stages and biodiversity is currently an important of issue concern in nature conservation of the Wadden Sea Area. In the 1960s, concerns regarding the decrease of species diversity due to domination by competitive grasses were already stated by Neuteboom (1963). However, the main species of concern during that period was the low-statured *Festuca rubra*. The increase in domination by grasses on salt marshes has gradually been acknowledged by nature managers and conservationists. Today, the tall *E. atherica* is the commonly acknowledged candidate to take over grasslands and is assumed to be the climax stage of salt-marsh vegetation on mainland and back-barrier island salt marshes (Dijkema *et al.* 2005). In the present chapter I will discuss the rapid spread of *E. atherica* in the past decades on the salt marshes in the Wadden Sea area. Additionally I will discuss the dispersal strategies of *E. atherica* in relation to management and succession. Finally, I will indicate the future challenges of *E. atherica*.

Invasion of *E. atherica* on salt marshes
Internationally eutrophication as a result of increased nitrogen input from watersheds to estuaries has become a major environmental concern in coastal systems (Vitousek *et al.* 1997). The findings of **Chapter 2** showed that the rapid expansion of *E. atherica* along the Wadden Sea coast is not likely to be caused by increased anthropogenic nutrients. It is more likely a result of successional processes on young and/or natural marshes, and changes in vertical accretion and/or cessation of livestock grazing in older natural marshes and marshes established from sedimentation fields. In the earlier years of salt-marsh development on Schiermonnikoog and on the young marshes of Schiermonnikoog and Spiekeroog, a high intrinsic rate of spread was found (Table 2.3), which is consistent with the competitive and clonal nature of *E. atherica* and it's potential to spread rapidly. A similar rapid expansion of *E. atherica* has been observed at other study sites along the Wadden Sea coast (Leendertse *et al.* 1993; Dijkema *et al.* 2005). Young stands of *Phragmites australis* along the coast of Chesapeake Bay also showed a similar pattern (Rice, Rooth & Stevenson 2000). Concurrently with the development of these islands, a decrease in expansion rate with increasing salt-marsh age has been observed. This suggests that the rate of expansion of *E. atherica* stagnates or decreases as fewer suitable sites remain for *E. atherica* to colonise. In the oldest section of Schiermonnikoog, *E. atherica* occupied nearly 60% of salt marsh in 2004 (Fig. 2.5). A similar level-

ing off in expansion, as a result of reaching equilibrium or simply because of limited space, has been found in old and well-established stands of *Phragmites australis* (Rice, Rooth & Stevenson 2000) and *Spartina alterniflora* (Rice, Rooth & Stevenson 2000; Civille *et al.* 2005). Although the expansion rate stagnated at the older sites, the abundance of *E. atherica* actually increased. The establishment of the artificial dune ridge on Terschelling in 1934 initiated vegetation development over the entire eastern part of the salt marsh at the same time. Corresponding with this trajectory of vegetation development, little variation was found in the expansion rate between the different sections of the salt marsh on Terschelling (Table 2.3).

E. atherica is a successful clonal plant, able to spread extensive distances underground through rhizomes, and rapidly increases in dominance after(**Chapter 4**). Contradictory to our hypothesis that the incidence and dominance of *E. atherica* increases with number of years after cessation of grazing, we observed a negative relationship between the dominance of *E. atherica* and the number of years of cessation of grazing on mainland marshes (Fig. 3.4.A). This negative relation could be the result of the initial selection by farmers over which sedimentation fields would be abandoned along the coast. Economic and logistic reasons affect the preference for abandoning certain sedimentation fields. The first fields to be abandoned are most likely the sedimentations fields with the lowest production rate of palatable species or which are least suitable for livestock grazing due to poor drainage conditions. The abiotic conditions at these fields are also less optimal for *E. atherica*. Additionally, it is quite likely that the high-marsh sedimentation fields, more conveniently located adjacent to the sea wall, are maintained and grazed for a longer period. As a result of intensive grazing, the spread of *E. atherica* was controlled at these fields, and *E. atherica* remains present in low densities. Thus, suggesting that the increase of *E. atherica* is not affected by duration of abandonment, but more likely by the abiotic conditions of the abandoned sites. If the abiotic conditions, i.e. inundation frequency depending on elevation and drainage, are optimal for the species, it is merely a matter of a couple of years for *E. atherica* to dominate the marsh, but if elevation is low and the artificial drainage of the marsh is diminishing, *E. atherica* will not be able to dominate the vegetation.

Optimal biotic and abiotic conditions for *E. atherica*

Surface elevation change encompasses two processes: it can increase the amount of nutrients in the soil, as suspended sediment transported by inundations (Olff *et al.* 1997); it can also increase elevation when sediment input is higher than auto compaction (Nolte *et al.* 2013). A high number of studies have shown that salt-marsh succession is induced by sedimentation, since increased sediment deposition leads to increased nitrogen (N) accumulation (Roozen & Westhoff 1985; Leendertse, Rozema, & Andrea 1997; Olff *et al.* 1997). Van Wijnen & Bakker (1997) showed that on back-barrier marshes, clay thickness is highly correlated with N-pool, and N-mineralisation (Bakker, Bouma, & Van Wijnen 2005), but overestimated at sites with thicker clay layers. However, the above mentioned studies were conducted on back-barrier marshes with a relative thin clay layer on top of a sandy sub-soil.
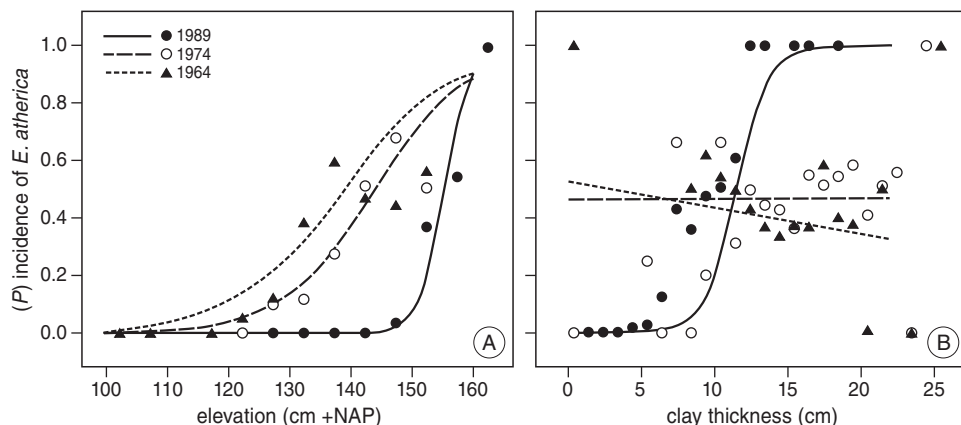
Figure 7.1 Probability of incidence of *E. atherica* as isolated patches along a successional gradient (years in legend indicate year when vegetation start to develop on the saltmarsh) (A) in relation to elevation and (B) in relation to clay thickness.

Studies on mainland marshes did not find such a response of *E. atherica* to fertiliser application (Kiehl *et al.* 1997; Bockelmann & Neuhaus 1999, personal communications Kai Jensen 2010). It is likely that biomass production on mainland marshes cannot increase due to fertilisation as a result of self-shading (Kiehl, Esselink, & Bakker 1997). Personal observations on isolated patches of *E. atherica* on the lower marsh along a successional gradient on the back-barrier marsh of Schiermonnikoog showed that the incidence of *E. atherica* is strongly correlated with elevation at all successional gradient of the marsh (Fig. 7.1A). Strikingly, the incidence of *E. atherica* was only correlated with clay thickness on the younger marsh (Fig. 7.1B). However, a significant positive correlation was found between the patch size of *E. atherica* and clay thickness (Pearson Correlation 0.530, P < 0.001) (Fig. 7.2). Thus, suggesting that in later successional stages, with a clay thickness of approximately 10cm or more, the clay (as nutrient supplier) has no additive effect on the incidence of *E. atherica*, but may have an effect on the competitive ability of the species. In accordance with the latter studies (on mainland marshes), our results suggest that nitrogen limitation does not play a role on mainland or older back-barrier marshes and sedimentation as a nitrogen source is not a determinant of the incidence of *E. atherica*.

