

# The assembly of a saltmarsh ecosystem

THE INTERPLAY OF GREEN AND BROWN FOOD WEBS

Maarten Schrama



rijksuniversiteit  
 groningen



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RIJKSUNIVERSITEIT GRONINGEN

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## THE INTERPLAY OF GREEN AND BROWN FOOD WEBS

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A black and white photograph of a coastal landscape. A winding, muddy path or stream flows through a flat, marshy area. The path starts from the bottom right and curves towards the left, eventually disappearing into the distance. The ground is uneven and appears to be covered in mud or sand. In the background, a flat horizon line separates the land from a sky filled with large, fluffy clouds. The overall scene is desolate and expansive.

# *Chapter 1*

# 1

## **General introduction**

Maarten Schrama

## General Introduction

Understanding species interactions constitutes a central topic in ecology. Ecological succession is one of the most powerful ecological processes in which species interactions are played out over time. In the past, research into succession has merely concentrated on primary producers and associated changes in soil nutrients (Clements 1916; Olson 1958b; Tilman 1982; Miles & Walton 1993; Tilman & Pacala 1993). More recently, the focus has shifted towards understanding ecosystem dynamics at multiple trophic levels and how their respective roles change over the course of vegetation succession (e.g. Van de Koppel *et al.* 1996; Van Der Wal *et al.* 2000a; Milner *et al.* 2007). Rather than perceiving succession as one ‘straightforward idea’ with plants playing the pivotal role (Clements 1916), ecological succession should be perceived as the result of complex processes at the ecosystem level. These involve interactions between numerous different species at various trophic levels and associated changes in abiotic factors (*sensu* Connell & Slatyer 1977). A more detailed overview of how food web ecology and vegetation succession ecology are now conceptually merging is provided in Box 1. Put differently, investigating successional dynamics can inform us about how communities assemble. This research can also tell us which trophic levels play decisive roles, and which abiotic factors are driving species changes (Odum 1969; Connell & Slatyer 1977). This does not necessarily conflict with the idea that there is a considerable degree of directionality in many successional series. But the challenge is to go beyond describing the changes in plant species composition and instead provide a more integrated view on the interplay of biotic and abiotic factors that yield different food web configurations. Chronosequences, as found for instance at retreating glaciers and after volcanic eruptions form a powerful tool for studying these long-term changes (e.g. Lindroth 1973; Whittaker *et al.* 1989; Edwards & Sugg 1993; Chapin *et al.* 1994; Kaufmann 2001; Hodkinson *et al.* 2004; Wardle *et al.* 2004b), but also chronosequences in secondary successions have intensively studied for this purpose (Payne 1965; Tilman 1988; Brown & Gange 1992; Scheu & Schulz 1996; Kardol *et al.* 2006).

In order to achieve a more integrated overview of the important processes and mechanisms that influence succession, a number of interesting avenues have been proposed. Firstly, even in the most remote successions, herbivore and carnivore species are already present in early succession (Sugg & Edwards 1998; Hodkinson *et al.* 2001; Kaufmann 2001), conflicting with the idea that plants alone initiate and drive the dynamics of early succession. Secondly, keystone interactions with selective herbivores can modify the course of succession (Van Der Wal *et al.* 2000a; Kuijper & Bakker 2005).



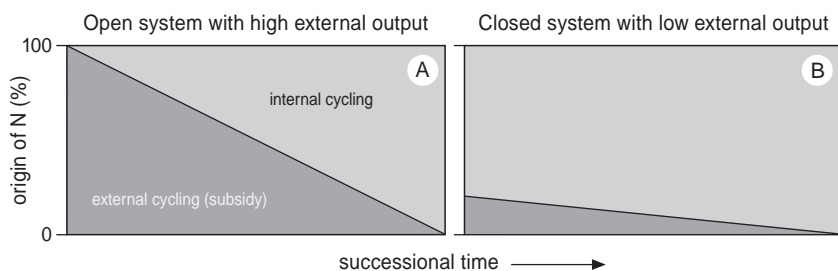
Thirdly, changes in aboveground food web components are not necessarily reflected in belowground food webs (Brown & Ewel 1987; Brown & Gange 1989; Scheu & Schulz 1996). So, an integrated analysis of the changes in ecosystem configuration during ecological succession is required to generate a more unified view on the role of the different food web components and their interplay with plants and abiotics. However, an integrated analysis of vegetation dynamics, ecosystem changes and above and below-ground food web and species diversity changes and (herbivores, carnivores, decomposers) in the different stages of succession has rarely been performed. This is likely caused by specialization of researchers on specific groups, and the general challenging nature of the task due to the massive number of species involved in many ecosystems. However, in some systems it may be possible, specifically in more extreme habitats with lower numbers of species in different groups. The principal aim of this thesis is therefore to study ecosystem assembly during succession in an integrated way, using a well-described salt marsh chronosequence on the island of Schiermonnikoog.

## **The role of external nutrient input during early succession**

In many ecosystems, a considerable part of the incoming energy and nutrients is derived from external resources. For instance, on desert islands in the Baja California, Mexico, most of the nutrients that enter into the ecosystem originate from the ocean (Stapp & Polis 2003). Other islands in oceanic regions profit greatly from guano input by nesting seabirds (Maron *et al.* 2006). Inland ecosystems in the arctic regions in Canada, receive large amounts of nutrients from dead and decaying salmon (Naiman *et al.* 2002; Yixin *et al.* 2003). And in many estuaries rivers are a the dominant inputs of nutrients and energy (Heip *et al.* 1995).

As successions are often characterized by nutrient limitation (Marrs & Bradshaw 1993), external nutrient input may play a major role in ‘subsidizing’ ecosystem development, especially during early stages of succession. Some authors have shown that early successional habitats receive large amounts of nutrients in the form of insect fallout (Sugg & Edwards 1998; Hodkinson *et al.* 2001), while others put a lot of emphasis on N-fixation by cyanobacteria and legumes during early succession (Crocker & Major 1955; del Moral 1993; Sprent 1993; Walker 1993).

Obviously, many ecosystems receive external nutrients, but to what extent *these* nutrients actually ‘fuel’ early vegetation succession and food web assembly is unknown, i.e., do they play a prime role in initiating and driving initial ecological dynamics?. While glacial primary successions and

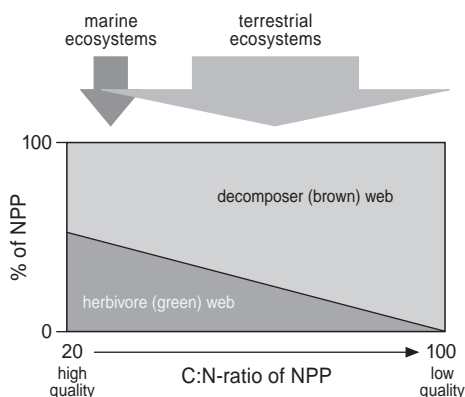


**Figure 1.1** Hypothetical origins of mineral nitrogen content along a successional sequence in relatively ‘open’ ecosystems such as salt marshes and dunes, and relatively ‘closed’ ecosystems such as glaciers and volcanoes. Along succession the importance of these external inputs diminishes in both systems, but the relative importance is higher in ‘open’ ecosystems.

volcanoes are often located in isolated landscapes, consequently receiving less nutrients from external sources (Whittaker *et al.* 1989; Figure 1.1A, Chapin *et al.* 1994; but see Hodkinson *et al.* 2001), early terrestrial successions of regularly flooded, coastal areas receive large amounts of external nutrient input. Although different successional habitats may differ in this respect and one successional ecosystem may be more ‘open’ to nutrient exchange than other systems, I hypothesize that external input can be important in ‘fuelling’, or initiating early succession, see Figure 1.1. Furthermore, I hypothesize that along a primary successional sequence, external input becomes relatively less important compared to internal production due to nutrient build-up. While others have also highlighted the importance of external nutrient input for ecosystem development (Hodkinson *et al.* 2001; Milner *et al.* 2007), no quantification has yet been made on the relative importance of these inputs to the different parts of the food web in the different stages of succession.

## Changes in food web configuration along successional food webs

While some ecosystems are herbivore dominated, others are clearly detritivore-dominated (Cebrian & Lartigue 2004; Moore *et al.* 2004; Shurin *et al.* 2006; Kaspari & Yanoviak 2009). These two configurations represent the two main pathways for the fate of primary production in ecosystems: the “green route”; from plants to herbivores and their respective predators, and the “brown route”; from plants to detritus, to detritivores and their respective predators. The balance between these two components has been argued to



**Figure 1.2** How average C:N-ratio of primary production affects large-scale food web configurations in marine and terrestrial ecosystems. Depending on its quality, primary production either gets processed through the ‘green’ (plant-herbivore-based) food web or through the brown (detritus-detritivore-dominated) food web. Marine systems are characterized by high quality production of organic matter, of which an (almost) equal proportion is taken up by the both parts of the food web, while terrestrial ecosystems are often characterized by much lower quality of organic matter, resulting in a brown-web-based food web. Such large-scale changes in food web configuration are also expected along successional sequences where vegetation quality decreases. After: Cebrian *et al.* 2004, Shurin *et al.* 2006.

strongly depend on the C:N-ratio of the primary production (Cebrian 1999). Food webs in ecosystems with very high plant quality (low C:N-ratio), such as in aquatic environments, appear to have similar sized green and brown web components, while food webs in ecosystems with low quality primary production (high C:N; peatbogs, spruce forests) have relatively small green web components (Figure 1.2; Cebrian & Lartigue 2004).

The quality of primary production (C:N, but also C:P-ratios of plant material) generally decreases over the course of succession (Odum 1969). Early successional plant communities are often characterized by annual species and algae with low cellulose and lignin content, while late succession stages are often characterized by larger amounts of structural material and lignin-rich biomass (Marrs & Bradshaw 1993), making it of lower quality to herbivores and decomposers. It can therefore be expected that these changes are reflected in a shift in food web configuration: from a more herbivore-dominated (green) web in early succession towards a more decomposer-dominated food web in late succession (Figure 1.2). Deviations from this general pattern can be expected in both early and late successional stages. If early successional stages receive a lot of external, high quality (dead) organic matter, a

fully decomposer-dominated (brown) food web may still be expected (*sensu* Polis & Hurd 1995), as the organic matter cannot be consumed by herbivores. Another deviation from this pattern may be expected in the later stages of succession, if internal (e.g., large herbivores) or external forces (e.g., fire) prevents a structural decrease of the quality of primary production. In other words, the recurrent influence of either of such internal or external disturbances may prevent the colonization of shrubs and trees, persistence of high quality vegetation and an overall maintenance of both the green and brown routes within the food web (McNaughton 1984; Holdo *et al.* 2009).

So, while studies of food web organization have revealed some very general predictions on the configuration of food webs between biomes and how these are driven by vegetation quality (Cebrian & Lartigue 2004; Shurin *et al.* 2006), it is remarkable that no research has been devoted to the large scale changes in food web configuration along a gradient of primary succession, *within* one ecosystem.

I hypothesize that in one ecosystem, there are at least two distinguishable, clear changes in brown/green food web configuration along a successional gradient. Firstly, I expect that along succession within one ecosystem, organic matter in early stages is processed via both the green and brown routes in the food web, but becomes almost entirely processed through the brown web towards late succession. Secondly, I expect that in the very first (unvegetated) stage of succession, regardless of its quality, most organic material is processed through the brown web. In order to gain a deeper understanding of how food web organization is shaped along gradients of succession, these hypotheses deserve further investigation.

## Diversity changes over succession

Since the last century, the rate of species extinctions has swiftly accelerated, mainly due to human interference (Ozinga *et al.* 2009; Steffen *et al.* 2011). Unfortunately, we still do not fully understand how biodiversity is regulated or maintained in natural communities. One of the major difficulties for understanding biodiversity is the inherent dynamic character of ecosystems, which results in extensive variation in species richness in time and space. The dynamical nature of ecosystems not only leads to great practical difficulties for researchers who attempt to understanding diversity, it may also be the very driver of diversity in natural ecosystems (Huisman & Weissing 1999). And because ecosystems are rarely in a stable state, the study of sufficient spatial and temporal variation is required to obtain fundamental comprehension of the important processes that drive species diversity.

Studying species diversity changes along gradients of productivity can aid to this fundamental comprehension. Along these gradients, especially along chronosequences, many different points in time and spaces can be used to provide an integrated view of long-term changes in diversity and the potentially important drivers within a relatively short time span of investigation. From the paradigm of Clements (1916), stating that plants are setting the stage for other species along successional sequences, it would logically follow that diversity of other species should follow similar patterns. Evidence from a number of studies supports this hypothesis (Brown & Southwood 1983; Knops *et al.* 1999; Siemann *et al.* 1999b; Symstad *et al.* 2000), although different patterns are found for other trophic levels or taxa. Especially for belowground organisms or detritivores (Payne 1965; Scheu & Schulz 1996), microorganisms (Ohtonen *et al.* 1999) or carnivores (Dobel *et al.* 1990), plant species richness did not explain much of the variation in diversity. At least in these trophic groups, vegetation structure, diversity in other trophic groups and soil litter stratification may be much more important for understanding species diversity such trophic groups. The main determinants of diversity thus differ considerably between different trophic groups (Hawkins & Porter 2003; Berg & Bengtsson 2007; Rickert *et al.* 2012).

Some studies have approached this problem from an experimental angle (Knops *et al.* 1999; Siemann *et al.* 1999b; Symstad *et al.* 2000), by determining arthropod richness in response to an a priori determined higher or lower plant species diversity, while controlling for all other variables. Along natural ecological and successional gradients, such other variables (vegetation structure, litter layer thickness) will be inevitable linked to plant species diversity and are inherently difficult to separate out. For instance, a high vertical structure and structured litter layer, which results in for instance high spider and macro-detritivore diversity (Uetz 1979; Dobel *et al.* 1990) often coincides with low plant species diversity.

Given the many contradicting findings for different trophic groups, a fundamental investigation of changes in diversity within different trophic groups along a full successional sequence is needed. This is expected to yield fundamental understanding of 1) the driving environmental variables that underly diversity in a range of trophic groups and 2) how diversity in one trophic group acts on diversity in other trophic groups.

## **Keystone Interactions during ecological succession**

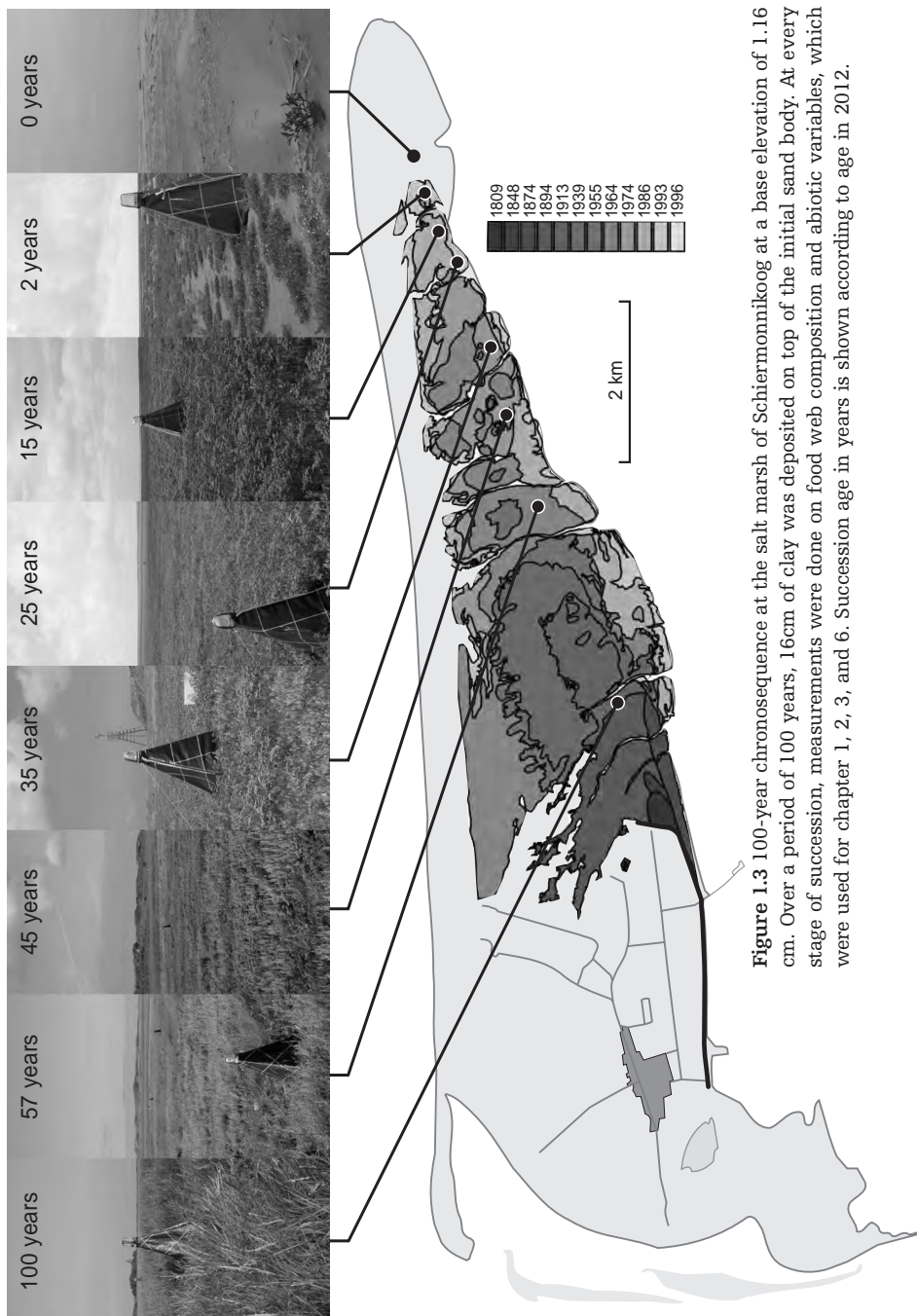
It has long been recognized that some species have a greater influence on community organization and ecosystem functioning than others, either

because of their biomass contribution (dominant species) or because they have a disproportionate effect of relative to their size (keystone species, Paine 1969; Power *et al.* 1996). Large herbivores for instance have been found to create a positive feedback on their own food quality by promoting fast growing palatable grasses (McNaughton 1984; Georgiadis *et al.* 1989; McNaughton *et al.* 1997a). Similarly, moose grazing on Isle Royale have been shown to lead to a negative feedback on soil fertility by promotion the dominance of slow growing low-quality conifers over deciduous trees (Pastor *et al.* 1993). Examples of belowground species having disproportionate impacts on ecosystem properties have been documented in places where earthworms were introduced. When in New Zealand European earthworm species were introduced, the soil nitrogen mineralization was increased with 10-30% and large shifts in plant and soil fauna were observed (Stockdill 1982; Yeates 1991). Interactions between animals and vegetation succession can be expected to play an important role in driving ecological succession, both above and belowground. So, while most studies have focused on the role of plants along a gradient of succession, an in-depth research on the interactions between dominant animals, both above and belowground and vegetation succession is lacking at the moment.

## Study system

The research reported in this thesis was performed on a back-barrier salt marsh on the island of Schiermonnikoog (NL). This salt marsh is one of the largest and best preserved natural salt marshes in Western Europe. From the west to the east, a complete range of salt marsh succession stages, a chronosequence, is found (Olff *et al.* 1997). This chronosequence encompasses more than 100 year of salt marsh accretion, thus presenting 'a natural laboratory' (Figure 1.3). It makes the area highly suitable for studying longer-term ecosystem dynamics within the timespan of a PhD project. At present, the new successional stages are still found on the east side, where the salt marsh is still extending. This happens because the main currents and winds are directed west-east and thus fresh sandy sediment is deposited on the eastern side of the island.

On top of the underlying sandflats, nutrient-rich sediment accumulates (Olff *et al.* 1997), of which the mineral fraction contains fractions of clay (18%), silt (30%) and sand (52%) (De Groot 2009). This mainly originates from the surrounding marine ecosystems and is associated with the establishment of plants. Sediment accumulation happens with a rate of 0.2–1.2 cm yearly, which declines towards late succession (Olff *et al.* 1997).



**Figure 1.3** 100-year chronosequence at the salt marsh of Schiermonnikoog at a base elevation of 1.16 cm. Over a period of 100 years, 16cm of clay was deposited on top of the initial sand body. At every stage of succession, measurements were done on food web composition and abiotic variables, which were used for chapter 1, 2, 3, and 6. Succession age in years is shown according to age in 2012.





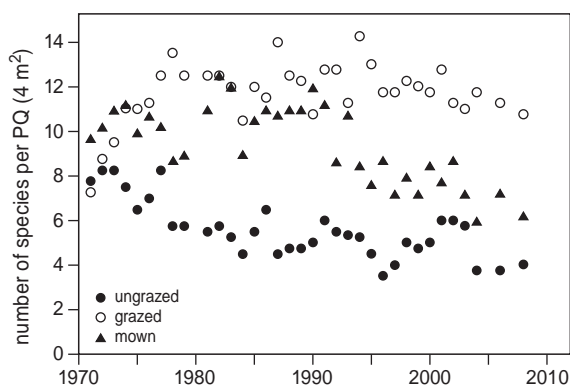
**Figure 1.4** Cattle enclosure (since 1973) on the cattle grazed marsh of Schiermonnikoog. Outside the enclosure, species-rich vegetation is present. Inside the fence, vegetation is species-poor and dominated by *Elytrigia atherica*. The fence is electrified between May and November. A mown treatment can be seen in the lower right corner.

In early successional stages, salt marshes become vegetated with a halophytic plant community. Intermediate successional stages on the salt marsh have high local plant diversity of about 10–12 species per square meter. Most species are dispersed by water and wind, which results in on average low dispersal limitations. This simplifies the analyses of the patterns in species richness, as the colonization/competition trade-off plays a less important role (Tilman 1988; Olff *et al.* 1997). Towards late succession, diversity of plants declines to 3–4 plant species per square meter (Van Der Wal *et al.* 2000a). Due to the multitude of stress factors (inundations, salt-stress, etc), a relatively simple (but well-adapted) animal species community is present (Cheng 1976).

### Role of small herbivores

European hares (*Lepus europeus*) have been shown to play an important role in maintaining species rich early successions (Kuijper & Bakker 2005), because they prevent light competition by preferentially feeding on late successional grass species like *Festuca rubra*, and *Elytrigia atherica* in the summer season and the small shrub *Atriplex portulacoides* (potential climax species on low marsh) in winter. Grazing these plant species by hares slows down succession with 30–40 years (Kuijper & Bakker 2005), but as soils





**Figure 1.5** Average species richness in grazed, mown and ungrazed plots on the 150 year-old salt marsh. Mown plots are hand-mown twice yearly; cattle grazed plots are grazed from May until November at a stocking rate between 0.5 – 1 cow ha<sup>-1</sup>.

become richer during later succession, declining forage palatability of the increasingly taller vegetation is thought to force hares to move to different areas (Van de Koppel *et al.* 1996; Huisman & Olff 1998). Interestingly, hare grazing prevents an increase in nitrogen mineralization while maintaining a high-quality vegetation (van Wijnen *et al.* 1999), thus contrasting with the paradigm that grazing on fertile soils increases nitrogen mineralization and availability (McNaughton 1984; Hobbs 1996; Pastor *et al.* 2006).

### Cattle grazing on old parts of the salt marsh

The oldest parts of the salt marsh (100-200 years) are grazed by cattle in summer, where a fence across the island prevents their access to earlier successional stages. Cattle grazing is used as a management tool to prevent the encroachment of late successional tall grass species (Figure 1.4). Low-density cattle grazing has resulted in the formation of local grazing lawns with a species rich plant community (Figure 1.5, Bos *et al.* 2002). Geese and hare also utilize these lawns; cattle grazing thus facilitates their return in the successional system (Bos *et al.* 2002).

### Outline of this thesis

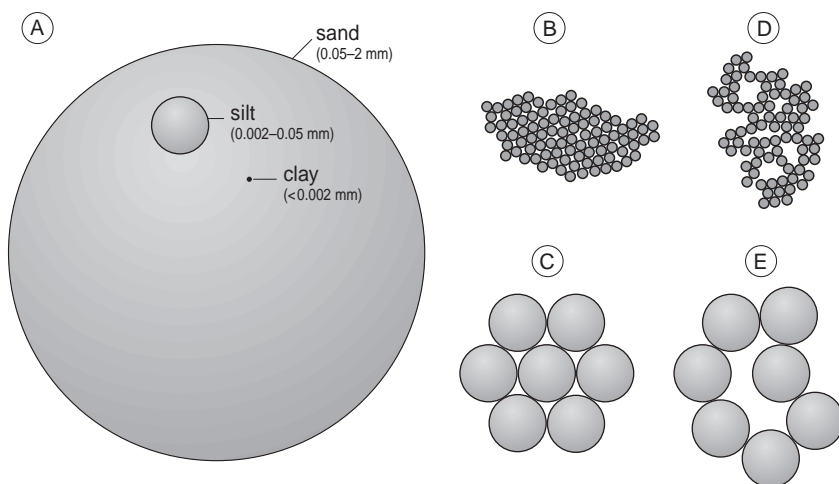
I will start with an analysis of the changes in the main trophic compartments of the food web, in the various stages of salt marsh succession (Chapter 2). As I expect strong changes in external nutrient input and changes in vege-

tation quality, I focus on changes in total biomass of the decomposer-dominated (brown) web and the herbivore-dominated (green) web. In chapter 3 I will investigate the hypothesis that early successional stages are more subsidized by external nutrients and organic matter than later stages of succession, using a stable isotope approach (Chapter 3). With this approach, external (marine) carbon sources can be separated from internally cycling carbon sources, while nitrogen isotopes can give information about changes in trophic structure over succession (Post 2002, also see box 2).

To gain more insight in the dynamics of species richness in the brown and green part of the food web along a successional sequence, I explored the following two alternative hypotheses in Chapter 3: 1) species richness dynamics in the green and brown web will be positively correlated as they covary simultaneously with the main drivers in ecosystem processes or, 2) they are uncoupled as they are subject to independent drivers and insensitive to each other.

In chapter 5 and 6 I explore an interesting situation where keystone species in the green and brown web affect the dynamics of succession. By operating in opposing ways on soil abiotics, they strongly affect plant and animal species composition. Specifically, I hypothesize that bioturbating fauna in late succession increases oxygen diffusion into the soil (Chapter 5), which is expected to result in higher N mineralization and therefore to act on the competitive balance between plants. In many terrestrial ecosystems, earthworms (mainly *Lumbricus* spp.) play an important role in the carbon cycle (Lee 1985; Lavelle 1988; Edwards & Bohlen 1996). By means of continuous litter processing, they create a layer of 'vegetable mould' which not only causes gradual burial of Greek Tempels (Darwin 1890), but also results in a speeding up of nutrient mineralization and soil aeration (Lavelle 1988; Lavelle 1997). Interestingly, the behavior of termites is very similar in this respect. Their influence on the decomposition process (especially of the fungus-growing termites Macrotermitinae) has a great influence on the local rates of nutrient mineralization, and their digging activities also cause rocks to sink into deeper soil layers and results in a fine textured upper soil layer (Bignell & Eggleton 2000). In soft-bottom marine ecosystems, actions of lugworms (*Arenicola* spp.) also result in 'bioturbation' of the soil. Their constant processing of soil results in a filtering out of smaller soil particles, subsequently leading to a sandy soil and a hard shell layer at 25 cm deep (Volkenborn *et al.* 2007; Volkenborn *et al.* 2009).

Interestingly, both 'ecosystem engineering' groups of Annelidae and Macrotermitinae are absent in salt marshes. I hypothesize that their functional role is replaced by *Orchestia gammarellus*, an amphipod species adapted to marine conditions (Chapter 5). In order to prevent desiccation,



**Figure 1.6** Relative soil particle sizes and effects of compaction on different soil textures. Sand has a soil particle diameter of 25–1000 larger than clay (A). When clay is compacted (B), a much lower diffusion of nutrients and oxygen can be expected than when silt or sand is compacted (C), because the space between the particles is much more reduced in fine textured soils. Similarly, bioturbation activity by a similar size animal can be expected to result in a greater increase in porosity in a fine-textured soil (D) than in a coarse textured soil (E).

this ‘earthworm of the saltmarsh’ can create its own little burrows (Moore & Francis 1986). I argue that both the digging and litter fragmenting (decomposing) behavior have strong influence on the competitive balance between early and late successional plant species.

Large grazers are hypothesized to exert an opposite force on the system by causing ‘biocompaction’ instead of bioturbation. I hypothesized that they decrease oxygen diffusion into the soil by means of trampling induced soil compaction. Thus N mineralization is predicted to decrease, which once more results in a shift in the competitive balance between plant species, but is also hypothesized to (negatively) affect soil fauna by a reduced pore space (Chapter 6). In chapter 7, I predict that this effect occurs in a variety of ecosystems, but the strength of the effect is expected to depend on soil texture and soil moisture. Due to the larger grain size, sand can be expected to be much less compactable than clay and silt (Figure 1.6). I therefore hypothesize that the negative effect of large herbivores on N mineralization only operates in compactable, fine textured clay-rich soils both in extremely wet and extremely dry systems and to a much lesser extent in coarse textured sandy soils (Chapter 7).

The effect of soil compaction on soil aeration need not necessarily be exerted by large herbivores. In chapter 8 I hypothesize that soil compaction by mowing machines in the managed grasslands of the Drentse Aa valley can also exert a strong influence on vegetation composition through similar mechanisms.

In chapter 9, I aim at synthesizing the various insights from this thesis. I first consider the findings from earlier chapters in the context of the process of ecosystem assembly. Then I combine these in an overall framework in which the important changes in food web configuration along a successional sequence are put together.

### **BOX 1: Vegetation succession as a driving force behind food web assembly?**

The view that interactions between primary producers are the main driving force of vegetation succession and ecosystem development has a long history (Clements 1916; Olson 1958; Tilman 1988; Miles & Walton 1993). In some systems (old fields) the trade-off between plant colonization and competition has been argued to be the driving force in the composition of a plant community (Tilman 1988). Plant species that are good colonizers appear to be poor in competing for nutrients, so they are in time outcompeted by plants that are later to arrive but better in extracting nutrients from the soil. Also, it has been shown that vegetation succession results in modification of the abiotic environment, specifically the accumulation of nutrients in the soil (Crocker & Major 1955; Olff *et al.* 1993) and the biotic environment, the accumulation of pathogens (Van der Putten *et al.* 2001; Kardol & Wardle 2010) and development of mutualistic interactions (van der Heijden *et al.* 2008). As a result, changes in vegetation composition, and subsequently in organic matter accumulation, soil fertility and microclimate are thought to drive changes in the animal diversity. In this view, plants provide food, safety from predators and buffers against climatic conditions (Horn 1974; Berg & Bengtsson 2007).

Food web ecology specifically aims at understanding trophic complexity, but has been developed relatively independently from work on vegetation succession, ever since the pioneering work by Elton (1927), Lindeman (1942), Hairston *et al.* (1960) and May (1973). In most food web studies, plants species are viewed as just one of the

many trophic groups that interact with other trophic groups. Many food web concepts, such as trophic diversity (Banasek-Richter *et al.* 2009), structural complexity (May 1973) connectedness and robustness (Pimm 1982; Williams & Martinez 2000; Dunne *et al.* 2002; Bascompte 2007; Petchey *et al.* 2009), and compartmentalization (Moore & Hunt 1988; Neutel *et al.* 2002; Neutel *et al.* 2007; Rezende *et al.* 2009) seem highly relevant at first sight for understanding ecosystem functioning in general, and therefore also for the understanding of ecosystem succession. However, most of these concepts have been studied only in modeling approaches and/or in ecosystems that are assumed to be in equilibrium (e.g. Moore & Hunt 1988; de Ruiter *et al.* 1998; Berg & Bengtsson 2007). Equilibrium assumptions by definition do not hold in ecosystems in which vegetation succession takes place, which is a very common phenomenon. Moreover, various studies have stressed the importance of higher trophic levels in stabilizing community structure, especially made in the case of detritivores in brown webs (de Ruiter *et al.* 1995; Moore *et al.* 2004) and herbivores in green webs (Olf & Ritchie 1998; Owen-Smith & Mills 2008; Hopcraft *et al.* 2010) but links to the dynamics of whole food webs are yet poor.

The few available studies that do focus on changes in main food web compartments along successional sequences find a progressive shift towards a more fungal-dominated soil food web (Ohtonen *et al.* 1999), and a clear order of arrival of trophic groups (Kaufmann 2001). Two other studies on just the brown web at a relatively high level of taxonomic aggregation have suggested that compartmentalization (sets of highly interacting groups) plays an important role in successional food webs (Neutel *et al.* 2002; Neutel *et al.* 2007). However, these studies lack the integrated perspective on how the different trophic groups in the green and brown web are interacting with each other along succession and/or even control or drive succession.

## **BOX 2: Stable isotopes in ecological research**

During the last decades, flows of nutrients across ecosystem boundaries have become increasingly studied (Polis *et al.* 1997). Stable isotopes proved to be a powerful tool for this in providing researchers

with a method that can discriminate between different food sources, different topographical origin of nutrients and structure and length of a trophic chain.

Most of the studies on food webs use stable isotopes of carbon ( $\delta^{13}\text{C}/\delta^{12}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}/\delta^{14}\text{N}$ ) and to a lesser extent sulfur ( $\delta^{34}\text{S}/\delta^{32}\text{S}$ ). Every organism consists of different isotopes of these elements, which may also differ considerably between tissues (Peterson & Fry 1987). In order to obtain information on diet, ratios of light and heavy stable isotopes of soft tissues (no bones) are compared to a standard reference. The heavy isotope is always the rare one. For  $\delta^{13}\text{C}/\delta^{12}\text{C}$ , PeeDee limestone is the reference, for  $\delta^{15}\text{N}/\delta^{14}\text{N}$  atmospheric nitrogen is the reference and for sulfur it is the Canyon Diablo meteorite (Peterson & Fry 1987).

In 1978 it was first shown that plant carbon had a 20‰ lower  $\delta^{13}\text{C}$  than atmospheric carbon dioxide (DeNiro & Epstein 1978). This indicates that metabolic processes, in this case photosynthesis, can have the tendency to use more of the light isotope. Another important finding was the difference between atmospheric  $\text{CO}_2$  (-7‰) and marine dissolved  $\text{HCO}_3$  (0‰), which is also reflected in organismal tissues. The  $\delta^{13}\text{C}$  of marine primary producers typically ranges between 19 – 24‰, compared around 28‰ for terrestrial primary producers (Williams & Gordon 1970).

Stable isotopes of nitrogen are especially useful to study the trophic position of species in food webs. In each trophic level  $\delta^{15}\text{N}$  accumulates in a more or less predictable amount of  $\delta^{15}\text{N}$ , which is usually set at 3.4‰ (Hobson & Welch 1992). This is due to the preferential excretion of the lighter isotope in nitrogen in urea and ureic acid.

Studies aiming at determining specific food sources and pathways of organic matter often make use of mixing models. Programs with specific software, such as IsoSource and Source/Step have been designed to deal with multiple sources and a number of different isotopes (Phillips & Gregg 2003; Lubetkin & Simenstad 2004). However, as they can only deal with a limited number of possible sources and diffraction constants, these programs are less useful for resolving complicated food web dynamics.



An aerial photograph of a coastal landscape. A winding river or estuary flows from the bottom center towards the top left, eventually meeting the ocean. The land is a mix of dark, textured areas (possibly marshes or forests) and lighter, sandy or silty areas. The ocean is visible in the top left, with a few small boats or structures on the horizon. The sky is a uniform light gray.

# *Chapter 2*



# 2

## Ecosystem assembly rules: the interplay of green and brown webs during salt marsh succession

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*Ecology, in press*

### Abstract

Current theories on vegetation succession and food web assembly are poorly compatible, as food webs are generally viewed to be static, and succession is usually analysed without the inclusion of higher trophic levels. In this study we present results from a detailed analysis of ecosystem assembly rules over a chronosequence of 100 years of salt marsh succession. First, using 13-year long observations on vegetation and soil parameters in different successional stages, we show that the space-for-time substitution is valid for this chronosequence. We then quantified biomass changes for all dominant invertebrate and vertebrate species, across all main trophic groups of plants and animals. All invertebrate and vertebrate species were assigned to a trophic group according to feeding preference and changes in trophic group abundance were quantified for seven different successional stages of the ecosystem. We find changes from a marine-fuelled, decomposer-based (brown) food web in early stages to a more terrestrial plant-based, herbivore-driven (green) food web in intermediate succession stages, and finally to a decomposer-based, terrestrial driven food web in the latest stages. Our results show that the structure and dynamics of salt marsh food webs cannot be understood except in the light of vegetation succession, and *vice versa*.

## Introduction

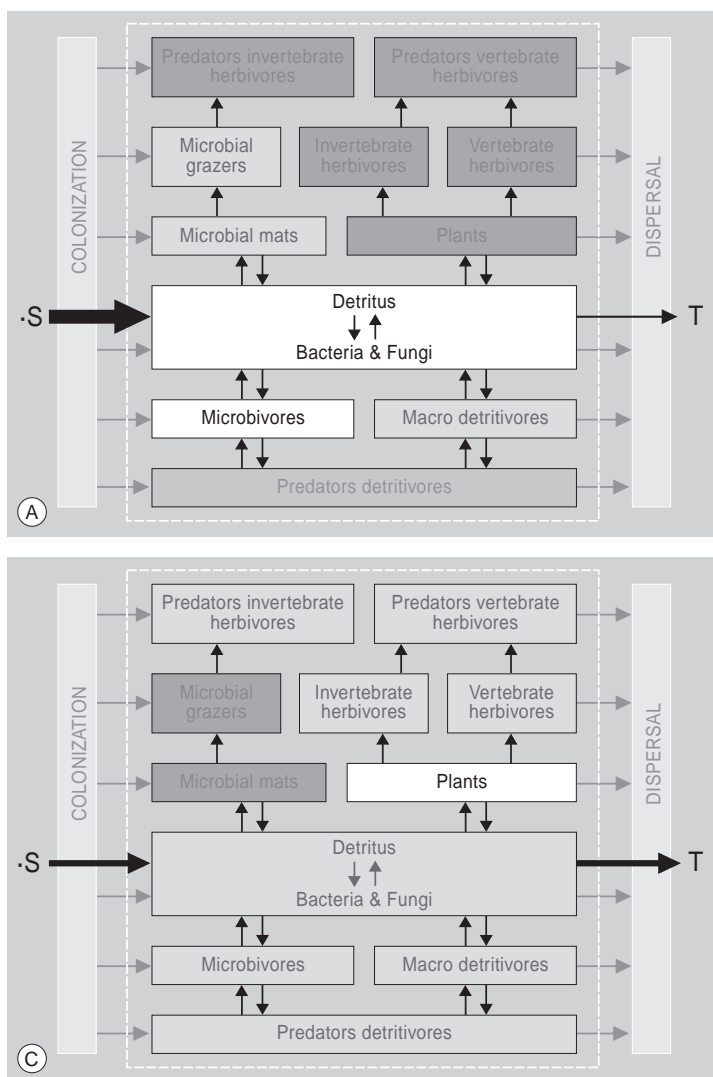
One of the central issues in ecology is how ecological forces shape communities of species that interact across multiple trophic levels. Studies of vegetation succession and food web assembly have generated many insights on this topic. Although quite separate, vegetation succession has generally been viewed as a linked plant- and soil-driven process in which the focus is on plant colonization, facilitation (soil modification), and interspecific competition/exclusion among plant species as driving forces, all within this one trophic level (Clements 1916; Miles & Walton 1993). More recently, this is combined with research on plant traits into the study of community assembly rules. Compositional changes in connected trophic levels (herbivores, predators, decomposers) in such successional ecosystems have long been assumed to be the derivative of such plant-driven succession (Connell & Slatyer 1977). Food web ecologists on the other hand have mostly viewed communities as static entities in which plants are just one of the trophic levels and the interplay among trophic levels will set the equilibrium community composition of each (Schoener 1983; de Ruiter *et al.* 1995; Berlow *et al.* 2004; Moore *et al.* 2004; Rooney *et al.* 2008). This means that current theories on vegetation succession and food web assembly are poorly compatible, which calls for studies that better integrate food web structure with the dynamics of vegetation succession (Schmitz *et al.* 2006). This requires new insights in how entire ecosystems assemble over time, not only communities at specific trophic levels. Specifically, the interplay of green (herbivore-based, with abundant predators of herbivores) and brown (detritivore-based, with abundant predators of detritivores) webs needs attention in this respect (Moore *et al.* 2004; De Deyn & Van der Putten 2005; van der Putten *et al.* 2009).

Several authors have raised objections to the strongly plant-centered view on community assembly and succession (Odum 1969; Connell & Slatyer 1977) and have argued for a more integrative approach that explicitly takes plants and higher trophic levels from both the 'green web' and 'brown web' into account (Connell & Slatyer 1977; Moore *et al.* 2004). Initial studies on multi-trophic community organization along gradients of primary productivity predict that herbivore densities first increase with more plant productivity and control plant standing biomass at the same level (green web interactions), until predation (Oksanen *et al.* 1981) or plant quality (Van de Koppel *et al.* 1996) limits further growth of the herbivore population. Other studies have focused more on the interactions between plants, organic matter production and decomposer densities (brown web interactions; Berendse *et al.* 1994; Moore *et al.* 2004; Wardle *et al.* 2004b; Berg & Bengtsson 2007).

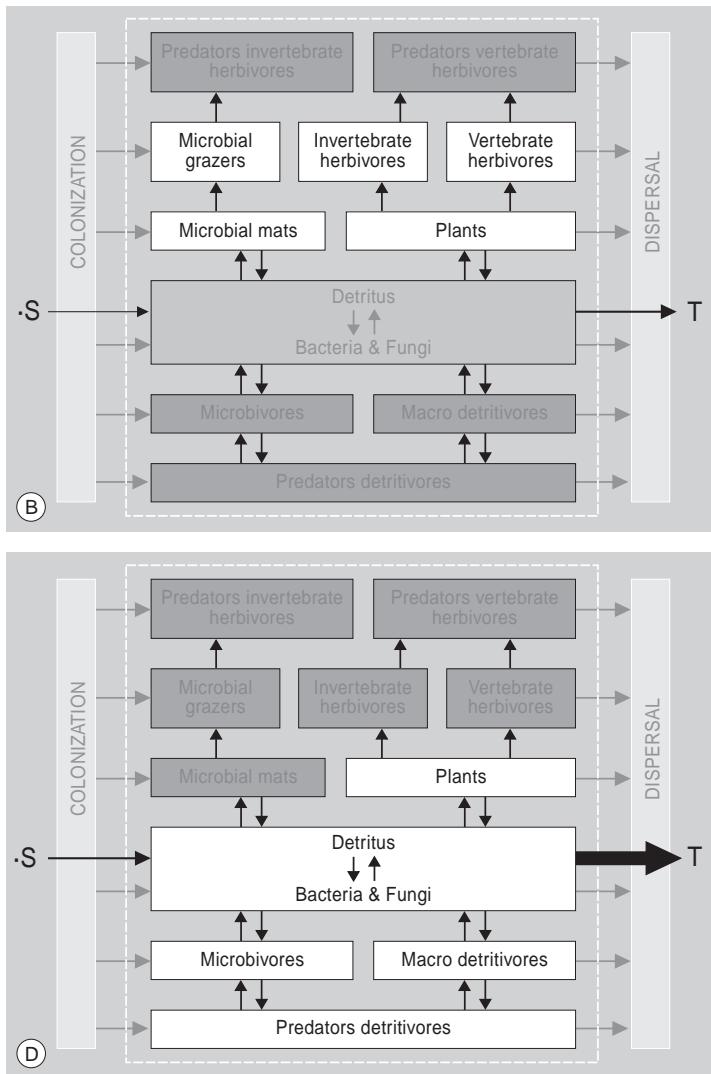
However, in some of the most intensively studied successions, plants were found to play a minor role in the first stages of succession (Edwards & Sugg 1993; Kaufmann 2001; Hodkinson *et al.* 2004) or were not even present at all (Payne 1965; Polis & Hurd 1995; Hodkinson *et al.* 2001). This raises general questions about the extent to which the green and the brown food web determine the onset and outcome of succession, and how the importance of these food web components changes over the course of primary succession.

The challenge then is to show how the interplay between vegetation succession and food web ecology leads to ecosystem assembly over time. In particular, can we identify 'ecosystem assembly rules', based on variation in average traits among trophic groups, that govern ecosystem development over time? Thereby, we expand on the current agenda of 'community assembly rules', which are based on variation in traits among species *within* trophic groups (Levin *et al.* 2001; Mutshinda *et al.* 2009). As mentioned above, many have stressed the importance of the interactions between detritivores and herbivores in shaping the trophic structure of ecosystems, but how both the brown web (detritivore-based web, *sensu* Moore *et al.* (2004)) and the 'green (herbivore-based) web exactly interact over succession is still poorly understood. This requires an integrated approach in which also the temporal component is taken into account (Bardgett *et al.* 2005). As the long-term dynamics in this type of long-term ecosystem assembly are poorly known, good descriptions in validated chronosequences are needed first. These can then form the basis for theoretical and experimental work to further explain the observed patterns.

Ecosystems can persist in alternative food web configurations, characterized by the number and identities of important pools and fluxes, their relative sizes and the connections among them (Shurin *et al.* 2006). In Figure 2.1 we suggest four typical food web configurations that may be observed both in different ecosystems and in different successional stages of the same ecosystem. The first theoretically possible configuration (Figure 2.1A) is dominated by the brown web, and thrives on input of organic material (S), as e.g. observed in coastal desert by Polis and Strong (1996). The second possible state (Figure 2.1B) is characterized by a self-sustaining small green web and a small detritus pool with high turnover and low inputs, as e.g. observed in early stages of sand dune succession by Olff *et al.* (1993). The third state (Figure 2.1C) can be characterized by green and brown webs that are both well-developed, and which 'compete' with each other for the available nutrients, as e.g. observed in grazing ecosystems (Cebrian & Lartigue 2004) small external input of subsidy as well as a small internal output of material is often present in this state. The last state (Figure 2.1D) is fundamentally different from Figure 2.1A as it is characterized as a large, but low quality



**Figure 2.1** Alternative main food web configurations between ecosystems, or successional stages within an ecosystem, with the differences between configurations determined by the number and identities of important pools and fluxes, and their relative sizes. The upper seven compartments within each subfigure represent the green web while the lower five compartments represent the brown web. Food web compartments with a higher biomass and flux are indicated with a lighter shade. During primary succession, either configuration (A) or (B) can be a precursor for (C) after which (D) represents the last stage. In configuration (A) primary production is very low but with a high external input of dead organic matter (S), which is depicted as a thick arrow pointing



into the box 'Detritus'. As a result, brown web dominates the food web. Exports (T) from the ecosystem are very small, which is depicted by the thin arrow pointing out of the box 'Detritus'. Succession state (B) consists mostly of a green web, which is based on nutrients and energy locally captured by the vegetation. Imports and exports of organic matter from the ecosystem are small. In configuration (C) both an abundant green and brown web occur, in which all trophic groups are common, which mostly rely on locally produced primary production. In configuration (D) the majority of energy and nutrients produced by plants is used by the brown web, with a sizable amount of organic matter export.

plant production (high C:N ratio), which leads to a small green web and a large brown web, as e.g. observed in boreal forests (Hilli *et al.* 2010). In such situations, decomposition by fungi and bacteria of dead plant material is essential to facilitate higher trophic levels (macro-detritivores and their predators), as the quality of the undecomposed plant material is insufficient to support most herbivores. Not only may these different configurations represent different types of ecosystems, we propose that they can also represent different stages of ecosystem succession, thus revealing processes of ecosystem assembly.

In this study, a thorough documentation of the long-term succession of a salt marsh food web is presented as a first step in this research agenda on ecosystem assembly rules which links succession and food web structure, with equal attention to its green and brown parts, resulting in such ecosystem assembly rules. We chose a salt marsh ecosystem for this study because this habitat is characterized by a limited number of species that are all able to cope with the generic physical and chemical stress factors (like flooding, salinity, waterlogging, anaerobic conditions, H<sub>2</sub>S toxicity). We performed a complete analysis of the dynamics of all main trophic groups of both the green and brown web along a 100 year old salt marsh chronosequence. First, we quantified the biomass for each group in seven different successional stages. Second, we related the observed ecosystem assembly to the main environmental changes during succession.

We finish by providing future directions in the analysis of ecosystem assembly rules and the consequences it may have for general understanding of how 'lots of things are put together' in ecosystems, i.e., when organisms both modify their environment, and interact within and across trophic levels and among the green and brown part of food webs (*sensu* Olff *et al.* 2009).

## Methods

### Description of salt marsh chronosequence

Measurements on ecosystem assembly food web composition were performed on the salt marsh of the island of Schiermonnikoog (53°30' N, 6°10' E), the Netherlands, in July 2008. The average yearly temperature on the island is 10.2 degrees Celcius ( $\pm$  SD 0.72), average rainfall is 824 mm ( $\pm$  SD 149.1) (data from the Royal Netherlands Meteorological Instituut KNMI at [www.knmi.nl](http://www.knmi.nl)). On the salt marsh of Schiermonnikoog a chronosequence is present, which spans one hundred years of succession (Olff *et al.* 1997). The earliest stages are formed on the east side of the island, late succession stages are situated 8 kilometers to the west. Over succession, vegetation composi-

tion changes from a state with very sparse vegetation towards a species rich vegetation in intermediate succession stages and a tall grass monoculture in late succession stages (Olff *et al.* 1997). The justification of the space-for-time replacement of this succession was checked by monitoring permanent plots in different successional stages during the last 13 years. At locations of successional age 0, 14, 27, 37, 60 and 85 years (age in 1994, Olff *et al.* 1997), biotic and abiotic parameters were collected over a 13-year period. From these six locations, we analyzed data on sediment layer depth (to the nearest 0.5 cm) and changes in cover of three dominant plant species: *Plantago maritima* (an early successional species, highly preferred by geese (Stahl *et al.* 2006)), *Artemisia maritima* and *Elytrigia atherica* (species of the intermediate and late succession, respectively (Olff *et al.* 1997)).

### Sampling design

Seven successional stages were identified, estimated as 0, 10, 25, 35, 45, 55 and 100 years of developmental age in 2008. More information on vegetation composition in the different successional stages can be found in the (Appendix I, Table 1). Salt-marsh age at each successional stage was estimated from topographic maps, aerial photographs, and the thickness of the sediment layer accumulated on top of the underlying sand layer (Olff *et al.* 1997). The sites were selected to have a similar base elevation (position at the initial elevation gradient on the bare sand flats with a base elevation of 1.16 m ( $\pm$  SE 2.2cm above Dutch Ordnance Level)). Different base elevations will have different inundation regimes, and therefore each has its characteristic vegetation succession (Olff *et al.* 1997). As salt marsh matures, sediment trapping by the vegetation at this base elevation increases the elevation of the soil surface with approximately 16 cm of clay in addition to the base elevation over 100 years over salt marsh succession, reducing the inundation frequency by floods, such as during spring tide.

Five sampling points within each of the seven successional stages were used. At these 35 sampling points, five different collection/sampling techniques were used to characterize the different components of the above- and belowground food web. Enclosed pitfall traps ( $\varnothing$  10 cm in 50  $\times$  50 cm Perspex enclosures) were used to gain quantitative estimates on soil inhabiting invertebrates such as snails, spiders, beetles and beach hoppers (Appendix I, Figure 3A). This method is especially suitable to estimate abundances of surface-active species and was adapted after Lang (2000). The combination of the long sampling period (18 days) and the small sampling area ensured that there was no bias towards the more active species, which is a fundamental problem of non-enclosed pitfalls (Lang 2000). Closed insect-emergence traps of 50  $\times$  50 cm, 80 cm height were used to collect flying insects emerging from

the soil and vegetation (Ausden 2000) with the same rationale (Appendix I, Figure 3A). These two methods were used in the period 4–22 July and were emptied every third day in order to completely 'empty' the plot. Accumulation curves for common species indeed showed a strong leveling off towards the end of this period (including the snail species *Ovatella myosotis*), suggesting that the time of sampling was sufficient to catch most specimen (Appendix I, Figure 2).

Soil macro- and larger mesofauna (e.g., springtails, oribatid mites, enchytraeid worms) were extracted from soil cores (Ø10 cm, 5 cm height) using a Tullgren funnel extraction methods (Van Straalen & Rijninks 1982). Nematodes were not sampled.

Hare and goose densities were estimated using pellet counts by averaging counts of two biweekly accumulations of droppings during 1–14 of May and 1–15 of July 2008 (Kuijper (Kuijper & Bakker 2005; Stahl *et al.* 2006).

Plant biomass was estimated by clipping the vegetation in 50 × 50 cm squares, to 1 cm height. Living and dead plant biomass were carefully separated and afterwards dried at 70°C for 48 hours. To account for attached clay and sand, ash free dry weight was determined as loss on ignition at 550°C for 3 hours (Van Wijnen & Bakker 1999). Vegetation height was determined three times, two meters apart, at each of the sampling points, using a 20 gram drop-disc. Soil temperature was measured continuously, using iButtons (Maxim Corp.). Soil moisture was measured using a ThetaProbe® moisture meter twice during the measuring period. In order to characterize external (marine) input, biomass of macroalgal driftline material (mainly *Fucus* spp.) was estimated during the months April, May and June 2009, inside the a 5 × 5 m square at each of the 35 sampling points. A clay core was used to determine the increase in sediment depth over succession (to the nearest 0.5 cm).

### **Biomass calculations and feeding guilds**

Invertebrates were identified up to species level and afterwards merged into feeding groups according to the classification in Table 2 of Appendix I, using feeding preferences for each species of invertebrate according to Remmert (1983), Irmiler and Heydemann (1986), Berg *et al.* (2004) Caballero *et al.* (2004) and Krantz and Walter (2009). The biomass for each species at each successional stage was estimated using the species-specific number to biomass corrections of Petersen and Luxton (1982), Caballero *et al.* (2004), Krantz and Walter (2009) and own measurements (for details, see Appendix I, Table 2). For some of the dominant species, namely *Orchestia gammarellus* (Macro-detritivores; Amphipoda), *Fucellia maritima* (Macro-detritivores; Diptera), *Ovatella myosotis* (Algal feeders; Gastropoda) and *Pardosa purbeckensis*



(Aboveground predators; Aranea), the density to biomass conversion was determined experimentally by calculating dry weight from 200 randomly selected individuals.

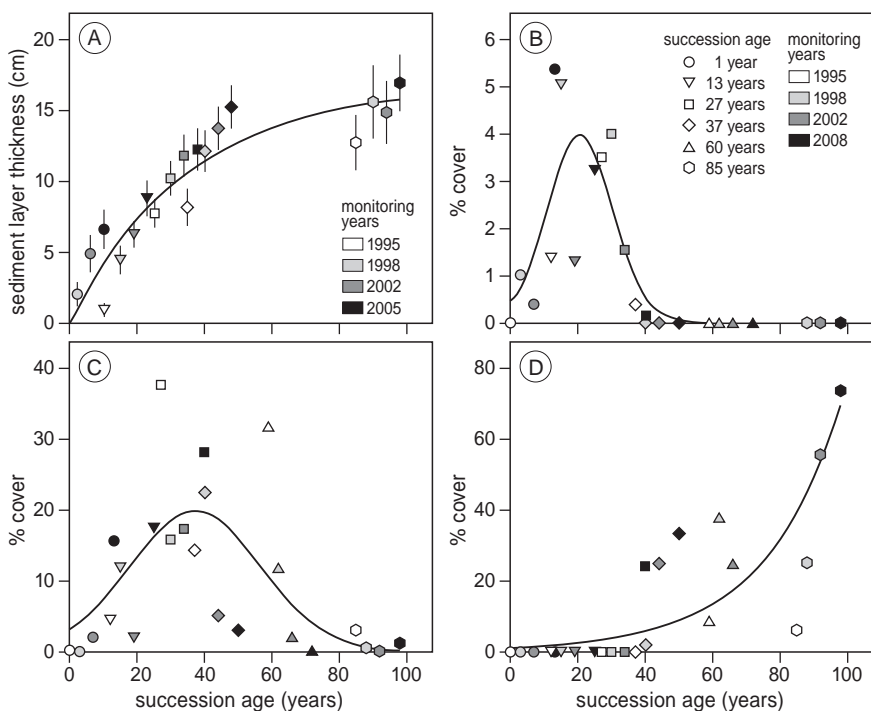
Hares are estimated to produce about 400 droppings per day (estimated allometrically from rabbit dropping count of Lockley 1962), while geese produce about 200 pellets per day (Bazely & Jefferies 1985). Dropping counts are commonly regarded as good indicators of grazing activity, both for geese (Owen 1971) and hare (Langbein *et al.* 1999).

