Chapter 6 The effect of successional stage and salinity on the vertical distribution of seeds in salt-marsh soils[5]

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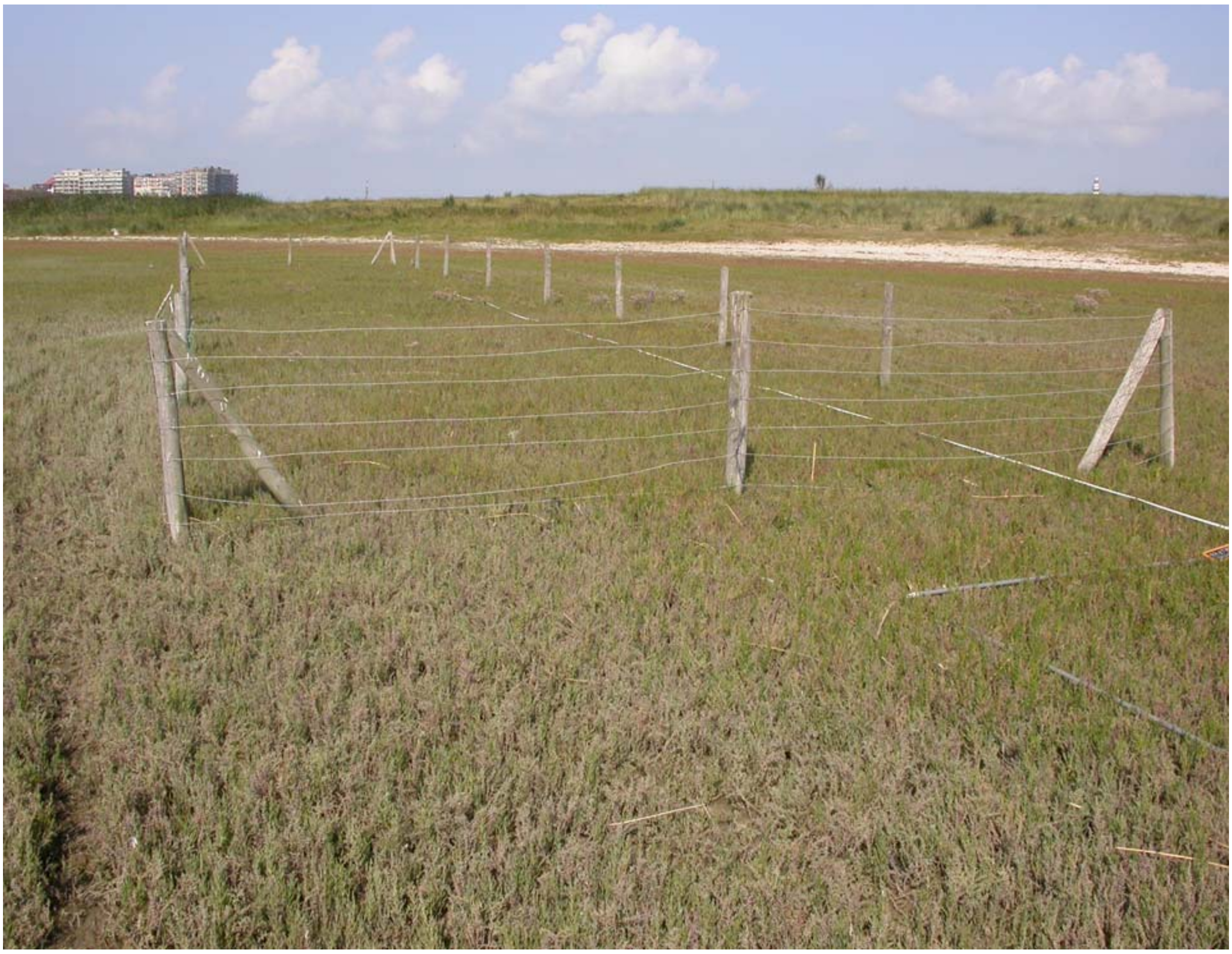

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6.1 Abstract

Seed bank density and similarity between seed bank and above-ground vegetation along depth were compared between two salt-marshes different in age. In addition, the effect of salinity on the variation in seed bank density and similarity between seed bank and above-ground vegetation along depth was investigated. The study was conducted in an euhaline salt-marsh that contained both old and newly created habitats. In addition, two other old salt-marshes were selected to study the effect of salinity on the distribution pattern of seed bank and similarity between seed bank and above-ground vegetation at different soil depths. One of them was mesohaline, the other euhaline.

Ten plots of 2m × 2m were situated in the new salt-marsh (existing since 2002) and 80 plots in the three old salt-marshes. Soil samples were collected at three different depths (0-5cm, 5-10cm and 10-15cm) in spring 2006. After washing by fresh water, the soil samples were spread in the greenhouse to allow viable seeds to germinate. Germination experiments lasted 6 months and all seedlings were identified and removed after identification. Above-ground vegetation composition was determined during the growing season in all plots.

Viable seed density was calculated for each plot and for the three different depths; the similarity between seed bank and floristic composition of the above-ground vegetation was calculated. A general linear model was used to investigate the effect of soil depth, age and salinity of the salt-marsh on density and similarity between seed bank and above-ground vegetation.

The results showed that seed density decreased with depth in all salt-marshes irrespective of their age and soil salinity.

Seed density and similarity between seed bank and above-ground vegetation were higher in the new salt-marsh than in the old one in the same study area. This is because in young as well as in old successional stages, the seed bank was mostly composed of new colonizers, while most perennial species were absent from the soil seed bank, although they were dominant in the above-ground vegetation of the old salt-marsh.

The characteristics of the seed bank of a mesohaline salt-marsh were found not to be fundamentally different from that of both euhaline salt-marshes.

Key words: Succession, Seed bank, Salinity, Salt-marsh, Vegetation, Mesohaline, Euhaline

6.2 **Introduction**

The persistence of seeds is a fundamental aspect of plant biology. It plays a key role in the conservation and restoration of plant communities and is important for the potential response of plant species to changing land use and climate (Thompson et al. 1997). Seed banks are crucial for the restoration of plant communities (Bossuyt et al. 2005), the recovery of endangered plant species and the conservation of genetic diversity (Wolters & Bakker 2002).