Even though sediment input may not directly affect the occurrence *E. atherica*, as mainland marshes and older back-barrier marshes are not nitrogen limited (Kiehl *et al.* 2001), the rate of surface elevation change does affect the elevation of the mainland marshes and therefore indirectly the occurrence of *E. atherica*. Stock (2011) measured positive surface elevation change rates of 20 mm/year close to the intertidal flats and hardly any change in surface elevation at a distance of 1 km from the intertidal flats. Concurrent to this, *E. atherica* did spread close to the intertidal flats, but not at sites with no surface elevation change on ungrazed parts of the salt marsh (Schröder, Kiehl,
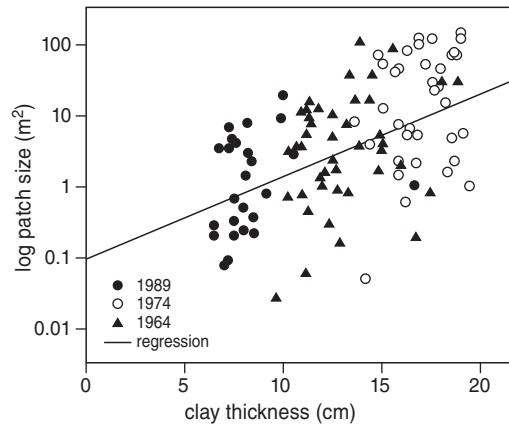
Figure 7.2 Correlation between size of isolated patches of *E. atherica* and clay thickness along a successional gradient. The years in the legend indicate the initial year when vegetation started to develop on the salt marsh.

& Stock 2002). The rate of surface elevation change is thus one predictor to estimate when a salt marsh will reach the elevation suitable for *E. atherica*.

The occurrence and dominance of *E. atherica* is strongly related to the drainage conditions of a salt marsh (Fig. 3.5). The main purpose of a drainage system is to divert flooding seawater in order to promote soil aeration, which is one of the major factors controlling plant growth in salt marshes (Armstrong *et al.* 1985; Adam 1990; Bakker *et al.* 2002; Marani *et al.* 2006). On a back-barrier marsh at great distance from the intertidal flats, a decrease of *E. atherica* was recorded (Fig. 2.5). The species was succeeded by *Phragmites australis*, indicating waterlogged conditions. The low incidence of *E. atherica* on sites with waterlogged conditions, as observed by Schröder *et al.* (2002) and in **Chapter 2**, suggests that *E. atherica* does not like 'wet feet'. Bockelmann *et al.* (2002) also suggest that besides inundation frequency, inundation duration may be of great importance for plants. Esselink *et al.* (2000) has shown that the neglect of artificial drainage systems clearly resulted in a lower drainage index of the permanent plots in later years. Personal observations in German and Dutch salt marshes have shown that on plots with low elevation, the artificial drainage system has partly filled up with sediment and drainage is blocked whereas on higher elevated locations, the drainage system is maintaining itself and functioning well. Thus, the cessation of ditch maintenance on the German mainland salt marshes is one of the important factors explaining the slow invasion of *E. atherica* on those salt marshes compared to the Dutch mainland marshes.

Dispersal strategies
The seed production and seed rain (deposition of seeds at a site) of *E. atherica* increases with age of the salt marsh (Chang 2006). As seeds of *E. atherica* are missing from the soil seed bank on the young salt marsh, and only occurring in low quantities in autumn

(after seed rain) on the older salt-marsh sites, seeds need to disperse to the right place in the right time for successful establishment. The dispersal of *E. atherica* seeds is significantly related to the distance from the sea and seeds from low marshes were found in abundance on high marshes, but not vice versa (Chang, Veeneklaas, & Bakker 2009). This corresponds well with other studies that found upward movements of seeds on vegetated marsh surface (Bakker, Dijkstra, & Russchen 1985; Huiskes *et al.* 1995). However, large quantities of *E. atherica* seeds are found in seed trapping on sea, suggesting a high export of seeds to other marshes (Wolters *et al.* 2006). Most likely, a great number of these seeds are deposited as driftline on the foot of a dune after winter storms (Wolters & Bakker 2002) and have a minor chance to successfully establish (Veeneklaas and Chang personal observations).

Seeds face two different challenges with respect to movement (or lack of movement). Firstly, they must disperse in order to colonize new areas, escape from higher mortality near con-specifics and/or find microsites that enhance establishment probability (Howe & Smallwood 1982). Secondly, once reaching a suitable microsite, they must stay long enough to imbibe water, germinate and establish. The results of **chapter 6** provide us with an insight into how the balance of dispersal and retention affect seed distribution over time in our open, temperate, hydrochorous systems. Seeds are likely driest in summer and early autumn during the period of initial detachment from parent plants. Both dispersal probability and distance would be enhanced by higher buoyancy of initially dry seeds and strong tidal action is not necessary to dislodge seeds as they float up even with a gentle upcoming tide (Fig. 6.2). Redistribution of seeds, when they are able to dry out between tidal inundations and rain fall, will also be greatly influenced by buoyancy traits of seeds during this period. Seeds are more likely to be waterlogged from late autumn to early spring when further secondary dispersal by tidal water will continue to redistribute seeds. Tides are generally higher; storms also occur more frequently and there is more standing water present on the marsh surface during this period. Once waterlogged, many seeds will tend to remain at a microsite unless they are dislodged by wave action, as occurs during storms. On the salt marsh of Schiermonnikoog, the highest density and diversity of seeds captured by traps during secondary dispersal were found during a stormy period (Chang, Veeneklaas, & Bakker 2009). After the last storm of the season, there may be some localized dispersal but many seeds will be retained at microsites, especially if located in dense vegetation, thus setting the template for seedling establishment.

Sexual and vegetative propagation in *E. atherica* are both important in colonizing new sites. The establishments of *E. atherica* seedlings increases with succession of the salt marsh as environmental conditions improve (Kuijper, Nijhoff, & Bakker 2004). Seedling establishment, i.e. sexual reproduction, therefore, becomes increasingly dominant, thereby affecting genetic and demographic structure of *E. atherica* on the older salt marshes. However, severe clonal competition may decrease the clonal diversity in the long run, tipping the balance to vegetative reproduction. On a late successional salt marsh a significantly higher clonal diversity was found in comparison to an early successional marsh (Fig. 5.2). On the contrary, studies on *P. maritima* (Gray, Parsell, & Scott

1979) and *Agrostis stolonifera* (Kik, Vanandel, & Joenje 1990), found a lower number of clones in the mature populations. Gray et al (1979) suggested two possible processes affecting the population clonal structure. On the one hand, during development of a marsh a decrease in the number of genotypes is expected, as individuals may be eliminated by competition. On the other hand individuals in the pioneer marsh may be continuously replaced in the mature marsh by new individuals, in which case the number of individuals per unit area between pioneer and mature sites may be similar. However, there is a third possibility; the number of clones could increase over time due to increasingly favourable conditions on the marsh. The results of **chapter 5** learn that both the first and third option occurs in the studied populations of *E. atherica*, as explained below.