In the results, we presented metabolic weight instead of biomass for all animals, but not for plants. Metabolic weight is calculated as biomass<sup>(3/4)</sup> (Kleiber 1947) and corrects for the fact that small animals have a higher metabolic rate per gram body weight than larger organisms, so the importance to the ecosystem also scales with that (Peters 1986).

## Results

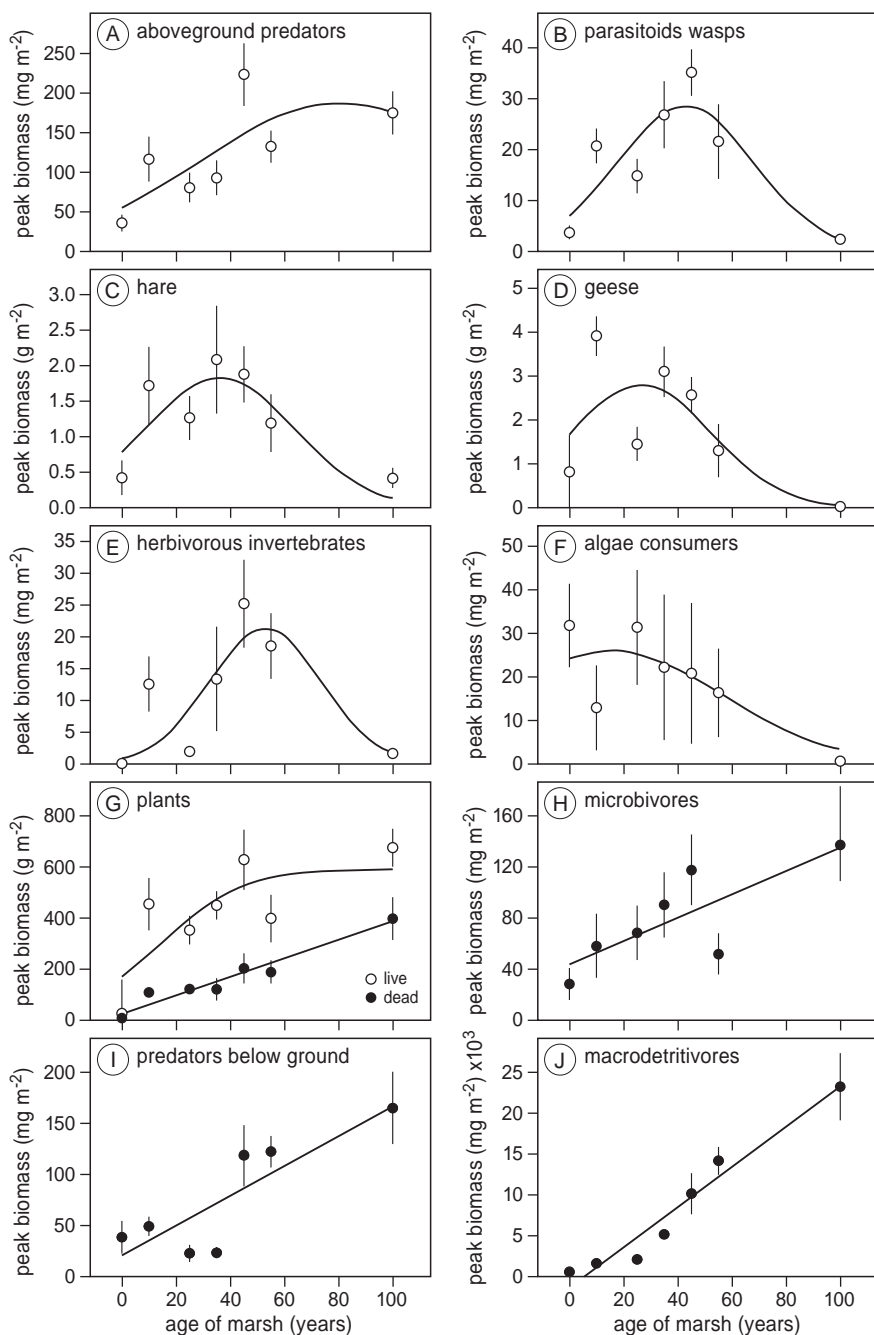
The thickness of the sediment layer at the permanent plots increased predictably with time (Figure 2.2A), both within and between locations. Along the same successional time axis, dominant plant species showed predictable changes in cover (Figure 2.2B-D). *Plantago maritima* showed a pronounced peak after 20 years of succession (Figure 2.2B). *Artemisia maritima* showed a peak at intermediate succession (40 years, Figure 2.2C), while the late successional dominant species *Elytrigia atherica* only started to increase after 40 years of vegetation succession (Figure 2.2D) and became dominant at 100 years. These patterns were reflected at each of the individual permanent plots, which indicates that our successional sequence is indeed a proper space-for-time substitution.

In the earliest stage of salt marsh succession, we found every trophic group from Figure 2.1 except invertebrate herbivores. We observed low plant biomass, covering only 8% ( $\pm$  3%) of the soil. Total dry biomass of organisms relative to total plant biomass (live + dead) in the youngest stage of succession was 14.6 % ( $\pm$ 3%). This is 5–16 times higher than in any of the other succession stages between 10 and 100 years, which ranged between 0.89 - 3% (mean 1.86  $\pm$  0.7%). This indicates that other sources of organic matter may support the food web as well and/or the turnover of the plant biomass is really high. Green web biomass formed by predators of herbivores and herbivores of plants was low in the earliest stage of succession, and consisted mainly of biomass of herbivorous vertebrates (Figure 2.2C-D). Also microbial mat feeding invertebrates were present with low biomass. A species of Diptera, *Fucellia maritima* and to a lesser extend *Enchytreae* spp. made up



**Figure 2.2** Illustrations of the space-for-time substitution for the 100-year chronosequence on the salt marsh of Schiermonnikoog. A) Increase in the sediment layer (Exp. rise to max.,  $R^2$  0.86;  $P < 0.0001$ ). B) Cover of *Plantago maritima* (Gauss,  $R^2$  0.65;  $P < 0.001$ ). C) Cover of *Artemisia maritima* (Gauss,  $R^2$  0.39,  $P < 0.001$ ). D) Cover of *Elytrigia atherica* (Exp. Increase,  $R^2$  0.73;  $P < 0.001$ ). Sediment layer measurements were done in 1995, 1998, 2002 and 2005 and vegetation cover was determined in 1995, 1998, 2002 and 2008. Different shadings of grey indicate the different years in which the measurements were done.

**Figure 2.3 (right)** Biomass (in mg DW or g DW  $m^{-2}$ ) of dominant trophic groups along the chronosequence (age of marsh in years). Bars represent standard errors. Fits indicate significant correlations. "Green" (herbivore-based) part of the food web is shown in open circles, "brown" (detritivore based) part of the web indicated by closed circles. A): above-ground predators (Gauss,  $R^2$  0.46;  $P < 0.001$ ). B): parasitoid wasps (Gauss,  $R^2$  0.46;  $P < 0.001$ ). C): hare (Gauss,  $R^2$  0.19;  $P = 0.014$ ) D) geese (Gauss,  $R^2$  0.27;  $P = 0.003$ ), E) herbivorous invertebrates (Gauss,  $R^2$  0.19;  $P = 0.014$ ), F) algae consumers (Gauss,  $R^2$  0.27;  $P = 0.002$ ). G) live (Power,  $R^2$  0.51;  $P < 0.001$ ) and dead standing biomass (Linear,  $R^2$  0.78;  $P < 0.001$ ) H) microbivores (Linear,  $R^2$  0.24;  $P = 0.002$ ), I) predators belowground (Linear,  $R^2$  0.48;  $P < 0.001$ ), J) macro-detritivores (Linear,  $R^2$  0.78;  $P < 0.001$ ).



the majority of the invertebrate biomass in this early succession. The first species is known to occur predominantly in decaying algae (Remmert 1983) and also the second species was found under decaying algae (personal observation). Microbivores were present in high biomass as well as their predators.

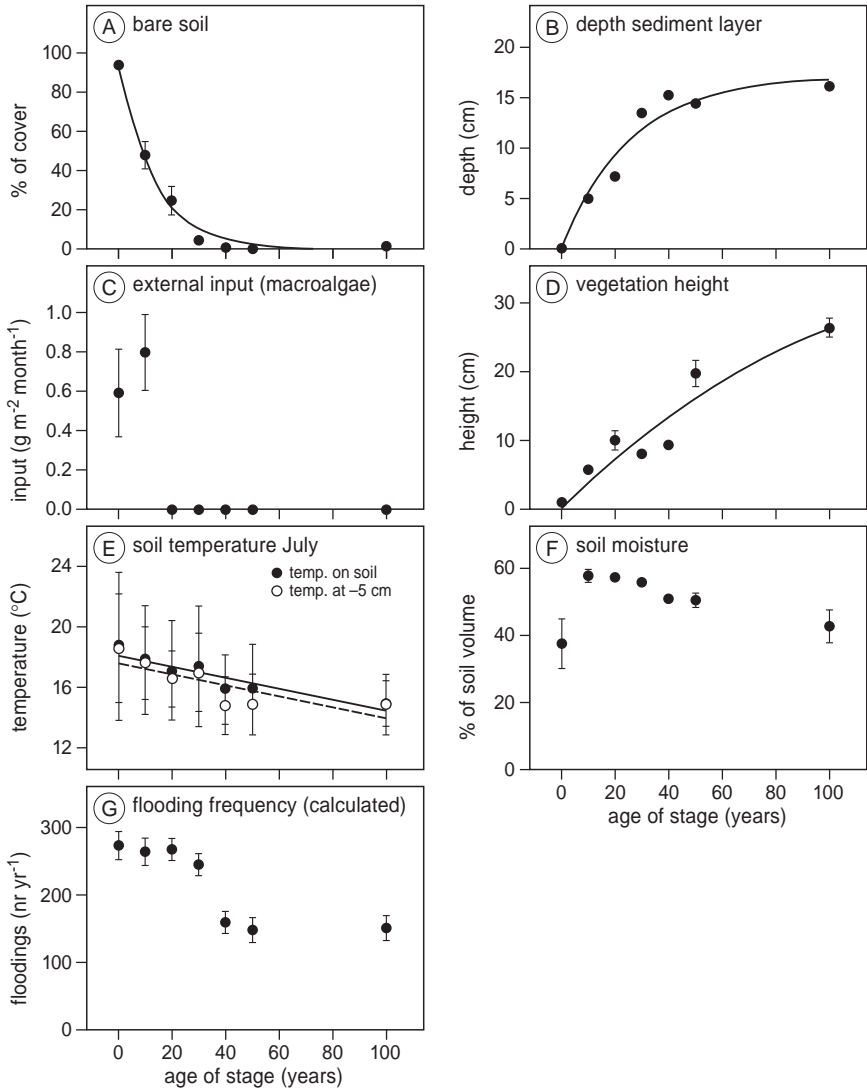
The change from an early (0 yrs) to an intermediate stage of succession (45 yrs) is characterized by a strong increase in aboveground standing biomass (Figure 2.3G), although live biomass increases more than standing dead biomass. Trophic groups in the green web (Figure 2.3A-F) which depend on live biomass also show a strong increase towards intermediate succession stages. Vertebrate herbivore biomass for geese and hare both showed a clear biomass optimum at respectively succession stage 35 and 45 years (Figure 2.3C and 2.3D). Invertebrate herbivores reach their highest biomass in the stages 45 years of succession (Figure 2.3A). Parasitoid wasps (Figure 2.3B), which predate on aboveground species (Quicke 1997), also show a clear biomass optimum in intermediate succession, at 45 yrs.

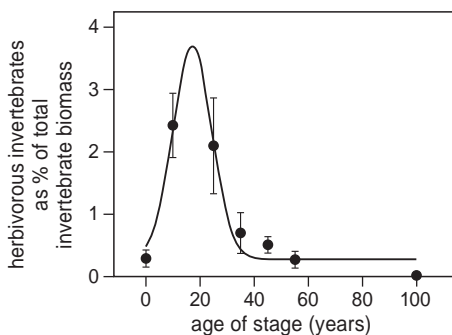
We also observed a clear continuous increase in the biomass of brown web groups as well, although less strong than the increase in the biomass of green web groups (Figure 2.3G-J). This increase was observed throughout all trophic groups in the brown web. The most abundant species in the brown web at intermediate successional stages, *Orchestia gammarellus* replaces *Fucellia maritima* between 10 yrs and 25 yrs as the macro-detritivore species with the highest biomass, increasing to up to 90% of the invertebrate biomass in the final successional stage.

From intermediate (45 yrs) to late succession (100 yrs), live vegetation biomass did not increase while standing dead biomass seemed to show a steady increase towards late succession (Figure 2.3G). Biomass of first order consumers in the green web biomass showed a sharp decline towards late

**Figure 2.4 (right)** Changes in environmental and abiotic parameters over the chronosequence. Bars represent standard errors. Fits indicate significant correlations. A) The percentage of bare soil ( $\pm$  SE) decreases strongly with age of succession. (Exp. decrease,  $R^2$  0.89;  $P < 0.001$ ). B) Sediment layer ( $\pm$  SE) increases with succession to about 16 cm thick (Exp. rise to max,  $R^2$  0.88;  $P < 0.001$ ). C External input of macroalgae is only present in first stages of succession. D) Vegetation height increases strongly towards late succession (Exp. rise to max,  $R^2$  0.87;  $P = 0.002$ ). E) Soil temperature ( $\pm$  SD) on the soil (closed symbols: Linear,  $R^2$  0.81;  $P = 0.003$ ) and soil temperatures at 5 cm deep (open symbols: Linear,  $R^2$  0.57;  $P = 0.03$ ) are on average 0.5 °C higher (GLM,  $F = 90.8$ ;  $P < 0.002$ ). Vegetation height increases towards late succession F) Soil moisture as a percentage of soil volume ( $\pm$  SE) first shows a strong increase and a gradual decreasing trend towards late succession. G) Flooding frequency decreases with increasing sediment layer thickness towards later stages of succession (after Olff *et al.* 1997).

succession, both for vertebrates and invertebrates, while the biomass of first order consumers in the brown web showed a pronounced increase towards late succession (Figure 2.5). Second order consumers mirrored these patterns, except for aboveground predators, which did not decrease towards late succession, but rather seemed to level off.





**Figure 2.5** Average biomass of herbivorous invertebrates as a percentage of the total invertebrate biomass (Gauss,  $R^2 = 0.57$ ;  $P < 0.001$ ).

### Changes in important environmental factors

Over the succession gradient, many environmental and abiotic properties change (Figure 2.4). We found a clear saturating decrease in the amount of bare soil (Figure 2.4A). Furthermore, we found an increase in the sediment layer over succession (Figure 2.4B), which reflects a similar increase in availability and pool of soil nutrients in the system (Olff *et al.* 1997), and a decrease in inundation frequency (Figure 2.4C). The input of macroalgae in the early stages of succession is much higher than in the older stages (Figure 2.4D). Vegetation height starts below 1cm in the first stage of succession, but increases up to 26cm towards the last successional stage (Figure 2.4E). Soil surface temperatures (Figure 2.4F) and also temperature fluctuations are highest in early, barely vegetated stages and show a mean decrease of 4 °C towards the late successional stage. Soil moisture decreases over succession (Figure 4G) but we found a much lower value for the earliest stage of succession where the clay layer is absent.

### Discussion

Our results suggest that the first successional stage is dominated by external detritus inputs, thus resembling food web configuration A in Figure 2.1. This is different from the classic view of early primary succession dominated by colonizing plants as depicted as configuration B. Over succession, we found the food web configuration to change towards configuration C, with an increasingly abundant green web, but still a sizeable brown web. Towards the late stages of succession, the brown web became again overwhelmingly abundant, with high plant biomass which is hardly used by herbivores, corresponding to configuration D in Figure 2.1.

We expect that our sampling methods appropriately captured the changes in main food web configuration during this succession sequence. As the growing season on the salt marsh is relatively short, plant and animal biomass and activity shows a pronounced peak in July (Irmeler & Heydemann 1986). We expect that sampling in more seasons would not affect our conclusions. In addition, it should be noted that the use of biomass of trophic groups has limitations in quantifying food web structure, as it does not fully capture the interaction strength between groups. For instance trophic groups with a high turnover rate may contribute stronger to trophic dynamics than expected from its biomass. Also, non-trophic (e.g. perceived predation risk) interactions are not captured in the estimated biomass. Nevertheless, we expect that our main conclusions uphold also upon more detailed analysis of trophic dynamics in this ecosystems.

In Figure 2.1 we propose that ecosystem assembly can either start as a green or a brown-dominated food web (Figure 2.1A vs Figure 2.1B). The high biomass of the brown web in early succession, attended with low observed plant biomass strongly suggests that the initial buildup of organic material in the earliest stages of this primary succession does not depend much on locally produced plant material. This is therefore an example of a marine subsidy to a terrestrial ecosystem as first observed by Polis *et al.* (1997). Also, Boschker *et al.* (1999) show that bacteria on the young salt marsh of Schiermonnikoog are mostly dependent on marine inputs as an energy source. If this is correct, we would conclude that the food web configuration A in Figure 2.1 captures the start of this succession better than configuration B; or said otherwise, the succession is started up by the brown web, not by the green web.

Our results show that the next stage in the succession can be characterized by configuration C in Figure 2.1: a food web that is mostly fuelled by local primary production, with a substantial green web. Between the stages of 10 and 45 years, we observed an increase in the importance of the green web relative to the brown web. This increase in herbivore biomass during succession is in line with classical exploitation theory (Hairston *et al.* 1960; Rosenzweig 1973; Oksanen *et al.* 1981; Van de Koppel *et al.* 1996). Increasing primary production of high quality, thus more food likely explains the increasing abundance of vertebrate herbivores over early succession. Previous work in this system has indicated that these vertebrate herbivores not only respond to, but also affect the course of vegetation succession (Olff *et al.* 1997; Van Wijnen & Bakker 1999; Kuijper & Bakker 2005). As a consequence, vertebrate herbivores may strongly affect various groups of invertebrates in the intermediate stages of this succession (interactions within the green web).

From intermediate to late succession (from 45 to 100 years), we observed a shift from a mixed green/brown food web configuration (Figure 2.1C) towards a configuration with high plant production, few herbivores, and dominance of the brown web (Figure 2.1D). Although we base this conclusion only on the last three successional stages, our space-for-time justification shows that the 75-year old stage is, at least regarding clay layer and vegetation composition, intermediate to the 55 and 100 year old stage. The shift in food web configuration may be explained by a decrease in forage quality for herbivores during these stages in succession (Van der Wal *et al.* 2000b), caused by increased nutrient availability, primary productivity, and associated plant competition for light (Van de Koppel *et al.* 1996; Olff *et al.* 1997). In turn, this has led to dominance of plant species with high structural support and hence low palatability (Van Der Wal *et al.* 2000a; Bos *et al.* 2005). In intermediate successional stages, small vertebrate herbivores still interact strongly with the course of succession by increasing forage quality (Kuijper & Bakker 2005), while in later stages, the ongoing vegetation succession causes herbivores to 'lose control' over vegetation composition (Van der Wal *et al.* 2000b; Kuijper & Bakker 2005). As a result, macro-detritivores make up most of the animal biomass in late succession, with the terrestrial amphipod *Orchestia gammarellus* as a dominant species, comprising about 90% of the total faunal biomass. The dominance of this species is a common phenomenon on mature productive salt marshes (Dias & Sprung 2003; Petillon *et al.* 2005). As *Orchestia gammarellus* produces large amounts of excrements on the surface (turnover of organic matter) and exhibits extensive digging behaviour (improving soil aeration) (Moore & Francis 1986; Dias & Sprung 2003) it can be expected to strongly act on nutrient cycling (Bardgett & Wardle 2010). We therefore speculate that this brown web dominance of the nutrient recycling is associated with late successional taller plant species, which in turn may promote macro-detritivores, while repelling herbivores.

Towards late succession, the biomass of aboveground predators declines less steeply than other aboveground trophic groups such as parasitoid wasps and invertebrate herbivores. This suggests that many of the ground active species either do not feed specifically on green web prey or may change their diet along succession from green to brown web prey. This is supported by other studies that find predators to connect different compartments across green and brown webs (Berg *et al.* 2001; Schmitz 2007; Birkhofer *et al.* 2008), which has been suggested to stabilize food webs (Neutel *et al.* 2007). To what extent aboveground predatory species indeed shift their diet from herbivorous to detritivorous species deserved further investigation.



### A general pattern?

We are inclined to think that our observed succession of main food web configurations represents a general pattern of ecosystem assembly. Although salt stress and stress from waterlogging are critical determinants of the salt marsh ecosystem which prevent final succession into forest, we argue that the principles underlying the observed changes in food web configuration can also apply to other ecosystems. For example, decreasing vegetation quality (Van der Wal *et al.* 2000b) and increasing structural complexity (increasing amount of dead organic material, standing biomass and increasing vegetation height) are typical for many successional sequences (Odum 1969). Therefore, food web configuration D in Figure 2.1 is suggested to be the end stage for many ecosystems including forests: a state with a relatively high C:N ratio of locally produced organic matter, a high standing stock of species belonging to the brown web and a low biomass of species belonging to the green web. However, some terrestrial ecosystems such as savannas which are dominated by large grazers and/or fire pose an obvious exception to this, as these agents prevent low quality plant tissues to dominate ecosystem processes in these systems (McNaughton 1984; Holdo *et al.* 2009). If this is correct, food web configuration C in Figure 2.1 is merely the end point of succession in these ecosystems. An interesting parallel to this can be found in salt marsh where large herbivores (cattle, horses) are introduced as grazers. Also in this system, they seem to shift the system back towards configuration C with higher vegetation diversity, improvement of plant quality and return of other (smaller) herbivores (Olf *et al.* 1997; Bos *et al.* 2002; Loucugaray *et al.* 2004).

Another critical difference between a salt marsh succession and other ecosystem successions arises from the amount of external nutrient input by sedimentation that governs the speed with which succession takes place (Olf *et al.* 1997). Due to the high external input and the short maturation time salt marsh plant species need, salt marshes are allowed to develop much faster than any forest will ever do. Mainland salt marshes which receive up to 10 times higher organic input develop much faster than this back-barrier island marsh (Bakker *et al.* 2002b). Succession goes much slower on primary successions like Glacier Bay (Crocker & Major 1955), Mount St. Helens (Sugg & Edwards 1998), the retreating Rotmoos Glacier (Kaufmann 2001). The amount of nutrients that an ecosystem receives during its first stages of succession may be of critical importance to the question whether succession will start with a food web configuration A or B, and hence for the progress of further ecosystem assembly. In the examples above with a slow rate of succession, low external input may force the ecosystem to accumulate nutrients by a slowly increasing primary production. In these ecosystems typi-

cally configuration B is a much more appropriate start situation than configuration A.

### **Conclusion: ecosystem assembly as merger of succession and food web theory?**

Ecological theory regarding succession has mostly focused on vegetation being the primary determinant that shapes the ecosystem. Our results show that in early stages with little vegetation, decomposers set the scene. That indicates that early successional development may not at all be driven by vegetation alone. During intermediate succession, when vertebrate herbivores are present in high numbers, herbivores seem to react strongly to the much higher biomass. Earlier experiments have already shown that these very same herbivores change the course of succession strongly but fail to halt it completely (Van de Koppel *et al.* 1996; Kuijper & Bakker 2005). We conclude that vegetation succession can only be understood in the light of food web dynamics, and vice versa.

Appendix I

**Table I.1** Additional plot characteristics for all sites. Soil coverage (in %  $\pm$  SD) for all plant species and vegetation measurements (based on five subplots). Plant cover for each species was estimated using the Londo scale (Londo 1976). Plant nomenclature follows van der Meijden (1990). Total number of plant species also represents an average ( $\pm$  SD). Vegetation height (cm) is an average ( $\pm$  SD) of 30 measurements with a 20 gram pyrofoam drop disc.

Age of succ. stage	<i>Artemisia maritima</i>		<i>Aster tripolium</i>		<i>Atriplex portulacoides</i>		<i>Atriplex prostrata</i>		<i>Elymus farctus</i>	
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.4
10	0.2	0.4	1.0	1.7	1.2	1.6	0.0	0.0	0.0	0.0
25	0.2	0.4	2.6	4.2	18.8	18.4	0.0	0.0	0.0	0.0
35	1.0	1.7	5.6	8.3	3.0	2.5	0.0	0.0	0.0	0.0
45	24.0	15.2	0.2	0.4	0.6	0.5	0.0	0.0	0.0	0.0
55	14.8	7.4	0.0	0.0	0.2	0.4	0.0	0.0	0.0	0.0
100	5.4	3.8	0.4	0.5	6.0	13.4	1.4	1.7	0.0	0.0

Age of succ. stage	<i>Elytrigia atherica</i>		<i>Festuca rubra</i>		<i>Glaux maritima</i>		<i>Juncus gerardi</i>		<i>Limonium vulgare</i>	
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	1.2	0.8	0.0	0.0	32.0	19.2
25	0.0	0.0	0.0	0.0	1.2	1.6	0.0	0.0	38.0	4.5
35	0.0	0.0	0.0	0.0	2.0	1.2	0.0	0.0	66.0	8.9
45	0.0	0.0	30.0	20.0	4.0	5.5	0.0	0.0	28.4	21.0
55	4.8	8.7	30.8	21.1	0.0	0.0	30.0	22.4	16.2	8.5
100	58.0	16.4	0.6	0.9	0.0	0.0	6.4	13.2	0.0	0.0

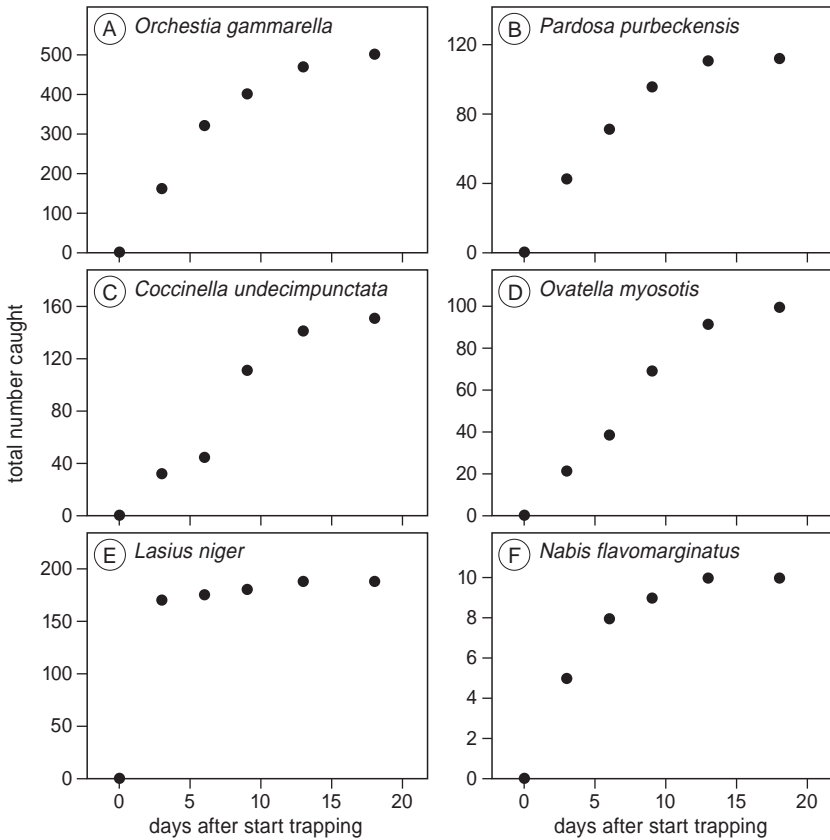
Age of succ. stage	<i>Plantago maritima</i>		<i>Puccinellia maritima</i>		<i>Salicornia europaea</i>		<i>Spartina anglica</i>		<i>Spergularia media</i>	
0	0.0	0.0	2.4	2.2	0.2	0.4	0.0	0.0	3.6	3.8
10	1.0	0.7	1.0	0.7	0.8	0.4	0.2	0.4	2.2	1.1
25	10.4	11.8	1.0	0.0	1.2	0.4	1.2	0.4	1.6	1.3
35	4.0	8.9	2.4	2.2	1.0	0.0	0.0	0.0	0.6	0.5
45	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
55	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Age of succ. stage	<i>Suaeda maritima</i>		<i>Triglochin maritima</i>		Total cover (%)		Number of species	
0	0.0	0.0	0.0	0.0	8.0	5.8	2.0	0.7
10	0.0	0.0	0.0	0.0	38.4	22.7	6.4	3.4
25	0.0	0.0	2.4	2.7	72.0	11.2	9.2	0.4
35	0.4	0.5	1.0	0.0	101.4	7.9	8.0	1.6
45	0.0	0.0	0.2	0.4	112.6	8.9	4.2	0.8
55	0.0	0.0	0.0	0.0	114.0	10.4	4.4	1.1
100	0.0	0.0	0.0	0.0	87.0	17.1	4.2	1.8

Londo, G. 1976. The decimal scale for relevés of permanent quadrats. Vegetatio 33: 61-64.  
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**Figure I.1** Pictures from each of the sites, July 2008



**Figure I.2** Numbers of trapped invertebrates in Perspex traps increase asymptotically with time. A and D represent two common detritivore species, a gammarid (A) and a snail (D), B (Lycosidae), C (Cocciidae) and E (Formicidae) and F (Nabidae) represent predators of invertebrates.

**Table I.2** Species specific weight, metabolic weight and feeding group. Reference for every biomass is presented in last column.

Taxa	Biomass (mg)	Biomass (3/4)	Feeding group	Reference
<b>Coleoptera</b>				
<b>Carabidae</b>				
<i>Bembidion minimum</i>	0.469	0.567	Invertebrate predator	Persson & Lohm 1977
<i>Dicheirotichus gustavii</i>	0.900	0.924	Invertebrate predator	Persson & Lohm 1977
<i>Dyschirius globus</i>	0.364	0.468	Invertebrate predator	Persson & Lohm 1977
<i>Dyschirius salinus</i>	0.364	0.468	Invertebrate predator	Persson & Lohm 1977
<i>Dyschirius thoracicus</i>	0.364	0.468	Invertebrate predator	Persson & Lohm 1977
<i>Pogonus chalceus</i>	0.900	0.924	Invertebrate predator	Persson & Lohm 1977
<b>Staphylinidae</b>				
<i>Bledius fergussoni</i>	0.180	0.276	Algae consumer	Persson & Lohm 1977
<i>Bledius subniger</i>	0.180	0.276	Algae consumer	Persson & Lohm 1977
<i>Brundinia marina</i>	0.180	0.276	Algae consumer	Persson & Lohm 1977
<i>Diglotta</i> sp.	0.180	0.276	Algae consumer	Persson & Lohm 1977
<i>Drusilla canaliculata</i>	0.180	0.276	Algae consumer	Persson & Lohm 1977
<i>Streptanus aemulans</i>	0.180	0.276	Algae consumer	Persson & Lohm 1977
<b>other Coleoptera</b>				
<i>Cassida vittata</i>	0.900	0.924	Invertebrate herbivore	Persson & Lohm 1977
<i>Coccinella undecimpunctata</i>	0.900	0.924	Invertebrate predator	Persson & Lohm 1977
<i>Heterocerus flexuosus</i>	0.364	0.468	Algae consumer	Persson & Lohm 1977
<i>Ochthebius auriculatus</i>	0.075	0.143	Macrodetritivore	Haugerud, NJ MS thesis 2003
<i>Ochthebius marinus</i>	0.075	0.143	Macrodetritivore	Haugerud, NJ MS thesis 2003
<i>Pelenomus zumpti</i>	0.099	0.177	Invertebrate herbivore	Persson & Lohm 1977
<i>Tytthasp.is sedecimpunctata</i>	0.900	0.924	Invertebrate predator	Persson & Lohm 1977
<b>Araneae</b>				
<i>Baryphyma duffeyi</i>	0.133	0.221	Invertebrate predator	Persson & Lohm 1977
<i>Clubiona stagnatilis</i>	1.415	1.297	Invertebrate predator	Persson & Lohm 1977
<i>Erigone atra</i>	0.132	0.219	Invertebrate predator	Persson & Lohm 1977
<i>Erigone longipalpis</i>	0.132	0.219	Invertebrate predator	Persson & Lohm 1977
<i>Oedothorax retusus</i>	0.132	0.219	Invertebrate predator	Persson & Lohm 1977
<i>Pachygnatha clercki</i>	1.415	1.297	Invertebrate predator	Persson & Lohm 1977
<i>Pachygnatha degeeri</i>	1.415	1.297	Invertebrate predator	Persson & Lohm 1977
<i>Pardosa purbeckensis</i>	1.415	1.297	Invertebrate predator	Persson & Lohm 1977
<i>Stemonyphantes lineatus</i>	1.415	1.297	Invertebrate predator	Persson & Lohm 1977
<i>Walkaenaria kochi</i>	0.133	0.221	Invertebrate predator	Persson & Lohm 1977
<b>Crustacea</b>				
<i>Carcinus maenas</i>	20.155	9.512	Macrodetritivore	Bessa <i>et al.</i> 2010
<i>Orchestia gammarellus</i>	3.300	2.448	Macrodetritivore	Own measurements

**Table I.2** Continued

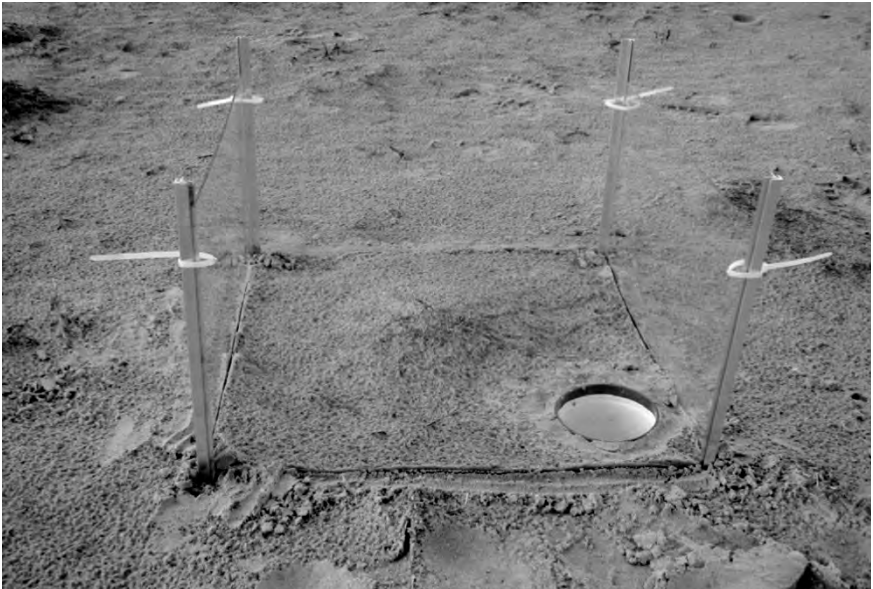
Taxa	Biomass (mg)	Biomass (3/4)	Feeding group	Reference
<b>Diptera</b>				
<b>Brachycera (flies)</b>				
<i>Fucellia</i> sp.	1.100	1.074	Macrodetritivore	Own measuments
<i>Nemotelus</i> sp.	0.620	0.699	Macrodetritivore	Own measuments
<i>Scaptomyza pallida</i>	0.069	0.134	Macrodetritivore	Persson & Lohm 1977
<i>Chloropidae</i> spp.	0.069	0.134	Invertebrate herbivore	Persson & Lohm 1977
<i>Dolichopodidae</i> spp.	0.082	0.153	Macrodetritivore	Persson & Lohm 1977
<i>Ephydriidae</i> spp.	0.069	0.134	Invertebrate herbivore	Persson & Lohm 1977
<i>Sphaerophoria</i> sp.	1.151	1.111	Invertebrate predator	Persson & Lohm 1977
<i>Sepsidae</i> spp.	0.069	0.134	Macrodetritivore	Persson & Lohm 1977
<i>Nematocera</i> (midges)				
<i>Symplecta stictica</i>	0.620	0.699	Macrodetritivore	Todd & Schmidt 2009
<i>Phoridae</i> spp.	0.074	0.141	Macrodetritivore	Persson & Lohm 1977
<i>Cecidomyiidae</i> spp.	0.017	0.048	Invertebrate herbivore	Persson & Lohm 1977
<i>Ceratopogonidae</i> spp.	0.079	0.149	Macrodetritivore	Persson & Lohm 1977
<i>Sciaridae</i> spp.	0.049	0.104	Macrodetritivore	Persson & Lohm 1977
<i>Scatopsidae</i> spp.	0.074	0.141	Macrodetritivore	Persson & Lohm 1977
<i>Sphaeroceridae</i> spp.	0.069	0.134	Macrodetritivore	Persson & Lohm 1977
<b>Hymenoptera</b>				
<i>Ichneumonoidae</i> spp.	0.133	0.221	Parasitoid	Persson & Lohm 1977
<i>Lasius niger</i>	1.091	1.067	NA	Persson & Lohm 1977
<i>Myrmica rubra</i>	1.091	1.067	NA	Persson & Lohm 1977
<b>Mollusca</b>				
<i>Ovatella myosotis</i>	8.101	4.802	Macrodetritivore	Own measuments
<i>Assimineae grayana</i>	6.200	3.929	Algae consumer	Own measuments
<b>Hemiptera</b>				
<i>Miridae</i> spp.	0.099	0.177	Invertebrate herbivore	Persson & Lohm 1977
<i>Salda maritima</i>	0.099	0.177	Invertebrate predator	Persson & Lohm 1977
<i>Aphididae</i> spp.	0.010	0.031	Invertebrate herbivore	Persson & Lohm 1977
<i>Cicadellidae</i> spp.	0.099	0.177	Invertebrate herbivore	Persson & Lohm 1977

**Table I.2** Continued

Taxa	Biomass (mg)	Biomass (3/4)	Feeding group	Reference
<b>Acari</b>				
<i>Platynothrus</i> sp.	0.013	0.039	microbivore	Krantz & Walker 2009
<i>Hermannia subglabra</i>	0.003	0.013	microbivore	Krantz & Walker 2009
<i>Zachvatkinibates</i> cf <i>quadrivertex</i>	0.003	0.013	microbivore	Krantz & Walker 2009
<i>Oribatella</i> cf <i>litoralis</i>	0.003	0.013	microbivore	Krantz & Walker 2009
<i>Liebstadia</i> cf <i>similis</i>	0.003	0.013	microbivore	Krantz & Walker 2009
<i>Oppiidae</i> spp.	0.001	0.003	microbivore	Krantz & Walker 2009
<i>Ameronothrus</i> spec.	0.005	0.020	microbivore	Persson & Lohm 1977
<i>Oribatida</i> spec.	0.005	0.020	microbivore	Persson & Lohm 1977
Predatory mite morph 1	0.011	0.033	below ground predator	Krantz & Walker 2009
Predatory mite morph 2	0.011	0.033	below ground predator	Krantz & Walker 2009
Predatory mite morph 3	0.011	0.033	below ground predator	Krantz & Walker 2009
Predatory mite morph 4	0.011	0.033	below ground predator	Krantz & Walker 2009
Predatory mite morph 5	0.008	0.026	below ground predator	Persson & Lohm 1977
<i>Uropodidae</i> sp.	0.011	0.033	below ground predator	Krantz & Walker 2009
<i>Trombidiidae</i> sp.	0.059	0.120	below ground predator	Persson & Lohm 1977
<i>Pyemephorus</i> sp.	0.008	0.026	below ground predator	Persson & Lohm 1977
<i>Veigaioides</i> sp.	0.008	0.026	below ground predator	Persson & Lohm 1977
Predatory mite morph 6	0.001	0.006	below ground predator	Krantz & Walker 2009
<b>Collembola</b>				
<i>Thalassaphorura debilis</i>	0.002	0.010	microbivore	Caballero 2004
<i>Mesaphorura</i> cf <i>macrochaeta</i>	0.001	0.004	microbivore	Caballero 2004
<i>Friezea mirabilis</i>	0.005	0.017	microbivore	Caballero 2004
<i>Archisotoma</i> spec.	0.003	0.012	microbivore	Caballero 2004
<i>Halisotoma maritima</i>	0.003	0.012	microbivore	Caballero 2004
<i>Entomobrya lanuginosa</i>	0.013	0.039	microbivore	Caballero 2004
<i>Isotoma riparia</i>	0.041	0.091	microbivore	Caballero 2004
<i>Folsomia sexoculata</i>	0.006	0.021	microbivore	Caballero 2004
<i>Brachystomella parvula</i>	0.005	0.017	microbivore	Caballero 2004
<b>Other taxa</b>				
<i>Coleophora</i> sp.	1.12	1.089	Herbivore invertebrate	Ganihar 1997
<i>Enchytraeidae</i> spp.	0.059	0.120	Detritivore	Nowak 2004
<i>Pseudococcidae</i> spp.	0.002	0.009	Invertebrate herbivore	Persson & Lohm 1977
<i>Lepus europeus</i>	4.3E+06	94428.120	Herbivore	Frylestam 1980
<i>Branta</i> spp.	1.9E+06	51175.866	Herbivore	Portugal <i>et al.</i> 2007



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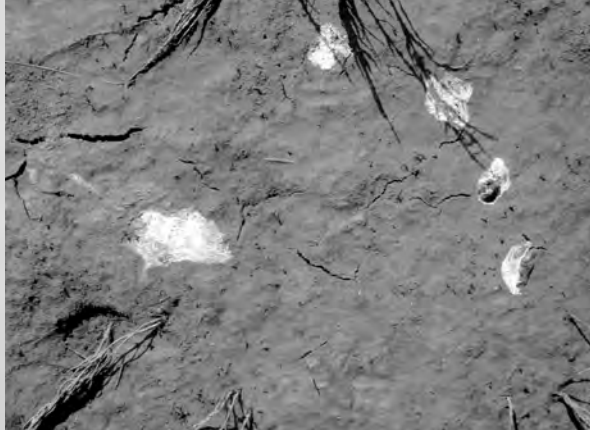


### Figure I.3 Left

Top. Enclosed pitfall trap in early succession (succession stage 0 years), designed to catch surface-active invertebrates (adapted after Lang 2000). The Perspex sheets (50 cm wide, 35 cm high, 3 mm thick), which prevented animals to escape from the traps (or enter the trap area), were slid along 2 aluminium rails into the soil, to a depth of 8 cm. The pitfall trap itself was placed in the southwest corner of the enclosure, and a 2 cm layer of water to which a detergent was added, was added to the pitfall trap.

Bottom. Enclosed pyramid photo-elector trap (50×50 cm) in late succession (succession stage 100 years), designed to catch winged invertebrates invertebrates (which can emerge from the soil) and web-building spiders (after Ausden 2000). The glass jar on top served as a 'light bulb', thereby attracting invertebrates. The glass jar was put upside-down in a PVC ring, and was filled with a 3 cm layer of ethanol. A tube in the middle of the ring (5 cm diameter) connected the PVC ring with the shaded compartment of the trap. A plastic cover was put around the jar and the PVC ring to prevent evapotranspiration of the ethanol. The black cloth was tightly nailed to the ground to prevent escape from animals within the trap and entrance of animals from outside.

# Chapter 3



# 3

## Food web assembly at the landscape scale: using stable isotopes to reveal changes in trophic structure during succession

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*Submitted to Ecosystems*

### Abstract

Food webs are increasingly evaluated at the landscape scale, accounting for spatial interactions involving different nutrient and energy channels. Also, while long viewed as static, food webs are increasingly seen as dynamic entities that assemble during vegetation succession. The next necessary step is, therefore, to link nutrient flows between ecosystems to local food web assembly processes.

In this study, we used a 100 year salt marsh succession in which we investigated the long-term changes in food web organization, especially focusing on the balance between internal vs. external nutrient sources. We found that during food web assembly, the importance of internal (terrestrial) nutrient cycling increases at the expense of external (marine) inputs. This change from external to internal nutrient cycling is associated with strong shifts in the basis of energy channels within the food web. In early succession, detritivores are mostly fuelled by marine inputs while in later succession they thrive on locally produced plant litter, with consequences for their carnivores. We conclude that this 100 years of food web assembly proceeds by gradual decoupling of terrestrial nutrient cycling from the marine environment, and by associated rearrangements in the herbivore and detritivore energy channels. Food web assembly thus interacts with nutrient and energy flows across ecosystem boundaries.

## Introduction

Food web studies have so far revealed many informative and repeatable patterns in trophic structure (e.g. Pimm 1982; Cohen & Briand 1984; Cohen & Newman 1985; Dunne *et al.* 2002), body size distribution, and topology (Cohen *et al.* 2003; Woodward *et al.* 2005). In addition, it is increasingly recognized that the understanding of the architecture of many food webs requires the inclusion of above- and belowground parts of ecosystems, as well as spatial interactions and/or temporal dynamics between food webs (Winemiller & Polis 1996; Moore *et al.* 2004; Berg & Bengtsson 2007; Rooney *et al.* 2008). These authors have shown that food webs are comprised of different energy channels based on discrete resources, which are often not homogeneously spread in space and time, thus influencing the different components of the food web in their own way. Moreover, spatial interactions among neighbouring ecosystems, such as between ocean and desert, cause some food webs to connect to others on a landscape scale (Winemiller 1990; Polis & Hurd 1995; Rooney *et al.* 2008). Specifically, spatial subsidies into the detrital part of the food web have been shown to have far-reaching consequences for food web structure and ecosystem functioning. They often lead to higher primary production, which in turn may cascade up trophic chains (Polis & Hurd 1995; Polis & Strong 1996).

While the role of spatial interactions in food web organizations are increasingly studied, good case studies of food web assembly over sufficient time are still poorly available, especially for food webs that are connected to others at the landscape scale. This lack is possibly explained by the large effort required in the quantification of the food web components in the first place, combined with the additional complications involved in following food web structure over sufficient time. One of the most obvious changes in food web structure that explicitly focuses on this temporal component can be found during primary succession. However, succession research has merely focused on vegetation and soil changes (Clements 1916; Miles & Walton 1993). The few notable exceptions (Van de Koppel *et al.* 1996; Kaufmann 2001; Neutel *et al.* 2007) have addressed only parts of food webs, rather than providing an integrated analysis of their structure.

Given the importance of spatial subsidies and temporal changes in food webs for understanding the processes of ecosystem assembly, it is now needed to combine insights from food web ecology on a landscape scale, i.e. combine vegetation succession research and food web assembly in single study systems. More precisely, we need to find out how different food web components, i.e. the green component of food webs, consisting of plants and herbivores and their predators, and the brown component of food webs, consisting of plant litter, detritivores and their predators interact over a successional

sequence by taking the spatial aspects of food webs into account. This can be done by building on recent insights in factors structuring different energy channels in food webs: specifically the balance between the brown (detritivore-driven) and green (herbivore-driven) parts of food webs (Cebrian & Lartigue 2004; Moore *et al.* 2004; Rooney *et al.* 2006; Shurin *et al.* 2006).

In this study, we used a chronosequence reflecting 100 years of primary succession on a coastal salt marsh to study food web assembly on a landscape scale. Previous work on our study system has quantified in detail the dynamics of soil and vegetation succession (Olff *et al.* 1997) and its interaction with vertebrate herbivores (Van de Koppel *et al.* 1996; Van Wijnen & Bakker 1999; Kuijper & Bakker 2005). This chronosequence has been carefully validated by 35 years of study of permanent plots, which has justified its space-for-time replacement (Figure 2.2; Olff *et al.* 1997). This means that 100 years of successional dynamics can be studied within a single year. We now add analyses of the trophic dynamics, by explicitly focusing on the brown and green part of the food web along the chronosequence to study changes in food web organization over succession. In addition, we use stable isotope analyses to study the landscape origin of the carbon and nitrogen used by different compartments.

Stable isotopes are excellent tools to study temporal changes in food web organization, and nutrient and carbon sources for the different energy channels, especially in coastal environments. Carbon isotopes are useful in discriminating marine from terrestrial sources (Polis & Hurd 1995; Maron *et al.* 2006), especially when the terrestrial plant species are predominantly C3 plants, which is the case in our system. Nitrogen isotopes are useful in studying the trophic position of different groups (Hobson & Welch 1992; Post 2002), and can thus also be used to study changes in trophic position and structure throughout succession.

Specifically, we unravel which nutrient and energy sources fuel the food web in the various stages of vegetation succession. Then we study how the main food web organization in this ecosystem changes over 100 years of primary succession. For this, we combine information on animal diets with measured nitrogen isotope values for each main trophic group in different stages of succession.

## Methods

### Study area and sampling design

The study area was located on the island of Schiermonnikoog, (53°30' N, 6°10' E), the Netherlands. A sequence of vegetation succession stages on the salt



marsh was present, where the earliest stages are formed on the east side of the island, and later stages of succession were situated 8 kilometers to the west (Oloff *et al.* 1997). The sampling locations get inundated regularly at high tides. Marine algae and other organic material wash ashore during these inundations. At high tide, many resting waders are present in the area, especially during spring and autumn migration, but many non-migrating waders are also present during the whole year.

Seven succession stages were identified, estimated as 0, 10, 25, 35, 45, 55 and 100 years of primary succession in 2010 (Oloff *et al.* 1997). Salt-marsh age at each successional stage was estimated from topographic maps, aerial photographs, and the thickness of the sediment layer accumulated on top of the underlying sand layer (Oloff *et al.* 1997; Van Wijnen & Bakker 1999), and calibrated using long-term observations of permanent plots (Figure 2.2; Oloff *et al.* 1997). The sites were selected to have a similar base elevation (vertical position with respect to mean sea level at the initial elevation gradient on the bare sand flats, before additional sedimentation happened due to vegetation succession). A base elevation of 1.20 m (+/- SE 2.2 cm) above Dutch Ordnance Level (N.A.P.) was used to select the sites for this study. Details on every sampling site can be found in Appendix II, Table 1. Along our succession gradient, inundation frequency declines due to ongoing sedimentation. The earliest stage of succession had an average inundation frequency of 184 times  $\text{yr}^{-1}$  (26% of all tidal cycles), while the last stage of succession has an inundation frequency of 131 times  $\text{yr}^{-1}$  (18% of all tidal cycles).

At every succession stage (site) five 25 m spaced plots (5 × 5 m each) were placed in which the main trophic groups from the green and brown part of the food web were sampled. Between April 23 and May 14 2010, field collections of all dominant animals, plants and marine deposits (species that represent > 90% of the biomass in their respective trophic group) were done in each of these plots. The changes in abundances of the different species, and their aggregations into trophic groups, are reported in a previous study (Chapter 2). Sampling methods for all trophic groups for the stable isotope analysis are described in the following paragraphs. For all details on the dominant species that were collected and sample size for every species or organic matter source, see Appendix II, Table 2.

### Estimations of marine input

As both bird guano and macro-algae that drift ashore can constitute an important source of nutrients (Polis *et al.* 1997; Maron *et al.* 2006), we made an estimation of the input of both components using different methods. Macroalgal dry weight was estimated in March and April 2010 by collecting all macro-algae from each 5 × 5 m plots which was subsequently rinsed and



dried at 70°C for 48 hours and weight again to determine dry weight per square meter. Bird densities were estimated by doing weekly counts on the numbers of resting birds were done at all sampling sites during the months March and April. Every location was visited four times. At every site, we used one 1-hectare plot in which the total number of resting birds per species was estimated between one hour before and three hours after high tide. Per species, the total number of bird minutes was calculated by multiplying the number of resting birds at every location times the number of minutes spend in each of the hectare plots.

### **Sampling for isotope analysis**

#### **TERRESTRIAL PLANTS**

For each plot, we collected one sample from each of the dominant terrestrial salt marsh plant species, each of which contained ten leaves: one random fresh leaf from ten individual plants. In total five samples for every plant species were collected at each site. These plant samples together represented at least 90% of the locally produced biomass in each of the sites. In order to standardize samples, only fresh plant leaves were collected. Stable isotope analysis of plant leaves were done on samples from succession stages 0, 10, 45 and 100 years, because we expected no strong differences in isotopic signals between sites. At each plot, a sample of local litter material was collected by taking five random samples of 10 gram of dead local vegetation, which were not overgrown with algae or covered in clay. These samples were well-mixed in a bowl from which a subsample of about 5 grams was taken for analysis.

#### **MARINE POM**

As we experienced no high tides during the sampling period, we sampled marine particulate organic matter (POM) at high tide at a distance of 150 m south of succession stages 10, 45 and 100 yrs. At five points per site, spaced 25 metres apart, we used a plastic hand-held net (30 × 30 cm) with fine mesh (500  $\mu$ m) which was manually moved five times over a distance of a meter through the upper 50 cm of the water column to catch drifting material. The content of the net was deposited on a clean plastic sheet and funnelled into a 2 ml plastic tube with demi water and stored at -20°C. Because we expected no large differences for marine POM stable isotope signals for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between sampling locations, no additional sampling was done in other succession stages.

#### **DIATOMS**

As marine diatoms represent a significant fraction of the organic matter that is deposited during floodings (Boschker *et al.* 1999), we made collections of

diatoms at locations close to the plots. Diatoms were collected from the marine sediment at low tide at a distance of 150m south of succession stages 0, 10, 45 and 100 years. The five sampling plots per site were spaced 25 m apart. From each of the five samples per site, we took one sediment sample of  $10 \times 10 \times 1$  cm deep ( $100 \text{ cm}^3$ ) from which diatoms were extracted in the laboratory. In order to separate the diatoms from the sediment, we applied the 'lens-tissue method' (Eaton & Moss 1966). After the diatoms migrated onto a GF/F Whatman filter (average pore size =  $0.7 \mu\text{m}$ ), the content was deposited on a plastic sheet and funnelled into a 2 ml plastic tube using demineralized water and stored at  $-20^\circ\text{C}$ . As we expected no strong differences between sites for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope signals, we did not sample any of the other sites.

#### MARINE MACRO-ALGAE

We collected the macro-algae species *Fucus* spp. because this species comprises the majority of the deposited marine macro-algae (>90%, see Table 3.1). Samples were collected in all  $5 \times 5$  m plots at succession stages 0, 10, 45 and 100 yrs. Every sample contained five random leaves of individual *Fucus* spp. deposits, which were at least 2 metres apart. After collection, all samples were brought to the laboratory where they were carefully rinsed with demineralised water to remove other organic material and mineral sediments and stored in 2 ml tubes at  $-20^\circ\text{C}$ .

#### INVERTEBRATES

Dominant invertebrate species (identified as dominants in Schrama *et al.* in prep) were collected in all subplots using a modified leaf blower (Echo Shred<sup>®</sup> Vac, net build inside with a mesh size of 1 mm). After applying the leaf blower for two minutes in each  $5 \times 5$  m plot, the net was emptied in a white plastic container and living specimens were collected by hand and put into 2 ml plastic tubes. Each sampling tube contained at least four individuals of each species. After transportation back to the field station, all tubes were immediately stored at  $-20^\circ\text{C}$ . Literature and personal observations on feeding preference were used to assign species to different trophic groups: herbivores, detritivores, herbivore feeding carnivores and carnivores feeding on both herbivores and detritivores (hereafter called omnivorous carnivores). In total, 10 species of invertebrate herbivores were collected, but not the same species from all succession stages. In total 14 species of carnivores were collected from which most species were collected in all stages. The group of carnivores feeding on herbivores and detritivores contained mostly spiders (Erigonidae and Lycosidae), beetles (Carabidae) and ants (Formicidae). Carnivores that fed on herbivores were different species of ladybugs

(Coccinellidae) and parasitoid wasps (Ichneumonidae). In total 10 species of detritivores were collected, mainly Amphipoda, Isopoda and beetles. Because many detritivore species cover the whole spectrum of food sources and little is known about their exact feeding preference, no a priori subdivision of this group was made.