The stage of succession (age) is an important factor, which may significantly affect seed bank characteristics (density, composition and similarity with above-ground vegetation). The seed bank richness and density declined from initial to terminal successional stages in fallow land (a habitat heavily anthropogenically influenced) in which the number of species in the seed bank decreased over the course of succession from 38 to 25 (Falinska 1999). Succession in semi-natural grassy or meadow communities tends to show other types of change (Donelan & Thompson 1980; Patridge 1989; Milberg 1995). Chambers (1995) found in an alpine herb field that for late successional stages the seed bank was dominated by species producing less persistent seeds. In sandy dune slack habitat, Bossuyt & Hermy (2004) found that similarity between vegetation and the 0-5cm layer in the seed bank increased with increasing slack age. Similarity between vegetation and the deeper soil layer was much lower and showed no significant correlation with age. In addition, they stated that during the course of succession there is a sharp increase in seed density with time and that the seed longevity index decreases during succession. Seed bank composition followed above-ground vegetation in time. As far as we know, the seed bank characteristics of young versus old salt-marshes have not been yet compared in detail.

The knowledge of vertical distribution of seed density in soil and the ability to predict the vertical distribution and emergence of viable seeds can be vital for vegetation restoration

(Espinar et al. 2005). The density of soil seed banks is normally highest at the soil surface and declines monotonically with depth. This pattern is assumed to reflect regular seed input at the surface and a more or less gradual decline in viability as seeds move vertically down the soil profile. Sometimes, for a variety of reason, peak density occurs below the surface or the density of soil seed bank remains constant along the soil depth gradient. For instance, Espinar et al. (2005) found that the total seed density at a depth of 15cm is higher at more shallow soil depths in the non-tidal part of the Doñana salt-marsh (southwest Spain), due to seasonal disturbance processes (soil cracking during the dry season).

In salt-marsh habitat, salinity is a determining factor for seed germination (Adam 1990). Increasing soil salinity decreased the proportion of germinating seeds and slowed down or delayed the seed germination and many seeds remained dormant due to low water potentials under high salinity levels (Ungar 1962; Chapman 1974; Noe & Zedler 2000; Tobe et al. 2000; Ungar 2001; Rubio-Casal et al. 2003; Qu et al. 2007). Therefore, it can be hypothesized that higher salinity gives seeds a higher chance and opportunity to penetrate deeper. Consequently, in high salinity habitat the decrease of seed density with soil depth is expected to be lower than in a less saline habitat.

It can also be expected that the similarity between seed bank and vegetation (hereafter called similarity) in higher salinity condition remains constant with depth while in less saline conditions, it decreases with depth. Similarity between seed bank and above-ground vegetation and seed density has frequently been investigated in salt-marsh habitat in the past. Grazing for example has been proven to be an important factor influencing the above-mentioned seed bank characteristics, although the conclusions are often contradictory (Ungar & Woodel 1993; Ungar & Woodell 1996; Erkkila & Heli 1998; Chang et al. 2001). Study on the variation of seed density and similarity was not investigated yet in relation to different degrees of salinity. Hereafter we compare euhaline and mesohaline salt-marsh in that respect.

Our study area consists of three salt-marshes with different environmental conditions. Saeftinghe is a mesohaline salt-marsh and the other two salt-marshes are euhaline (IJzermonding and Zwin). In addition, the IJzermonding consists of both old and recently created salt-marsh habitats. We tested the following two hypotheses: 1- the seed bank in both old and new salt-marshes is composed of early successional species, since early successional species tend to form persistent seed banks and late successional species tend to show a more transient seed bank (e.g. Bossuyt & Hermy, 2004), 2- in mesohaline salt-marsh, seed density and similarity with above-ground vegetation decrease stronger with depth as compared with euhaline salt-marsh, where a more constant seed bank density is expected, due to better preservation circumstances in more saline environment, combined with the worse germination conditions in the latter environment.

6.3 Material and Methods

6.3.1 Study areas

Soil cores were sampled at three salt-marsh sites, the mesohaline salt-marsh ‘Verdronken Land van Saeftinghe’ in the Netherlands (further on called: Saeftinghe) with a west-east salt gradient of 5.5 – 8psu of the river water along the Scheldt estuary, and the euhaline salt-marshes IJzermonding and Zwin (salinity>15psu) in Belgium. All sites are protected as nature reserves. Although mesohaline and euhaline salt-marshes are also different in size, management regime (grazed and ungrazed), landscape (estuary and coastline salt-marsh) and dominant plant species, using relatively seed characteristic along depth decreased the effect of these environmental conditions (covariates) on the results. In addition, there is no previous research and pre-assumption that showed these environmental factors could affect on vertical distribution of seeds.

The area of IJzermonding has recently (2002) been increased during a large-scale restoration project by creation of a new salt-marsh. Large amounts of sand and slurry were removed in the period 2000-2002 (hereafter called ‘new’ salt-marsh). In other parts of the IJzermonding, two smaller salt-marsh relicts remained intact for centuries (hereafter called ‘old salt-marsh’) (see Fig. 1.4).

6.3.2 Data collection

Data were collected in 90 plots (2m × 2m), 50 of which were established in the Saeftinghe, 26 in the IJzermonding (10 in the new and 16 in the old salt-marsh) and 14 in the Zwin. Seed bank composition was assessed by seedling germination under greenhouse conditions. Soil samples were collected in March 2006 when natural stratification had already taken place in the field. With an auger with a diameter of 3cm, an average of 25 soil cores were collected at random up to a depth of 15cm in each plot. These samples were divided in three subsamples (0-5cm, 5-10cm and 10-15cm) and the sub-samples were then pooled per soil layer for each plot. This resulted in an average of 0.88 litre soil sample in each plot for each layer, which exceeds the recommended 0.8 litre soil necessary to determine the species composition of the seed bank (Hutchings 1986). The methodology of Ter Heerdt et al. (1996) was used to concentrate the soil seed bank samples, which were washed through a coarse (2mm mesh width) and a fine (0.18mm mesh width) sieve. The first sieve removes most roots and coarse vegetative parts. The high water pressure together with finger pressure was used to make sure that all seeds were able to cross through the first sieve. The second sieve withholds all of seeds while the majority of silty soil material is washed out. The concentrated samples were then spread in a thin layer (maximum 0.4cm thick) in 40cm × 40cm trays filled with sterilized potting soil. In March 2006, the trays were randomly placed on shelves with a natural light regime and were kept moist by regular rain water spraying. Air temperature varied between 14°C and 25°C. 24 control trays, filled with the same sterilized potting soil were also placed randomly on the shelves to test for possible greenhouse and potting soil contamination.