Two important environmental variables affecting clonal structure can explain the differences in clonal diversity between the young and old sites. First, herbivory can have a profound negative effect on seedling establishment (Hangelbroek *et al.* 2002; Kuijper, Nijhoff, & Bakker 2004). Colonisation of new sites by *E. atherica* happens mainly through seedling recruitment (Bockelmann, Wels, & Bakker 2011). Seeds of *E. atherica* are able to establish successfully on 10-year-old salt marsh when hares and geese are excluded, whereas under natural conditions, grazing by hares and geese prevent or at least slow down invasion of *E. atherica* on young successional stages. On older successional stages, however, herbivory on seedlings is less severe (Kuijper & Bakker 2003; Kuijper, Nijhoff, & Bakker 2004; Kuijper, Dubbeld, & Bakker 2005). Hence, the probability for successful establishment will be higher at the older site in comparison to the younger site.

The second variable potentially affecting the clonal structure of *E. atherica* is clay thickness. Clay thickness is, to a certain extent, positively correlated with soil nitrogen content and increases over time due to tidal sediment deposition (Olff *et al.* 1997; Van Wijnen & Bakker 1997). Nitrogen is the limiting factor to early successional stages of salt-marsh succession and succession of *E. atherica* in particular (Kiehl, Esselink, & Bakker 1997; Leendertse, Rozema, & Andrea 1997) and is also likely to be involved in salt tolerance of salt-marsh plants (Stewart & Lee 1974; Mansour 2000). With an increasing age of the salt marsh, increasingly favourable conditions for seedling recruitment of *E. atherica* are present along the chronosequence. Whereas extensive clones can redistribute nutrients (D'Hertefeldt & Jonsdottir 1999) and thus are less affected by locally thin clay layers, seedlings cannot cope with a nitrogen deficit. Seedlings are, therefore, highly dependent on the site conditions where they germinate. Seedlings on the older site are less likely to face a nutrient constraint and probably have a higher salt tolerance. On the younger site *E. atherica* is more strongly confined to areas with a relatively higher elevation (Table 5.2 and Fig. 7.1), possibly to reduce inundation and salt stress.

As patches or subpopulations of *E. atherica* age and increase in abundance and density, intra-specific competition is likely to play a counteracting role (Bengtson 2003), resulting in a loss of genets and therefore a decrease in clonal diversity (Gray, Parsell, & Scott 1979; Watkinson & Powell 1993). Empirical evidence for intra-specific competition decreasing clonal diversity has been found for various species at different time-

scale: *Lolium perenne* (Mcneilly & Roose 1984), *Calamagrostis epigejos* (Lehmann 1997), *Spartina alterniflora* (Travis & Hester 2005). Vegetation density could facilitate for an input of new individuals as vegetation density is positively correlated with seed entrapment (Fig. 6.2). Though vegetation density or, more importantly, the tall and dense canopy could limit seedling recruitment, as competition for light will be high (Wilson & Tilman 1991; Aspinwall & Christian 1992; Bakker & De Vries 1992; Piquot *et al.* 1998). In many clonal plant species seedling recruitment is highly dependent on disturbance, e.g. removal of above-ground biomass (Watkinson & Powell 1993; Eriksson & Jakobsson 1998). Although the sample size is too small, the three largest sampled patches, in Fig. 5.2, hint a negative trend in clonal diversity with increasing patch size. Travis *et al.* (2002) found a high genetic diversity within young populations of *S. alterniflora*. Apparently, the seedling recruitment in populations of *S. alterniflora* is limited to the first 30 years after initial colonisation, whereafter clonal diversity will decrease very slowly (Travis & Hester 2005). As *E. atherica* resembles the clonal salt marsh species, Spartina alterniflora, the same structural patterns could be found for *E. atherica* when populations get older, as in the three large patches in our study. Thus, aging of the population will possibly lead to a decrease of seedling recruitment and a severe clonal competition may decrease the clonal diversity (Gray, Parsell, & Scott 1979; Kik *et al.* 1990).

A comparable low clonal diversity was found within abandoned, *E. atherica* dominated plots in an experiment on a late successional grazed marsh (Fig. 4.3). In grazed and mown plots a higher clonal diversity was observed. Disturbance, like mowing and grazing, may enhance the transport of seeds and bulbils (Mouissie, Lengkeek, & Van Diggelen 2005; Reisch & Scheitler 2009) and thus increasing the clonal diversity. A higher clonal diversity was expected in the grazed than in the mown areas as large herbivores often have positive effects on seedling recruitment by opening up the vegetation through grazing and creating gaps through trampling (Bakker 1987; Silvertown & Smith 1988; Bullock *et al.* 1994). But despite the higher germination recorded in the grazed plots, seedling survival was higher in mown plots at the present study site (Bakker & De Vries 1992). In grasslands, small-scale disturbances may enhance germination but not necessarily seedling survival (Eriksson 1997). In *Trifolium repens*, disturbance enhances germination but the survival of seedlings was higher in undisturbed vegetation (Barrett & Silander 1992). Kuijper *et al.* (2004) showed that the survival of seedlings of *E. atherica* was lower when neighbouring plants are removed, than when subject to competition from neighbouring plants. The single annual mowing event reduces competition and creates a window of opportunity for seedling recruitment. The presence of large numbers of small sized genets and the highest total number of genets in the mown plots support this. Although disturbance by cattle regularly creates windows of opportunity for seedling recruitment the continuous nature of the disturbance reduces the chance of successful recruitment. Disturbance can enhance seedling recruitment but too much disturbance will enhance clonal dispersal.

The data in Table 5.3 revealed low differentiation between sites indicating habitat differentiation between the studied lower-marsh sites. This is consistent with the study by Bockelmann *et al.* (2003) that showed low but significant differentiation between
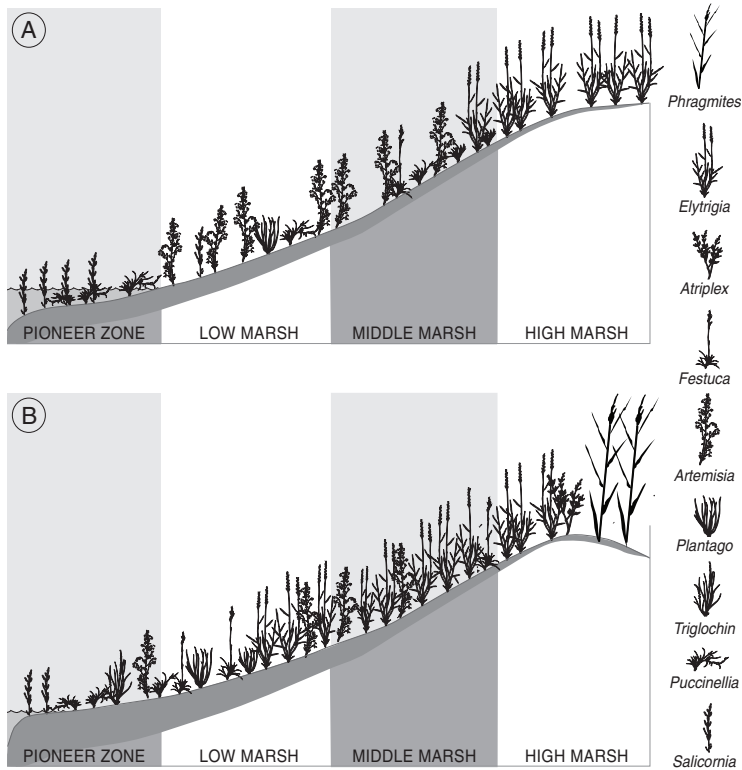
Figure 7.3 Changes in salt marsh succession on a salt marsh. In earlier succession (A) *E. atherica* occurs only at higher elevations. Over time (B) *E. atherica* expands to the lower marsh, but decreases on marshes far from the sea.

sites at comparable distances on the same island of Schiermonnikoog. In addition, Refoufi & Esnault (2006) found a comparable average genetic differentiation among populations at the same spatial scale in Mont Saint-Michel Bay, France. In both studies, this genetic differentiation between sites with distances <60 km could not be attributed to isolation-by-distance. Bockelmann *et al.* (2003) therefore attributed this differentiation to measured differences in biotic or abiotic conditions between sites (lower marsh vs. higher marsh). The isolated patches on the lower salt marsh established most likely independent from each other, as there was no correlation between genetic similarity and geographic distance. The observed high genetic similarity within patches suggests that seedlings establishing in the patch mainly originate from within the patch.

