Chapter 1 General introduction

1.1 What is succession?

Succession is a fundamental concept in ecology. Many definitions are given in the literature. Simple but straightforward definitions are: the change in species composition or three-dimensional architecture of the plant cover of a specified place through time (Pickett & Cadenasso 2005) or the changes observed in an ecological community following a perturbation that opens up a relatively large space (Connel & Slatyer 1977). In fact, succession is the continuous change in the species composition of natural communities that results from many causes and processes (Glenn-Lewin et al. 1992), particularly the colonization, growth and mortality of organisms under environmental conditions that are continuously changing as a result of either the actions of the organisms themselves (autogenic succession) or of externally imposed processes (allogenic succession) or both (Huston 1994). Grime (1979) considers succession as the change in predominant kinds of life histories of the plant species, succession being a shift in the relative importance of ruderal, competitive and stress-tolerant species. Succession occurs, with different rates and patterns, in all natural communities, and is the fundamental process of vegetation dynamics (Huston 1994).

1.1.1 Temporal and spatial aspects of succession

Vegetation succession has temporal and spatial aspects (e.g. Southall et al. 2003). Indeed, time and space are related, in that forcing functions for vegetation change over large areas tend to be the same as those causing change over long time periods, and likewise for small areas and short time spans the causes of change are supposed to be similar (Shugart & Urban 1988; Falinski 1988). An inherent property of succession that is responsible for much of the confusion surrounding the interpretation of successional patterns and the development of a theory of succession is that succession creates both temporal and spatial patterns (Huston 1994). Temporal succession refers to vegetation changing (progressive or retrogressive) in time (Glenn-Lewin & van der Maarel 1992), while spatial variation in vegetation can result from a single successional sequence (sere) that occurs under similar conditions (i.e. resource availability, soil characteristics, differentiated disturbance; e.g. Olff et al. 1999) and follows the same pattern of species composition at different locations (Huston 1994). Spatial variation in vegetation can also be caused by succession that occurs under different conditions in different locations (Ferreira et al. 2007) and follows different patterns of species composition toward different endpoints (Huston 1994).

Succession, particularly plant succession, was one of the first major research topics of the field of ecology (Huston 1994). The observation of temporal and spatial changes in plant communities has a long history, and use of the term 'Succession' dates back at least to Thoreau, 1860 (cf. Huston 1994) and Clements, 1916 (cf. Pickett & Cadenasso 2005) and has been a challenging problem in ecological studies up to the present (Drury & Nisbet 1973; West et al. 1981; Smith & Huston 1989; Prach et al. 2001; Kahmen & Poschlod 2004; Wolters et al. 2008). The spatio-temporal variation in vegetation patterns and its driving forces are of great and ongoing concern in ecology and there have been studies dealing with the theme of succession in various habitats and ecosystems, of which the books and papers of Connell & Slatyer (1977), Tilman (1985), Huston & Smith (1987), Turner et al. (1998) and Kahmen & Poschlod (2004) are among the more conceptual ones.

1.2 General considerations on succession in salt-marsh habitat

The succession on salt-marshes, whose pioneer vegetation is regularly submerged by seawater, is often described as consecutive stages, known as haloseres. Succession may start in a newly created salt-marsh (primary succession). On the other hand, vegetation may undergo succession after being damaged but not destroyed, called secondary succession (Adam 1990). Secondary succession occurs after salt-marsh vegetation has been damaged by clipping or by excessive trampling and grazing. Wash-over processes in salt-marshes may completely destroy the existing vegetation by covering it by storm-driven sediment, but in other cases it can grow through a thin layer of fresh sediment. Secondary succession can also start on a mature soil if plant propagules are left after the former vegetation has been virtually destroyed. In such succession most of the plant species are either present from the outset as buried seeds, bulbs and rhizomes or invade shortly afterwards (Packham & Willis 1997).

Of the three major mechanisms of succession outlined by Connell & Slatyer (1977), none by itself can account for the complete range of floristic replacements found in salt-marshes.

1.2.1 The facilitation mechanism

In the facilitation mechanism, species replacement is assisted by environmental changes brought about by organisms in earlier stages in the succession (Packham & Willis 1997). In this mechanism, the early-succession species modify the environment so that it is more suitable for late successional species to invade and grow to maturity (Connell & Slatyer 1977). Whittaker (1975) stated that in the facilitation mechanism "one dominant species modified the soil and microclimate in ways that made the entry of a second species possible, which then became dominant and modified the environment in ways that suppressed the first and made the entry of a third dominant possible, which in turn altered its environment." This sequence continues until the resident species no longer modifies the site in ways that facilitate the invasion and growth of a different species (Castellanos et al. 1994; Huckle et al. 2000).

1.2.2 The tolerance mechanism

The tolerance mechanism does not depend on the initial presence of early successional species. Species, which occupy a site early, have little or no influence on the recruitment of other species, which grow to maturity despite their presence (Burrows 1990). Any species can start the succession but those which establish first are replaced by others that are more tolerant (competitively superior) and usually longer-lived at the habitat. In this mechanism species that appear later are simply those that arrived either later or at the very beginning, but germinated and/or grew more slowly. The sequence of species is determined solely by their life-history characteristics. In contrast to the early species, the propagules of the later ones are dispersed more slowly and their juveniles grow more slowly to maturity. They are able to survive and grow despite the presence of early-succession species that are healthy and undamaged (MacArthur & Connell 1966; Farrell 1991).

1.2.3 The inhibition mechanism

In contrast to the first mechanism, in inhibition mechanism, once earlier colonists colonize a habitat, they secure the space and/or other resources, and subsequently inhibit the invasion of new colonists or suppress the growth of those already present. The latter invade or grow only when the dominating residents are damaged or killed, thus releasing resources (Connel & Slatyer 1977). In this mechanism, invasion is prevented by the present occupants of the site, perhaps through heavy shading or allelopathic mechanisms; replacement will occur only when previous colonists are removed. The species, which colonize the site first, thus gain a major advantage (Packham & Willis 1997).

In salt-marsh conditions, it is generally expected that vegetation changes in time are driven by the facilitation mechanism. Soil trapped by early colonizers, that themselves are able to endure the harsh pioneer stage environmental conditions, elevates the substrate, thus facilitating the colonization by mid- and late-successional species, which were not able to endure the early succession conditions of daily inundation with salt water. Beeftink (1965) and Hoffmann (1993) stated that *Salicornia* spp. or *Spartina townsendii*-dominant communities would eventually change to *Elymus athericus* (on levees) or *Halimione portulacoides*-dominant (on backlands) communities through the facilitation mechanism by sedimentation (Fig. 1.1). However, inhibition mechanism through spatial competition could also occur in salt-marsh habitat, as it has been reported by a few authors for *Spartina* (e.g. Packham & Willis 1997).

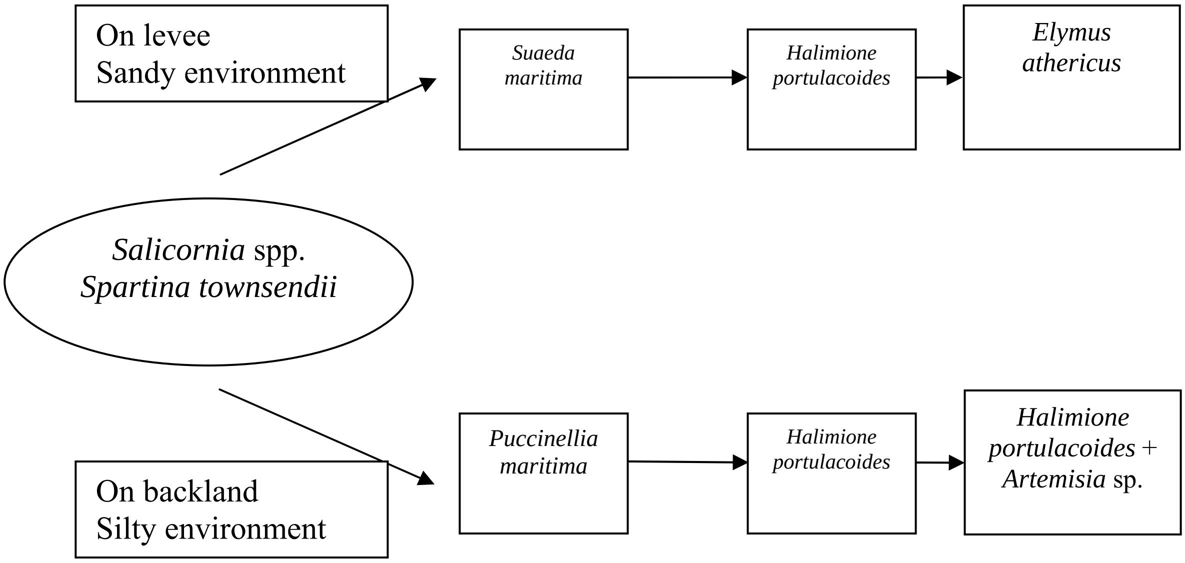

Fig. 1.1. Vegetation succession illustrated with the dominant plant species in consecutive vegetations in backland and levee in SW- Netherlands (reconstructed from Beeftink 1965; Hoffmann 1993); grazing is believed to set back succession to vegetations dominated by low-sward grasses, predominantly *Puccinellia maritima.*