#### MARINE BIRDS

Because Oystercatchers (*Haematopus ostralegus*) and Lesser black-backed gulls (*Larus rudibundus*) were found to be dominant in our succession sequence, five samples of fresh faeces of both bird species were collected in five 25 m spaced 3 × 3 m plots. These plots were located 50 m south of the plots at which the other collections were done. Per species per sample we took one individual pallet of guano from 5 individual birds, directly after defecation, using a pair of tweezers.

#### STABLE ISOTOPE ANALYSIS

All invertebrate, plants and marine macro-algae samples were stored frozen at -20 °C and processed by freeze-drying and grinding with a pebble mill (1000 rotations per minute for two minutes using a Retsch MM2). The  $\delta^{13}\text{C}$ - and  $\delta^{15}\text{N}$ - isotopes values were determined by using a Thermo Flash 2000 elemental analyser coupled to a Thermo Delta V isotope ratio mass spectrometer. Isotope values were calibrated to a laboratory acetanilide standard ( $\delta^{13}\text{C}$  -26.1 ‰ and  $\delta^{15}\text{N}$  1.3‰ calibrated on NBS-22 and IAEA-N1, respectively) and corrected for blank contribution. The samples were mostly analyzed in duplicate and the reported data represents the mean of these analyses. The results are reported on the per mill scale with respect to Vienna Pee Dee Belemnite [VPDB] and graphically presented in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopes diagrams, to visualize changes in carbon and nutrient sources and trophic structure during succession. The results were graphically presented in isotope ratio diagrams to analyse changes in carbon and nutrient sources and trophic structure during succession.

#### STATISTICS

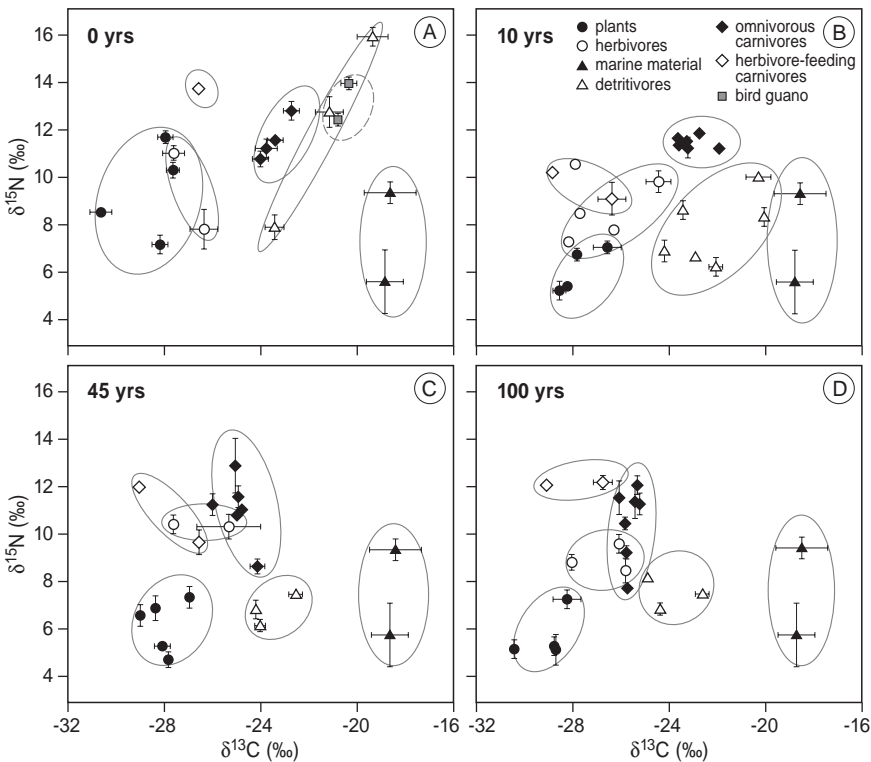
For the analysis of changes in food web stable isotopes composition estimations for species were averaged within trophic groups. To test for differences between trophic groups or within trophic groups between succession stages, general linear models with post-hoc Tukey HSD tests were done as assumptions needed for doing parametric analysis were met. Linear least square regressions were done within each trophic group to find changes in stable isotope ratios for both nitrogen and carbon over succession. All statistical analyses were done in Statistica 9.0.

# Results

## Food web composition

Plotting all species in the various plant successional stages in a  $\delta^{15}\text{N} - \delta^{13}\text{C}$  plane reveals major changes in food web structure (Figure 3.1A-D). A strong difference between  $\delta^{13}\text{C}$  values of salt marsh plants and marine diatoms was observed, of about 10‰ of  $\delta^{13}\text{C}$  on average.

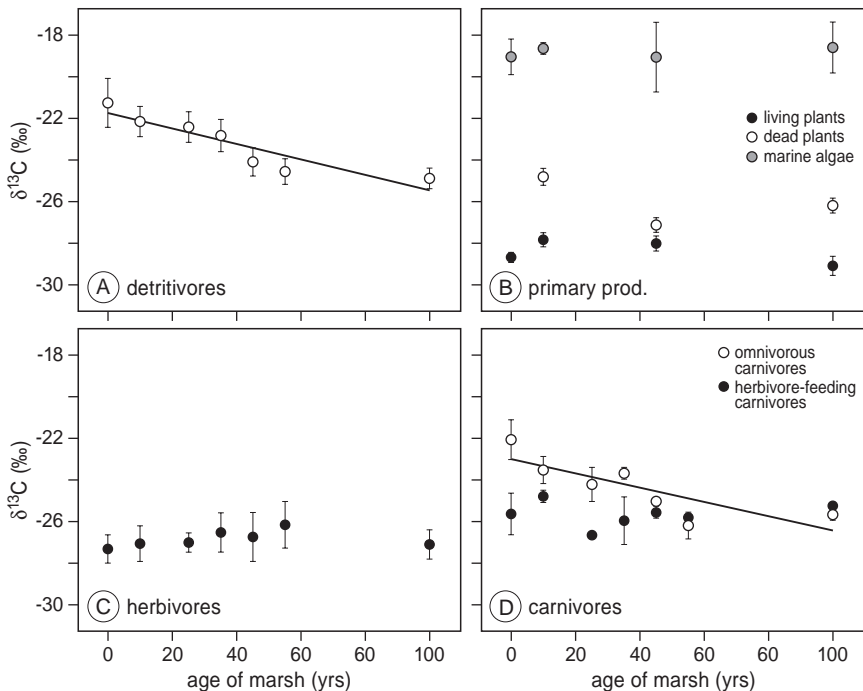
On average herbivores had 2–3 ‰ higher  $\delta^{15}\text{N}$  signatures than primary producers (Figure 3.2A & 3.2C). Also, the  $\delta^{15}\text{N}$  values for carnivores were 2–3 ‰ above values for herbivores and 3–4 ‰ above those for detritivores, except in the first stage of succession.



**Figure 3.1** All species in a CN plane depicted in four successional stages: A: 0 years, B: 10 years, C: 45 years and D: 100 years. Circles with different shadings represent different trophic groups, indicated in the legend. Note that primary producers show only very small shifts on the carbon-axis over time. Mark the widening gap between marine primary producers and other trophic groups (particularly carnivores and detritivores) over successional time.

### From early to intermediate succession

To obtain insight on how various trophic groups that either belong to the green web (terrestrial plants and herbivores) or the brown web (detritus and detritivores) are fuelled during early succession and how this changes towards intermediate succession stages, we graphed trends of average  $\delta^{13}\text{C}$  values (Figure 3.2, mostly reflecting changes in base levels) and  $\delta^{15}\text{N}$  (Figure 3.3, mostly reflecting trophic changes) over time. Differences between  $\delta^{13}\text{C}$  values of the green web and the brown web were greatest during early succession (Figure 3.2A-D). The low carbon isotopic value for detritivores ( $-21.2 \pm 0.42$ ) at the start of succession is much lower than for herbivores ( $-27.4 \pm 0.21$ ; Tukey HSD;  $N = 5$ ,  $P = 0.002$ ) and herbivorous carnivores ( $-26.0 \pm 0.9$ ; Tukey HSD;  $N = 5$ ;  $P < 0.05$ ). This suggests that detritivores derived its energy initially mostly from marine sources. Marine sources such



**Figure 3.2**  $\delta^{13}\text{C}/^{12}\text{C}$  values for all trophic groups. Symbols show averages  $\pm$  SE. Groups belonging to the brown web are depicted with open circles; groups belonging to the green web are depicted with black circles. A) Detritivores (linear regression:  $R^2$  0.41;  $P < 0.001$ ) B) marine and terrestrial primary producers C) herbivorous invertebrates D) herbivore feeding carnivores and carnivores feeding both on herbivores and detritivores (linear regression:  $R^2$  0.66;  $P = 0.02$ ).

as diatoms, bird guano and marine macro-algae had average  $\delta^{13}\text{C}$  levels between  $-21\text{‰}$  and  $-18\text{‰}$  and were most abundant in early succession. Numbers of resting marine birds and input of marine macro-algae (isotope value) were highest in the first two stages of succession (Table 3.1), with Oystercatchers (*Haematopus ostralegus*) making up the majority of resting birds. Interestingly, omnivorous carnivores (i.e., feeding on herbivores, detritivores and other carnivores, such as spiders) had similarly elevated  $\delta^{13}\text{C}$  values as detritivores ( $21.2 \pm 0.42$  vs  $-23.0 \pm 0.52$ , Tukey HSD;  $N = 5$ ;  $P = 0.5$ ), while herbivore-feeding carnivores, such as ladybugs ( $-27.4 \pm 0.21$  vs  $-26.0 \pm 0.9$ ; ) resembled herbivore  $\delta^{13}\text{C}$ -values in these early stages of succession (Tukey HSD;  $N = 5$ ;  $P = 0.5$ ). This suggests that the omnivorous carnivores were mostly feeding on the marine-subsidized detritivores during this stage of succession.

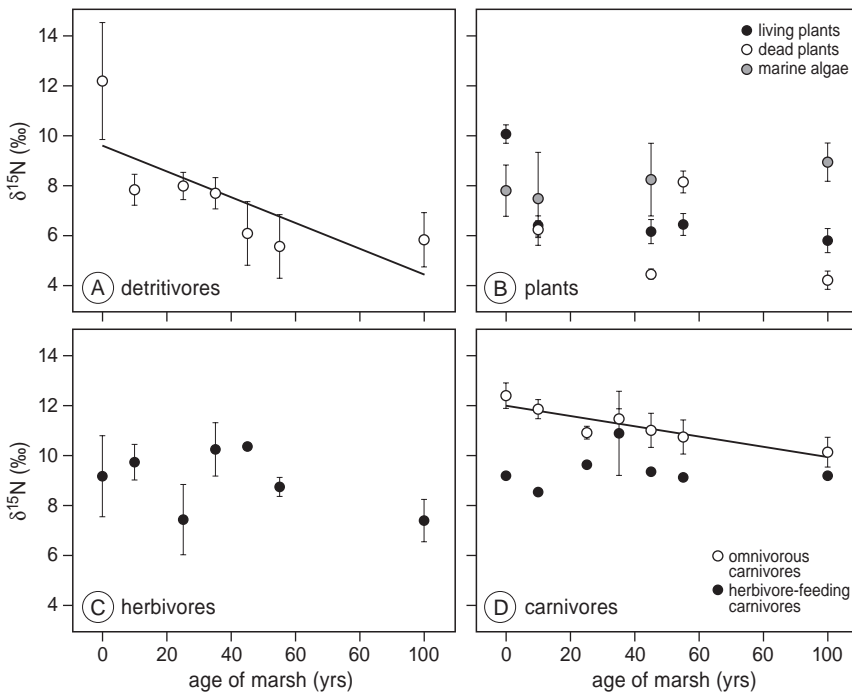
We found very high  $\delta^{15}\text{N}$  for detritivores (average  $\delta^{15}\text{N}$  value  $12.5 \pm 0.2$ ) during the first stage of succession (Figure 3.3A-D), which were especially high for Enchytraeds ( $\delta^{15}\text{N}$  between  $15.2 - 16.5$ , Figure 3.1A). This again suggests that at least some dominant species in this trophic group feed on high trophic marine sources ( $\delta^{15}\text{N}$  values  $5.6 \pm 1.1$  for macro-algae and  $8.6 \pm 0.2$  for diatoms vs  $13.0 \pm 0.3$  for bird guano). Omnivorous carnivores (average  $\delta^{15}\text{N}$  value  $11.5 \pm 0.2$ ) also yielded high  $\delta^{15}\text{N}$  values in early successional stages, indicating again a trophic link between omnivorous carnivores and the detritivore part of the web. Interestingly, the few terrestrial plant species that we sampled in early succession also had slightly elevated  $\delta^{15}\text{N}$  values compared to same plant species the next stage of succession (Tukey HSD;  $N = 5$ ;  $P < 0.001$ ; Figure 3.3B), which indicates that their nitrogen may have come from marine sources.

#### From intermediate to late succession

For herbivores, we observed no change in  $\delta^{13}\text{C}$  between early and late successional stages, while detritivores and omnivorous carnivores show a strong decrease in  $\delta^{13}\text{C}$  isotopic signal (Detritivores:  $R^2$  0.41;  $P < 0.001$ , Figure 3.2A; Omnivorous carnivores:  $R^2$  0.66;  $P = 0.02$ , Figure 3.2D). Patterns for  $\delta^{15}\text{N}$  were similar, but somewhat less pronounced. Both detritivores and omnivorous carnivores show a gradual and significant decrease in  $\delta^{15}\text{N}$  levels over succession (Detritivores:  $R^2$  0.48;  $P < 0.05$ , Figure 3.3A; Omnivorous carnivores:  $R^2$  0.81;  $P < 0.005$ ; Figure 3.3D), while herbivores and herbivore feeding carnivores remain stable over succession (Figures 3.3C, 3.3D). Both marine and terrestrial primary producers have the same  $\delta^{15}\text{N}$  signal in the later stages of succession. The clear observed differences in  $\delta^{13}\text{C}$  signal between herbivores and detritivores during early succession disappeared towards later successional stages. This was accompanied by a gradual

**Table 3.1** Resting marine birds (in bird minutes  $\text{hr}^{-1} \pm \text{SD}$ ) and amount of marine macro-algal input ( $\text{mg m}^{-2} \text{ month}^{-1}$ ) in each of the stages of succession. Bird minutes were calculated by multiplying the number of birds times the number of minutes birds spend in each hourly observation in each hectare plot.

Species	Number of bird minutes per hour per hectare succession stage (years)																	
	0	SD	10	SD	25	SD	35	SD	45	SD	55	SD	100	SD				
Oystercatcher ( <i>Haematopus ostralegus</i> )	32572	30840	40	44	0	0	0	0	0	0	0	0	0	0				
Dunlin ( <i>Calidris alpina</i> )	1800	2080	692	760	0	0	0	0	0	0	0	0	0	0				
Brent goose ( <i>Branta bernicla</i> )	452	520	420	460	0	0	0	0	0	0	0	0	0	0				
Herring gull ( <i>Larus argentatus</i> )	152	172	20	20	0	0	0	0	0	0	16	16	0	0				
Black-headed gull ( <i>Chroicocephalus ridibundus</i> )	60	68	0	0	0	0	0	0	0	0	0	0	0	0				
Eider duck ( <i>Somateria mollissima</i> )	0	0	360	396	0	0	0	0	0	0	0	0	0	0				
Grey plover ( <i>Pluvialis squatarola</i> )	0	0	20	20	0	0	0	0	0	0	220	200	0	0				
Lesser black-backed gull ( <i>Larus fuscus</i> )	0	0	40	44	0	0	0	0	0	0	0	0	0	0				
Redshank ( <i>Tringa totanus</i> )	0	0	32	36	0	0	0	0	0	0	0	0	0	0				
Total Marine Birds	32572	30840	1624	1304	0	0	0	0	0	0	236	216	0	0				
Marine macro-algae ( $\text{mg m}^{-2} \text{ month}^{-1}$ )	594	223	797	196	5	3	2	1	2	0	3	0	6	1				



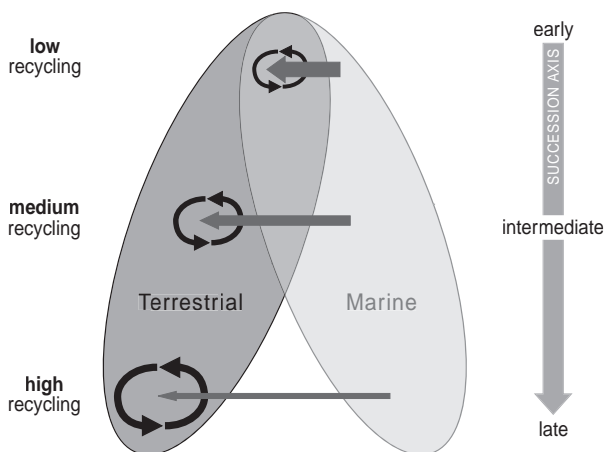
**Figure 3.3**  $\delta^{15}\text{N}/^{14}\text{N}$  values for all four trophic groups. Symbols show averages  $\pm$  SEM. A) Detritivores (linear regression:  $R^2$  0.48;  $P < 0.05$ ), B) terrestrial and marine primary producers C) herbivores D) carnivores feeding on herbivores and carnivores feeding both on herbivores and detritivores (linear regression:  $R^2$  0.81;  $P < 0.005$ ). Note the high value for  $\delta^{15}\text{N}$  in the first succession stage for both A and B.

replacement in detritivore species. While in early succession *Fucellia maritima* and Enchtraeid worms are dominant, beach hoppers (*Orchestia gammarellus*) comprise most of the detritivore biomass in later stages of succession.

## Discussion

Our results strongly suggest that during this primary succession over a period of more than 100 years, the energy and nutrient sources fuelling this terrestrial food web changed from mostly driven by external, marine inputs towards a dependence on internal, terrestrial nutrient cycling (Figure 3.4). Both macro-detritivores and omnivorous carnivorous invertebrates (i.e., the





**Figure 3.4** Conceptual overview of changes in nutrient supply to the food web over a gradient of successional stages. The two circles depict two ecosystems: in this case the intertidal marine and the terrestrial salt marsh ecosystem. Dark grey arrows indicate the magnitude of nutrient flow from the marine towards the terrestrial ecosystem and the circular black arrows indicate the magnitude of local nutrient cycling. We distinguish three distinct phases during succession: early, intermediate and late succession. In early succession, external (marine) input of nutrients supports a brown web (detritus-detritivore dominated, indicated by a thick grey straight arrow from marine to terrestrial) in which local internal (terrestrial) production is very low (shows as small black circular arrows indicating low cycling). Intermediate succession is characterized by a substantially lower external input of nutrients and a fairly high local production. The local production sustains a sizable green web (plant-herbivore dominated) and fuels most of the brown web although some species still rely on external input for nutrients. Late succession is characterized by very low external input and high local production of low quality plant material. Most of the primary production is turned into litter as herbivores are virtually absent from the system. Most of the biomass in the food web is therefore dependent on terrestrially produced litter.

brown food web component) showed a remarkable shift in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values along the studied chronosequence, while other components of the food web, such as plants and their herbivores (i.e., the green web food component) remained more similar in this respect. This pattern is likely caused by both a decline in marine inputs during succession and an increase in nutrient pools and nitrogen mineralization due to the development of a litter layer as observed in earlier work (Van Wijnen & Bakker 1999). So, the role of the brown web along this successional chronosequence changes from being vectors of external nutrients in early succession towards agents of internal cycling in late succession, while the green part of the food web remains dependent on local production all along the successional gradient.

### **What causes succession to start?**

The main sources of energy and nutrients for the food web assembly in the first stage of succession shows many similarities to the Baja California islands that were described by Polis and Hurd (1995). Both have a high marine input, a low local primary production and a high abundance of invertebrates that do not rely on locally produced organic material, but rather on detritus. Peak standing biomass of living vegetation in our earliest stage varies between 5–50 g m<sup>-2</sup> and covers only up to 2% of the soil surface, while in later stages of succession peak standing biomass is between 830–1050 g m<sup>-2</sup> (Schrama *et al.* in prep). A difference, however, between our first succession stage and the desert islands described by Polis & Hurd (1995), is the much higher rainfall and nutrient inputs by inundations and guano from waders at our study site, which allows the onset of long-term succession towards dense vegetation.

Therefore, we hypothesize that nutrients that enter in the first stage of succession operate as a ‘kick-start’ to long-term food web assembly. Which of the different components of marine subsidy, i.e., guano, macro-algae, diatoms or carrion is the most important source for fuelling the food web at this stage of succession was not the focus of our study. However, our bird count data suggests that nutrients from bird guano may play a very important role in starting vegetation succession and associated food web assembly. Using data from (Zwarts & Blomert 1996) on fecal nutrient concentrations of marine birds, combined with our observed densities, we can roughly estimate that the first stages of succession may receive up to 30 kg of N per ha per year, while macro-algae yield only 8–10 kg N ha<sup>-1</sup> yr<sup>-2</sup>. As the main bird species responsible for this are non-migratory oystercatchers, this input is relatively constant throughout the year, not limited to specific seasons. Furthermore, this nitrogen input by high trophic level marine-feeding predators provides a good explanation for the relatively heavy isotope signals that we found for early successional plants and detritivores. So, we conclude that our earliest successional stages can be seen as marine-subsidized food webs, both at the trophic level of the primary producers as well as on higher trophic levels.

### **Causes of declining marine inputs**

The suggested reduction of marine inputs towards later successional stages is likely caused by a combination of factors: marine birds avoid roosting in denser vegetation, and a ‘sieving effect’ of taller vegetation in older stages at the direct edge of the salt marsh, which often prevents drifting of larger marine material onto the marsh at high tides. The consequences of the observed decrease in external nutrient inputs of marine origin are most clearly observed in the isotopic signal for detritivores and omnivorous carni-

vores. While in early succession these groups exhibit a strong external (marine) signal, the diminishing external marine input -not only in relative but also in absolute terms - causes the brown part of the food web to rely more on the local production of plant litter as succession proceeds. Local plant biomass production is high in late successional stages and so is local litter input (Chapter 2, Olff *et al.* 1997). Detritivore species now have isotopic signals similar to plants, carnivores and herbivore species, which indicates that both the green and the brown part of the food web mainly use terrestrially produced organic matter as a source of nutrients. The increase in N mineralization towards later successional stages is driven by the enhanced total pool of soil nutrients which accumulates during succession due to vegetation development, a cumulative effect of more nutrient inputs than losses during all stages (Olff *et al.* 1997; Van Wijnen & Bakker 1999). Therefore, we conclude that the combined decrease in the input of marine, external organic material and an increase of the primary production causes the both the green and brown parts of the food web to become increasingly 'fuelled' by local primary production towards late successional stages.

### **The role of carnivorous and detritivorous invertebrates in early succession**

From other studies on primary succession it is known that spiders and ground beetles are amongst the first carnivorous species to invade an area (Hodkinson *et al.* 2001; Kaufmann 2001; Coulson *et al.* 2003). We also observe this in our salt marsh food web assembly, where omnivorous carnivores and detritivores thrive on external inputs in the earliest successional stages. On early successions at glacier forelands and volcanic sediments, external inputs of windblown insects are also an important organic source for early food web assembly (Edwards & Sugg 1993; Coulson *et al.* 2003). This will also happen in our system, but we suggest that on salt marshes the importance of this input is minor compared to marine nutrient inputs. Although the relative importance of local primary production versus the external input of energy and nutrients as a food source for these early soil dwelling carnivores and detritivores has seldomly been addressed, our findings emphasize the importance of external marine inputs of nutrients and energy to the first stage of succession. This upsets the idea further that colonizing plants are required to 'get nutrient cycling and vegetation succession going'.

### **Consequences of declining detritus quality**

The quality of the coarse detritus that is decomposed by the brown part of the food web in early succession is likely much higher than the later stages (Olff *et al.* 1997), even though both stages of succession are dominated by

members of the brown web (Chapter 2). Enchytraeid worms and *Fucellia maritima* are dominant species in the earliest succession stage, and they likely both feed on marine sources of high resource quality. The intermediate to later stages of succession have larger stocks of lower quality organic material locally produced by terrestrial plants. This decline in litter quality is most likely driven by increasing importance of light competition among plants, which results in dominance of grass species with high investments in structural tissues of the dominant plant species (Huisman & Olff 1998) and resulting low litter quality (Olff *et al.* 1997). The macro-detritivores in this part of the salt marsh consist almost entirely of the species *Orchestia gammarellus*, a semi-terrestrial amphipod. Its litter processing as well as its digging behavior make litter more easily accessible and decomposable for bacteria and fungi (Moore & Francis 1986). We suggest that because of the lower quality of litter in late succession, litter preprocessing by these macro-detritivores may be essential for nutrient mineralization. So, where the first stage of succession resembles a marine food web, in the sense that it has high quality organic material, high turnover of organic matter and low standing plant biomass (Cebrian & Lartigue 2004; Shurin *et al.* 2006), it gradually changes into a typical terrestrial food web with lower organic matter quality, slower cycling of nutrients and higher standing plant biomass.

## Conclusions

We suggest that our findings represent a general pattern of ecosystem assembly, which is schematically represented in Figure 3.4. The earliest successional stages are subsidized with external, high quality organic material which 'kick starts' early successional vegetation development. After the initial kick start, internal cycling of nutrients becomes progressively more important, where the brown part of the food web increasingly depends on this internal cycling of nutrients. The amount of subsidy of organic matter will determine the subsequent rate of food web assembly. From several other ecosystems it is known that primary succession is happening much slower, which is likely caused by lower initial inputs. However, several recent studies on primary succession (Sugg & Edwards 1998; Kaufmann 2001; Hodkinson *et al.* 2004), now seem to agree on the importance of some form of external input of nutrients to the system to initiate long-term developments. For our ecosystem we conclude that 100 years of food web assembly proceeds by gradual decoupling of terrestrial nutrient cycling from the marine environment, by associated rearrangements in energy channels between the brown and green part of food webs, and increasing importance of macro-detritivores for nutrient cycling during later successional stages. Food web assembly thus interacts with nutrient and energy flows across ecosystem boundaries.

## Appendix II

**Table II.1** General characteristics for each of the sampling sites.

Age succ. stage (yrs)	Elevation (cm above NAP $\pm$ SE)		Flooding freq. (yearly average 1998-2008 $\pm$ SE)*		Sediment layer thickness (cm $\pm$ SE)		Vegetation height (cm $\pm$ SE)	
0	115.2	2.3	184.2	5.8	0.0	0.0	1.2	0.3
10	116.2	2.0	178.3	5.9	4.9	0.3	5.9	0.7
25	116.0	3.2	178.3	5.9	7.2	0.2	10.2	1.3
35	118.4	1.9	164.8	6.5	13.5	0.6	8.1	1.7
45	123.4	1.9	136.9	6.5	15.3	0.2	9.4	0.8
55	125.0	2.1	127.7	7.0	14.4	0.3	19.7	3.8
100	124.4	1.5	131.8	6.8	16.2	0.8	26.1	2.0

\* Flooding data was taken from an online archive with freely available measurements, which can be downloaded from [http://live.waterbase.nl/waterbase\\_wns.cfm?taal=en](http://live.waterbase.nl/waterbase_wns.cfm?taal=en)

**Table II.2** The amount of subsamples per site for all collected species. Every subsample is composed of at least 5 individuals. Not all species were identified up to the species level, those were given a 'sp.' behind the genus name. When more than one species was collected per taxa, 'spp.' is given behind the genus name.

Group	Species	Age of succession stage						
		0	10	25	35	45	55	100
Primary production								
Terrestrial plants	<i>Artemisia maritima</i>					5		5
	<i>Atriplex portulacoides</i>		5			5		5
	<i>Elytrigia atherica</i>							5
	<i>Festuca rubra</i>		5			5		5
	<i>Limonium vulgare</i>	5	5			5		
	<i>Puccinellia maritima</i>	5	5			5		
	<i>Salicornia europaea</i>	5	5					
	Soil organic matter		2			5		5
	Terrestrial organic matter		5			5		6
Marine prim. Prod	Bacillariophyceae (Diatoms)	5	5			5		4
	Particulate organic matter		5			4		3
	<i>Fucus vesiculosus</i>		4			5		4
Herbivores	<i>Bledius</i> sp.	5						
	<i>Cassida vittata</i>	4	4	3	4			
	<i>Auchenorrhyncha</i> spp.							1
	<i>Chrysomelidae</i> sp.		1	1	1	3	1	1
	<i>Elateridae</i> sp.	1		1			2	
	<i>Curculionidea</i> sp.		2	1	3	2	4	
Carnivores	<i>Bembidion minimum</i>		3	2	3	5	2	5
	<i>Clubiona stagnatilis</i>	5	1	2	1	2	5	1
	<i>Coccinella sedecumpunctata</i>	5		3	2			
	<i>Dyscherius globus</i>	1	3	5	4	2	2	
	<i>Ichneumonoidea</i> spp.	1	1	1			1	
	<i>Erigonidae</i> spp.	4	5	3	5	2	3	3
	<i>Pardosa pubeckensis</i>	5	4	5	4	5	5	4
	<i>Pogonus chalceus</i>	1		2	1			
	<i>Salda littoralis</i>	1	1	4	4	3	3	1
	<i>Tytthaspis sedecumpunctata</i>	1	2		2	4	5	1
Detrivores	<i>Helophorus brevipalpis</i>							4
	<i>Isotoma riparia</i>		1			4	2	2
	<i>Platynothrus</i> sp.			1				
	<i>Symplecta stictica</i>		2	4				
	<i>Nemotelus</i> sp.		3	2	1	4	3	
	<i>Ochthebius marinus</i>		4	4	3	3	1	1
	<i>Orchestia gammarellus</i>	4	5	4	5	5	5	5
	<i>Ovatella myosotis</i>		4	5	5	5	5	5
	<i>Phyllocia moscorum</i>							1
	<i>Fucellia maritima</i>	4						
	<i>Enchytraea</i> sp.	5						





# Chapter 4





# 4

## Decoupled diversity dynamics of green and brown food web components during salt marsh primary succession

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*Manuscript in preparation*

### Abstract

Most terrestrial ecosystems are characterised by a strong functional connection between the green (plant-herbivore-based) and brown (detritus-detritivore-based) parts of the food web. Also, the strong relationship between species diversity and ecosystem functioning is increasingly recognized. However, the linked changes of diversity within green and brown food web compartments in relation to key ecosystem processes are rarely studied. Here, we report changes in species richness and diversity over a wide range of invertebrate green and brown trophic groups during 100 years of primary succession on a salt marsh, using a calibrated chronosequence. We contrast two hypotheses on the relationship between green and brown food web diversity: coupled versus decoupled dynamics. We found that while species richness for plants and invertebrate herbivores (green web groups) peaked at intermediate productivity, the diversity of macro-detritivores, microbivores and secondary consumers (brown web groups) continuously increased towards the latest successional stages. We explain this decoupling of diversity between green web and brown web trophic groups by different drivers of their diversity. While green web trophic groups are found to be mainly driven by vegetation parameters, such as the amount of bare soil, vegetation biomass production, and vegetation height, brown web trophic groups are mostly driven by production and standing stock of dead organic material and soil development. These results suggest that the understanding of terrestrial ecosystem functioning from their community structure requires the simultaneous study of green and brown food web compartments.

## Introduction

Plant-herbivore-based and detritus-detritivore-based parts of food webs have traditionally been studied in separation. But there is now increasing recognition that green and brown, and above- and belowground components of ecosystems strongly affect each other (Moore *et al.* 2004; Wardle *et al.* 2004a; Bardgett & Wardle 2010). This calls for the study of their joint changes in structure and function along environmental gradients. Relatively independently from this notion, the relationship between species diversity and key components of ecosystem functioning is increasingly studied for many species groups, including soil biota (Nielsen *et al.* 2011), plants (Tilman *et al.* 1996), and aboveground predators (Griffin *et al.* 2008). In general, these studies find a positive relation between diversity and productivity, with the magnitude of effects determined by the identity of the species involved (Hooper *et al.* 2005; Cardinale *et al.* 2006). A third (and older) related line of scientific exploration has investigated how and why species diversity changes in space and time, including along productivity gradients (reviewed by Rosenzweig 1995). However, the causal directionality and ecosystem importance of this relationship is still hotly debated (Grace *et al.* 2007; Adler *et al.* 2011).

Most of these studies typically focus on one particular trophic group or food web compartment (either plants, decomposers, herbivores, or predators), due to the specific interest or (taxonomic) expertise of the investigators involved and due to logistic limitations. When multiple species groups are studied simultaneously in food webs, then the taxonomy (and thus diversity) of the food web compartments is generally poorly resolved (Cohen *et al.* 1993; Berlow *et al.* 2004; Berg & Bengtsson 2007). Only in the later stage of meta-analysis, different species groups are jointly taken into account, but with the strong downside that data on different species groups often originate from different types of ecosystems, or within ecosystems are derived from spatially separated locations. Therefore, it is still unclear what the relationships are between the diversity patterns of the main different trophic groups (green and brown) in one and the same ecosystem along spatial and temporal gradients. This is what we aim to unravel in the current study.

Interestingly, to what extent the changes in species richness among green and brown trophic groups follow similar patterns over time and space and to what extent they react to the same environmental variables is a topic that has not received much attention in terrestrial ecosystems. Herbivores have been argued to follow plant species richness patterns closely (Hutchinson 1959) and evidence for this has been found by a number of studies (Brown & Southwood 1983; Edwards-Jones & Brown 1993; Knops *et al.* 1999; Siemann *et al.* 1999a). Others have argued herbivore diversity rather to depend on

plant productivity and vegetation structure (Olff & Ritchie 1998; Mittelbach *et al.* 2001; Hawkins & Porter 2003). For secondary consumers, few attempts have been made to link species richness to changes in plant species richness, and only limited evidence exists that this is actually the case (Arim & Jaksic 2005; but see Eisenhauer *et al.* 2011). For spiders, vertical vegetation structure has been argued as the principal component governing species richness (Greenstone 1984; Langellotto & Denno 2004), but information is scarce. For birds foraging on leaf-dwelling invertebrates, the structure of the vegetation and substrate also has been argued to be the principal determinant of resource partitioning and thus diversity of consumers (Root 1967).

A higher diversity of plant species can be expected to result in a larger variety of litter resources and therefore a more diverse assemblage of detritivores. While some have shown this to be the case for herbivorous nematodes (Bardgett & Wardle 2010) and for a number of other soil fauna groups (Eisenhauer *et al.* 2011), others have argued that species richness for detritivores may be regulated by a soil vertical structure, rather than by plant diversity (Berg & Bengtsson 2007; Krab *et al.* 2010). For instance, a monoculture of Scots pine forest was found to harbour high species diversity for soil fauna due to a strong vertical stratification of litter quality (Berg *et al.* 2001; Berg & Bengtsson 2007). This would imply that belowground/brown diversity is regulated differently than, and independent from aboveground/green web diversity.

Two main hypotheses can be formulated for the general relationship between green and brown food web components: i) the diversity of the green and brown web are coupled as they covary simultaneously with the main drivers in ecosystem processes or ii) their diversity will be uncoupled as they are subject to independent drivers and insensitive to each other's level of diversity. A possible line of argumentation leading to the first hypothesis would be if plant diversity plays a central role in the consumer diversity of both green and brown food web compartments (bottom-up regulation of diversity). For example, this would occur when more plant diversity leads to more resource diversity for herbivores, decomposers and in turn to more diversity in their enemies (Srivastava *et al.* 2009). Uncoupling the diversity of green and brown trophic groups can instead occur if one is more bottom-up regulated and the other more top-down, or if different ecosystem processes determine the diversity of each group of organisms independently. Uncoupled dynamics of food web components will also occur if herbivores and herbivore-feeding carnivores are expected to react stronger to plant diversity (following Brown & Southwood), while detritivores are reacting primarily to litter layer development and vertical stratifications therein (following Scheu & Schultz 1996, Bengtson & Berg 2006, Berg *et al.* 2010).

The aim of this study was to discriminate between both hypotheses using a salt marsh primary succession chronosequence, in which the diversity dynamics of dominant green and brown trophic groups was studied to the species level. This chronosequence has been calibrated to a period of 100 years (Olff *et al.* 1997). Primary succession leads to (directional) build-up of plant productivity and corresponding changes in plant species diversity (Tilman 1982, Olsen 1958, Clements 1916, Olff *et al.* 1997), as well as to changes in invertebrate and vertebrate species composition (Edwards & Sugg 1998, Kaufmann 2001, Chapin III 1994). The advantages of the particular successional sequence that we used are that 1) the different stages are spatially close together (within 1–3 km), 2) the rather low species richness makes the study logistically feasible, and 3) most plant species, and to a lesser extent invertebrates, are dispersed by water, as the area is frequently flooded. Therefore, we can exclude dispersal limitation as a possible cause of changes in ecosystem structure, making the interpretation of observed patterns in species richness easier (Wolters & Bakker 2002). It therefore provides an ideal setting to study species richness patterns for different trophic groups in response to biotic and abiotic drivers, and in response to each other.

## Methods

### Description of salt marsh chronosequence

The species diversity of the different brown and green trophic groups were performed on the salt marsh of the island of Schiermonnikoog (53°30' N, 6°10' E), the Netherlands, in July 2008. The average yearly temperature on the island is 10.2°C ( $\pm$  SD 0.72°C), average annual rainfall is 824 mm ( $\pm$  SD 149.1 mm) (data from the Royal Netherlands Meteorological Institute KNMI at [www.knmi.nl](http://www.knmi.nl)). On this salt marsh a well-described chronosequence is present, which spans one hundred years of succession (Chapter 2; Olff *et al.* 1997). The earliest stages are formed on the east side of the island, late succession stages are situated 8 km to the west. The justification of the space-for-time replacement of this succession has been checked by monitoring permanent vegetation plots and key soil parameters in different successional stages during the last 20 years (Chapter 2; Olff *et al.* 1997). Along this gradient of successional stages, vegetation composition changes from a state with very sparse vegetation (dominated by *Salicornia europaea*, *Puccinellia maritima*) towards a species-rich vegetation in intermediate succession stages (dominated by *Festuca rubra*, *Limonium vulgare* and *Plantago maritima*) and finally a tall, species poor canopy (dominated by the grass *Elytrigia atherica*) in late succession stages.

## Sampling design

Seven successional stages were identified, estimated as 0, 10, 25, 35, 45, 55 and 100 years of succession age in 2008. More details on vegetation composition in the different successional stages can be found in Appendix I, table 1. Salt-marsh age at each successional stage was estimated from topographic maps, aerial photographs, and the thickness of the sediment layer accumulated on top of the underlying, fixed sand layer (Oloff *et al.* 1997). The sites were selected to have a equal base elevation (position at the initial elevation gradient on the bare sand flats with a base elevation of 1.16m ( $\pm$  SE 0.02m above Amsterdam Ordnance Level (NAP)). Different base elevations will have different inundation regimes, and therefore each elevation has its characteristic vegetation succession (Oloff *et al.* 1997). As the salt marsh matures, sediment trapping by the vegetation at a base elevation of 1.16m increases the elevation of the soil surface with 16cm of clay over 100 years of salt marsh succession, reducing the inundation frequency by floods at spring tide (Appendix I, Table 1).

In each of the seven successional stages, five sampling points were selected. At the in total 35 sampling points, five different collection techniques were used to characterize the different components of the above- and belowground food web. Plant diversity was estimated in 2×2 m quadrats using the cover estimation method (Londo 1976). Enclosed pitfall traps ( $\varnothing$  0.1 m in 0.5×0.5 m Perspex enclosures, which were adapted after Lang (2000)) were used to gain quantitative estimates on soil inhabiting macro-invertebrates, such as spiders, beetles and bugs (Appendix I, Figure 3). Closed insect-emergence traps of 0.5×0.5 cm, 0.8 m height were used to collect flying insects emerging from the soil and vegetation (Ausden 2000). These two methods were used in the period 4–22 July and were emptied every third day in order to completely 'empty' the plot. Accumulation curves for common species indeed showed a strong leveling off towards the end of this period, suggesting that the time of sampling was sufficient to catch most specimens (Appendix I, Figure 2). Soil macro- and larger mesofauna (e.g., springtails, oribatid mites, and enchytraeid worms) was extracted from soil cores ( $\varnothing$ 10 cm, 5 cm height) using a Tullgren funnel extraction method (Van Straalen & Rijninks 1982).

## Environmental parameters

Plant biomass was used as a measure of productivity and was estimated by clipping the vegetation in 0.5×0.5 m squares, up to 1 cm above the soil. Living and dead plant biomass were carefully separated and afterwards dried at 70°C for 48 hours. To account for attached clay and sand, ash free dry weight was determined as loss on ignition at 550°C for 3 hours (Van Wijnen

and Bakker 1999). Soil temperature was measured continuously during the measurement period, using iButtons (Maxim Corp.). Soil moisture was measured using a ThetaProbe® moisture meter twice during the measuring period, respectively 7 and 14 days after the traps were put in the field.

Vegetation height was determined at three sites, two meters apart, at each of the sampling points, using a 20 gr drop-disc. In order to obtain a proxy for soil aeration, we took soil redox potential measurements at 13 November 2009. For this we used four measuring Platinum-electrodes (Pt) and one AgCl-reference electrode (Cole-Palmer®), which were all connected to a Graphtec GL200 Datalogger (Graphtec GB Ltd). Pt-electrodes were placed in a square design, 10 cm from the reference-electrode, at a depth of 2 cm in the soil. To standardize the measurements, we read out redox potential values 2 min after the electrodes were placed. Averages of the four Pt-electrodes were corrected for the value of the AgCl-reference electrode (+197 mV at pH7). Although this measurement does not provide absolute values of the amount of oxygen in situ, it gives a reasonable estimate for the relative differences in anoxia between locations measured on the same day (van Bochove *et al.* 2002).

### Division of species into feeding guilds

Invertebrates were identified up to the species level and afterwards merged into feeding groups according to the classification in Appendix I, Table 1, using feeding preferences for each species of invertebrate according to Remmert (1983), Irmiler & Heydemann (1986), Berg *et al.* (2004) Krantz & Walter(2009), Caballero *et al.* (2004) We distinguished the following trophic groups. Herbivores (sap suckers and leaf chewers: weevils (Coleoptera: Curculionidae), plant-and leafhoppers (Hemiptera: Auchenorrhyncha), aphids (Hemiptera: Aphididae), leaf beetles (Coleoptera: Chrysomelidae), moths (Lepidoptera); Algae consuming species (mainly snails (Mollusca) and rove beetles(Coleoptera: Staphylinidae); Microbivores (small soil fauna (<4 mm) feeding on litter or on litter colonizing microorganisms, such as springtails (Collembola) and oribatid mites (Acari: Oribatida); Macro-detritivores (larger fauna feeding on litter or litter colonizing microorganisms; such as diptera-larvae, *Orchestia gammarellus*, and snails); Belowground carnivores (predatory mites (Acari : Mesostigmata, Acari: Prostigmata) and other belowground predatory species, feeding preferably on microbivores) and aboveground carnivores (spiders (Araneae), ground beetles (Coleoptera: Carabidae), ladybird beetles (Coleoptera: Coccinellidae and bugs (Hemiptera:Miridae)).

### Statistical analysis

We conducted nonmetric multidimensional scaling (NMDS) analysis to examine changes in species composition over succession and we calculated

correlations of these ordinations with environmental variables. The dissimilarity matrices in the three different analyses were based on within trophic group diversity. The first analysis was based on species richness, the second was based on Shannon diversity and the third was based on Pielou's evenness. A Bray-Curtis dissimilarity metric was used to determine distances between the sampling points, for which we used metaMDS function in the R vegan package (Oksanen *et al.* 2011; RDCT 2011).

A generalized regression model was used to determine the key ecological variables with which the species richness of trophic groups correlated. In the first analysis, we used species richness of the different groups as dependent factors and environmental factors as independent variables. In the second analysis, we also used species richness of the other trophic groups as independent variables.

A general linear model was used to assess the relationship between above-ground vegetation parameters and species richness, vegetation height, live plant biomass and dead plant biomass in all other trophic groups. All statistics were done in R (RDCT 2011) and Statistica 9.0.

## Results

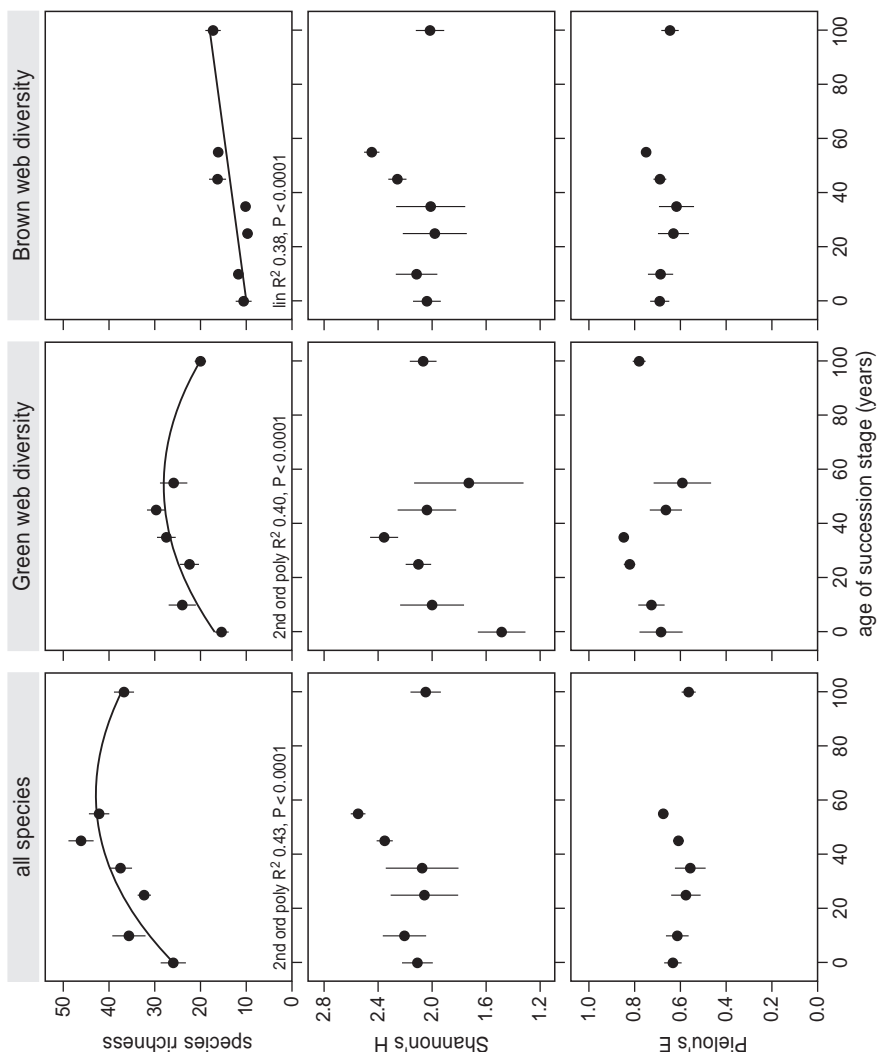
### Overall diversity

Overall, we found a clear optimum for species richness at the intermediate succession stages (Figure 4.1A). Within the complete food web, we observed strong differences between patterns of species richness for the green and brown part of the food web. Species richness in the green web showed a clear optimum at intermediate succession, while the brown web species richness showed a steady increase towards late succession (Figure 4.1B and 4.1C).

### Patterns of species richness over succession

For vascular plants, species richness first increased, but towards late succession exhibited a strong decline in the number of species (Figure 4.2D). This indicates that the species composition not only becomes less rich, but also becomes dominated by fewer species, in this case the grass *Elytrigia atherica*.

Within the green web, we found different patterns in species richness for algae consuming species, invertebrate herbivores and aboveground predators. Species richness for algae consuming species decreased with successional stage (Figure 4.2A), whereas species richness for invertebrate herbivores that feed on live plant material showed a peak at intermediate succession stages (Figure 4.2B). Both trophic groups had a very low number of species in the latest successional stage. In contrast to the former trophic



**Figure 4.1** Changes in overall species richness, diversity and evenness along the successional gradient. From left to right, the panels give the total green web and brown web species richness. From top to bottom, the panels show patterns for species richness, Shannon diversity ( $H'$ ) and Pielou's evenness ( $E$ ). Only significant correlations are shown, statistics are shown in each of the panels.



groups, species richness for aboveground predators showed a clear increase towards late succession (Figure 4.2C).

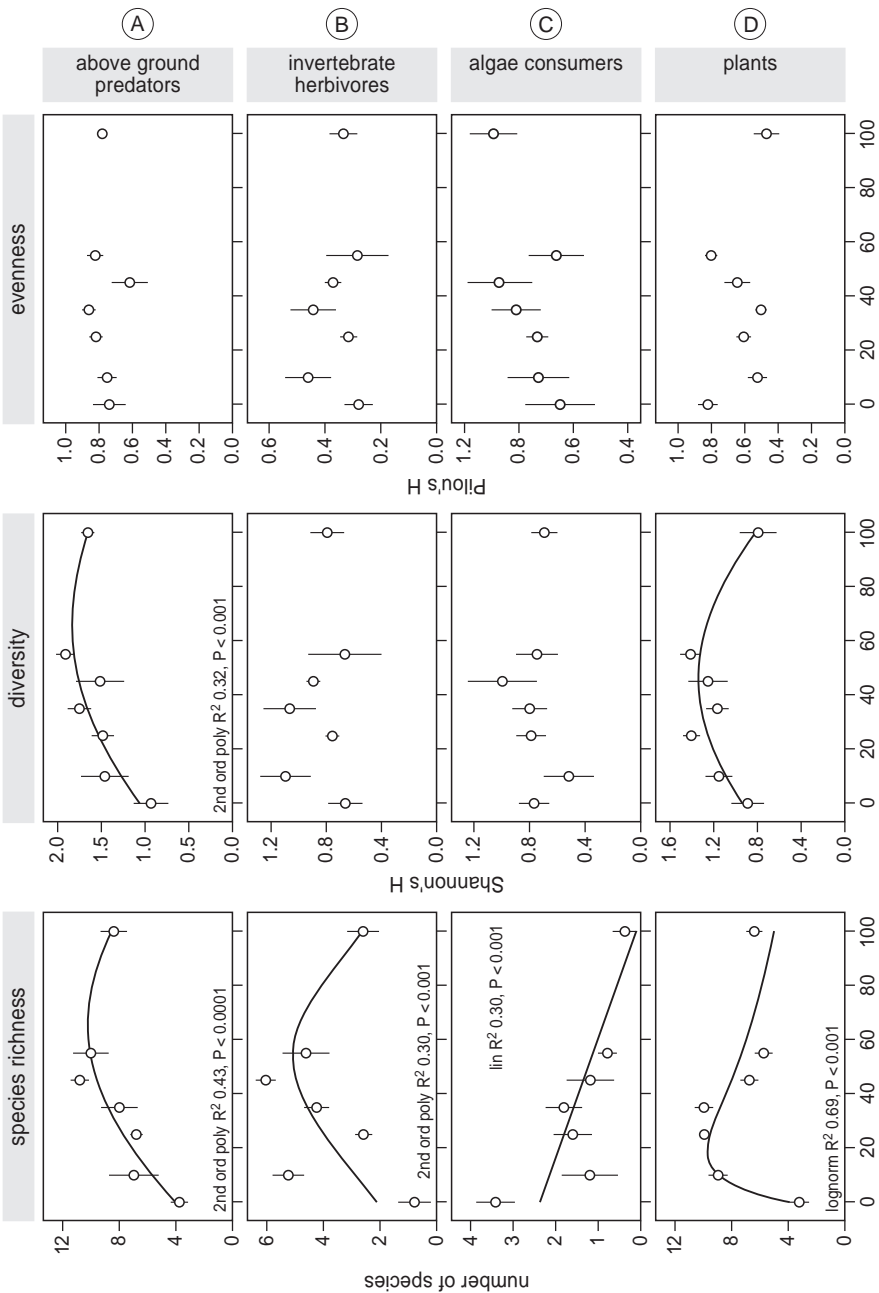
Species richness for trophic groups in the brown web showed a strikingly different pattern. Especially microbivores showed a strong increase in species richness along the succession gradient (Figure 4.2E). Also macro-detritivores (Diptera, *Orchestia*) showed an increase in species richness, which leveled off towards late succession (Figure 4.2F). Belowground predators, mainly predatory mites also showed a steady increase in species richness along succession, with a remarkable sudden increase after 45 years (Figure 4.2G).

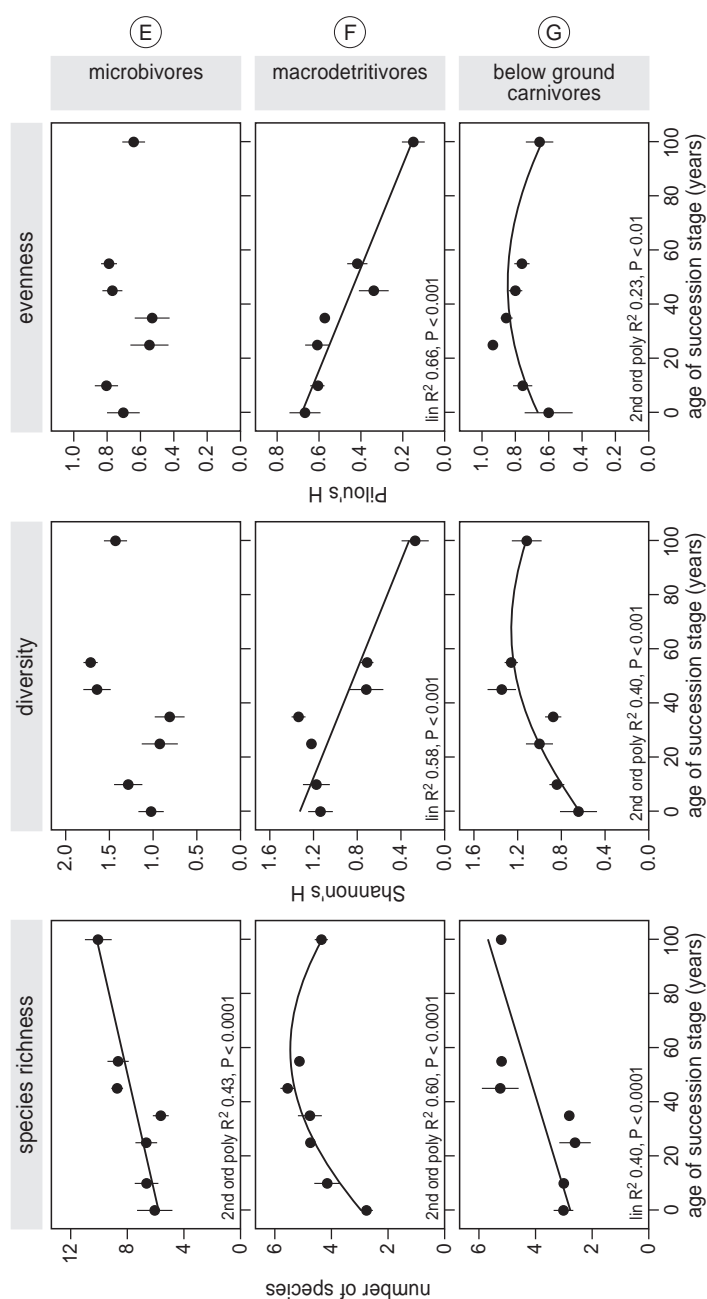
Overall, few trophic groups showed strong patterns for both Pielou's evenness and Shannon diversity ( $H'$ ). Significant correlations were found for diversity of plants and belowground carnivores, which showed a clear optimum at intermediate succession (Figure 4.2D & 4.2G). Macro-detritivore diversity and evenness showed a marked decrease towards late succession. This strong trend can be ascribed to the overwhelming dominance of *Orchestia gammarellus* in the later stages of succession.