Conclusion
The rapid spread of *E. atherica* on mainland and back-barrier marshes is for a great extent a consequence of succession. At young successional stages the incidence of *E. atherica* is restricted the high marsh (Fig. 7.3A). In time of succession *E. atherica* gradu-
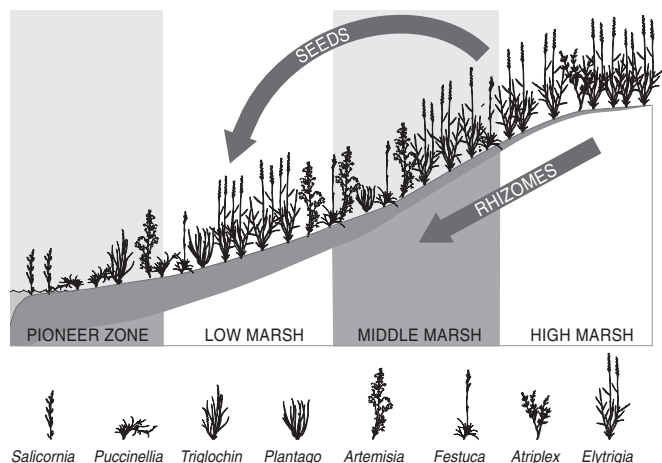
Figure 7.4 Rapid spread of *E. atherica* to lower marsh and new habitats occurs by means of seed dispersal and rhizomes.

ally spreads to lower elevation of the salt marsh. But additionally, in time of succession, the incidence of *E. atherica* decreases on the salt marshes far from the sea, due to decrease of sediment input and accelerated sea-level rise (Fig. 7.3B).

Dispersal through seedlings and rhizomes are both important in the rapid spread of *E. atherica* (Fig. 7.4). At young successional stages and lower salt marshes *E. atherica* establishes through seedlings. Successful seedling establishment is strongly related to suitable abiotic and biotic factors, such as nutrient availability and gaps. Once successfully established as a seedling the spread of *E. atherica* can occur through rhizomes and rapidly out compete other species. *E. atherica* can spread and invade clonally from high marsh to lower marsh, or spread clonally as an isolate patch on a lower marsh.

Future of *E. atherica*

Although the *E. atherica* community has been described as the climax stage of succession, a decrease of the *E. atherica* community was observed on the oldest part of Schiermonnikoog and Terschelling at a large distance from the intertidal flats (Fig.2.5 and App S2.2). The decrease of the *E. atherica* community is most likely related to local environmental changes. On Schiermonnikoog and Terschelling, the former *E. atherica* stands have recently been replaced by stands of *Phragmites australis* and *Juncus gerardii*, suggesting waterlogging conditions. A comparable process, leading to the decline of *Elytrigia repens*, was caused by waterlogging on brackish mainland salt marshes (Esselink *et al.* 2000). Esselink *et al.* (2000) and Schröder *et al.* (2002) have shown that there is a decreasing gradient of annual vertical accretion in relation to distance from the intertidal flat. Stock (2011) and Temmerman et al (2005) found that sediment is mainly supplied via the creek/drainage system and mainly deposited close to the source and along the levees. The input of sediments is related to the frequency, height and duration of

tidal inundations and, along with the sediments, organic matter including nitrogen is imported (Olff *et al.* 1997; Wijnen, Bakker, & Vries 1997; Esselink & Chang 2010). A decrease of sediment input on older salt-marsh site may lead to a decrease of available nitrogen. Moreover, a decrease of sediment input accompanied by soil autocompaction may also lead to the formation of depressions with stagnant water. This process may be further enhanced by sea-level rise, and coinciding with poor drainage, will lead to water-logged conditions after tidal inundations and rainfalls. Predictive models of sea-level rise for back-barrier marshes suggest that the surface elevation of the salt marsh close to the source will be able to keep up with the pace, however an increasing inundation frequency is expected (Wijnen & Bakker 2001; Stock 2011). The more inland salt marsh area, with low accretion rate will not be able to keep up with sea-level rise. This will lead to rewetting of the marsh, and thus lead to unsuitable conditions for *E. atherica*.

In this project the question where and when *E. atherica* will dominate the salt marsh remains partially unanswered. The answer to this question will help us to understand the consequences of future accelerated sea-level rise, but also the implications of management choices. In this project I have unravelled the abiotic and biotic factors which affect the successfulness of the rapid spread of *E. atherica*. Further spatial modelling should be done in order to predict when and how fast *E. atherica* will dominate or disappear from the salt marsh. The most important factors in such a spatial model should be elevation in relation to Mean High Tide, surface elevation surface, accelerated sea-level rise and drainage conditions.

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Summary
Samenvatting

Summary

All salt marshes in the Wadden Sea are protected under national nature legislation and within EU Habitats Directives. The aim of the Trilateral Wadden Sea Plan, under joined management of the Netherlands, Germany and Denmark, is to maintain and where possible to extend the area of salt marshes and increase the biodiversity. The observed increase of late-successional salt-marsh communities at the expense of young successional stages and biodiversity is currently an issue of concern in nature conservation of the Wadden Sea Area. Additionally, the increased dominance of *Elytrigia atherica* on salt marshes has gradually been acknowledged by nature managers and conservationists. In the past decades the tall native grass, *E. atherica* has increased in frequency and dominance on the salt marshes along the North Sea coast. The rapid spread and dominance of *E. atherica* has led to monospecific stands of the tall grass, and hence a low species and structural diversity. Around 1980, approximately 8% of the salt marsh along the Dutch coast was covered by *E. atherica* and this expanded to 17% coverage around 2000. This rapid expansion does not only affect the vegetation community but also the invertebrate fauna and bird populations. Concurrently it is assumed that *E. atherica* will be the climax stage of salt-marsh vegetation on the mainland and back-barrier island salt marshes.

The main aim of this thesis is to study how *E. atherica* has been able to spread so rapidly in the past decades on the salt marshes in the Wadden Sea with focus on the possible dispersal strategies which apply for this species: through rhizomes and/or through seeds. In particular, it is interesting to know whether *E. atherica* has adapted its dispersal strategy in relation to management and succession.

Invasion of *E. atherica* on the salt marsh

Natural succession is an important factor in the increase of *E. atherica* dominance. In the later successional stages of salt marshes, more nutrients are available as due to silt accumulation. Increase of anthropogenic eutrophication may lead to an increased nitrogen availability on the overall salt marsh, independent of the successional stage of the marsh. This would enable a late-successional species, such as *E. atherica*, to expand more rapidly and spread to lower and younger marshes. It is difficult to identify the relative influences of natural succession and anthropogenically induced processes on the rapid spread of *E. atherica*. Therefore, I used a combination of time series of vegetation maps and chronosequence data of four naturally developed back-barrier salt-marshes (Terschelling (NL), Schiermonnikoog (NL), Norderney (D) and Spiekeroog (D)) to address this problem (**chapter 2**). The selected natural salt marshes have not been grazed by livestock or been subjected to other management regimes. I found different rates of expansion of *E. atherica*. The expansion occurred at higher rates on young marshes than on older marshes. Young marshes have relatively high levels of vertical accretion, which leads to greater nitrogen input into the soil, whereas older marshes have low levels of nutrient input. The expansion rates were lowest on the oldest salt marshes suggesting that the rate of expansion of *E. atherica* stagnates or decreases as

fewer suitable sites remain for *E. atherica* to colonise. Since the amount of atmospheric deposition of nitrogen is similar on young and older marshes, we conclude that the spread of *E. atherica* is more likely due to natural succession than caused by anthropogenic input of nutrients.