Seedlings were identified as soon as possible after germination, counted and removed or, if they could not be identified immediately, transplanted to pots to allow further growth and identification.

After 6 months, when no further seedlings germinated, the trays were left to dry for two weeks. This allowed the samples to be crumbled to expose deeper buried seeds to the light. After watering the samples for another 3 weeks and changing the light into a light regime of 8 hour dark/16 hour light, no new seedlings emerged. Nevertheless, the residual soil was checked for remaining seeds by viewing small random samples taking from trays under a microscope and probing seeds with a needle in order to distinguish between firm and empty seeds. Since, the number of seeds that remained in the investigated soil samples was very low, this procedure was not repeated for all trays.

Above-ground vegetation composition was determined during the growing season of 2006 by estimating the cover of all vascular plant species with a decimal scale (Londo 1976) in the 90 plot (2m × 2m), used earlier for the soil seed bank sampling.

6.3.3 Data analysis

Seedling counts were recalculated to germinable seed density expressed per m² for each 5cm soil depth layer and for each species separately. Similarity between the species composition of the above-ground vegetation and the soil seed bank was assessed with the quantitative Czekanowski similarity index (Kent & Coker 1995) in each depth separately.

Seed density was transformed to $\log_{10}(x+1)$ to meet the normal distribution. General linear model and post-hoc tests were used to compare the seed density and similarity between new and old salt-marsh for IJzermonding data. Seed density and similarity were introduced as dependent variables while, depth categories (0-5cm, 5-10cm and 10-15cm) and age (old and new) were introduced as fixed factors. An interaction between depth and age was also considered in the model. Furthermore, the data were divided into two groups: Zwin and IJzermonding as euhaline plots and Saeftinghe as mesohaline plots. Seed density and similarity were introduced as dependent variables and depth and salinity (euhaline and mesohaline) as fixed factors. Interactions between fixed factors were also entered in the model.

In addition, an analysis was performed to compare the seed density and similarity among three salt-marshes. In this analysis, the data collected in Zwin, Saefinghe and the old salt-marsh in IJzermonding were used. Again seed density and similarity were introduced as dependent variable and depth and different salt-marshes as fixed factors. Interaction between fixed factors were also entered the model. In all analyses, the impact of depth is assumed to be put into evidence by a significant interaction between depth and salt-marsh factors (old and new; euhaline and mesohaline).

6.4 Results

6.4.1 Seed bank properties and succession stage

Seed densities in old and new salt-marshes at IJzermonding were significantly different, respectively 3656 seeds/m² and 6604 seeds/m² (df= 1, F= 2.84 and *p*= 0.04). In both old and new salt-marshes, the seed density was highest in the upper layer. The interaction between depth and age was not significant (df= 2, F= 2.11 and *p*= 0.13) (Fig. 6.1).

Similarity in the new salt-marsh was higher than in the old salt-marsh (df= 1, F= 13.74 and *p*<0.01). Highest similarity was observed in the upper 0-5cm soil layer; although this was not significant as indicated by a non significant interaction between depth and age (df= 2, F= 1.47 and *p*<0.05; df= 2, F= 2.01 and *p*= 0.14 respectively) (Fig. 6.2).