1.3 What is known about salt-marsh succession

Vegetation succession in salt-marshes in Europe was described by Beeftink (1962; 1965; 1966; 1977). He distinguished two kinds of vegetation succession in European salt-marsh: progressive and retrogressive. Changes in the vegetation which involve an increasing number of species and an increasing complexity of structure are considered to be progressive succession e.g. the sere *Puccinellietum maritimae*, *Juncetum gerardii*, *Agropyro-Rumicion crispi*. The sere in the opposite direction is called retrogressive succession. He stated that the causes of retrogressive succession can be both natural (e.g. erosion and grazing) and man-made (e.g. grazing and mowing). After Beeftink's thorough studies of salt-marshes in the south-western part of the Netherlands (Beeftink 1965; 1966), the effects of biotic and abiotic factors on vegetation succession in salt-marsh habitats were further investigated, e.g. tidal inundation (Egan & Ungar 2000; Wolters et al. 2005), grazing (Bakker & Ruyter 1981; Pehrsson 1988; van Wijnen et al. 1997; Schroder et al. 2002; Kleyer et al. 2003; Kiehl et al. 2007) and soil factors (Tessier et al. 2000; Schroder et al. 2002; Tessier et al. 2003) (see details of these and other studies in the introduction of each of the following chapters). Very generally speaking, the results of these diverse studies showed that the impact of biotic and abiotic factors on vegetation succession often depends on the study area where the observations were made. Hence, general conclusions on succession determining process are difficult to make.

1.3.1 Which species are able to be the first colonizers in a salt-marsh?

The variables that control plant species co-occurrence (Gotelli & McCabe 2002) and primary succession (Kalliola et al. 1991; Walker et al. 2006; Shiels et al. 2008) are manifold and interactive (van der Valk 1992; Wiegleb & Felinks 2001).

Initial substrates are colonized by a species through recruitment from viable seeds. Seed can be available by seed dispersal or from seed buried in the soil (Rand 2001). Seed (or diaspores in general) availability is only the first step needed to establish a population on a new substrate; seed availability alone only makes a species a member of the potential flora of the new site, not necessarily of its actual flora (Major & Pyott 1966). The probability of successful establishment from dispersed seed depends on several factors, i.e. seed production, seed dispersion and seed germination (van der Valk 1992). Unfortunately, these factors have never been examined together in detail in any single study. Many secondary factors affect each of these processes. For instance, seed production (the number of seeds produced by a plant or a population during a given growing season or year) depends on such factors as the age or size of the plant, the abundance of the population in the community or at a higher scale, environmental conditions during the previous and current growing season, availability of pollinators, predispersal predation and energetic trade-off between vegetative propagation and seed production (Willson 1983; Price & Jenkins 1986; Zammit & Westoby 1988; Howe & Westly 1988; Louda 1989).

Seed dispersal depends on the vector of seed transport (van der Valk 1992) and the ability of seeds for dispersion (Adam 1990). In coastal marshes, dispersal of seeds by tidal currents is suggested as the main pathway, as most seeds are able to float in the water column (Koutstaal et al. 1987; Huiskes et al. 1995). The ability of seeds for dispersal by tidal currents is affected by seed mass and seed shape (length and width) (Poschlod et al. 2005).

Only part of the colonization process has been completed when seeds are produced, transported and have reached a site: they must still germinate. Seed germination is a complex physiological process influenced by many environmental conditions (Mayer & Poljakoff-Mayber 1982). In salt-marshes, seed germination can be affected by soil salinity, moisture and nutrition level (Woodell 1985; Shumway & Bertness 1992; Noe & Zedler 2000).

In general, a species can be an early successful colonizer if its parents produce sufficient seeds at local or regional scale, disperse to new areas very well and germinate successfully. Fig. 1.2 shows the conceptual framework for exploring constraints in the colonization of salt-marsh species. At the first step, total seed production can be affected by the abundance of adults in the old salt-marsh. In chapter 2, the relationship between the frequency of species in newly created salt-marsh and its adjacent old salt-marsh (functioning as a seed source) will be examined. It is hypothesized that a species occurring in high abundance in the adjacent old salt-marsh (the local seed source) has a higher chance of becoming an initial colonizer in the newly created salt-marsh. In addition, some seed traits (seed shape and mass) which could affect seed dispersal will be investigated for the new colonizers. Although several studies dealt with mechanisms of succession in salt-marsh habitat, there are few studies on the species traits which help species to be initial colonizers. The hypothesis is that initial colonizers have shorter and lighter seeds than later colonizers. Moreover, it is hypothesised that initial colonizers are more salt and poor-nutrient tolerant than later colonizers.