### Relations with abiotic parameters

Plant species diversity was positively correlated to soil parameters; both soil redox potential and soil moisture contribute significantly to plant richness (Table 4.1A). Plant species richness increased with decreasing soil aeration and increased when soil moisture is higher. Together, these parameters explained 76 % of the variation in plant species diversity (Table 4.1A). Species richness of algae consumers was positively related to the amount of bare soil and negatively correlated to soil redox potential (Table 4.1A). These factors together explained 66% of the variation in algae consumer species richness. Invertebrate herbivore species richness was correlated with the amount of living plant biomass (positive) and litter biomass (negative), and soil parameters did not explain any additional variation (Table 4.1A). These two factors explained a mere 40% of the variation.

Species richness of macro-detritivores was strongly and negatively correlated to the amount of bare soil and strongly and positively correlated to the amount of dead organic material (Table 4.1A). Moreover, macro-detritivore species richness was positively correlated to soil redox potential (Table 4.1A), indicating that we found more species of macro-detritivores in well-aerated soils. Species richness of microbivores was negatively correlated to soil moisture, but positively correlated to the amount of litter and vegetation height (Table 4.1A). Species richness of belowground predators was also positively correlated to vegetation height and soil redox potential, which together explained 68% of the variation (Table 4.1A).





**Figure 4.2** Diversity for all trophic groups along the succession gradient. From left to right, the panels show patterns for species richness, Shannon diversity ( $H'$ ) and Pielou's evenness (E). Each row (A-G) gives results for a different trophic group. Open symbols indicate green web trophic groups ( $\pm$  SE), closed symbols indicate brown web trophic groups ( $\pm$  SE). Fits were calculated using all 5 sampling points per location, with  $R^2$  and  $P$ -value at the bottom of each of the graphs. Only significant fits are shown ( $\alpha < 0.05$ ).

**Table 4.1** Results from multiple regression analysis (with backwards stepwise analysis) with factors explaining variation in species in trophic groups. *A*) only includes abiotic factors *B*) includes abiotic factors and trophic groups. Results in *B*) are only presented when results were different than in *A*).

Trophic group	A			B			F	model R <sup>2</sup>	Factor	B	P	F	model R <sup>2</sup>
	Factor	B	P	B	P	F							
Plants	Whole model		0.0000			28.28	<b>0.76</b>						
	Soil moisture	0.44	0.0003			21.32							
	Soil redox potential	-0.61	0.0047			11.01							
Algae consumers	Whole model		0.0001			17.81	0.66		Whole model		0.0004	19.54	<b>0.83</b>
	% bare soil,	0.81	<0.0001			33.09			Bare soil (%)	1.39	0.0000	54.31	
	Soil redox potential	-0.32	0.0400			5.12			Soil redox potential	-0.63	0.0015	16.08	
									Vegetation height	0.55	0.0241	6.51	
									Herbivore species richness	0.57	0.0171	16.71	
Herbivores	Whole model		0.0003			14.39	0.40		Whole model		0.0001	14.62	<b>0.80</b>
	Living veg. (gram)	0.76	0.0029			5.66			Living veg. (grams)	0.38	0.0172	14.85	
	Dead veg (gram)	-0.51	0.0311			12.50			Veg height (cm)	-0.36	0.0355	15.78	
									Bare soil (%)	-0.62	0.0068	10.63	
									Aboveground predators	0.36	0.0008	6.07	
Macro-detritivores									Algae consumers	0.69	0.0034	13.21	
	Whole model		0.0002			13.99	0.70		Whole model		0.0000	32.20	<b>0.79</b>
	Bare soil (%)	-1.14	0.0000			37.33			Bare soil (%)	-0.43	0.0044	11.25	
	Dead veg (gram)	-0.62	0.0097			8.94			Aboveground predators	0.61	0.0002	22.80	
	Soil redox potential (mV)	0.58	0.0046			11.35							

Table 4.1 Continued

Tropic group	A				B					
	Factor	B	P	F	model R <sup>2</sup>	Factor	B	P	F	model R <sup>2</sup>
Microbivores	Whole model		0.0008	10.29	0.62					
	Soil moisture	-0.43	0.0133	8.03						
	Dead veg (gram)	-1.16	0.0208	6.78						
	Vegetation height (cm)	1.74	0.0016	15.19						
Abovegr. carn.	Whole model		0.0010	9.65	0.60	Whole model		0.0000	32.41	0.65
	Bare soil (%)	-1.08	0.0007	18.66		Macro-detritivore richness	0.82	0.0000	32.41	
	Vegetation height (cm)	-0.85	0.0047	11.29						
	Soil redox potential (mV)	0.90	0.0003	23.23						
Belowgr. carn.	Whole model		0.0001	19.48	0.68	Whole model		0.0001	15.47	0.72
	Vegetation height (cm)	0.59	0.0017	14.50		Macro-detritivore richness	0.30	0.0442	4.89	
	Soil redox potential	0.39	0.0255	6.15		Microbivore richness	0.43	0.0099	8.89	
						Dead veg (gram)	0.45	0.0086	9.31	

## Correlations with abiotics and species richness in other trophic groups

### GREEN WEB

Variation in plant species richness was explained by abiotic factors, not by changes in species richness of other trophic groups (Table 4.1B). It has to be noted that grazing effects of aboveground herbivores were not taken into account in this analysis. In the case of algae consumers, additional variation in species richness was explained by herbivore species richness and vegetation height, with which it was negatively correlated (Table 4.1B).

A much higher proportion of the variation in invertebrate herbivore species richness was explained when species richness of other trophic groups was taken into account (Table 4.1B). Apart from the vegetation parameters and the amount of bare soil explaining significant amounts of variation, algae consumer species richness and aboveground predator richness were positively correlated to herbivore species richness. All factors together explained 80 % of the variation (Table 4.1B). Interestingly, aboveground predator species richness correlated positively with macro-detritivore species richness. This model explained more variation (65% of the variation, Table 1) than a model that only took abiotic factors into account.

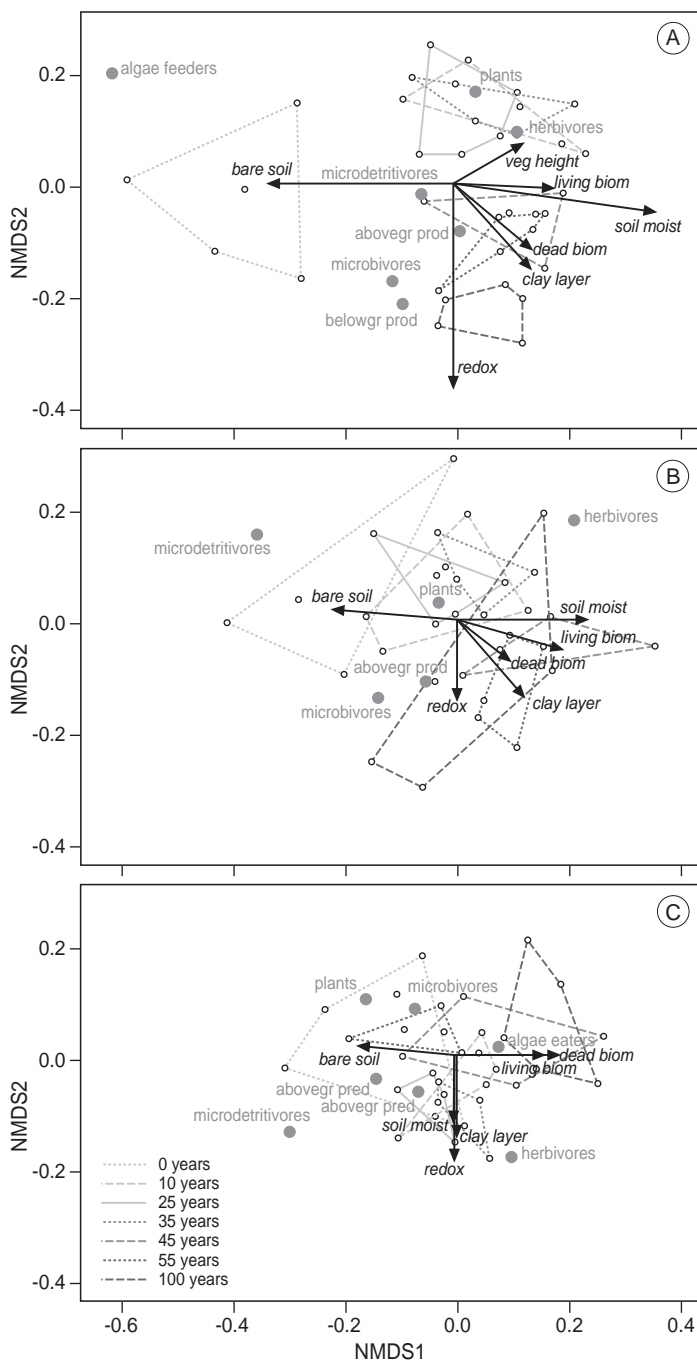
### BROWN WEB

Additional variation in macro-detritivore richness was explained by aboveground predator species richness. Taking this factor into account resulted in a model with only two parameters: percentage of bare soil (negative correlation) and aboveground predator richness (positive correlation), which together explained 79% of the variation (Table 4.1B). For microbivores, we found no relationship with species richness in any other trophic group (Table 4.1B).

Additional variation in belowground predator species richness was explained by including microbivore richness and macro-detritivore richness. Together with variation in litter material, these three factors explained 72% of the variation in species richness in this group (Table 4.1B).

The NMDS analysis based on species richness resulted in clear compositional differences in the subsequent stages of succession (Figure 4.3A). NMDS

**Figure 4.3 (right)** NMDS plots of trophic group diversity along the successional gradient. A) NMDS based on species richness in each of the trophic groups, B) NMDS based on species evenness (Pielou's E) in each of the trophic groups, and C) NMDS based on the Shannon diversity index. Age of the different successional stages is shown in given in each polygon and is indicated with a different color. Vectors indicate different environmental variables and are scaled proportionally in each of the graphs. Closed brown circles depict centroids for of the trophic groups, of which text is shown in grey.



axis 1 was most strongly correlated with the percentage of bare soil ( $r = -0.85$ ;  $p < 0.001$ ) and soil moisture ( $r = 0.77$ ;  $p < 0.001$ ), and NMDS axis 2 was most strongly correlated with soil redox potential ( $r = -0.79$ ,  $p < 0.001$ ), litter accumulation ( $r = -0.54$ ,  $p < 0.001$ ) and thickness of the clay layer ( $r = -0.47$ ,  $p < 0.001$ ). Centroids for the species richness of the trophic groups clearly aggregate with different stages of succession. Algae consuming species richness was aggregated with the earliest stage of succession, while plant species richness and herbivore species richness aggregated with the intermediate succession stages. Macro-detritivore species richness, microbiore species richness and above- and belowground predator species richness clearly aggregated with the later stages of succession. The stress-value for this analysis was strong ( $K = 0.133$ ).

NMDS analyses based on Pielou's evenness and Shannon diversity explained a much smaller amount of variation in compositional differences between the different successional stages (Figure 4.3B & 4.3C) and correlations with the environmental factors were also much weaker. Also stress values for these analyses were less strong, 0.215 and 0.200 for the analysis based on Shannon-diversity and 0.200 Pielou's evenness, respectively.

## Discussion

Along the investigated primary succession, our results show strikingly different patterns for species richness in the green and brown compartment of the food web. Trophic groups in the green web showed an apparent increase from early successional stage followed by a decrease in species richness towards later stages of succession, whereas trophic groups in the brown web showed a has the highest species richness at later stages. Our results therefore support the hypothesis that brown web species diversity becomes decoupled from green web species diversity along the studied chronosequence.

Results from our multiple regression analysis and NMDS analysis indicated that this discrepancy in green and brown web species richness along the chronosequence is likely to be caused by different factors driving species diversity, by aboveground vegetation parameters, and soil variables and dead organic matter accumulation, respectively.

## Problems regarding space for time-substitutions

A common problem with studies on community assembly along chronosequences is that current conditions at early successional stages may not necessarily reflect past conditions at older locations locations (Pickett 1989;

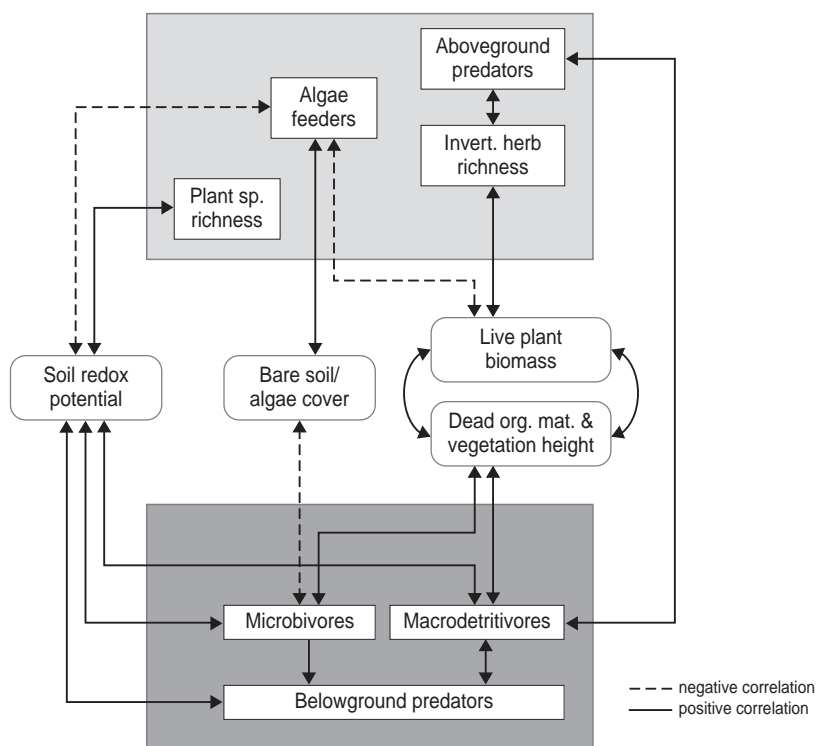


Mattews 1992). However, the development of vegetation patterns along the chronosequence on Schiermonnikoog has been tested extensively (Chapter 2, Olff *et al.* 1997). Also, the lack of large topographic variation on this salt marsh prevents variation in climatic conditions associated with small differences in elevation, which is often the case in chronosequences on glaciers and volcanic substrates (Kaufmann 2001). While species colonisations from adjacent areas are often a confounding factor in diversity measurements along chronosequences, our aboveground sampling methods (closed traps) were specifically designed to minimize such effects. By using soil samples to characterise belowground communities, such confounding effects are expected to be negligible as dispersal of belowground invertebrates is generally assumed to be a very slow process (Van der Putten *et al.* 2001).

The time scales along which community assembly operates along our studied chronosequence were relatively short (100 years), much faster than for most other studies on community assembly (Mattews 1992; Kaufmann 2001; Hodkinson *et al.* 2004). This probably reflects the high rates of external nutrient input in our study system by regular flooding with slightly eutrophicated seawater. Rates of ecological succession may be even higher in mainland salt marshes (Bakker *et al.* 2002b), where clay accretion often happens at a much faster rate. Nonetheless, many of the measured environmental variables show similar and directional trends to those in other studies on chronosequences. For example, the amount of bare soil and soil moisture clearly decreased over the chronosequence. On the other hand, soil organic matter build-up, standing live biomass and vegetation height showed strongly increased along the successional stages. These findings correspond with many other chronosequence studies (del Moral 1993; Kaufmann 2001; Hodkinson *et al.* 2004), and suggest that the observed changes in species diversity patterns along our chronosequence are not different from other succession sequences.

### **Drivers of green web diversity**

Our multiple regression analyses showed a positive relationship between vegetation biomass-related components and the invertebrate herbivore richness, but no correlations were found between species richness in these trophic groups and plant species richness. Other studies have found that higher species richness of plants leads to increased species richness in higher trophic levels (Brown & Southwood 1983; Brown & Ewel 1987; Steffan-Dewenter & Tscharntke 1997; Siemann *et al.* 1999a). Instead, our study suggest that the plant species richness may be of much less importance for herbivore species richness than the structure of the vegetation, which has also been argued by others (Hawkins & Porter 2003; Rickert *et al.* 2012, in



**Figure 4.4** Species diversity in the different trophic groups of the green and brown compartment of the salt marsh food web together with the dominant environmental drivers (rounded boxes) and biotic drivers (black outlined boxes). The dark grey box illustrates the brown part of the food web and the light grey box illustrates the green part of the food web.

press). Interestingly, most studies that report a positive effect of plant species richness on aboveground arthropod richness, were done at experimental sites with artificial plant communities, where vegetation height and biomass were tried to keep constant (Knops *et al.* 1999; Siemann *et al.* 1999b; Symstad *et al.* 2000). On the other hand, studies that report a strong influence of vegetation structure on herbivore arthropod richness were mostly done in natural communities, where vegetation structure and plant species richness often show a hump shaped relationship with productivity (Hawkins & Porter 2003; Rickert *et al.* 2012). Therefore, we speculate that, although plant species richness may beget herbivore species richness in experimental situations, along natural primary successions, other factors that represent vegetation structure are of overwhelming importance for the explanation of invertebrate

herbivore species richness. Also for algae consumer richness, we found an abiotic factor that explained most of the variance in species richness along the chronosequence, in this case the amount of bare soil. We hypothesize that this relationship is caused by the higher availability of algae on bare sand as opposed to vegetated parts of the chronosequence (Figure 4.4). Other studies have also reported high species richness of algae consuming species in early successional stages (Kaufmann 2001; Hodkinson *et al.* 2004), where the amount of bare soil was high. Algae consuming species richness also reacted negatively to increasing redox potential (Figure 4.4), which probably reflects the disappearance of the algal mat when the soil becomes more well-aerated (due to for instance activity of bioturbating *Macrofauna*).

This leads us to the conclusion that green web species richness on the salt marsh is driven primarily by abiotic factors, such as vegetation structure and resource availability, rather than by vegetation diversity, thus strongly contrasting with findings from other studies.

### Drivers of brown web diversity

In contrast to the drivers of green web species richness, we find brown web species richness to be primarily driven by belowground abiotic parameters and soil organic matter production (Figure 4.4). This is in agreement with other studies (Scheu & Schulz 1996; Carlson *et al.* 2010), but appears to be in contrast to Eisenhauer *et al.* (2011), who reports higher diversity of soil fauna to be associated with higher plant species richness. However, the latter study has not distinguished between soil biota belonging to the green and brown part of the food web, which complicates the comparison.

We speculate that the observed increase in dead organic matter and the development of a clear litter layer in later stages of succession may be the result of a decrease in vegetation quality and a coinciding decrease of (vertebrate) herbivory (Van de Koppel *et al.* 1996; Olff *et al.* 1997; Van der Wal *et al.* 2000b). Later stages of succession are dominated by the grass *E. atherica*, which indeed is presumed to be of low quality for both herbivores (Kuijper *et al.* 2004) and detritivores (Hemminga (Hemminga & Buth 1991). If this hypothesis is correct, we would predict that a less dominant green web in late succession may lead to a low amount of leaf consumption and a corresponding increase in litter C:N-ratio, followed by an increase in dead organic matter production. The different steps that are needed to remineralize recalcitrant litter may in turn result in an increase in the amount of niches and may therefore be accompanied by an increase in species richness in the brown web.

Especially for microbivore richness and belowground predator species richness, we found a strong increase in species richness over succession. This

agrees with other studies which show that belowground species diversity is more regulated by vertical soil stratification than by vegetation diversity (Scheu & Schulz 1996; Frouz *et al.* 2001; Berg & Bengtsson 2007). In our study system, vertical stratification of the soil litter layer may increase due to activity the burrowing amphipod *Orchestia gammarellus*. This species has its highest abundances in late successional mat-forming grasses, such as *Elytrigia atherica* and *Festuca rubra* (Chapter 5). We speculate that the increased soil aeration as a result of the burrowing behaviour may lead to a well-aerated soil and hence a more pronounced and deeper vertical soil stratification with possibilities for more species to co-occur. Additional support for this effect of amphipod presence was given by the strong positive correlation between belowground trophic group richness and soil redox potential on which these animals have been shown to have a considerable effect (Chapter 5). So, in contrast to species richness in the green web, we find brown web diversity to increase towards late succession which we suggest to be driven by an increase in litter production and an increased vertical stratification of the soil (Figure 4.4).

### Drivers of diversity of secondary consumers

Our results indicate that species richness in predators both above- and belowground was strongly correlated to changes in species richness in primary consumers, rather than to changes in plant species richness or abiotic parameters. Interestingly, both groups of predators showed a strong relationship with species richness in first order consumers in the brown web and less so with changes in species richness in the green web. This may point at stronger feeding relationships (Figure 4.4) of predators with the brown web prey species than with the green web species. Some of the dominant aboveground predators, for instance the spider *Pardosa purbeckensis*, have been shown to be highly opportunistic (Schaefer 1974). As abundances of brown web species are often found to be considerably higher than abundances of green web species (Irmler & Heydemann 1986), opportunistic aboveground predators may actually feed on species in the brown web rather than on species from the green web. According to the results of our multiple regression analysis, aboveground predators are correlated best to macro-detritivore species richness, while belowground predators species richness is much stronger related to microbivore species richness. This difference in consumption pattern is explained by the smaller size of belowground predators (generally a lot smaller (<4 mm) than the aboveground predators). However, this does not indicate that aboveground predators do not feed on green web invertebrates, but additional studies are needed to show this. So, our data suggests that diversity in both groups of secondary consumers is

mainly driven by species richness dynamics in primary consumers in the brown web and to a lesser extent also by dead organic matter availability and soil aeration.

## **Conclusion**

Our results highlight the importance of the joined study of both aboveground and belowground species richness and their environmental drivers. We demonstrate a striking divergence in species richness between the green and brown part of the food web over the course of salt marsh succession. Our analyses indicate that dissimilarity in diversity patterns can be explained by differences in the driving environmental variables for the green and brown part of the food web rather than by changes in plant species diversity. While green web species diversity is mostly driven by aboveground abiotics and vegetation structure, brown web species diversity is regulated by below-ground abiotic variables and litter production (Figure 4.4).

So we conclude that on the long term, different ecosystem processes independently drive the diversity of the green and brown part of the food web. Still, both parts of the food web are bottom-up regulated in terms of resources and are therefore connected by the balance between the amount of plant live and dead organic material and its quality.

Our results question the paradigm that aboveground and belowground communities are most strongly affected by plant species diversity. This thus highlights the importance of conservation of both early and late successional stages. While early successional stages with low standing biomass may be much more important to green web diversity, late successional stages with high standing biomass and high litter production may be of prime importance to brown web richness.

# Chapter 5



# 5

## Amelioration of stress for plants on a salt marsh by the macro-detritivore *Orchestia gammarellus*

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*Manuscript in preparation*

### Abstract

*Orchestia gammarellus* is a highly common, soil dwelling and burrowing amphipod species on European tidal marshes, where it acts as a key macro-detritivore. In this study, we investigated the hypothesis that activity of this species results in stress alleviation for salt marsh plants. More specifically, we hypothesized that bioturbation and litter fragmentation by *Orchestia* enhances soil aeration, redox potential, litter decomposition and nitrogen mineralization, thus altering the competitive balance between plant species and speeding up vegetation productivity. Through this, *Orchestia* seems to functionally replace earthworms in this ecosystem, as earthworms cannot cope with soil salinity. In order to test this, we performed field measurements to relate *Orchestia* presence to environmental factors, and we performed field and lab experiments in which we manipulated its abundances to investigate its influence on soil aeration, litter decomposition and nitrogen mineralization. Measures on environmental characteristics showed that *Orchestia* abundance is strongly related to soil redox potential, which can either indicate that the animal prefers aerated localities on the salt marsh, or creates these conditions by itself. In our laboratory experiment, we found strong support for the idea that *Orchestia* itself promotes soil aeration. Our field experiment showed that *Orchestia* stimulates nutrient mineralization, which we suggest to be the result of enhancing litter fragmentation rate and increase soil aeration. Moreover, *Orchestia* exhibits strong negative effects on seedling survival, hereby influencing the plant community composition. To summarize, *Orchestia* exhibits a number of important characteristics typical for an ecosystem engineer, in acting as the 'earthworms of the salt marsh' with respect to bioturbation and nutrient cycling. In addition, its strong influences on hypoxia-stress amelioration and seedling survival are expected to be a major determinant of the competitive balance between hypoxia-tolerant and hypoxia-intolerant plant species, thus affecting the plant species composition.



## Introduction

Plants species that grow in stressful, anoxic environments require specific adaptations to cope with the lack of oxygen and the potential negative effects of oxygen depletion to root tissue (Justin & Armstrong 1987; Vartapetian & Jackson 1997; Bailey-Serres & Voesenek 2008). Some of the most stressful soil conditions occur in salt marshes (Bertness & Leonard 1997), where anaerobic organic matter decomposition by sulphate-reducing bacteria leads to harmful sulfide levels for many plant species (Laanbroek 1990). And in addition, the plants need to deal with anoxic conditions which restricts root functioning. Currently, described ways how plants cope with permanent hypoxia-stress and associated negative effects include the formation of aerenchyma and resulting radial oxygen loss (Armstrong *et al.* 1991; Vartapetian & Jackson 1997), very shallow rooting (Armstrong *et al.* 1991), the facilitation from adjacent plant species with radial oxygen loss (Bertness & Leonard 1997), or facilitation by Fiddler crabs via bioturbation (Bertness & Leonard 1997). Strikingly, dominant late successional grass species on European salt marshes, such as *Elytrigia atherica* and *Festuca rubra* produce only little aerenchyma (Justin & Armstrong 1987). Moreover, they generally occur in monospecific stands (Olf *et al.* 1997; Van der Wal *et al.* 2000a), which indicates that facilitation by (nursing) plant species is not important. Recently, it was illustrated that these plant species can only colonize well-aerated circumstances (Davy *et al.* 2011), which makes their success on poorly drained salt marshes with a low elevation (Kuijper *et al.* 2005) a mystery.

From intertidal soft-sediment ecosystems, it is known that macrofauna plays an important role in aerating the sediment (Bouma *et al.* 2009). Lugworms (*Arenicola marina*) for instance bioturbate and promote oxygen diffusion in the upper 10 cm of the sediment, which results in sediment destabilization and a decrease in organic particles (Volkenborn *et al.* 2009). In addition, a bivalve (*Loripes lacteus*) has been observed to alleviate hydrogen sulfide toxicity stress to seagrass (*Zostera marina*). By promoting chemical reduction processes (Van der Heide *et al.* in prep), it can reduce levels of hydrogen sulfide and promote nutrient mineralization, thus facilitating the establishment and growth of *Zostera*. Another interesting parallel is found in terrestrial ecosystems, in which earthworms are an important factor in promote soil aeration (Lavelle 1988), and enhancement of nitrogen mineralization (O'Brien & Stout 1978; Lavelle 1988). Diminished activity of earthworms as a result of a parasite in Scotland has resulted in a less aerated soils and a related shift in vegetation composition (Boag & Yeates 2001). Also in New Zealand, where earthworms were only recently introduced, nutrient



cycling was found to be greatly increased by the amelioration of soil aeration and litter fragmentation (Stockdill 1982; Yeates 1991).

However, can macrofauna on salt marshes also alleviate abiotic stress levels to plants via bioturbation? In European salt marshes bioturbating annelid species are lacking, possibly because of the combination of high salt stress, periodic droughts and frequent floodings. Other well-adapted macro-detritivores occur in high densities, of which *Orchestia gammarellus* (Pallas 1776; hereafter *Orchestia*) is the most important macro-detritivore species in salt marshes with a low to intermediate elevation (Moore & Francis 1986; Meyer *et al.* 1995; Dias & Sprung 2003), where salt and hypoxia-stress levels for plants are also highest. It can occur in densities up to 3500 ind. m<sup>-2</sup> (Dias & Sprung 2003), but typically occurs in densities around 700 ind. m<sup>-2</sup> (Dias & Sprung 2003; Laffaille *et al.* 2005). It has been shown to exhibit extensive bioturbation activity, presumably to avoid dessication stress (Moore & Francis 1986; Dias & Sprung 2003) and/or to avoid predation. Additionally, this species and its relatives have been shown to act positively on decomposition rates of plant litter and rates of microbial growth (Fenchel 1970; Lopez *et al.* 1977; Buth & De Wolf 1985), likely a result of the combination of litter fragmentation, promotion of microbial growth on the resulting smaller fragments, and bioturbation, while only a small fraction of the litter itself is consumed (Fenchel 1970; Lopez *et al.* 1977).

In order to understand how *Orchestia* modifies its environment and whether its bioturbating activities alleviate soil anoxia stress and its associated negative effects to late successional salt marsh plant species, we addressed the following questions: 1) To what extent does the species improve soil aeration (as measured through soil redox potential)? 1) What is the influence of *Orchestia* on the rate of litter decomposition and nitrogen mineralization? 3) And to what extent does the activity of *Orchestia* affect plant species composition and productivity? We hypothesize that actions of *Orchestia* on salt marshes have two important effects. Firstly, bioturbation by *Orchestia* results in increased soil aeration. Secondly, the promotion of soil aeration and the facilitation of litter decomposition by *Orchestia* enhances N mineralization, resulting in a higher availability of N to plants.

We tested these predictions by 1) field measurements to relate *Orchestia* presence to environmental factors, 2) performing a laboratory and a field experiment in which we varied the abundances of *Orchestia* to study its effect on soil hypoxia and soil N mineralization.

## Methods

### Study site

The field measurements on the relationship between *Orchestia* abundance and environmental variables, as well as the field experiment were done at the salt marsh of the coastal barrier island of Schiermonnikoog, located in the Dutch part of Wadden Sea (53°30' N, 6°10' E), in October 2008.

Sampling locations for estimations of abundances of *Orchestia* were taken along a gradient of vegetation succession (Van de Koppel *et al.* 1996; Olff *et al.* 1997). To keep inundation frequency constant, plots were all located at a base elevation of 1.16 m ( $\pm$  SD 2.2 cm) above Dutch Ordnance level (approximate mean sea level). Sampling locations were spread evenly over the 8-kilometer succession gradient and were spaced at least 100 m apart.

The field experiment, in which we varied densities of *Orchestia*, was done at a part of the salt marsh where vegetation succession started on intertidal sand flats in 1967 (Olff *et al.* 1997). In this stage, vegetation patches were dominated by *Elytrigia atherica* and were interspersed with patches dominated by *Limonium vulgare*.

Soil samples for the bioturbation experiment in the laboratory were taken from a 120-year old part of the salt marsh chronosequence (Olff *et al.* 1997), which was grazed by cattle at a stocking density of 0.5 cow ha<sup>-1</sup> (Bos *et al.* 2002). Individual of *Orchestia* and *Elytrigia* were collected in an ungrazed part of the salt marsh, which was located 500 meters eastward.

### Field measurements; the correspondence of *Orchestia* and relevant environmental factors

In order to investigate how abundances of *Orchestia* vary with environmental variables, we sampled *Orchestia* densities and relevant environmental variables at 18 locations on the salt marsh. Quantitative estimations of *Orchestia* abundances were made using enclosed pitfall traps ( $\emptyset$  0.1 cm in 0.5×0.5 m Perspex enclosures, after Lang (2000)). Pitfalls were emptied every 3 days over a period of 18 days, between between 16 October and 4 November 2008 (the season of the highest densities) until no more *Orchestia* specimens were found in the traps.

In order to obtain a proxy for shallow soil aeration, we took soil redox potential measurements at 13 November 2009 at each sampling site. For this we used four measuring Platinum-electrodes (Pt) and one AgCl -reference electrode (Cole-Palmer®), which were connected to a Graphtec GL200 Datalogger (Graphtec GB Ltd). Pt-electrodes were placed in a square design, 10 cm from the reference-electrode, at a depth of 2 cm in the top soil. To standardize the measurements, we read out redox potential values 2 min

after the electrodes were placed in the soil. Averages of the four Pt-electrodes were corrected for the value of the AgCl-reference electrode (+197 mV). Although this measurement does not provide absolute values of the amount of oxygen in situ, it gives a reasonable proxy for the relative differences in soil aeration between locations measured on the same day (van Bochove *et al.* 2002).

We determined total aboveground plant peak biomass (including standing dead leaves and litter) by clipping a 0.5×0.5 cm square at 1 cm above the soil surface, which was dried at 70°C for 48 hours.

The thickness of the accumulated sediment (clay + organic matter + sand) layer on the sand layer is a good proxy for the total soil N pool (Olff *et al.* 1997). We took a sample of the sediment layer thickness with a sediment core (1 cm Ø), from which we determined the layer thickness to the nearest mm.

Absolute elevation (to the nearest cm) was determined using the Dutch Ordnance Survey map (©AHN; ahn.nl, 2008), using the coordinates from the field and the point intersect tool in ArcGIS 9.0. The percentage cover for every plant species was determined using the Londo cover estimation method (Londo 1976).

### ***Orchestia* effects on nutrient cycling and vegetation productivity**

To investigate the hypothesis that *Orchestia* speeds up nutrient cycling, modifies soil physical properties and changes vegetation composition, we performed a manipulative field experiment in which we varied the *Orchestia* abundance. Enclosures consisted of 1×1 m mesh fence (1 mm mesh size), which was dug into the soil to a depth of 0.25 m, because detailed personal observations showed *Orchestia* to be mostly active in top soil layer. Each enclosure was subject to a single treatment: either an *Orchestia* addition treatment, an *Orchestia* removal treatment, or a control treatment in which no *Orchestia* were added or removed. The three treatments were replicated five times, and were spaced at least 50 m apart. Monthly, during five-days *Orchestia* was collected in the removal plots using pitfalls in the south west corner in each of the enclosures, as monthly springtides were suspected to cause redistribution of *Orchestia* over the salt marsh. During this five-day period, all *Orchestia* from the removal treatment were transported to the addition treatment in the same replicate, each day the traps were emptied. The *Orchestia* from the control and addition treatment were caught, counted and released back into the same plot. An additional 500 individuals were added to the addition treatment at the start of the experiment. For an overview of the monthly numbers, see Appendix III, Figure 1. The inside of the enclosures were protected from mice and voles by a 1 cm iron mesh

which was dug in 10 cm into the soil and raised 10 cm above the soil. During the experiment, no mice or vole damage was observed in the enclosures. To find out whether effects of *Orchestia* would differ between vegetation types, treatments were placed into a *Puccinellia maritima*-dominated vegetation type in November 2008, and into an *Elytrigia*-dominated vegetation type in July 2009.

To investigate the effect of *Orchestia* abundance on leaf litter decomposition, litter bags were placed in the *Puccinellia*-dominated plots between 13 December 2008 and 3 March 2009. We used 5×12 cm litterbags (mesh size 1.5 mm), which exclude *Orchestia*, but allowed in most other soil animals (such as springtails, nematodes, and microbes). Another set of litter bags with similar mesh size, but with 20 additional holes (Ø 5 mm), were added to allow *Orchestia* to enter the litterbags and measure their effect (see Buth & Wolf 1989). All litter bags were filled with 10 g of fragmented (fragment size  $\pm$  2 cm) and dried (70°C, for 48 hours) *Elytrigia* litter, which was collected as dead standing biomass from a patch approximately 100 m from the plots. After the field incubation, litterbags were brought back to the lab, where clay and sand was carefully rinsed off on a sieve after which the litter was dried (70°C, 48 hours), and weighted to the nearest 0.01 g.

Nitrogen mineralization was measured *in situ* between 2 November 2008 and 16 July 2010, using mineralization tubes (Berendse *et al.* 1998; van Wijnen *et al.* 1999). The tubes (Ø 5 cm; 10 cm high) were put in the soil to a depth of 10 cm and were sealed with a lid on top to prevent plants from growing in. Water was allowed to enter and leave via the small holes at ground level (0.5 cm). Nitrogen-free Miracloth® was put on the bottom to allow water levels to rise and drop inside the tube as well as to prevent ingrowth of roots. At the start of each incubation period for each sample location, we collected one reference tube and we put an incubation tube at the same moment, right next to it. This procedure was continued throughout the experiment. The reference samples were transported to the laboratory directly and were handled the same way as the field-incubated samples. In the laboratory, fresh roots were removed, the samples were well-mixed by hand and 25 g of fresh soil was taken for analysis. All mobile nitrate and ammonium was dissolved into 60 ml of 1M KCl, which was left shaking for 16h. Afterwards, samples were manually filtered with nitrogen-free filters and analysed on a Skalar 5100 (Skalar BV) autoanalyser.

### ***Orchestia* affecting soil aeration?**

To study the hypothesis that *Orchestia* can ameliorate soil anoxia stress to plants by means of its bioturbation activity, we performed a laboratory experiment. For this, intact salt marsh clay soil samples without *Orchestia*

were collected in PVC rings ( $\varnothing$  25 cm, 8 cm height,  $N = 20$ ), in the field at the grazed marsh of the island of Schiermonnikoog ( $53^{\circ}28'46N$   $6^{\circ}13'48E$ ), by pushing them into the soil. This kept the soil structure generally intact. To half of these rings, 100 *Orchestia* individuals were added, which resembles a density of 2000 *Orchestia* individuals per  $m^{-2}$ , which are high, but certainly not unrealistically high densities (Dias & Sprung 2003; Laffaille *et al.* 2005). To mimic the very moist salt marsh conditions as observed in the field, the 8cm tall rings were put in trays with a shallow layer of 2 cm of artificial sea water, which was made using demineralized water with  $34\text{ g l}^{-1}$  Instant-Ocean®. To avoid algal growth, the salt water was replaced every two weeks. The experiment was done in a climate chamber at controlled climate conditions: 70% RH,  $17^{\circ}C$ , during the day (6AM-8PM) and  $14^{\circ}C$  at night. To prevent the *Orchestia* from escaping, every ring was fenced with a 25 cm high transparent plastic sheet. To study direct effects of *Orchestia* on plant growth, four living *Elytrigia* plants were planted in the soil in every ring. Thirty days prior to the experiment, these plants were taken from the same field site and were potted in their own soil in the greenhouse under the same environmental conditions. The mean wet weight of the planted *Elytrigia* plants in each mesocosm was  $13.7\text{ g}$  ( $\pm$  SE 1.2) without *Orchestia* and  $12.4\text{ g}$  ( $\pm$  SE 0.6) in the presence of *Orchestia*. To mimic rainfall, mesocosms were sprayed with 10ml fresh water every 3-4 days, using a sprayer. To quantify effects on litter decomposition, we added leaf litter to the rings, one day before the *Orchestia* individuals were put in. The first  $10\text{ g}$  ( $\pm$  SE 0.1) of dried ( $70^{\circ}C$ , for 48hr) *Elytrigia* litter was added at the start of the experiment and another  $10\text{ g}$  was added after exactly 4 weeks, which was halfway the experiment. At the end of the experiment, the remaining litter was carefully removed from the soil, carefully rinsed in a sieve (mesh size  $300\text{ }\mu\text{m}$ ), dried ( $70^{\circ}C$ , 48hr) and weighed to determine dry weight of the remaining litter, after which mass loss was measured.

In order to estimate effects of addition of *Orchestia* on the soil aeration we measured soil redox potential inside the pots biweekly, as a proxy for the level of oxygen in the soil. The Pt-electrodes used for this were again placed in a square design; 10 cm from the reference-electrode. All four measurement electrodes were first placed at 1cm depth and subsequently at 4cm depth. Values for each depth were read out after 2 minutes. We used the average value of the four Pt-electrodes for each ring in the statistical analysis, to avoid pseudo-replication.

At the end of the experiment, the number of newly formed shoots on every individual of *Elytrigia* was counted. Live aboveground biomass was clipped at soil level, and was subsequently dried ( $70^{\circ}C$ , 48hr). The remaining number of living *Orchestia* individuals was counted by careful inspection of

all mesocosms. The effects of *Orchestia* exclusion on spontaneous seedling performance (from seeds that emerged from the seed bank from the field) was investigated by counting the number of surviving seedlings for every species and the total biomass (aboveground + belowground) of seedlings.

## Statistics

To analyse the abundance of *Orchestia* in relation to environmental factors in the field, we used a correlation matrix and a generalized linear model with backwards stepwise regression. In the latter analysis, we took the number of *Orchestia* as a Poisson distributed response variable.

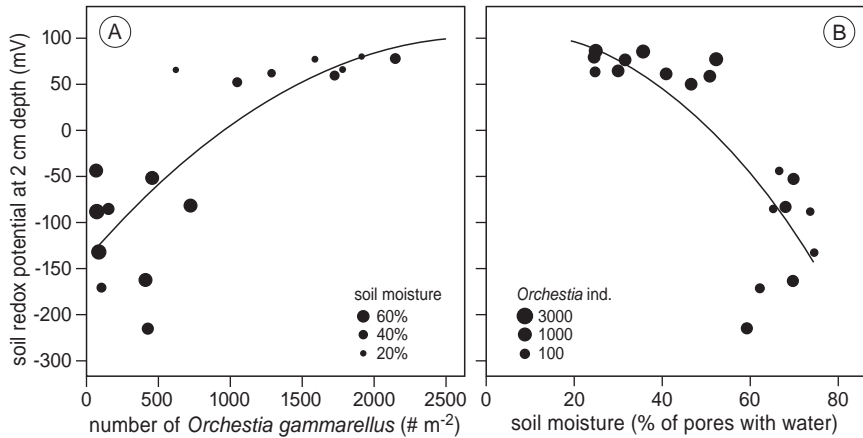
Results from the climate chamber experiment were tested using a general linear model with *Orchestia* treatment as a fixed factor. Non-normally distributed dependent variables were tested between the two *Orchestia* treatments using a non-parametric MWU-test. Differences in soil redox potentials between *Orchestia* treatments were tested using a generalized linear model with treatment and depth as categorical predictors. Differences in nutrient mineralization between *Orchestia* treatments was analysed using a general linear model with treatment and replicate as categorical predictors. All statistics were done using Statistica 9.0.

## Results

### *Orchestia* abundance and environmental variables

We found that *Orchestia* is a highly common species on our study locations on the salt marsh, with densities varying between 68 ind. m<sup>-2</sup> and 3492 ind. m<sup>-2</sup>, and an average of 1002 ind. m<sup>-2</sup>. The correlation matrix showed that densities of *Orchestia* were positively correlated to soil redox potential ( $R^2 = 0.57$ ,  $P < 0.001$ ), standing dead plant biomass ( $R^2 = 0.28$ ,  $P = 0.02$ ), thickness of the clay layer ( $R^2 = 0.52$ ,  $P < 0.001$ ), and vegetation height ( $R^2 = 0.48$ ,  $P = 0.001$ ). Densities were negatively correlated to soil moisture content ( $R^2 = 0.51$ ,  $P < 0.001$ ), and we found no correlation with the amount of living plant biomass.

The generalized multiple regression analysis showed that only soil redox potential significantly explained variation in the spatial distribution in *Orchestia* density (Wald Stat 6.84;  $P < 0.001$ ), while variation in vegetation structure and age of succession stage did not explain additional variation. *Orchestia* abundances were highest at high redox-values (soil redox potential >50 mV, Figure 5.1A). The correlation between the number of *Orchestia* and soil redox was best described by a 2<sup>nd</sup> order polynomial ( $R^2 = 0.84$ ,  $F_{42.36}$ ,  $P < 0.001$ ; Figure 5.1A). In turn, soil redox potential values correlated



**Figure 5.1** Three-way relationship between soil redox potential, numbers of *Orchestia gammarellus* and soil moisture in the field. A) positive correlation between *Orchestia* numbers and soil moisture (2<sup>nd</sup> order polynomial:  $F = 18.1$ ,  $R^2 = 0.67$ ,  $P < 0.001$ ). Size of dots corresponds to soil moisture (% pores saturated). B) Negative correlation between soil moisture and soil redox potential (2<sup>nd</sup> order polynomial:  $F = 42.4$ ,  $R^2 = 0.84$ ,  $P < 0.001$ ). Sizes of dots correspond to the abundance of *Orchestia*. Dimensions of dots are depicted in the legend.

strongly with soil moisture ( $R^2 = 0.68$ ,  $N=18$ ;  $P < 0.0001$ , Figure 5.1B). Soils were found to be very anoxic ( $< -0$  mV) when soil moisture values were above 54% of the total soil volume.

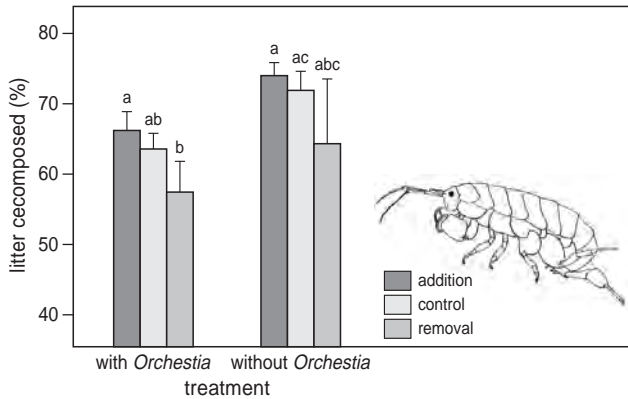
## Results field experiment

### *Orchestia*-additions

Our manipulations of the densities of *Orchestia* in the experimental plots were effective. In the *Puccinellia*-dominated vegetation, on average 1341 *Orchestia* individuals were collected from the *Orchestia*-removal treatment and added to the *Orchestia*-addition treatment (Appendix II, Figure 1). In the *Orchestia*-addition and control treatment, on average 1968 and 1993 individuals were caught, respectively, which was higher than in the removal treatment (GLM:  $F_{(2)} = 6.5$ ,  $N = 5$ ,  $P = 0.021$ ), but not significantly different from each other.

In the *Elytrigia*-dominated vegetation, the abundances of *Orchestia* were about three times higher as in the *Puccinellia*-dominated vegetation. On average 3011 individuals were collected from the removal treatment and





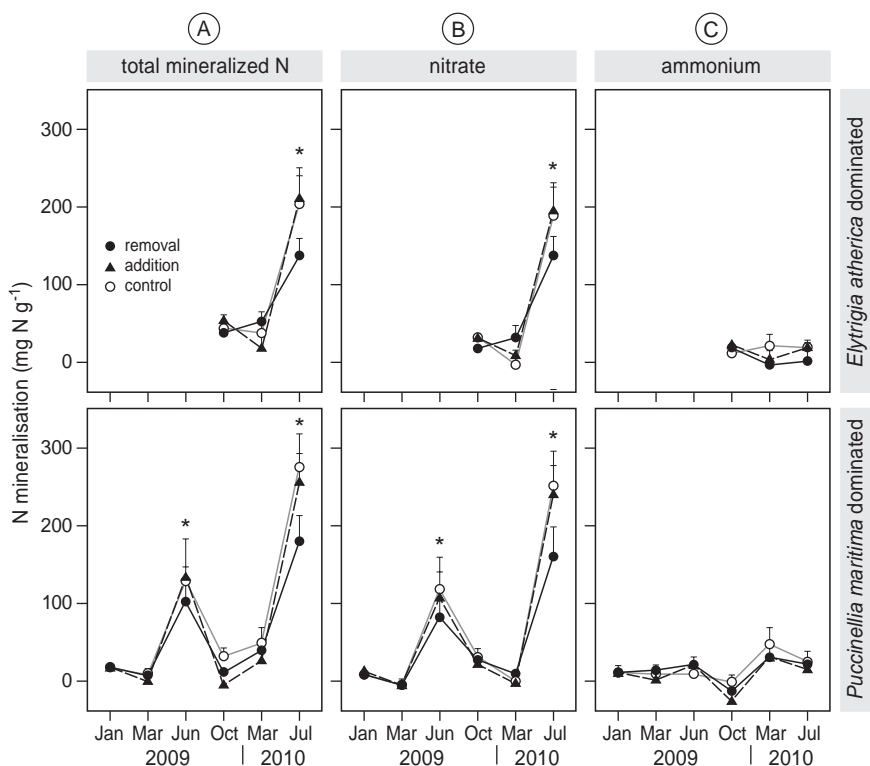
**Figure 5.2** Litter decomposition in litterbags after 12 weeks of incubation ( $N = 5$ ) with different mesh sizes (light grey: *Orchestia* had access, dark grey: without *Orchestia*), incubated in *Orchestia*-removal and addition plots. Bars denote averages ( $\pm$  SE) of litterbags. Letters denote significant differences between treatments  $\alpha < 0.05$ .

added to the addition treatment. The average number of *Orchestia* that was caught in the *Orchestia*-addition treatments in this vegetation type was 3857 individuals, which was not different from the 3525 individuals that were caught in the control treatment, nor was it significantly different from the removal treatment.

### Litter decomposition

We found a higher decomposition of *Elytrigia*-litter in the litterbags to which *Orchestia* had access. In the control and *Orchestia*-addition treatment, 68% and 72% of mass loss occurred over a period of 12 weeks, respectively, while only 59% mass loss was observed in the *Orchestia*-removal treatment (GLM:  $F_{(2)} = 3.6$ ,  $N = 5$ ;  $P < 0.05$ , Figure 5.2). In the litterbags open to *Orchestia*, litter decomposition was on average 10% higher than in litterbags where *Orchestia* was excluded (GLM:  $F_{(1)} = 4.4$ ,  $N = 5$ ,  $P < 0.05$ ). A few juvenile *Orchestia* were found in the litterbags that were intended to exclude *Orchestia*, which indicates that bags were not fully excluding *Orchestia*. In the *Orchestia*-addition treatment, we found a 12% difference between litterbags with and without *Orchestia* (Figure 5.2). Mass loss in the control treatment was not different from the *Orchestia*-addition treatment. In the *Orchestia*-removal treatment, remaining weight in litterbags with and without access to *Orchestia* were not significantly different from each other (Figure 5.2).

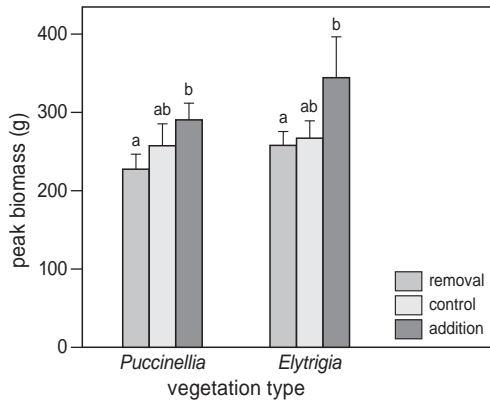




**Figure 5.3** Nitrogen mineralisation ( $\pm$  SE) as a result of removal of *Orchestia*, *Orchestia*-additions and a control treatment. A) shows total mineralization of nitrogen, B) shows mineralization of nitrate and C) depict mineralization of ammonium. The upper three graphs show results from manipulation of *Orchestia* abundance in *Elytrigia atherica*-dominated vegetation. The lower three graphs depict results from the *Puccinellia maritima*-dominated vegetation. \*  $P < 0.05$ .

### Effect of *Orchestia* on N mineralization in field experiment

We found that *Orchestia*-removal consistently reduced N mineralization in both vegetation types and in all summer measurements (Figure 5.3). In the *Puccinellia*-dominated vegetation, we found a 32% and 43% higher N mineralization in the *Orchestia*-addition treatment in June 2009 and July 2010, respectively. In the *Elytrigia*-dominated vegetation, we found a 42% higher N mineralization in the *Orchestia*-addition treatment in July 2010. The N mineralization in the *Orchestia*-addition treatment was also significantly higher than in the *Orchestia*-removal treatment (GLM:  $F_{(2)} = 3.8$ ,  $N = 5$ ,  $P < 0.05$ ), but not compared to the control, indicating that removal rather



**Figure 5.4** Above-ground plant biomass in response to *Orchestia*-removal, *Orchestia*-addition and a control treatment, in both *Puccinellia maritima* and *Elytrigia atherica* dominated vegetation. Letters indicate significant differences of a post-hoc tukey test at  $\alpha < 0.05$  within vegetation type.

than addition of *Orchestia* results in effects on N mineralization. The higher total N mineralization at higher abundances of *Orchestria* was all in the form of  $\text{NO}_3^-$  (Figure 5.3B), indicating high nitrification rates at higher abundances of this species.

### Effect of *Orchestia*-addition on peak standing biomass

We found a positive effect of *Orchestia*-addition on aboveground peak plant biomass (GLM,  $F_{(2)} = 7.0$ ,  $N = 5$ ,  $P = 0.006$ , Figure 5.4), both in *Elytrigia*-dominated vegetation and *Puccinellia*-dominated vegetation. *Orchestia*-addition resulted in 27% more aboveground peak plant biomass in the *Puccinellia*-dominated vegetation, and in 34% more aboveground peak plant biomass in the *Elytrigia*-dominated vegetation. Values in the control treatment did not differ from the two treatments, although values are between the addition and removal treatment.

## Results climate chamber experiment

### Effect of addition of *Orchestia* on seedling establishment in the climate chamber experiment

On average 72% (+/- SE) of all *Orchestia* survived to the end of the climate chamber experiment, with some variation between experimental pots (maximum 84 individuals, minimum 62 individuals). In 8 out of 10 meso-

cosms where *Orchestia* were absent, a fungal layer formed on top of the leaf litter. In the mesocosms without *Orchestia*, no fungal layer was present, suggesting that *Orchestia* consumed fungi.

Although no plant seeds were added, spontaneous recruitment from the soil seed bank inside the mesocosms occurred. In absence of *Orchestia*, we found a much higher number of established seedlings at the end the experiment ( $24.7 \pm 6.7$  vs.  $0.7 \pm 0.3$ , respectively; Student t-test:  $t = 3.8$ ,  $N = 10$ ,  $P < 0.002$ ), as well as a higher seedling total biomass ( $0.76 \text{ g} \pm 0.15$  vs  $0.03 \text{ g} \pm 0.02$ , respectively; Student T-test  $t = 5.0$ ,  $N = 10$ ,  $P < 0.001$ ). The majority of the seedlings in absence of *Orchestia* were *Salicornia* spp. (68%) and *Suaeda maritima* (12%) and only a small fraction belonged to *E. atherica* (2.4%). In the *Orchestia*-addition treatment the majority of the seedlings were *E. atherica* (57% of all seedlings). We found no significant difference between treatments in the absolute numbers of *E. atherica* seedlings at the end of the experiment. Finally, we did not find a significant correlation between seedling biomass and the number of surviving individuals of *Orchestia* ( $R = 0.21$ ;  $R^2 = 0.04$ ;  $P > 0.5$ ).

### **Effect of addition of *Orchestia* on growth of planted individuals of *E. atherica***

Of the *Elytrigia* plants that were added at the beginning of the experiment, 20% and 17.5% died within the first week, in presence and absence of *Orchestia*, respectively. These plants were left out of the analysis. No more plants died in the remaining seven weeks of the experiment.

Overall, we found a 40% higher weight per ramet tiller of *Elytrigia* in the presence of *Orchestia* (Table 5.1). The number of newly formed tillers was not affected (Table 5.1). Moreover, total dry weight of all dead plant material per mesocosm was 58% lower in the *Orchestia*-addition treatment, which was most likely due to consumption of the dead plant parts by *Orchestia*. We also observed some grazing by *Orchestia* on the lower living parts of the plant, which may have impaired plant production somewhat, but we did not quantify this.