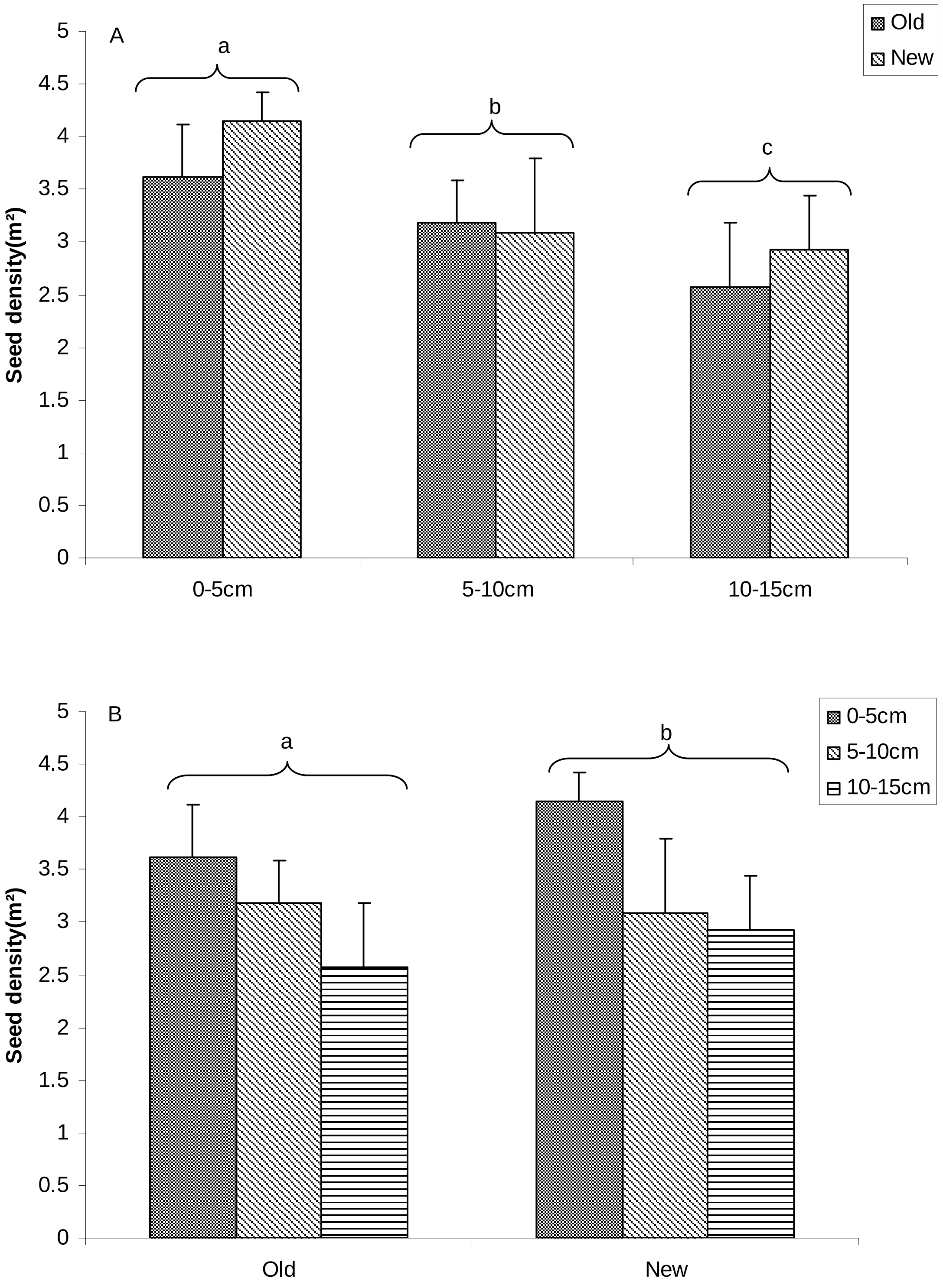

Fig. 6.1. The variation of seed density according to depths (A) and succession stages (B) at IJzermonding (Y axis shows the $\log_{10}(x + 1)$ seed density/m²). Different successive letters indicate significant differences (at $p<0.05$) among layers (A) and between stages (B).

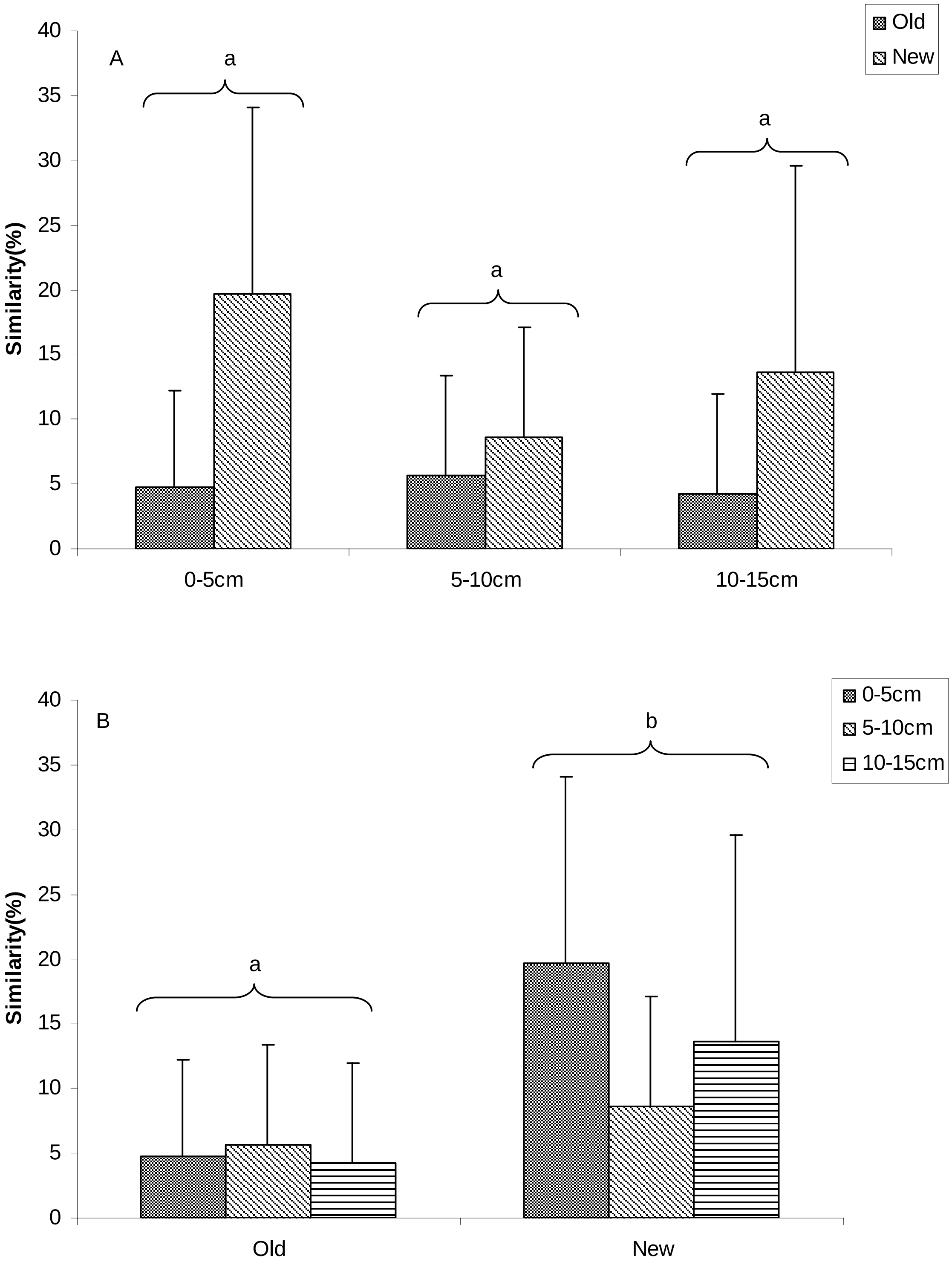

Fig. 6.2. The variation of similarity according to depths (A) and succession stages (B) at IJzermonding. Different successive letters indicate significant differences (at *p*<0.05) among layers (A) and between stages (B).