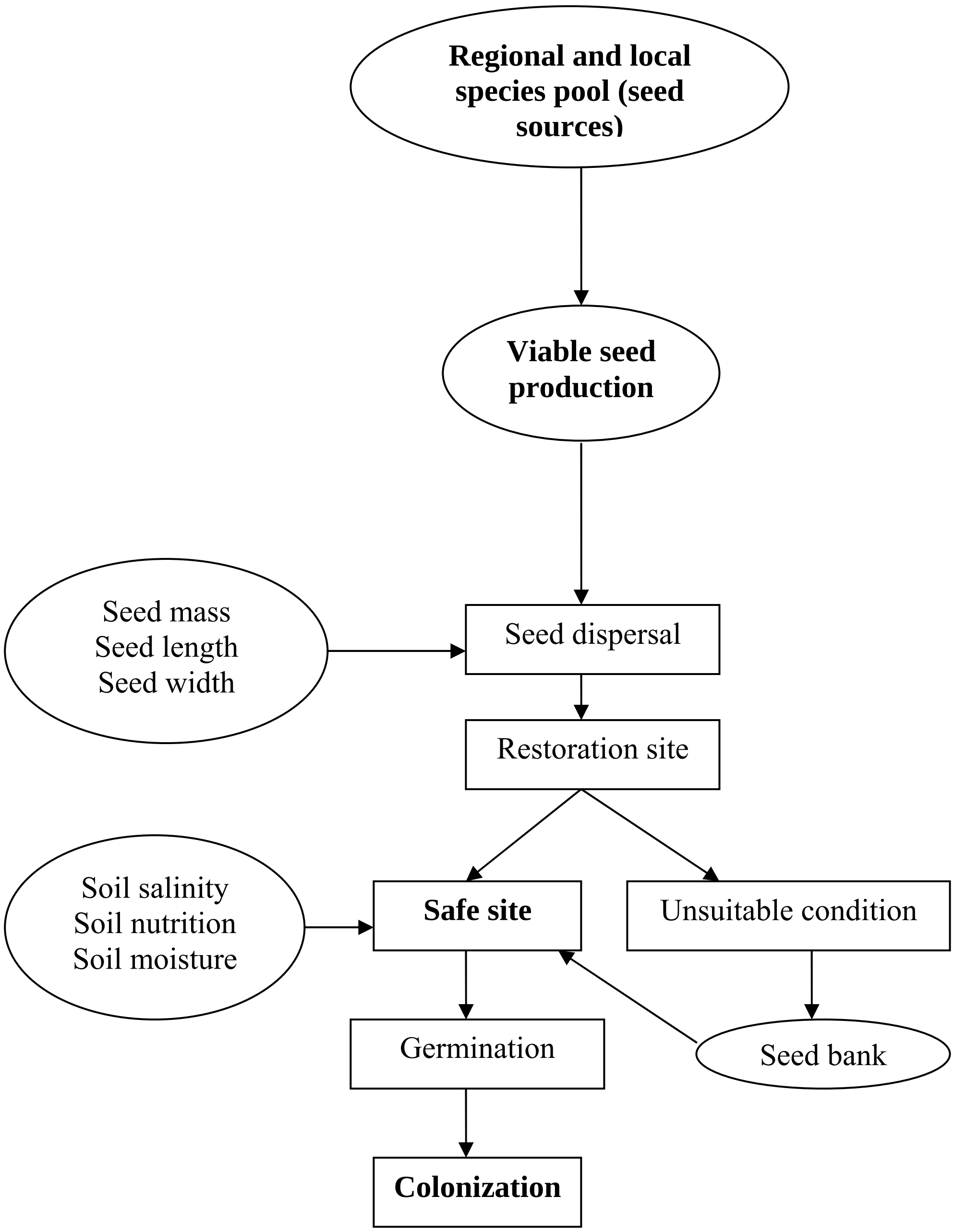

Fig. 1.2. Framework for exploring constraints in the initial colonization of salt-marsh species

1.3.2 Inundation, vegetation and succession

Once species have colonized a new substrate, the growing and replacement of different species can be influenced by biotic and abiotic factors (e.g. Bernhardt & Koch 2003). In salt-marsh habitat, inundation frequency is one of the most important abiotic factors. Inundation by the tide affects halophytes largely through changes in oxygen availability (or soil aeration) and soil chemistry (Silvestri & Marani 2004). Additionally, tidal currents may have mechanical effects on plants, particularly in the lower part of salt-marshes and affect the growth rate and survival. As the presence of thick cuticles in most salt-marsh species is likely to prevent direct entry of salt into the plant, the immediate effect on halophyte physiology could be the flooding of soil pores affecting the availability of oxygen for aerobic root processes and producing reduced toxic ions (Packham & Willis 1997). The physiology of water-logging tolerance has been quite widely studied in plant species in salt-marshes (e.g. Anderson 1974; Cooper 1982 ; Armstrong et al. 1985; Naidoo et al. 1992; Varty & Zedler 2008) and different strategies were reported for salt-marsh plants to adopt and survive in periodic soil saturation (Visser et al. 2000). Given the complexity and the variability in the combination of strategies to cope with anaerobic conditions, the effects of tidal flooding on germination and respiration are also species-dependent. Cooper (1982) found that species such as *Plantago maritima*, *Puccinellia maritima* and *Salicornia europaea* were particularly tolerant to water-logging, whereas species generally found higher on the marsh, including *Festuca rubra*, *Juncus gerardii* and *Armeria maritima*, were less so. As a result, the survival and stability of different species might be different in relation to inundation frequency; the rate of species replacement is influenced by inundation frequency.

Classical concepts of succession emphasize the role of organisms in modifying the environment; the operation of such autogenic factor leads to the gradual development and replacement of plant species and communities (Clements 1916), which is considered to exhibit increased species diversity, yield and amounts of organic matter with time (Odum 1969). Succession processes such as "facilitation" and "inhibition" are associated with it (Connel & Slatyer 1977). Such features (autogenic plant succession) are indeed likely to predominate at higher elevations, where tidal influence is reduced. Lower elevations, however, experience frequent tidal inundation, associated with more complete litter removal and greater likelihood of inorganic sedimentation. This enhancement of the allogenic (environmental) influence leads to replacement of species by the tides, as for instance Eilers (1979) found in the lower salt-marshes. Consequently, inundation could influence not only the germination and growth of species but also the stability and dynamics of different species and communities. Previous studies have shown that the speed of vegetation succession in salt-marshes depends on drainage conditions and on sedimentation rates in relation to sea level rise (Leendertse et al. 1997; Olff et al. 1997; Schröder et al. 2002). Nevertheless, there is a lack of studies on vegetation replacement and succession related to inundation frequency. The effect of inundation frequency on vegetation succession (species turnover) will be studied in chapter 3. We hypothesize there that the rate of species succession and turnover at higher inundation frequencies is lower.

1.3.3 Grazing and succession

Grazing is one of the most important biotic disturbance factors, affecting the growth rate of species and the rate of replacement. In terrestrial habitats, animal grazing has been reported to be an important succession factor (Olff et al. 1999) and nature management tool for poor and low-productivity habitats (Provoost et al. 2002; Stroh et al. 2002; Hellström et al. 2003). Moderate grazing was beneficial to annual species, whereas ungrazed grassland was dominated by tall perennial grasses (Noy-Meir et al. 1989). Species richness increased with increasing grazing pressure, but decreased sharply when the grazing pressure was severe (Taddese et al. 2002). Although, grazing increased the number of rare species, it negatively affected plant species richness in acidic, extremely nutrient poor coastal grasslands, while species richness was positively affected in more basic, nutrient richer coastal dune grasslands (Tahmasebi Kohyani et al. 2008). As a result, the effects of livestock on the environment are numerous and depend on the habitat, grazing intensity and herbivore species (see also Gough & Grace 1998). Probably the most obvious and most important grazing effect is the selective phytomass extraction by herbivores. Some plant species or certain plant functional types were preserved during the course of succession; others were discriminated against by a grazing impact, depending on the preferences of the livestock species (Hülber et al. 2005). Additionally, grazing animals influenced their environment when they created gaps by trampling, scratching or rolling (Lamoot et al. 2004). These micro sites were extremely important for mid-successional stages, because they represented spots where retrogressive succession and a regeneration of niches for low-competitive plant species (Bakker et al. 2003) occur. As a result, it can be expected that during succession some species disappear or decrease in abundance and some new species appear or increase in abundance by grazing.

For the management of dry and nutrient-poor sandy ecosystems, mainly sheep grazing is used as a measure of nature management. The effectiveness of sheep grazing as a tool for protection and restoration has been proven in various studies (Stroh et al. 2002; Hellström et al. 2003). There are, however, few studies that investigated the mechanism of plant succession induced by sheep grazing in salt-marsh habitat (but see, e.g. Jensen 1985); most researches have been done about the effect of cattle grazing. Effects of five years of cattle grazing on a salt-marsh vegetation were investigated by Bakker & Ruyter (1981). They demonstrated that the ungrazed area showed a progressive succession while in some parts of the grazed area retrogressive succession took place, i.e. the vegetation became more open and diversity increased by grazing. Intensive grazing of salt-marshes by cattle can lead to a downward shift of vegetation zones (Bakker 1989) towards a pioneer succession stage and to a loss of grazing-sensitive species, because only a few plant species (e.g. *Salicornia* spp. and *Puccinellia maritima*) tolerate frequent biomass loss and trampling (Kiehl et al. 1996). Gibson & Brown (1992) showed that the impact of herbivores is considered to induce regressional trends against successional development.

Short-term investigations showed that plant species density was lowest in intensively grazed low salt-marshes but did not differ between moderately grazed and ungrazed plots (Kiehl 1997). A meta-analysis of long-term vegetation changes in the Wadden-Sea salt-marshes showed, however, that grazing abandonment would have a negative effect on species density due to the increasing dominance of competitive plant species such as *Elymus* spp. in the high marsh (levees) or *Halimione portulacoides* in the low marsh (backland) (Bos et al. 2002). In contrast, some examples from long-term grazed salt-marshes indicate that species-rich vegetation mosaics can also persist over long periods (Kiehl et al. 2000). This was already proven for the study area (see Fig. 1.5) in 1904, when Massart (1908) described the species rich vegetation of the salt-marsh (salt-marsh with *Salicornia europaea, Suaeda maritima, Glaux maritima, Puccinellia maritima, Armeria maritima* and other salt-marsh species), that was grazed by cattle, horses and hinnies.