In the past decades economic motivations of farmers and changes in nature management goals has lead to cessation of grazing with livestock on a great number of mainland salt marshes along the Wadden Sea coast. Concurrent with this management change *E. atherica* increased in both incidence and dominance. However, this has not been observed on all abandoned mainland marshes. I used long-term data on vegetation, elevation, sedimentation and drainage conditions to study which factors apart from grazing management may affect the rate of increase of *E. atherica* on mainland salt marshes (**chapter 3**). Contrary to expectation, I found that the dominance of *E. atherica* did not increase with the number of years since cessation of grazing. The incidence and dominance of *E. atherica* on mainland marshes was mainly related to elevation and drainage conditions. Better drainage conditions had a positive effect on the incidence and dominance of *E. atherica*. When abiotic conditions, namely inundation frequency and drainage, are optimal for *E. atherica*, this species can dominate the marsh within a few years. However, if drainage is neglected, this dominance does not occur. Observations in German and Dutch salt marshes have shown that on plots with low elevation, the artificial drainage system has partly filled up with sediment and drainage is blocked whereas on higher elevated locations, the drainage system is maintaining itself and functioning well. Thus, the cessation of ditch maintenance on the German mainland salt marshes is one of the important factors explaining the slow invasion of *E. atherica* on those salt marshes compared to the Dutch mainland marshes.

Optimal site conditions for *E. atherica*

A large number of studies have shown that salt-marsh succession is induced by sedimentation, since increased sediment deposition leads to increased amounts of nutrients in the soil and to increase of the elevation of the marsh, when the sediment input is higher than the auto compaction. To assess the optimal abiotic conditions for *E. atherica* to establish on the salt marsh, I measured the incidence of *E. atherica*, clay thickness and elevation in isolated patches of *E. atherica* on the lower marsh along a successional gradient on the back-barrier marsh of Schiermonnikoog. I found that the incidence of *E. atherica* is strongly correlated with elevation at all successional gradients of the marsh. Also, a positive correlation was found between the patch size of *E. atherica* and clay thickness at all successional gradients of the marsh. Strikingly, the incidence of *E. atherica* was only correlated with clay thickness on the younger marsh. Thus, suggesting that in later successional stages, with a clay thickness of approximately 10 cm or more, the clay (as nutrient supplier) has no additive effect on the incidence of *E. atherica*, but may have an effect on the competitive ability of the species.

The rate of sedimentation is very variable in space and also affects the spatial distribution of *E. atherica*. Close to the intertidal flats sedimentation results in surface elevation changes of approximately 20 mm/year, whereas at a distance of 1 km from the

intertidal flats hardly any change in surface elevation is observed. Concurrent to this, on ungrazed areas of the salt marsh, *E. atherica* did spread close to the intertidal flats, but not at sites with no surface elevation change. The rate of surface elevation change is thus one predictor to estimate when a salt marsh will reach the elevation level suitable for *E. atherica*. Furthermore, the occurrence and dominance of *E. atherica* is strongly related to the drainage conditions of a salt marsh (**chapter 2 & 3**). My findings suggest that *E. atherica* does not like 'wet feet'.

Dispersal strategies

The seed production and seed rain (deposition of seeds at a site) of *E. atherica* increases with age of the salt marsh. As seeds of *E. atherica* are missing from the soil seed bank on the young salt marsh, and only occurring in low quantities in autumn (after seed rain) on the older salt-marsh sites, seeds need to disperse to the right place in the right time for successful establishment. The dispersal of *E. atherica* seeds is strongly related to the distance from the sea and seeds from low marshes were found in abundance on high marshes, but not vice versa. However, large quantities of *E. atherica* seeds are found in seed trapping on sea, suggesting a high export of seeds to other salt marshes. Most likely, a great number of these seeds are deposited as driftline on the foot of a dune after winter storms and have a smaller chance to establish successfully. In **chapter 6** I studied the effects of moisture conditions, seed morphology, vegetation structure and hydrodynamic variables on seed retention and movement. I compared the seed traits of three species, *Plantago maritima*, *Suaeda maritima* and *E. atherica* . Additionally I studied the effect of three different vegetation types on the retention of these seed on the salt marsh and in a flume facility where hydrodynamic variables could be controlled. In all these experiments, the least number of seeds of *E. atherica* were retained in the vegetation, in comparison to the other species. Dry *E. atherica* seeds had a higher floating capacity and dislodged more easily. Wave action had dislodged more seeds than flow velocity alone. Denser, less rigid vegetation types, such as *Festuca*-vegetation retained greater numbers of seeds than more open, more rigid vegetation types, as *Limonium*-vegetation. These results suggest that buoyancy traits appear to determine whether seeds move in the drier summer and autumn months, after initial detachment from parent plants. However, the intensity of wave action will determine whether waterlogged seeds stay in a microsite during the wetter months of late autumn to early spring.

In **chapter 5**, I assed what mode of propagation, sexual or vegetative, governs the successful spread of the species in new habitats. I used microsatellite fingerprint patterns to determine genetic identities and study the clonal diversities of differently sized patches of *E. atherica* on two lower salt-marsh sites of different age, 25 and 35 years respectively. On the late successional salt marsh a significantly higher clonal diversity was found in comparison to an early successional marsh. Two important environmental variables affecting clonal structure can explain the differences in clonal diversity between the young and old sites. First, herbivory can have a profound negative effect on seedling establishment. On older successional marshes, however, herbivory on seedlings is less severe. Hence, the probability for successful establishment will be

higher at the older site in comparison to the younger site. The second variable potentially affecting the clonal structure of *E. atherica* is nutrient availability. The lack of nitrogen, a nutrient and an enzyme compound for salt tolerance, is the limiting factor in early successional stages of salt-marsh and succession of *E. atherica* in particular. Whereas extensive clones can redistribute nutrients and thus are less affected by locally thin clay layers, seedlings cannot cope with a nitrogen deficit. Seedlings are, therefore, highly dependent on the site conditions where they germinate. With an increasing age of the salt marsh, increasingly favourable conditions for seedling recruitment of *E. atherica* are present and thus seedlings on the older site are less likely to face a nutrient constraint. On younger sites, due to nutrient constraints, *E. atherica* seedlings are more strongly confined to areas with a relatively higher elevation, to reduced avoid frequent inundation and salt stress. As patches or subpopulations of *E. atherica* age and increase in abundance and density, intra-specific competition is likely to play a counteracting role, resulting in a loss of genets and therefore a decrease in clonal diversity. Vegetation density could facilitate for an input of new individuals as vegetation density is positively correlated with seed entrapment. Though vegetation density or, more importantly, the tall and dense canopy could limit seedling recruitment, as competition for light will be high. Three largest sampled patches hint a negative trend in clonal diversity with increasing patch size.