Plant species in the above-ground vegetation and seed bank of old and new salt-marsh at the IJzermonding are compared in Table 6.1 (only typical salt-marsh species with Ellenberg salinity index higher than 3 and *Juncus bufonius* were shown). Species not found in the vegetation relevés of the plots and only sparsely represented in the whole area are *Artemisia maritima, Atriplex littoralis, Chenopodium rubrum, Juncus bufonius, Juncus gerardii* and *Plantago maritima*. The dominant species of the standing vegetation of the old salt-marsh are the perennial species *Elymus athericus, Limonium vulgare* and *Spartina townsendii*. They are absent or nearly absent in the seed banks of new and old salt-marshes. Dominant in the vegetation of the new salt-marsh are the annuals *Salicornia* spp., *Suaeda maritima* and *Spergularia* spp. They are all well represented in all local seed banks.

Table 6.1. Relative abundance of salt-marsh species in seed bank and vegetation in new and old salt-marshes in IJzermonding. S_{Ell} shows salinity Ellenberg index value. Species which are expected to appear in salt-marshes (Ellenberg index>3, Ellenberg 1991) are shown (see Appendix F for all species)[6]

Species		Seed bank								above-ground vegetation	
		New				Old					
	S_{Ell}	0-5cm	5-10cm	10-15cm	Total	0-5cm	5-10cm	10-15cm	Total	New	Old
Aster tripolium	8	0.06	0.00	0.00	0.05	0.14	0.15	0.00	0.13	0.00	0.54
Atriplex littoralis	7	0.84	0.55	0.00	0.73	0.81	1.09	0.36	0.84	0.00	0.00
Elymus athericus	6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.37	55.82
Glaux maritima	7	0.00	0.61	0.00	0.08	1.28	0.20	0.00	0.88	0.00	0.22
Halimione portulacoides	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.33
Juncus bufonius agg.	0	0.26	0.61	4.16	0.60	4.19	1.92	6.80	3.91	0.00	0.00
Limonium vulgare	8	0.00	0.00	0.00	0.00	0.21	0.16	0.00	0.17	3.33	12.19
Parapholis strigosa	5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.41	0.11
Puccinellia maritima	8	0.00	0.00	0.00	0.00	0.31	0.16	0.00	0.24	1.85	4.57
Sagina maritima	4	0.00	0.00	0.60	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Salicornia spp.	9	55.36	39.38	39.43	51.96	35.40	49.74	50.99	40.56	13.70	2.94
Spartina townsendii	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.81
Spergularia spp.	8	11.18	1.77	11.32	9.87	26.34	26.41	20.96	25.79	8.15	1.85
Suaeda maritima	8	3.20	3.29	1.06	3.05	5.38	2.24	2.97	4.36	36.30	0.98
Triglochin maritimum	8	0.00	0.00	0.51	0.04	3.65	6.57	3.42	4.35	0.00	0.22

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[6] The Ellenberg indicator value of 3 was chosen as threshold for salt-marsh species, because of the general description given by Ellenberg (1992): S= 3: species indicative of (at least) β-mesohaline conditions; S= 2, meaning ‘from oligohaline conditions’, cannot be considered as typical of salt-marsh conditions anymore.