Particularly strong competitive species such as *Elymus athericus* tend to increase their dominance during progressive succession, resulting in a strong decrease in species diversity in late successional stages. But there is no study of the effect of sheep grazing on this species. Whether sheep grazing can reduce the expansion of *Elymus athericus* is a question which has not been answered so far.

In addition, studies on the effects of livestock grazing on forage quality can be important in salt-marsh habitat since forage quality is relevant in the attraction of other herbivorous animals such as geese (Hupp et al. 1996). Studies on the effect of sheep grazing on forage quality in salt-marsh habitat are not reported so far.

In chapter 4, the effects of sheep grazing on vegetation succession will be analyzed from 2004 to 2007 after the establishment of exclosures in different plant communities in the salt-marsh and in the ecotone between salt-marsh and sand dune. We will focus on the variation in abundance of *Elymus athericus* with and without sheep grazing. In addition, the effect of sheep grazing on forage quality parameters of some salt tolerant species will also be investigated in chapter 4. We hypothesize that grazing by sheep in salt-marsh habitat can be an adequate management method to avoid ruderalisation of salt-marsh habitat by *Elymus athericus*, i.e. to maintain pioneer and species rich saline grasslands through selective grazing on the more competitive species, such as *Elymus athericus*. Moreover, it is hypothesized that sheep grazing has a positive influence on forage quality.

1.3.4 The effect of soil conditions on vegetation in different successional stages

Soil characteristics constrain plant performance and community composition (Grime 2001; Pywell et al. 2003), and attempts to restore plant communities are likely to fail if they do not consider the limitations imposed by soil conditions (Eviner 2008). Understanding the effects of soil conditions on above-ground vegetation is critical since it can help to predict plant responses to soil conditions, determining which species can thrive at a given site and which will outcompete others (Eviner 2008).

Salt-marshes are habitats with extreme environmental conditions due to regular flooding by saline water. Gradients of water-logging and salinity from the pioneer zone over the low to the high marsh induce a distinct vegetation zonation depending on the tolerance ranges of different plant species (Adam 1990). Soil is the substrate from which the roots of plants absorb water and mineral salts. Plant community distribution and species composition are known to be related to specific soil properties such as soil texture, pH, salinity and toxic influences (e.g. Funk et al. 2004).

The ability of certain plants to survive in particular salt-marsh environments is often related to water and mineral nutrient availability, soil aeration and oxygen diffusion rates, redox potential and the presence or absence of toxic ions (Adam 2002). As soil aeration and oxygen diffusion rates are correlated with soil texture, the distribution, frequency and abundance of plant species will be strongly affected by soil texture. Another soil factor, salinity, is one of the most important edaphic factors governing the distribution of salt-marsh plant species (Packham & Willis 1997). Elevation is also one the most important indirect abiotic factors, influencing the distribution and occurrence of species in salt-marsh habitat through other ecological determinants associated with elevation, and hence with inundation frequency. Indeed, several studies were carried out on the effect of different abiotic factors on distribution and occurrence of species in salt-marshes (e.g. Huckle et al. 2000). Nevertheless, vegetation-soil relationships for different successional stages in salt-marshes have not been addressed so far.

It has been demonstrated that stochastic factors, such as seed availability, are important in plant distribution and occurrence in early successional stages (Walker et al. 2006), while in late successional stages, more deterministic factors such as abiotic characteristics play a more important role in the distribution and occurrence of species (Lepš & Rejmánek 1991). In other words, early in the succession process, species establishment would be largely stochastic (Økland 1999; del Moral et al. 2005), and eventually, deterministic processes should produce predictable relationships between species and their environments (del Moral & Lacher 2005). Community structure in mature systems is often assumed to result from deterministic links between plants and their environment (del Moral 1999). Therefore, it is to be expected that species distribution and occurrence are more predictable by abiotic factors in late successional stages in comparison to early successional stages. In chapter 5, we hypothesize that the relationship between soil characteristics and vegetation is stronger in late successional than in early successional stages.

1.3.5 Seed bank and succession

Depth distribution of viable seeds is not merely of academic interest (Espinar et al. 2005). Indeed, for some restoration measures the knowledge of the depth distribution of viable seeds in soil is indispensable, e.g. for topsoil removal, a regularly applied restoration measure in some habitats (Grootjans et al. 2001), has a significant impact on seed availability. The soil removal treatment dramatically decreased the availability of seeds in the seed bank in dune slacks (Grootjans et al. 2001), flood meadows (Holzel & Otte 2003) and fen meadows (Ramseier 2000), indicating that soil seed bank density and composition decline monotonically with soil depth. Some studies conversely stated that viable seed density in deeper layers is higher than in shallow layers or that the distribution of seeds along depth is binomial (Espinar et al. 2005). Depth distribution is often a reasonably good indicator of seed longevity (Thompson et al. 1997; Bekker et al. 1998).

Salt-marshes can differ in salt concentration (Adam 1990). Several studies have proven that salinity can delay or hamper the germination (Rubio-Casal et al. 2003). As a result, in higher salinity conditions it can be expected that a relatively higher number of seeds may penetrate to the soil compared with less saline conditions. In chapter 6, we will compare the depth distribution of seeds in two salt-marshes differing in salt concentration (euhaline and mesohaline).

Seed bank and above-ground vegetation can have a mutual interaction. This interaction can be influenced by age (Wolters et al. 2002), as the above-ground vegetation changes during succession. In terrestrial habitats, several studies reported on the vertical and horizontal distribution of seeds in soil with respect to age (e.g. Bossuyt & Hermy 2003), while knowledge is scant for salt-marsh habitats (but see Wolters & Bakker 2002). Early successional species tend to form persistent seed banks and late successional species tend to show a more transient seed bank (Bossuyt & Hermy, 2004). Therefore it can be expected that seed density and similarity between seed bank and above ground vegetation decrease along time. Seed bank density and similarity with above-ground vegetation will be compared for early and late successional stages in chapter 6. It is hypothesized that the vertical distribution of seeds is different for euhaline and mesohaline salt-marshes. In addition, it is further hypothesized that seed density and similarity between seed bank and above-ground vegetation in old salt-marsh is lower than in new salt-marsh.

1.4 The reasons why to study succession in a restoration program

Successional vegetation processes are an important aspect in ecological restoration, because they determine the type and timing of restoration measures used and affect the final success. Therefore, vegetation succession and its study should be taken into account in virtually any restoration program. On the other hand, results of both spontaneous processes and restoration measures are usually intermingled and influence each other (Luken 1990). In some restoration programs, we can completely rely on spontaneous succession (Prach et al. 2001). Spontaneous succession is affected by many local biotic and abiotic factors and their interactions and, hence, will be different in different places. It is not possible to extrapolate the results of a study in a particular location to other locations (Eviner & Hawkes 2008). Many restoration failures can be attributed to site-specific issues that were not taken into account (Wassenaar et al. 2007), indicating the importance of studies on natural plant succession in each site separately.

Beside the importance of vegetation succession studies in restoration programs, newly (by man or naturally) created substrates offer us unique opportunities to study plant succession fundamentally, in which salt-marshes are a remarkable habitat. Salt-marsh communities normally arise on bare substrate and when mature are often eroded, creating bare areas which are re-colonized. They are thus very suitable habitats to study processes of vegetation change (Packham & Willis 1997). Previous studies showed that the kind of pioneer species, the way of succession and the speed of species turnover are different in different salt-marshes. For instance, Wolters et al. (2008) showed that annual species were the new colonizers in salt-marshes in the north of the Netherlands and perennial species only started to colonize or increase notably in abundance after a relative long time after restoration. In contrast, Odland & del Moral (2002) demonstrated that in wetland conditions perennial herbs soon invaded and came to dominance, although annuals together with acrocarpous mosses were the first colonizers in this case also. Sometimes vegetation changed and replaced quickly and in other cases vegetation was stable for several years. Therefore, plant succession will be unique in each (salt-marsh) habitat, indicating again the necessity to study plant succession in each salt-marsh separately.