A long-term (30-year) field experiment where populations of *E. atherica* were subjected to three different management regimes, grazing by cattle, mowing and abandonment enabled me to study the effect of management on the clonal structure of *E. atherica* (**chapter 4**). Abundance and phenotypic traits were affected by the management regimes, resulting in a higher abundance in abandoned fields and plants having shorter and narrower leaves in managed fields. I found that biomass removal affected the clonal structure of populations and increased the genetic diversity compared to that in abandoned fields. Within the abandoned, *E. atherica* dominated plots, a low clonal diversity was found, comparable to the large patches as described in **chapter 5**. In grazed and mown plots a higher clonal diversity was observed. A higher clonal diversity was expected in the grazed and the mown areas, as disturbance, like mowing and grazing, enhance the transport of seeds and bulbils and thus increasing the clonal diversity. Although disturbance by cattle regularly creates windows of opportunity for seedling recruitment, thus resulting in a high clonal diversity, the continuous nature of the disturbance reduces the chance of successful recruitment. The single annual mowing event reduces competition and creates a window of opportunity for seedling recruitment. Thus, disturbance can enhance seedling recruitment but too much disturbance will enhance clonal dispersal.

Future of *E. atherica*

Although the *E. atherica* community has been described as the climax stage of succession, a decrease of the *E. atherica* community was observed on the oldest part of Schiermonnikoog and Terschelling at a large distance from the intertidal flats. The decrease of the *E. atherica* community is most likely related to local environmental changes. On

Schiermonnikoog and Terschelling, the former *E. atherica* stands have recently been replaced by stands of *Phragmites australis* and *Juncus gerardii*, suggesting waterlogging conditions. There is a decrease of sedimentation and vertical accretion at a greater distance to the sea, as sediment is mainly supplied via the creek/drainage system and mainly deposited close to the source and along the levees. A decrease of sediment input on older salt-marsh site or at greater distance to the mudflats may lead to a decrease of available nitrogen. Moreover, a decrease of sediment input accompanied by soil auto compaction may also lead to the formation of depressions with stagnant water. This process may be further enhanced by sea-level rise, and coinciding with poor drainage, will lead to waterlogged conditions after tidal inundations and rainfalls. Predictive models of sea-level rise for back-barrier marshes suggest that the surface elevation of the salt marsh close to the source will be able to keep up with the pace, however an increasing inundation frequency is expected. The more inland salt marsh area with low accretion rate will not be able to keep up with sea-level rise. This will lead to rewetting of the marsh, and thus lead to unsuitable conditions for *E. atherica*. Decrease of *E. atherica* community at the oldest and/or waterlogged marshes suggest that this community is not the climax stage and that there is a world beyond *E. atherica*.

Conclusion

The rapid spread of *E. atherica* on mainland and back-barrier marshes is to a great extent a consequence of succession. At young successional stages the incidence of *E. atherica* is restricted to the high marsh. In time of succession *E. atherica* gradually spreads to lower elevation of the salt marsh. But additionally, in time of succession, the incidence of *E. atherica* decreases on the salt marshes far from the sea, due to decrease of sediment input and accelerated sea level rise. Dispersal through seedlings and rhizomes are both important in the rapid spread of *E. atherica*. At young successional stages and lower salt marshes *E. atherica* establishes through seedlings. Successful seedling establishment is strongly related to suitable abiotic and biotic factors, such as gaps and nutrient availability. Once successfully established as a seedling the spread of *E. atherica* can occur through rhizomes and rapidly out compete other species. *E. atherica* can spread and invade clonally from high marsh to lower marsh, or spread clonally as an isolate patch on a lower marsh. When abiotic conditions, namely inundation frequency and drainage, are optimal for *E. atherica*, this species can dominate the marsh within a few years. But when drainage conditions decrease, as a result of succession, maintenance or climate change, *E. atherica* will disappear, as this species does not like 'wet feet'.

Samenvatting

Alle kwelders in de Waddenzee zijn beschermd onder nationale natuurwetgeving en onder de Europese Habitatrichtlijnen. Het doel van het Trilateral Wadden Sea Plan, een samenwerkingsverband van Nederland, Duitsland en Denemarken, is om het huidige kwelderareaal te behouden en waar mogelijk uit te breiden en de biodiversiteit te vergroten. Momenteel wordt een toename van areaal van oudere kwelders in de Waddenzee waargenomen. Deze toename gaat echter wel ten koste van het areaal van de jong ontwikkelde kwelders en de biodiversiteit. Dit is verontrustend voor het natuurbehoud van het Waddenzeegebied. Ook het probleem van de toenemende dominantie van zeekweek (*Elytrigia atherica*) wordt geleidelijk internationaal door natuurbeschermers en natuurbeheerders erkend. De afgelopen decennia is zeekweek zowel in frequentie als dominantie op de kwelders langs de Noordzeekust sterk toegenomen. Rond 1980 was ongeveer 8% van de kwelder langs de Nederlandse kust bedekt met zeekweek. In 2000 was deze soort al toegenomen tot 17% van de bedekking. Deze snelle verspreiding en dominantie van zeekweek - ook wel verkweking genoemd - heeft geleid tot een eentonige ruige vegetatie met een lage diversiteit van planten en structuur. Deze verkweking heeft niet alleen invloed op de plantengemeenschap maar ook op de insecten- en vogelpopulaties op de kwelder. Aansluitend wordt aangenomen dat, zowel op de eiland- als de vastlandkwelders, dominantie van zeekweek de climax van de kweldervegetatie zal zijn.

Het doel van dit proefschrift is om te onderzoeken hoe zeekweek zich in de laatste decennia zo snel heeft kunnen verspreiden op de kwelders in de Waddenzee. Interessant daarbij is vooral welke verspreidingsstrategieën bij deze soort van toepassing zijn: verspreiden via wortelstokken of via zaden. Daarnaast is het van belang te weten of de verspreidingsstrategie van zeekweek is aangepast in relatie tot beheer en successie.

Invasie van zeekweek op de kwelder

Natuurlijke successie is een belangrijke factor bij de toename van de zeekweek. Als gevolg van overstromingen vindt er sedimentafzetting plaats op de kwelders. Naarmate de kwelder ouder wordt, neemt de sedimentlaag toe en wordt de kwelder voedselrijker. Toename van antropogene eutrofiëring kan leiden tot een toenemende voedselrijkdom over de gehele kwelder, ongeacht de leeftijd van de kwelder. Hierdoor kan een 'late successiesoort', zoals zeekweek, sneller toenemen en zich verspreiden naar een lager gelegen en/of jongere kwelder. Het is lastig onderscheid te maken tussen de invloeden van natuurlijke successie en antropogene eutrofiëring op de snelle verspreiding van zeekweek. Om dit onderscheid te kunnen maken, heb ik gebruik gemaakt van een combinatie van tijdreeksen van vegetatiekaarten en chronosequentiedata van vier natuurlijk ontwikkelde eilandkwelders (Terschelling, Schiermonnikoog, Nordeney en Spiekeroog) (**hoofdstuk 2**). Deze kwelders hebben zich natuurlijk ontwikkeld als gevolg van overheersende zeestromingen, sedimentafzettingen en ecologische omstandigheden zonder menselijke ingrepen. Verder heeft op de geselecteerde kwelders geen begrazing of een andere beheermaatregel plaatsgehad. Er werden verschillende verspreidingsnelheden van zeekweek op deze kwelders gevonden. De hoogste relatieve snelheid van versprei-

ding werd waargenomen op de jonge kwelders. Hier wordt per jaar een grotere hoeveelheid sediment afgezet dan op de oudere kwelders. Dit resulteert ook in een grotere inbreng van stikstof in de bodem op jonge kwelders. De snelheid van verspreiding van zeekweek was het laagste op de oude kwelder. Mogelijk doordat op een oude kwelder nog maar een beperkt aantal geschikte locaties beschikbaar zijn om te koloniseren, stagneert of neemt de verspreiding van zeekweek zelfs af. Gezien de hoeveelheid atmosferische stikstofdepositie (antropogene eutrofiëring) gelijk is op jonge en oude kwelders, concluderen we dat de toename van zeekweek eerder een gevolg is van natuurlijke successie dan van antropogene eutrofiëring.