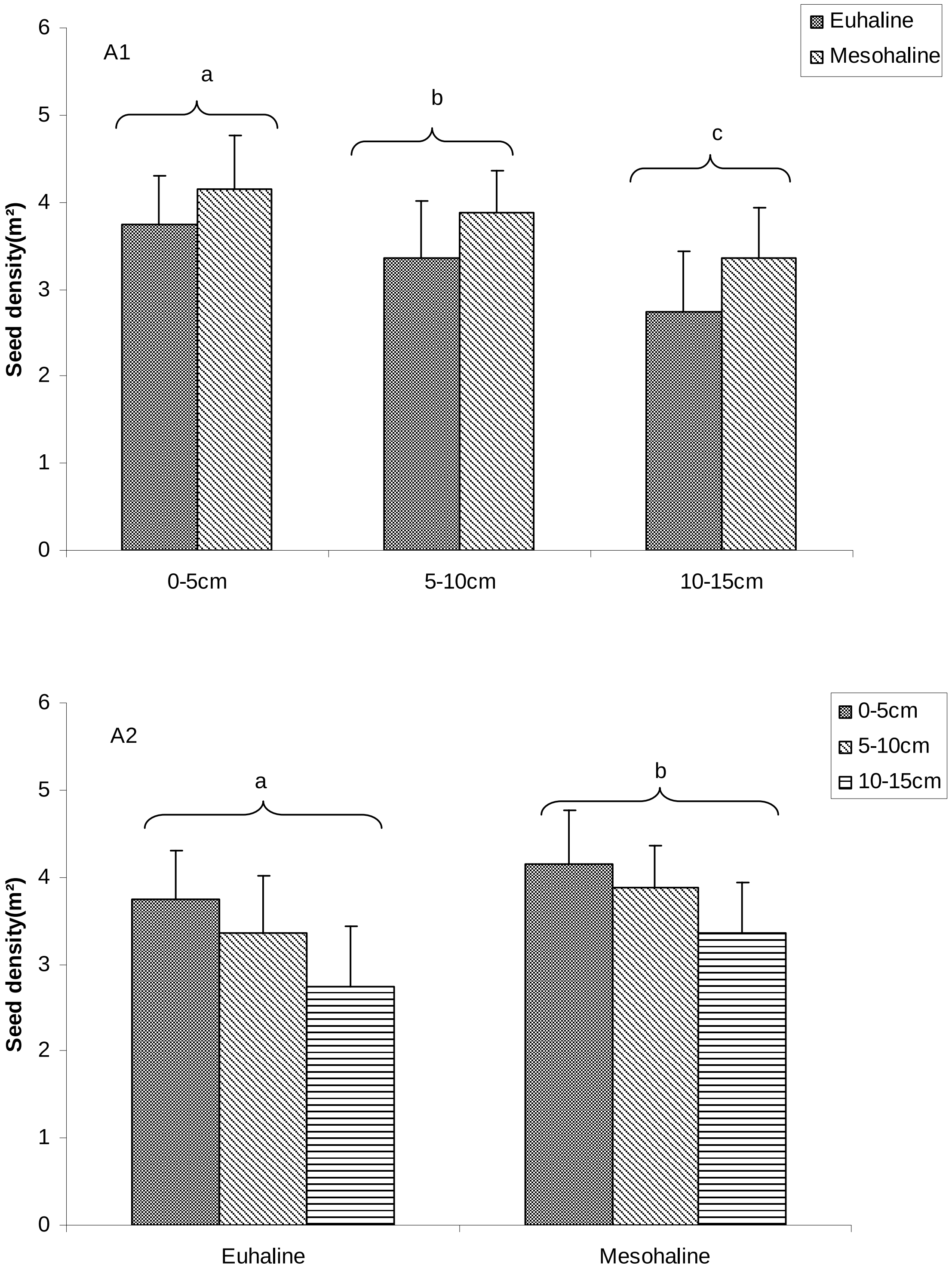

Fig. 6.3. The variation of seed density in different depths (A1) in euhaline and mesohaline salt-marshes (A2) (Y axis shows the $\log_{10}(x+1)$ seed density/m²). Different successive letters indicate significant differences (at $p<0.05$) among layers (A) and between salinity (B).

6.4.2 Seed bank properties and salinity

Seed density was significantly different in two euhaline and mesohaline salt-marshes (df= 1, F= 32.19 and p<0.01) with highest in mesohaline. Although the effect of depth was significant (df= 2, F= 32.76 and p<0.01), there was no significant interaction between depth and salinity (df= 2, F= 0.54 and p= 0.58) (Fig. 6.3).

Similarity was significantly different in two euhaline and mesohaline salt-marshes (df= 1, F= 4.94 and p<0.05) with highest in euhaline area. Lowest similarity was observed in deepest layer while the interaction of depth and salinity had no significant effect on similarity (df= 2, F= 4.26 and p<0.05; df= 2, F= 0.049 and p= 0.95 respectively).

6.4.3 Seed bank properties in different three salt-marshes

Seed density was significantly different in three (old) salt-marshes (df= 2, F= 23.92, p<0.05). The effect of depth was also significant (df= 2, F= 23.92 and p<0.01) while the interaction between depth and different salt-marshes was not significant (df= 4, F= 0.45 and p= 0.79). The highest seed densities were found in the Saeftinghe and the Zwin, with an average of 20318 and 16048 seeds per m^2, respectively, while the lowest seed density was found in IJzermonding with an average of only 3656 seed per m^2 (Fig. 6.4).

Similarity between seed bank and above ground vegetation was very low in all salt-marshes. Nevertheless, similarity was highest in Saeftinghe (df= 2, F= 2.47 and p<0.05). Lowest similarity was observed in deepest layer and the interaction of depth and salt-marshes had no significant effect on similarity (df= 2, F= 2.46 and p<0.05; df= 4, F= 0.35 and p= 0.84 respectively).

Table 6.2. Relative abundance of species in seed bank and above-ground vegetation in the old salt-marshes (Saeftinghe and Zwin). S_{Ell} shows salinity Ellenberg index. Species which are expected to appear in salt-marshes (Ellenberg index>3, Ellenberg 1991) and *Juncus bufonius* are shown (see Appendix G for all species)

Species		Seed bank								Above-ground vegetation	
		Saeftinghe				Zwin					
	S_{Ell}	0-5cm	5-10cm	10-15cm	Total	0-5cm	5-10cm	10-15cm	Total	Saeftinghe	Zwin
Aster tripolium	8	0.00	0.28	0.05	0.07	0.32	0.05	0.00	0.46	3.82	4.05
Atriplex littoralis	7	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00
Chenopodium glaucum	3	0.03	0.08	0.00	0.04	0.10	0.00	1.34	0.12	0.00	0.00
Elymus athericus	6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.43	14.76
Glaux maritima	7	0.90	3.37	1.76	1.55	5.08	3.21	10.54	4.30	3.55	0.71
Halimione portulacoides	8	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.02	0.00	53.81
Juncus bufonius agg.	0	62.69	39.31	33.30	55.08	47.19	78.56	57.24	66.99	0.00	0.00
Juncus gerardii	7	9.44	6.95	6.29	8.63	11.98	7.78	10.41	9.32	3.71	0.12
Limonium vulgare	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.48
Parapholis strigosa	5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.00
Puccinellia maritima	8	0.01	0.07	0.08	0.03	0.19	0.00	0.00	0.06	9.77	2.38
Salicornia spp.	9	6.13	11.84	17.30	8.26	6.15	0.59	0.77	2.40	6.11	2.14
Spartina townsendii	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.34	0.83
Spergularia spp.	8	7.14	24.78	25.35	12.61	14.30	1.29	4.56	5.72	2.73	2.38
Suaeda maritima	8	0.00	0.02	0.00	0.00	0.64	0.10	0.39	0.29	0.98	4.64
Triglochin maritimum	8	0.22	0.70	1.66	0.43	0.00	0.05	0.00	0.03	1.04	0.00