1.5 Material and methods

1.5.1 The recently history of the study area

The study area is part of the Flemish Nature Reserve the IJzermonding and is located along the right hand bank of the IJzermonding in the city of Nieuwpoort (Prov. West-Flanders), Belgium (Fig. 1.3). It consists of two parts: an old, more or less untouched salt-marsh and a recently created intertidal area, where a new salt-marsh came to development (Fig. 1.4). The IJzermonding salt-marshes are one of the only four daily-inundated salt-marsh areas in Belgium. The other three are the Baai van Heist, the Zwin with true salt-marshes and the Scheldt estuary with brackish salt-marshes. The history of the old salt-marsh area during the last century is one of constant deterioration and regression, caused by many kinds of destruction.

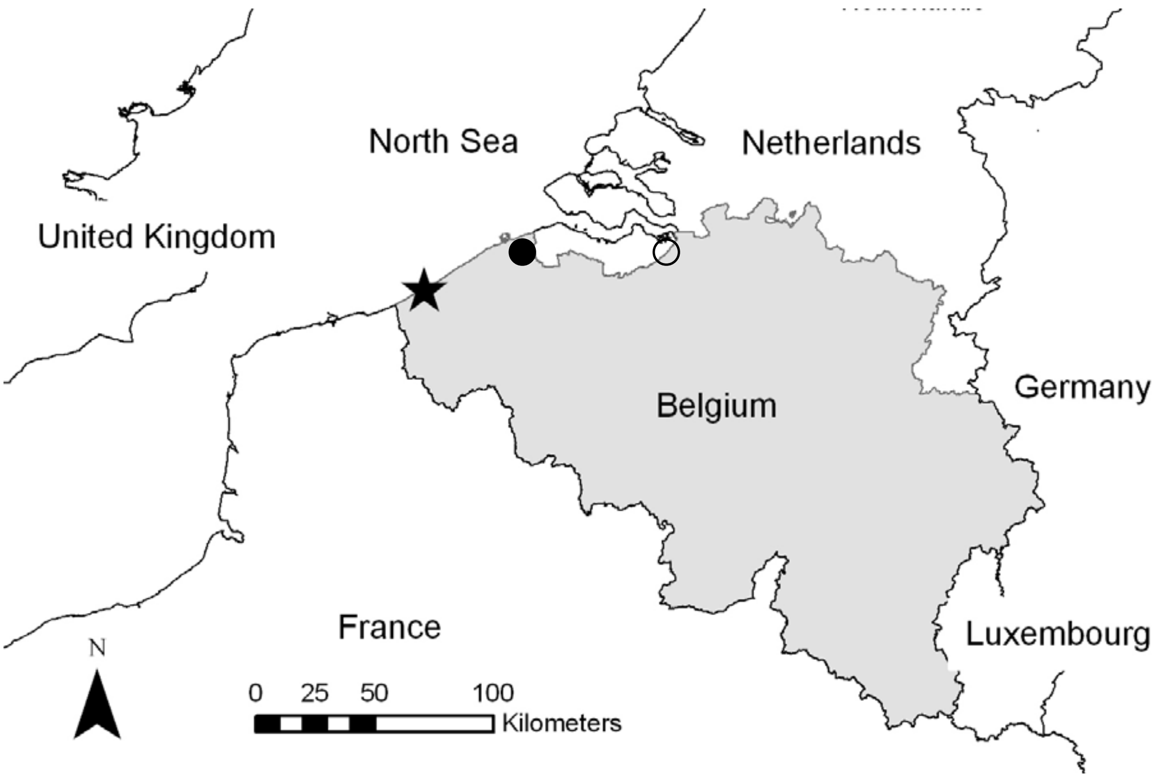

Fig. 1.3. The position of the study area (the asterisk). The study area is located in the city of Nieuwpoort, Belgium. Two other study areas are used in this PhD to study seed bank characteristics (chapter 6): the Zwin area is located at the filled circle; the Verdronken Land van Saeftinghe is located at the open circle.

Fig. 1.4. The position of the newly (2002) created salt-marsh (N) and the old salt-marsh (O1 and O2) in the Flemish Nature Reserve "De IJzermonding" at Nieuwpoort.

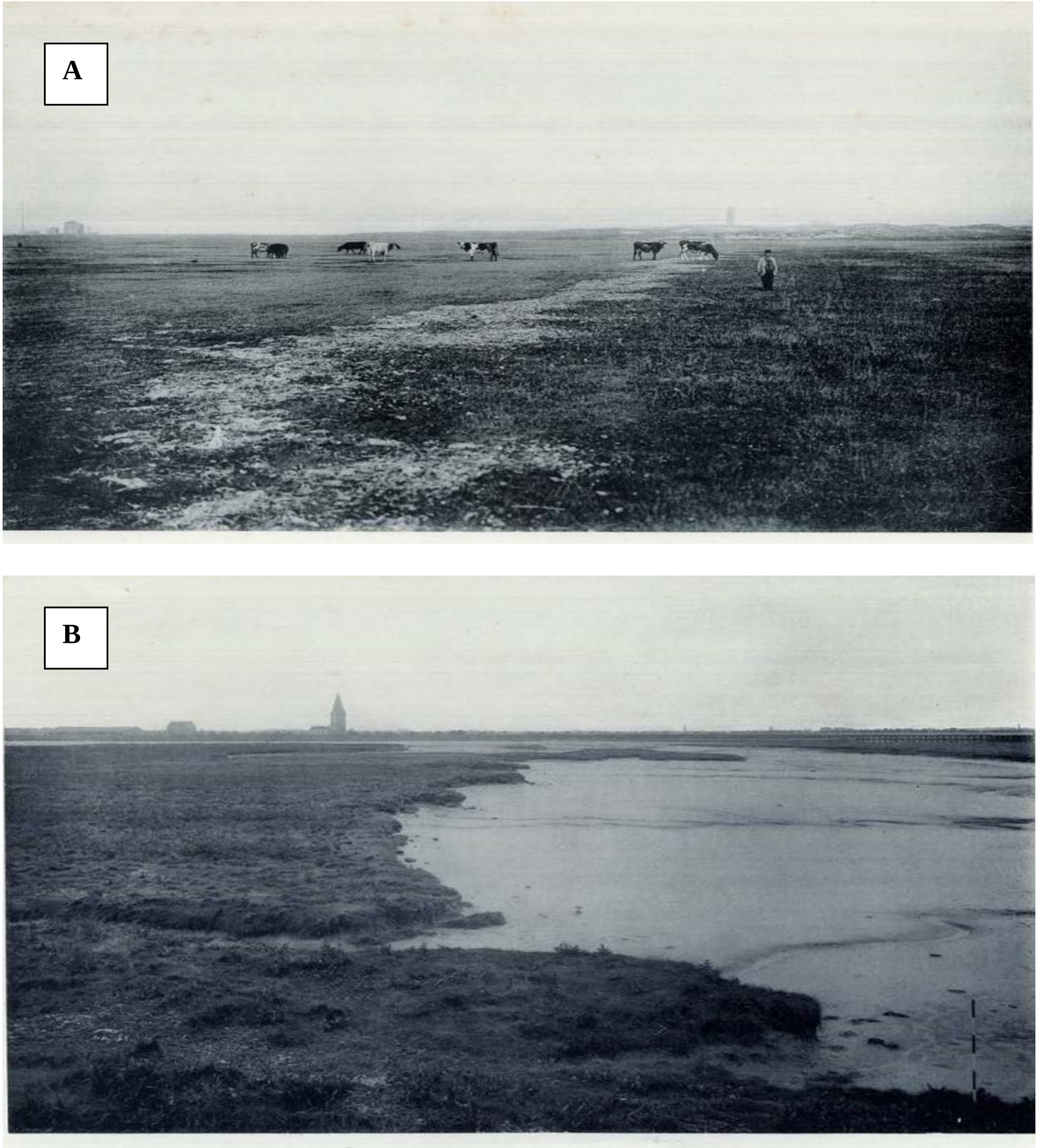

Fig. 1.5. Photos taken by Massart in 1904 (Massart 1908). (A) The surprisingly narrow ecotone between salt-marsh (left) and coastal dune (right) with floodmark conditions in between in the former IJzer estuary, before this area was raised with slurry and sand in the mid-20th century; the picture was taken in northern direction, in between the middle part of transect 3 and 4 (Fig. 1.6) in the present study; the vegetation was described as follows: “left of a central floodmark, a salt-marsh vegetation, grazed by cows, horses and hinnies (both latter not in the picture), appears with *Salicornia europaea, Suaeda maritima, Glaux maritima, Puccinellia maritima, Armeria maritima* and other salt-marsh species, right of the floodmark a classical dune grassland appears, with species like *Festuca rubra, Agrostis* sp., mosses and lichens (descriptions, translated from Vanhecke et al. 1981). (B) Creek of Lombartsyde in the IJzer estuary at low tide with a non-grazed vegetation of *Puccinellia maritima, Aster tripolium, Triglochin maritimum, Plantago maritima, Halimione portulacoides, Suaeda maritima* and other salt-marsh species; the picture was taken in a western direction (descriptions, translated from Vanhecke et al. 1981).