### **Effect of *Orchestia* on soil redox potential**

Initial measurements on soil redox potential showed that soils were highly reduced at the start of the experiment. Over time, we observed a gradual oxidation of the soil in both treatments in the experiment, but the increase in redox potential in mesocosms with *Orchestia* present was much stronger than in those without *Orchestia* (Figure 5.5). Positive redox potentials were only found in presence of *Orchestia* (Figure 5.5). Overall, aeration effect of *Orchestia* was found both at 1 and 4 cm depth (Figure 5.5). At 1 cm depth,

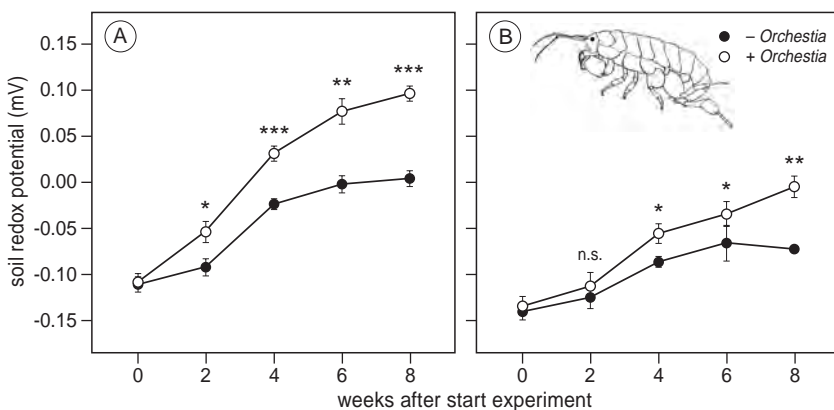
the difference in soil redox potentials between treatments with and without *Orchestia* increased with 250% between 14 and 56 days, (from 37.9 to 93.7; GLM:  $P < 0.001$ , Figure 5.5A). At 4 cm depth, this difference increased with 562% between 14 and 56 days (from 12.1 mV to 68.1 mV;  $P < 0.005$ , Figure 5.5B). The high survival rates of *Orchestia* in our experimental pots indicated that the initial anaerobic soil conditions were not of great influence on the survival of *Orchestia*.

### Effect of *Orchestia* on decomposition and nitrogen availability

We observed that litter decomposed faster in presence of *Orchestia*. Of the initial 20 g of leaf litter in each bag, 43% was decomposed after 8 weeks in presence of *Orchestia*, while 31% of the litter was decomposed without *Orchestia* (Table 5.1). We observed a small increase in total extractable mineral nitrogen (8%) in the *Orchestia*-addition treatment, but this difference was not significant (Table 5.1), suggesting that the plants mostly used the additional N mineralization. Total extractable  $\text{NO}_3^-$  was 164% higher in presence of *Orchestia* ( $t = 4.10$ ;  $N = 10$ ;  $P < 0.001$ ), but we find no significant effect on the availability of  $\text{NH}_4^+$  (Table 5.1). The ratio of available  $\text{NO}_3^-:\text{NH}_4^+$  is also higher in the *Orchestia*-addition treatment ( $t = 3.11$ ;  $N = 10$ ;  $P = 0.006$ ). As higher  $\text{NO}_3^-$  levels may be the result of a higher litter decomposition and subsequent nitrification, we tested the correlation between the

**Table 5.1** Soil physical properties and vegetation response in response to presence and absence of *Orchestia gammarellus* in the climate chamber experiment after 8 weeks.

	Without <i>Orchestia</i>		With <i>Orchestia</i>		Test value		P
	Mean	SE	Mean	SE			
Soil moisture (%)	34.55	0.78	32.09	0.48	F	1.0	0.34
Total number of <i>Elytrigia</i> tillers	2.23	0.38	3.03	0.43	F	2.6	0.14
Number of newly formed <i>Elytrigia</i> tillers	0.63	0.18	1.37	0.32	F	4.8	0.05
Dry weight of live aboground parts of <i>Elytrigia</i> (g. ind <sup>-1</sup> )	0.18	0.04	0.25	0.03	X <sup>2</sup>	1.4	0.15
Dry weight of dead <i>Elytrigia</i> (g. ind <sup>-1</sup> )	0.26	0.04	0.11	0.02	X <sup>2</sup>	12.8	<0.001
Number of <i>Orchestia</i> after experiment			67.8	2.65			
% of Litter decomposed	31.75	1.23	43.2	0.88	F	72.0	<0.001
Soil extractable $\text{NH}_4^+$ (g kg <sup>-1</sup> )	7.97	0.74	8.06	0.74	t	0.92	0.67
Soil extractable $\text{NO}_3^{2-}$ (g kg <sup>-1</sup> )	0.49	0.08	1.32	0.19	t	4.20	<0.001
Soil extractable total mineral N (g kg <sup>-1</sup> )	8.54	0.7	9.27	0.8	t	0.69	0.49
Total biomass of seedlings (g per pot)	0.76	0.15	0.03	0.02	X <sup>2</sup>	20.0	<0.001

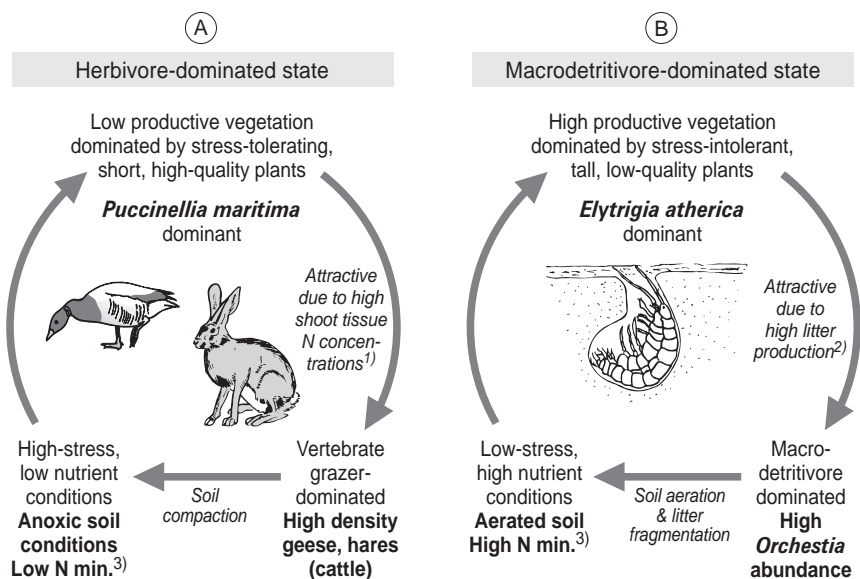


**Figure 5.5** Soil redox potential ( $\pm$  SE) over time at depth 1 cm (A) and 4 cm (B) in treatments with and without *Orchestia gammarella*. \*  $P < 0.05$ . \*\*  $P < 0.005$  \*\*\*  $P < 0.001$ .

amount of decomposed litter and the different components of extractable N, and found a positive relation between the rate of litter decomposition and the extractable  $\text{NO}_3^-$  levels ( $N = 10$ ;  $R^2 = 0.38$ ;  $P = 0.004$ ), while the correlations between decomposition and extractable levels of  $\text{NH}_4^+$  or total mineral  $\text{NO}_3^-$  were not significant.

## Discussion

In this study, we aimed at understanding the consequences of the activity of *Orchestia gammarellus* on primary productivity, nutrient cycling and plant community composition on salt marshes. Our results strongly suggest that *Orchestia* enhances soil aeration, leaf litter decomposition, and N mineralization, confirming our expectations. As a result of these effects, we find higher vegetation productivity, and a well-aerated soil on sites and in treatments with higher abundances of *Orchestia*. We summarize these effects in Figure 5.6. In absence of macro-detritivores, the accumulating silt and organic layer on the middle and low marsh results in a poorly aerated, anoxic soil, where microbes consume all free oxygen, and plant species are favored that are adapted to the resulting stressful circumstances, such as *Puccinellia maritima* (Davy *et al.* 2011b) (Figure 5.6A). Actions of macro-detritivores can offset these negative effects by ameliorating soil aeration, at the same time speeding up N mineralization through litter decomposition and by promoting oxygen availability to microorganisms (Figure 5.6B). We argue that alter-



**Figure 5.6** Conceptual scheme showing two different ecosystem states on the middle salt marsh, which are dominated by different parts of the food web and coincide with different vegetation types. A) Herbivore dominated state with dominance of low productive, high quality plant species (main species: *Puccinellia maritima*), with low litter input, low soil aeration and anoxic soil conditions, hence low numbers of macro-detritivores. B) Macro-detritivore dominated state with dominance of high productive, low quality plant species (main species: *Elytrigia atherica* has great difficulties in sustaining itself in a nutrient-poor, badly aerated soil. In this state, *Orchestia gammarellus* is the dominant macro-detritivore which, by increasing soil aeration litter fragmentation, speeds up nutrient cycling and ameliorates anoxia-resulting stress to dominant plant species. <sup>1)</sup> Van der Graaf *et al.* 2002; Bos *et al.* 2005; <sup>2)</sup> Van Wijnen & Bakker 2000 <sup>3)</sup> Van Wijnen *et al.* 1999.

ations in abiotic stress and nutrient availability favor productive, tall, late successional plant species with few adaptations to cope with prolonged periods of soil anoxia. Combined with the negative effects of *Orchestia* on seedling survival of forbs, a feedback loop is created in which non-hypoxia-tolerant species such as *E. atherica* and *F. rubra* profit and hypoxia-tolerant species are outcompeted through light competition (Figure 5.6B).

Even though results for the laboratory and field experiment showed results in a similar direction, but some differences existed as well. The method of adding and removing of *Orchestia* in field enclosures was less effective than expected, as shown by the high numbers that were caught in the removal treatment each month and the small difference between

numbers in the control and *Orchestia*-addition treatments (see Appendix III, Figure 1). Moreover, the results from the control treatment are quite similar to the addition treatment, indicating that local redistribution of *Orchestia* may have occurred, possibly in response to the unnaturally high densities. These redistributions may have taken place during high tides spilling over the enclosure boundaries, which occurred 28 times between November 2008 and July 2010 (live.waterbase.nl). Also, small crabs and small fish were found in the enclosures after such tides. Furthermore, density-dependent responses may have occurred, like higher recruitment and survival of *Orchestia* in the treatments where the densities were initially reduced. Nevertheless, we do find strong negative effects of *Orchestia* abundance reduction on plant peak biomass, N mineralization and litter decomposition rates in the field, showing the potential importance of *Orchestia* for ecosystem processes.

### Effects on soil aeration

Late successional, tall grass species were expected to benefit from presence of *Orchestia*, due to its positive influence on soil aeration and N mineralization. The strong effect of *Orchestia* on the soil redox potential in the laboratory experiment shows that presence of *Orchestia* indeed leads to higher levels of soil aeration, both in shallow soil and in deeper soil layers. The effect in deeper soil layers is interesting as it possibly ameliorate anoxia stress to anoxia-intolerant plant species rooting in deeper soil layers. Earlier studies have already pointed out the potential importance of the digging and burrowing behaviour of this species (Moore & Francis 1986; Dias & Sprung 2003), but none had yet related this behavioural characteristic to changes in soil physical and biological properties. Other bioturbating animals, such as earthworms (*Lumbricus* sp.; Lavelle *et al.* 1997), lugworms (*Arenicola marina*; Volkenborn *et al.* 2007), Fiddler crabs (Bertness 1985) and other crabs (Boyer & Fong 2005; Gutierrez *et al.* 2006) have earlier been mentioned as important actors on soil aeration (Bardgett & Wardle 2010). In New Zealand, where earthworms were introduced in the 19<sup>th</sup> century, nutrient cycling increased with 30%, because of higher soil aeration and enhanced microbial activity (Stockdill 1982; Yeates 1991). Another study on earthworm pathogens in Scotland has revealed that a pathogen-induced lower density of earthworms leads to a more anoxic soil, hereby favouring plant species such as rushes and sedges, which roots contain large amounts of aerenchyma in order to deal with increased hypoxia (Boag & Yeates 2001). Our field measurements showed that abundance of *Orchestia* at a given location correlates strongly with soil redox potential at a given location. Moore and Francis (1986) have pointed out that *Orchestia* could be sensitive to low oxygen levels and high redox values, while *Diptera* larvae seem better able to cope with

these circumstances. The burrows that are created by *Orchestia* are shallow (1–2 cm deep), but seem nevertheless appropriate to meet both its oxygen and moisture preferences (Moore & Francis 1986). This may operate via a strong increase in the soil-air exchange area. So *Orchestia* acts strongly on soil aeration by bioturbation, which we suggest to be beneficial to its own presence and plant species with low tolerance to reduced soil oxygen levels.

### Effect on vegetation production and N mineralization

The observed positive effect of *Orchestia* on vegetation productivity presumably operates via enhanced mineralization of nitrogen in combination with aeration effects. Positive effects of other macro-detritivores, especially earthworms on vegetation productivity have been observed in various crops (Lee 1985; Edwards & Bohlen 1996), which is often attributed to their positive influence on N mineralization by litter fragmentation and consumption of microbial biomass (Bardgett & Wardle 2010). As the vegetation on a temperate salt marsh is generally nitrogen limited (Olff *et al.* 1997; Van Wijnen & Bakker 1999; Kuijper *et al.* 2005), enhancement of N mineralization is likely to result in higher vegetation productivity. Results from our field experiment showed that *Orchestia* presence increased N mineralization most strongly in spring and summer measurements, when vegetation has its highest growth rate. We observed no strong increase in N availability in the laboratory experiment. This may be due to the fact that we only measured availability of extractable N, not the increase in N mineralization. However, we did observe a strong increase in plant dry weight in the presence of *Orchestia*, suggesting that extra N was mineralized and was taken up by the plants. Apart from the increase in availability of  $\text{NO}_3^-$ , no other conclusions can therefore be drawn from these numbers.

We suggest that positive effects of *Orchestia* on N mineralization occurs both via a positive effect on litter decomposition (see Hemminga & Buth 1991), and via the promotion of soil aeration, as the higher production of  $\text{NO}_3^-$  in presence of *Orchestia* can only occur when oxygen is abundant (Laanbroek 1990). Our results show structurally higher levels of litter decomposition in presence of *Orchestia*. Fenchel (1970) showed that the amphipod *Parhyalella whelpleyi* does not consume large amounts litter directly, but increases metabolism of micro-organisms by 110% by its litter fragmenting activity and consumption of micro-organisms and metazoans. A similar influence on litter decomposition of a congeneric amphipod species *Orchestia grillus* was suggested by Lopez & Levinton (1977). Therefore, we suggest that it is very likely that *Orchestia* plays major active role in the decomposition of litter, mostly by means of enhanced litter fragmentation, which results in enhanced litter decomposition by micro-organisms.



Yet, our estimations on influence of *Orchestia* may represent an under-representation, as the litterbags without *Orchestia* were obviously not fully excluding *Orchestia* juveniles.

Another hypothesis for the positive effect of *Orchestia* on N mineralization is that addition of *Orchestia* itself resulted in higher N mineralization. Assuming that all *Orchestia* died after addition,  $0.03 \text{ g N m}^{-2}$  would have been added between April and June 2009, while the actual observed extra total mineralization was  $1.22 \text{ g N m}^{-2}$ . In the months before July 2010,  $0.06 \text{ g N m}^{-2}$  was added in the form of living *Orchestia*, while the actual extra mineralization in the addition treatment was  $3.88 \text{ g N m}^{-2}$  (for details on calculation, see Appendix III, Table 1). We therefore conclude that the addition of *Orchestia* itself does not account for the strong increase in N mineralization in the addition treatment, but we cannot exclude the possibility that it accounted for a minor part of it. So, we conclude that *Orchestia* has positive effects on N mineralization, *via* enhanced litter fragmentation, litter decomposition and soil aeration.

### Effects on seedling predation

The effect of *Orchestia* on vegetation composition may partly be due to its striking influence on seedling survival. In our laboratory experiment, many seeds established in absence of *Orchestia*, while in presence of *Orchestia* only a very small fraction of the seeds survived. *Orchestia* is generally characterised as a detritivore (Moore & Francis 1986), although predation on living tissues may be a way to supplement its diet with nitrogen (White 1993). Since the increase in litter decomposition that results from litter consumption ranges between 30 and 40%, we suspect that food limitation cannot be an explanation for this behavior. *Orchestia* has been observed to feed on *Atriplex portulacoides* leaves in autumn (personal observation), but to our knowledge no other study reports similar observations. Strikingly, the majority of the surviving seeds in the treatment with added *Orchestia* belonged to *Elytrigia*. Studies by Hulme (1994), Brown & Gange (1992) and Allan & Crawley (2011) on seedling predation by invertebrates also point to a more negative impact of invertebrate grazing on dicots than on graminoids. This strengthened our notion that *Orchestia*, which is often found in the litter layer of dense stands of *Elytrigia*, has a relatively positive influence on grass species and a relatively negative influence on emerging forbs. We suggest that this selective foraging on living tissue is motivated by plant quality. Although no conclusive data on quality differences between seedlings exists, earlier studies have shown that the late-successional grass *Elytrigia* is not preferred by geese (Van der Wal 2000) and hare (Kuijper *et al.* 2005). Thus we conclude that *Orchestia* has a strong structuring impact on vegetation community compo-

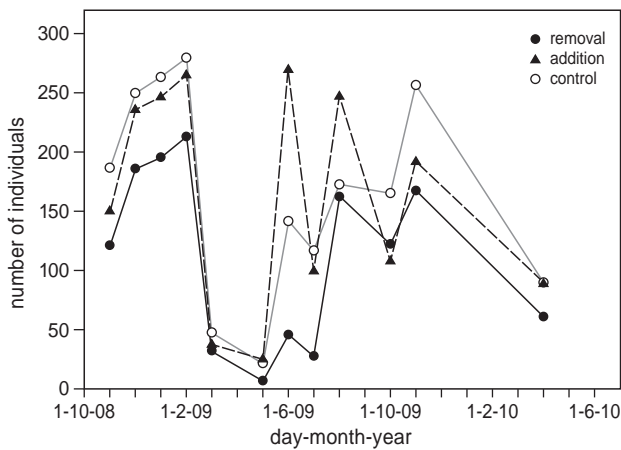
sition, especially (indirectly) favoring late successional grasses such as *Elytrichia*.

In conclusion, we found strong indications that the macro-detritivore *O. gammarellus* can be responsible for anoxia stress alleviation to plants due to its bioturbation activity. Moreover, we found strong evidence that this may have strong, positive consequences to plant species with few physiological capacities to cope with this anoxia stress. By acting on soil aeration, plant productivity and plant species composition, we argue that *O. gammarellus* plays a key role in community structure and ecosystem functioning of European salt marshes.

### **Acknowledgements**

Daan Schrama and Gerard Schrama are gratefully acknowledged for help during the laboratory and field experiment. Fons van der Plas, Roel van Klink and Ruth Howison are thanked for their input on earlier versions of the manuscript.

Appendix III



**Figure III.1** Monthly numbers of added *Orchestia*, removed *Orchestia* and numbers of *Orchestia* in the control treatment. Statistics mentioned in main text.

**Table III.1** Calculations of amount of N added in the form of *Orchestia gammarellus*.

Nr. ind. added between March& Summer		<i>Orchestia</i> weight (indiv)	N m <sup>-2</sup> added (in Orch) March-Summer	Extra N m <sup>-2</sup> mineralized	Added <i>Orchestia</i> as % of total Nmin
2009	57	3.3 mg ind. <sup>-1</sup> (~20% N = 0.66 mg N)	0.038 g	1.22 g	3.1%
2010	89	3.3 mg ind. <sup>-1</sup> (~20% N = 0.66 mg N)	0.059 g	3.88 g	4.8%



# Chapter 6



# 6

## Understanding variation in nitrogen mineralization in moist grasslands: the importance of herbivore trampling

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*In revision for Oecologia*

### Abstract

Large herbivores can have a major impact on soil nitrogen cycling in grasslands. Via selection for high quality plant species and input of dung and urine, large herbivores can speed up nitrogen cycling in fertile grassland soils while slowing down nitrogen cycling in unfertile soils. However, recent studies show that large herbivores instead reduce nitrogen mineralization in some temperate fertile soils, but not in others. Hence, key processes related to large herbivores affecting soil nitrogen cycling may not have been taken into account so far.

We hypothesize that large herbivores can reduce nitrogen mineralization in loamy or clay soils through soil compaction, but not in sandy soils. Especially under wet conditions, strong compaction in clay soils can lead to periods of soil anoxia, which reduces decomposition of soil organic matter and hence, restricts N mineralization. This would suggest that the net effect of herbivores on N cycling can be positive or negative, depending on soil texture.

In this study, we use a long-term (37 year) field experiment on a salt marsh to determine the effect of large herbivores on nitrogen mineralization, and how it depends on soil texture. Our results confirm that large herbivore presence decreased nitrogen mineralization on a clay soil, but not on a sandy soil. By comparing a hand mown treatment with a herbivore-grazed treatment, we show that these differences can be explained by herbivore-induced changes in soil physical properties rather than to above-ground biomass removal. On clay soil, we find that large herbivores reduce the soil air-filled porosity, induce more negative soil redox potentials, reduce soil macrofauna abundance and reduce decomposition activity. On sandy soil, we observe no changes in these variables in response to grazing. We conclude that effects of large herbivores on nitrogen mineralization cannot be understood without taking soil texture, soil moisture and feedbacks through soil macrofauna into account.

## Introduction

Large vertebrate herbivores play a key role in determining the community structure and functioning of grassland ecosystems. They act on plant diversity (Olff & Ritchie 1998), vegetation heterogeneity (McNaughton 1984; Frank *et al.* 2000) and can have a major influence on the local food web, both above-ground (Samways & Kreuzinger 2001; de Visser *et al.* 2011) and belowground (King & Hutchinson 1976; Bardgett & Wardle 2003). Two main mechanisms have been proposed via which large herbivores affect the vegetation composition. Firstly, grazers alter the light competition balance for plants by removing plant biomass and selective foraging (Huisman & Olff 1998; e.g. Pastor *et al.* 2006). Secondly, grazers promote nutrient cycling by production of dung and urine, which stimulates soil microbial activity (e.g. McNaughton *et al.* 1997a), leading to dominance of fast-growing grazing-tolerant plants (Olff & Ritchie 1998). Both mechanisms may stimulate high quality plant regrowth resulting in high quality litter production, higher rates of litter decomposition and rapid cycling of nutrients, thus creating a positive feedback loop on large herbivore densities (Fryxell 1991; McNaughton *et al.* 1997a; Pastor *et al.* 2006). Evidence for this mechanism has been found in several grassland ecosystems, where it leads to the formation of grazing lawns (McNaughton 1984; Frank & McNaughton 1993; Knapp *et al.* 1999). Soil fertility, however, seems a key aspect (Hobbs 1996). While grazing on fertile soils results in dominance of palatable plant species and high nitrogen mineralization, grazing on infertile soils promotes dominance of plant species with low palatability (and low N concentration in tissue and litter), resulting in lower decomposition and lower nitrogen mineralization rates (Pastor *et al.* 1993; Hobbs 1996; Pastor *et al.* 2006).

However, on some fertile soils, large herbivores reduce rates of nitrogen mineralization instead of promoting it, while vegetation quality was not reduced. For instance, in coastal salt marshes and river flood plains, herbivores have been found to reduce nitrogen mineralization rates (van Wijnen *et al.* 1999; Bakker *et al.* 2004), despite the dominance of highly palatable plant species. Also, a recent large cross-site study questions the generality of the idea that large herbivores promote nutrient cycling on fertile soils. Some of the sites show clear increases in nitrogen mineralization in response to grazing while other sites show no effect or decreases in nitrogen mineralization in response to grazing (Bakker *et al.* 2006; Bakker *et al.* 2009). Apparently, other factors than soil fertility also moderate the effect of grazers on nutrient mineralization.

One aspect that has received little attention in this controversy, and which may explain the contrasting results outlined above, is the effect of large

herbivores on soil physical properties (Hamza & Anderson 2005). In temperate agricultural grasslands, soil compaction via trampling of large herbivores is frequently reported to have a negative effect on nitrogen mineralization (Hamza & Anderson 2005 and references therein), where the strength and direction of the effect largely depend on soil texture and soil moisture (Rasiah & Kay 1998; Hamza & Anderson 2005). On fine textured clay soils, herbivore trampling has been found to strongly reduce soil porosity, which negatively affects diffusion of water, nutrients and oxygen content (Rasiah & Kay 1998). This negatively affects the nitrogen mineralization rate in two different ways. Firstly, the reduction in soil porosity can lead to a lower soil fauna activity which results in a reduction in the rate of nitrogen mineralization (Breland & Hansen 1996). Secondly, the decrease in oxygen diffusion can result in a higher denitrification, resulting in a lower amount of available nitrogen to plants (Le Roux *et al.* 2003; Menneer *et al.* 2005). On coarse textured, sandier soils, where soil particles are on average 100–1000 times larger, herbivore trampling is not likely to decrease soil porosity to the extent that nutrient mineralization is affected. In fact, herbivore trampling on sandy soils may lead to a higher nutrient diffusion rate (Voorhees *et al.* 1985; Rasiah & Kay 1998) and better water retention (Xu *et al.* 2008). Although these are well described effects in many agricultural systems (Hamza & Anderson 2005; Mikola *et al.* 2009), few studies have addressed the impact of trampling-induced soil compaction in natural ecosystems, but with some notable exceptions (Kiehl *et al.* 2001; Gass & Binkley 2011). Nevertheless, in none of these studies the effects of large herbivores were assessed on different soil types, nor were effects on the decomposer community taken into account.

In the present study, we have investigated the effects of large herbivore trampling on soil nitrogen mineralization in two types of soils, a clay soil and an adjacent sandy soil. Following the literature discussed above, we expected that herbivore trampling affects nitrogen mineralization differently in the two different soils. On the fine textured clay soil we expected herbivore trampling to result in an array of strong effects: 1) a decrease in soil porosity; 2) an increase in soil moisture level; 3) a decrease in soil aeration; 4) a decrease in decomposition activity; 5) a decrease in nitrogen mineralization. Moreover, we expected a change in the soil macrofauna community composition towards smaller and fewer species. On the sandy, coarse textured soil we expected a much smaller effect of large herbivores on soil physical properties, fauna composition and nitrogen mineralization.

## Methods

### Study area

A long term (37-year, starting in 1973) grazing experiment on the salt marsh was used for this study. The salt marsh on the coastal barrier island of Schiermonnikoog, the Netherlands (53°48'N, 6°22'E) has developed on a sandy beach plain with small dunes. Clay-rich sediment (clay + silt fraction ( $<0.74\ \mu\text{m}$ )  $\sim 47\%$ , hereafter *clay soil*) is deposited on the lower parts of the salt marsh on top of the underlying sandy substrate (De Groot 2009). The higher parts have not been covered by sediment, so soils have remained sandy (clay + silt fraction  $<3\%$ , hereafter *sandy soil* (De Groot 2009). The grazed part of the study area received a long-term stable grazing management since 1973, with a relatively constant stocking density of  $0.5\ \text{cows ha}^{-1}$  (Bakker *et al.* 2002a). Both on clay and on sandy soil, parts of the area were left ungrazed. Since grazing was installed, vegetation in the grazed and ungrazed parts of the marsh has undergone quite different development (see Bos *et al.* 2002).

### Study design

We selected eleven locations on clay soil and six locations on sandy soil. All locations were spaced at least 30 m apart. For a detailed map of the locations, see Appendix IV, Figure 1A. All locations had a  $3\times 3\ \text{m}$  grazed plot and a similar-sized ungrazed plot on opposite sides of a grazing fence (Appendix IV, Figure 1B). To avoid edge effects plots were located 2 m away from the fence. Additionally, a  $3\times 3\ \text{m}$  mown plot was present at eight of these locations, however, only present in the clay soil treatment. It was laid-out on the ungrazed part of the locations, at least 2 m from the other treatments and was hand-mown with a bush-cutter twice yearly (in June/July and August/September) since 1973. Details on canopy height, root mass and abiotic soil parameters can be found in Appendix IV, Table 1.

### Soil physical parameters

Measurements on the degree of soil compaction, soil macro-porosity, water content, root mass, organic matter content, nitrogen mineralization, decomposer activity, sediment (clay) layer thickness and soil fauna composition were done in all  $3\times 3\ \text{m}$  plots for all treatments on both sandy and clay soil in July 2010.

Bulk density, soil moisture and organic matter content were determined by taking the average of three soil samples. Soil samples were taken 2 m from each other using a soil corer ( $\varnothing\ 10\ \text{cm}$ , 10 cm height) which is also used to take soil micro-arthropod samples (Van Straalen & Rijninks 1982). Thus care



was taken to avoid compaction of the sample. Prior to sampling, above-ground vegetation was carefully cut (1 cm above the soil surface) using a pair of scissors and removed from the sample. The cores were weighed, dried (at 70°C, for 48 hours) and weighed again to calculate bulk density and to determine water content (% of initial fresh weight). After removal of living roots, which were weighed and dried to determine roots mass, organic matter content (OM) was determined as loss on ignition at 550°C for 3 hours.

Bulk density is a commonly used measure to assess the effects of large herbivores on soil structure (Bakker *et al.* 2009). However, it does not take into account differences in specific weight of the different soil components. For example, a soil with low porosity, a high organic matter content (with a low specific weight) and low mineral content (with a high specific weight) can have a similar bulk density as a soil with high porosity, a low organic content and a high mineral content. However, the effect on soil aeration could potentially be large, which may result in a very different soil functioning. Therefore, we also compared total soil porosity, air filled porosity and water filled porosity between the different treatments and soil types. In order to calculate these parameters, we used the specific weights of sand and clay (2.65 g cm<sup>-3</sup> (LNV 1970)) and organic matter (1.47 g cm<sup>-3</sup> (LNV 1970)) and the water content of the different soil samples.

As a proxy for the saturation of oxygen in the soil and the different microbial processes that happen therein, we measured the soil redox potential. We chose a set up in which five electrodes with a platinum tip and one Ag/AgCl calomel reference electrode (Cole-Palmer®) were connected to a Graphtec GL200 Datalogger (Graphtec GB Ltd). All redox measurements were taken at the 13<sup>th</sup> of November 2010. In order to obtain a depth-profile of the soil redox potential, all electrodes were placed into the soil first at 2 cm, and subsequently at 5 cm and 10 cm depth. Measurements were read out exactly two minutes after the electrodes were placed at a certain depth. Averages of these five electrodes were used for graphs and statistics, after correction for reference electrode (+192 mV), temperature and soil pH. Quick measurements on redox may not necessarily reflect absolute values but are suitable for making comparisons between treatments (van Bochove *et al.* 2002) and are an important predictor for plant species distribution on salt marshes (Davy *et al.* 2011a).

### Soil fauna

Soil fauna was extracted from soil cores taken with a soil corer (see above) from the top 5 cm of the soil in all treatments using the Tullgren-method (Van Straalen & Rijninks 1982). Above-ground plant biomass was removed prior to sampling. All invertebrate species were identified to the species level

for Collembola, the important detritivorous amphipod *Orchestia gamma-rellus* and to morphospecies for other taxa. In order to check whether differences in soil fauna composition were larger between treatments at deeper soil layers, we also took samples from 5–10 cm deep at locations 4, 5, 6, 8 and 9 on clay soil, but not from locations at sandy soil. At these locations, clay layer was thicker than 10 cm. In the analyses, we distinguished between meso (<4mm) and macrofauna ( $\geq 4$ mm) as we expected stronger effects of a decrease in pore volume on macrofauna than on mesofauna.

### **Decomposer activity**

We measured decomposer activity using one bait lamina strip per plot (Kratz 1998). This method is widely used as a proxy for decomposer activity, especially to allow comparisons between treatments (see Rozen *et al.* 2010). Bait lamina sticks (8 cm long, 0.5 cm wide) contained 16 holes spaced 5mm apart, filled with a standard mixture of cellulose, bran flakes and active coal in a ratio of 70:27:3. Sticks were put into the soil with the top most hole 0.5cm below soil surface, at the 28<sup>th</sup> of August 2010 and were taken out at the 4<sup>th</sup> of October 2010. Afterwards, sticks were carefully rinsed and holes subsequently checked on activity of decomposers. When the light of a lamp (60 W) penetrated the hole successfully it was recorded as ‘active decomposers’ while unsuccessful light penetration was recorded as ‘inactive decomposers’.

### **Nitrogen mineralization**

The net nitrogen mineralization was measured in situ during a 5-week period using the incubation method described by Berendse *et al.* (1989). On June 16 2010, a pair of intact soil cores was taken at every plot using a PVC tube ( $\varnothing$  4.3 cm, 10 cm height). Of each pair, one sample was transported to the laboratory in a cooling box, after which extractable nitrogen ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) was extracted within 24 h. The second tube was incubated *in situ*, of which the bottom was sealed off with a nitrogen free Philitron<sup>®</sup> filter to prevent root ingrowth and to facilitate in and outflow of water and air. A plastic lid was placed on top to prevent plant growth. After five weeks, the incubated soil tube was brought to the laboratory for analysis. From both samples,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were extracted from 12.5g of field-moist soil, which was mixed with 30 ml 1m KCl for eight hours and centrifugated afterwards. The extract was analysed using a Skalar 5100 autoanalyzer (Skalar BV, The Netherlands). Net nitrogen mineralization, expressed as mg of extractable  $\text{NH}_4^+$  and  $\text{NO}_3^-$  produced per month, was calculated by taking the difference between the nitrogen content in the incubated soil cores and first paired sample. Samples disturbed by cows were excluded from the analysis.

## Statistics

Statistical testing on soil fauna species and specimen numbers was done using a non-parametric Kruskal-Wallis test as data were not Poisson nor normally distributed. Decomposer activity data from the bait lamina sticks was analyzed using a logistic regression with log-link function and treatments as independent variables. A general linear model was done on all soil variables to test for treatment effects. A multiple regression with backwards stepwise removal was used to investigate the different variables explaining differences in macro-porosity on different soils with macro-porosity as a dependent variable and organic matter content, water content and treatment as independent variables. We used a general regression model to test for independent effects of treatment and soil type on nitrogen mineralization, and we took the interaction effect between these to test whether the treatment showed differences between soil types. Additionally, we took water and air filled porosity, total porosity, bulk density and organic matter content into account as covariates. To meet the assumption of homogeneity of variance, a Levene's test was used; to meet the assumption of normality of residuals, we inspected the standardized residuals visually and we tested those on normality using a KS-test. All statistics were done using Statistica 9.0.

## Results

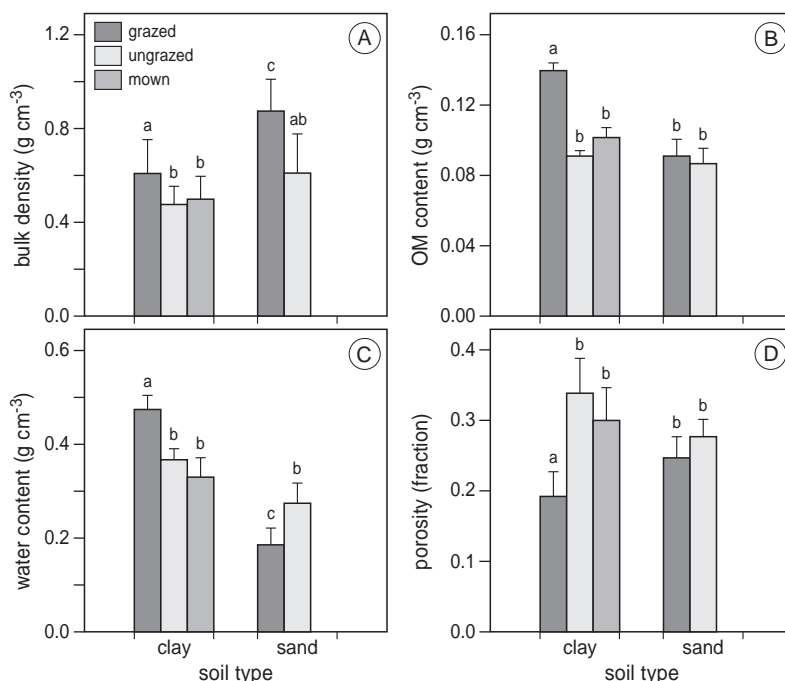
### Soil physical parameters

In accordance with our expectations, soil bulk density was significantly higher in the grazed plots, both on clay and sandy soil (Figure 6.1A). On clay soil, grazing resulted in a 30% higher bulk density. No significant differences were found between the bulk density of mown and ungrazed plots on clay soil. On sandy soil, bulk density was 41% higher in the grazed treatment than in the ungrazed treatment. Bulk density was 32% higher in the grazed sandy soil compared to the grazed clay soil.

On clay soil, SOM content was 31% higher in the grazed plots than in the ungrazed plots, and 27% higher than in the mown plots (Figure 6.1B). SOM content was not different between treatments on sandy soil.

On clay soil, the grazed plots had 31% higher water content than the ungrazed plots and 43% higher water content than in mown plots. We found the opposite for sandy soil where water content was 31% lower in the grazed plots than in the ungrazed plots (Figure 6.1C).

Grazing on clay soil resulted in 8 – 10% lower total soil porosity compared to mown and ungrazed treatment (Figure 6.1D). For sandy soil, grazing resulted in a 12% lower total porosity (Figure 6.1D).

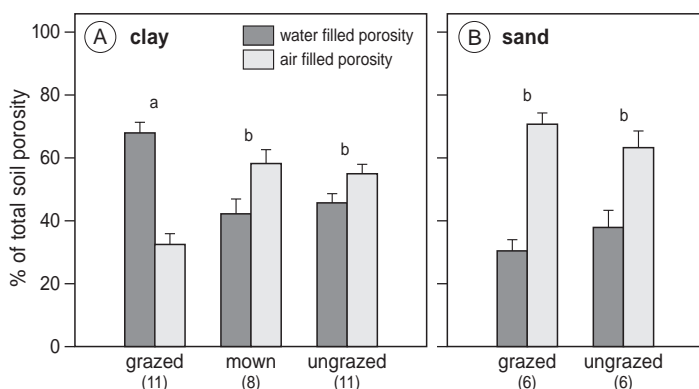


**Figure 6.1** Soil physical properties in the grazed, ungrazed and mown treatments. Different letters indicate significant differences at  $\alpha = 0.05$  A) Bulk density, B) organic matter (OM) content as fraction of total weight, C) Water content as fraction of total wet weight, D) macro-porosity as a fraction of the total volume.

On grazed clay soil, water filled porosity was on average more than 20% higher than in the mown and ungrazed treatment and well above 60% (Figure 6.2A). On sandy soil, no differences between the percentages of water filled porosity were found (Figure 6.2B).

### Factors influencing air filled soil porosity

On clay soil, differences in air filled porosity was explained by four factors: grazing treatment, SOM content, water content and soil water content (Table 6.1). This indicates that the porosity decrease on clay soil was clearly influenced by the grazing treatment. On sandy soil, variation in air filled soil porosity was best explained by variation in bulk density and organic matter fraction (Table 6.1), but not by grazing treatment and water content.



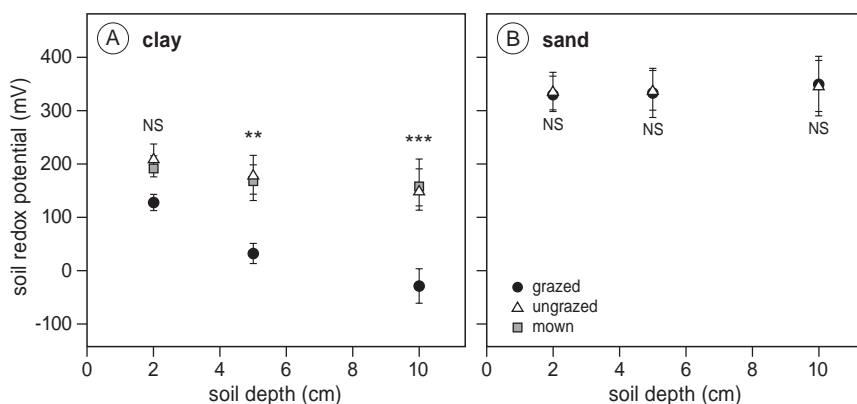
**Figure 6.2** Air filled porosity and water filled porosity in grazed, ungrazed and mown treatments. A) grazed, ungrazed and mown treatments on clay soil, B) grazed and ungrazed treatments on sandy soil. Different letters indicate significant differences at  $\alpha = 0.05$ .

### Redox potential

In clay soil we found the redox potential at 2 cm depth to be 83 mV lower in the grazed treatment compared to the ungrazed and mown treatment, but this difference was not significant (Figure 6.3A). This difference increased to 140 mV at 5cm depth ( $P = 0.002$ ) and 205 mV at 10 cm depth ( $P = 0.0009$ ). Soil redox potential values on sandy soil did not differ between treatments ( $P > 0.10$ ), nor did we find lower soil redox values with increasing depth (Figure 6.3B). Moreover, soil redox potentials on sandy soil were on average between 120–160 mV higher than on clay soil ( $F = 115.6$ ;  $P < 0.001$ ).

**Table 6.1** Factors explaining air filled porosity.

Factors	Clay		Sand	
	F	P	F	P
Intercept	1.77	NS	129.33	< 0.001
Bulk density	4.75	0.039	38.92	< 0.001
OM content	8.66	0.007	18.36	0.004
Water content	76.76	< 0.001		NS
Treatment	12.66	< 0.0012		NS

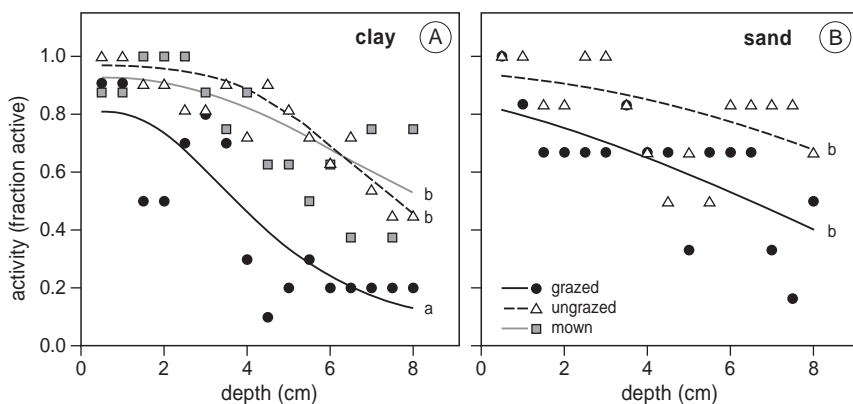


**Figure 6.3** Redox potentials in grazed, ungrazed, and mown treatment. A) Grazed treatment on clay soil is significantly different from other treatments at 5 and 10 cm depth. B) Treatments on sandy soil are not significantly different. Bars denote standard errors. \*\*  $p < 0.01$ ; \*\*\*  $p < 0.005$ ; NS: not significant.

## Soil fauna

Overall, we found  $21.7 (\pm \text{SD } 4.2)$  species and  $207.5 (\pm \text{SD } 88.4)$  specimen in the upper 5 cm of clay soil and  $27.1 (\pm \text{SD } 5.7)$  species and  $221.6 (\pm \text{SD } 94.3)$  specimen in sandy plots (Appendix IV, Figure 3). If we subdivide the soil fauna compartment into mesofauna ( $< 4$  mm) and macrofauna ( $\geq 4$  mm), we found no differences between treatments on sandy soil, but strong differences between treatments on clay soil. Abundances of macrofauna in the top 5 cm in ungrazed clay soil were 91% higher than in the grazed treatment ( $N = 11$ ;  $Z = 2.53$ ;  $P = 0.033$ ). We did not observe differences between numbers of individuals of mesofauna between grazed and ungrazed clay soil ( $N = 30$ ;  $H = 1.81$ ;  $P = 0.412$ ; Appendix IV, Figure 3). Community composition of the mown treatments was intermediate, both for macro- and mesofauna and not statistically different from either of the other treatments.

In the soil layer of 5–10 cm deep differences between soil fauna compartments were much more pronounced. We found significantly more specimen of mesofauna in the ungrazed treatment ( $N = 5$ ;  $Z = 2.02$ ;  $P = 0.04$ ), and three times more specimen of macrofauna in the ungrazed treatment ( $N = 5$ ;  $Z = 2.02$ ;  $P = 0.04$ ). Mown plots were intermediate, and did not differ significantly from both the grazed and the ungrazed plots. The detritus-feeding crustacean *Orchestia gammarellus*, which exhibits digging behaviour, was only observed in the ungrazed plots ( $3.4 \pm \text{SE } 1.0$  indiv. per sample) and in the mown plots on clay soil ( $0.6 \pm \text{SE } 0.4$  indiv. per sample), but was not found in the grazed plots on clay soil nor in any of the sandy plots.



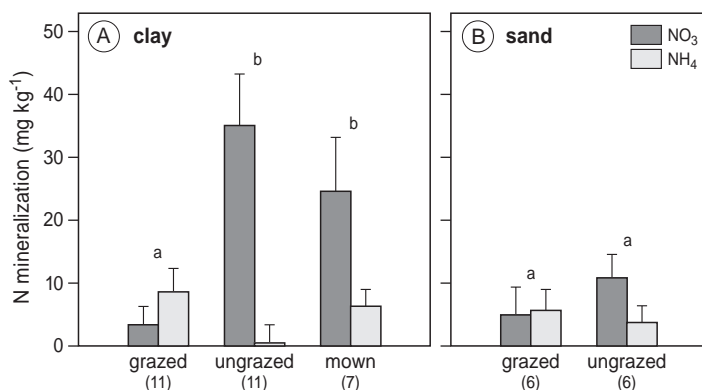
**Figure 6.4** Decomposer activity as measured with bait lamina sticks with depth in grazed, ungrazed and mown treatments. A) clay soil, B) sandy soil. Symbols indicate fraction of empty (active) holes at each depth. Letters indicate significantly different slopes. Statistics and regressions are mentioned in the results section.

### Decomposer activity

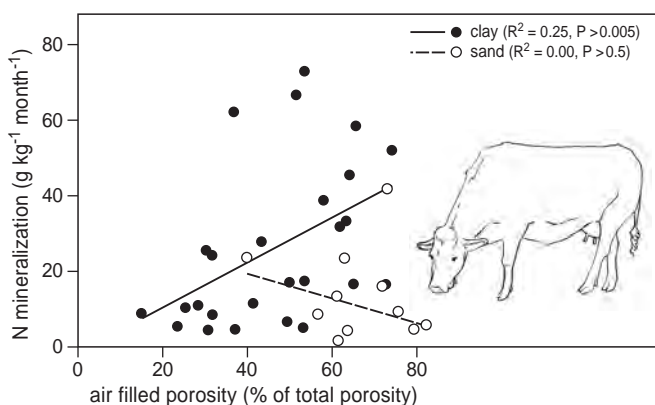
Overall, decomposer activity was found to be highest in ungrazed treatments on both clay and sandy soil which also have the highest soil porosity (Figure 6.4). In both clay soil (Wald Stat=26.91;  $P < 0.001$ ; Figure 6.4A) and sandy soil (Wald Stat = 6.85;  $P = 0.008$ ), we observed a decrease in decomposition activity with depth, but the decrease in decomposer activity with depth is much stronger in clay soil. In clay soil, we found a steep decrease in decomposer activity with depth in all treatments (Wald Stat = 64.55,  $P < 0.001$ ). Grazed clay soil showed a stronger decrease in decomposer activity, where we found only 10% decomposer activity at 8 cm depth, versus a 45–51% decomposer activity at 8cm depth in ungrazed and mown treatments. In sandy soil, we only found a slight decrease with depth in the grazed sandy treatment (Wald Stat = 4.8;  $P = 0.027$ ; Figure 6.4B), but no effect of depth in the ungrazed treatment (Wald Stat = 2.16;  $P = 0.14$ ). Furthermore, decomposition activity in the grazed clay soil was lower than in grazed sandy soil (Wald Stat = 8.3;  $P = 0.004$ )

### Nitrogen mineralization

Paired t-tests within soil type showed a lower total N mineralization in the grazed plots on clay soil compared to the mown plots ( $t = 2.6$ ;  $P = 0.04$ ) and ungrazed plots ( $t = 4.1$ ;  $P = 0.004$ ) (Figure 6.5A). On sandy soil, no difference was found between grazed and ungrazed treatments (Figure 6.5B). The multiple regression analysis showed that both grazing treatment ( $F_{(1,26)} =$



**Figure 6.5** N-mineralization (in mg kg<sup>-1</sup>) in grazed, ungrazed and mown plots on clay (A) and sand (B). Numbers in brackets behind treatments indicate sample size. Statistical testing was done on total nitrogen mineralized. Different letters indicate significant differences at  $\alpha = 0.05$ .



**Figure 6.6** Estimate of macro-porosity versus total nitrogen mineralization between 18 June and 26 July 2010. Closed circles represent samples from clay soil ( $R^2 = 0.25$ ;  $P = 0.005$ ), open circles represent results for sandy soil (NS).

8.77;  $P = 0.006$ ) and the interaction between treatment and soil type had a significant effect on total N mineralization. The interaction effect ( $F_{(1,26)} = 10.02$ ;  $P = 0.004$ ) between treatment and soil indicated that the effect of grazing treatment depended on soil type. Of all covariates that were taken in the analysis, only air filled porosity explained additional variation ( $F_{(1,26)} = 4.71$ ;  $P = 0.04$ ). Net N mineralization was higher with lower air saturation



on clay soils, but no relationship was found on sandy soil (Figure 6.6). In turn, air filled porosity was negatively correlated to redox potential ( $R^2 = 0.44$ ;  $P < 0.001$ ) and to OM content ( $R^2 = 0.15$ ;  $P = 0.01$ ).

We also found a remarkable difference in the ratio of  $\text{NH}_4\text{:NO}_3$  between the treatments. On grazed clay soil, 73% of the mineralized nitrogen was available as  $\text{NH}_4$ , while in the mown treatment only 20% was available as  $\text{NH}_4$  and in the ungrazed treatment only 2% was available as  $\text{NH}_4$ . On sandy soil, we found no differences in the ratio  $\text{NH}_4\text{:NO}_3$  between the grazing treatments (Figure 6.5B). Both absolute values for the amount of mineralized N and the difference between the proportions were not significantly different.

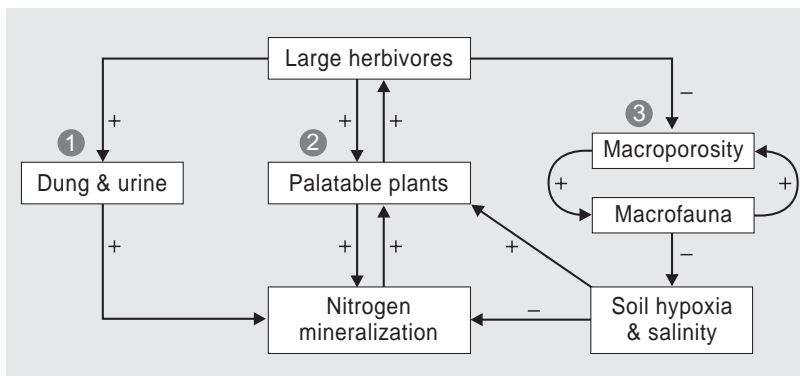
## Discussion

### Long-term effects of grazing on soils with different texture

The results of the present study show that herbivore trampling and the associated effects on soil functioning may provide an important third route via which herbivores can create long-standing effects on nitrogen mineralization and soil macrofauna (Figure 6.7). This figure shows the paradox of how increased soil hypoxia through compaction can in turn promote the palatability of the vegetation (Pathway 3). This is only possible when direct effects of stress on vegetation composition (with dominance of species with high palatability, see Bakker 1989) are more important than the negative effect of hypoxia on nitrogen mineralization.

Our findings may therefore also shed light on some of the conflicting results from earlier studies on the impact of large herbivores on N cycling, which were also done in study areas with fine textured soils (van Wijnjen *et al.* 1999; Kiehl *et al.* 2001; Bakker *et al.* 2006; Bakker *et al.* 2009).

An essential element of this conceptual diagram is that herbivores affect nitrogen mineralization via compaction in some soils, but not in others (Pathway 3, Figure 6.7). Our results indicate that large herbivores reduce nitrogen mineralization and associated reduction in soil fauna through soil compaction on a clay soil, but not on sandy soil. The general idea that trampling leads to soil compaction on both sand and clay is confirmed by several other studies on cattle (Mwendera & Saleem 1997; Menneer *et al.* 2005), savannah herbivore communities (Belsky 1986), sheep (Kiehl *et al.* 2001) and for human trampling (Hsu *et al.* 2009). However, the consequences of soil compaction for porosity are much larger on clay than on sand, which we attribute to the physical structure of clay soils that makes them more compactable, a finding that is also supported by other studies (Rasiah & Kay 1998; Di *et al.* 2001; Hsu *et al.* 2009; Appendix IV, Figure 2 for an X-ray



**Figure 6.7** Conceptual model illustrating the three pathways via which large herbivores can influence nitrogen mineralization in fertile soils. Pathway (1) reflects the input from dung and urine from large grazers which can increase nitrogen mineralization (McNaughton *et al.* 1997b; Mikola *et al.* 2009). Pathway (2) reflects the effect of herbivores on vegetation palatability and hereby on quality of litter inputs, which in effect stimulates nitrogen mineralization (McNaughton 1983; Frank *et al.* 2000). Pathway (3) illustrates the effect of large herbivores on nitrogen mineralization via their negative effect on macro-porosity and macrofauna abundance and increased soil anoxia. The resulting positive effect of soil anoxia on plant quality is only possible when direct effects of stress on vegetation composition is more important than the negative effect of hypoxia on nitrogen mineralization.

picture of a compacted and a non-compacted clay soil). We suggest that the observed negative effects on net nitrogen mineralization in grazed clay soils operate both via adverse effects on soil fauna and via increased gaseous losses of nitrogen. When pores get smaller, organic matter may be physically protected against microbial attack, and microorganisms may be inaccessible to predating soil fauna (Bouwman & Arts 2000). We found considerable reductions in larger soil fauna in grazed clay soil, especially in deeper soil layers, which may indeed have retarded decomposition due to lower grazing on microbial biomass and lower degrees of litter fragmentation (Bouwman & Arts 2000; Osler & Sommerkorn 2007). The high water filled porosity (WFP) in the grazed clay soil (67%) compared to the ungrazed clay soil (45% WFP), indicated that denitrification may also have played a role in losses of inorganic nitrogen. Reported threshold values of WFP causing denitrification differ according to soil type, but for sandy and clay soils, threshold WFP ranging from 50 to 74% have been reported (e.g. Sexstone *et al.* 1988; Nelson & Terry 1996; Menneer *et al.* 2005). The observed differences in soil redox potential also indicate a much higher potential for denitrification in grazed

clay soil (Laanbroek 1990). Both results therefore strongly suggest that in grazed clay soil, denitrification contributed to the lower net mineralization inorganic N, while we found little indication that denitrification may have played a role in the ungrazed and mown treatments on clay soil and the treatments on sandy soil. Thus we conclude that grazing by large herbivores may have a stronger effect on nitrogen mineralization on clay soils than on sandy soils due to their difference in sensitivity to compaction and the resulting changes in soil porosity and WFP. In other words: the magnitude of the arrow from 'Grazers' to 'Macroporosity' in Figure 6.7 is moderated by soil type.

### **Alternative explanations for explaining differences in N mineralization**

Differences in root mass have previously been suggested to explain differences in N mineralization. Fornara *et al.* (2011) showed that higher root mass lead to higher N mineralization. However, our results show the opposite result: in grazed clay soil, where root mass was higher (Appendix IV, table 1), N mineralization was observed to be lower. So, we suggest that differences in root mass do not satisfactorily explain the observed differences in N mineralization.

Differences in grazing intensity have also been argued to explain differences in N mineralization. In general, higher grazing intensity results in a larger effect on N mineralization (e.g. Le Roux *et al.* 2003). Grazing intensities on the different soil types have not been measured during the 37 years of the experiment, but our measurements on canopy height show that grazing on clay may have been more intensive than on sand (data in Appendix IV, table 1). Firstly, the vegetation in grazed plots on sand had a higher canopy than the grazed plots on clay (6.8 cm vs 3.1 cm;  $P < 0.05$ ). Secondly, within plot variation in vegetation height is also higher at the sandy grazed plots than at the grazed clay plots (29.1% vs. 19.5%). However, though a lower grazing intensity on sandy soil may partly explain the small observed effect of grazing on N mineralization, it does not provide an explanation for the strong adverse effects of grazing on N mineralization on grazed clay soil.