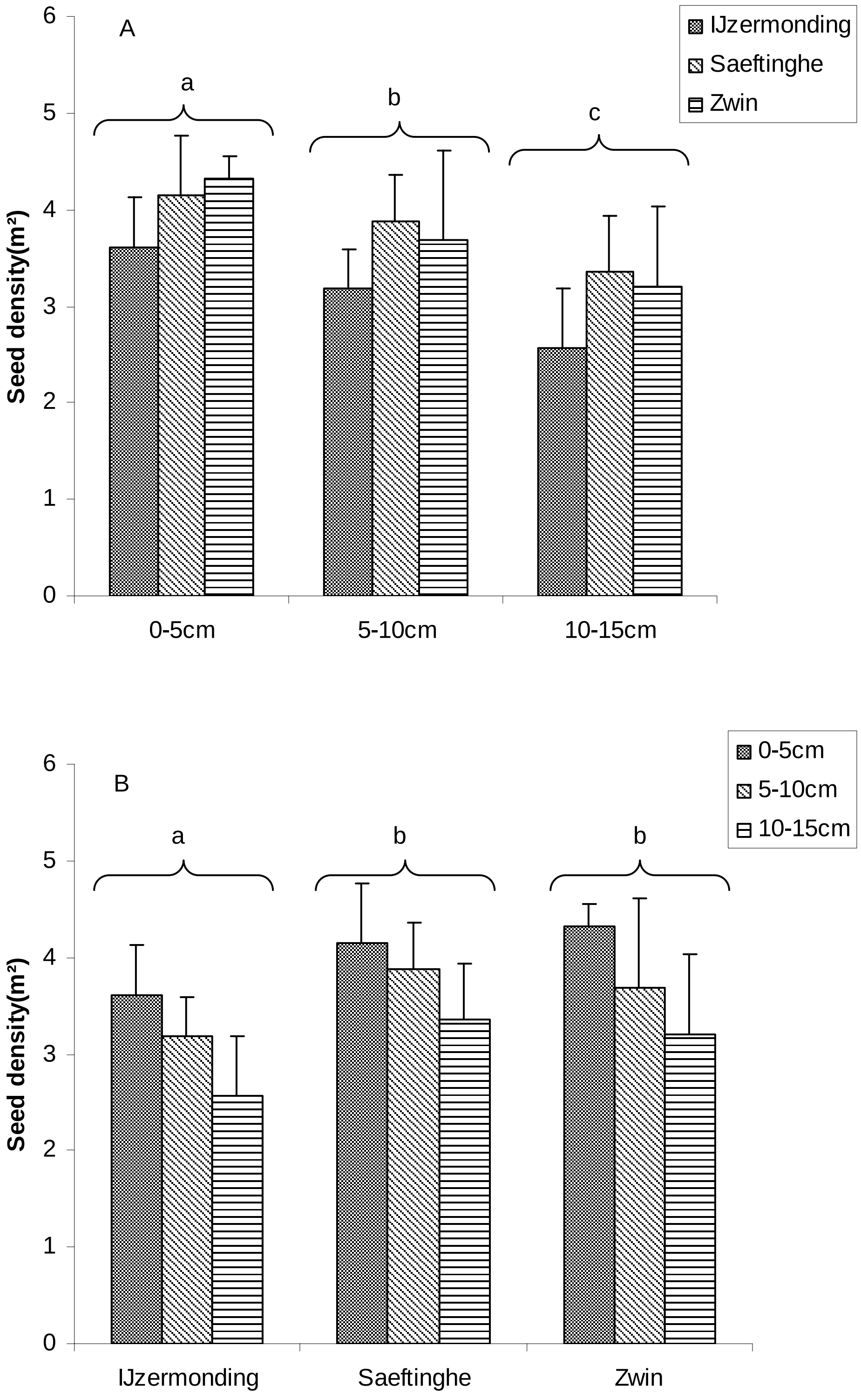

Fig. 6.4. The variation of seed density according to depths (A) and salt-marsh (B). Y axis shows the $\log_{10}(x+1)$ seed density/m². Different successive letters indicate significant differences (at $p<0.05$) among layers (A) and salt-marshes (B).

As shown in Table 6.2, Saeftinghe and Zwin have as common difference with IJzermonding with the massive presence of *Juncus* spp. in their seed banks. The dominant perennials for Saeftinghe (*Elymus athericus, Puccinellia maritima* and *Spartina townsendii*) and for Zwin (*Elymus athericus, Halimione portulacoides* and *Limonium vulgare*) are absent or nearly absent in the respective seed banks. The annuals *Chenopodium rubrum, Salicornia* spp. and *Spergularia* spp. occur in high number in the seed banks, and are not present or relatively sparse in above-ground vegetation. Only typical salt-marsh species with Ellenberg salinity index higher than 3 and *Juncus bufonius* were shown in Table 6.2.

6.5 Discussion

There was a pronounced difference between the seed density in the new and old salt-marsh of the IJzermonding nature reserve. Since seed bank density is the result of the balance between seed input and output (Fenner 1985), the high seed density in the first successional stages can be attributed to a high seed input by most pioneer species (e.g. *Salicornia* spp., *Suaeda maritima* and *Spergularia* spp.) and to a low seed output by seed decay i.e. a high longevity of these species. Pioneer species are known to produce high densities of seeds (Davy et al. 2001; Wolters & Bakker 2002), that remain viable for a long period (Thompson et al. 1997). Similar patterns were found in grass and heathland communities, where there was a decrease in total seed density with increasing age (Bossuyt & Hermy 2003). Remarkably, seed density was higher during early stage for the three different soil layers. If the seeds are persistent enough, they will also become incorporated in the deeper soil layer (Thompson et al. 1997; Grandin 2001).

Despite the dominance of *Suaeda maritima* in the vegetation of the recently created salt-marsh, the seed of this species was not dominant in the soil. Since seed production per individual plant of *Suaeda* is higher than of *Salicornia* plants (see chapter 2, Table 2.4) and most probably also of individual *Spergularia* plants (not counted though), we can indirectly conclude that *Suaeda* seeds are most probably less persistent than *Salicornia* and *Spergularia* seeds. Early germination is a possible cause for its low abundance in the soil (Tessier et al. 2000).

Three distinct relationships between vegetation and soil seed bank can be recognized along vegetation succession in relation to underlying ecological processes of community assembly (Chang et al. 2005): 1) the seed bank composition varies with the established vegetation along vegetation succession seres, 2) the seed bank composition remains rather constant and is composed of early successional species that produce many seeds that persist along the succession sere, and 3) the seed bank composition remains constant but contains a matrix of species found in the different stages. Results of this study mainly coincide with the second relationship, in which colonizing species of the early successional stage i.e. *Salicornia* spp., *Suaeda maritima* and *Spergularia* spp. produced many persistent seeds. In late successional stages the seed bank mostly consisted of the same species as in the early succession stages, despite of perennial dominance (e.g. *Elymus athericus*) in above-ground vegetation. A study on the seed bank in IJzermonding of old salt-marsh in 2001-02 (Stichelmans 2002 cit. in Hoffmann & Stichelmans 2006) also showed that *Salicornia* spp. *Spergularia* spp. *Chenopodium rubrum*, *Atriplex prostrata* and *Suaeda maritima* were dominant in the seed bank. They remained dominant after five years. Although frequently found in this former seed bank analysis of the old marsh at the same study site (Stichelmans 2002 cit. In Hoffmann & Stichelmans 2006), we did not detect any seedlings of *Sagina apetala* and few for *S. maritima* this time. Our results are in accordance with studies of primary succession in an uplifted seashore (Grandin 2001) and of secondary succession in species-rich meadows (Kalamees & Zobel 1998) and old fields (Lavoral et al. 1993), on which the seed bank composition is mostly composed of early successional species. In contrast, some authors demonstrated that the seed bank of early successional stages disappeared as species disappeared in the vegetation (Bossuyt & Hermy 2004).