There are some photos available dating from the turn of the last century (Massart 1908) from the old salt-marshes (e.g. Fig. 1.5). A comparison of the vegetation between Massarts's photos and vegetation in 1974 by Goetghebeur (1976) showed that the surface of salt-marsh vegetation steadily diminished in time. There were 60-70ha of salt-marsh vegetation in 1913, while it decreased to less than 5ha in 1974. On the other hand, some species were always reported as occurring in high abundance, e.g. *Aster tripolium* in the higher parts. *Spartina townsendii* was only present from the mid-20th century onwards; it was not yet described by Hocquette (1927) and Isaäcson & Magnel (1929), while Duvigneaud & Lambinon 1963 described it for the first time from the area in 1963. Some species were reported as being rare and only present in small spots, e.g. *Armeria maritima*. Several species have disappeared since 1913; the vegetation has totally changed (Goetghebeur 1976). From 1976 onwards, the vegetation was further disturbed by military, agricultural and fishery activities.

The new intertidal area was created between 1999 and 2002 within the framework of a large-scale LIFE restoration project. General aim of the initiative was to restore or create beach-salt-marsh-dune ecotones with gradual salt-fresh, dynamic-stable, wet-dry and mud-sand gradients. In order to reach this goal, several large buildings and roads were broken down, an entire tidal dock was restructured and some 500,000m^3 of dredging material was removed to restore or create intertidal and coastal dune habitats and their intermediate ecotones.

It was decided to monitor changes from the very start of the restoration process in both habitats giving the opportunity to study vascular plant succession in old and new salt-marshes. Investigations were multidisciplinary and were realized in a partnership between several scientific institutes: Ghent University, Catholic University of Louvain, Royal Belgian Institute of Natural Science and the Institute of Nature Conservation with facility support of VLIZ (Hoffmann 2006a). Studies included the most relevant abiotic conditions, such as sedimentation and erosion, topography and ground water fluctuations, biotic conditions, particularly available seed bank and biological response variables, i.e. flora and vegetation, benthic macrofauna, terrestrial arthropods and birds. To study vegetation succession, data

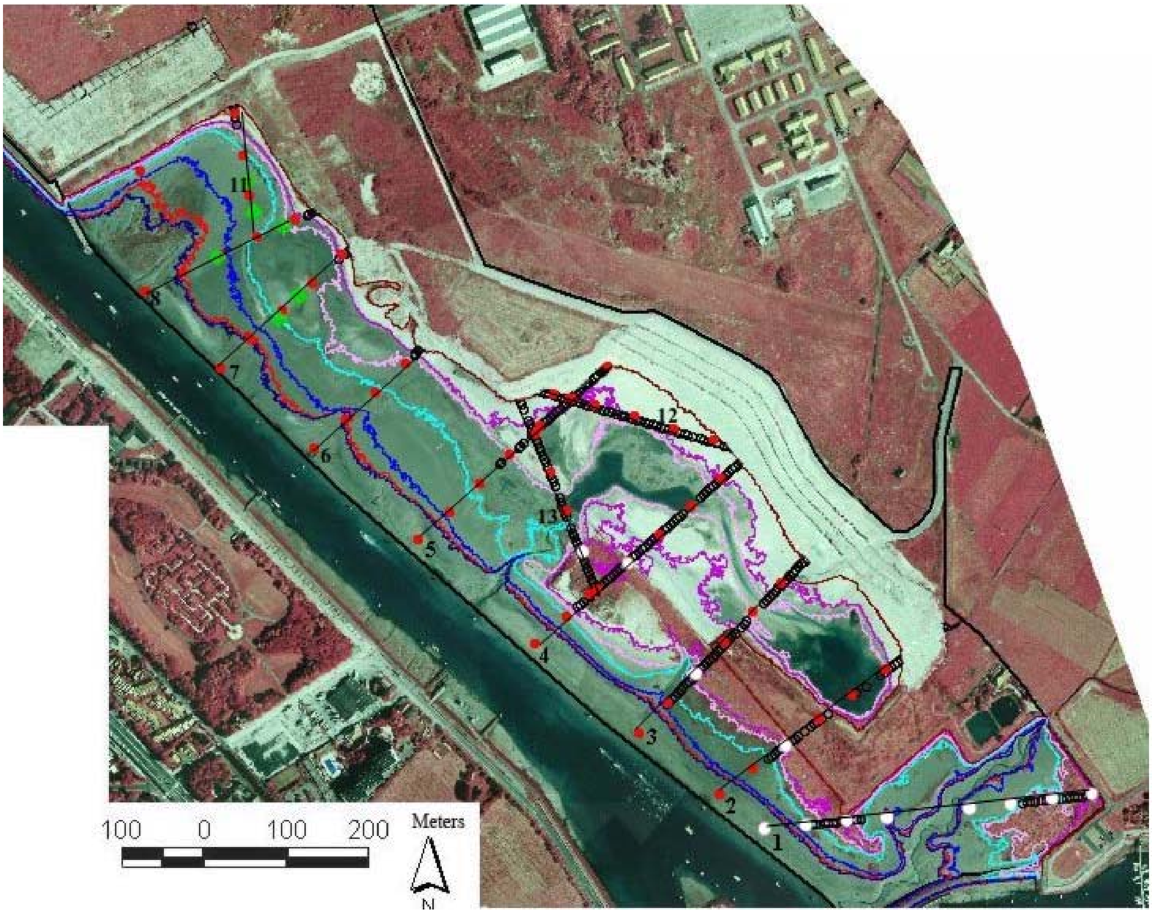

Fig. 1.6. The positioning of the quadrates in the old and new salt-marshes. Black circles show the quadrates which were sampled in 2003; white and red circles show the poles located along transects with 50m distance between each other, white poles are located in the old salt-marsh and red poles are located in the newly created salt-marsh or on the mud flats, which have no macrophytic vegetation. The aerial photo has been taken at low tide on the 28th July, 2002, after the majority of restoration works were realised in early spring of 2002; in the summer of 2002 the first colonizers (*Salicornia* spp., *Suaeda maritima* and others) were already observed in the newly created intertidal area. The numbers show different transects.

were collected systematically along transects perpendicular to the most important gradients in both habitats, new and old salt-marshes. Vegetation changes were followed through yearly sampling of permanent plots in the growing season. Dune vegetation was sampled in July and August; salt-marsh vegetation was sampled in late August and September. Dune data are not considered in this study, since we here focus on succession processes in estuarine intertidal conditions. Within this intertidal part of the nature reserve, 90 permanent plots were laid out in 4 transects in 2001; in 2002 the number of plots was 94 and the number of transects was 4. In 2003, the number of intertidal plots was raised to 279 and the number of transects to 11, while in 2005 and 2007, the number of intertidal sampled plots was 214 and 267, respectively and the number of transects was 11 (Fig. 1.6). Evaluation of early successional trends was done after the last sampling in the summer of 2007 of some biotic and abiotic factors relevant for this study, i.e. inundation frequency and sheep grazing.

1.5.2 Materials

Not all vegetation or seed bank sample plots were used in all chapters. Selection of plots depended on the environmental or biological data available for every plot, or from the specific questions raised per chapter.