How large grazers slow down net nitrogen mineralization on clay soil in our study is in marked contrast to the effect of moose browsing in boreal forests (Pastor *et al.* 2006), with very different consequences for the vegetation composition. Moose browsing shifts the vegetation to species of higher litter C:N ratio, resulting in lower decomposition and N mineralization (Pathway 2, Figure 6.7). In our salt marsh study site, we find that despite the slowing down of nitrogen mineralization on clay soil, plant quality for herbivores is strongly enhanced by grazing. Crude protein ratios in a grazed community ranges between 12.5% (*Festuca rubra* dominated) and 20% (*Puccinellia maritima* dominated), while in the ungrazed community, crude

protein is between 9.25% (*Juncus maritimus* dominated) and 10.5% (*Elytrigia atherica* dominated) (data from Bakker 1989). Also cell wall content of the ungrazed plant community is 10-30% lower than in the grazed communities (data from Bakker 1989). We suggest that this may reflect plant responses to increased abiotic stress (Pathway 3, Figure 6.7). In our case, large grazers promote plant species that are tolerant to both herbivory and soil abiotic stress and have high tissue N concentrations. This soil abiotic stress results from the earlier mentioned hypoxia, but may also arise as a result of increased salinity on compacted soils due to lower leaching of salt and higher evapotranspiration of grazed vegetation (Chaneton & Lavado 1996; Olff & Ritchie 1998). However, the increase in litter quality resulting from a shift to high quality plants does not enhance mineralization in our study system, precisely because of the same unfavourable abiotic conditions for mineralization. Yet, the enhanced plant quality as a result of grazing leads to the formation of grazing lawns and, therefore, creates a positive feedback of large herbivores on their own presence (Bos *et al.* 2002).

### **Contrast and consistency with previous studies**

The strong difference between nitrogen mineralization in the mown and grazed treatments on clay soil indicates that the breakdown of litter is the limiting step for nitrogen mineralization in our study system, not its inputs. We argue that this mechanism works via the herbivore-induced abiotic stresses on nitrogen mineralization as a result of soil compaction (Pathway 3, Figure 6.7). Both the build-up of organic matter and the low decomposition activity in the grazed plots on clay soil also indicate a long-term decrease in nitrogen mineralization in the grazed plots on clay soil. This provides an explanation for the contrast between our results and results from studies in which an increased nitrogen mineralization rate was found in response to grazing (Ruess & Seagle 1994; McNaughton *et al.* 1997a; Frank & Groffman 1998). In dry savanna and steppe ecosystems, grazers do not restrict decomposition and no organic matter accumulation occurs (Sankaran & Augustine 2004). Hence, nutrient mineralization can be sped up according to Pathway 1 & 2 (Figure 6.7): large herbivores increase nutrient mineralization by defecation and increase of quality of litter into the decomposer food web. In our study system herbivores do restrict decomposition in clay soil, resulting in a dominant effect of Pathway 3 (Figure 6.7), and a decrease in nitrogen mineralization. In hindsight, this may also explain the inexplicable results by Bakker *et al.* (2004) in a floodplain grassland. Here, cow grazing decreased nitrogen mineralization, but rabbits and vole grazing increases nitrogen mineralization, while in all treatments vegetation quality was enhanced. By taking the soil compacting effects by cows (which rabbits and voles do not

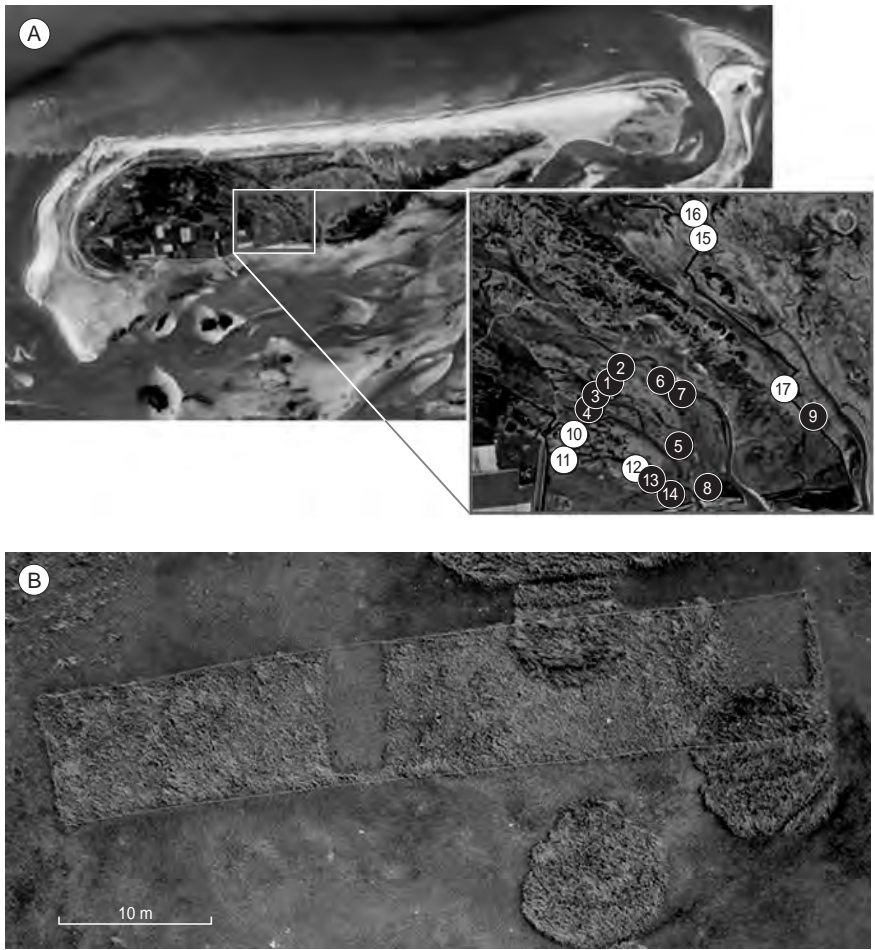
have; Pathway 3 in Figure 6.7) on this fine textured soil into account this seems a classic example of how Pathway 3 and Pathway 1 & 2 separate out in the same study system.

In order to track effects of herbivores on soil porosity, our results suggest that bulk density is a less appropriate measure than the soil air filled porosity. The reason for this is that the measure of bulk density is highly influenced by differences in specific weight between mineral substances (clay, sand) and organic matter content (leaf litter). Organic matter is light but fills many pores and has a high water holding capacity (White 2006). Grazing by herbivores on clay soil results in a higher organic matter accumulation compared to the ungrazed treatments as well as the grazed sandy soil treatment. Hence, comparing bulk density between these treatments results in an underestimation of the compactive effect on clay soils, something which can be captured by measuring air filled porosity.

Ultimately, we argue that effects of large herbivores on coarse textured soils may operate predominantly through the classic Pathways 1 and 2 in Figure 6.7, by increasing or decreasing N mineralization either through the promotion or reduction of litter quality inputs. In contrast, on fine textured soils, effects of large herbivores may be strongly moderated by soil compaction (Pathway 3 in Figure 6.7). In wet, saline soils these effects are expected to be the most extreme. As a result of that, the apparent presence of grazing lawns on the clay soils on salt marshes shows that grazing lawns can develop despite the negative effect of large grazers on soil nitrogen mineralization.



# Appendix IV

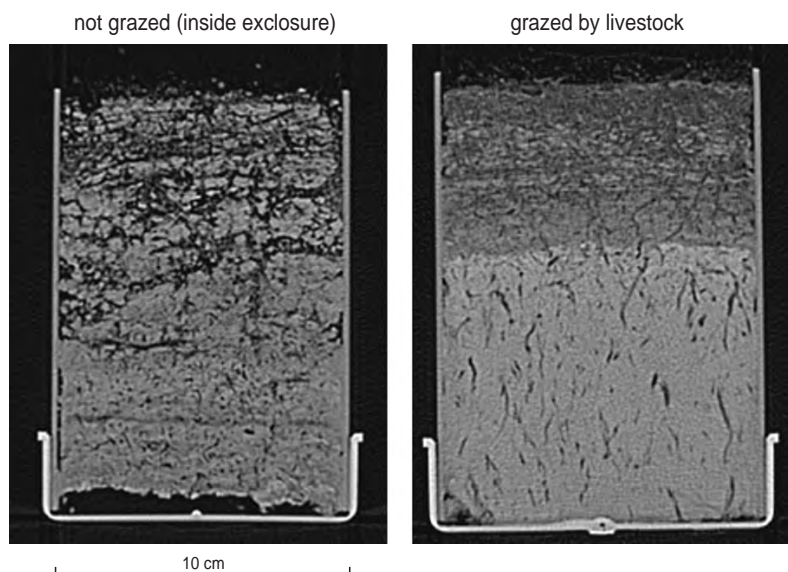


**Figure IV.1** A) Research locations at the grazed salt marsh of the island of Schiermonnikoog. Black dots depict locations on clay ( $N = 11$ ), white dots depict research plots on sand ( $N = 6$ ). Numbers correspond to appendix IV, table 1 with the general plot characteristics. B) Aerial picture of an experimental enclosure which has been ungrazed since 37 years. The enclosure is surrounded by a grazing lawn, which has been created by grazing livestock. Hand mown treatments can be seen at the right and in the middle of the enclosure. The circular (non-grazing lawn) tall vegetation structure is dominated by *Juncus maritimus*.

**Table IV.1** General plot characteristics for all plots and all treatments. Thickness of sediment layer shows the thickness of the clay layer which is deposited on the sandy subsoil. On the sandy plots no clay sediment layer is present. \* NAP stands for Dutch Ordnance level.

Plot nr	Soil type	Treatment	Sediment layer (cm)	Vegetation height (cm)	Abs. elevation above NAP (cm)	Root weight (g/100cm <sup>3</sup> )
1	Clay	Grazed	11.0	2.17	87.2	6.24
2	Clay	Grazed	8.3	2.00	88.8	5.40
3	Clay	Grazed	16.7	1.40	60.3	1.81
4	Clay	Grazed	16.8	3.00	69.9	2.93
5	Clay	Grazed	16.5	3.43	76.7	3.55
6	Clay	Grazed	10.2	7.93	80.2	1.88
7	Clay	Grazed	10.5	3.60	72.8	3.30
8	Clay	Grazed	35.5	1.63	69.0	2.97
9	Clay	Grazed	15.5	3.07	147.5	4.58
13	Clay	Grazed	11.2	3.07	178.7	5.85
14	Clay	Grazed	32.7	2.30	143.9	3.64
1	Clay	Mown	11.8	5.50	88.2	2.54
2	Clay	Mown	11.0	3.87	91.6	2.16
3	Clay	Mown	20.0	5.07	68.3	1.99
4	Clay	Mown	20.0	6.10	76.4	1.20
5	Clay	Mown	22.2	4.07	79.1	1.49
6	Clay	Mown	16.2	7.83	83.9	1.08
7	Clay	Mown	13.2	9.00	76.4	1.24
8	Clay	Mown	37.7	2.60	71.8	1.45
1	Clay	Ungrazed	10.0	21.40	89.1	0.70
2	Clay	Ungrazed	11.2	11.57	99.6	2.41
3	Clay	Ungrazed	20.3	15.90	66.4	0.72
4	Clay	Ungrazed	21.5	15.33	75.0	0.71
5	Clay	Ungrazed	24.2	25.67	78.8	0.73
6	Clay	Ungrazed	16.7	15.17	83.8	0.74
7	Clay	Ungrazed	15.2	19.83	84.0	0.87
8	Clay	Ungrazed	38.3	24.60	73.1	1.05
9	Clay	Ungrazed	12.9	21.90	147.8	1.31
13	Clay	Ungrazed	12.0	16.83	180.7	1.44
14	Clay	Ungrazed	33.7	19.83	143.6	1.25
10	Sand	Grazed	0	8.03	191.3	3.24
11	Sand	Grazed	0	5.20	198.5	3.66
12	Sand	Grazed	0	5.40	294.4	2.96
15	Sand	Grazed	0	7.60	152.3	2.34
16	Sand	Grazed	0	11.33	154.8	2.70
17	Sand	Grazed	0	3.13	197.1	2.11
10	Sand	Ungrazed	0	12.33	187.4	0.97
11	Sand	Ungrazed	0	15.07	193.2	2.29
12	Sand	Ungrazed	0	11.40	294.0	1.40
15	Sand	Ungrazed	0	19.00	161.9	0.90
16	Sand	Ungrazed	0	14.83	151.6	1.78
17	Sand	Ungrazed	0	18.00	201.6	1.08

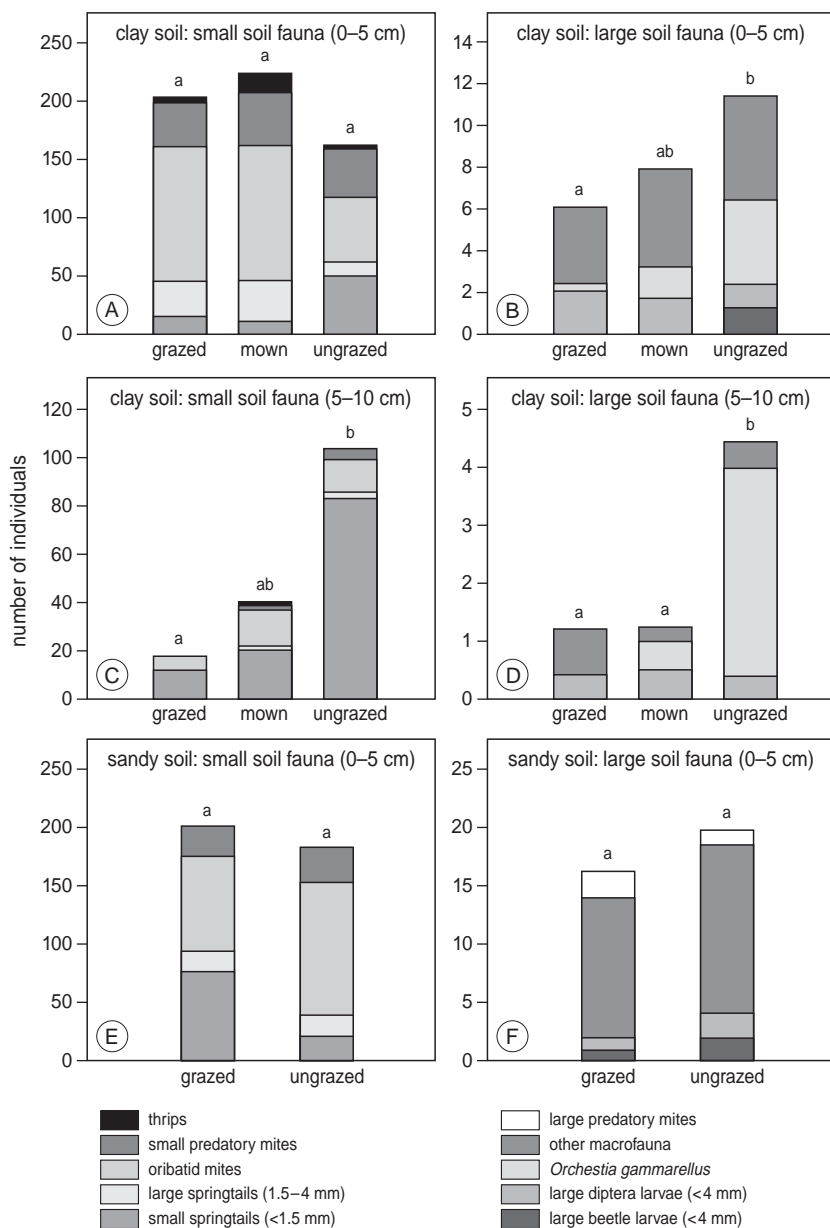




**Figure IV.2** X-ray tomography pictures of cross sections of soil samples from the clay soil. Left panel shows an ungrazed soil sample with lots of macropores shown in black (both air and water filled pores appear as black). Right panel shows a sample from grazed soil with few macropores.

**Figure IV.3 (right)** Number of individuals for fourteen groups of soil invertebrates in all treatments on clay (A-D) and sandy soil (E-F) G: grazed, M: mown, U: ungrazed. A) mesofauna (0-4 mm) in upper 5 cm of soil B) macrofauna (>4 mm) in upper 5 cm of soil C) mesofauna from soil samples of 5-10 cm deep D) macrofauna from soil samples of 5-10 cm deep E) mesofauna in sandy soil (0-5 cm deep) F) macrofauna in sandy soil (5-10 cm deep). Eight groups of invertebrates belong to mesofauna: small Collembola (<1.5 mm), larger Collembola (1.5-4 mm), oribatid mites, small predatory mites, small beetle larvae, Thrips (Thysanoptera), small beetles (<4 mm). Five groups belong to Macrofauna: Larger beetle larvae (>4 mm), Diptera larvae (>4 mm), *Orchestia gammarellus*, Larger predatory mites (>2 mm) and other larger soil invertebrates. Different letters indicate significant differences at  $\alpha = 0.05$ .





**Table IV.2** Number of plant species ( $\pm$  SD) and cover for every plant species ( $\pm$  SE).

Clay Soil						
	total nr. of plant species ( $\pm$ SD)					
Grazed	11.45 (1.69)					
Mown	6.75 (2.05)					
Ungrazed	3.55 (0.69)					
	<i>Agrostis stolonifera</i>	<i>Armeria maritima</i>	<i>Artemisia maritima</i>	<i>Aster tripolium</i>	<i>Atriplex prostrata</i>	<i>Elytrigia atherica</i>
Grazed	6.5 (3.1)	1.5 (0.4)	1.5 (0.7)	1.8 (0.7)	0.3 (0.1)	1.4 (1.1)
Mown	2.3 (1.6)	1.6 (0.6)	25.4 (6.1)	0.1 (0.1)	0.8 (0.3)	12.8 (6.5)
Ungrazed	0.0 (0.0)	0.0 (0.0)	3.3 (0.9)	0.0 (0.0)	2.5 (0.8)	45.3 (3.3)
	<i>Festuca filliformis</i>	<i>Festuca rubra</i>	<i>Glaux maritima</i>	<i>Juncus maritimus</i>	<i>Juncus gerardii</i>	<i>Limonium vulgare</i>
Grazed	0.0 (0.0)	17.6 (4.3)	6.1 (0.9)	0.9 (0.7)	25.9 (5.1)	2.8 (1.3)
Mown	3.8 (4.0)	34.0 (5.8)	0.0 (0.0)	0.0 (0.0)	0.1 (0.1)	1.8 (1.2)
Ungrazed	0.0 (8.0)	7.3 (3.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	<i>Plantago coronopus</i>	<i>Plantago maritima</i>	<i>Potentilla anserina</i>	<i>Puccinellia maritima</i>	<i>Trifolium repens</i>	<i>Triglochin maritima</i>
Grazed	1.5 (1.1)	14.5 (4.3)	0.8 (0.7)	12.5 (5.6)	2.9 (1.9)	2.5 (1.1)
Mown	0.1 (0.1)	2.6 (1.7)	0.5 (0.5)	0.0 (0.0)	0.3 (0.2)	0.1 (0.1)
Ungrazed	0.0 (0.0)	0.0 (0.0)	0.2 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Table IV.2 Continued

Sandy Soil						
	total nr. of plant species ( $\pm$ SD)					
Grazed	17.67 (3.44)					
Ungrazed	10.33 (3.83)					
	<i>Achillea millefolium</i>	<i>Ammophila arenaria</i>	<i>Anthoxanthum odoratum</i>	<i>Armeria maritima</i>	<i>Bolboschoenus maritimus</i>	<i>Bromus hordeaceus</i>
Grazed	1.0 (0.7)	1.8 (1.3)	0.3 (0.2)	7.3 (7.2)	2.0 (2.2)	2.3 (1.2)
Ungrazed	0.3 (0.4)	3.3 (2.3)	2.2 (2.2)	0.0 (0.0)	0.2 (0.2)	0.0 (0.0)
	<i>Calamagrostis epigejos</i>	<i>Carex arenaria</i>	<i>Cerastium fontanum</i>	<i>Elytrigia atherica</i>	<i>Festuca filliformis</i>	<i>Festuca rubra</i>
Grazed	2.0 (2.2)	1.8 (1.2)	1.0 (0.3)	2.2 (0.7)	2.0 (2.2)	24.3 (7.7)
Ungrazed	2.2 (2.2)	0.5 (0.2)	0.8 (0.2)	8.7 (4.1)	0.0 (0.0)	22.0 (4.2)
	<i>Plantago coronopus</i>	<i>Poa pratensis</i>	<i>Rumex acetosella</i>	<i>Rumex crispus</i>	<i>Trifolium repens</i>	
Grazed	1.0 (0.3)	1.0 (0.0)	1.2 (0.5)	1.0 (0.7)	3.2 (1.3)	
Ungrazed	0.0 (0.0)	0.7 (0.2)	2.2 (2.2)	0.7 (0.2)	0.0 (0.0)	

# Chapter 7



# 7

## An integrated framework to explain nitrogen mineralization in grazed ecosystems

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*In revision for Perspectives in Plant Ecology, Evolution and Systematics*

### Abstract

Large herbivores are key drivers of nutrient cycling in ecosystems world-wide, and hence they have an important influence on the productivity and species composition in plant communities. Classical theories describe that large herbivores can accelerate or decelerate nutrient cycling by altering the quality and quantity of resource input into the soil food web. However, different empirical studies find contrasting impacts of herbivores on nitrogen availability that cannot be explained by changes in resource quality and quantity alone. We argue that it is important to integrate alternative pathways that include herbivore-induced changes in soil physical conditions into classical theories. Herbivores can have a major impact on N availability through their influence on soil physical conditions. Specifically, trampling can increase soil compaction and reduce water infiltration and soil aeration, which subsequently alters the activity and species composition of soil decomposers which may affect N mineralization.

In this paper we reconcile observations of the impact of herbivores on N availability that were previously regarded as contradictory by including alterations of soil physical properties. In very wet and very dry soils, particularly those with a fine texture, herbivores may limit soil N availability through compaction, which can outweigh the effects of herbivore-induced changes in resource quality and quantity. In soils of intermediate moisture levels and soils with a coarse texture, compaction does not limit N mineralization and herbivore-induced changes in resource quality and quantity are the major factors that determine N mineralization. These ideas are supported by case studies from the literature. Therefore, we conclude that our novel integrated framework will help us to understand when herbivores accelerate or decelerate soil nutrient cycling across environmental gradients and improves our understanding of the functioning of grazed ecosystems.

## Classical theories on nutrient cycling in grazed ecosystems

Large vertebrate herbivores are key determinants of plant community composition, productivity and the functioning of ecosystems worldwide (Olff & Ritchie 1998; Knapp *et al.* 1999; Bardgett & Wardle 2003; Cromsigt & Kuijper 2011). One of the major pathways through which herbivores affect the plant community is via their influence on nutrient cycling and soil nutrient availability (McNaughton 1984; Georgiadis *et al.* 1989; Hobbs 1996; Frank *et al.* 2000; Bardgett & Wardle 2003). Herbivores can either speed up or slow down rates of nitrogen mineralization (Hobbs 1996; Bardgett & Wardle 2003). Classical theories that explain the contrasting impact of herbivores on nutrient cycling primarily focus on herbivore-induced changes in the quality and quantity of resources that are recycled through the soil food web (McNaughton 1984; McNaughton *et al.* 1997b; Bardgett & Wardle 2003; Pastor *et al.* 2006).

In high-fertility ecosystems, herbivores indeed have been found to speed up soil nutrient cycling through the deposition of dung and urine which provide easily available nutrients and stimulate the activity of soil microbes (Frank & McNaughton 1993; McNaughton *et al.* 1997b; Frank & Groffman 1998; Augustine *et al.* 2003). Moreover, herbivores can increase the allocation of nutrients to roots and they may stimulate root exudation in plants (Holland & Detling 1990), which may favour soil microbial activity (Hamilton and Frank 2001). Accelerated nutrient cycling enhances plant nutrient uptake and leaf nutrient concentrations (e.g. Holland & Detling 1990; Hamilton & Frank 2001) and increases the quality of plant litter. This results in a positive feedback loop (McNaughton 1984; McNaughton *et al.* 1997a) where herbivores enhance soil nutrient availability, which leads to higher plant litter quality, which in turn increases rates of soil organic matter decomposition (Cornwell *et al.* 2008). In high-fertility ecosystems, herbivores may further accelerate nutrient cycling by favouring the proportion of fast-growing, palatable plant species in a community (Augustine & McNaughton 1998; Cromsigt & Kuijper 2011).

Negative effects of herbivores on nutrient cycling are mainly explained by a decrease in the proportion of palatable plant species in a community (Pastor *et al.* 1993; Hobbs 1996). In systems where the overall plant quality is low, herbivores selectively feed on high-quality subordinate species (as legumes vs. grasses in grasslands, or deciduous tree saplings vs. conifers in forests). This results in a shift in community composition towards less palatable species with lower litter quality and decelerated decomposition rates (Augustine & McNaughton 1998; Ritchie *et al.* 1998; Wardle *et al.* 2002; Harrison & Bardgett 2004; Persson *et al.* 2005; Pastor *et al.* 2006).

Although changes in the quality of resource input into the soil food web can explain the impact of herbivores on nutrient cycling in some ecosystems (McNaughton 1984; Pastor *et al.* 1993; Ritchie *et al.* 1998; Wardle *et al.* 2002; Harrison & Bardgett 2004; Persson *et al.* 2005), they still cannot explain contrasting effects of herbivores on nutrient mineralization in many other situations (Biondini *et al.* 1998; van Wijnen *et al.* 1999; Kiehl *et al.* 2001; Bakker *et al.* 2004; Su *et al.* 2004; Pei *et al.* 2008; Wang *et al.* 2010; Gass & Binkley 2011; Shan *et al.* 2011). Even in a large-scale comparison across different sites herbivore effects on soil N cycling could not be understood from changes in plant quality (Bakker *et al.* 2006; Bakker *et al.* 2009). Therefore, herbivore effects on nutrient cycling are still not fully understood and there is a need to explore additional mechanisms that can help us to explain herbivore-induced changes in nutrient cycling (Gass & Binkley 2011).

In this paper, we aim to integrate alternative pathways with classical theories that explain under which conditions herbivores speed up or slow down nutrient cycling to be able to understand the impact of herbivores on nutrient cycling across a wide range of ecosystems. Here, we focus on the nitrogen (N) cycle, as N is an important limiting nutrient for plant production (Vitousek & Howarth 1991) and has been studied extensively in many terrestrial ecosystems. We first integrate the important drivers of N mineralization into the key components and pathways of herbivore effects on soil N availability. We then use a literature review to show how the alternative proposed pathways can affect nutrient cycling. Lastly, we use this knowledge to predict how herbivores modify N mineralization across a wide range of grazed ecosystems and verify whether our conceptual framework can help us to better understand contradictory impacts of herbivores on N mineralization by comparing our predictions with published case studies.

## Understanding N mineralization in grazed ecosystems

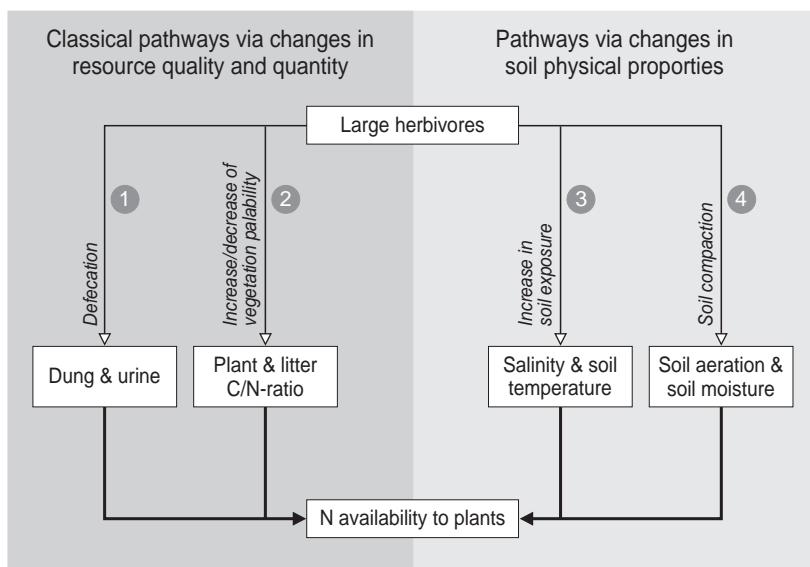
The net soil N mineralization rate is defined as the rate at which mineral forms of N (ammonium and nitrate) become available for uptake by plants through a complex of biological decomposition and transformation processes (Swift *et al.* 1979; Chapin *et al.* 2002). Mineral N is mainly released in the form of ammonium through decomposition of plant litter by soil organisms. Ammonium can be transformed into nitrate. Mineral N is used by soil microbes, and it can be lost from a system through leaching and denitrification. The rate at which soil organisms mineralize N is primarily determined by the quality of the plant litter that enters the soil food web (Parton *et al.* 2007; Cornwell *et al.* 2008). High quality litter with low C/N ratios is decom-

posed faster (Cornelissen 1996). In addition, soil physical conditions, such as temperature, moisture and aeration can be major drivers of the activity of soil organisms and therefore are important determinants of mineralization (Hamza & Anderson 2005; Powers *et al.* 2005; Bardgett & Wardle 2010) and denitrification (Laanbroek 1990). Hence, the rate at which organic forms of N are transformed into inorganic forms is affected by two key drivers, i.e. the quality of resources for the soil food web and the soil physical conditions.

Herbivores can strongly modify both resource quality (Bardgett & Wardle 2003; Pastor *et al.* 2006), as well as soil physical conditions (Asner *et al.* 2004; Gass & Binkley 2011). However, theories on grazing and nitrogen mineralization emphasize herbivore-induced changes in resource quality and quantity (McNaughton *et al.* 1997a; Bardgett & Wardle 2003), while impacts that run via soil physical conditions have received much less attention (Gass & Binkley 2011).

We propose that in order to understand the impact of herbivores on nutrient cycling across a wide range of ecosystems it will be highly relevant to include the two major components that affect N mineralization into one integrated framework (Figure 7.1). The first, i.e. the classical, component includes herbivore effects on the quality of resources for the soil food web via the addition of dung and urine and via changes in the C/N ratios of litter (Pathway 1 and 2 in Figure 7.1; McNaughton *et al.* 1997b; Bardgett & Wardle 2003). The second component represents herbivore effects on soil physical conditions which are important for rates of nitrogen mineralization (Gass & Binkley 2011). This component is composed of at least two key parameters that drive N mineralization. First, herbivores can increase the exposure of bare soil by removing the aboveground vegetation (Pathway 3 in Figure 7.1). This can enhance N mineralization rates in ecosystems where soil temperature is limiting decomposition, because soil temperature may increase due to reduced insulating capacities of the vegetation (Olofsson *et al.* 2004; Gornall *et al.* 2009). Alternatively, it can also reduce N mineralization when evaporation increases salinity at the soil surface (Srivastava & Jefferies 1996; Buckeridge & Jefferies 2007). Secondly, trampling by herbivores can compact the soil (Pathway 4 in Figure 7.1). In compacted soils aeration and water filled porosity are altered, which has major consequences for N mineralization rates (Hamza & Anderson 2005; Powers *et al.* 2005; Drewry *et al.* 2008). However, the impact of herbivore-induced soil compaction strongly depends on the interactions between soil moisture conditions and soil texture (Hamza & Anderson 2005; Drewry *et al.* 2008). It will be highly relevant to understand this interaction in more details, because compaction and soil moisture are important determinants of nutrient cycling irrespective of litter quality (Powers *et al.* 2005). Therefore, in the following paragraph we assess how





**Figure 7.1** Overview of the influence of large herbivores on the nitrogen (N) cycle in grasslands. This diagram depicts four main pathways via which herbivores can influence soil N mineralization. Prevailing theories mainly focus on Pathways 1 and 2 via which herbivores alter the quality of resources entering the soil food web, i.e., by excreting dung and urine and by changing plant quality. Pathways 3 and 4 operate through soil physical properties. Pathway 3 describes the effects of herbivores on the exposure of bare soil, thereby increasing soil temperature or soil salinity (e.g. Gornall 2009, Buckeridge & Jefferies 2007). Pathway 4 represents effect of large herbivore trampling via soil compaction on soil moisture and soil aeration, which consequently affects soil N mineralization (this article).

herbivore induced soil compaction influences N mineralization in both wet and dry ecosystems and in soils with fine and coarse texture, of which the results are summarized in Table 7.1. In this table, we have attempted to be as complete as possible for studies on both the wet and dry end of the gradient. In the intermediate region of soil moisture (between 10%-60%), we have only used the most important studies.

## How soil compaction by herbivores interacts with soil moisture and soil texture

Soil water availability is one of the main parameters that determine nutrient mineralization, because it drives the amount of water and oxygen availability

for soil microbes (Paul 2003). The amount of water a soil can hold mainly depends on texture and pore size distribution. Water filled porosity, i.e., the proportion of pores filled with water, is increased when clay and organic matter content are high or when the fraction of small pores is large. Herbivores can alter water filled porosity through trampling, because this may induce soil compaction which in turn enhances the fraction of small pores in the soil and thereby limits water infiltration rates (Wolkowski 1990). Consequently, herbivores can have a strong impact on N mineralization through trampling. Whether the effect of grazing induced soil compaction on N mineralization is positive or negative may vary along a gradient of soil moisture and soil texture (Table 7.1; Hamza & Anderson 2005). In wet soils, increased soil moisture may reduce oxygen availability and inhibit mineralization (Drewry *et al.* 2008), whereas in water-limited soils, it enhances N mineralization. Fine-texture soils are composed of fine particles and thus sensitive to compaction. Hence, compaction effects on water filled porosity may be stronger in fine-textured soils than coarse-textured soils (Figure 7.2; Rasiah & Kay 1998). Thus, the strength and direction of the effects of herbivore trampling on N mineralization will be highly dependent on the interaction between soil moisture and soil texture (Table 7.1; Hamza & Anderson 2005).

### **Wet end of the soil spectrum**

In wet soils, compaction can result in strongly decreased nitrogen mineralization (Breland & Hansen 1996; De Neve & Hofman 2000). Torbert & Wood (1992) reported a 65% decrease in N mineralization in a strongly compacted soil. These negative effects can originate from reduced porosity (Breland & Hansen 1996) and reduced oxygen and nutrient diffusion (Lipiec & Stepniewski 1995; Startsev & McNabb 2007). Both processes can lead to lower activity of soil organisms and increased denitrification (Laanbroek 1990). When pores become smaller, organic material and microbial biomass become physically protected against attack by soil fauna and microbial feeders, respectively (Breland & Hansen 1996). Consequently, numbers of soil fauna are found to decrease in more compacted soils (Bouwman & Arts 2000; Sorensen *et al.* 2009). This reduces the potential to stimulate soil microbial growth (i.e. breakdown of the 'microbial loop'; Verhoef & Brussaard 1990; Berg *et al.* 2001; Osler & Sommerkorn 2007), and may thus lead to negative effects on the rate of N mineralization.

Denitrification occurs when oxygen diffusion into the soil is hampered, usually when the water filled porosity is higher than 60% (Haunz *et al.* 1992; Lipiec & Stepniewski 1995; Menneer *et al.* 2005). Consequently, oxygen becomes limiting for aerobic decomposition, after which nitrate can be used

as an alternative electron acceptor, subsequently leading to loss of nitrate from the soil (Wollersheim *et al.* 1987; Laanbroek 1990). Compaction induced denitrification can greatly affect soil N levels. An experiment by Menneer *et al.* (2005) showed that loss of inorganic N by denitrification in response to severe trampling of large herbivores can be up to 593 g N ha<sup>-1</sup> yr<sup>-1</sup>.

Compaction on fine textured soils is more likely to lead to high levels of water filled porosity (>60% water filled porosity) in response to compaction (Hamza & Anderson 2005; Drewry *et al.* 2008). Hence, negative effects on N mineralization are more often found on wet, fine-textured soils, (Hamza & Anderson 2005; Powers *et al.* 2005). Several studies on these soils report a decrease in N mineralization in response to large herbivore grazing (Table 7.1; van Wijnen *et al.* 1999; Kiehl *et al.* 2001; Schrama *et al.* in prep). Although one would expect herbivore grazing on coarse textured soils also to affect N mineralization in (very) wet conditions, we have not found any studies that report on effects of large herbivores on coarse textured, wet soils.

**Table 7.1** Reported effects of herbivore grazing on N mineralization, categorized by soil type and soil moisture. At very high (> 60%) and very low (<10%) soil moisture, negative effects of grazing on net N mineralization as a result of denitrification and reduced mineralization were expected. \*No studies found.

Soil type	Soil moisture content in response to grazing		Reported effect on N Mineralization	References
<b>Fine textured soil</b>	High	>60 %	--/-	1) 2) 3)
	Intermediate	40-60 %	-/0	2) 3) 4) 5) 6) 7)
	Low	10-40 %	0/+	3) 8) 9)
	Very low	<10%	-	10) 11) 12)13)
<b>Coarse textured soil</b>	High	>60 %	NA*	
	Intermediate	40-60 %	+	14) 15)
	Low	10-40 %	+	8) 16) 17) 18)19)
	Very low	<10%	-/+	20) 21) 22) 23) 24)25)

Van Wijnen *et al.* (1999); 2) Kiehl *et al.* (2001); 3) Menneer *et al.* (2005); 4) Bakker *et al.* (2004); 5) Olsen *et al.* (2011); 6) Lavado and Alconada (1994); 7) Chaneton and Lavado (1996); 8) Sankaran and Augustine (2004); 9) Gass and Binkley (2011); 10) Zhang *et al.* (2008); 11) Tessema *et al.* (2011); 12) Biondini *et al.* (1998); 13) Shan *et al.* (2011); 14) Mikola *et al.* (2009); 15) Le Roux *et al.* (Le Roux *et al.* 2003); 16) Augustine *et al.* (2003); 17) McNaughton (1997a); 18) Peco *et al.* (2006); 19) Frank and Groffman (2000), 20) Wang *et al.* (2010); 21) Su *et al.* (2004); 22) Barger *et al.* (2004); 23) Giese *et al.* (2011); 24) Bradley *et al.* (2006); 25) Pei *et al.* (2008).

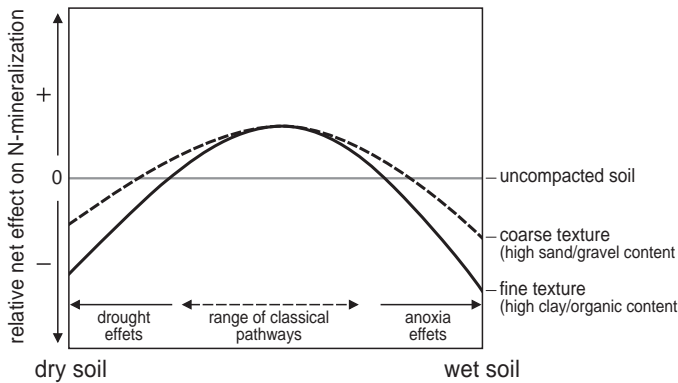
### Dry end of the spectrum

In arid, warmer ecosystems, soil moisture is often the main limiting factor for decomposition and thus also for N mineralization (van de Koppel *et al.* 1997; Austin *et al.* 2004). In dry ecosystems the effect of compaction is not consistently negative or positive. Compaction reduces water infiltration, which limits water availability. At the same time it may increase the proportion of small pores which enhances the capacity of a soil to retain water. The balance of these effects therefore determines the effect on N mineralization. If the infiltration rate is strongly decreased by compaction, this may lead to lower water filled porosity (Castellano & Valone 2007; Allington & Valone 2010), and to a decrease in N mineralization (Pei *et al.* 2008). Alternatively, when water filled porosity increases due to a compaction-induced reduction in pore size, N mineralization can be enhanced (Peco *et al.* 2006).

Similarly to wet soils, the impact of compaction on water availability in dry soils depends on the soil texture (Figure 7.2). In fine-textured soils, compaction may reduce the pore-size and enhance the capacity of the soil to retain water. Under these conditions herbivores have the potential to increase decomposition and N mineralization (Table 7.1; Peco *et al.* 2006; Zhang *et al.* 2008). However, when fine-textured soils become extremely dry (soil moisture below 10%), compaction reduces water infiltration and increases evaporation to such an extent that it becomes the limiting process for N mineralization (Table 7.1; Pei *et al.* 2008; Giese *et al.* 2011; Tessema *et al.* 2011). Moreover, in some dry, fine-textured soils with high levels of soluble salts, the combination of reduced water infiltration due to trampling and high evaporation at the soil surface result in salinization or calcification. Increased levels of soil salinity can impose an additional constraint on N mineralization (Lavado & Alconada 1994; Chaneton & Lavado 1996; Srivastava & Jefferies 1996).

In dry coarse-textured soils, compaction effects are generally less strong than in fine-textured soils. In such soils, compaction does not strongly enhance the proportion of small pores, while infiltration into the soil is can become limited through trampling effects in the top layer. As a result, compaction leads to lower water filled porosity (Castellano & Valone 2007; Allington & Valone 2010) and hence to decreased decomposition and lower N mineralization (Table 7.1; Su *et al.* 2004; Jeddi & Chaieb 2010; Wang *et al.* 2010; Shan *et al.* 2011).

To summarize, trampling-induced soil compaction on N mineralization varies across along gradients of soil moisture and soil texture (Table 7.1). Particularly in dry and wet soils with fine texture trampling-induced compaction may have an important (negative) influence on N mineralization, which is illustrated in Figure 7.2. In more coarse-grained soils and soils with



**Figure 7.2** Hypothetical changes in N mineralization as a result of herbivore trampling in soils with different texture. On a fine-textured soil (clay) herbivore compaction may lead to soil anoxia (wet end of the gradient) and strongly reduced water infiltration (dry end of the gradient), which both affect N mineralization negatively. These effects hence outweigh the positive effects on N mineralization via litter quality and quantity. On a coarse texture soil (sand), herbivore trampling induced effects on N mineralization are less pronounced, and may therefore be outweighed by effects through excreta, litter quantity and quality.

an intermediate soil moisture trampling effects may be less pronounced (Figure 7.2). Here, herbivore-induced changes in resource quality and quantity (Pathway 1 and 2 in Figure 7.1) determine the outcome of the effect of large herbivores on N mineralization.

## **Towards an integrated understanding of herbivore impact on N mineralization**

Our integrated framework that combines the modification of soil physical properties by herbivores with the longer acknowledged effects of quality of litter input helps us to explain contradictory results on N mineralization (Figure 7.1). Whether overall effects of herbivores on N mineralization are positive or negative depends on the balance between the different pathways. From the literature review (see Table 7.1), it appeared that the impact of herbivores on N mineralization via the soil physical pathway is particularly strong in ecosystems with extreme soil moisture conditions or fine soil texture. In wet and dry ecosystems, trampling-induced soil compaction can create suboptimal soil moisture conditions for N mineralization. These effects are more likely to occur in fine-textured soils that are more sensitive to

compaction than coarse-textured soils. Therefore, our framework suggests that in these systems trampling effects outweigh the effects via dung and urine deposition and enhanced litter quality. Indeed, in ecosystems with extreme soil moisture conditions a reduction in N mineralization under grazing is often reported (Lavado & Alconada 1994; Chaneton & Lavado 1996; Jeddi & Chaieb 2010; Wang *et al.* 2010; Giese *et al.* 2011; Shan *et al.* 2011), even when plant quality was enhanced by grazing (van Wijnen *et al.* 1999; Kiehl *et al.* 2001; Bakker *et al.* 2004).

In contrast, in ecosystems with intermediate soil moisture or coarse-grained soils, negative effects on N mineralization through soil compaction are less pronounced and may therefore be outweighed by effects via excreta and litter quality. Studies from ecosystems with such soil conditions report an overall increase in mineralization rates when herbivores enhance the quality of resource input into the soil (such as in Georgiadis *et al.* 1989; McNaughton *et al.* 1997a; Bardgett & Wardle 2003; Cromsigt & Kuijper 2011), and a decrease when herbivore reduce plant and litter quality through selective grazing or browsing (Pastor (Pastor *et al.* 1993; Ritchie *et al.* 1998; Augustine 2003).

## Conclusion

Our synthesis aims to clarify the effects of herbivores on N mineralization, by explicitly taking soil physical properties into account. We show that our model explains contrasting effects of large herbivores on nitrogen mineralization along gradients of soil moisture and soil texture. In very wet and dry systems with fine soil texture, effects of herbivores on N mineralization via the alteration of soil physical conditions outweigh effects of herbivores via changes in resource quality.

## Acknowledgements

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## Appendix V

**Table V.1** Overview of processes determining nutrient mineralization. Direction in which these processes are influenced by soil compaction is indicated using signs.

Factors affecting mineralization	main direction <sup>a</sup>	explanation
<b>Plants</b>		
Plant quality	+	enhanced availability of nutrients stimulates decomposer activity a)
Oxygen transport through roots	+	marsh plants use aerenchym to transport oxygen, locally increasing bacterial production b)
Root exudates	+	input of carbon into the soil causes higher microbial production c)
<b>Large herbivores</b>		
Defoliation of vegetation	+	defoliation results in higher root exudation and litter quality causing higher decomposition d)e)
Dung and urine production	+	nitrogen in wastes becomes easily available to microorganisms and plants f) g)
Changing plant species community	+/-	preferential feeding on high/low quality results in high/low quality vegetation and litter i)
Trampling	+/-	+ litter availability to decomposers increases h) - high trampling results in anoxic soil which inhibits decomposition (this paper)
<b>Soil fauna</b>		
Increased organic matter availability to microbes	+	soil fauna pellets serve as favourable mineralization setting j)
Stimulating mineralization in gut	+	guts of earthworms, termites, microarthropods stimulate soil microbial decomposition k), l)
Excreting excess nutrients	+	by foraging on microbes, soil fauna accelerates nutrient mineralization ("microbial loop") m)
Bioturbation	+	termites, mice, earthworms, ants create macropores by digging and burrowing activities n), o)
<b>Soil parameters</b>		
Soil fertility	+/-	high soil fertility yields high mineralization, low soil fertility leads to lower mineralization p)
Soil moisture	+/-	mineralization works optimal under intermediate moisture conditions. Both in dry and very wet soils mineralization is impeded. q)

<sup>a</sup>Indicates whether mineralization is generally enhanced (+) or decreased (-)

a) (Wardle 1998); b) (Table 1, Jackson and Armstrong 1999); c) (Bardgett *et al.* 1998); d) (Briske *et al.* 1996); e) (Ferraro and Oesterheld 2002); f) (Afzal and Adams 1992); g) (Bogaert *et al.* 2000); h) (Zacheis *et al.* 2002); i) (Ritchie *et al.* 1998, Wardle 2002); k) (Trigo *et al.* 1999); l) (Daniel and Anderson 1992); m) (Clarholm 1985); n) (Lavelle *et al.* 1997); o) (Brown *et al.* 2000); p) (Sankaran and Augustine 2004); q) (Bardgett 2005)

# Chapter 8





# 8

## Soil compaction by a cutting regime affects below-ground plant traits and vegetation composition

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*In revision for Plant and Soil*

### Abstract

To maintain grassland biodiversity machine mowing, mimicking the traditional hand mowing, is often used as a successful management tool. Few studies have investigated the long-term differences between traditional and mechanical mowing. Machine mowing causes soil compaction and thus reduction of soil aeration. In response, we expected strong effects on below-ground plant traits: root aerenchyma formation and superficial root growth. Effects are expected to be more pronounced in soils vulnerable to compaction.

We evaluated a long-term (38-year) experiment with constant hand-mowing and machine-mowing management regimes on two different soil types: a coarse textured (sandy) soil and a finer textured (sandy-organic) soil.

Long-term machine mowing leads to soil compaction and lower soil aeration, but only on the fine textured soil. Here we find a higher cover of superficially rooting species and a lower cover of species without adaptations to soil hypoxia, but no effect on species with a high capacity of forming aerenchyma.

Mowing with heavy machines on soils vulnerable to compaction changes the vegetation community as a result of changes in soil physical conditions. This is reflected in a shift towards a community with a greater proportion of superficially rooting species.

## Introduction

Low-intensity farming has disappeared in large parts of Western Europe. This has resulted in a decline of species-rich grasslands (Lawton & May 1995; Bignal & McCracken 1996; Pywell *et al.* 2002). Characteristic plant communities linked to centuries of hay-making practices on nutrient-poor soil conditions have therefore declined markedly (McKinney & Lockwood 1999; Walker *et al.* 2004). In an effort to maintain or restore valuable grassland plant communities that are of nature conservation interest, management regimes are often installed. Extensive cutting and grazing management measures have been effective in maintaining species-rich grassland communities (Walker *et al.* 2004).

Cutting in these grasslands is often done with heavy machinery, as traditional hand cutting is costly (Liira *et al.* 2009). Few studies have evaluated how these different management practices affect above- and belowground plant traits, however, some notable exceptions are Huhta (2001) and Liira *et al.* (2009).

From agricultural practices it is known that wheel traffic from heavy agricultural machines has a strong soil compacting effect (Hamza & Anderson 2005), which is not expected as a result of hand mowing. Soil physical properties, such as soil moisture, soil aeration and denitrification, can be strongly affected by soil compaction (Soane *et al.* 1982; Jensen *et al.* 1996; Nevens & Reheul 2003), especially in wet soils with a high organic fraction or soils with fine texture (Rasiah & Kay 1998). Strong negative effects of soil compaction on plant performance are frequently observed when soils are wet during compaction (Voorhees *et al.* 1985; Nevens & Reheul 2003). This happens because of two reasons. Firstly, root growth may be physically impeded by soil density (Engelaar *et al.* 1993; Striker *et al.* 2007) and secondly, compaction increases the chance of oxygen-deficient conditions because of smaller pore size and increased water content (Vartapetian & Jackson 1997; Jackson & Armstrong 1999). On the other hand, under dry conditions soil compaction may even be favourable to plant performance, as compaction may increase capillary action and thus improves access to water and nutrients (Voorhees *et al.* 1985).

When managed with heavy machines, compaction can be expected to act on plant community composition, especially if soils are fine textured and thus sensitive to soil compaction. If soil compaction leads to hypoxia in the rooting zone, aerenchyma formation in roots and species with superficial rooting can be expected (Vartapetian & Jackson 1997; Elzenga & van Veen 2010) and species that are unable to produce such adaptations may be selected against. However, species with aerenchyma in strongly compacted

soil may also have a disadvantage due to reduced physical strength of these roots (Engelaar *et al.* 1993; Striker *et al.* 2007). So, while machine mowing and hand mowing have similar aboveground effects in terms of reducing light competition, belowground responses to the different management regimes may be strongly differing.

In this study, we evaluated a long-term (38 year) experiment in which a machine-cutting regime and a hand-cutting regime were installed on two previously similar sites. The first research field was located on a sandy soil with a relatively high content of organic matter, which adds fine texture, thus leading to higher capillary forces and higher water holding capacity (hereafter: fine textured soil). The second research field was located on an adjacent field with sandy soil with less organic matter and thus coarser texture and lower water holding capacity (hereafter: coarse textured soil).

Firstly, we expected a divergence in vegetation development and species composition between the machine mown and hand mown treatments. We expected differences in the root traits, in particular an increasing cover of species with aerenchyma formation or superficial root growth in the machine mown treatment. Furthermore, we expected that differences between machine mown and hand mown treatments are stronger on a fine textured soil than on a coarse textured soil. In contrast to belowground traits, aboveground traits are not directly affected by changing soil physical properties. Thus, a priori we expected no treatment effect on canopy height, specific leaf area, and leaf dry matter content.

## Methods

### Study site and treatments

The study site Loefvledder (53°05'N, 6°40'E) is located in the brook valley of the Drentsche Aa, The Netherlands. It is in the upper course of the brook valley at 7m above Dutch Ordnance Level, and is characterized by a sandy subsoil. The study site includes two fields with different agricultural history before they were transformed into a nature reserve. The first field (with a wet, organic top layer, hereafter called fine textured soil) was acquired in 1967 ('old field' in Bakker *et al.* (2002a)) and fertilizer application was stopped in the same year. The other field (with a sandy top layer, hereafter called coarse textured soil) was acquired in 1972 ('new field' in Bakker *et al.* (2002a)) and fertilizer application stopped in that year. The cessation of fertilizer application lead to a decline in biomass production in both fields from a yield of about 400 to 650 g/m<sup>2</sup> in the 1970s to 250 to 350 g/m<sup>2</sup> nowadays for the old field and of about 600 to 900 g/m<sup>2</sup> to 200 to 500 g/m<sup>2</sup> in the new field

(Bakker *et al.* 2002a). At the start of the experiment, both fields had a similar groundwater level of  $56.3\text{ cm} \pm \text{SD } 16.1\text{ cm}$  for the fine textured soil and  $58.7 \pm \text{SD } 23.9$  for the coarse textured soil (yearly average) below ground level.

We used data from two mowing experiments in each field. Hence, both experiments were carried out within a single field with the same agricultural history and soil type. The experiments started in 1972, after which the vegetation was mown once every year in July by means of machines in one treatment and by hand using a petrol scythe in the other treatment. Hay was also removed with machines in the machine mown treatment and raked by hand in the hand mown treatment. Hence, on both mowing treatments aboveground biomass was removed. More details on the setup of the field experiment can be found in Bakker (1989).

Each treatment included six permanent plots of  $2 \times 2$  m. Dynamics of the species composition of the plant communities were recorded in most years between 1972 and 2010 by estimating cover of individual species according to the decimal scale (Londo 1976). The unique long-term character of the experiment is necessary to reveal long-standing effects on community development of vascular plants. Unfortunately the low sample size is the downside of this.

### Measurements on soil physical properties

Bulk density was determined near each permanent plot by taking the average of two soil samples. Soil samples were taken in July 2010 using a Tullgren soil core device with a diameter of 10 cm, which is often used to sample soil fauna (Van Straalen & Rijninks 1982) and minimizes soil compaction while taking the core. From these cores, living aboveground vegetation was carefully cut and removed before the soil sample was taken. The samples were weighed, dried ( $70^\circ\text{C}$ , 48 hours) and weighed again to determine bulk density. Air filled porosity was determined in the field, using a method that was first described by Jafarzadeh (2006) and which is based on 'dynamic compaction'. According to this method, the volume of soil on which a weight is dropped decreases asymptotically with the number of times that a weight is dropped on the soil, until all air is pushed out. Maximal compaction is typically reached after dropping the weight ten times. These samples were taken pairwise with the other soil samples using the same soil corer as described above. We used a 1.5 m long PVC cylinder ( $\varnothing$  10 cm) to compact a 15 cm high soil core with equal diameter, which was placed at one end of the PVC cylinder. A lid was put on the bottom of the PVC cylinder, in order to avoid loss of sample. From the top of the vertically placed cylinder, we applied a force of  $6.5\text{ kN m}^{-2}$  by dropping a weight of 5 kg, ten times. As a measure for the total amount of air-filled porosity, we measured the decrease in volume of the sample.

As a proxy for the oxygen level in the soil we measured the soil redox potential. We chose a set up in which four electrodes with a platinum tip and one Ag/AgCl reference electrode (Cole-Palmer®) were connected to a Graphtec GL200 Datalogger (Graphtec GB Ltd). All measurements were taken at the 23<sup>rd</sup> of June 2011. In order to obtain a depth-profile of the soil redox potential, all electrodes were placed into the soil first at 5 cm and subsequently at 10 cm depth. Measurements were read out exactly two minutes after the electrodes were placed at a certain depth. Readings were corrected for the potential of the reference electrode (+197 mV) and pH. Averages of the four platinum electrodes were used for further analyses. Quick in situ measurements on redox may not necessarily reflect absolute values and are highly variable throughout the year, but are suitable for making comparisons between treatments (van Bochove *et al.* 2002).