Door economische motieven van boeren en door veranderingen van natuurbeheervisies, is de afgelopen decennia de beweiding op een groot aantal vastelandkwelders langs de Waddenzeekust stopgezet. In samenhang met deze verandering in beheer, is zeekweek gaan toenemen in frequentie en dominantie op deze kwelders. Maar deze toename is niet op alle verlaten kwelders waargenomen. Ik heb langetermijngegevens van vegetatie, hoogteligging, sedimentatie en drainageomstandigheden gebruikt om te onderzoeken welke factoren, naast begrazingsbeheer, bepalend zijn voor de toename van zeekweek op de vastelandkwelders (**hoofdstuk 3**). Tegen de verwachting in, nam de dominantie van zeekweek niet toe met het aantal jaren dat de kwelder uit beweiding was genomen. De frequentie en de dominantie van zeekweek hing vooral samen met de hoogteligging en de mate van drainage. Goede afwatering had een positief effect op de zeekweek. Uit de studie blijkt dat wanneer abiotische omstandigheden - vooral overstromingsfrequentie en afwatering - optimaal zijn voor zeekweek, deze soort binnen een paar jaar de kwelder domineert. Als het afwateringsysteem in verval raakt, vindt deze toename niet plaats. Waarnemingen op Duitse en Nederlandse kwelders hebben laten zien dat zonder onderhoud aan de greppels, deze op de lage kwelder dichtslibben en er water langer op de kwelder blijft staan, terwijl op de hogere kwelder deze greppels uit zichzelf openblijven en water afvoeren. Dit verklaart de langzamere toename van zeekweek op de lage Duitse kwelders ten opzichte van de hogere Nederlandse kwelders.

Optimale standplaatscondities voor zeekweek

Een groot aantal studies heeft laten zien dat kweldersuccessie wordt aangezet door sedimentatie. Een toename van sedimentafzetting leidt tot toename van nutriënten in de bodem. Ook zorgt sedimentafzetting voor een toename van de hoogteligging van de kwelder ten opzichte van de zeespiegel, mits de sedimentafzetting hoger is dan de inklinking van de bodem. Om te bepalen wat de optimale abiotische standplaatscondities zijn voor zeekweek om zich te vestigen op de kwelder, heb ik geïsoleerde zeekweekvlakken (0,5 m2 tot 90 m2) op de lage kwelder van Schiermonnikoog onderzocht. Ik heb op drie locaties langs de successiegradiënt in deze vlakken, het voorkomen van zeekweek, de kleidikte en de hoogteligging gemeten. Uit dit onderzoek blijkt dat over de gehele successiegradiënt het voorkomen van zeekweek sterk gerelateerd is aan de hoogteligging ten opzichte van de zeespiegel en dus overstromingsfrequentie. Verder is, over de gehele successiegradiënt, een relatie gevonden tussen de grootte van een vlak zeekweek en de kleidikte. Bijzonder is dat er alleen op de jonge kwelder een relatie gevon-

den wordt tussen kleidikte en het voorkomen van zeekweek. Deze bevinding suggereert dat op oudere kwelders, met een kleidikte van >10cm, de klei (als voedingsbron) geen effect heeft op de mate van voorkomen van zeekweek, maar mogelijk wel aanvullende waarde heeft voor het competitieve vermogen van zeekweek.

De mate van sedimentafzetting is van plaats tot plaats variabel en daarmee ook bepalend voor de ruimtelijke verspreiding van zeekweek. Dicht bij het wad kan sedimentafzetting resulteren in bodemverhoging van bijna 20 mm/jaar, terwijl op meer dan een kilometer van de wadrand er bijna geen verhoging van de bodem plaatsvindt. Tegelijk vindt op onbegraste kwelders dicht bij de wadrand verspreiding van zeekweek plaats, behalve op locaties waar de bodemhoogte niet toeneemt. De verandering van bodemhoogte is dus een van de belangrijke voorspellende factoren om te bepalen wanneer de kwelder een hoogte bereikt waarop zeekweek kan voorkomen. Daarnaast wordt de frequentie en dominantie van zeekweek sterk bepaald door de afwateringcondities van de kwelder (**hoofdstuk 2 & 3**). Mijn conclusie is dat zeekweek niet van natte voeten houdt!

Dispersiestrategieën

De zaadproductie en zaadregen (het vallen van zaden op een locatie) van zeekweek neemt toe naarmate de kwelder ouder wordt. Maar uit eerder onderzoek blijkt dat deze zaden niet teruggevonden worden in de zaadbank (zaadvoorraad in de bodem). Op de jonge kwelder zijn er geen zaden van zeekweek in de zaadbank en op de oudere kwelder alleen in de herfst (na zaadregen) in geringe aantallen. Het is daarom van belang dat zaden zich verspreiden naar de juiste plek op het juiste tijdstip om zich succesvol te kunnen vestigen. De verspreiding van zeekweekzaden wordt sterk bepaald door de afstand ten opzichte van de zee. Zaden worden vooral van de lage kwelder naar de hoge kwelder verspreid, maar niet andersom. Desondanks, worden grote hoeveelheden zeekweekzaden op zee gevangen, wat suggereert dat er een grote export van zaden is naar andere kwelders. Een groot deel van deze zaden eindigt hun reis als vloedmerk aan de rand van een duin na een winterstorm en hebben weinig kans om uit te groeien tot een volwassen plant. In **hoofdstuk 6** heb ik de effecten van vochtigheid, zaadvorm, vegetatiestructuur en hydrodynamische variabelen op het vasthoudend vermogen van zaden en de verplaatsing van zaden onderzocht. Dit is gedaan aan drie kweldersoorten: zeeweegbree, schorrenkruid en zeekweek. Daarnaast heb ik het effect van drie verschillende vegetatietypen op het vasthoudend vermogen voor zaden getoetst. Dit heb ik zowel gedaan in een experiment op de kwelder als in een gootopstelling waar verschillende hydrodynamische variabelen gecreëerd konden worden. Uit alle experimenten kwam naar voren dat van zeekweek de minste zaden in de vegetatie werden vastgehouden. Wel hadden droge zeekweekzaden een groter drijfvermogen en raakten ze het makkelijkst los uit de vegetatie. Door toevoeging van golfslag werden er meer zaden losgeslagen uit de vegetatie, dan bij waterstroming alleen. De zaden werden beter vastgehouden in een dichte, minder stijve vegetatie, zoals roodzwenkgraspollen, dan in een meer open en stugge vegetatie, zoals lamsoorplanten. Deze resultaten duiden erop dat het drijfvermogen, na het loskomen van de ouderplant, bepaalt of zaden zich verspreiden

in de droge zomer en herfstmaanden. Maar de intensiteit van de golfslag bepaald of natte zaden op hun microlocatie blijven tijdens de natte maanden van het late najaar tot aan de lente.

In **hoofdstuk 5** heb ik bekeken welke vorm van voortplanting bepalend is voor de succesvolle verspreiding van deze soort naar nieuwe leefgebieden: seksueel door middel van zaden of vegetatief door middel van wortelstokken. Daarbij heb ik gebruik gemaakt van DNA-technieken om genetische individuen te onderscheiden en de genetische diversiteit in zeekweekvlakken te onderzoeken. Ik heb hierbij vlakken van verschillende grootte op een 25- en 35-jaar oude lage kwelder met elkaar vergeleken. Op de oudere kwelder bleken significant meer genetische individuen in één vlak te zitten dan op de jongere kwelder.