In chapter 2, we used plots that were sampled in 2003, 2005 and 2007. The number of plots is therefore restricted to those that were sampled in all three study periods. This means that 175 plots were used from the newly created salt-marsh and 66 plots from the old salt-marsh. Plot positions are visualized in Appendix A. Additionally, in 2008 vegetation was sampled in 30 quadrates to estimate seed production (these plots are not indicated on the map in Appendix A); these 30 quadrates were concentrated in three different subsites (10 quadrates each) and were situated within the old salt-marsh (reference site). Seed bank data used in chapter 2 are collected from the same plots as were used in chapter 6 from the newly created salt-marsh; the position of these seed bank plots are visualized in Appendix E.1.

In chapter 3, all plots of which inundation frequency data were available and vegetation data were sampled in 2003, 2005 as well as 2007 were included; this concerns a total of 119 plots, all situated in the newly created salt-marsh area (Appendix B).

In chapter 4, data collected in five exclosure and five neighbouring enclosures in 2005 and 2007 were used (Appendix C). Each exclosure and each enclosure consisted of 5 permanent plots, leading to a total of 40 subplots, in which vegetation composition, plant species cover, and plant species forage quality determinants were measured (for further explanation, we refer to the method-part of chapter 4).

In chapter 5, we used 155 plots that were sampled in 2005, of which soil factors and elevation were measured. Of these, 95 were situated in the newly created salt-marsh and 60 in the adjacent old salt-marsh (Appendix D).

Finally, *in chapter 6*, vegetation and soil seed bank were measured in 90 plots; they were sampled in 2006. These plots are located in four salt-marsh entities, i.e. IJzer estuary-old marsh, IJzer estuary-new marsh, Zwin and Saeftinghe (see Appendix E.1, E.2 and E.3).

Table 1.1 shows the material (mostly vegetation) and methods (mostly permanent plots) used in each chapter, separately.

Table 1.1. Materials and methods used in different chapters. The size of plots is 2m × 2m and quadrates is 50cm × 50cm.

Chapter	Parameters to be estimated	Location	Year	Method	Number (plots or quadrates)
2	Vegetation data	Newly created and old (reference) salt-marshes (IJzermonding)	2003, 2005 and 2007	Permanent plots	175+66
	Seed production	Old (reference) salt-marsh (IJzermonding)	2008	Quadrates	3×10
	Seed bank	Newly created salt-marsh (IJzermonding)	2007	Soil cores	10
3	Vegetation data	Newly created salt-marsh (IJzermonding)	2003, 2005 and 2007	Permanent plots	119
	Inundation frequency	Newly created salt-marsh (IJzermonding)	2003	Tide and plot level	119
4	Vegetation data	Newly created salt-marsh (IJzermonding)	2005 and 2007	Permanent plots	4×5×2
	Forage quality	Newly created salt-marsh (IJzermonding)	2006 and 2007	Quadrate	1516
5	Vegetation data	Newly created and old (reference) salt-marshes (IJzermonding)	2005	Permanent plots	95+60
	Soil factors	Newly created and old (reference) salt-marshes (IJzermonding)	2005	Soil cores	95+60
	Elevation	Newly created and old (reference) salt-marshes (IJzermonding)	2005	Plot level	95+60
6	Vegetation data	(IJzermonding-new, IJzermonding-old, Zwin, Saeftinghe, respectively)	2007	Temporary plots	10+16+ 14+50
	Soil seed bank	(IJzermonding-new, IJzermonding-old, Zwin, Saeftinghe, respectively)	2007	Soil cores	10+16+ 14+50

1.6 The restoration interest of salt-marshes

Coastal salt-marshes are defined as areas, vegetated by herbs, grasses or low shrubs, bordering saline water bodies. They are subject to periodic flooding as a result of fluctuating water levels of the adjacent saline water bodies (Adam 1990). Salt-marshes are restricted to a narrow zone between land and sea, many salt-marsh plant species and their associated plant communities are considered rare (Doody et al. 1993) or vulnerable to extinction (Westhoff et al. 1993).

Salt-marshes are considered particularly important for migratory birds and waterfowl (see e.g. Rowcliffe et al. 1995; Zedler & Callaway 1999; van der Wal et al. 2000; Dierschke & Bairlein 2004). This importance is reflected in the national and international policies on the conservation and restoration of salt-marshes (Doody et al. 1993; Janssen & Schaminée 2003; Ozinga & Schaminée 2005). Apart from their nature conservation interest, salt-marshes are important as a natural flood control, dissipating wave energy (Möller et al. 1999). It has been estimated that with a 6m wide salt-marsh in front, a 6m high seawall would be sufficient to protect the hinterland, whereas in the absence of a salt-marsh the seawall should be 12m high (King & Lester 1995). As building and maintaining seawalls is expensive there are obviously great economic advantages in having a salt-marsh in front of coastal embankments (Wolters et al. 2005).

Recently, large losses of salt-marsh area have been reported globally (Dijkema 1987; Cox et al. 2003). These losses have been attributed to several factors associated with human development and climate change, dredging, coastal squeeze, land claim for farming or building, pollution from land or sea (Allen 2000, Goodwin et al. 2001; Adam 2002, Doody 2004). Consequently, in an attempt to restore the former salt-marsh area and to promote salt-marsh development, to enlarge salt-marsh-dune ecotones, a restoration project was started along the right bank of the IJzer estuary. The main goal of the nature restoration project was to enlarge the intertidal salt-marsh and mud flat area, creating an ecotone between river and land, since ecotones are potentially important hot spots of biodiversity, both at large scale (Smith et al. 2001) at regional and at local scale (van Leeuwen 1966). Restoration measures were taken in such a way that gradual gradients of inundation were created, along which a vegetation development from vegetation free sand or mud flat to vegetated salt-marsh habitat was expected. Salt-marsh vegetation development was assumed to be possible; thanks to the presence of salt-marsh species in adjacent old salt-marsh, the newly created intertidal area and the old salt-marsh were only a few meters apart and were hydrologically connected by tidal inundations.

Although, there is much debate on the question of how to define restoration success and in many cases there are no clearly defined targets, one of the possibilities for assessing success is to compare the ecological structure (richness and composition) of a restored site with one or more reference sites (Thom et al. 2002; Edwards & Proffitt 2003). In the present study, the rate of appearance of salt-marsh species present on the adjacent old salt-marsh could be used as an evaluation criterion for restoration success.

1.7 Why permanent plots?

Permanent plot studies have a long history (Austin 1981) and long-term permanent plots are important as they can help in separating trends and seasonal fluctuations (Huisman et al. 1993), and are needed to test ecological models that are often based on assumptions and not derived from solid field studies (Bakker et al. 1996). The study of long-term permanent plots has made it clear that vegetation development in many ecosystems under restoration was different from the final state that was anticipated. This may generate new hypotheses (Klotzli & Grootjans 2001). Furthermore, long-term recordings are needed to validate the effects of management measures. Even, experimental changes in salt-marsh management (Bos et al. 2002) revealed clear changes after some years, stressing the importance of long-term monitoring (Bakker 2005).

Permanent plots are useful in studies of climate change (Petriccione 2005), in field experiments (Brys et al. 2005) and in the restoration projects (Bekker et al. 1996; Kiehl & Wagner 2006). Measuring sequential percent cover and species composition in permanent plots allows the trajectory of vegetation change to be quantified by multivariate methods and similarity measures (del Moral 2007). Bakker et al. (1996) stressed that permanent plot studies permit both internal and external driving forces to be explored. Such studies can lead to new hypotheses and offer clues to appropriate experiments to test these hypotheses (Odland & del Moral 2002).

The size of vegetation relevés may differ according to the structural characteristics of the vegetation. Dutch ecologists have used permanent plots 2m × 2m in size for decades to show cyclical and directional vegetation dynamics in salt-marshes (Leendertse et al. 1997; Smits et al. 2002). Consequently, we also used plots 2m × 2m in our study.