### Plant Traits

To study the potential impact of soil compaction on species composition in the studied grasslands, we looked at species aboveground traits and species belowground traits. We used the ability to produce aerenchyma and maximal rooting depth as species belowground traits. According to these two characteristics, species were assigned to three groups: Species with a maximal rooting depth less than 10 cm were assigned to group 1. Species with less than 15% aerenchyma and rooting depth deeper than 10 cm were assigned to Group 2. Species with the ability to produce more than 15% aerenchyma and rooting depth more than 10 cm were assigned to Group 3. As previous analyses have shown that the threshold for the minimum amount of aerenchyma (porosity) that is functionally significant in oxygen-deficient soil can also be defined as 10% (De Willigen & Van Noordwijk 1989), we re-ran the analyses on aerenchyma with a threshold between group 2 and 3 at 10% aerenchyma. All frequent species (species with cover >5% in any year or occurring for at least five consecutive years in a plot) at the research locations were assigned to one of the groups. Values for maximal rooting depth were taken from literature (von Kutschera *et al.* 1982) and values for aerenchyma (root porosity) were either taken from literature (von Kutschera *et al.* 1982; Justin & Armstrong 1987) or were determined from plants grown on hydroculture. For details on species root traits and division into groups, see Appendix VI, Table 1.

All species in which we experimentally determined root porosity were taken from the Loefvledder research location. Three specimen for every species were taken on the 9th of August 2010. Every specimen was carefully rinsed and placed on hydroculture (Appendix VI, Figure 1). Plants were grown at 0.25 strength Hoagland-solution (Hoagland & Arnon 1950). After two

weeks, all plants were put in a bath of 0.05 % agar in nutrient solution (w:v) to minimize oxygen diffusion in the water column, hereby mimicking a hypoxic environment (Wiengweera *et al.* 1997). Acidity of the solution was kept constant at pH6 ( $\pm 0.2$ ) and the hydroculture medium was replaced biweekly to avoid algal growth. After another four weeks, plants were harvested. From every individual, five 1cm root parts were carefully cut from the adventitious roots, 2cm from the root tip. As a proxy for porosity, we used the percentual volume of air inside the aerenchyma of these root segments (Visser & Bogemann 2003). The air-filled aerenchyma in the submerged root particles was filled with water using a vacuum pump (Emergo BV, Landsmeer, the Netherlands) by applying a vacuum of 0.04 Bar for 5 min, which was repeated three times. To calculate the increase in water in the pores, weight increase of root particles was determined by weighing before and after the aerenchyma was infiltrated. As a species specific proxy for porosity, we used the average of all individuals of one species.

We used specific leaf area (SLA), leaf dry matter content (LDMC) and canopy height (CH) as species aboveground traits. For details on species traits, see Appendix VI, Table 2. All three traits are commonly used to indicate responses to a multitude of changes in environmental circumstances as they contribute information on interspecific variation (Wilson *et al.* 1999; Westoby & Wright 2006). The specific leaf area is known to be correlated with photosynthetic capacity and leaf lifespan (Ryser & Urbas 2000; Wright *et al.* 2004; Shipley 2006). Leaf dry matter content is associated with leaf lifespan (Weiher *et al.* 1999; Ryser & Urbas 2000) and community litter decomposition rates (Quested *et al.* 2007; Laughlin *et al.* 2010). Canopy height is a good surrogate for the competitive vigour of a species (Gaudet & Keddy 1988; Tilman 1988). For most of the species we used trait data from own measurements on individuals sampled in the surroundings of the study site. The values of the few missing species were taken from the trait database LEDA (Kleyer *et al.* 2008). We measured canopy height from the base of the plant to the highest photosynthetic tissue in metres. Sampled leafs were scanned with the HP Image & Scanning Program (2009) and afterwards leaf fresh weight was determined. After drying in the oven at 70°C for 24 hours finally leaf dry weight was determined. Leaf area (mm<sup>2</sup>) was determined by analyzing the leaf scans using the software Lafore (Lehsten 2004). Leaf dry matter content was calculated as the ratio of dry to fresh leaf weight (mg g<sup>-1</sup>) and specific leaf area was calculated by dividing the area of the fresh leaf by the dry weight (mm<sup>2</sup> mg<sup>-1</sup>).

## Data Analysis & Statistical analysis

To evaluate the two mowing treatments and whether the treatment effect was different in the two fields, we used Bray-Curtis dissimilarity index and mixed models. Firstly, we calculated how the floristic similarity between treatments changed over time. In order to calculate Bray-Curtis floristic dissimilarity index between treatments within fields, plant species cover data of every permanent plot in a given treatment was compared to cover data for all plots in the other treatment. The yearly average of these 36 comparisons was used as the dissimilarity between treatments in a given year. We calculated the Bray-Curtis dissimilarity index using R (RDCT 2011) with package 'vegan' (Oksanen *et al.* 2008).

Secondly, we applied mixed models to test for differences between treatments in below- and aboveground traits. Analysis were done in R (RDCT 2011) with package 'lme4' (Bates *et al.* 2011) to calculate models and package 'languageR' (Baayen 2011) to calculate p-values.

For the belowground traits the model was structured as followed:  
$$\text{lmer}(\text{sqrt}(\text{data.prop.cover}) \sim \text{data.year} * \text{data.treat} + (1 | \text{data.plot}) + (1 | \text{data.year}), \text{family} = \text{"gaussian"}).$$

Using the summed proportional cover of all species in a permanent plot belonging to the same root group (data.prop.cover) as dependent variable, the year of recording (data.year) and the treatment (data.treat) including their interaction as fixed effects and the plot (data.plot) as well as the year of recording as random effects. A gaussian distribution was assumed for this data. The cover data entered the model square root transformed, because of heteroscedascity (see Gotelli & Ellison 2004). The treatment effect was evaluated by looking at the year-treatment interaction effect.

For the aboveground traits: specific leaf area (SLA), leaf dry matter content (LDMC) and canopy height, we used log transformed cover weighted and unweighted community means as response variable and used the same model structure as for the belowground traits.

According to our hypothesis, the compactive machine mown treatments should result in lower soil aeration and higher soil moisture, to which species with adaptations to high levels of soil moisture should respond positively. This reaction is expected to be captured in the Ellenberg-moisture values for these species (Ellenberg *et al.* 1992). Therefore, we performed an analysis on the weighted community average of the Ellenberg moisture values, using the same mixed model structure as for the plant traits, but with community Ellenberg moisture values as response variable.



## Results

We found divergent succession between treatments on fine textured soil: species composition became more dissimilar over time ( $R^2 = 0.67$ ,  $P < 0.001$ ; Figure 8.1). On the coarse textured soil, a less steep increase in dissimilarity between the treatments was observed ( $R^2 = 0.38$ ,  $P < 0.001$ ; Figure 8.1). For a similar analysis on the Bray-Curtis dissimilarity on presence-absence, we found no increase in dissimilarity over time. The number of plant species showed a gradual decline on the fine-textured soil, for both the hand mown ( $R^2 = 0.65$ ,  $P < 0.001$ ; Appendix VI, Figure 2) as well as for the machine mown treatment ( $R^2 = 0.74$ ,  $P < 0.001$ ). On the coarse textured soil, no decrease in species richness was noted (Appendix VI, Figure 2).

### Effects on soil properties

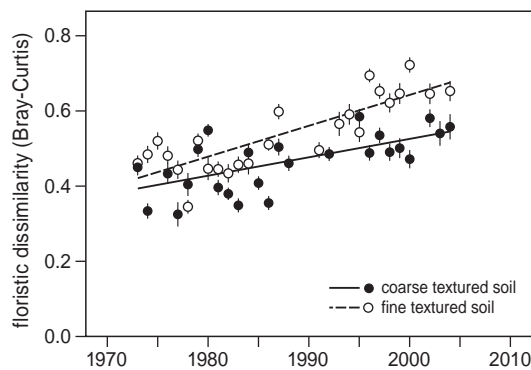
We found a significantly more compacted, less porous soil in the machine mown treatment on both soil types (Table 8.1). However, compaction on fine textured soil had a much stronger effect on soil physical properties than on coarse textured soil. Soil redox potentials were significantly lower in the machine mown treatment on fine textured soil, indicating lower oxygen availability (Table 8.1), but this effect was not found on the coarse textured soil. Mixed models on community Ellenberg moisture values show a significant treatment-year interaction effect on fine textured soil, with higher Ellenberg moisture values in the machine mown treatment (Table 8.2). No year or treatment-year effect was found on Ellenberg moisture values on coarse textured soil (Table 8.2; Appendix VI, Figure 3).

### Effects on belowground traits

Functional belowground traits in coarse textured soil showed no divergence between treatments (Figure 8.2A-C), but we found a major divergence between treatments on the fine textured soil (Figure 8.2D-F). This is in agreement with the changes in soil properties and the increasing floristic dissimilarity between treatments on fine textured soil. Species with superficial root growth (group 1) showed a significantly stronger increase in response to the machine mown treatment (Table 8.2, Figure 8.2D). Then again, species with minor or no root adaptations (group 2) showed a significantly stronger decrease in cover in the machine mown treatment (Table 8.2; Figure 8.2E). A steady increase in cover of aerenchyma-forming species was observed in the fine-textured soil (Figure 8.2F), but we found no significant treatment-effect (Table 8.2). This indicates that the increase in the cover of aerenchyma-forming species occurred regardless of mowing regime. For none of the root groups on coarse textured soil, we found a significant difference between



treatments over time (Table 8.2). In addition to the absence of a treatment-year effect on the coarse textured soil, we also could not detect any trend over time (Figure 8.2A-C). In addition to the results on root traits, we found higher root volumes in the upper 10 cm in the fine textured soil in response to machinemowing (56.6 vs 11.5 ml l<sup>-1</sup> P < 0.001), but no such differences between treatments were found in the coarse textured soil (10.3 vs 3.1 ml l<sup>-1</sup> P = NS; Table 8.1).



**Figure 8.1** Floristic dissimilarity  $\pm$ SE between treatments on coarse textured soil and on fine textured soil. Coarse textured soil:  $R^2 = 0.67$ ;  $P < 0.001$ ; Fine textured soil:  $R^2 = 0.38$ ;  $P < 0.001$ ). Mean values are given  $\pm$  SE.

**Table 8.1** Soil physical properties in all treatments after 38 years. A paired t-test was done to identify significant differences. Mean values are given  $\pm$  SE. NS Not Significant, \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

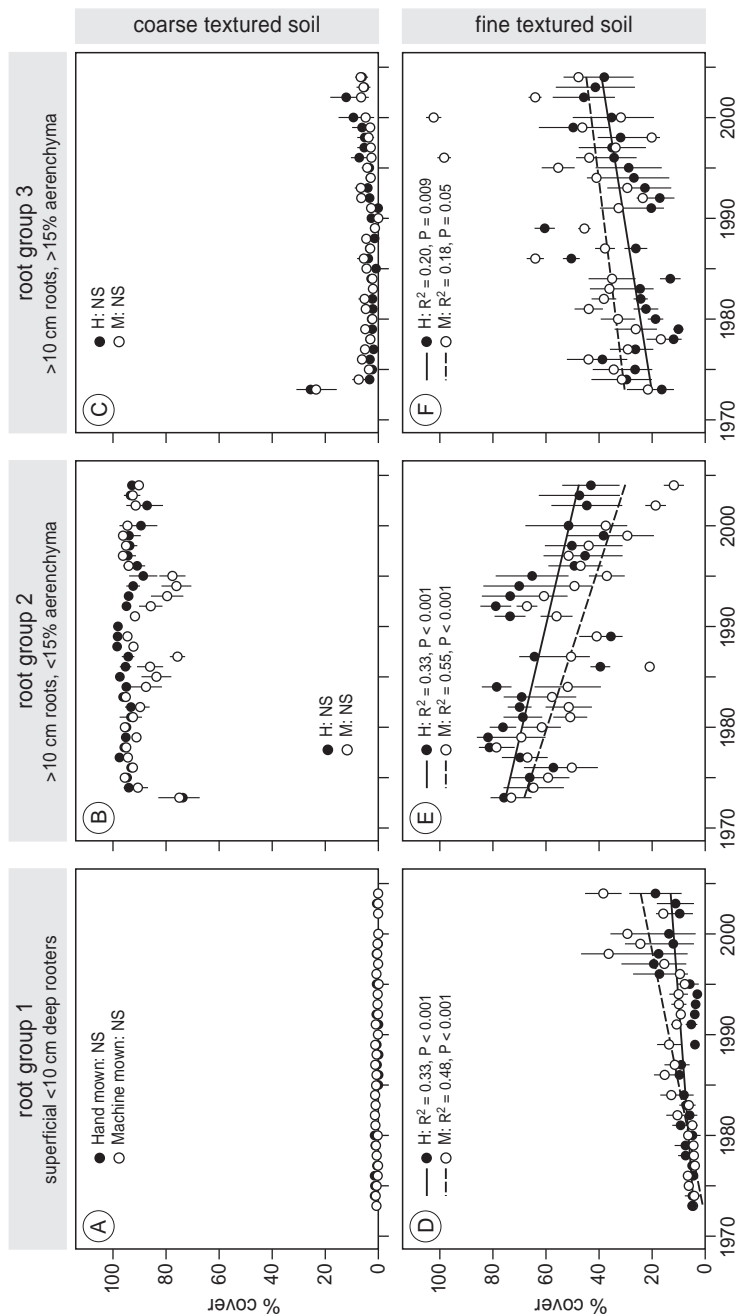
	Machine mown	Hand mown	N	T	P
<b>Fine textured soil</b>					
Bulk density (g cm <sup>-3</sup> dry weight volume)	0.42 $\pm$ 0.05	0.41 $\pm$ 0.03	6	0.6	NS
Air filled porosity (%; v:v)	11.0 $\pm$ 0.84	21.3 $\pm$ 1.14	8	7.8	***
Soil redox potential (mV) 5 cm	322.5 $\pm$ 18.6	431.2 $\pm$ 18.3	5	28.3	***
Soil redox potential (mV) 10 cm	271.4 $\pm$ 41.2	416.1 $\pm$ 20.5	5	5.6	**
<b>Coarse textured soil</b>					
Bulk density (g cm <sup>-3</sup> dry weight volume)	0.67 $\pm$ 0.05	0.68 $\pm$ 0.05	6	0	NS
Air filled porosity (%; v:v)	17.7 $\pm$ 0.69	23.2 $\pm$ 0.68	8	5.9	***
Soil Redox Potential (mV) 5 cm	429.8 $\pm$ 7.9	455.2 $\pm$ 12.3	5	1.7	NS
Soil Redox Potential (mV) 10 cm	410.3 $\pm$ 11.0	451.6 $\pm$ 20.0	5	2.2	NS

### Effects on aboveground traits

Of all aboveground traits, we found that the weighted community mean for canopy height reacted strongest to our treatment. Values are clearly lower in the machine mown treatment on both soils (Table 8.2). The community leaf traits showed a far less, and less consistent, divergence between treatments over time. Nevertheless, the weighted community mean for SLA was different between treatments in fine textured soil and the weighted community mean for LDMC was different between treatments on coarse textured soil (Table 8.2). A re-analysis performed on presence/absence data for all plant species showed an even stronger effect of treatment on the community mean for canopy height in fine textured soil, but not on the coarse textured soil (Table 8.2). Using presence/absence data we also found no treatment effect on the community means for SLA and LDMC (Table 8.2).

**Table 8.2** Different effects on aboveground and belowground traits as a result of compacting and non-compacting mowing treatments on the fine and coarse textured soil. Abbreviations behind traits depict the different statistical method used. CV: calculated using plant species cover, P/A: calculated using presence/absence of plant species in the plots.

Factor	Treatment-Year interaction effect: fine textured soil				Treatment-Year interaction effect: coarse textured soil			
		Estimate ± SE	F/t-value	P		Estimate ± SE	F/t-value	P
<b>Aboveground traits</b>								
Specific leaf area (SLA)	CV			NS		-0.0053 ± 0.001	t = -5.2	<0.001
	P/A			NS				NS
Canopy height (CH)	CV	-0.0104 ± 0.002	t = -6.5	<0.001		-0.0065 ± 0.001	t = -6.5	<0.001
	P/A	0.007 ± 0.0008	t = -8.1	<0.001				NS
Leaf Dry Matter Content (LDMC)	CV	0.0064 ± 0.001	t = -6.5	<0.001				NS
	P/A			NS				NS
<b>Belowground traits</b>								
Rhizomal volume (ml 100ml <sup>-1</sup> )			F = 7.4	<0.01				NS
Gr. 1 Superficial <10cm deep rooters	CV	0.008 ± 0.001	t = 5.6	<0.001				NS
Gr. 2 > 10cm roots, <15% aerenchyma	CV	-0.003 ± 0.001	t = -2.3	0.02				NS
Gr. 3 > 10cm roots, >15% aerenchyma.	CV			NS				NS
<b>Ellenberg indicator values</b>								
Ellenberg Moisture Value	CV	0.0301 ± 0.007	t = 4.2	<0.001				
	P/A			NS				NS



**Figure 8.2** Response of the three categories of plant rooting strategies in different long-term mowing regimes on coarse textured (A-C) and fine textured soil (D-F). Significant shifts for the different root groups and treatments are depicted  $\alpha < 0.05$ . Mean values are given  $\pm$  SE.

## Discussion

Our results show that long-term machine mowing leads to soil compaction and lower soil aeration, but mainly on the fine textured soil. This is reflected in a treatment effect on plant species composition and in belowground plant traits. Our results thus suggest that differences in root response to mowing treatments are highly related to abiotic soil conditions.

### The effect of regular machine mowing

It is generally accepted that mowing with heavy machines leads to compacted soils (Soane *et al.* 1982; Hamza & Anderson 2005). We could detect these effects in both fine and coarse textured soil. The resulting changes in the soil physical properties may have consequences for the growing conditions for plants. Especially soils with a fine pore structure are vulnerable to compaction with heavy machines (Voorhees *et al.* 1985). A greater proportion of pores become water-filled and as a result, oxygen diffusion into the soil decreases. Consequently, the soil redox potential is lower in the fine textured soil in response to machine mowing. Also our results on air-filled porosity and soil moisture show that soil aeration was significantly reduced in the machine mown fine textured soil. In waterlogged soils plant roots face oxygen shortage, which leads to reduced root performance and nutrient uptake (Colmer 2003; Bailey-Serres & Voesenek 2008). Prolonged negative effects of oxygen shortage can be mitigated by plants in several ways of which aerenchyma formation and superficial root growth are the most prominent (Armstrong *et al.* 1991). At shallower soil depth, we found that soil was less reduced, indicating that superficial root growth may be beneficial to plant species that are less tolerant to soil hypoxia (Armstrong *et al.* 1991).

One can expect that a species is either able to cope with soil hypoxia, or is selected against, as Huber *et al.* (2009) show for genotypes of *Trifolium repens*. On the other hand, new colonizers with a tolerance for coping with soil hypoxia can be expected to have a selective advantage. Indeed, we find a much greater replacement of species between treatments on the fine textured soil, as the floristic dissimilarity increases over time. This is in contrast to previous studies which reported no strong evidence that mowing with heavy machines results in an other vegetation composition than traditional hand-mowing (Huhta *et al.* 2001; Liira *et al.* 2009). Our results indicate that this can be explained by the variation in sensitivity of soils to compaction.

So, machine mowing on fine textured soils can aggravate waterlogging effects and thereby affect plant performance. Consequently, this leads to an advantage to species that can cope with periodic hypoxia, and thus to changes in plant species composition.

### Explaining treatment effects by differences in traits

Along with the increasing dissimilarity, we also found differences in plant traits between treatments. In the literature, monitoring the aboveground plant traits is the prevailing method of evaluating plant community responses to environmental gradients (Lavorel *et al.* 2008). However, *a priori*, we only expected a treatment effect on belowground traits.

We found a strong increase in abundance of species with superficial root growth in the machine mown treatment on fine textured soil and a decrease in species that are less tolerant to hypoxia. This was also reflected in the high root biomass in the upper 10 cm of the soil layer in the machine mown treatment on fine textured soil. Surprisingly, we found no increase in cover of plant species with the ability to produce at least 15% aerenchyma. While on the one hand, soil aerenchyma formation is a good strategy to cope with soil hypoxia, on the other hand production of aerenchyma may be restricted by the physical pressure in compacted soils (Engelaar *et al.* 1993; Striker *et al.* 2007). This may put constraints on the spread of aerenchyma-forming species and provide an explanation for the lack of dominance of aerenchyma-forming species.

Coinciding with the increasing floristic dissimilarity on the fine textured soil, we found a consistent treatment effect on the commonly used aboveground trait canopy height, but not on leaf traits (SLA and LDMC). We suggest that the reaction of aboveground maximum canopy height in response to machine mowing on fine textured soil could be related to the changing belowground conditions. Canopy height and rooting depth have been mentioned as highly correlated traits (Douma *et al.* 2011). Therefore, we argue that a lower canopy height could be an indirect effect that plays via effects on rooting depth, imposed by the increase of superficially rooting species.

The response of root growing strategies to the different mowing treatments is in agreement with an effect on the Ellenberg-moisture values in the different mowing treatments. Machine mowing on a fine textured soil resulted in lower soil aeration, consequently leading to plant species communities with higher average Ellenberg-moisture values. On coarse textured soil, no clear effect of machine mowing on soil aeration was found. Consequently, we also found no treatment effect on Ellenberg-moisture values on this soil type. This thus shows that the species replacement in the machine mown treatment on fine textured soil is directed towards the wetter end of the spectrum (Ellenberg *et al.* 1992).

To conclude, species-rich grasslands on fine textured soil, but not on coarse textured soil, can be expected to diverge from historical grassland communities which were traditionally hand mown. We suggest that this

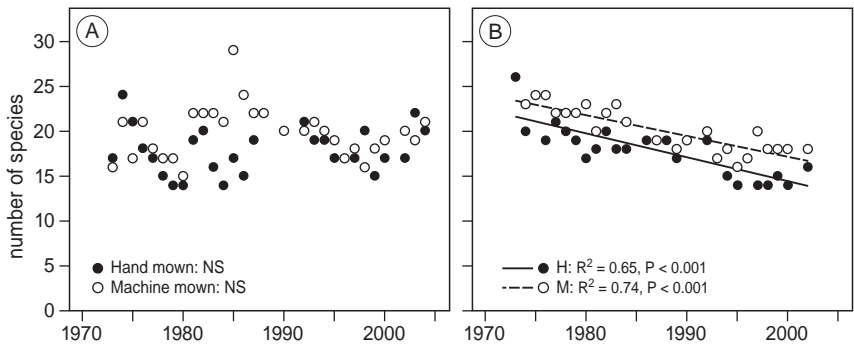
happens in response to changing soil physical conditions induced by soil compacting machine mowing. As a result of the altered belowground growing conditions, we found shifts in community belowground traits. This may be, as in the case of canopy height, reflected in the aboveground traits. It thus additionally emphasizes the need for evaluation both aboveground and belowground plant traits when belowground plant organs are expected to respond to the studied environmental gradient. From the perspective of nature conservation, the impact of heavy machines may not necessarily lead to an undesired and less valuable plant species community. On the contrary, as in the case of our study site, many valuable yet different plant species can be found in both treatments.



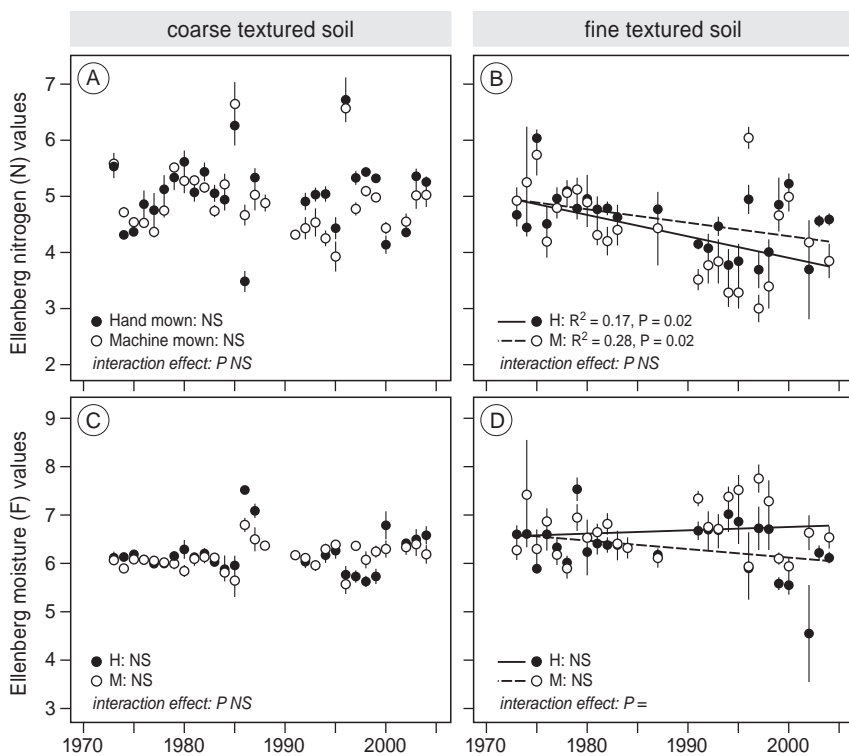
Appendix VI



**Figure VI.1** Set up of hydroculture-experiment for measuring ability to produce aerenchyma. Every container contains 20 plants; one individual for every species. Plants were assigned random locations in the containers.



**Figure VI.2** Changes in total plant species numbers (averages of six plots of 2×2 m) per treatment over time. A) denotes plant species numbers on coarse textured soil. B) denotes species richness on fine textured soil. Open symbols indicate machine mown treatments, closed symbols indicate hand mown treatments.



**Figure VI.3** Community weighted means for Ellenberg (N) nitrogen (A,B) and Ellenberg (F) moisture values (C, D). Closed symbols indicate hand mown treatments, open symbols indicate machine mown treatments. Linear fits are shown when significant or if interaction effect between treatments was significant,  $R^2$  and  $P$  are given in figure legend.



**Table VI.1** Details on cover, presence and belowground parameters for all species.  
Part 1: belowground traits of species used in the analyses.

Species	Freq. Years Coarse textured HandMown				max freq years	Freq. Plots Coarse textured HandMown				max freq plots	Max Cover Coarse textured HandMown				max max cover	root grouping 10 percent		root grouping 15 percent	porosity (after Justin & Armstrong 1987)	Porosity own measurements28.8
	Freq.	Years	Coarse	textured HandMown		Freq.	Plots	Coarse	textured HandMown		Max Cover	Coarse	textured HandMown	Max Cover		Coarse	textured HandMown			
<i>Cerastium fontanum</i>	17	29	1		29	6	6	1		6	2	2	1		2	gr1	gr1			
<i>Galium saxatile</i>			14	14	14			4	6	6			40	20	40	gr1	gr1	1.1		
<i>Hydrocotyle vulgaris</i>			17	28	28			4	6	6			4	52	52	gr1	gr1	2.3		
<i>Leontodon autumnalis</i>	11	7	3	1	11	5	5	3	1	5	2	1	1	1	2	gr1	gr1	1.2		
<i>Luzula campestris</i>			12	28	28			2	5	5			2	12	12	gr1	gr1	3.6	3	
<i>Potentilla erecta</i>				20	20				6	6				20	20	gr1	gr1		6	
<i>Viola palustris</i>			24	27	27			6	6	6			15	15	15	gr1	gr1		1	
<i>Agrostis capillaris</i>	19	21	28	26	28	5	5	6	6	6	20	47	60	40	60	gr2	gr2		9	
<i>Anthoxanthum odoratum</i>	22	24	28	28	28	6	6	6	6	6	60	52	30	47	60	gr2	gr2		7	
<i>Bromus hordeaceus</i>			1		1			1		1		1			1	gr2	gr2			
<i>Cardamine pratensis</i>	31	32	9	6	32	6	6	5	4	6	7	2	1	1	7	gr2	gr2	9.9		
<i>Cirsium arvense</i>	12	8			12	5	4			5	20	2			20	gr2	gr2			
<i>Festuca rubra</i>	32	32	29	28	32	6	6	6	6	6	50	47	40	40	50	gr2	gr2	1.9	2	
<i>Juncus bufonius</i>	3	1			3	2	1			2	1	1			1	gr2	gr2		10	
<i>Lychnis flos-cuculi</i>		1	9	9	9		1	3	2	3		1	2	1	2	gr2	gr2	2.1		
<i>Poa pratensis</i>	32	32	27	10	32	6	6	6	6	6	30	20	10	10	30	gr2	gr2		7	
<i>Poa trivialis</i>	30	32	11	9	32	6	6	5	5	6	30	20	10	4	30	gr2	gr2	8.6		
<i>Agrostis canina</i>		2	26	27	27		2	6	6	6		1	52	30	52	gr3	gr3		22	
<i>Agrostis stolonifera</i>	17	19	7	4	19	6	6	6	6	6	20	70	20	20	70	gr3	gr2		13	
<i>Alopecurus geniculatus</i>	5	9			9	6	6			6	10	4			10	gr3	gr3			
<i>Carex curta</i>			1	23	23			1	3	3			1	12	12	gr3	gr3			
<i>Carex nigra</i>			29	28	29			6	6	6			20	30	30	gr3	gr3	36.1	18	
<i>Carex ovalis</i>	3	4	18	23	23	1	2	6	6	6	1	1	10	4	10	gr3	gr3		29	
<i>Carex panicea</i>			2		2				1	1				1	1	gr3	gr3			
<i>Cirsium palustre</i>	18	13	17	16	18	5	4	5	5	5	20	12	5	10	20	gr3	gr3		16	
<i>Cynosurus cristatus</i>	2	2			2	3	1			3	1	1			1	gr3	gr3			
<i>Dactylis glomerata</i>		2			2		1			1		1			1	gr3	gr3			
<i>Deschampsia cespitosa</i>		2			2		2			2		4			4	gr3	gr3		38	
<i>Festuca pratensis</i>	24	31	5	2	31	6	6	4	2	6	20	30	1	1	30	gr3	gr3		19	
<i>Galium palustre</i>	6	17	9		17	2		4	2	4	1		10	2	10	gr3	gr1		10	
<i>Holcus lanatus</i>	32	32	27	25	32	6	6	6	6	6	90	80	10	20	90	gr3	gr2		13	
<i>Juncus acutiflorus</i>			1	16	16			1	3	3			1	30	30	gr3	gr3		17	
<i>Juncus articulatus</i>				1	1				1	1				20	20	gr3	gr3			

**Table VI.1** Continued. Part 1: belowground traits of species used in the analyses.

Species	Freq. Years Coarse textured HandMown				max freq years	Freq. Plots Coarse textured HandMown				max freq plots	Max Cover Coarse textured HandMown				max max cover	root grouping 10 percent		root grouping 15 percent	porosity (after Justin & Armstrong 1987)	Porosity own measurements
	Freq.	Years	Coarse textured	HandMown		Freq.	Plots	Coarse textured	HandMown		Max Cover	Coarse textured	HandMown	Max Cover		root grouping	10 percent			
<i>Juncus conglomeratus</i>	7	11	16	1	16	2	3	3	1	3	4	4	1	1	4	gr3	gr3			49
<i>Juncus effusus</i>	17	20	29	28	29	4	6	6	6	6	20	50	30	40	50	gr3	gr3	36.1		
<i>Lolium perenne</i>	3	6			6	5	6			6	30	20			30	gr3	gr3			
<i>Lotus pedunculatus</i>	3	1	17	8	17	2	1	6	5	6	1	1	1	3	3	gr3	gr1	10.7		
<i>Nardus stricta</i>				13	13				1	1				4	4	gr3	gr3			
<i>Phleum pratense</i>		6			6		1			1		1			1	gr3	gr3			
<i>Plantago lanceolata</i>	14	19		3	19	6	6		1	6	47	47		1	47	gr3	gr2			13
<i>Potentilla anserina</i>	27	13		1	27	4	3		1	4	20	2		1	20	gr3	gr3			
<i>Ranunculus acris</i>	6	27	7	9	27	4	6	6	4	6	1	2	2	1	2	gr3	gr2			10
<i>Ranunculus repens</i>	32	32	14	13	32	6	6	6	6	6	60	52	20	20	60	gr3	gr2			10
<i>Rumex acetosa</i>	32	32	29	27	32	6	6	6	6	6	40	40	30	30	40	gr3	gr2	9		13
<i>Rumex crispus</i>	6	6			6	2	3			3	2	1			2	gr3	gr3	39.1		
<i>Rumex obtusifolius</i>	2	13			13	1	4			4	2	4			4	gr3	gr3	28.8		

**Table VI.1** Continued. Part 2: Species not used in the analyses.

Species	Freq.	Years	Sand	HandMown	Freq.	Years	Sand	Machine	Freq.	Years	Organic	HandMown	Freq.	Years	Organic	Machine	max	freq	years	Freq.	Plots	Sand	HandMown	Freq.	Plots	Sand	Machine	Freq.	Plots	Organic	HandMown	Freq.	Plots	Organic	Machine	max	freq	plots	Max	Cover	Sand	HandMown	Max	Cover	Sand	Machine	Max	Cover	Organic	HandMown	Max	Cover	Organic	Machine	max	max	cover																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Agrostis vinealis				3					3				2				2			3				6			2				1			1				2			1			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2			2

**Table VI.2** Details on aboveground traits for all species; CH = canopy height in m, LDMC = leaf dry matter content in  $\text{mg g}^{-1}$ , SLA = specific leaf area in  $\text{mm}^2 \text{mg}^{-1}$ , NA = not available; trait values were derived by own measurements or if marked with a \* were taken from the LEDA trait-base (Kleyer *et al.* 2008).

Species	CH	LDMC	SLA
<i>Agrostis canina</i>	0.18	263	44
<i>Agrostis capillaris</i>	0.31	231	44
<i>Agrostis stolonifera</i>	0.32	177	58
<i>Agrostis vinealis</i>	0.11 *	334 *	19 *
<i>Alopecurus geniculatus</i>	0.18	210	35
<i>Anthoxanthum odoratum</i>	0.33	147	56
<i>Anthriscus sylvestris</i>	0.75 *	207 *	30 *
<i>Bromus hordeaceus</i>	0.28 *	290 *	26 *
<i>Calamagrostis canescens</i>	0.93 *	361 *	22 *
<i>Callitriche cophocarpa</i>	NA	NA	NA
<i>Cardamine pratensis</i>	0.18	140	40
<i>Carex curta</i>	0.33 *	261 *	27 *
<i>Carex nigra</i>	0.22	258	26
<i>Carex ovalis</i>	0.14	297	26
<i>Carex panicea</i>	0.26	NA	NA
<i>Cerastium fontanum</i>	0.27	97	40
<i>Cirsium arvense</i>	0.74 *	153 *	14 *
<i>Cirsium palustre</i>	0.81	124	15
<i>Cynosurus cristatus</i>	0.47	185	40
<i>Dactylis glomerata</i>	0.51 *	263 *	25 *
<i>Dactylorhiza majalis</i>	0.24	122	25
<i>Deschampsia cespitosa</i>	0.48	228	17
<i>Elytrigia repens</i>	NA	NA	NA
<i>Equisetum arvense</i>	0.33 *	238 *	11 *
<i>Equisetum fluviatile</i>	0.49	158	8
<i>Equisetum palustre</i>	0.49	179	12
<i>Festuca pratensis</i>	0.61	179	29
<i>Festuca rubra</i>	0.37	229	24
<i>Galium palustre</i>	0.28	208	NA
<i>Galium saxatile</i>	0.30	216	46
<i>Glyceria fluitans</i>	0.41	216	44
<i>Holcus lanatus</i>	0.48	156	48
<i>Holcus mollis</i>	NA	NA	NA
<i>Hydrocotyle vulgaris</i>	0.02	107	28
<i>Hypochaeris radicata</i>	0.05 *	NA *	21 *
<i>Juncus acutiflorus</i>	0.52	230	8
<i>Juncus articulatus</i>	NA	NA	NA
<i>Juncus bufonius</i>	NA	NA	NA

**Table VI.2** Continued

Species	CH	LDMC	SLA
<i>Juncus conglomeratus</i>	0.68	332	5
<i>Juncus effusus</i>	0.66	316	4
<i>Juncus subnodulosus</i>	0.73*	189 *	7 *
<i>Leontodon autumnalis</i>	0.09*	148 *	26 *
<i>Lolium perenne</i>	0.18*	219 *	25 *
<i>Lotus pedunculatus</i>	0.43	148	42
<i>Luzula campestris</i>	0.05	185	35
<i>Luzula multiflora</i>	0.18	193	33
<i>Lychnis flos-cuculi</i>	0.35	115	33
<i>Lythrum portula</i>	0.11*	277 *	24 *
<i>Nardus stricta</i>	0.12*	399 *	10 *
<i>Persicaria hydropiper</i>	0.39*	161 *	44 *
<i>Persicaria maculosa</i>	0.38*	203 *	22 *
<i>Persicaria mitis</i>	0.33*	173 *	33 *
<i>Phleum pratense</i>	0.59	290	26
<i>Plantago lanceolata</i>	0.23	129	26
<i>Plantago major</i>	0.10*	107 *	21 *
<i>Poa pratensis</i>	0.20	303	31
<i>Poa trivialis</i>	0.29	160	NA
<i>Potentilla anserina</i>	0.08*	228 *	22 *
<i>Potentilla erecta</i>	0.13	240	28
<i>Ranunculus acris</i>	0.33	140	26
<i>Ranunculus flammula</i>	0.28	149	25
<i>Ranunculus repens</i>	0.18	112	30
<i>Rhinanthus angustifolius</i>	0.43	156	40
<i>Rumex acetosa</i>	0.45	122	25
<i>Rumex crispus</i>	0.60*	105 *	20 *
<i>Rumex obtusifolius</i>	0.53*	150 *	29 *
<i>Sagina procumbens</i>	0.13*	217 *	19 *
<i>Senecio sylvaticus</i>	0.38*	114 *	30 *
<i>Stellaria graminea</i>	0.27	147	55
<i>Stellaria media</i>	0.18*	84 *	49 *
<i>Stellaria pallida</i>	0.30*	119 *	43 *
<i>Stellaria palustris</i>	0.25*	NA	23 *
<i>Stellaria uliginosa</i>	0.18	125	69
<i>Trifolium repens</i>	0.17	134	41
<i>Viola palustris</i>	0.06	119	41

# Chapter 9



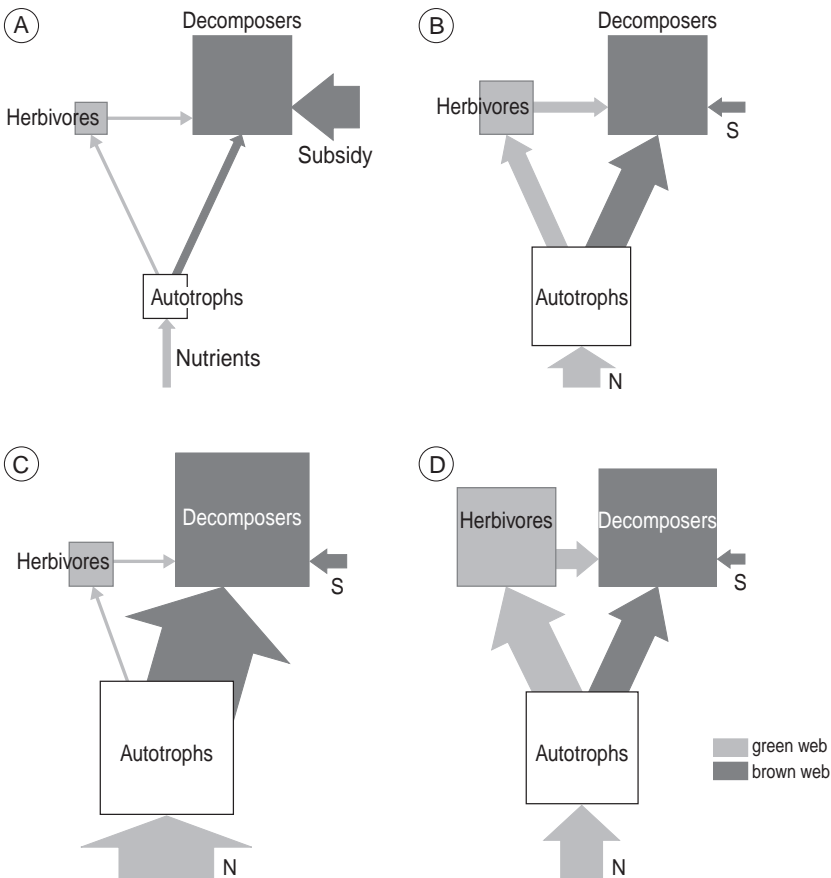
# 9

## **Ecosystem assembly on a Salt Marsh, a synthesis**

Maarten J.J. Schrama

# Introduction

Primary succession yields a fascinating process of ecosystem assembly, from which many ecological generalities can be drawn (Clements 1916; Olson 1958; Lindroth 1973; Whittaker *et al.* 1989; Wardle *et al.* 2004b; Milner *et al.* 2007; Neutel *et al.* 2007). These studies hint at the exciting and creative dynamics of natural processes in building complete and complex ecosystems from combinations of simple beginnings, basic principles and historical contingencies. But despite this, hardly any studies so far were able to give an account of the bigger picture of overall changes in food web composition that occurs during primary succession. Well into the 1990's, a BES-organized symposium on primary succession had trouble finding a researcher to discuss development of early successional animal communities, and indeed, was not





successful in finding such a person (Miles & Walton 1993). However, during the last decade, more and more authors are now shifting or developing their attention for the subject of the built-up of early successional communities, taking animals explicitly into account (e.g. Hodkinson *et al.* 2001; Kaufmann 2001; Neutel *et al.* 2007). These studies have provided much of the knowledge on which this thesis is building forth.

In the previous chapters, I have discussed insights in and drivers of ecosystem assembly. Rather than being fixed in time and space, I have found the salt marsh food web to go through a number of distinct phases during ecological succession. During each of these phases, I identified key processes that govern the configuration and diversity of the food web. In this synthesis, I will try to connect the different processes and the key players from the perspective of the different configurations of the food web, and attempt to link these to observed changes in other ecosystems. I will argue that these processes are ubiquitous and yield an insightful understanding of the process of ecological succession that we can see everywhere around us.

Four stages along the studied salt marsh successional gradient described in this thesis can be distinguished, which are (Figure 9.1). Stage 1): early succession, external (marine) input drives early salt marsh development, especially the development of the brown web. Stage 2): intermediate stages of succession, hare grazing slows down vegetation succession and both the green part of the food web (with invertebrate herbivores such as microlepidoptera and herbivorous beetles) and the brown part of the food web (with detritivores such as amphipods and diptera larvae) are represented. Stage 3a): late succession with a dominant brown food web and very little grazing by vertebrate herbivores. This results in fast nutrient recycling and a species-poor plant community. Stage 3b) can develop from stage 3a) when large herbivores are introduced. Here a green web dominates with a species-rich plant community and a small brown web.

**Figure 9.1 (left)** Proposed differences in carbon flow between the green and brown part of the food web among the different states of the salt marsh ecosystem. The **first** state (A) is characterized by an abundant brown web, which is sustained by external subsidy (S) and has a small pool of internally recycled nutrients (N). The **second** state (B) can be characterized by a fairly high plant production, of high quality (palatability) which is highly preferred by geese and hare, and a relatively small brown web. In the **third** state (C), the food web is strongly detritivore-dominated with herbivores consuming maximally 5% of the annual primary production. *Orchestia gammarellus* plays a dominant role in this state. The **fourth** state (D) is principally typified by the dominant vertebrate herbivore presence. High densities of cattle, hare and high densities of herbivorous insects can be found in this state and few (macro)detritivores as well as a considerable built-up of organic matter.

### Stage 1: External input during early salt marsh development.

In the first stage of vegetation succession where the higher plants are sparse or absent, a sizeable brown web was found to be already present (chapter 2). Both Enchytraidea and Diptera larvae had high abundances. Brown web species which are typical of later successional stages (the amphipod *Orchestia gammarellus*, and the gastropod *Ovatella myosotis*) were not present or were found in much lower numbers.

Results from the carbon stable isotope analysis (Chapter 3) indicate that most of the organisms in the brown web in these early successional stages derive their nutrients (and energy) from marine sources. Therefore, the food web in this stage resembles a classic subsidized food web (figure 9.1A, sensu Polis *et al.* 1999). On the other hand, herbivorous invertebrates in this stage do not show higher  $\delta^{13}\text{C}$ -values, indicating that they rely completely on terrestrial plants for obtaining their energy, carbon and possibly nutrients.

In order to discover the origin of the marine-derived nutrients, I analysed nitrogen stable-isotopes. I identified two possible sources of nutrient input: drift line material (mainly macro-algae *Fucus* and *Ulva* spp. in early successional stages), and bird faeces (guano). Studies from other small island ecosystems have shown that these are two prominent sources of nutrient input (Polis & Hurd 1995; Maron *et al.* 2006). Compared to later stages of succession, I found extremely high  $\delta^{15}\text{N}$ -values in species dominating the first stage of succession. Strikingly, this was the case for both decomposers and to a lesser extent also for plants. This suggests that most food for organisms in early successional stages is of marine origin, and of a high trophic position (Post 2002). Therefore, I speculate that non-herbivorous birds (feeding on for instance fish, bivalves, worms or crustaceans) are one of the most important vectors in early stages of food web assembly of the investigated salt marsh succession sequence, rather than macroalgae or bacterial N-fixation. Where Van Wijnen (1999) estimated the input of marine birds to be maximally  $1 \text{ g m}^{-2} \text{ yr}^{-1}$ , my results show that these figures may be much higher: up to  $34 \text{ g m}^{-2} \text{ yr}^{-1}$ , but only in open, bare, early successional stages. This value is much higher than the previously estimated atmospheric deposition in early successional stages:  $1.5\text{--}2 \text{ g m}^{-2} \text{ yr}^{-1}$  (van Wijnen & Bakker 2000) and is also higher than the input of macroalgal drift:  $0.5\text{--}1 \text{ g m}^{-2} \text{ yr}^{-1}$ . The data on bird counts in the various successional stages as well as the stable isotope analysis indicate that the Oystercatcher (*Haematopus ostralegus*) is one of the most prominent vectors for transport of marine nutrients onto the land.

Also other studies point at external marine input in playing an important role in fuelling early successional stages on sheltered beaches, and also on places where no large resident populations of shorebirds are present. Lee *et al.* (1983) show that the growth of two pioneer beach plants, *Salsola kali* and



**Figure 9.2** *Atriplex prostrata* growing on the beaches of Mull (Hebrides, UK) within patches of decaying *Fucus* sp. As shorebirds are rare in these places, macroalgal beach wrack is likely to be the primary source of nitrogen to the food web in these early stages of dune succession (photo M. Schrama).

*Cakile maritime*, was vastly greater in the drift zone than above or below it. On isolated islands, marine macroalgal driftline material was documented to be the most important food source for animals at the basis of the terrestrial food web (Polis & Hurd 1995). Other authors have argued that this phenomenon also plays an important role in early dune succession (Davy & Figueroa 1993; Walker 1993, see figure 9.2).

Microbial mats and N-fixation by early successional cyanobacteria have earlier been mentioned to play important roles during ecological succession on sandy shores. Stal (1994) has shown that these mats can fix maximally about  $15 \text{ g N m}^{-2} \text{ yr}^{-1}$ , which was calculated to support an aboveground peak standing biomass of  $150 \text{ g}$  of vegetation per square meter (Sprent 1993). That is, when all decaying cyanobacteria would become available to growing plants. In North-American salt marshes, which receive a much lower input of nutrients from the marine environment, N-fixation by *Spartina alterniflora* and benthic microalgae can also fulfill a large proportion of plant demand (Tyler *et al.* 2003). In the chronosequence on the salt marsh of Schiermonnikoog, N-fixation was unlikely to be of great importance as  $\delta^{15}\text{N}$ -values were very high in early successional stages (Figure 3.3) and no clearly developed microbial mats or high densities of *Spartina* sp. were observed in the earliest stages of succession.

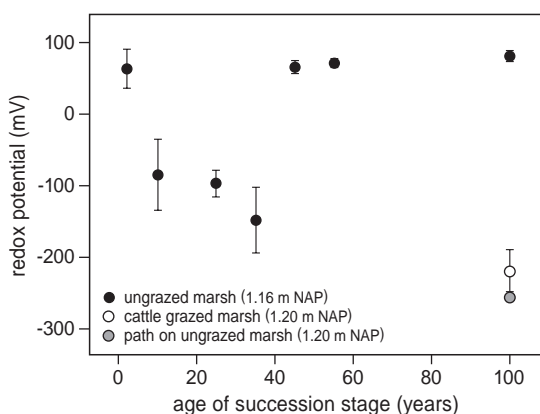
The high rates of nutrient input in the Schiermonnikoog chronosequence are in sharp contrast with the rates of community assembly in 'slow' primary successions such as on glacier forefronts and recent lava flows (Whittaker *et al.* 1989; Chapin *et al.* 1994; Kaufmann 2001). In such successions, N-fixation by cyanobacteria and plant symbionts are often reported to be the prime source of nutrients (Whittaker *et al.* 1989; Chapin *et al.* 1994), although invertebrate fallout has also been mentioned as a nutrient source to early successional food webs (Ashmole & Ashmole 1987; Sugg & Edwards 1998; Hodkinson *et al.* 2001; Coulson *et al.* 2003). However, the relatively small amount of nitrogen input of  $0.26 \text{ g N m}^{-2} \text{ yr}^{-1}$  at retreating glaciers (Hodkinson *et al.* 2001) and  $0.512 \text{ g N m}^{-2} \text{ yr}^{-1}$  for the pumice plains on Mt St Helens (Edwards & Sugg 1993) results in a very slow accumulation of nutrients and a correspondingly slow rate of succession.

To summarize, bird guano and marine input are increasingly reported to play an important role in fuelling early successional coastal food webs, thereby facilitating early successional vegetation development. Irrespective of the exact origin of the nutrient sources in the primary stages of the studied chronosequence, I argue that the open character of the salt marsh ecosystem results in a fast rate of ecosystem assembly, where early successional brown webs, fuelled by marine inputs, are important in 'kick-starting' early successional nutrient accumulation, according to Figure 1.1.

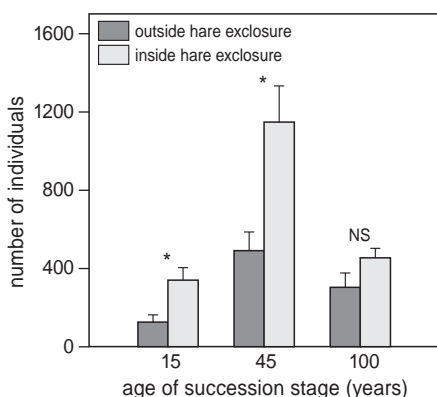
## **Stage 2: Effects of small vertebrate herbivores on intermediate successional stages**

From early to intermediate successional stages, both plant species richness and invertebrate species richness were found to increase (chapter 4). However, if hares are not present in these early successional salt marsh vegetations, plant species composition would rapidly become dominated by *Elytrigia atherica* (Kuijper & Bakker 2005), a species that dominates the late successional stages. Examples of this have been found on hare-free islands of Rottumerplaat (NL) and Mellum (Germany) (Kuijper *et al.* 2003). On Schiermonnikoog, this mechanism was shown by fencing out hares from exclosures. This means that hares significantly slow down the speed of vegetation succession in these barrier island salt marshes, mainly as a result of selective grazing on late successional grasses, thus prolonging the dominance of early successional plant communities (Kuijper & Bakker 2005).

Van Wijnen (1999) showed that the exclusion of hares leads to a rapid increase in both litter accumulation and N mineralization within fences. This is in strong contrast to theories assuming that vertebrate herbivore grazing speeds up N cycling (McNaughton 1984). My results show that when hares become absent towards late succession (Chapter 2), macro-detritivores

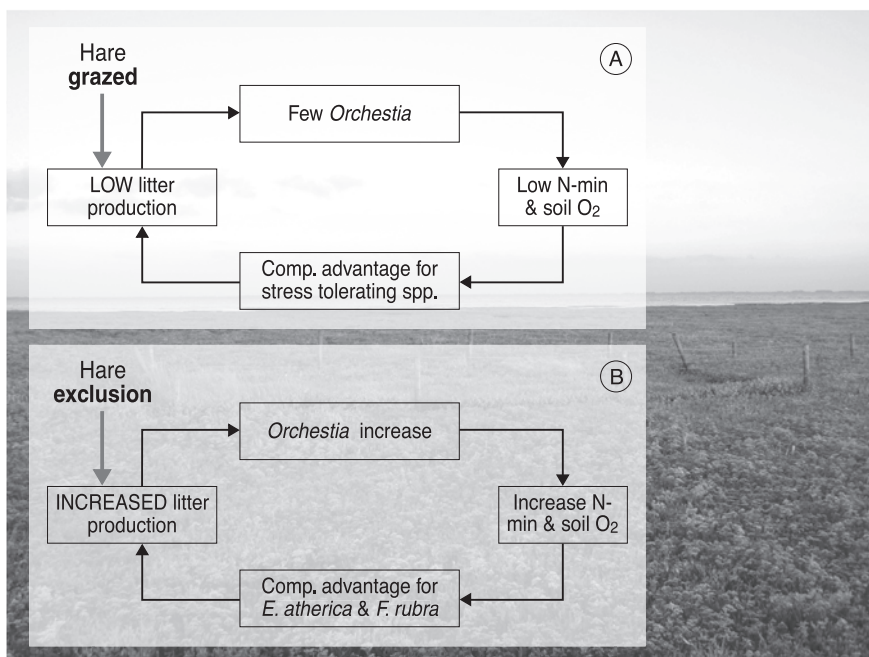


**Figure 9.3** Redox values over the succession gradient, as measured on 13 November 2010. Redox values become higher in later successional (grass-dominated) stages, but cattle grazing and human trampling on a mown path result in a significant lowering of the soil redox potential.



**Figure 9.4** Abundances of *Orchestia gammarellus* inside (grey bars) and outside (black bars) hare enclosures (N = 4) in different stages of succession. Measurements were done in November 2009. \*P < 0.05; NS: Not Significant.

become highly numerous (Chapter 2). Moreover, a strong increase in soil redox potential (Figure 9.3) and an increase in N cycling in these late successional stages was observed (van Wijnen *et al.* 1999; van Wijnen & Bakker 2000). My measurements in enclosures suggest that detritivore abundance and increased soil redox potential may be causally related (Figure 9.4). In the light of these findings, the effects of hares on vegetation dynamics and



**Figure 9.5** Summary of the effects of A) hare grazing and B) hare exclusion in intermediate succession stages. With hares, there is suppression of litter production and selective grazing, which result in low densities of decomposers, subsequently suppressing N mineralization and soil aeration. Consequently, a species rich (stress-tolerant) plant community is maintained. B) Without hares, there is no suppression of litter production and no selective grazing on late successional plant species. This leads to increased abundance of *Orchestia*, an increased N mineralization and soil aeration. As a result, a shift in plant community composition occurs with *E. atherica* as a dominant species.

ecosystem processes can be summarized as follows (Figure 9.5): Through the prevention of successful establishment of late successional grasses, hares (indirectly) suppress the brown food web, as the hares consume plant material of tall, productive species, before it can turn to litter and be the food source for macro-detritivores (van Wijnen *et al.* 1999). According to van Wijnen (1999), vertebrate herbivores consume approximately 30% of the NPP in this stage). When hares are excluded, litter production goes up, subsequently resulting in a strong increase in abundances of *Orchestia gammarellus*, a soil burrowing and litter fragmenting amphipod. Moreover, a tall and dense vegetation promotes shelter for this negative phototactic species during day time. When this animal establishes, oxygen diffusion into the top

soil and net N mineralization are suggested to be strongly enhanced (Chapter 5, Lopez *et al.* 1977). Consequently, this promotes the development of a late-successional, nitrophilic plant species community, with low palatability. Once a shift has occurred, it cannot be set back by hares or other small vertebrate herbivores. In other words: hares lose control because they stop suppressing the brown web. And it seems that when the brown web gains control over the nutrient cycling, it speeds up its own dominance.