While in early successional stages new colonizers become to a large extent incorporated in the seed bank leading to a relatively high similarity between seed bank and above-ground vegetation, in the late successional stage most dominant species have very transient seed bank or no seed input (e.g. *Elymus athericus* and *Spartina towsendii*; see chapter 2). Immediate germination (Tessier et al. 2000), absence of seed production due to pathogen infection (Chang et al. 2005; the results of this study) and pollen limitation (Bertness & Shumway 1992), might explain the absence of seeds of a particular species in the seed bank. Furthermore, seeds of some species (e.g. *Elymus athericus*) are relatively large and heavy and remain attached to bracts after detachment from the spike. Due to their relatively large size, seeds of these species are unlikely to percolate downwards through the soil; they readily germinate in the soil or become concentrated in the driftline (Peart 1984; Thompson et al. 1994; Chang et al. 2005).

As a consequence of the above discussed differences in seed production and longevity, our results also showed that similarity between seed bank composition and standing vegetation decreased during succession.

Although salinity can promote seed dormancy (Tobe et al. 2000) and give seed a higher chance to penetrate into soil deeper and deeper, but two salt-marshes (euhaline and mesohaline) are the same in vertical seed distribution with highest densities in topsoil. This pattern indicates the general rules of seed distribution in which regular seed input at the surface by seed rain increase the number of seeds in the top soil; as the seeds penetrate in deeper soil layers, they probably lose part of their viability. Espinar et al. (2005) showed that seeds of some salt-marsh species suffer a rapid loss of viability when buried e.g. *Scirpus maritimus*.

Both the similarity between standing vegetation and seed bank composition and seed density show a similar pattern: both decrease with increasing soil depth. The decreasing similarity with increasing depth could be related to decreasing seed density with depth. This result is in accordance with previous studies in terrestrial habitats (e.g. Bossuyt & Hermy 2004). However, the similarity between seed bank and above-ground vegetation in the three salt-marshes was different. The lower similarity in the Zwin and IJzermonding (old) was mainly caused by the dominance of *Halimione portulacoides* and *Elymus athericus* in the vegetation, species that were never found in the seed bank.

Seed density in Saeftinghe and the Zwin was significantly higher than at the IJzermonding. *Juncus bufonius* had the highest seed density in the greenhouse and the highest density of emerging seeds were found in samples from Saeftinghe and the Zwin, indicating that the high seed density is probably due to the high abundance of *Juncus bufonius*.

Juncus bufonius was the most abundant species in the seed bank. This species has been reported as dominant representative in the seed bank of many, environmentally quite diverse terrestrial ecosystems in and outside Europe (e.g. in alkaline marsh vegetation: Vyvey 1983; as an non-indigenous species in temperate rain forest: Sem et al. 1996; coastal subarctic ecosystems: Staniforth et al. 1998; forest: Devlaeminck et al. 2005; dry acidic dune grasslands: Bossuyt et al. 2007); it is one of the most abundant seed bank species in Baltic seashore meadows (Erikkila & Heli 1998). In a Mediterranean salt-marsh *Juncus bufonius* produced a large number of seeds that persist in the soil seed bank with a mean density of 5083 seeds/m² at a depth of 0-10cm (Marañón 1998). This species is 17th in frequency appearance in NW European studies on seed banks (Thompson et al. 1997). *Juncus bufonius* is a weedy, widely distributed and low salt tolerant annual species (the Ellenberg index for salinity is 0; the Ellenberg index for salinity for the closely related, hard to distinguish *Juncus ambiguus* is 4; on the salt-marshes we might be dealing with the latter species in most cases;

seedlings of both can not be distinguished though). Probably, salinity prevents the appearance of this species in the vegetation (Marañón 1998). This is apparently also the case for the other species only sparsely present in the standing vegetation but abundant in the seed bank (*Atriplex littoralis, Chenopodium rubrum* and *Juncus gerardii*). When seeds of species of low to moderate salt tolerance are dispersed into a region of high salinity, the salt concentration of the soil will prevent the germination of the seeds and hence their establishment in the vegetation (Shumway & Bertness 1992). Therefore, seeds of *Juncus bufonius* can remain viable in the soil and seed density may increase in time. After washing the soil by fresh water in the laboratory and watering by fresh water in the greenhouse, this species appears abundantly.

The three investigated seed banks of old salt-marshes do not fundamentally differ from each other. The mesohaline has the same vertical profile as both euhaline seed banks. In all three the dominant salt-marsh plant seeds are of annuals; these of perennials are largely lacking.

6.6 Conclusion

The age of salt-marsh could affect on density and composition of seeds buried in soil. We have provided evidence that seed bank of young as well as old salt-marsh is composed of seeds of new colonizers (cf. hypothesis 1). This explains the very low similarity between seed bank and above-ground vegetation in that habitat, particularly in its late successional stage. This implies that restoration is possible from a long-term persistent seed bank if the target species would be pioneer colonizers. Late successional species largely need to colonize from (often sparsely present; see chapter 2) seed sources in surrounding salt-marshes or by vegetative propagules, such as rhizome parts.

In salt-marsh habitats, salinity is not able to change (or inverse) the monotonically distribution of seeds along depth. The characteristics of the seed bank of the mesohaline salt-marsh were found not to be fundamentally different from that of both euhaline salt-marshes (cf. hypothesis 2).