1.8 Objectives and outline of the thesis

Understanding the driving factors of succession is still a matter of great interest in ecological science, despite countless studies that deal with succession in all kinds of habitats. One of those habitats is salt-marsh environment in which drivers determine plant species turnover in time and changes in spatial plant arrangement during the succession process. General primary succession determinants in naturally developing salt-marshes are relatively well described, although the vast literature on this matter shows that local plant succession is unique for each salt-marsh environment, since biotic and abiotic factors always differ among localities. Additionally, relatively little knowledge is available on the specific drivers in restoration conditions, which will always differ from natural conditions, in which spontaneous salt-marsh vegetation would develop. Knowledge of succession patterns and processes in these restoration conditions is essential though, when one wants to be able to estimate potential success of restoration initiatives that aim at the creation or restoration of a diverse salt-marsh environment in general. This thesis uses the restoration initiative taken in 1999-2004 along the right bank of the IJzer estuary (province of West-Flanders, Belgium) to learn about the specificity of succession processes at a salt-marsh restoration site.

Primary succession on a newly created salt-marsh starts with colonization of species from the local and regional species pool from the seed bank or from seed rain. Seed dispersal from the local species pool is more important than from the regional species pool, since it may take several years to disperse seeds from the regional species pool to newly created salt-marsh (Wolters et al. 2008). Nevertheless, species present in the local species pool show different colonization abilities. In the study area, no seed bank existed because of the huge quantities of slurry material deposited on the former salt-marshes, removed in 2001. This was proven in a pilot study performed in the area in 2001 (Stichelmans 2002 cit. in Hoffmann & Stichelmans 2006). Nevertheless, the colonization by a few species was enormous and fast, i.e. immediately after creation of the new intertidal area in 2002. Among the different species existing in the adjacent area, only few species were able to colonize during the first growing season, while most species appeared more slowly. More surprisingly is that some species have not yet appeared in the newly created salt-marsh, five years after its creation. In chapter 2, we will describe the characteristics of the initial colonizers in the new salt-marsh environment, which may help a species to be an initial colonizer. Which species trait could be important for a plant species to appear itself as a new colonizer? The abundance of the first colonizers in the local salt-marsh, the salt and nutrient limitation tolerance and seed dispersal traits of new colonizers will be dealt with.

Once populations colonize and establish on a new substrate, vegetation succession starts. Both biotic and abiotic factors could influence vegetation succession (Olff et al. 1997). The effect of some abiotic factors on plant succession will be investigated in chapter 3, in which inundation frequency is one of the most important abiotic factors, affecting vegetation dynamics.

One of the most important biotic factors affecting vegetation succession is grazing. The new salt-marsh was accessible for sheep from 2004 onwards. The effect of grazing on early plant succession and succession trajectory was investigated by the establishment of exclosures and will be described in chapter 4. Particularly, the effect of sheep grazing on the strong competitor *Elymus athericus* will be discussed in this chapter, since it has been shown to be a problematic species for general species richness in European salt-marshes. In addition, it will be tried to relate the grazing of different plant species (particularly *Elymus athericus*) to their forage quality in this chapter.

The relationships between biotic and abiotic factors with vegetation can vary according to stage of succession (Lepš & Rejmánek 1991). For example, the relationship between abiotic soil factors and vegetation could be different in different stages of succession. Another example, the relationship between seed bank (as a biotic factor) with vegetation, could be different in different successional stages (Milberg 1995). Having an old salt-marsh adjacent to a newly created salt-marsh gave us an unique opportunity to compare some aspects of vegetation succession in different stages. The relationship between abiotic factors (soil factors) with vegetation in both new and old successional stages will be compared in chapter 5.

Temporal and spatial distribution of seeds buried in soil, seed density and similarity with above-ground vegetation will be compared between new and old salt-marshes in chapter 6.

Fig. 1.7 shows the conceptual framework of the different chapters in this thesis, and tries to illustrate the connections between the chapters and the relevance of each in explaining the general questions on succession determinants.

Successful restoration of plant communities depends on the availability of target species, the ability of the species to reach a target area and the presence of suitable environmental conditions that allow the species to germinate and establish. Succession starts with colonization of some species available by seed dispersal from the reference site close to the restoration site. However the rate of this succession could be impacted by a very strong abiotic factor, inundation frequency or a biotic factor, sheep grazing. In a given time along successional stages, the relationship between abiotic factors and vegetation could be different between early stages and lately stages. In addition, the relationship between seeds buried in soil and above-ground vegetation could also be different in early and late stages of succession.

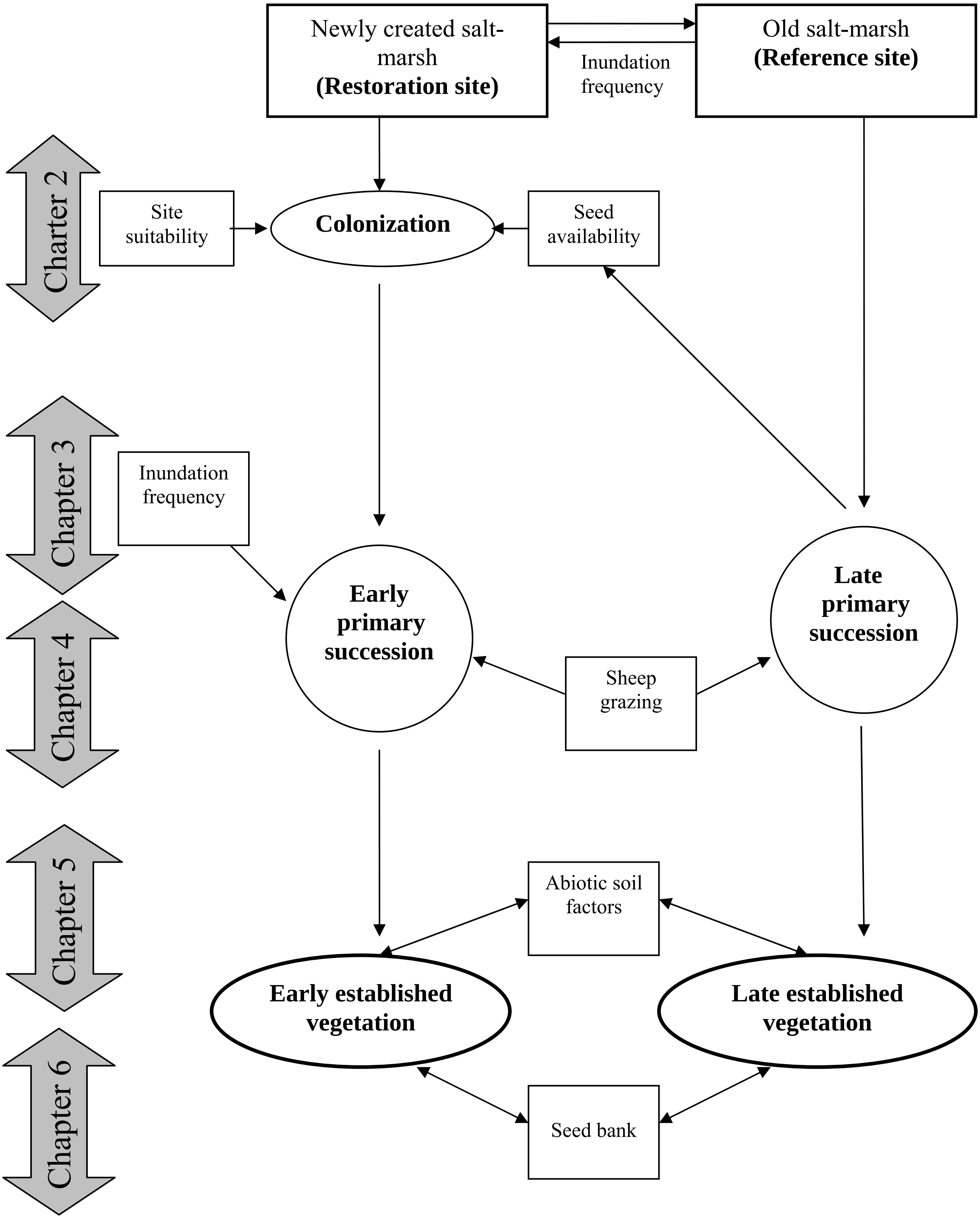

Fig. 1.7. The conceptual framework for exploring the constraints for early vegetation succession compared with late vegetation succession.