So, I conclude that hares prolong the intermediate stages of vegetation succession by selectively removing late successional grass species, which prevents litter built-up, and the development of the associated soil fauna community. In doing so, they maintain (stressful) soil abiotics, low N-mineralization and a herbivore-driven food web (Figure 9.5A). On the other hand, when hares are excluded, late successional grasses rapidly invade and a detritivore-dominated food web develops, the soil becomes well-aerated and the net N mineralization strongly increases (Figure 9.5B).

### **Stage 3a: Brown web dominates late succession.**

Towards late succession (50 – 100 years), I found a strong increase in macro-detritivore abundance, at the expense of green web representants, such as microlepidoptera, herbivorous bugs (*Miridae*) and herbivorous beetles (Chapter 2). This was also reflected in patterns of species diversity (Chapter 4). My abundance estimations shows that densities of *Orchestia* are comparable to those of earthworms in grasslands: estimations of *Orchestia* ranged between 0.3 and 11 g DW m<sup>-2</sup>, assuming that *Orchestia* weigh approximately 3.3 mg each (own measurements), while earthworms biomass estimations range between 0.5–10 g—DW m<sup>-2</sup> (Lavelle 1997). Given the smaller body weight of *Orchestia* – their metabolic activity per unit area may even outweigh that of earthworms.

The late successional salt marsh is therefore characterized by an abundant brown web and a high primary production of relatively low quality leafs to vertebrate herbivores (Chapter 2, Van Wijnen & Bakker 1999; van Wijnen & Bakker 2000). The primary source of nutrients for the brown web in these stages of succession is locally produced plant litter material, while a much smaller portion is of marine origin (Chapter 2). The plant community itself is characterized by a species-poor nitrophilic species community with a relatively low tolerance to soil anoxia (Davy *et al.* 2011), featuring *Elytrigia atherica* as a dominant species and *Festuca rubra* and *Atriplex prostrata* as co-dominant species.

Other terrestrial ecosystems in late succession often have a large consumption of decomposers relative to that of herbivores. This has been argued to be an effect of high C:N-ratios (Cebrian 1999). For instance, herbi-



vore consumption is usually (much) lower than 10% of the total primary production in forests (which are often climax successional stages) and herbivores thus play a subordinate role in these systems. On salt marshes, van Wijnen (1999) estimated that total herbivore consumption declines from 30% of total primary production in early succession, to less than 5% in late succession. However, such figures have not been gathered for many different successional sequences, especially not for early stages. The generality of this therefore needs additional testing.

My results show that *Orchestia gammarellus* has a positive effect on litter fragmentation and soil aeration. In chapter 5, I show that this results in an increase in N mineralization. These results are in agreement with others who show positive effects of litter fragmenting macrofauna on N mineralization (Fenchel 1970; Trigo *et al.* 1999; Bardgett & Wardle 2010). For instance, earthworm addition experiments have shown considerable increases in nutrient cycling. In parts of New Zealand where these animals were previously not found, introduction of earthworms has resulted increased yields of plants (up to 30%; Stockdill 1982). This increase is supposed to be mainly a result of an increase in the growth of micro-organisms. Bacteria have been shown to benefit from the production of earthworm-casts (Trigo *et al.* 1999). Lopez & Levinton (1977) have shown a similar increase in bacterial growth in excrements of a conspecific amphipod: *Orchestia grillus*. In yet another amphipod species, *Parhyalella whelpleyi*, Fenchel (1970) showed that none of the litter that went through the gut was decomposed, but all organisms that lived (and fed) on the litter were consumed. Consequently, microbial respiration was increased with 110%. So, promotion of microbial growth is clearly one mechanism via which the rate of N cycling is increased in soils with high densities of *Orchestia*. Conversely, Hemminga & Buth (1991) did not find a strong positive effect of litter fragmenting fauna on decomposition rates on salt marshes. This result may be explained by the fact that *Orchestia gammarellus* could not enter the litterbags which they used. Additionally, I find strong evidence that N mineralization is increased in places with high densities of *Orchestia*, as a result of increased soil aeration due to bioturbation. Burrowing by earthworms has been shown to lead to higher soil porosity and promote the water infiltration rate (Stockdill 1966). Removal of earthworms (due to an invasive parasitic flatworm) was previously reported to lead to lower oxygen diffusion rates and lower rates of decomposition (Boag & Yeates 2001). Higher oxygen availability because of increased bioturbation may thus promote N mineralization and denitrification rates (Laanbroek 1990; Breland & Hansen 1996).

In summary, higher *Orchestia* abundance may lead to higher N availability to plants via two pathways. First, presence of *Orchestia* may speed up

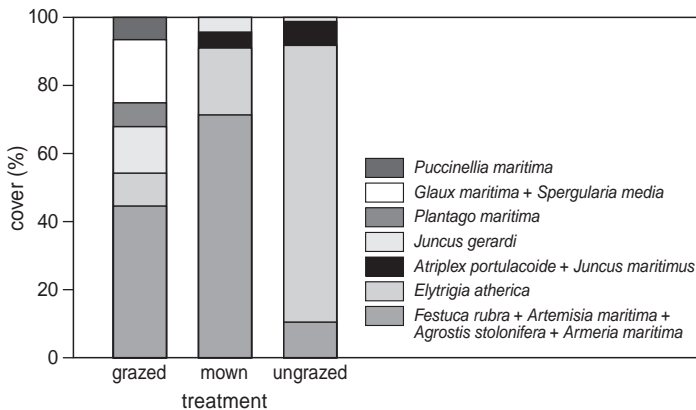


N mineralization via an increase in litter fragmentation. Secondly, *Orchestia* may speed up net N mineralization via a positive effect on soil aeration.

Changes in soil aeration may not only affect decomposition, but also vegetation composition. Differences in soil redox potential have recently been shown to be one of the major drivers of vegetation composition on salt marshes (Davy *et al.* 2011). I find that soil aeration increases over the gradient of succession stages (Figure 9.3), coinciding with an increase in abundance of bioturbating soil macrofauna (Chapter 2). Results from the laboratory experiment with *Orchestia* (Chapter 5) indeed confirm the hypothesis that soil aeration in salt marsh soil increases in response to *Orchestia*-addition. Consequently, an *Orchestia*-mediated increase in soil aeration could potentially be responsible for a shift in the competitive balance between plant species towards a species composition that is less well adapted to periods of prolonged soil anoxia. Additionally, I speculate that this increase in soil aeration may (partly) underlay the observed increase in belowground species richness (Chapter 4). An increase in the depth of the oxic layer may be responsible for a more pronounced vertical stratification of the soil profile, thus providing a greater diversity of niches (Berg & Bengtsson 2007). In grassland ecosystems in New Zealand, earthworm introductions were found to similarly affect soil fauna composition (Stockdill 1982). Also in forests, greater vertical stratification promotes soil fauna diversity (Berg & Bengtsson 2007). Nevertheless, addition (long-term) experiments are needed to show such effects of *Orchestia* on other soil biota in salt marshes.

### **Stage 3b: Large herbivore presence results in a green web dominated state**

While high abundances of bioturbating soil macrofauna (in this case *Orchestia gammarellus*) promote beneficial conditions for nutrient mineralization, large herbivore trampling can reverse this, by inducing unfavourable conditions for nutrient mineralization (Chapter 6, 7), especially on the dry and wet end of the moisture gradient. Moreover, the negative effect on N mineralization seems stronger in fine textured soils than in coarse textured soils (Chapter 7). Some studies have shown that N mineralization can also increase in response to trampling. Goose trampling may result in increased leaf litter incorporation into the soil (Zacheis *et al.* 2002). Moderate trampling by cattle may also enhance water availability to soil microbes in ecosystems where N mineralization is water-limited (Sankaran & Augustine 2004), and rates of cyanobacteria-promoted N fixation are enhanced due to creation of open patches of soil (Zacheis *et al.* 2001). However, in salt marshes the negative effects of compaction on N mineralization were found to override these mildly positive effects of trampling on N mineralization. Thus, on salt

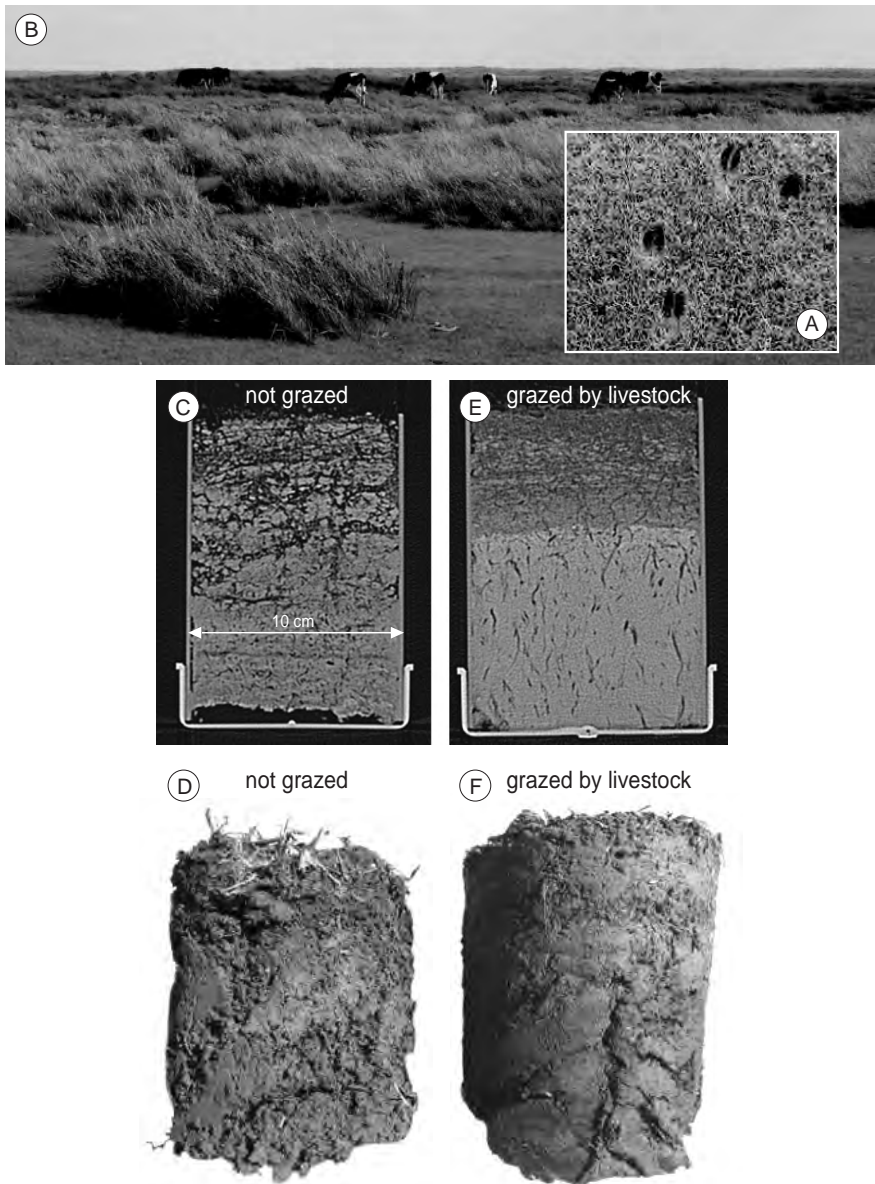


**Figure 9.6** Plant species cover in a 100-year old salt marsh in three treatments: grazed by cattle, hand mown and not grazed by cattle. Cover of plant species from early successional dominants is much higher in the grazed treatment. Cover percentages were determined in June 2010.

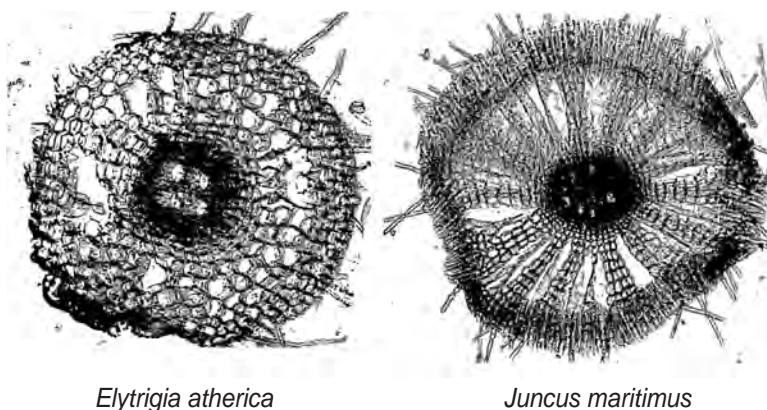
marshes, with its wet, fine-textured soils, a trampling induced decrease in N mineralization outweighs positive effects of large herbivores on N mineralization.

Irrespective of the effect of soil fauna, salt marsh successions are characterized by a gradual increase in N mineralization due to the build-up of nutrient pools in the soil (Olf *et al.* 1997, Van Wijnen 1999) and an increase in soil redox potential (Figure 9.3). A sudden decrease in N mineralization and soil aeration in late succession in response to grazing and trampling can, therefore, be expected to lead to a shift in plant species composition towards those in early successional stages. Indeed, the grazed meadows on the late-successional salt marsh of Schiermonnikoog (grazing lawns) have a plant species community that shows a number of similarities with an early successional plant community (Figure 9.6). In contrast, hand-mown plant communities lack most of the species of the early successional middle marsh (Figure 9.6).

The question is why exactly such a shift in plant community occurs in response to grazing and whether the effect is caused by biomass removal or by the physical trampling impact by the cows. Comparing a cattle-grazed (and trampled) treatment to a hand-mown treatment shows that the shift can be attributed both to effects of trampling plus the removal of above-ground light competition (Chapter 6). Trampling leads to lower soil porosity and hence a lower redox potential (Figure 9.7), which consequently leads to



**Figure 9.7** Trampling (A) as a result of livestock grazing (B) on salt marshes causes soil compaction. The soil in an ungrazed part of the salt marsh has a loose structure (C) and is well aerated (D), whereas the grazed marsh soil is very compact and has a (very) low macroporosity (E) and low soil aeration (F). Note the black color in deeper soil layers indicating reduced iron sulphide.



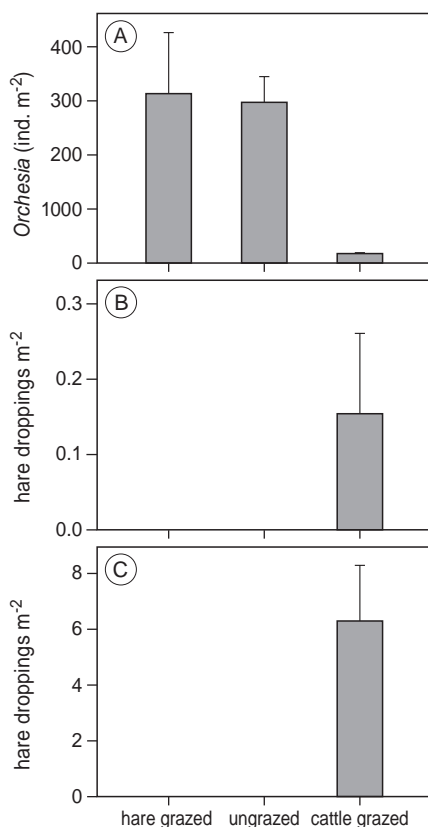
**Figure 9.8** Root cross sections of A) *Elytrigia atherica* and B) *Juncus maritimus*. *E. atherica* creates aerenchyma through 'random' cell death in response to anaerobic conditions, while *J. maritimus* creates 'spokes' and 'pipes', through which air can diffuse. The latter root structure is much less vulnerable to soil compaction (sensu Striker *et al.* 2007).

lower soil aeration and increased soil physical pressure. These are especially important stress factors to plants which root systems are not designed to deal with these multiple stresses, which may be the case for most late successional salt marsh plant species (Davy *et al.* 2011). For instance, even though roots of *Elytrigia atherica* are potentially able to produce a small amount of aerenchyma in order to cope with low soil aeration (Justin & Armstrong 1987), they have not been found able to do so in strongly compacted soil (personal communication T. Elzenga). I hypothesize that the structural integrity of the root vertical structure in the adventitious roots of *J. maritimus* may play an important role in dealing with the two stress-factors: soil anoxia and a highly compacted soil (Striker *et al.* 2007). To illustrate this, Figure 9.8 shows cross-sections of roots of *Juncus maritimus* and *Elytrigia atherica*. The first species increases in response to grazing, while the latter species decreases in response to grazing. I suggest that this may be a result of the differences in structural strength of the roots of these two species. Applying a vertical pressure (for instance exerted by the hoof of a cow) is more likely to result in the collapse of the relatively weak root structure of *Elytrigia atherica*, but not of the architecturally strong root of *Juncus maritimus* (Figure 9.8, Striker *et al.* 2007). Therefore, I speculate that *Elytrigia atherica* suffers from the physical impossibility of extending its roots in strongly compacted soil.

To conclude, a combination of the low nutrient availability and the stress resulting from grazing induced soil compaction may be responsible for the observed decline in cover of late successional plant species in response to grazing. The decline in this dominant late successional species, together with the herbivore-induced changes in soil physical conditions thus results in a plant species community that very much resembles communities in early successional stages.

Following my results in chapter 6, I want to argue that the mechanism described above may be a general mechanism by which large herbivores can increase vegetation diversity. I want to emphasize that it differs from classical theories on how large herbivores alter vegetation diversity. These classical theories focus on mechanisms via which large herbivores influence vegetation community directly (biotic-biotic interactions, for instance by enhancing seed dispersal, selectivity of grazing and creation of heterogeneity (Ritchie & Olff 1998), while the above mechanism focuses on indirect influence of large herbivores (biotic-abiotic-biotic interaction). By grazing on the salt marsh, large herbivores alter the vegetation in an indirect way, by changing the soil abiotics, which in turn affects the vegetation community and the soil fauna community. Other examples of similar abiotic consequences of grazing can be found in steppes, tundras and on savannas. While large herbivore grazing on the salt marsh leads to anoxia stress, grazing in steppe systems and on tundras may lead to soil salinization (Lavadon & Chaneton 1996, Buckeridge & Jefferies 1997), and may lead to very low soil moisture in dry steppes as a result of strongly reduced water infiltration (Allington & Valone 2010; Jeddi & Chaieb 2010). In response, the vegetation composition changes according to the change in abiotics. Therefore, we suggest that large herbivores can affect the vegetation community via induced changes in the abiotics, rather than via selection of palatable or unpalatable species, and that this may be a ubiquitous mechanism that may add to our understanding of the functioning of large herbivores in ecosystems.

So, the late succession stage on the salt marsh can persist in two alternative states. Either it is dominated by the brown web, when large herbivores are absent, or by the green web when large herbivores are present. In the first case, the succession stage is dominated by *Elytrigia atherica* with high amounts of dead organic matter and high densities of soil burrowing amphipods, which promote soil aeration and N mineralization. In the latter case, the food web is strongly herbivore-dominated, while decomposing and bioturbating macrofauna are only present in very low densities (Fig 9.9). The plant species community in this state is characterized by a highly palatable species rich meadow composed of species that are adapted to prolonged



**Figure 9.9** Densities of some of the dominant representants of the brown and green web in a late successional stage (100 yrs) in May 2009, and their change in abundance in response to introduction of large herbivores. (A) Densities of *Orchestia gammarellus* are high in late succession, but are very low after introduction of large herbivores. (B,C) Abundances of small vertebrate herbivores are low in late succession, but are much higher after introduction of large herbivores .

periods of soil anoxia. So, the species composition is relatively similar to the early successional stages, where the periods of soil anoxia were also present. However, I suggest that the reason for these conditions to be present in the early successional stages is very different. The low primary production and high consumption by small herbivores in these stages does not allow for a sufficient production of litter to sustain high densities of bioturbating macro-detritivores, which result in a relatively compact, anoxic soil.

## Conclusion

I argue that the combined food web configurations of the different stages of succession, which were presented in this thesis, provide a coherent view on the dominant changes in food web components that take place during ecosystem assembly in general. Along primary succession on the salt marsh, the food web advances through four distinctly different phases, which are illustrated in Figure 9.1. Classical succession research has mostly focused on the perspective of the plant. In order to be able to give a more integrated account of the characteristic changes along succession, I have chosen a more ecosystem-based approach. This bears importantly on other facets of succession: nutrient accumulation and control of the nitrogen cycle. Classically, nitrogen has been considered a strongly limiting nutrient during succession. I have shown that in all of the above-mentioned stages some groups of organisms have an important role in regulation of the nitrogen cycle. In early succession, N-limitation is (at least partly) reduced by input of external nutrients, supported by activity of the brown web. In intermediate succession, small vertebrate herbivores prevent the brown web to become dominant, thus preventing an increase in the rate of nutrient cycling despite internal accumulation in the system. Towards late succession, small vertebrate herbivores lose control due to a decrease in forage quality. This leads to a dominant brown web and a high turnover of nutrients, probably amended by the overwhelming dominance of *Orchestia gammarellus*. This system can shift back to an early successional community composition, if cattle grazing is introduced into the ecosystem. This cattle grazing leads to slower cycling of N, a restoration of early successional abiotic and biotic conditions and a major decline in brown web biomass.

## Future directions

In my analyses of the changes along the successional gradient of the salt marsh on Schiermonnikoog, I have tried to be as complete as possible. However, some remarks have to be made. There are always constraints in food web and succession ecology, and in this case the constraints were in the microbes and the microfauna, such as nematodes. These are two parts of the food web that are often overlooked, but have been argued to be of strong importance to below-ground food web functioning (de Ruiter *et al.* 1998; Van der Putten *et al.* 2001; De Deyn *et al.* 2003; Veen *et al.* 2010).

In his dissertation, Van der Wal (1998) showed some analyses of the nematode diversity and abundance over the different stages of grassland succession. He shows nematodes to be highly numerous in several stages of succession on the salt marsh, which is also shown by Hemminga & Buth



(1991). An in-depth research of the changes in the microfauna community and its drivers, such as *Orchestia*, could add to a more complete understanding of the successional dynamics of the salt marsh.

Microbial ecology is a fast-developing field, and some of the predictions and conclusions of this thesis can be tested using recently developed techniques for estimating production, diversity and microbial biomass (Christen 2008). For one, does microbial diversity resemble the dynamics of the brown - green web changes along the successional gradient? Is microbial activity increasing along succession? Is there a shift in the microbial community towards ammonium-producing, denitrifying bacteria, hydrogen-sulphide producing bacteria? Is there a shift from bacteria dominated litter decomposition towards fungal dominated decomposition over the course of salt marsh succession, similar to what has been found on retreating glaciers (Ohtonen *et al.* 1999). Or, does the bioturbation and litter fragmentation by *Orchestia* keep the microbial web bacteria-dominated? In that case, will a shift back towards bacterial dominated decomposition occur after cattle introduction?

And last but not least, the hypotheses and ideas in this thesis require rigorous testing in similar and other ecosystems to test their generality. So, whether the brown-green-brown-green shift can be found throughout a range of ecosystems, whether food-web wide patterns of diversity resemble those on the salt marsh and whether the predicted compaction framework holds, remains to be tested.





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# Summary



# Summary

## Summary

Ecological succession is a fascinating process where communities of organisms are successively replaced by other communities. All newly exposed substrates exhibit a unique successional arrival of organisms. Whether it takes place on the pumice plains of Mount St Helens or on a decaying carcass, with enough patience and observational skills, one can track the successional changes from the very beginning to the complicated network of species interactions.

Although no two successional sequences are equal, many researchers have the tendency to try and find generalizable patterns in them. To try and find basic principles that underly 'ecosystem assembly'. I am no exception to this. And I used one of the finest chronosequences for this aim: the salt marsh of the island of Schiermonnikoog.

### **Vegetation succession and Food web ecology: merging two fields**

For a number of decades, research on vegetation succession and food web ecology have been performed in relative isolation. While vegetation succession has been all about describing changes in plant species composition and related abiotic changes, food web ecology has focused merely on 'static species interactions' (often done because of methodological constraints—constructing a food web takes a lot of time). However, both clearly present an oversimplified version of reality: plants interact with animals and vice versa, in space and time. In this thesis I make an attempt to link these two disciplines. By doing so, I demonstrate that many of the changes along a successional gradient can only be understood by integrating knowledge on vegetation succession with changes in food web composition. In the above-ground food web (spiders, beetles etc) as well as the belowground food web (springtails, mites).

Chronosequences (sequences of chronological successional stages) provide an ideal setting for studying changes in food webs. In a relatively short period one can track the changes in all the different stages without having to wait for a hundred or a thousand years before the late successional stages are reached.

### **The chronosequence on Schiermonnikoog**

At the research site of the salt marsh of Schiermonnikoog, the salt-march chronosequence spans a 100 years of succession, from bare sand-flats (0 years) to a mature, *Elytrigia*-dominated marsh (100 years). This makes it possible to study successional changes from the very beginning to the late succession within the time period of a PhD. Other advantages of this salt

marsh chronosequence are that it only has a single layer of vegetation, a rather limited species diversity and last but not least, a long history of research was done in this chronosequence, which provides detailed information.

In the first three chapters (2,3 and 4) I make an attempt to characterize the food web in the different stages of succession. In Chapter 5 and 6 I discuss how dominant species act on the course of succession and in chapter 7 and 8 I try and generalize some of the findings to other ecosystems. Chapter 2 focuses on the large scale changes in food web configuration, by focusing on the herbivore-based and the detritivore-based compartments of the food web. In early succession, when little vegetation is present, the food web appears to be mainly detritivore-based. This food web configuration closely resembles the food web at desert islands in the Baya California (USA). Towards intermediate succession more and more herbivore biomass was added to the food web, while the brown web biomass increased less strong. After 45 years of succession, the food web had an almost equal green and brown web. This food web configuration seems to resemble those in grazed savanna ecosystems. Towards late succession, green web biomass showed a sharp decrease (both for vertebrate and invertebrate herbivores) at the expense of brown web biomass. At the last succession stage (100 years) the food web seems completely brown web dominated, with the amphipod *Orchestia gammarellus* highly dominant in these stages. The food web configuration in this stage appears similar to other late-successional ecosystems such as Oak and Beech-dominated late succession stages, which also have a large brown web and a very small green web. Based on literature, we speculate that in many other ecosystems, successional changes in food web configuration might resemble this distinct 'brown-green-brown shift' in food web configuration. However, some ecosystems may remain in an early stage, such as the earlier mentioned desert islands (which have a very low primary production due to the extremely low amount of precipitation), or in an intermediate stage such as savannas (under the influence of fire and large herbivores).

### **What is the origin of the nutrients that 'fuel' the food web?**

The second question of this thesis concerns the origin of the nutrients that drive the food web in the different stages of succession. Classical theories expect local fixation of N (via for instance cyanobacteria) to fuel early food webs, but other studies suggest that in early succession, many nutrients are derived from external sources. Using stable isotopes, we test whether the nutrients in the different stages of succession are locally generated (fixed via photosynthesis) or whether they are derived from external sources? In all stages of succession and of all the dominant species in the food web, we

measured the ratio of stable isotopes of carbon ( $\delta^{13}\text{C}/^{12}\text{C}$ ) to get an indication of the amount of 'internally' (locally –terrestrially- produced) vs externally derived nutrients (from the external marine ecosystem) in each of the stages of succession. My results from chapter 3 show that early in succession, organisms in the brown web are mainly externally fuelled. Along the successional gradient, the marine signal gets weaker, indicating that the food web becomes internally fuelled during succession. Because herbivores are always dependent on local primary production, they do not show this shift over succession. These results therefore show that in early succession the food web is externally fuelled, over the course of succession changing towards an internally fuelled both web. These results corroborate the results from chapter 2, which indicated that a large proportion of the biomass in the youngest successional stages consists of detritivores and not of herbivores. The conclusion from this study is that the food web in early stages is heavily subsidized with marine-derived nutrients ('open'), but becomes gradually more dependent on internal cycling of nutrients ('closed'). The influx of external nutrients may also be an important factor contributing to the rapid successful establishment of pioneer plants in the early successional stages as well as an ultimate cause for the relatively fast rate with which the salt marsh succession proceeds compared to other successions.

### **Early stages of succession fertilized by birds?**

Nutrients can be transported between ecosystems in different ways, of which the most important are via aerial transport, through floodings and via animals. To find out which of these modes of transport is the most significant source that subsidized early successional food webs is important, because it sheds light on the crucial links between different ecosystems. That knowledge can be necessary when aiming to conserve ecosystems. My results from the analysis of nitrogen stable isotopes showed that the nutrients were of high trophic origin, not from algae. This may indicate that birds may play an important role as a vector for these nutrients: they forage at sea, and often prey on animals and rest in early successional stages during high water. Indeed, the bird excrements that I used to test this hypothesis had a comparable value for the nitrogen stable isotope ratios. I conclude from these measurements that birds play a crucial role in 'kick-starting' early succession, thereby providing an important link between the ecosystem in the sea and the ecosystem on the land.

### **The impact of keystone species on successional dynamics**

Apart from birds in early succession, how are other dominant species in the food web interacting with the successional dynamics? Are there representa-



tives in both parts of the food web that cause the ecosystem to assemble in one way or another, and if so how? Successional dynamics has historically been characterized by an increase in primary production and vegetation composition. It seems reasonable to expect that the influence of keystone species is noticeable in these dynamics. Chapter 5 and 6 are concentrating on these issues.

### ***Orchestia gammarellus* as the earthworm of the salt marsh?**

In Chapter 5, I approach this issue using the most important delegate of the brown web: *Orchestia gammarellus*. This animal (size 0.5–1.5 cm) can occur in densities up to 4000 ind m<sup>-2</sup> in late successional stages, although numbers are much lower in early successional stages. This may indicate that, especially in late successional stages, the species may serve an important role in driving some of the successional dynamics. In many ecosystems, earthworms are dominant large detritivores, but in salt marshes earthworms do not occur. In this chapter I hypothesize that *Orchestia*, by means of its foraging and digging activities may present a functional equivalent of earthworms on salt marshes, thus affecting the ecosystem configuration. Because earthworm densities are difficult to manipulate, the salt marsh provides an ideal study system to study the effect of the presence of bioturbating macrofauna on the development of the ecosystem. My results show that activity of *Orchestia* results in a more oxic soil, presumably as a result of a combination of digging activity and foraging on algae. Moreover, I found that activity of *Orchestia* can speed up nutrient mineralization and litter decomposition. Both may have a significant influence on the successional dynamics. Early successional soils are more anoxic and late successional plant species (notably sea-couch grass *Elytrigia atherica*) have a high nutrient demand. The numerical increase of *Orchestia gammarellus* over the course of succession may thus be tightly linked to the changes in plant species composition.

### **Large herbivores set back the successional clock by compacting the soil**

Large herbivores (young cattle in the case of Schiermonnikoog) have the exact opposite effect on the configuration of the ecosystem. While a higher activity of soil fauna towards late succession leads to a more granular, loose soil structure, introduction of large herbivores in late successional stages results in a more compact soil with little activity of soil macrofauna. Trampling leads to lower soil porosity and a wetter, less well aerated soil. In effect, it results in a slowing down of the rate of nutrient cycling. These changes in soil physical conditions in turn affect the plant species composition. Species like *Elytrigia atherica* that are not adapted to these changing soil conditions, are replaced by plant species that are more characteristic of early succes-

sional stages. Other studies have previously described this latter phenomenon, but they attributed it to changes in light competition.

By comparing effects of large herbivores on clay and on sandy soil, I show that these herbivore effects on soil physical properties are highly texture specific. On a coarser sandy soil, no strong effects of herbivores on soil moisture, oxygen levels and nutrient cycling were found. Also no strong effect on the plant species community was noted. This difference can be attributed to the fact that fine-grained soils (such as clay salt marsh soils) are highly compactable, while coarse grained soils (like sandy soils) are much less compactable. Therefore effects are smaller on coarse textured soils.

Dominant detritivores and dominant herbivores thus operate as counter-acting forces on the state of the ecosystem. Where the brown web dominates (like in late successional stages), I find a productive, nitrophilic vegetation with a high carbon to nitrogen ratio on a well-aerated soil. If large grazers are introduced, a shift towards a green web dominated ecosystem configuration on an anoxic soil occurs. The vegetation becomes less productive and halophytic, with a low carbon to nitrogen ratio.

### **Large grazers speeding up or slowing down nutrient cycling?**

Classical theory predicts that large herbivores affect nutrient cycling via changes in litter input into the soil. From this, it follows that in ecosystems where litter quality is increased, N mineralization is also increased, while in ecosystems where litter quality is decreased, N mineralization is also decreased. Results from my study on the salt marsh contrast with these predictions. Even though litter quality is enhanced, the rate of N mineralization is much lower. In a number of other studies, both on very wet soils and very dry soils, similar observations have been made. In chapter 7 I argue that both in very wet or very dry soils, compaction by large herbivores makes these soils even wetter or dryer, respectively, thus causing a decrease in N mineralization. Under these soil conditions, herbivore-induced changes in soil physical conditions can outweigh the above mentioned effects on litter quality, thus creating a decrease in N mineralization independent of the effects on vegetation quality. Also in soils that are rich in ions (calcium, boron, sodium), herbivore-induced soil compaction can result in a decrease in N mineralization. In this case, concentrations of these ions become very high in the upper layer of soil due to increased hydrolic conductivity and evaporation at the soil surface.

### **Consequences for nature management**

In the Netherlands, grazers are often used as a management tool. These results may therefore also be of interest for nature managers. As a result of

my study, I would argue that a decrease in N mineralization in Dutch nature reserves can only be expected 1) if litter quality goes down or 2) in very wet soils. The first would be expected in forests or in places where the soil is so nutrient poor that only slowly growing, heavily defended species manage to withstand grazing. This would result in a shift towards a lower quality of litter input in the soil and could be expected in nutrient-poor heathlands and coastal dunes. The second would be expected in salt marshes, or in floodlands adjacent to rivers, where soils can become more anoxic due to herbivore-induced soil compaction. In all other areas a decrease in N mineralization due to grazing by large herbivores may be difficult to achieve, also because most soils in the Netherlands are well drained and fairly nutrient-rich.

### **Concluding remarks**

Between early and late succession, the salt marsh food undergoes a number of major structural changes. My study shows large scale patterns in ecosystem assembly and shows how a number of some of the representatives of the brown and green food web modify the configuration of the ecosystem. Hence, it sheds light on the assembly of many other ecosystems.

In order to study these interactions in a successional context, the availability of an undisturbed salt marsh like the one at Schiermonnikoog is crucial. I hope that this beautiful ecosystem yields many more insights on the fascinating topic of ecosystem assembly.

# *Samenvatting*



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Successie heeft de afgelopen 150 jaar vele onderzoekers gefascineerd. Of dat nu een langzaam vergaand karkas of een zojuist uitgebarstte vulkaan is, alle nieuwe substraten vertonen vormen van successie. Mits er goed wordt opgelet en er bovenal genoeg geduld wordt opgebracht kan de onderzoeker alle subtiele veranderingen in de tijd waarnemen, die ervoor zorgen dat een ecosysteem zich ontwikkelt van een dorre vlakte tot een 'volwassen' ecosysteem.

Na het waarnemen van de volgorde waarin veranderingen plaatsvinden volgt het uitzoomen. De onbedwingbare neiging van de onderzoeker om over een proces 'waarin alles in elkaar gezet lijkt te worden', generaliseerbare wetten te formuleren. De wetten waarnaar deze 'ecosystem assembly' zich schikt. Ook ik heb het geluk gehad dat ik dit vier jaar lang heb mogen doen op de kwelder van Schiermonnikoog. Daarbij probeer ik een brug te slaan tussen vegetatie-successie en de studie van voedselwebben.

Vreemd genoeg zijn onderzoek aan vegetatie-successie en onderzoek aan voedselwebben lange tijd gebeurd in relatieve isolatie. In dit proefschrift tracht ik deze vakgebieden te verbinden en ik hoop duidelijk te maken dat de integratie van essentieel belang is voor het begrijpen van de sturende processen gedurende ecosysteem-successie. Dus in plaats van voedselwebben als statische eenheden te zien (wat vaak om methodologische redenen gebeurt – het samenstellen van een voedselweb kost nu eenmaal veel tijd) bekijken we in deze studie juist de grootschalige veranderingen in voedselwebben in zeven verschillende stadia tijdens een 100-jarige kweldersuccessie. Deze bespreek ik in hoofdstuk 2,3 en 4. Naast de grootschalige veranderingen heb ik mij in hoofdstuk 5 en 6 ook bezig gehouden met de vraag hoe een aantal verschillende dominante diersoorten invloed uitoefenen op het verloop van de successie. Vervolgens heb ik in hoofdstuk 7 en 8 deze resultaten gebruikt om soortgelijke processen in andere ecosystemen te begrijpen. Hieronder bespreek ik eerst de grootschalige veranderingen tijdens de successie en daarna bespreek ik de invloed van de dominante soorten op het voedselweb.

### Onderzoek aan successie met behulp van chronosequenties

In veel chronosequenties (een opeenvolging van successiestadia van verschillende leeftijd) zijn dergelijke veranderingen zeer lastig te onderzoeken. Vaak liggen deze chronosequenties op zich terugtrekkende gletsjermorenes, of in vulkanische gebieden, waarbij het vaak honderden jaren duurt voordat laat-successionele gemeenschappen zich vestigen. De chronosequentie van Schiermonnikoog is mede daarom zo geschikt voor deze studie: ze heeft slechts 1

laag vegetatie (geen onbereikbare boomkruinen in de latere stadia van successie), een relatief geringe diversiteit aan planten en dieren, geen rotsige ondergrond en de successie is na 100 jaar nagenoeg tot een onveranderbaar stadium gekomen, wat in het verleden ook wel climax genoemd werd. Dit is een enigszins misleidende term, want een ecosysteem blijft dynamisch, ook in deze latere stadia van successie.

### **Grootschalige veranderingen in voedselwebben langs een successiegradient**

De eerste vraag die zich opdringt bij het integreren van voedselwebben en successie, is of het mogelijk is om een karakterisering van de verschillende configuraties van het voedselweb in de verschillende stadia te maken? Hiervoor hebben we alle aanwezige plant- en diersoorten in de zeven onderzochte stadia van successie ingedeeld in verschillende trofische groepen, die behoren tot het herbivore deel van het voedselweb (het groene web) met daarin alle fauna die direct en indirect afhankelijk is van planten en het detritivore deel (het bruine web) van het voedselweb met daarin alle fauna die direct en indirect afhankelijk is van dood organisch materiaal.

Mijn onderzoek in hoofdstuk 2 en 4 laat zien dat er inderdaad zeer duidelijke patronen zijn in de verandering van de biomassa in de verschillende delen van het voedselweb. Bovendien komen deze veranderingen vaak opvallen overeen komen met voedselwebben in studies in andere ecosystemen.

In de allerjongste stadia van de kwelder, wanneer er nog nauwelijks planten aanwezig zijn, blijkt het grootste deel van het voedselweb tot de bruine deel van het voedselweb te behoren: veel vliegen, spinnen en potwormen. Dit stadium komt verrassend goed overeen met de beschrijvingen van woenstijn-eilanden in de Baya California (USA) waar het voedselweb sterk leunt op de inkomsten van nutriënten uit aanspoelsel uit zee. Ook op zandige (noordzee)stranden is het vaak een drukte van belang in het vloedmerk. Ook hier betreft het vaak louter detritivoren of predatoren daarvan. Zodra op de kwelder deze allervroegste successiestadia begroeid raken met hogere planten, neemt de herbivoren-biomassa in het systeem sterk toe, zowel voor gewervelden (hazen, ganzen) als de ongewervelden (rupsen, kevers, wantsen). De biomassa van het bruine web neemt dan ook wel toe, maar minder sterk. Dit midden-stadium van successie lijkt bijvoorbeeld sterk op de door grote herbivoren begraaide savannes zoals de Serengeti: een grote biomassa in het groene web, een geringe plantenbiomassa van hoge kwaliteit en een relatief geringe biomassa in het bruine web.

Naarmate de successie vordert daalt de biomassa van het groene web, maar neemt de biomassa van het bruine web juist extra sterk toe. In het

laatste successiestadium vonden we nauwelijks meer biomassa in het groene web. Zowel gewervelde als ongewervelde herbivoren hebben het toneel dan verlaten. Hier is bijna alle biomassa toe te schrijven aan het bruine web. Met name *Orchestia gammarellus*, een vlokreeft-achtige die voornamelijk op het land leeft is hier zeer dominant. Ook dit lijkt een algemeenheid te zijn: veel laat-successionele ecosystemen met een hoge biomassa (zoals bijvoorbeeld eiken- en beukenbossen, maar ook tropische regenwouden) worden gedomineerd door het bruine web. Dit gaat vaak gepaard met een grote afname in vegetatiekwaliteit: de aanwezige biomassa heeft een relatief hogere koolstof-stikstof-ratio. Dat levert vooral problemen op voor planteneters, terwijl detritus-eters daar veel minder problemen mee hebben. Ik suggereer dat het voedselweb in veel andere ecosysteem-successies ook deze bruin-groen-bruine verandering doormaken, maar dat sommige systemen in het groene stadium blijven hangen, onder invloed van bijvoorbeeld vuur (zoals in savannes). Andere systemen blijven wellicht in het eerste 'bruin-gedomineerde' stadium hangen, zoals eerdergenoemde woestijn-eilanden. Dat komt dan vaak omdat er niet of nauwelijks lokale productie van planten is. Overigens worden de beschreven patronen ook in termen van soortendiversiteit gevonden: terwijl de diversiteit van de organismen in het bruine web gestaag toeneemt, vertoont die van de organismen in het groene web eerst een toename en daarna weer een afname.

### **Waar komen de voedingsstoffen voor het voedselweb vandaan?**

De tweede grote vraag is waar de energie voor het voedselweb in de verschillende stadia van successie vandaan komt. Wordt het voedsel volledig lokaal gegenereerd? Of wordt het voedselweb van buiten 'gesubsidieerd', waardoor het als het ware een 'kick-start' krijgt? En wordt het voedselweb naar de latere stadia van successie minder of juist meer 'gesubsidieerd'?

Om de hypothese te testen dat het voedselweb in deze vroege successiestadia echt vanuit de zee gesubsidieerd wordt (extern 'gestuurd' wordt) hebben we voor de belangrijkste soorten in het voedselweb de stabiele isotopen-ratio's van koolstof en stikstof bepaald, waarmee onderscheid gemaakt kan worden tussen externe 'subsidie' en lokaal geproduceerd organisch materiaal.

Mijn resultaten in hoofdstuk 3 laten zeer duidelijk zien dat de voedselwebben in de vroege successie grotendeels extern gedreven worden. Naarmate de successie vordert neemt de sterkte van het signaal van het externe input af. Hier vind ik ook een groot verschil tussen de vertegenwoordigers van het groene en bruine web: herbivoren laten geen verandering over de successie zien (dat klopt met de theorie: zij zijn dan ook afhankelijk van plantenmateriaal en daar zit theoretisch geen verandering in koolstofisotopen



in), terwijl de vertegenwoordigers van het bruine web (dat afhankelijk is van dood organisch materiaal) deze verandering van extern gesubsidieerd naar 'afhankelijk van lokaal geproduceerde nutriënten' wel heel duidelijk laten zien. Dit klopt ook met de resultaten uit hoofdstuk 2. De aanwezige biomassa in de heel jonge successie-stadia bestaat bijna geheel uit detritivoren (consumenten van dood organisch materiaal) en niet uit planten en/of planteneters. De conclusie is dan ook dat het voedselweb in vroege successie zeer sterk gesubsidieerd wordt, hiermee mogelijk de nutriënten voor de aldaar aanwezige plantengroei vastleggend en naar latere successiestadia steeds meer door interne nutriënten wordt gedreven.

### **Jonge successie-stadia bemest door vogels?**

De resultaten uit de analyse van de stikstof-isotopen geeft een sterke aanwijzing dat de influx van nutriënten in deze vroege successiestadia voor een groot deel van vogelpoep (guano) afkomstig is. Deze resultaten sluiten daarmee aan bij studies die beschrijven dat vestiging of verdwijnen van vogels op een eiland voor grote veranderingen in het ecosysteem kan zorgen, juist vaak op kleine, vaak geïsoleerde eilanden. Deze resultaten zijn ook interessant omdat zij laten zien dat de verschillende ecosystemen (in dit geval waddenzee en kwelder) nog veel nauwer met elkaar verweven zijn dan tot op heden vaak gedacht werd. In dit geval wordt de kweldersuccessie 'aangeslingerd' door materiaal uit zee, wat voor een deel waarschijnlijk door de aldaar aanwezige vogels geleverd wordt. Dit heeft ook belangrijke implicaties voor het behoud van deze gebieden; alles staat of valt met de verwevenheid van deze ecosystemen.

### **Invloed van individuele soorten op het verloop van successie:**

#### **Kwelderspringers en Grote Grazers**

Hoe beïnvloeden dominante soorten in het groene en bruine deel van het voedselweb het verloop van successie? Welke representanten in het groene en bruine web hebben een sturende rol, zodanig dat door hun acties het verloop van successie beïnvloed wordt? En hoe zorgen deze soorten daarvoor? Successionele dynamiek wordt in veel systemen gekenmerkt door een toename van de primaire productie van vegetatie. Het ligt voor de hand dat deze beesten juist daarop een sterke invloed hebben. Hoofdstuk 5 en 6 draaien om deze vragen.

#### **Kwelderspringers als de regenwormen van de kwelder?**

Hoofdstuk 5 benadert deze vraag vanuit de belangrijkste vertegenwoordiger van het bruine web, de Kwelderspringer *Orchestia gammarellus*. Dit beest (grootte: 0.5–1.5 cm) kan in grote getale aanwezig zijn op kwelders, tot wel

4000 individuen per vierkante meter. Deze aantallen nemen sterk toe richting de latere stadia van successie, wat zou betekenen dat deze soort hier juist relatief belangrijk zou kunnen zijn. Dat maakt deze soort een goede kandidaat voor het beantwoorden van de vraag: speelt de kwelderspringer een belangrijke rol voor het sturen van de successie, en zo ja op welke manier? In veel ecosystemen zijn regenwormen dominante detritivoren, waarvan gezegd wordt dat een doorslaggevende rol spelen voor de productiviteit en structuur van een bodem. Echter, op kwelders komen regenwormen (gek genoeg) niet voor. Dat maakt het ook een ideaal ecosysteem om deze vragen te beantwoorden: omdat regenwormen heel diep in de grond kunnen zitten is het lastig om experimenten te doen om hun invloed te kwantificeren, maar met de redelijk oppervlakkig actieve kwelderspringers kun je dergelijke manipulatie-experimenten wel doen: zowel in het veld als in het lab. In hoofdstuk 5 wordt beschreven hoe activiteit van kwelderspringers voor een beter doorluchtte bodem zorgt, waarschijnlijk door een combinatie van graafgedrag en graasgedrag op algen. Tevens leidt activiteit van kwelderspringers tot een hogere strooisel-afbraak en een hogere nutriënten-mineralisatie. De invloed hiervan op de dynamiek van de successie is waarschijnlijk aanzienlijk: vooral de plantensoorten (strandkweek) van de late successie kunnen niet goed tegen slecht doorluchtte bodems en hebben veel nutriënten nodig om de concurrentie te overschaduwen. Zo lijkt de toename van deze detritivoren naar de latere successiestadia sterk gekoppeld aan de toename van met name laat-successionele grassen als strandkweek.

### **Grote herbivoren zorgen voor het terugzetten van de successie**

Grote herbivoren (jonge koeien in het geval van Schiermonnikoog) lijken juist het tegenovergestelde effect op het ecosysteem te hebben. Terwijl de bodemfauna zorgt voor het losmaken van de structuur van de bodem, hiermee de zuurstofgraad verhogend en de nutriëntenkringloop versnellend, zorgt bodemcompactie door grote herbivoren juist voor het tegenovergestelde effect. Door het op de bodem stampen neemt het aantal volume van bodemporen af. Dat zorgt ervoor dat de bodem natter en minder goed doorlucht wordt. Dit blijkt grote gevolgen te hebben voor de nutriënten-kringloop. Tezamen met de door compactie veroorzaakte lagere zuurstofbeschikbaarheid en hogere zoutgehaltes resulteert dit in een totaal verschillende bodemfauna en plantengroei. De plantensoort die zo kenmerkend is voor late stadia van successie, strandkweek, lijkt daarmee grotendeels uit het begraasde ecosysteem te verdwijnen, om plaats te maken voor een meer zoutminnende, soortenrijke flora die goed aangepast lijkt aan anoxia in de bodem. Deze lijkt overigens verrassend veel op jonge stadia van de kweldersuccessie. Dit is iets dat ook al eerder door andere onderzoekers werd opgemerkt, maar wat tot

nu toe voornamelijk aan bovengrondse processen geweten werd, zoals licht-competitie tussen plantensoorten. Dit onderzoek laat zien dat het voor de hand ligt dat soortgelijke bodemomstandigheden (lage zuurstofbeschikbaarheid, hoge zoutgehaltes) zorgen voor een soortgelijke plantengroei.

Terwijl veel klassieke studies het belang van de invloed van graasgedrag op de nutrientencycling van het ecosysteem benadrukken, lijken de fysische effecten van grote herbivoren op een natte kwelderbodem van doorslaggevend belang. In hoofdstuk 6 worden deze effecten vergeleken met die een zandige bodems, die net iets hoger liggen. Hier zijn de effecten van compactie veel minder duidelijk. De doorluchting van de bodem neemt niet af en wordt de bodem wordt ook niet natter. Bovendien vind ik daar ook geen vertraging van de nutrienten-cyclus en een veel minder sterke reactie van de vegetatiegemeenschap.

### **Kwelderspringers en koeien hebben een tegengesteld effect op het verloop van successie**

De dominante detritivoren op de kwelder: kwelderspringers en de dominante herbivoren: koeien lijken een tegenovergestelde invloed het ecosysteem te hebben. Wanneer het systeem detritivoor-gedomineerd is resulteert dat in een hoog-productieve vegetatie met een relatief hoge koolstof:stikstof-ratio (lage vegetatiekwaliteit) op een goed doorluchtte bodem, gepaard gaand met een hoge diversiteit van het 'bruine deel van het voedselweb'. Wanneer daar grote grazers in worden toegelaten vind ik een vegetatie met een veel lagere productiviteit en een lagere koolstof:stikstof-ratio (hogere vegetatiekwaliteit) op een slecht doorluchtte bodem. Ook dit lijkt gekoppeld aan een verandering van het voedselweb: een lagere diversiteit in het bruine web en een hogere diversiteit in het groene web.

### **Grote Herbivoren als versnellers of vertragers van de stikstofkringloop?**

Terwijl de klassieke theorie voorschrijft dat dat herbivoren in de meeste gevallen op nutriëntrijke bodems de nutrienten-cyclus opstuwten, omdat ze ervoor zorgen dat het afbreekbare materiaal van een hogere kwaliteit is (of afremmen als ze ervoor zorgen dat het afbreekbare materiaal van lagere kwaliteit wordt), lijkt dit niet overal op te gaan. In hoofdstuk 7 laat ik zien dat dit principe juist op heel natte en heel droge bodems niet opgaat. Begrazing door herbivoren op heel natte bodems leidt tot verhoogde bodemvocht-gehaltes, terwijl de door herbivores veroorzaakte compactie op zeer droge bodems ervoor zorgt dat deze nog droger worden. In beide gevallen kunnen herbivoren ervoor zorgen dat de mineralisatie geremd wordt, omdat deze zowel geremd kan worden door een teveel aan bodemvocht, waardoor zuurstofloosheid ontstaat, als door droogte, waardoor decompositie groten-

deels tot stilstand komt. In beide gevallen vindt deze verlaging van de nutriënten-mineralisatie in aanwezigheid van grazers plaats, ondanks dat de vegetatiekwaliteit vaak toeneemt. Daarbij komt dat compactie in bodems die rijk zijn aan ionen vaak voor ophoping van zouten aan de oppervlakte die voor veranderingen in de selectiedrukken op de plantengemeenschap kan zorgen. Op de kwelder uit zich dat in een begraasde gemeenschap die rijk is aan halofyten met aanpassingen tegen zuurstofloosheid in de bodem, die overigens veel overeenkomsten vertoont met de vroeg-successionele vegetatiegemeenschap. Een dergelijk fenomeen is ook beschreven op begraasde overstromingsgraslanden in Zuid-Amerika.

### **Gevolgen voor natuurbeheerders**

In Nederland worden grazers vaak ingezet als natuurbeherende om natuurgebieden te beherenverschralen. Daarom zijn de resultaten van dit onderzoek ook voor op begrazing gericht natuurbeheer interessant. Naar aanleiding van ons hierboven beschreven onderzoek is een echte verarming van het ecosysteem alleen te verwachten als 1) de bodem al zo arm is dat snelgroeiende grassen het verliezen van langzamer groeiende houtige gewassen (in droge grijze duinen of arme heidegronden), of 2) als de bodem zeer compacteerbaar is (dwz met een fijne textuur) en droog of juist heel nat, waardoor mineralisatie van stikstof door een overvloed aan vocht of juist een tekort aan vocht vertraagd wordt. In de meeste andere gevallen zullen grazers de nutriënten-kringloop vaak juist versnellen (door de hergroei te stimuleren en met uitwerpselen de snelheid van nutriëntencyclus nog verder op te stuwten), waardoor planten aangepast aan arme milieus juist versneld het onderspit zullen delven. Daarom is het te verwachten dat grote grazers in Nederland (waar het grootste deel van de bodems rijk is en niet extreem nat of droog) de stikstofkringloop eerder versneller dan afremmen.

### **Concluderende woorden**

Deze studie aan de successie van de kwelder maakt duidelijk dat de verschillende componenten van het voedselweb nauw met elkaar samenhangen. Hiermee biedt het inzicht voor vele andere systemen die misschien minder makkelijk te ontleden zijn of waar geen uitgebreide successiereeksen zijn.

Voor het bestuderen van al deze interacties is het van groot belang dat een ongestoorde kwelder als die van Schiermonnikoog behouden blijft en dat de universiteit daar de faciliteiten biedt om een dergelijk onderzoek te kunnen doen. Opdat deze plek in de toekomst nog veel mooie inzichten op zal leveren.



# Dankwoord



# Dankwoord

Een werk als dit komt niet zomaar tot stand. Wanneer zo'n boekje over de samenhang der dingen af is en je kijkt over je schouder, wordt opeens duidelijk hoe lang de weg is die je hebt afgelegd. Al op jonge leeftijd was ik veel buiten. Bramen plukken met opa en ondertussen genieten van de vossen en herten die vaak tot op minder dan 10 meter te benaderen waren. Later bij de NJN leer je al die beesten benoemen, eerst de grootste (bevers *Castor fiber* - toen alleen nog in de Biesbosch), ver voordat je deze als ecosystem engineer kunt benoemen. Later steeds kleiner: libellen, zweefvliegen (*Spilomya saltuum*!), loopkevers, waterwantsen. Zouden die mensen die mij al die kennis hebben bijgebracht beseffen dat ze mij in staat gesteld hebben om voedselwebben te bestuderen? In ieder geval wil ik ze hier bedanken.

Het duurde bij mij uiteindelijk tot ver in mijn studie voordat de overduidelijke samenhang tussen al die componenten duidelijk werd. 'Alles heeft met alles te maken' is misschien soms een beetje een cliché, maar desalniettemin zeer waar. Maar het is zaak om vervolgens in die brei van connecties de cruciale links te zien. Om de juiste zaken met elkaar te verbinden. Alle kritische commentaren van Jan Bakker, Joost Tinbergen, Theo Elzenga, Marten Staal en Richard Ubels, vooral tijdens de cursussen op schier, zijn van onmisbaar belang geweest voor het maken van deze connecties en het scherpen van de geest.

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*Maarten Schrama*

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