Deze verschillen in genetische diversiteit, die de klonale structuur kunnen beïnvloeden, worden bepaald door twee belangrijke omgevingsfactoren. Ten eerste kan begrazing een negatief effect hebben op de vestiging van zaailingen. Op oudere kwelders is begrazing van zaailingen minder hevig. Daarom zal de kans op succesvolle vestiging van zaailingen hoger zijn op de oudere kwelder dan op de jongere kwelder. Een tweede factor die mogelijk invloed heeft op de klonale structuur van zeekweek is de voedselbeschikbaarheid. Stikstof, een nutriënt en een bouwsteen van een enzym t.b.v. zouttolerantie, is een beperkende factor op de zeer jonge kwelder. Grote klonen kunnen nutriënten via hun wortelstokken herverdelen, zaailingen kunnen niet op dergelijke wijze omgaan met een stikstoftekort. Zaailingen zijn daarom afhankelijk van de standplaatscondities waarin zij kiemen en zijn sterker aangewezen op standplaatsen met een relatief hoge ligging ten opzichte van de zeespiegel om de kans op overstroming en zoutstress te beperken. Wanneer vlakken of subpopulaties van zeekweek ouder worden en, gepaard daarmee, toenemen in aantal en dichtheid, kan de hoeveelheid van nieuwe zaailingen toenemen. Dit komt doordat de invang van zaden toeneemt met de dichtheid van de vegetatie. Maar vegetatiedichtheid of nog belangrijker, de hoge en dichte bedekking, beperkt ook de vestiging van zaailingen omdat de competitie voor licht groter wordt. Daarnaast zal in oudere vlakken op den duur ook intraspecifieke competitie gaan optreden. Genetische individuen concurreren elkaar weg, waardoor de genetische diversiteit weer afneemt. De waargenomen lage genetische diversiteit in de drie grootste vlakken geven de suggestie dat genetische diversiteit afneemt met toename van de grootte van de vlakken.

Op Schiermonnikoog vindt een lange termijn (> 30 jaar) veldexperiment plaats, waarin populaties van zeekweek tot ontwikkeling zijn gekomen onder invloed van drie verschillende beheermaatregelen: beweiding door runderen, jaarlijks maaien en geen beheer. Dit experiment heeft mij de mogelijkheid gegeven om het effect van beheer op de genetische structuur van zeekweek te bestuderen (**hoofdstuk 4**). De beheermaatregelen hadden een effect op de dichtheid en de fenotypische kenmerken van zeekweek, waarbij in de velden zonder beheer zeekweek de hoogste bedekking had en de planten in de beheerde velden kortere en smallere bladeren hadden. Mijn bevinding was dat het verwijderen van biomassa door begrazing en/of maaien een effect had op de genetische structuur van de populaties. Binnen de onbeheerde velden, waar zeekweek domineerde,

werd een vergelijkbaar lage genetische diversiteit gevonden als in de grote vlakken in **hoofdstuk 5**. In de begraasde en gemaaide velden werd een hogere genetische diversiteit gevonden. Dit komt, zoals verwacht, door verstoringen als maaien en grazen en door het transport van zaden en knollen, dat hier nieuwe genetische identiteiten inbrengt. Deze verstoringen zorgen ook voor nieuwe gaten in de vegetatie waar zaailingen zich kunnen vestigen en daarmee ook de genetisch diversiteit verhogen. Maar begrazing door runderen zorgt ook voor een continue kans voor zaailingen om vertrapt of opgegeten te worden. Maaien daarentegen gebeurt maar één keer per jaar, waarna de zaailing in rust tot kieming kan komen. Concluderend: verstoring kan vestiging van zaailingen bevorderen, maar teveel verstoring bevordert verspreiding door middel van wortelstokken.

Toekomst van zeekweek

Ondanks dat de zeekweekvegetatiegemeenschap wordt omschreven als het climaxstadium van de successie, wordt op de oudere kwelders van Schiermonnikoog en Terschelling een afname van deze vegetatiegemeenschap waargenomen. Deze afname is zeer waarschijnlijk het gevolg van lokale veranderingen van de milieucondities. Op Schiermonnikoog en Terschelling zijn de voormalige zeekweekhalmen vervangen door grassoorten als riet en zilte rus, soorten van zeer natte standplaatsen. Op grotere afstand van de zee vindt er een afname van sedimentafzetting en ophoging van de bodem plaats, doordat sediment vooral wordt aangevoerd via kreken en/of greppels en voor het grootste deel wordt afgezet dicht bij de bron en op de banken. Een afname van sedimentinbreng op de oudere kwelder of op grotere afstand van zee kan zorgen voor een afname van beschikbare stikstof. Bovendien kan een afname van sedimentinbreng samen met inklinking van de bodem leiden tot de ontwikkeling van een depressie met stagnerend water. Dit proces kan verder worden versterkt door zeespiegelstijging, en samenvallend met slechte afwatering, leiden tot langdurige plasdrasomstandigheden na een overstroming of een regenbui. Zeespiegelstijgingmodellen van eilandkwelders voorspellen dat de kwelders dicht bij de zee mee kunnen groeien met de zeespiegelstijging, al wordt wel een hogere overstromingsfrequentie verwacht. De meer inlandse kweldergebieden, met weinig sedimentafzetting kunnen niet mee groeien met de zeespiegelstijging. Hierdoor zal deze kwelder natter en ongeschikt worden als standplaats voor zeekweek. De afname van de zeekweekvegetatiegemeenschap op de oudere en/of nattere kwelders laten zien dat deze plantengemeenschap niet het climaxstadium is en er een wereld is voorbij dit zeekweekstadium.

Conclusie

De snelle toename van zeekweek op de vasteland- en eilandkwelders is grotendeels een gevolg van successie. In vroege successiestadia is zeekweek gebonden aan standplaatsen op de hoge kwelder. Tijdens de successie kan zeekweek geleidelijk verspreiden naar de lagere delen van de kwelder. Maar ook zal, gedurende de successie, het voorkomen van

zeekweek afnemen op de kwelders ver van zee door afname van sedimentafzetting en zeespiegelstijging. Verspreiding door middel van zaden en wortelstokken zijn even belangrijke dispersiestrategieën in de snelle toename van zeekweek. Op jonge en/of lage kwelders vestigt zeekweek zich door middel van zaden. Succesvolle vestiging van zaden wordt sterk bepaald door geschikte abiotische en biotische omstandigheden, zoals voedselbeschikbaarheid en ruimte in de bestaande vegetatie. Eenmaal succesvol gevestigd als zaailing kan zeekweek zich vegetatief (d.m.v. wortelstokken) verbreiden en spoedig andere soorten verdrijven. Ook kan zeekweek zich vanaf hoge kwelders indringen op de lage kwelders door vegetatieve verbreiding. Wanneer de abiotische omstandigheden, met name overstromingsfrequentie en afwatering, optimaal zijn voor zeekweek kan deze soort de kwelder binnen enkele jaren domineren. Maar als de afwateringcondities afnemen, door successie, onderhoud of klimaatverandering zal deze soort verdwijnen omdat zeekweek niet van natte voeten houdt.

Dankwoord

Poeh, het boekje is af!!! De totstandkoming van dit boekje heeft letterlijk en figuurlijk heel wat voeten in de aarde gehad. Het is dan ook niet alleen een afronding van mijn promotieonderzoek maar ook de afronding van een periode van mijn leven. Op deze plek wil ik iedereen bedanken die op welke wijze dan ook heeft bijgedragen aan dit product; alleen had ik het niet kunnen doen! Een aantal mensen wil ik in het bijzonder noemen. Maar eerst een kleine voorgeschiedenis.

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